Immunological peculiarities in a “traditional” worm infested Papua New Guinean society and possible implications for the increase of allergies in fast changing “modern” societies

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Keywords

Allergy; atopic disease; worms; specific IgE; total IgE; specific activity; change theory; Papua New Guinea; sensitization; Radio Allergo Sorbens Test (RAST); Skin Prick Test; mite; clonality; affinity; traditional society; hygiene hypothesis

Abbreviations

ADHD	attention deficit hyperactivity disorder
B. tr.	Blomia tropicalis
CI	Confidence interval
CL	Confidence limit
df	degrees of freedom
D. fa.	Dermatophagoides farinae
D. pt.	Dermatophagoides pteronyssinus
FEIA	fluorescent enzyme immuno assay
fig.	figure
HDM	house dust mite
IgE	immunoglobulin E
Il-10	interleukin-10
log	logarithm (to the base 10)
n	number
ns	not significant (on a 0.05 alpha level)
OR	odds ratio
PEFR	peak expiratory flow rate
PNG	Papua New Guinea
r	Pearson Product-Moment Correlation Coefficient
RAST	radio allergo sorbens test
s	(sample) standard deviation
SPT	skin prick test
sTot-IgE	serum total immunoglobulin E

Figures and tables

Summary

The objective of this study is to describe the peculiarities of allergic sensitization in a Papua New Guinean society largely following a traditional lifestyle and to develop a theory explaining the increase of allergies in "modern" industrialized societies.

At the beginning, we intend to give a coherent introduction to the pathophysiology of allergic reactions, particularly to the immunoglobulin E (IgE) composition, a crucial characteristic of the IgE system. Subsequently mites are identified as the clinically most relevant allergen source on Karkar Island. We confirmed the association of worm infestation and highly elevated total IgE levels. Moreover a significant positive correlation of total IgE and specific IgE (in-vitro sensitisation measured by RAST) was shown. Thus we deduce that the entire IgE system is triggered by helminth infections i.e. not only total IgE (contested allergy protective variable) but also allergen specific IgE (undisputed allergy mediating variable). Interestingly, triggering the allergy mediating part of the IgE system (specific IgE) dos not seem to lead to an increased prevalence of positive skin prick test (SPT) reactivity or allergy on Karkar as we have previously found that only 4.4% of the islanders suffer from allergic rhinoconjunctivitis/asthma (atopic dermatitis was absent). The worm induced elevated total IgE levels appear to protect the islanders from allergic reactions and may explain the striking epidemiological discrepancy between high in-vitro (RAST) and low in-vivo (SPT) sensitisation. We were able to show a significant protective effect of a low ratio of specific IgE to total IgE (i.e. a low allergen specific activity): The prevalence of positive skin prick test reactions upon contact with house dust mite extracts was significantly reduced when mite specific activity was lower than 0.1%.

The pattern of sensitization on Karkar is an image/picture/reflection of the allergens existing in the island's environment – irrespective of the allergological potency of the different allergens. The assumed "general/twofold IgE boost" in worm infested individuals seems to have two consequences: firstly we see the already described dissociation of RAST and SPT/allergy. Secondly it is possible that the IgE boost reveals a pre-existing clinically and serologically invisible (not measurable) "low level" immunological sensitization against multiple environmental antigens. Pushing levels of allergic sensitisation over the detection limit, worms may act like a magnifying glass which shows us that the immune system generally controls/samples a much larger array of antigens than previously thought – including many allergologically "unsuspicious" substances. Based on the hypothesis that there exists the same basically universal "subliminal" immunological "screening" of innumerable environmental substances in Western countries (just serologically invisible due to the lack of worm boosts), we developed our "change hypothesis of allergogenesis": In a "modern environment" with immensely accelerated change resulting in largely increased antigen diversity there is much more possibility for cross-reactivity than in a "traditional" society. Recent studies show that even low concentration/affinity cross-reactive IgE can induce considerable histamine

release/clinical reactivity. Thus the rise of allergies in industrialized societies may be a consequence of generally increased specific activities (disappearance of parasitism) and increased cross-reactivity (“confusion” of the immune system).

1 Background part one: Type I allergies and their low prevalence on Karkar Island

Allergies are an increasing cause for morbidity and high expenses[1] in health systems all over the world. It is forecast, that allergic problems will keep increasing in future and will be a major "global public health concern".[2] In "developed", industrialized, "modern", affluent countries (shortly yet inaccurately: in the "West"), allergic diseases represent the most common cause of chronic illness.[3] Meanwhile allergic diseases are so "omnipresent" in our "modern life" that the word allergy[4] itself has started to be used in a figurative sense like "to be allergic to ones work or a despised person". In the early 19th century however, e.g. hay fever was still largely unknown – or at least regarded as an "unusual illness".[5] There is broad consensus that "modern" countries are more affected by the rise of allergic disease than less affluent[6] "traditional" societies.[7] Genetic predisposition is doubtlessly a factor contributing to the development of allergies;[8] yet genetic factors can not explain the fast epidemiological changes.[9] Thus environmental influences seem to be far more important.[10] Despite our rapidly increasing knowledge on allergies and the existence of innumerable – often contradictory – theories[11] we nevertheless do not know for sure why "modern Western people" more and more often display an immune system which erroneously classifies harmless environmental substances (pollen, animal epithelia, house dust mite proteins, foods etc.) as worthwhile recognizing and attacking. On Karkar Island,[12] the "traditional lifestyle", especially the old communicable parasitic infestations

1 The yearly socio-economic costs of allergic diseases in Australia was estimated 9.4 billion US-Dollar (reference year: 2005) (Australasian Society of Clinical Immunology and Allergy, 2011: 158).
2 Pawankar R, Canonica GW, Holgate ST, Lockey RF, 2011: 12.
3 Wright RJ, Sternthal MJ, 2011: 91.
4 The etymological meaning of allergy is basically „to react differently" (in comparison with the "normal/non-allergic" population). In other words: allergy points to an "altered [immunological] reactivity".
5 Mutius E, 2000: 9.
6 Unfortunately, in some studies we still find the somewhat crude dichotomy *developed - less developed* countries/societies/parts of the world (e.g. van den Biggelaar AH et al., 2001: 231; Pearce EJ, 2007: 1288). This is an ethnocentric terminology which should be avoided.
7 Pawankar R, Canonica GW, Holgate ST, Lockey RF, 2011: 17. Leung R et al., 1997: 354-360. Schäfer T, Ring J, 1997: 14-22. Aberg N, Hesselmar B, Aberg B, Eriksson B, 1995: 815-819. Woolcock A, Peat J, Trevillion L, 1995: 935-940. Wüthrich B, Schindler C, Leuenberger P, Ackermann-Liebrich U, 1995: 149-156. Turner K, Dowse G, Steward G, Alpers M, Woolcock A, 1985: 158-162. Ring J, 1997: 7-10.
8 Von Hertzen LC, Haahtela T, 2004: 131.
9 Mutius E, 2000: 9ff.
10 In respect to the considerable increase of type I allergies in "developed" countries Blaser et al. remark: "A perturbation of such magnitude must be environmentally caused…" (Blaser MJ, Chen Y, Reibman J, 2008: 562).
11 Jackson rightfully remarks: "As allergy blossomed in the modern world, so too did the range of explanations for its presence" (Jackson M, 2006: 23).
12 For details see chapter 4.1.

in the absence of what the author calls "exaggerated change"[13], still seems to convey a certain protection against the "modern noncommunicable disease allergy". Thus at least in respect to parasites (communicable disease) and allergies (noncommunicable disease), the islands population might not have to pass through a wearing period of double disease burden.

Whenever we talk about "allergies" in this work, we actually refer to type I allergies, which are immunoglobulin E (IgE)[14] mediated allergies.[15] The three clinical manifestations of type I allergies are also called atopic[16] diseases (allergic rhinoconjunctivitis, allergic asthma and atopic dermatitis/eczema[17]). Probably as a consequence of the rise of allergies in Western societies more than the "classical" organ systems seem to get involved: recently even a kind of "allergy" of the oesophagus has been described (eosinophilic esophagitis) – as one might have expected also with increasing prevalence. In addition to the three "classical" type I allergies there exist type II, III and IV allergies as well as autoimmune diseases. The underlying pathophysiology and the clinical symptoms of these other allergies are different from type I allergies, yet all of them represent "dysregulated adaptive immune responses"[18]. To describe these "non type I allergies" would be out of the scope of this work. Here, we exclusively focus on type I allergy (= atopy), the form of allergy we may call "the typical allergologic plague of the 21st century"[19] – at least if we talk about industrialized countries. In a patient suffering from an atopic disease, contact with the relevant allergen, which is per se an "innocuous antigen"[20], causes inflammation. This inflammation mediated by a "mislead" immune system is no longer protective, but harmful to the organism. Because of the short latency (some minutes) between allergen contact and clinical symptoms,[21] type I allergies are also called "immediate type allergic disorders". Depending on the location of the inflammation, the allergy manifests itself as rhinoconjunctivitis allergica (inflammation of the conjunctivae or the naso-pharyngeal mucosa, e.g. hay fever), allergic asthma (inflammation of the bronchial mucosa) or atopic dermatitis/eczema (inflammation of the skin, especially head neck and the flexural sites of extremities).

13 We will address the topic "exaggerated change" in chapter 6.3 under the heading of "change theory" of allergogenesis.
14 For details on IgE see subsequent chapters, especially chapter 2.1.
15 Wu LC, Zarrin AA, 2014: 247.
16 A misnomer – not only from the etymological point of view.
17 The expression "atopic dermatitis" is more used in Anglo-Saxon countries, whereas e.g. in the German speaking counties "atopic eczema" ("atopisches Ekzem") is more common. The terminus "dermatitis" focuses on the acute inflammation of the skin, whereas the word "eczema" includes the whole continuum from acute inflammation to chronic stages.
18 Reuter S, Stassen M, Taube C, 2010: 799.
19 Ring and Bergmann pointed out that allergies (in general) are sometimes called "the epidemics of the 21st century" (Ring J, Bergmann KC, 2014: XVIII). Jackson called allergies "a modern plague" (Jackson, 2006: 10).
20 Winter defines allergens as "intrinsically innocuous antigens that produce hypersensitivity responses" (Winter WE, Hardt NS, Fuhrman S, 2000: 1382).
21 Galli SJ, Tsai M, 2012: 693.

One of the major findings of our previous study on Karkar was (data collected in 1997), that the overall prevalence of type I allergies is strikingly low on the Island. Only 4.4% of the examined population sample suffered from an atopic disease (allergic rhinoconjunctivitis and/or allergic asthma and/or atopic eczema).[22] Other studies on allergy prevalence on Karkar do not exist, yet observations in Gaubin Hospital – the only hospital on the island – confirmed that allergic diseases are indeed rare on Karkar. Unfortunately we can only give a rather broad 95% Confidence Interval (95% CI) for the Karkar population. Based on our raw data we infer that between 2.2% and 7.8% of the Islanders suffer from type I allergies.[23] Despite of the "relatively elevated" upper 95% Confidence Limit (CL) of 7.8% it is obvious that the prevalence is much higher in the affluent "modern western world" where allergic rhinitis alone can affect up to around one third of the population.[24] Having investigated the socioeconomic and environmental conditions prevailing on Karkar, we finished our previous study with the conclusion that the "traditional lifestyle" seems to protect the islanders against atopic diseases. Yet we could not name single factors out of the many characteristics of traditional lifestyle, which may be responsible for this potentially protective effect. There are different reasons why we decided to include and re-evaluate rather "old" data from 1997 in the current study and combine them with unpublished raw data from 2002 and 2009 (for details in respect to composition and properties of the study population see table 3): Firstly we wanted to look more closely into serological details and to apply broader descriptive and analytical statistics. Secondly, by connecting traditional lifestyle with low prevalence of atopic disease in the year 2000 we basically "just" confirmed the "jungle/bush" hypothesis, i.e. we detected an association without naming possible causalities. Thirdly, original allergological raw data from 1997 allow a rare and valuable insight into the earlier times of an ongoing period of transition from tradition to modernity;[25] in other words: the Karkar society was still a bit more "traditional" than in 2017. Fourthly, combining our older and newer data allowed us to give (to our knowledge) the most precise description of allergologic sensitization currently available for Papua New Guinea (see fig. 19). Finally – and probably most importantly –, molecular biological methods like surface plasmon

22 Herbert O, 2009: 134. We only found allergic rhinoconjunctivitis and allergic asthma within our Karkar study group, yet no atopic eczema. Woolcock AJ et al. mentioned already in 1978 that "hay fever and eczema" are "rarely observed in Papua New Guinea. No prevalence data are available". (Woolcock AJ, Colman MH, Jones MW, 1978: 155).

23 We calculated the exact 95% CI for the percentage in the Karkar population using the Clopper and Pearson Method, not the slightly less accurate normal approximation. This is also why the lower and upper confidence intervals are not symmetrical in respect to the sample percentage.

24 Prevalence of allergic rhinitis in Ghent, Belgium 30.9% (Blomme K et al., 2013: 200ff). Prevalence in adolescents in the United Kingdom 38.6% and in the United States 33.6% (Strachan D et al. 1997: 161-76).

25 ImmunoCAP, the second generation RAST, was introduced in 1989 and improved in 1995 (Phadia. Thermo scientific: no pagination). Thus we dispose of precise and reliable IgE data from a very early time.

resonance measurements[26] have become available, extending our knowledge about the effects of the composition of the IgE repertoire.[27] This allows new explanatory models.
To start with, a low prevalence of atopic diseases in a heavily worm infested society may be considered an immunological paradox. There is a well documented T helper cell (Th cell) dualism between Th1 vs Th2[28] (Th cells exert the main regulation of the acquired immune system). Generally, a shift towards a Th1 response (e.g. triggered by bacterial and viral infection) represents protection from allergy; a shift towards a Th2 response represents a risk for allergy. Atopic subjects normally show a Th2 response. Worm infestations are known to trigger a strong Th2 like response[29]– even in the presence of simultaneous bacterial and viral infections[30] – and according to the above mentioned simplified dualism we would have to expect even more allergies on Karkar than in the "West".[31]

2 Background part two: Pathophysiological principles of type I allergies

Allergology is a complicated field as its immunological basics are complex. Our molecular biological knowledge increases steadily as more and more sophisticated methods – e.g. surface plasmon resonance[32] – become available. Yet knowing more details does not simplify the task of understanding the interwoven pathways behind allergic reactions. This chapter "Pathophysiological principles of type I allergies" will be split into three sections. The first part "From allergen contact to allergic reaction – an overview" will give a short yet hopefully coherent explanation of what actually happens in type I allergies. The second part "Mast cells" presents some particularities of these important effector cells. Finally the third part "IgE properties: Crucial in determining the extent of allergic reactions" has to go more into depth, however only as far as it is necessary for understanding and interpreting our subsequent results from Karkar Island.

26 See chapter 2.3.5.
27 Admittedly, despite of these recent advances, much of the regulation and production of IgE remains "poorly understood" (Wu LC, Zarrin AA, 2014: 247).
28 "… type-1 and type-2 responses inhibit each other…" (Adams JF, Schölvinck EH, Gie RP, Potter PC, Beyers N, Beyers AD, 1999: 2030).
29 "Infections with helminth parasites generally induce a strong polarization towards a Th2 type response …" (Helmby H, 2009: 122). Erb et al. state: "A prominent feature of infections with helminths is that they selectively induce strong Th2 type immune responses …" (Erb KJ, 2007: 1170) and Gause et al. remark: "this response [to helminths] is largely defined by type-2 immunity" (Gause WC, Urban JF Jr, Stadecker MJ, 2003: 269).
30 As van den Biggelaar states: In developing countries helminth infections "… shift the overall responses towards Th2" (van den Biggelaar AH et al., 2000: 1723).
31 We agree with van den Biggelaar et al: "Paradoxically, in countries where helminth infections have high prevalences and the immunological balance is shifted towards Th2-type responses, allergic disorders are least prevalent" (van den Biggelaar AH et al., 2000: 1723). In this respect Smits et al. refer to "The Helminth Paradox" (Smits HH, Everts B, Hartgers FC, Yazdanbakhsh M, 2010: 5).
32 See chapter 2.3.5.

2.1 From allergen contact to allergic reaction – an overview

The more complex systems are, the more helpful it is to grasp the basic concepts by simplification. Yet under these circumstances avoiding falsification becomes a crucial task. So we try to put type I allergic reactions in the following single phrase nutshell: "When the allergen (e.g. house dust mite protein) activates (cross-links) the specific levers (two or more immunoglobulin E molecules on mast cells), the 'allergological bomb' (mast cell, see chapter 2.2) explodes (degranulates), releasing diverse pro inflammatory substances (e.g. histamine), responsible for the allergic symptoms (rhinoconjunctivitis, asthma or atopic dermatitis)" (see fig. 1).

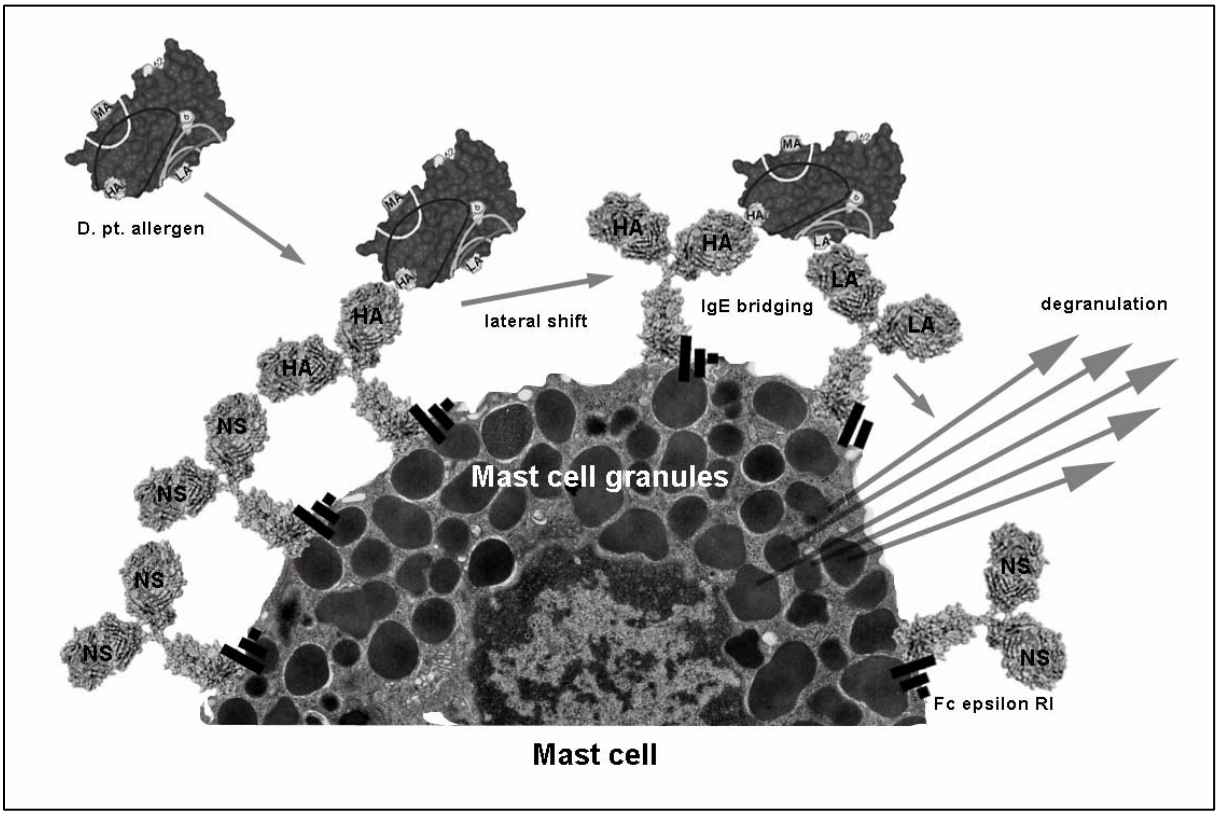


Fig. 1. Model of mast cell degranulation (proportions arbitrary): Mast cells contain multiple mediator filled (incl. histamine) granules.[33] The FcεRI receptors (black squares) bind non-specific (NS) and specific IgE to the cell surface.[34] The D. pt. allergen[35] binds with (preferably) a high affinity epitope (HA) to a D. pt. specific IgE with sub-specificity for the HA epitope (IgE labelled HA). The IgE-allergen complex probably drifts over the mast cell surface until it encounters an IgE with different D. pt. sub-specificity, e.g. a low affinity (LA) IgE. Cross-linking of two IgE activates an intracellular cascade resulting in degranulation and release of mediators.

33 Electron-microscopical picture of mast cell modified from Histology@yale. Mast cell EM: no pagination.
34 Crystal structure of IgE modified from Handlogten MW et al., 2013: 790. For FcεRI see chapter 2.2.
35 Three dimensional model of the major HDM allergen D. pt. 2 modified from Christensen et al., 300. A more detailed figure of this allergen including an epitope description may be found in chapter 2.3.4.

In a chronological order, the first thing to happen if the "bomb" shall explode on contact with a specific substance (allergen), the body must build the corresponding substance specific lever. This lever is the protein Immunoglobulin E (IgE). IgE only represents a tiny proportion of the serum proteins (50-200 ng/ml blood)[36]. Despite of the fact that the IgE fraction represents the smallest group of the five classes (isotypes) of immunoglobulins (antibodies) it conveys considerable effects.[37] Thus IgE may be called the "immunologic gatekeeper of the type I or immediate hypersensitivity response".[38] The very first i.e. initial production of "IgE specific to a certain substance" (in short: specific IgE) is called sensitization. Only a person who passed through this initial process can develop type I allergy mediated symptoms. Sensitization itself is a complex process involving the collaboration of different immune competent cells like macrophages/dendritic cells/Langerhans cells/B cells (processing of the original substance and subsequently presenting it), T helper cells (recognizing the processed and presented substance and building of cytokines like interleukin 4), B-cells (stimulated by interleukin 4 to undergo "immunoglobulin class switch"[39]) and plasma cells (activated B-cells, producing specific IgE).[40] At the end of the sensitization process, the immune system has learned to recognize a substance.[41] This acquired knowledge of the immune system is "embedded" in the specific IgE, which dock on the surface of mast cells, representing the specific "lever on the bomb".

2.2 Mast cells

In an allergic person, re-exposition to the relevant allergen (after sensitization) triggers the release of a broad array of pro-inflammatory mediators from effector cells (mast cells, basophils, eosinophils).[42] Mast cells, one of the major effector cells of allergy,[43] are mainly located in tissues[44] where the individual comes in close contact with external allergens,[45] e.g. the gastrointestinal mucosa (consequence: oral allergy syndrome, diarrhoea), the respiratory tract mucosa (consequence: rhinitis, asthma), the conjunctivae

36 Wu LC, Zarrin AA, 2014: 247.
37 "The normal concentration of IgE is only 0.05% of the IgG concentration." (Winter WE, Hardt NS, Fuhrman S, 2000: 1385). Data given by Wu indicate that concentration of free IgE is only about 0.001% to 0.01% of other immunoglobulin isotypes (Wu LC, Zarrin AA, 2014: 247).
38 Hamilton RG, MacGlashan DW Jr, Saini SS, 2010: 274.
39 Immunglobulin class switch is the cytokine (e.g. interleukin 4) triggered "reprogramming" of a B-cell/plasma-cell. Following class switch, the cell does not produce IgM, IgG or IgA (type I allergy irrelevant) but IgE (crucial for type I allergies) (Galli SJ, Tsai M, 2012: 693).
40 Galli SJ, Tsai M, 2012: 693.
41 The backbone of IgE memory is "… re-activation and differentiation of [IgE switched] memory B cells following a secondary encounter with the same antigen" (Wu LC, Zarrin AA, 2014: 252).
42 Christensen LH et al., 2008: 298.
43 Galli SJ, Tsai M, 2012: 693.
44 "Mast cells are tissue-based inflammatory cells …" (Stone KD, Prussin C, Metcalfe DD, 2010: S75).
45 Urb M, Sheppard DC, 2012: 1.

(consequence: conjunctivitis) and the skin (consequence: atopic dermatitis[46]). During skin prick testing (see chapter 4.2) activation of dermal mast cells by test allergens is used to demonstrate sensitization against the tested substances. One of the most important mediators is the pro-inflammatory histamine. Within minutes after mast cell activation[47] (mediated by mast cell bound IgE, see next chapter) the preformed cytoplasmic granules "fuse with the [mast cell] plasma membrane"[48] (i.e. surface) and histamine[49] is released.[50] This process is called degranulation. Within minutes the histamine causes inflammation[51] of the surrounding tissue. This is why type I allergies are also called immediate type allergic reactions. Mast cell activation resulting in histamine release occurs, when (theoretically) two surface bound IgE (= antibodies) are cross-linked/bridged by the respective allergen (= antigen).[52] Every mast cell displays of innumerable docking molecules for IgE embedded in its plasma-membrane/surface, the so called "high affinity IgE receptors" (FcεRI).[53] The high affinity of these receptors is supposed to represent one of the reasons for the very low concentrations of circulating free IgE.[54] The composition of IgE bound to the mast cell surface reflects the composition of IgE in the human serum.[55] This is because IgEs with different allergen specificities nevertheless display equal affinity to the IgE receptor. Perivascular mast cells are able to extend cell processes across the blood vessel wall to actively acquire IgE and sample the (currently) circulating IgE composition.[56] In summary, the severity of type I allergic reactions is defined by the degree of mast cell degranulation.[57] The extent of mast cell degranulation itself largely depends on the kind and composition of IgE bound to the mast cell surface. Therefore the following chapter exclusively focuses on IgE and IgE properties, or – as we put it before – we will have a closer look at the lever (IgE) of the allergological bomb (mast cell).

46 Triggering atopic dermatitis by direct allergen contact to the skin is possible (e.g. airborne contact dermatitis), yet more often systemic exposition to allergens causes skin aggravation (e.g. inhalation of pollen in spring often provokes dermatological deterioration).

47 Reuter S, Stassen M, Taube C, 2010: 798.

48 Stone KD, Prussin C, Metcalfe DD, 2010: S75.

49 We will focus on histamine though mast cell granula contain a large variety of mediators: tryptase, chymase, tumour necrosis factor alpha etc (Urb M, Sheppard DC, 2012: 1 and Reuter S, Stassen M, Taube C, 2010: 797).

50 Urb M, Sheppard DC, 2012: 1 and Reuter S, Stassen M, Taube C, 2010: 798.

51 As any inflammation allergic inflammation is characterized by: dolor, calor, rubor, functio laesa.

52 Christensen LH et al., 2008: 298. Actually, approximately 1,000 cross-linkings seem to be necessary to trigger effector cell degranulation. This number was estimated by MacGlashan DW et al. for basophils (MacGlashan DW et al., 1997: 1438-45).

53 FcεRI is also expressed on eosinophils (Gounni AS et al., 1994: 183ff), basophils, dendritic cells and macrophages. Besides of FcεRI there exists another IgE receptor: the low-affinity receptor for IgE (FcεRII = DC23) (Wu LC, Zarrin AA, 2014: 247).

54 Winter WE, Hardt NS, Fuhrman S, 2000: 1382.

55 Otherwise specific IgE measurements (RAST) in the serum would be quite useless.

56 Wu LC, Zarrin AA, 2014: 256.

57 „In fact, the acute symptoms associated with allergic pathologies largely result from the degranulation of mast cells" (Pearce EJ, 2007: 1288).

2.3 IgE properties: Crucial in determining the extent of allergic reactions

The Y-shaped IgE molecule (see fig. 1) disposes of two identical allergen-binding sites (variable regions at the "tips of the Y") and one Fc fragment (constant region at the "base of the Y")[58]. The Fc fragment binds to the FcεRI receptor on effector cells (mast cells, basophils)[59], the allergen-binding sites "define" the specificity of the IgE molecule. The reaction of a person having contact to a potential allergen largely depends on his/her IgE "composition/repertoire/profile".[60] This composition is reflected by the properties of IgE on mast cells (and other effector cells). The clinically most relevant IgE properties – generally and in respect to our work – are specificity, specific activity, clonality and affinity, which shall be explained in the following sections.

2.3.1 "Non"-specific IgE

"Non"-specific IgE is an imprecise expression. "Non"-specific in relation to what? We actually do not know whether certain IgE molecules we call non-specific are really non-specific to any allergen/antigen. The latter would mean, that this IgE does not bind/recognize any potential antigen existing. Maybe we just did not use the right serological allergy test. Or the IgE is directed against some structure not relevant in allergological respect and the antigen-antibody complex is simply not resulting in any pathologic reaction. Yet without an initial suspicion of pathology, a test is normally not developed. Thus we may just not be able to detect a specific binding even if we had used all the currently available allergy/antigen kits. Due to these considerations we put the "non" of "non"-specific in quotation marks as we may be speaking of specific IgE with (at present!) unknown specificity. Already in 1986 Turner KJ was precise enough to talk about IgE "of undefined specificity"[61]. Logically "non"-specific IgE can not be measured directly: we have to measure the total IgE in the sera (sTot-IgE) and subtract the sum of specific IgE levels. This simple fact points to the "paradox" that the more different IgE specificities are determined in a subject, the smaller the "non"-specific IgE fraction gets. Anticipating our results at the end of chapter 5.5 we shall give an estimate of the "IgE conditions" within our Karkar data base: "non"-specific IgE (i.e. IgE with unknown specificity) represents 99.4% of our sTot-IgE and specific IgE[62] represents only 0.6%.[63]

58 Rosenwasser LJ, 2011: 178.

59 Furthermore the FcεRI receptor is expressed on dendritic cells, (activated) macrophages and Langerhans cells (Rosenwasser LJ, 2011: 179). According to Stone et al. "eosinophile expression of FcεRI is minimal" (Stone KD, Prussin C, Metcalfe DD, 2010: S78).

60 This notion was reconfirmed by Christensen LH et al.: "We show that the composition of the allergen-specific IgE repertoire is of major importance for basophil degranulation…" (Christensen LH et al., 2008: 302f). Hamilton RG and Saito H used the expression "IgE antibody profile" for the above mentioned "IgE repertoire" (Hamilton RG, Saito H, 2008: 305).

61 Turner KJ referred to the large quantities of IgE produced in parasite infected subjects (Turner KJ, Dowse GK, Stewart GA, Alpers MP, 1986: 562).

62 Twenty pooled specific IgE vlaues.

2.3.2 Specific IgE

Specific IgE means that we refer to an IgE with known allergologic/antigenetic target. That is, if the IgE (antibody) exclusively binds to house dust mite allergen (antigen), we call this IgE house dust mite specific IgE. Specific IgE is clinically one of the most useful biomarkers, as its detection allows linkage of symptoms to particular allergens.[64] Furthermore, the likelihood of a clinical reaction to a certain allergen (normally) increases with the level of the corresponding specific IgE.[65] However, sometimes specific IgE can be detected in the absence of clinical allergy. The "reasons for this apparent lack of agreement … [between presence of specific IgE and absence of allergic symptoms] … have not been fully elucidated."[66] In the course of this work we intend to give some explications for the "lack of agreement" based on specific activity and clonality (see subsequent chapters).

In some cases the specific IgE antibody may be able to bind to two (or more) different (not necessarily related) antigens (yet we still consider this specific binding): e.g. a house dust mite specific IgE recognizes similar steric structures of a shrimp protein (cross-reactive tropomyosin)[67]. A possible consequence of the existence of these "cross-reacting specific IgEs" is that a house dust mite allergic patient may suddenly develop intolerance against shrimps. Specific IgE levels within a population – as IgE levels generally – are not normal distributed. They do not follow the typical bell-shaped symmetrical density function as part of the population shows extremely high IgE values. Yet normal distribution is necessary for performing parametric statistical tests. Fortunately normalization is "easily" achieved by using the logs of the IgE values (see chapter 5.2, figs. 14 and 15 in respect to sTot-IgE).[68] The so called "log normal distribution" of specific IgE is reflected in the six semi quantitative RAST-classes[69]. The RAST-classes are traditionally used to display the level of specific IgE, in other words, the degree of allergic sensitization of an individual against a certain substance. Corresponding to the log

63 All percentages refer to the respective geometric means.

64 Hamilton RG, MacGlashan DW Jr, Saini SS, 2010: 274.

65 Siles RI, Hsieh FH, 2011: 585.

66 Hamilton RG, MacGlashan DW Jr, Saini SS, 2010: 274.

67 Witteman AM, Akkerdaas JH, van Leeuwen J, van der Zee JS, Aalberse RC, 1994: 56ff.

68 Due to this fact, most studies log-transform sTot-IgE and specific IgE values. For example: "All the statistical analyses … used log IgE values" (Omenaas E et al., 1994: 532). Barbee RA et al. state: "… the skewed distribution of immunoglobulin E in all populations that have been studied has made it necessary to convert from arithmetic to logarithmic values … Following logarithmic conversion a normal distribution curve is evident…" (Barbee RA, Halonen M, Lebowitz M, Burrows B, 1981: 109). In agreement van den Biggelaar AH et al. note: "[IgE] Antibody levels were not normally distributed and [statistical] analysis was therefore performed on log-transformed data…" (van den Biggelaar AH et al., 2001: 233).

69 "RAST" (Radio Allergo Sorbent Test) is actually a misnomer as the test is not requiring radioactive labelling any more. Thus Hamilton RG and Williams PB suggest to "cease using the term "RAST" because it is outdated". Instead they propose "serologic IgE antibody assay" (Hamilton RG and Williams PB, 2010: 34). Yet in this study we keep using the expression "RAST" because of its shortness and for conventional reasons.

normal distribution of specific IgE, the higher the IgE level gets, the broader the RAST-class bins become (e.g. see y-axis in fig. 40).

2.3.3 The specific IgE to total IgE ratio: the so called "specific activity"

In allergology, (serum) specific IgE and (serum) total-IgE (sTot-IgE) have been measured for a long time (sTot-IgE is the sum of specific IgE and "non"-specific IgE). The detection of specific IgE indicates sensitization against the corresponding allergen. In Western societies, the total IgE level normally represents a more general indicator for an allergic diathesis (in allergic individuals often over 100 kU/l, 1U equivalent to 2.4 ng).[70] In recent years it became apparent that the "ratio of (one certain) specific IgE to total IgE" – i.e. the specific activity (SA) – is a very important allergological marker[71]. It was shown that SA even predicts the efficacy of "new" allergy treatments[72] and "old" allergy treatments[73]. We shall give a simple example for SA: if one out of 100 IgE molecules happens to be specific to house dust mite, the (house dust mite) SA is one percent. The smaller the SA is, the less likely it is, that two IgE molecules with the same allergen specificity are located (or "get located" in the case of lateral shift) in direct vicinity on the mast cell surface. Yet this vicinity is necessary for the bridging/cross-linking of the two specific IgE by the corresponding allergen.[74]

In 2008 Christensen LH et al. were the first to develop an elegant model[75] which allows describing with unprecedented precision and "unique flexibility"[76] how effector cell[77] degranulation is affected by the most important properties of the individual IgE repertoire:

70 Winter WE, Hardt NS, Fuhrman S, 2000: 1382. Other reasons for increased total IgE levels exist: immune system neoplasias (uncommon) or parasitic infections (in Western societies not very common any more).

71 For example Christensen LH et al., 2008: 289-304, Hamilton RG and Williams PB, 2010: 36 and Johansson SG et al., 2009: 1472-7.

72 Johansson SG et al. referred to the anti-IgE omalizumab (Xolair®) (Johansson SG et al., 2009: 1472-7). More information about omalizumab is given in chapter 6.1.

73 Di Lorenzo G et al. showed that SA is helpful in predicting clinical response to allergen-specific immunotherapy (Di Lorenzo G et al., 2009: 1103ff).

74 "The higher specific IgE/total IgE ratio increases the probability that 2 IgE molecules with the same allergen specificity will be docked in their receptor close to each other on an effector cell … and thus be more readily cross-linked by a given allergen molecule" (Hamilton RG, Saito H, 2008: 306).

75 "A panel of recombinant IgE (rIgE) antibodies specific for the major house dust mite allergen Der p 2 (see fig. 3, chapter 2.3.4) was developed and characterized in regard to Der p 2 affinity, as well as Der p 2 epitope specificity, by using surface plasmon resonance technology. Human basophils were sensitized with different combinations of rIgEs, and degranulation responses were measured by means of flow cytometry after challenge with Der p 2" (Christensen LH et al., 2008: 289). Surface plasmon resonance is described in chapter 2.3.5. "Sensitization of basophils" means, that the original native/physiological IgE repertoire is stripped of the cells and replaced by an exactly defined composition of predetermined "artificial" IgE (=sensitization). Flow cytometry allows to differentiate between degranulated and non-degranulated basophils and to determine the ratio of both states.

76 Hamilton RG, Saito H, 2008: 305.

77 Experiments were performed with basophils which are similar to mast cells in many respects (Christensen LH et al., 2008: 289-304).

SPECIFIC ACTIVITY, IgE CONCENTRATION, CLONALITY and AFFINITY.[78] Their estimations have become possible, combining advanced immunological techniques like antibody cloning, generation of recombinant chimeric (mouse/human) IgE, surface plasmon resonance measurements[79] for epitope mapping and flow cytometry to determine basophil activation. Hamilton RG and Saito H are certainly right when they underline that the "future diagnostic implications of these [Christensen's] observations are profound".[80] Figure 2 shows the effect of different SA levels on effector cell degranulation in vitro. Christensen's original figure has been modified to display only facts which will be relevant at later stages of our work.

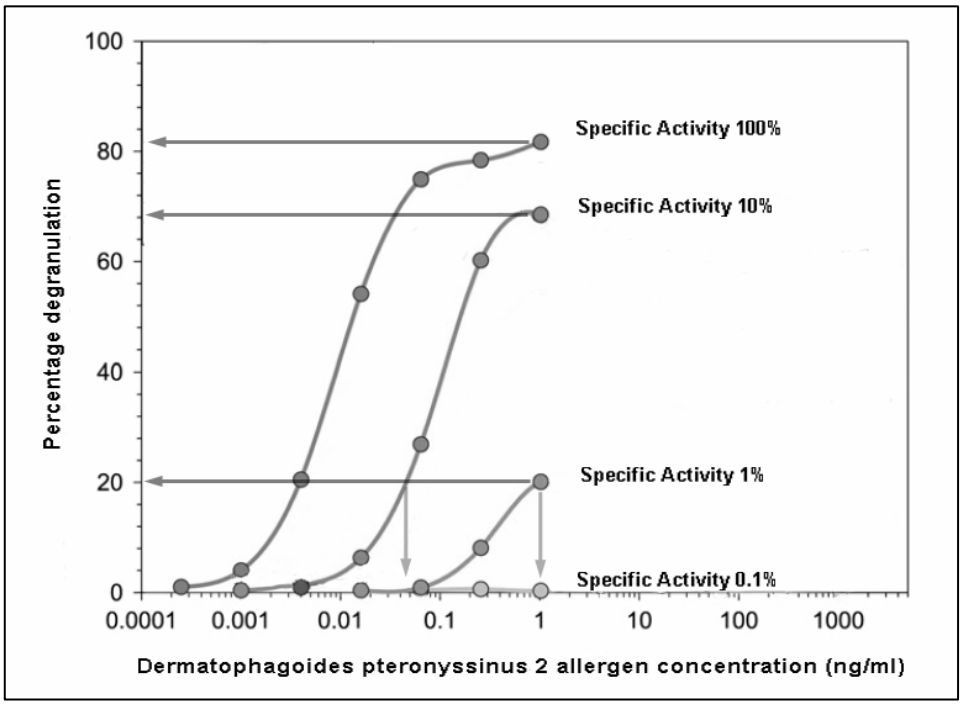


Fig. 2. Level of (in-vitro) effector cell degranulation triggered by (recombinant) Dermatophagoides pteronyssinus 2 challenge at different specific activities.[81] Lower than 0.1% SA: total suppression of degranulation (0% degranulation). Lower than 1% SA: suppression of degranulation (maximum 20% degranulation). At 10% SA 70% degranulation and at 100% SA 82% degranulation (in all cases degranulation percentage in respect to stimulation with 1 ng/ml recombinant major HDM allergen rDer p 2). The vertical arrows point to different allergen sensitivities at different SAs: at 1% SA 22 times more allergen is necessary (1 ng/ml) to generate 20% degranulation than at 10% SA (0.045 ng/ml allergen concentration is sufficient) (modified from Christensen LH et al., 2008: 302).

78 For details in respect to clonality and affinity see subsequent two chapters.
79 See chapter 2.3.5.
80 Hamilton RG, Saito H, 2008: 305.
81 At all different specific activities, the composition of the specific IgE mixture consisted of "equimolar quantities of 3 [high affinity] rIgE clones … recognizing nonoverlapping Der p 2 epitopes" (Christensen LH et al., 2008: 302, fig. 4).

Even with very high D. pt. allergen concentrations (1 ng/ml, x-axis) there is no degranulation if SA is under 0.1%. At SA levels between 0.1% and 1% degranulation is markedly reduced with a maximum of just 20%. The most pronounced raise of degranulation occurs between 1% and 10% SA: degranulation increases from 20% to 70%. A further tenfold increase of SA (from SA 10% to SA 100%) increases degranulation only marginally from 70% to 82%. Yet SA does not only affect maximum degranulation levels (horizontal arrows) but also "sensitivity" (vertical arrows) of effector cells. Our example shows that cells sensitised with 10% SA react already upon stimulation with 0.045 ng/ml allergen (consequence: 20% degranulation). Cells sensitised with only 1% SA however do not release histamine at this allergen concentration (they are not "sensitive" enough) but a 22 times higher allergen concentration is needed (1 ng/ml) to trigger 20% degranulation.

2.3.4 Properties of specific IgE: Clonality

Talking about IgE with one certain specificity (e.g. against HDM) does not imply, that all the antibodies forming this group are necessarily equal. On the contrary: for an allergic reaction to take place in vivo they have to be different in the following way: despite of the same "overall" specificity for a certain allergen they must show diverse "sub specificity". A house dust mite allergen for example – as any natural allergen – shows a unique steric structure, a surface formed like an uneven non-symmetrical piece of rock (see fig. 3).

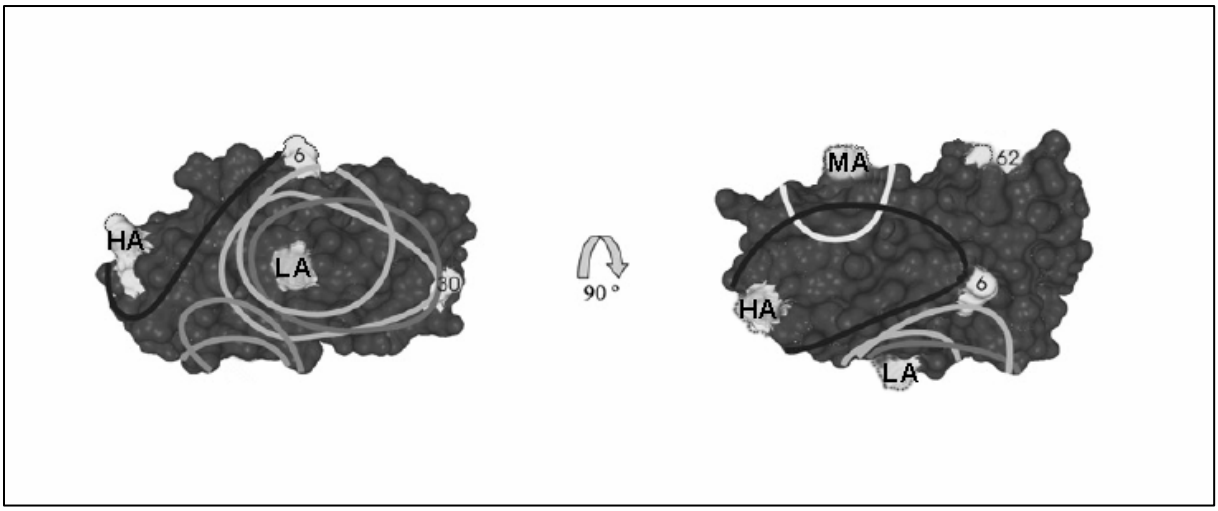


Fig. 3. Structure of Dermatophagoides pteronyssinus 2 allergen (Der p 2), a major dust mite allergen (Derewenda U et al., 2002: 189ff). Allergen mapping showing epitope positions: Each epitope is represented by an amino acid with central position in the epitope (light areas) and corresponding epitope binding regions for D. pt. specific IgE (elliptic markings). Epitopes have been arbitrarily labelled for illustrative reasons by the author: HA = high affinity epitope; MA = medium affinity epitope; LA = low affinity epitope. Due to overlaps of the HA and MA epitope-binding-regions no simultaneous binding of IgE from these two clones is possible (modified from Christensen LH et al., 2008: 300). Der p 2 may serve a biological function "involving binding and/or transport of a lipid-like molecule" (Derewenda U et al., 2002: 193).

Some of the surface structures, the (immunogenic) epitopes, are particularly prone to be recognized by specific IgE.[82] As there are several structures/epitopes on an allergen which can be recognized by antibodies (average: two to twelve[83]), allergens are said to be "multivalent" in respect to IgE binding.[84] When a specific IgE binds/recognizes a certain epitope, it occupies and accordingly blocks the corresponding surface region. Another IgE with exactly the same (or overlapping) allergen- and epitope-specificity can not bind/recognize the allergen simultaneously. Consequently two "totally" equal IgE (in respect to allergen specificity and epitope sub-specificity) can not be cross-linked by an allergen, thus no degranulation occurs.[85] Logically, cross-linking of two IgE molecules by an allergen is only possible, if the "appropriate" IgE are in direct vicinity on the mast cell surface. Yet the surface is covered by IgE with innumerable different specificities (representing the whole serum IgE repertoire). How is it possible that two (or more) IgE with the same specificity (and different sub-specificity) happen to be placed next to each other? As soon as a mast cell bound specific IgE molecule "captures" the corresponding epitope of an allergen, the FcεRI-IgE-allergen complex seems to start to diffuse "laterally across the surface of the mast cell until a second IgE [with the same specificity and different sub-specificity] is encountered" and cross-linking occurs.[86] In short: To allow histamine release two (or more) neighbouring IgE have to display the same allergen specificity yet different epitope specificity[87] (sub-specificity). Specific IgE with the same epitope specificity is said to have the same clonality. This is, because all these "totally identical" specific IgEs were generated by the same clone of plasma cells. Talking about increased "clonality" is a short form of referring to increased "number of epitopes [on a certain allergen], recognized by the IgE repertoire".[88] Picturing clonality in our lever-bomb system we could state: If only one kind of allergen specific lever exists (one sub specificity, i.e. one clonality) the bomb never goes off, if two types of lever exist (two sub

82 Handlogten MW, Kiziltepe T, Serezani AP, Kaplan MH, Bilgicer B, 2013: 789.

83 From the two to twelve epitopes only one to five seem to be "immunodominant, meaning they are involved in the degranulation" in the "majority" of subjects with that particular allergy (Handlogten MW, Kiziltepe T, Serezani AP, Kaplan MH, Bilgicer B, 2013: 789). In the case of Der p 2 three epitopes are currently known to be immunogenic (Derewenda U et al., 2002: 195).

84 Handlogten MW, Kiziltepe T, Serezani AP, Kaplan MH, Bilgicer B, 2013: 789.

85 Christensen et al. could prove this assumption in-vitro: "Basophils monosensitized with a single Der 2-specific rIgE … showed no degranulation, as expected, because of the requirement for the presence of IgEs having at least 2 different epitope specificities for productive cross-linking" (Christensen LH et al., 2008: 301).

86 Handlogten MW, Kiziltepe T, Serezani AP, Kaplan MH, Bilgicer B, 2013: 792. This theory is supported by results of Christensen et al., who state: "[Our] experimental observation is in agreement with a model previously put forth by Aalberse et al., who hypothesized that an allergen initially becomes captured by a high-affinity FcεRI-bound "anchor" IgE and is then dragged over the cell surface until this complex encounters other, even low-affinity, FcεRI-bound IgEs causing efficient cross-linking and effector cell degranulation" (Christensen LH et al., 2008: 303).

87 Non-overlapping allergen surface regions must be recognized.

88 Hamilton RG, Oppenheimer J, 2015: 834. From the "IgE point of view" one could as well follow Christensen who defined clonality as "the number of different IgE clones binding 1 allergen molecule simultaneously" (Christensen LH et al., 2008: 301).

specificities, i.e. two clonalities for the same allergen) the bomb can explode (the requirement of recognizing at least two different epitopes could be understood as an immunological safety mechanism). If three types of lever exist (three clonalities, consequence: increased FcεRI-IgE-allergen clustering) the bomb will go off at lower allergen concentrations than in the case of only two clonalities[89] (increased sensitivity) and so forth. Clonality may be regarded as an "expression of immunological confidence": the more clones recognize an allergen (higher clonality), the "more confident" the immune system seems in categorizing the respective antigen as a "dangerous intruder". This increased "immunological confidence" is expressed as increased effector cell sensitivity (in-vitro)[90] and increased skin prick test (SPT)[91] reactivity or clinical symptoms (in-vivo). The molecular biological reason behind the positive correlation of clonality and effector cell degranulation may be found in the three dimensional structure of the allergen: as soon as an allergen is captured by a mast cell bound IgE, the captured allergen is "fixed/immobilized" in a certain position (lateral shift seems possible, however). The position of the entire allergen should determine the position of the remaining free epitopes. Maybe a second mast cell bound IgE with clonality A can not reach/bind its corresponding epitope A on the fixed allergen (as epitope A sticks out in an inappropriate angle, pointing away from the mast cell). But a third IgE with clonality B may be able to cross-link with epitope B which happens to be in the right position.

2.3.5 Properties of specific IgE: Affinity

The concept of affinity is quite easy to grasp. The better a specific IgE molecule "fits" upon an epitope of a specific allergen, the stronger it will hold/bind to that region. If IgE were magnetic, affinity would in a way describe the strength of the magnet. Hamilton RG et al. defined affinity simply as "tightness of binding".[92] High affinity results in a strong tendency of a specific IgE to associate with the corresponding epitope and thereafter a low tendency to dissociate again. The affinity between specific IgE (antibody) and corresponding allergen/epitope (antigen) can be determined by surface plasmon resonance measurements.[93] Differences between high and low/weak affinity interactions are huge:

89 Provided – of course – that the total number of allergen specific IgE bound to the mast cell surface is kept equal (Christensen et al., 301ff).

90 The effect that "higher IgE clonality increases basophil sensitivity" was proved by Christensen's precise study: "Basophils sensitized with 3 different rIgE clones … showed 5- to 20-fold higher sensitivity than basophils sensitized with only 2 different rIgE clones". As generally in Christensen's study, all relevant "other parameters were kept constant" (Christensen et al., 301ff).

91 Skin prick tests represent a reliable and uncomplicated way of testing allergic sensitization in vivo. For details see chapter 4.2.

92 Hamilton RG, MacGlashan DW Jr, Saini SS, 2010: 275.

93 Christensen LH et al., 2008: 298ff.
Surface plasmon resonance is an extremely sensitive method to measure adsorption and the strength of adsorption (affinity) of molecules, proteins and (for our purposes) antibodies (e.g. IgE) onto an antigen (e.g. allergen) coated chip. Binding of particles leads to emission of light which is measured by the sensor chip.

Handlogten considered a 760-fold difference adequate to model natural allergy systems.[94] Christensen LH et al. found an approximately 300-fold affinity difference in specific IgE produced by nine different clones and directed against one specific HDM allergen (recombinant D. pt. 2) in one HDM allergic patient.[95] High affinity antibodies seem to be produced in germinal centres of lymph nodes (affinity maturation by accumulation of somatic mutations).[96] Like in the case of high clonality, high affinity also leads to increased mast cell sensitivity[97]. Yet things are slightly more complicated: as it is relevant for our later conclusions it shall be mentioned already at this point that recent biomolecular studies revealed that – quite contrary to earlier expectations – even weak affinity IgE play an essential role in mast cell degranulation. However a requirement for this is that a high affinity IgE clone is involved in the allergen-IgE clustering on the mast cell as well.[98] A reason for this finding may be that a high affinity IgE molecule is necessary to initially capture and hold the allergen (as already shown in fig. 1). After the FcεRI-IgE-allergen complex has diffused laterally across the mast cell surface an encounter with a low affinity IgE molecule [with the same specificity and different sub-specificity] may be sufficient to allow for cross-linking. Another fact in relation to affinity which will be relevant at the end of this work is the following: A minimal change in the allergen structure can have an immense effect on affinity. Christensen LH et al. demonstrated that the replacement (mutation) of just one amino acid in a central position of an epitope (compare fig. 3) of the HDM allergen D. pt. 2 "led to abolished antibody [IgE] binding"[99]. In other words: the smallest change which is possible in a protein (the exchange of only one amino acid) can turn high affinity into cero affinity – and vice versa.

94 Handlogten MW, Kiziltepe T, Serezani AP, Kaplan MH, Bilgicer B, 2013: 791.

95 Christensen LH et al., 2008: 302.

96 Wu LC, Zarrin AA, 2014: 256. (Early) low-affinity antibodies are probably produced at extrafollicular sites (Ibid., 251).

97 Christensen et al., 301ff.

98 Handlogten et al. designed a heterotetravalent artificial allergen (imitating a natural allergen's epitope heterogeneity) to prove that the selective inhibition of the low-affinity IgE (which recognized this allergen) was sufficient to suppress mast cell degranulation – even if high affinity IgE was still able to interact with the allergen (Handlogten MW, Kiziltepe T, Serezani AP, Kaplan MH, Bilgicer B, 2013: 789ff).
Christensen LH et al. showed that the combination of a high with a low affinity IgE clone directed against a recombinant HDM allergen triggered the same maximal degranulation in-vitro as two high affinity IgE clones; just sensitivity was slightly diminished when a high affinity clone was combined with a low affinity clone (Christensen LH et al., 2008: 303). The relevance of this finding for our work will be discussed in chapter 6.3.

99 Christensen LH et al., 2008: 299 and fig. E on page 300.

3 Objectives

The major two questions are: Why is there a positive correlation of Western lifestyle and prevalence of allergies and how can we avoid that "developing" traditional societies have to pay the price of an increasing prevalence of allergies in future? In order to answer these questions, we investigate the effects of a traditional Melanesian lifestyle (in particular helminth infections) on allergic sensitization and allergic disease on Karkar Island (see chapter 4.1), Papua New Guinea (PNG). Curiously – despite of the historically "short" period of independence from Australia (1975) – research activity at the University of Sydney seems rather limited in the former trust territory: Since 1975 only 78 out of 16,900 theses (0.46%) dealt with PNG and in the field of Public Health only 4/349 (1.1%).[100]

To start with, we intend to identify the clinically most relevant allergen(s) on Karkar Island. Based on our 1997 data, we already suspected mite antigens to represent the most important allergens on Karkar.[101] Yet this result was not too reliable, as the number of allergic subjects in the general population on Karkar is very low. Therefore we will now investigate the in vivo sensitization pattern of a deliberately selected group of 28 allergic (often asthmatic) patients (data collected in 2002, status of subjects re-evaluated in 2009; for more details in respect to composition and properties of the study population see table 3). In this context we want to measure the peak expiratory flow rates (PEFR) of asthmatics and give reference values for the female and male population on Karkar. Thereafter we will try to confirm high serum total-IgE (sTot-IgE) levels in a village society suffering from parasitic infestations. Subsequently we will investigate the relationship of worm infections and elevated sTot-IgE. Then we will complete our knowledge about allergic sensitization in Papua New Guinea incorporating more skin prick test (SPT) results in our pre-existing Karkar data base. Thereafter we compare serological (in vitro) sensitization (RAST) and clinical (in vivo) sensitization (SPT) on Karkar Island. As we found "unusual" discrepancies between high RAST and low SPT prevalences, we will investigate the correlation of sTot-IgE with RAST and sTot-IgE with SPT. In a next – quite laborious – step we try to exclude some possible/theoretical explanations for the RAST – SPT discrepancies. Subsequently we will concentrate on specific activity to show, why diverting the immune system towards Th2 responses in a worm infested population does not lead to an increase in SPT reactivity or allergic diseases. In this context we intend to investigate the possibility of mast cell blockage at low levels of allergen specific activity. In a last step we want to merge the multitude of different ideas about allergogenesis into a hopefully coherent concept of the "change theory of allergogenesis".

100 Moreover, the four Public Health theses exclusively addressed oral health problems in PNG (catalogue accessed Jan 29th 2013).

101 Herbert O, 2000: 59.

4 Methods

4.1 Karkar Island, traditional study population and allergological tests

Karkar is a small tropical volcanic island 50 miles in circumference (370 km²) lying about 20 miles from the north coast of the Papua New Guinea (PNG) mainland (see fig. 4).[102]

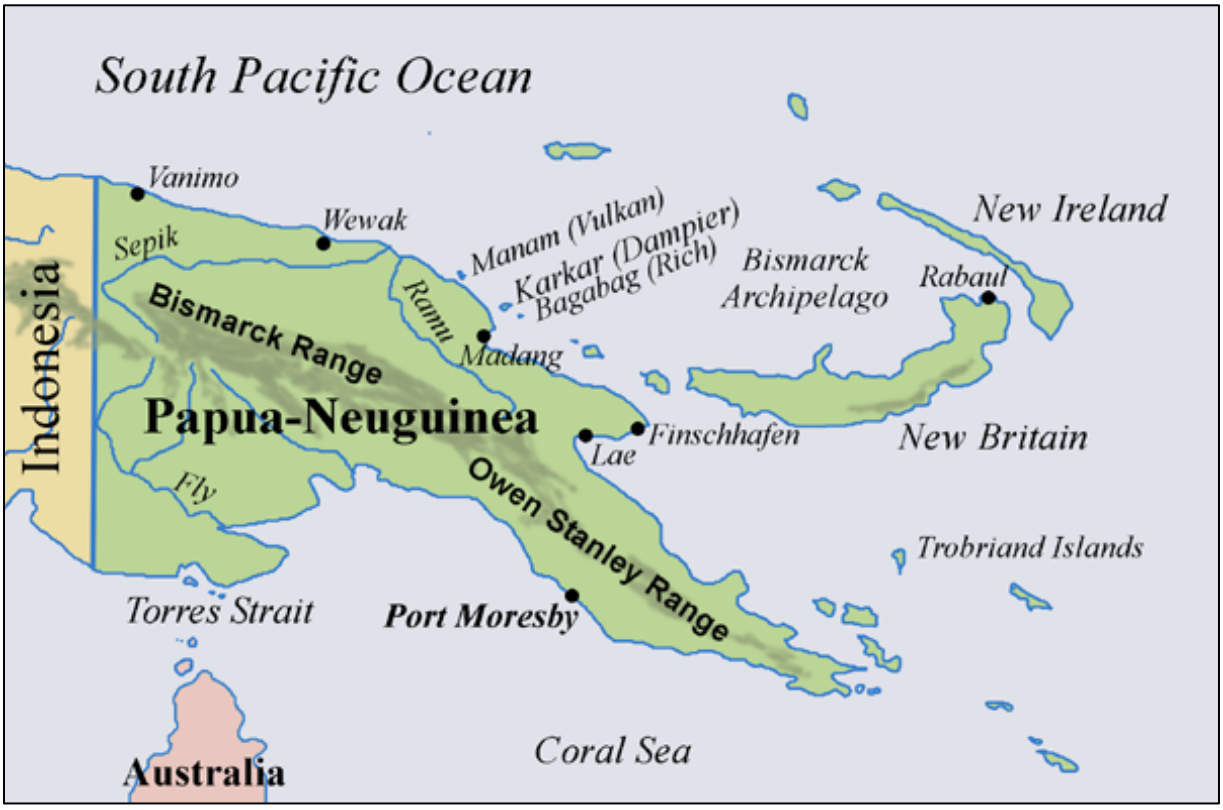


Fig. 4. Position of Karkar Island, Madang Province, 20 miles north from the Papua New Guinea mainland.

Karkar Island is separated by the Isumrud Strait from the mainland and dominated by Mount Kunugui (1832 meter, last eruption 1979) (see fig. 5).[103] Humidity is high, rainfall about 400 cm and temperature ranges between 20 and 30 degree Celsius.[104] The whole island is thickly wooded.[105]

102 Anderson HR, 1978: 63 and Walsh G, 1974: 223 ff.
103 Holdsworth D, 1984: 111.
104 Anderson HR, 1978: 64. According to Cotes JE et al.: mean maximum temperature 30ºC and mean minimum temperature 23ºC (Cotes JE, Saunders MJ, Adam JE, Anderson HR, Hall AM, 1973: 320).
105 Ryan P, 1972: 670.

Fig. 5. Karkar Island, view from the Papua New Guinea mainland over the Isumrud Strait.

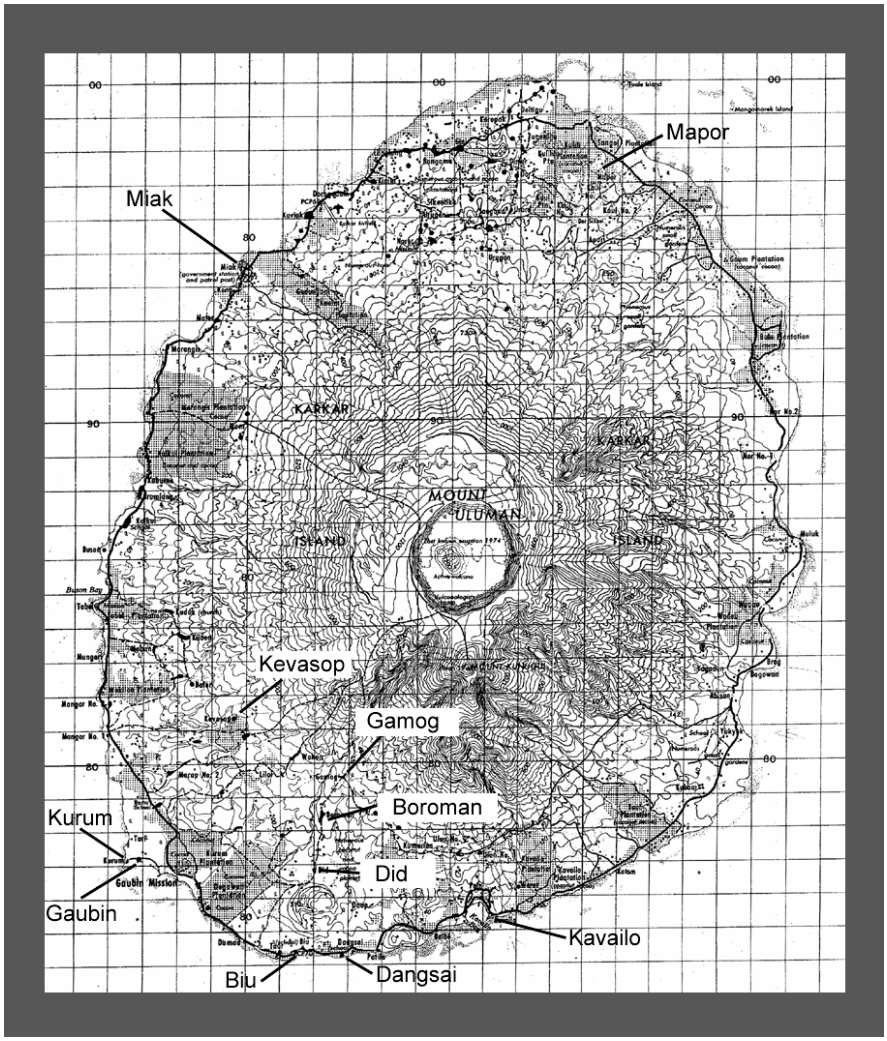


Fig. 6. Position of the eleven home villages of the study participants (for details in respect to composition and properties of the study population see table 3).

The study participants lived in the eleven following villages on Karkar Island: Biu, Boroman, Miak, Gaubin, Dangsai, Kurum, Kavailo, Did, Kevasop, Gamog and Mapor (see fig. 6). Most of the 70,000 Karkar live on traditional subsistence horticulture (83% of the adults within our study group)[106]; others work on coconut plantations[107] (see fig. 7) or cocoa plantations (plantation work represents the main source of cash income)[108]. Already pupils considered the introduction of Western medicine the reason for the high population density on the Island. Asked, what would be different on Karkar without Gaubin Hospital, Nathan answered lapidarily: "Much less people would live here".

Fig. 7. Karkar coconut plantation worker removing the husk from the hard shell.

Diet consists mainly of carbohydrate like taro, fruit, banana, nuts etc. The protein intake is low yet fortunately there is no general malnutrition on Karkar, as the island is very fertile.[109] Breast feeding is prolonged; solids are often introduced at an early age however

106 In this respect, there were no major changes since 1978 (Anderson HR, 1978: 64). Numbers very similar to ours were published by the Evangelic Lutheran Church in 1999 for the whole country of PNG: According to "Evangelisch Lutheranische Kirche" 85% of the PNG population live on subsistence horticulture (Evangelisch Lutheranische Kirche, 1999: no pagination).

107 As Ryan stated, Karkar is "… one of the richest copra-producing areas in the world" (Ryan P, 1972: 670).

108 Yongoe K, 1993: 18.

109 Holdsworth D, 1984: 111.

bottle-feeding is rare. Two different languages are spoken on Karkar: *Takia* and *Waskia*, yet basically all islanders are fluent in Neo Melanesian Tok Pidgin.[110] Families are large; the mean number of siblings was 4.8 (see chapter 6.3) and the clan (Pidgin *lain*) – a form of "extended" family (also representing the individual's security circle) – incorporates an even higher number of subjects. The average body height of the Karkar is small: we calculated a mean height of only 161.0 cm (s ±6.9; n=49) for males over 15 years and of only 152.4 cm (s±6.5; n=49) for females over 15 years[111], yet physical fitness is high (this is also reflected by the PEFR values, see chapter 5.1). Education is mainly at primary school level, yet a high school is available at Miak. Fortunately, atmospheric pollution is virtually inexistent (no car traffic) except for domestic wood smoke. However, we detected elevated lead concentrations in Karkar hair samples (see chapter 4.4). The traditional village structure is largely intact, urbanisation is absent. The village huts are still built with organic materials in the traditional way. Fig. 8 shows a hut in Did village.

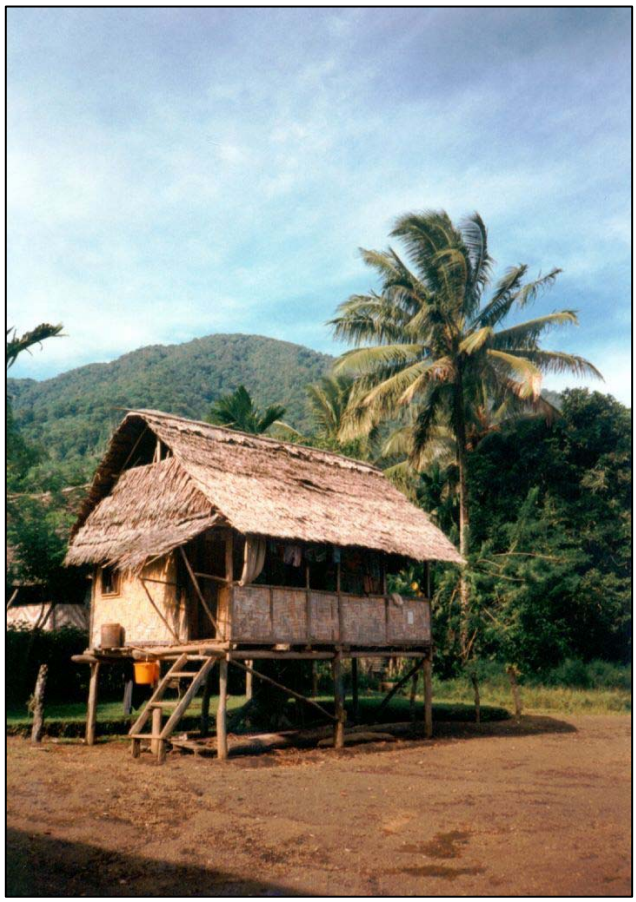


Fig. 8. Typical traditional Karkar hut in Did. The building material consists exclusively of organic substances.

110 All interviews with Islanders were conducted in Pidgin, as the author does neither speak *Takia* nor *Waskia*.

111 Cotes JE et al. reported mean heights for young Karkar men (Kaul Village) of 1.65 m (range 1.53-1.72) and for young women of 1.56 m (1.45-1.67) (Cotes JE, Saunders MJ, Adam JE et al., 1973: 322f). Houghton considered the Karkar an "extreme" in Polynesian body size (Houghton P, 1995: 355).

Probably one of the most striking aspects of traditional lifestyle is the high prevalence of communicable diseases: parasites (worms, malaria), bacteria (common skin infections, tuberculosis, gastrointestinal and respiratory tract infections) viral (respiratory, hepatitis) and fungal (tinea, pityriasis) infections play a pre-eminent role in the Karkar's life.

Altogether 282 different volunteers took part in the study (see table 3). In 1997 we randomly selected 248 Karkar Islanders in order to assess atopic sensitization (20 RAST, 10 SPT) in the general population[112]. In 2002 we performed a further convenient sample based on 129 subjects. For reasons we will explain later in detail, no RAST measurements were performed in 2002 and we based allergological diagnose exclusively on medical histories, skin prick tests (13 SPT) and Peak Expiratory Flow Rates (PEFR)[113]. In 2002 we additionally examined 28 Karkar suffering from allergic disease in order to elucidate the clinical relevance of different allergens in the causation of allergic symptoms. In 2009 the allergy status of 25 of the 28 confirmed allergic participants was re-evaluated clinically. In all occasions the participants were approached directly in their home villages. The median age at first contact was 24.0 years and the percentage of women was 52.5%, that of men 47.5%. In 2002 and 2009 we took the same detailed medical histories on general health, atopic manifestations and life conditions as we did in the 1997 investigation.[114] We recorded the reported atopic status, frequency and extent of symptoms as well as the circumstances under which patients developed clinical signs of allergy. The answers were translated from Neo-Melanesian Tok-Pidgin to German and English. Translation of biomedical terms – e.g. in the context of consent and medical histories – is problematic as there is often neither a similar concept in the indigenous culture, nor an expression in Melanesian Pidgin. Thus words like "allergy" had to be carefully transcribed. The difficulty of defining and communicating essential "Western" medical terms may be estimated when we look at the clumsy transcription of allergy in the "Guide to biological terms in Melanesian Pidgin": *Nem bilong sampela samting sapos sampela man i pulim win i gat samting nogut … dispela man i kamap sik long dispela sapos i gat ALLERGY long* dispela *samting.*[115] The literal translation would be that allergy is the "word for something when a person pulls air with something bad [inhales a problematic substance] … this person gets ill to this [upon contact with the substance] if she/he has an ALLERGY to this something."[116] The fact that there is no Pidgin word for "allergy" may also be a consequence of the low prevalence of this disease in Papua New Guinea. This reminds us of the situation in the Western world at the start of the twentieth century, when – as Jackson points out – "allergy [still] had no name"[117].

112 Theses accepted in 2000 at the Technical University, Munich.

113 For details on PEFR see chapter "4.3 Physical examination and Peak Expiratory Flow Rates". PEFR measurements were performed on 143 islanders. Results of the additional SPT were pre-published in Herbert O, 2009, PEFR data has not been published yet.

114 For details concerning the questions see Herbert O, 2009: 107f.

115 Simon M, 1977: 4.

116 Translation by the author.

117 Jackson M, 2006: 7.

4.2 The two tests for allergic sensitization: SPT and RAST

Skin Prick Tests (SPT) = "in vivo sensitization": Skin Prick Tests were performed with stainless steel lancets at the volar side of the lower forearm of the participants and the wheal diameters were read after 20 minutes. We used histamine as positive and sodium chloride 0.9% as negative control. Thirteen aeroallergens, four food allergens and four miscellaneous allergens were tested.[118] Wheal diameters of 3 mm or more were considered positive provided there was no response to the negative control.[119] During each of the two periods of data collection, the same batches of allergen extracts were used and all SPT tests were performed by one researcher.

Specific IgE measurements ("RAST") = "in vitro sensitization": Within a maximum time of four hours – depending on the location of the village – the collected blood samples were transported to Gaubin Hospital in a cooled container. The blood was centrifuged and sera were stored frozen until specific IgE measurements were performed at the Department of Dermatology, Technical University, Munich, Germany. The Pharmacia (now Phadia) CAP System RAST Fluorescent Enzyme Immuno Assay (FEIA) was used to evaluate specific IgE and results are given either in kU/l or in the semi-quantitative RAST classes from 1 to 6.[120] We measured 9 aeroallergens, 8 food allergens and 3 miscellaneous allergens.[121] The cutoff level for the detection of specific IgE was 0.35 kU/l.[122] When performing statistical tests on IgE it is important to know, that specific IgE is log normal distributed (just as total IgE, see figs. 14 and 15). This means we are able to achieve normal distribution using the logarithms of the original scale numbers. In our work we always use the base-10 logarithms instead of the natural logs (base-e) as the magnitude of the original value is easier to grasp. As logs are not defined for 0 yet in some of our

118 The 21 SPT measurements: House dust mite 1 (Dermatophagoides pteronyssinus), house dust mite 2 (Dermatophagoides farinae), storage mite (Blomia tropicalis), cockroach (Blattella germanica, Allergopharma), cockroach (Blattella germanica, Stallergene), mould (Cladosporium herbarum), grass pollen mixture, mugwort (Artemisia vulgaris), dog epithelium, rat epithelium, cat epithelium, pig epithelium, hen's feathers, hen's egg, cow's milk, codfish, banana, skin fungus (Epidermophyton floccosum), skin fungus (Trichophyton rubrum), skin fungus (Trichophyton mentagrophytes) and latex (Hevea brasiliensis).

119 SPT wheal diameters were obtained calculating the arithmetic mean of the longest wheal diameter and the diameter perpendicular to it.

120 The corresponding levels of the six RAST CAP classes in kU/l are as follows: Class 0 = <0.35 kU/l, class 1 = 0.35-<0.7 kU/l, class 2 = 0.7-<3.5 kU/l, class 3 = 3.5-<17.5 kU/l, class 4 = 17.5-<50 kU/l, class 5 = 50.0-<100.0 kU/l, class 6 = >100 kU/l. The increasing size of the RAST bins when IgE levels gets bigger is due to the log normal distribution of IgE values.

121 The 20 RAST measurements: House dust mite 1 (Dermatophagoides pteronyssinus), house dust mite 2 (Dermatophagoides farinae), cockroach (Blattella germanica), timothy grass (Phleum pratense), pig epithelium, mugwort (Artemisia vulgaris), dog epithelium, cat epithelium, rat epithelium, banana (Musa sapientum/paradisiaca), sweet potato (Ipomea batatas), papaya (Carica papaya), mango (Mangifera indica), mackerel (Trachurus japonicus), cow's milk protein, egg protein, codfish (Cadus morua), roundworm (Ascaris lumbricoides), arabic gum (Gummi arabicum) and latex (Hevea brasiliensis).

122 Meanwhile, tests have become available detecting specific IgE levels as low as 0.1 kU/l (Hamilton RG, MacGlashan DW Jr, Saini SS, 2010: 275).

RASTs specific IgE was not detectable, we had to add a constant to avoid cero values.[123] We added 0.175 kU/l only to the RAST negative results (not generally to all RAST results) as this may balance what we consider "cero artefacts" – consequence of limited test sensitivity – to a certain extent. Our considerations leading to choose 0.175 kU/l are laid down in the legend of figure 49 (addendum).

4.3 Physical examination and Peak Expiratory Flow Rates

The medical examination focused on skin manifestations of atopy and clinical signs of atopic airway diseases (such as rhinoconjunctivitis, rhinitis and stridor). In order to confirm bronchial asthma, routine chest auscultation was followed by measurement of Peak Expiratory Flow Rates (PEFR) before and after inhalation of Terbutaline. An increase in PEFR score after Terbutaline inhalation of 15% or more was considered proving bronchial asthma.[124] In the following, we want to give a short overview over PEFR measurements. PEFRs permit the detection of airway obstruction before wheezing is heard, or before the patient feels subjective symptoms. Measuring PEFRs can be considered an objective quantitative measurement of bronchial asthma. After explaining the purpose of the study, the correct manner of a PEFR measurement was shown to the participants. The participants were observed during their performance of the test (device: "The Mini-Wright Standard" Peak Flow Meter, Technipro, CAT Nr. 310 3001) in order to avoid faulty technique.[125] Disinfected mouthpieces were used for each person. Tests were performed in a standing position and it was made sure, that the Peak Flow Meter was kept horizontally. After inhaling deeply and fully (maximal inspiratory effort), the participants had to exhale as quickly and forcibly as possible (maximal expiratory effort) with their lips tightly closed around the mouthpiece. The score was read, the marker returned to zero and the procedure repeated twice. The highest of three satisfying scores was recorded. Subsequently we measured the PEFRs after inhalation of the short acting beta antagonist Terbutaline (Bricanyl, 250 µg Terbutaline-sulfate per application). Terbutaline was applied to reverse an eventually existing airway obstruction, in this case indicating bronchial asthma. In order to make sure, that the inhalation of the bronchodilating aerosol was performed correctly, a spacer was used (Nebuhaler, each individual was supplied with a new mouthpiece). The transparent plastic chamber allowed an optical control of the actual inhalation of the vapor. After applying one dose of Terbutaline in the spacer, the participants had to inhale deeply and hold their breath for 10 seconds. After exhaling, one more dose of Terbutaline was administered to the participants in the same way. Subsequently the individuals had to wait for 10 minutes before performing a second cycle

123 McDonald JH, 2008: 150f.

124 Admittedly the definition of reversibility (≥15% PEFR increase) is arbitrary (Worth H, 2007: 443).

125 Nunn and Gregg point to the importance that subjects make a maximum exhalation effort. Thus "close attention" has to be paid to the correct PEFR test technique (Nunn AJ, Gregg I, 1989: 1070).

consisting of three PFER measurements as described before. Again, the highest score was recorded.[126]

Fig. 9. Peak Expiratory Flow Rate (PEFR) measurement in Kavailo.

4.4 Measurement of lead concentrations in hair samples

We measured the lead contamination in hair samples (collected in 2002) of over 200 Karkar Islanders. As discussing the results is out of the scope of this work, we will only give a short overview and cover the topic entirely in this chapter. We determined the lead concentration as we wanted to prove that the "traditional lifestyle" on a "remote" island is associated with low/no heavy metal exposition of the human body. However, despite of the lack of anthropogenic environmental pollution (no cars, no artificial fertilizers, no plumbing system in houses) we unexpectedly detected elevated lead concentrations in hair. Subsequently we intended to discover the origin of the lead contamination: we thought of different possible sources of lead on Karkar and investigated water and soil samples, betel lime and newspapers (measurements May 2009). Soil from four different villages and water from five different wells showed no abnormal levels. The local newspaper (Post-Courier) is often used to roll cigarettes with homegrown *brus* (tobacco). The Post-Courier contained only less than half of the lead concentration of an arbitrarily selected German newspaper (Süddeutsche Zeitung). Lime (Pidgin *kambang*) and betel pepper (Pidgin *daka*) are chewed together with the popular betel nuts; the lime

126 Our Peak Expiratory Flow Rate measurements followed basically the description given by the National Health Service (National Health Service Forth Valley, 2009: 30f.).

releases the mild stimulant arecoline. Lead concentration in lime from six villages differed by a factor of ten (possible contamination of lime during the production process?). However, even the daily ingestion of 20g of the lime with the highest lead level would lead to a minor lead exposition (i.e. 1.5% of the allowed daily dose). To our knowledge, historically lead was brought to Karkar only in very limited amounts in the form of the rust proof primer paint "Mennigfarbe" (lead tetroxide or "red lead"). Missionary G. Kunze reported that in 1891 the Karkar were very keen on this colour to use it "… [um] sich damit in geradezu fratzenhafter, das menschliche Antlitz vollständig entstellender Weise [zu bemalen] …" ("… to paint themselves in a downright grotesque way, which entirely disfigures the human countenance …").[127] We conclude that the origin of the elevated lead concentrations in hair on Karkar remains in the dark. The only not investigated alternative source of lead we can think of are contaminated table- and/or cookware. It may be wise to exclude this option in future.

4.5 Statistics

Descriptive statistics: Central tendency of normally distributed data is displayed as arithmetic mean (AM) including the 95% Confidence Interval of the AM (CI); only if explicitly mentioned AM and standard deviation was given. IgE levels (total IgE as well as specific IgE) are log normal distributed i.e. they show a long tail of large values in their original, untransformed form. When logarithmically transformed[128], IgE values become normally distributed (see figs. 14 and 15). For log-normal distributed data the geometric mean (GM) is a more stable form of displaying central tendency than the widely used arithmetic mean (AM).[129] The AM would be too much subject to distortion by the fraction of exceptionally high values in such an originally positively skewed distribution. For data without normal distribution, medians and ranges were calculated.

Analytical statistics: Generally, statistical tests were performed as two sided tests and comparisons are considered significant at a P value of less than .05. Relationships between nominal data/percentage comparisons were investigated using the χ^2 test of independence (if the expected values were smaller than five, the Fisher exact test was applied). Differences between two arithmetic means of normally distributed measurement variables were investigated using the unpaired t-test (Student t-test); if more than two groups had to be compared the type I ANOVA was applied. Statistical tests on IgE levels were performed on log-transformed data to insure the bell-shaped/Gaussian distribution necessary for the creation of valid confidence intervals (CI) and for normality dependent

127 Kunze G, 1925 b: 6.

128 We used 10 as base for the logarithm, yet as stated by Olivier "any base for the logarithm" may be used (Olivier J, Johnson WD, Marshall GD, 2008: 333).

129 One way to look at the GM is as described by Olivier: "The antilog of the arithmetic mean for log-transformed data is the geometric mean…" (Olivier J, Johnson WD, Marshall GD, 2008: 335).

tests (t-test, analysis of variance [ANOVA]).[130] The CIs were back transformed from the log scale to the original scale before summarizing results. Characteristically a CI for log-normal distributed data is not symmetric about the sample GM, i.e. the CI "will itself be positively skewed"[131]. If normality assumptions were not met or unattainable by transformation the non-parametric Mann-Whithey-U test was applied to compare medians. The relationship between two normally distributed measurement variables was investigated using linear regression (if necessary log-log-transformed correlation), the relationship between one independent measurement and one dependent nominal variable was assessed by simple logistic regression.[132] In the case of ranked or non-normally distributed variables the non-parametric Spearmans rho (ρ) was applied to express correlation.

4.6 Conflicts of interest, funding, acknowledgements and consent

The author declares there is no conflict of interest. Expenses for skin prick test (SPT) solutions and RAST/sTot IgE measurement kits were generously covered by the Technical University Munich, Dept of Dermatology and Allergy Biederstein, Munich, Germany. We thank the head of department, Prof. J. Ring, for granting the use of the IgE measurement instrumentation and Johanna Grosch for her valuable support. A minor amount for SPT solutions was funded by the Royal Prince Alfred Hospital, Dept of Dermatology, University of Sydney, Australia. We thank Prof. Drasch and G. Roider (Forensic Institute at Ludwig Maximilians University, Munich) for measuring lead levels in hair, soil, water, newspaper and betel lime. Except of the funding of one flight to Papua New Guinea by "Deutsche Gesellschaft für Allergologie und Klinische Immunologie, Germany", and one by Channel Seven Network Australia, all other costs were covered by the author. The author thanks the officer in charge of the Gaubin Hospital, Karkar Island, Papua New Guinea, for approving the use of the Hospital laboratory. Statistician Dr. Mario D'Souza, University of Sydney, helped with the calculations. Informed consent was obtained (in written or oral form) from all participants; in the case of children, the consent was obtained from parents. Our greatest thanks, however, go to all the kind study participants and the many invaluable informants – without them this work would not have been possible. *Mi laik givim bikpela tok tenk yu igo long ol manmeri bilong Karkar!*

Before starting with the subsequent section we want to point to a peculiarity of the structure of chapter 5: In derogation of a common proceeding, we merged results and discussion in one chapter. Due to the large extent of the work, separating results and discussion would have led to reduplications and confusion.

130 "Statistical inferences in the log scale remain valid for the data" (Olivier J, Johnson WD, Marshall GD, 2008: 333).

131 Olivier J, Johnson WD, Marshall GD, 2008: 335.

132 McDonald JH, 2008: 247ff.

5 Results and Discussion

5.1 Mites – the clinically most relevant allergen on Karkar Island

The relevance of specific allergens in causing sensitization and atopic disease varies all over the world. This variation largely depends on environmental and socioeconomic conditions prevailing in different regions. Determining the most relevant allergen(s) on Karkar is important for different reasons. Clinically it allows us to recommend countermeasures against the respective allergen (basically allergen avoidance, as specific immunotherapy is normally still unavailable in Papua New Guinea). Academically and especially in respect to the current study it shows us, which allergen(s) is/are worthwhile examining in detail (like investigating the relationship between sTot-IgE, specific IgE and SPT)[133]. Based on the small number of eleven allergic subjects within the investigated general population (data collected in 1997), we already suspected mites to represent the clinically most relevant allergen on Karkar Island.[134] This assumption was supported to a certain extent by the fact that in vitro (RAST) sensitization within the general population was most prevalent against mites[135] followed by cockroaches (73% for D. pteronyssinus,[136] 70% for D. farinae and 57% for cockroaches).[137] RAST sensitization against the other allergens was distinctly lower. All the RAST (and SPT) sensitization values are displayed in detail in chapter 5.4, yet already at this point it is worthwhile mentioning that in vitro (RAST) sensitization has to be interpreted with caution as the clinical relevance is dubious in the traditional Karkar society.[138] Within the small number of 11 clinically atopic subjects of our first investigation, SPT results correlated much

133 See chapter 5.8 “The key to the differences between RAST and SPT: The Specific Activity”.

134 Herbert O, 2009: 57.

135 All participants had been screened for scabies, as scabies (also caused by a mite: Sarcoptes scabiei) is known to be able to trigger house dust mite RAST in some individuals (Falk ES, 1981: 167ff). In only three of the participants scabies could not be excluded, thus a major influence of scabies on our results is most unlikely.

136 To compare our mite RAST result with a similar study performed within a group of 520 schoolchildren at Lambaréné, Gabon, we calculated the rate of Karkar showing D. pt. antibody levels over 1 kU/l. On Karkar the percentage was 54.4% (135/248). This is even higher than the prevalence of 32% in Lambaréné (van den Biggelaar AH et al., 2000: 1725).
Part of the reactivity (RAST) against D. pt. could be due to cross-reactivity with Ascaris lumbricoides: tropomyosin of Ascaris cross-reacts with tropomyosin of HDM (Santiago HdaC, Ribeiro-Gomes FL, Bennuru S, Nutman TB, 2015: 93).

137 Prevalence of RAST sensitization against Ascaris lumbricoides was 82%, yet here we just refer to allergic sensitization and anti Ascaris IgE is an indicator for helminth infections and not for allergy.

138 For details see chapter 5.8. It shall just be mentioned shortly that we found high correlations between RAST sensitization against D. pteronyssinus and D. farinae (r^2=0.81; $p<0.01$); this is higher than the correlation between the same mites found by Tsai et al. who reported only r^2=0.46; $p<0.01$ (Tsai LC et al., 1988: 12). Another high correlation was found within our data between sweet potato and mango (r^2=0.77; $p<0.01$).

better with atopic symptoms than RAST results.[139] Thus in this chapter we will focus on SPT reactivity rather than RAST to evaluate clinically relevant sensitization. There are different ways of investigating the relevance of allergens: Firstly we will describe the pattern of SPT sensitization within the general population. Secondly – after describing the diagnostic process of 28 allergic subjects – we will draw information from the medical histories of the allergic Karkar and third we will compare the (SPT) sensitization rates in the allergic group with the general population.

To begin with, we will show that the pattern of skin prick test reactivity within the general Karkar population already suggests great importance of mite- and (to a lesser extent) cockroach allergens: Skin Prick Test reactivity ≥3 mm within the general population was far most common against mites (14% to 21%, depending on species and year of investigation) followed by cockroach (8% to 12%, depending on manufacturer of the SPT solutions).[140] All the other eight tested aeroallergens (grass pollen mixture, mugwort pollen, Cladosporium herbarum, dog epithelium, rat epithelium, cat epithelium, pig epithelium, hen's feathers) as well as the four investigated food allergens (hen's egg protein, cow's milk, codfish, banana) provoked positive SPT reactions in only 0-1% of the subjects. Furthermore no islander showed a positive SPT reaction to latex.[141] A comparable clear predominance of mite SPT in comparison to other aeroallergens has been described by van den Biggelaar AH et al. in a similar setting in Lambaréné, Gabon.[142]

To put our estimate that mites (and possibly cockroaches) represent the clinically most relevant allergen on Karkar on a more solid base, we intended to investigate the medical histories of a larger allergic group and compare the sensitization pattern of the allergic group with the general population. As the overall prevalence of allergic disease is low on the island[143], it is difficult to obtain sufficiently large numbers of allergic subjects in a

139 Interpreting their results from Recife, Brazil, Lopes MI et al. drew the same conclusion in respect to cockroaches: "The skin prick test is more appropriate for the detection of clinically relevant sensitivity to cockroaches than specific IgE determination" (Lopes MI, Miranda PJ, Sarinho E, 2006: 204).

140 For details see chapter 5.4.
We correlated the different SPT results. A high correlation was found between the two house dust mites species D. pteronyssinus and D. farinae (r^2=0.76). A lower correlation existed between D. pteronyssinus and the storage mite Blomia tropicalis (r^2=0.41). Skin reactions provoked by cockroach solutions produced by Allergopharma correlated highly with those produced by Stallergene (r^2=0.59). All correlations mentioned above were significant ($p<0.01$).

141 Within the "various" group, skin fungus (dermatophyte) antigens created elevated prevalence of positive SPT reactivity: 8% of the general population (year 2002) developed a positive SPT result to Trichophyton rubrum [Stallergene] antigen, 7% to Epidermophyton floccosum antigen, and 5% to Trichophyton mentagrophytes antigen [Allergopharma]. Yet this dermatophyte pattern reflects the high prevalcence of skin mycoses on the island: Thirteen percent (32/247) of the subjects controlled in 1997 were clinically diagnosed with Tinea corporis or faciei (skin mycosis of the body or face; pidgin: "grille"). Allergologically sensitization against dermatophyte antigens is most certainly irrelevant.

142 Exact Lambaréné positive SPT prevalence (≥3 mm) in the general population (children) according to data given in table 1 (n=520): D. pt = 11%, cat = 2.3%, grass pollen (Dactylis glomerata) = 0.8% and dog = 0.2% (van den Biggelaar AH et al., 2001: 235).

143 Prevalence of atopic diseases was 4.4% (Herbert et al., 2009: 130ff).

random sample. Thus we deliberately investigated islanders who complained about symptoms suggestive of allergic diseases (mainly asthma)[144] or islanders who had previously been diagnosed with allergic disease at Gaubin Hospital. Originally, we suspected allergies in 39 Karkar. Yet medical histories and/or symptoms of eleven subjects were not consistent with atopic disease thus we considered their problems of non-allergic origin (physical irritation of mucosae by fire smoke, airway/eye infection, chronic obstructive pulmonary disease[145]). Combining the medical histories with physical examinations including chest auscultations, peak expiratory flow measurements (PEFRs) and SPT, we were able to confirm atopic disease in 28 subjects (16 males, 12 females).

Measuring PEFRs can be considered an objective quantitative assessment of bronchial asthma; asthma in general, yet not necessarily allergic asthma.[146] Allergic asthma is an obstructive inflammatory disorder of the airways triggered by exposure to allergens. It is associated with bronchial hyperresponsiveness and (fully or partially) reversible (!) airflow obstruction.[147] Reversibility (spontaneous or drug induced) is the most important feature to differentiate asthma from chronic obstructive respiratory disease (COPD). COPD is – like allergic asthma – an obstructive respiratory disease, yet in contrast to allergic asthma COPD is normally not associated with allergy and there is no PEFR increase after inhalation of bronchodilators (i.e. reversibility). Instead COPD is often associated with smoking, productive cough, older age (over 50 years) and slowly but continuously progressive respiratory symptoms (over years and decades).[148] PEFR reference values were not available for the Karkar population. Due to the Karkars' extremely small body size (see chapter 4.1) and quite muscular constitution the application of European reference values may be misleading. Thus we first had to measure PEFR in the general population.[149] The PEFR values for 110 females and males are displayed in fig. 10.[150]

144 Little data is available on asthma for Karkar: In 1978 Anderson estimated the asthma prevalence within the general Karkar population 1.3% for boys and 0.3% for girls (Anderson HR, 1978: 68).

145 See subsequent comments on COPD.

146 The method of PEFR measurement has been described in chapter 4.3.

147 Pawankar R, Canonica GW, Holgate ST, Lockey RF, 2011: 13 and Reuter S, Stassen M, Taube C, 2010: 799.

148 Worth H, 2007: 441.

149 "Due to considerable ethnic variations in ventilatory capacity, approximate local norms should be used for interpretation (Zverev Y, Gondwe M, 2001: 14).

150 Smokers have not (!) been excluded, yet people with recent respiratory infections.

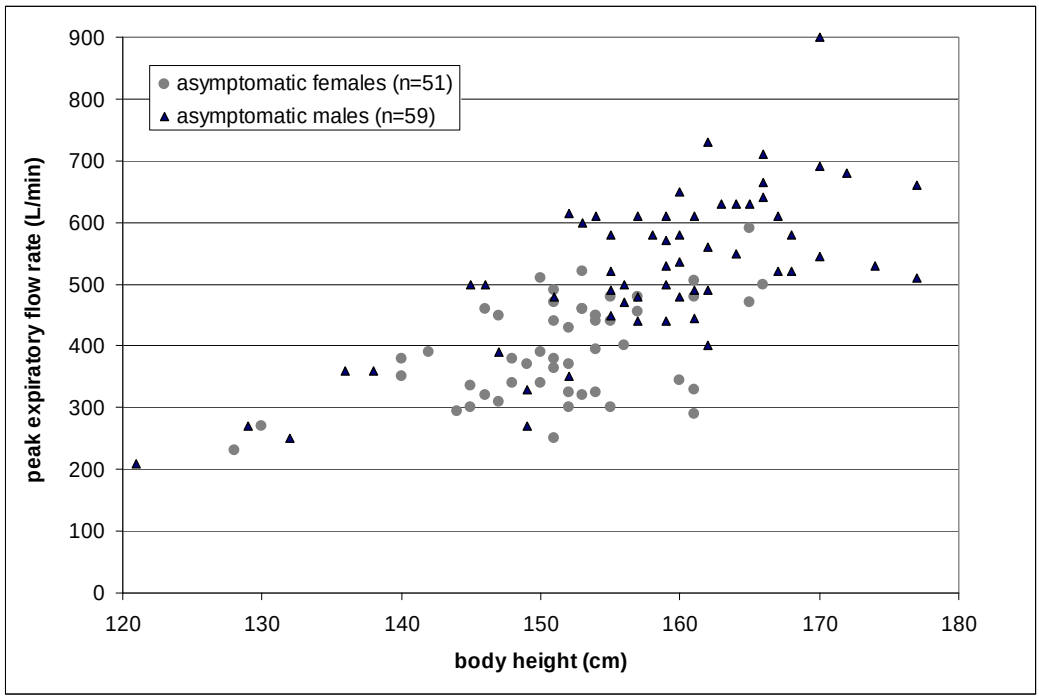


Fig. 10. Correlation of body height and peak expiratory flow rate (PEFR) in asymptomatic Karkar females and males.

We suspected allergic asthma in 25 Karkar. This estimation was based on pre-diagnose at Gaubin Hospital, medical history (especially paroxysmal dyspnea in the early morning, shortness of breath, wheezing, dry cough at night without a cold, coughing associated with rhinoconjunctivits) and chest auscultation (wheezing). In 14 of these 25 subjects (7 females and 7 males), asthma diagnose could be supported by an over 15% increase of PEFR after the inhalation of Terbutaline (thus COPD unlikely). The PEFR values of the seven asthmatic females and seven males in relation to the asymptomatic population are displayed in figs. 11 and 12. Within the asymptomatic population, the relationship between body height and PEFR was linear.[151]

151 Thus linear regression was used without previous logarithmic transformation of data.

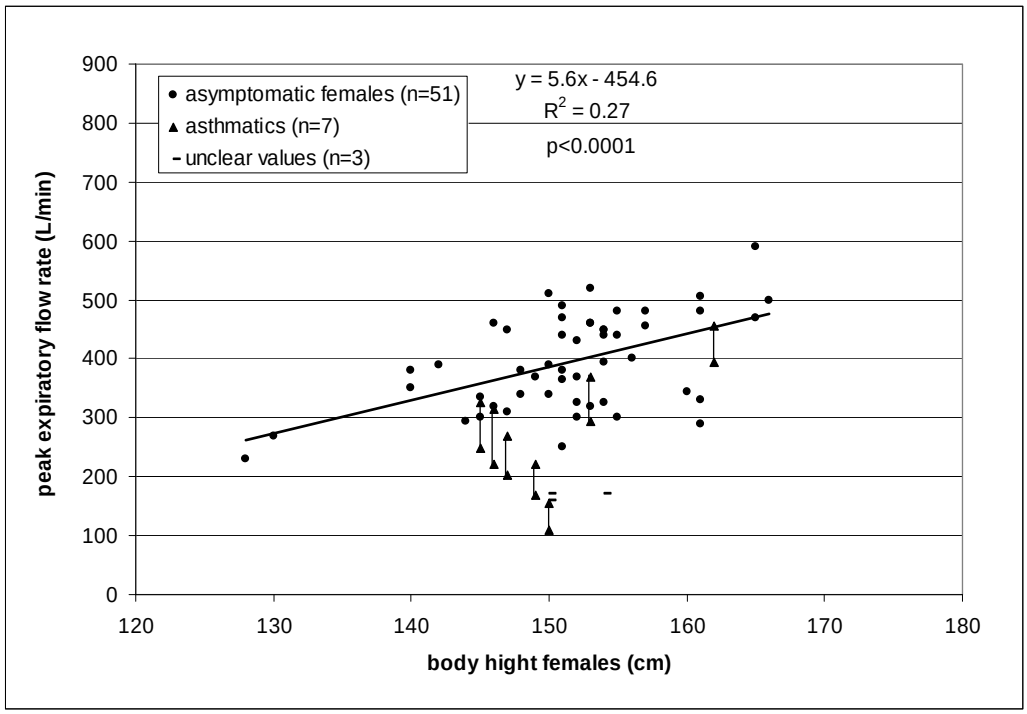


Fig. 11. Peak expiratory flow rates of seven asthmatic females: lower value before and higher value after inhalation of Terbutaline. Background: 51 asymptomatic females (with corresponding regression line and equation) and three unclear values.

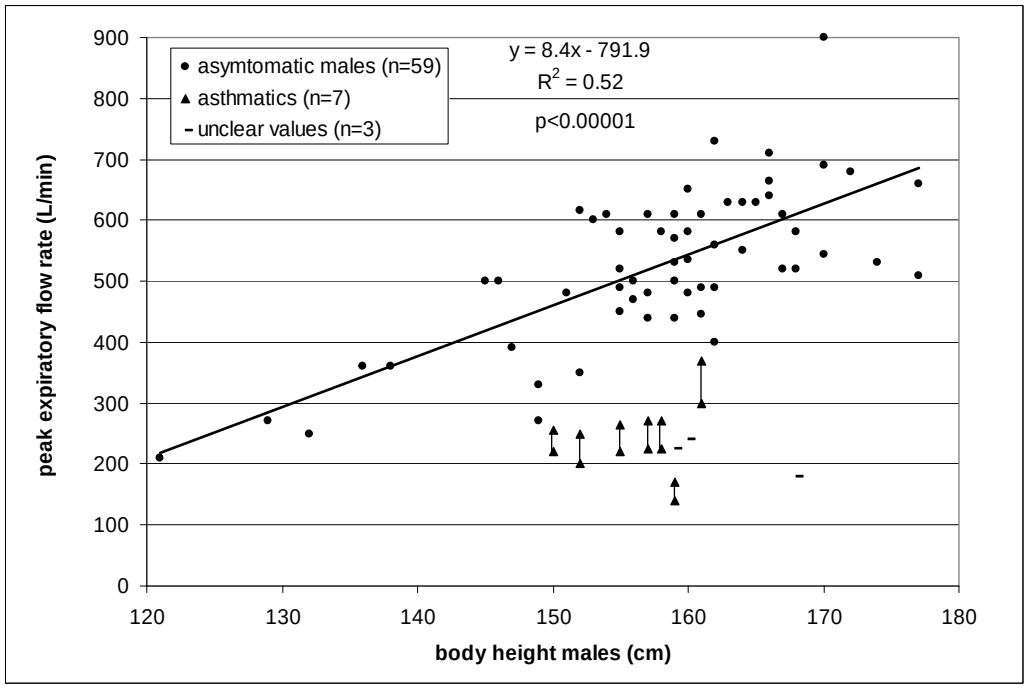


Fig. 12. Peak expiratory flow rates of seven asthmatic males: lower value before and higher value after inhalation of Terbutaline. Background: 59 asymptomatic males (with corresponding regression line and equation) and three unclear values.

At this point we may just insert some comments on the values displayed in the last two figures. In fig. 11, showing the PEFR of females, the seven asthmatics are represented by triangles. The connecting line illustrates the PEFR increase evoked by Terbutaline inhalation. The 51 asymptomatic females (round points) constitute the base for the regression line, corresponding equation and p value. The only two children are represented by the two values at the far left: one girl aged 10 (body height 128 cm) and one girl aged 12 (body height 130 cm). The remaining 49 asymptomatic subjects were adolescents and adults (range 14-60 years). The three "unclear values" (symbolised by minus) represent two women aged 53 and one aged 54. They had no history of respiratory problems and we consider their low results to be a consequence of incorrect test performance.[152] In comparison with Caucasian PEFR values the Karkars' results seem low, yet we have to consider their low body height. Using the regression equation we would predict a PEFR value of 504 l/min for a "European size" (171 cm) asymptomatic Karkar woman. This is higher than Caucasian normal values (400-450 l/min for age group 20-60 years)[153] and probably reflects the high level of habitual activity and superior fitness (including the power of expiratory muscles) of the islanders.[154] Corresponding to fig. 11 fig. 12 shows the PEFR of males. The seven male asthmatics are represented by triangles and – as before – the connecting line shows Terbutaline induced PEFR increase. The 59 asymptomatic males (round points) constitute the base for the regression line, corresponding equation and p value. Like in the female group, there are only two children in the asymptomatic male group: one boy aged 8 (body height 121 cm) and one boy aged 10 (body height 132 cm).[155] The remaining 57 asymptomatic subjects were adolescents and adults (range 14-79 years). The three "unclear values" (minus) represent one man aged 67 (body height 168 cm) who had problems performing the test correctly[156] and two men aged 67 (body height 159 cm) and 71 (body height 160 cm) who suffered from indefinable chronic respiratory problems. The two symptomatic males were strong smokers, complaining about chronic cough. They experienced no increase of PEFR after inhalation of Terbutaline. All in all, in the latter two cases, chronic obstructive respiratory disease (COPD) seemed to be a more likely diagnose than asthma. As before, we wanted to compare the Karkars' PEFR results with Caucasian normal values. Using the regression equation we would predict a PEFR value of 711 l/min for a "European size" (180 cm) asymptomatic Karkar man. Again this is quite higher than Caucasian reference values

152 Questionable low PEFR values often result from not exhaling with maximum force or not closing the lips tightly around the mouthpiece.

153 Caucasian female normal values for PEFR measurements with the Mini-Wright Peak Flow Meter given by the National Health Service (National Health Service Forth Valley, 2009: 32).

154 The predicted values for asymptomatic Karkar female would probably be even slightly higher if we had excluded smokers from the reference group: It has been shown that smokers (even if asymptomatic) have lower PEFRs than non-smokers (Nunn AJ, Gregg I, 1989: 1069). Of course, this argument would apply to the male values as well.

155 The subject with body height 129 cm (in between the two boys) is not a child, but a small adolescent aged 16.

156 He reported no history of respiratory problems.

(580-650 l/min for age group 20-60 years)[157] and is probably owed to the islanders' superior fitness. [158]

We recapitulate that we suspected asthma in 25 Karkar and were able to substantiate it by PEFR in 14. In the remaining nine islanders, PEFR were normal in respect to our Karkar reference values and there was no increase of PEFR after inhalation of Terbutaline. Nevertheless clinical history and SPT results lead us to diagnose them with allergic asthma. Normal PEFR values are possible even in asthmatic patients, as asthma symptoms are volatile, not only due to diurnal variability.[159] Therefore it is likely that we performed our measurements during symptom free intervals.[160] In three Karkar we were able to diagnose allergic rhinoconjunctivitis based on medical history, physical examination and SPT reactivity. All in all, combining the medical histories with physical examinations including chest auscultations, peak PEFRs and SPT, we were able to confirm atopic disease in 28 subjects. Twenty-five suffered simultaneously from allergic rhinitis/rhinoconjunctivitis and allergic asthma and three exclusively from allergic rhinitis/rhinoconjunctivitis. Atopic dermatitis was not observed on Karkar. In the 2009 reevaluation we were able to confirm the diagnosis in 25 of the 28 allergic participants, one patient had died from an unknown cause and two were absent from their home villages.

Having identified 28 allergic subjects, we may now describe their medical histories to answer our initial question about the clinical relevance of allergens. The allergy history revealed that 19 of 28 allergic subjects experienced clinical symptoms inside their huts, especially when resting at night. This points to the relevance of indoor allergens like mites[161] and cockroaches.[162] The 19 subjects denied symptoms during the day, e.g. when having contact with plants or animals. All of them were unaware of what might cause their problems. As symptoms occurred mostly when the allergic subjects rested at night, we may shortly describe the sleeping-conditions in the villages. The majority of Karkar rest directly on the wooden floors of their huts (Areca palm wood).[163] Figure 13 shows the

157 Caucasian male normal values for PEFR measurements with the Mini-Wright Peak Flow Meter given by the National Health Service (National Health Service Forth Valley, 2009: 32).

158 The Karkars' PEF rates are quite high: this is not only supported by the fact that we did not exclude smokers from our Karkar reference values but also by the fact that the European values are already high in comparison to other populations e.g. Africans, Indians and Chinese (corresponding age and height) (Nunn AJ, Gregg I, 1989: 1070). However, Cotes JE et al. found, that PNG highlanders had even higher lung function than Karkar people from Kaul Village (Cotes JE, Saunders MJ, Adam JE, Anderson HR, Hall AM, 1973: 320).

159 Worth H, 2007: 443.

160 Unfortunately we could not repeat measurements at different times during the day, as visits to the villages were quite time consuming. Especially PEFR values at night would have been informative.

161 Visitsunthorn N et al. showed in a Thai study: "Airborne levels of mite allergens were low compared to the high concentrations found n mattresses… " (Visitsunthorn N et al., 2010: 155).

162 Relying exclusively on allergy history it is not possible to distinguish between (storage/house dust) mite and cockroach allergy, as both allergen groups occur simultaneously as indoor allergens.

163 The traditional Areca palm flooring was already described by missionary G. Kunze in 1890 (Kunze G, 1897 a: 21).

sleeping place of an asthmatic patient, an old foam mat without bed linen in direct proximity to the fire place and ashes: a perfect habitat for mites.

Fig. 13. Sleeping berth of an asthmatic patient: an ideal habitat for mites.

The remaining nine of the 28 allergic subjects experienced symptoms inside and outside the huts. In two of them the medical history permitted to identify the following antigens as at least one cause of symptoms: grass pollen (1 case, high probability) and dog epithelia (1 case, very high probability). In the remaining seven allergic subjects the only anamnestic information is that indoor and/or outdoor allergens may be responsible for the clinical reactions. None of the 28 Karkar described signs suggestive of food allergy. All in all, in respect to the medical history mites and cockroaches were the most "suspicious" allergens within the group of allergic subjects: In 19 of 28 atopic islanders the medical history was highly suggestive of mite and/or cockroach allergy and in seven mites and cockroaches may have been involved as well.

Answering the question which allergens are clinically most relevant we are not only relying on the medical histories. Important information may be drawn especially from sensitization differences between allergic groups and the general population. Thus table 1

compares the skin prick test results (in vivo sensitization) of our allergic group (n=28)[164] with the general Karkar population (n=129; for D. pt. and D. fa. n=244).

Table 1. Significantly higher prevalence of positive skin prick test reactivity against mites and cockroaches in the allergic group than within the general population. Differences for the other allergens were not significant (for mites chi square test was applied, for cockroaches, grass and mould Fishers exact test).

Allergen	Allergic subjects positive SPT	General population positive SPT	p	OR
Blomia tropicalis	67% (16/24)	21% (27/129)	<0.00001	7.55
D. pteronyssinus	88% (21/24)	16% (40/244)	<0.00001	35.7
D. farinae	---	14% (34/244)	---	---
Cockroach (Allergopharma)	46% (11/24)	12% (16/129)	<0.0001	6.0
Cockroach (Stallergene)	25% (6/24)	8% (10/129)	<0.05	4.0
Grass pollen mixture	8% (2/24)	1% (1/129)	ns	---
Mould (Cladosporium h.)	8% (2/24)	1% (1/129)	ns	---
Pig epithelia	0% (0/24)	0% (0/129)	---	---
Hen's feathers	0% (0/24)	0% (0/129)	---	---
Banana	0% (0/24)	0% (0/129)	---	---
Latex	0% (0/24)	0% (0/129)	---	---

SPT reactivity against storage and house dust mites was significantly more prevalent in the allergic subjects than in the general population: In the allergic group 67% of the subjects were positive against Blomia tropicalis, in the general population only 21%. Values for D. pteronyssinus were 88% vs. 16% respectively (D. farinae was not measured in the allergic group). SPT reactivity against cockroach was generally lower but still significantly more prevalent in the allergic group as well: In the allergic group 46% of the subjects were positive against Blattella germanica extract produced by Allergopharma, in the general population only 12%. Values for Blattella germanica extract produced by Stallergene were 25% vs. 8% respectively. There was a tendency for increased prevalence of skin prick test reactivity against grass pollen mixture and Cladosporium herbarum in the allergic group, yet the differences to the general population were not significant. Furthermore the percentages of positive SPT reactivity against grass pollen mixture and Cladosporium herbarum in the allergic group were still low in comparison to mites and cockroaches: the prevalence of positive SPT reactivity was only 8% for both allergens (1% in the general population). The sensitization prevalence against pig epithelia, hen's feathers, banana and latex was identical in both groups (0%). All in all, the comparison of

164 SPT results are available only for 24 of the 28 allergic subjects, as four allergic Karkar received antihistamine and/or oral glucocorticoid therapy.

the SPT sensitization pattern in the allergic group and the general population points to an important role of mites and possibly cockroaches in the causation of symptomatic allergy on Karkar Island. The assumption that mite allergens are indeed more relevant than cockroach allergens is not only supported by the higher sensitization prevalence against mites but also by stronger SPT reactions: Within the allergic group, positive skin reactions were more pronounced against mites (D. pteronyssinus mean wheal diameter 6.5 mm, Blomia tropicalis 4.4 mm) than against cockroach (Allergopharma and Stallergene pooled mean wheal diameter 3.6 mm).[165] Moreover it can not be excluded that some of the cockroach sensitizations are due to cross-reactivity with mite allergens.[166]

The enormous immunologic importance of mites and cockroaches on Karkar is a consequence of climatic and traditional socioeconomic conditions prevailing on the tropical Island. The warm and humid climate on Karkar[167] and the way, in which traditional huts are constructed, create environmental conditions in which mites and cockroaches[168] multiply considerably.[169] Air temperature fluctuates around 28 degree Celcius[170] without much seasonal variation. Relative air humidity is very high (all year over 65%, rainfall 3550 mm p.a.) and the traditional, sago palm covered roofs start to leak considerably, if not replaced every five years. The many gaps in the wooden floors are not easily cleaned.[171] Consequently, organic material accumulates (especially human and to a lesser extent animal epithelia and detritus of the building material itself), is altered by fungal overgrowth and thus represents an ample nutritional source for mites and cockroaches.[172] The level of mite antigen exposure is known to represent a risk for the incidence of specific sensitization.[173] Compared to mites and cockroaches, other allergens obviously play a minor clinical role. Yet considering the environmental conditions, more frequent SPT / clinical reactivity may have been predicted for moulds/Cladosporium herbarum (mould on decaying organic substances, contamination of organic building

165 Positive SPT reactions against grass pollen and mould (Cladosporium herbarum) solutions were generally small: all wheal diameters within the allergic group were 3 mm.

166 Sun et al. performed IgE cross-inhibition assays (n=18) in China to prove that D. pt. sensitization is able to cause "positive SPT reactions against cockroach" (Sun BQ et al., 2010: 3540ff).

167 Hornabrook R, Kelly A, McMillan B, 1975: 590f.

168 Sun et al. showed in a large multicenter study in China that also cockroaches represent a bigger allergological problem in warmer and more humid climates: prevalence of positive cockroach SPT was higher in patients in southern than in northern China (Sun BQ et al., 2010: 3540).

169 Spieksma F, 1997: 360ff. Fernández-Caldas E, Puerta L, Caraballo L, Mercado D, Lockey R, 1996: 98f. Dotterud LK, Korsgaard J, Falk ES, 1995: 788ff. Munir A et al., 1995: 55ff.

170 Mean maximum temperature 30ºC and mean minimum temperature 23ºC (Cotes JE, Saunders MJ, Adam JE, Anderson HR, Hall AM, 1973: 320).

171 Admittedly, Western style carpets should be worse: "… the carpet contains sufficient allergen to replace the allergen in the air many thousands of times." (Erwin EA, Woodfolk JA, Custis N, Platts-Mills TA, 2003: 477).

172 "The quantity of dander that is dispersed by cats, dogs, or humans is sufficient to supply food for dust mites…" (Erwin EA, Woodfolk JA, Custis N, Platts-Mills TA, 2003: 478).

173 Kuehr J et al., 1994: 44ff.

materials),[174] dog and cat epithelia (60% of the participants kept at least one of these animals), rat epithelia (91% of the participants admitted they would regularly see rats in their huts)[175] and pig epithelia (pigs are omnipresent in the gardens and villages).[176]

Conclusions: Based on our 1997 data (SPT, RAST, medical history) we had already suspected mites and cockroaches to represent the clinically most important allergens on Karkar Island. Data from 2002 confirm our previous findings. Within the general population positive skin prick tests were most common against mites (between 14% and 21%) followed by cockroach (8% to 12%). The other 13 tested allergens provoked positive SPT reactions in only 0-1% of the subjects. A similar pattern has been reported from Lambaréné, Gabon, in 2000.[177] Medical histories of a group of 28 allergic Karkar pointed to a predominant role of mites and cockroaches as well: In 19 out of 28 Karkar suffering from atopic disease (allergic asthma and rhinoconjunctivits) the medical histories were highly suggestive of mite and/or cockroach allergy and in another seven these allergens may have been involved as well (in only one case the medical history pointed to a grass pollen allergy and in one case to a dog allergy). Generally it is difficult to determine the exact relevance of cockroach allergens in comparison to the relevance of mite allergens due to overlaps in cockroach-mite exposition and clinical manifestations. Yet skin prick test sensitization patterns indicate that mites play a more important role than cockroaches on Karkar Island. Sensitization prevalence against mites is higher than against cockroaches: this is not only true for the general population but also for the allergic group. Furthermore within the allergic group mean positive skin prick test responses (wheal diameters) were larger against mite solutions (4.4 or rather 6.5 mm) than against cockroach solutions (3.6 mm). We conclude that mites (not only house dust mites but also "storage" mites)[178] represent the clinically most important allergen source on

174 No statistical differences between indoor and outdoor prevalence were detected in Spain (de Ana SG et al., 2006: 357). Thus considering the structure of the open huts on Karkar, indoor and outdoor prevalence should definitely be similar on Karkar Island.

175 Actually, rat allergen is known to represent an important cause of hypersensitivity (Perry T, Matsui E, Merriman B, Duong T, Eggleston P, 2003: 346ff).

176 A possible explanation for the low positive SPT prevalence and low clinical reactivity may be tolerance as expounded by Erwin EA et al.: "… exposure to cats, dogs, rats, and other animals can induce a form of immunologic tolerance … this change occurs with dander allergens rather than with all allergens." (Erwin EA, Woodfolk JA, Custis N, Platts-Mills TA, 2003: 479).

177 Within a group of 520 schoolchildren positive skin prick test prevalence was highest for D. pt. with 11% (16% within our Karkar data) whereas positive skin prick test prevalence for cat, grass and dog was only between 0.2 and 2% (0-1% within our Karkar data) (van den Biggelaar AH et al., 2000: 1725).

178 As terminology is somewhat misleading we have to clarify: Blomia tropicalis is a (nonpyroglyphid) "storage" mite. Although these mites are mainly found in stored grain, they are present in house dust samples in tropical and subtropical parts of the world as well. Worldwide, the (pyroglyphid) mites D. pt. and D. fa. are the "most important allergy-causing HDMs" (Sade K et al., 2010: 849), yet our Karkar SPT sensitization pattern (see chapter 5.4) indicates, that storage mites should not be ignored on Karkar Island: In the unselected population SPT sensitization against Blomia tropicalis was higher (21%) than against D. pt. (16%) and D. fa. (14%).

Karkar Island,[179] followed by cockroach antigens. Pollen and animal epithelia seem to be comparatively irrelevant. The considerably higher relevance of mite allergens in relation to pollen and animal epithelia contrasts the conditions in many "modern" Western societies where the mentioned allergens are often of similar importance.[180]

Clinical implication: On Karkar the villagers' old foam mats are hardly ever replaced and control of mites by encasing or generally questionable chemical measures[181] is neither accessible nor affordable. At least for asthmatic patients it may be advisable to substitute the foam mats by basic coconut fibre mats. The raw material is readily available on Karkar, virtually free of cost and mats could be manufactured locally. This might encourage patients to replace them on a more regular base, thus achieving at least a temporary decrease of allergen exposition. A study from Thailand showed that after 12 months use, coconut fibre mattresses had lower mean mite allergen levels than polyurethane, synthetic fibre and kapok mattresses.[182] As the "supposed symptomatic level" of 10 µg allergen/g dust was reached around the sixth month of use,[183] it may be advisable for mite allergic subjects on Karkar to change the coconut fibre mats in half year intervals.

5.2 High serum total-IgE levels on Karkar

In Western countries atopy and allergies represent the most common reasons for elevated serum total-IgE (sTot-IgE) levels over 100 kU/l.[184] If allergy were the only reason for high sTot-IgE, we would expect quite low levels of sTot-IgE on Karkar as only 4.4% of the investigated general population showed signs of allergy. Yet parasitic infections are known to be the principal cause for increased sTot-IgE levels in many traditional societies,

179 Based on SPT results Woolcock AJ et al. concluded already in 1978 that house dust mites were the "commonest" allergens in two investigated PNG populations (Woolcock AJ, Colman MH, Jones MW, 1978: 155). In rural China, the situation seems to be similar as the "most common sensitizing aeroallergens were dust mite and cockroach" (Kim JS et al., 2008: 929ff).

180 For example, within a large convenient sample of the Belgian general population (n=2,320) 17.1% of allergic rhinitis was HDM-related, yet a slightly higher percentage (17.6%) was grass-related (Blomme K et al., 2013: 202).
Calabria CW et al. report similar sensitization rates for grass and dust mite in patients with rhinitis symptoms in Texas (Calabria CW, Dice JP, Hagan LL, 2007: 442ff).

181 Visitsunthorn N et al., 2010: 156. Furthermore Sidenius KE et al. considered the possibility that killing mites without removing them may not be wise: they measured rather increased and not decreased mite allergen concentrations in dust three months after freezing the samples. Allergens released from the killed mites could have caused the unexpected effect (Sidenius KE et al., 2002: 36).

182 The respective values were 20.2, 22.4, 28.9 and 32.2 µg allergen/g dust. (Visitsunthorn N et al., 2010: 155ff). The climatic conditions were similar to Karkar (page 157).

183 Visitsunthorn N et al., 2010: 155ff.

184 Winter WE, Hardt NS, Fuhrman S, 2000: 1383. Barbee RA et al. found for the general Anglo-white US population (Tucson, Arizona, n=2,743): "In both sexes at all ages atopic subjects had [geometric] mean levels [of sTot-IgE] several times those of their age-matched nonatopic counterparts". Atopy was defined as positive SPT reactivity (Barbee RA, Halonen M, Lebowitz M, Burrows B, 1981: 109).

especially in so called "third world" countries in the tropics.[185] The Karkar suffer from multiple communicable diseases, including diverse parasitic worm infestations. Factors attributing to the immense importance of helminths on Karkar are the traditional lifestyle (sharing of food, low hand hygiene, unavailability/non-use of toilets, walking barefoot etc.) and the warm and humid climate which favours the survival of eggs and larvae (for details on worm infections see next chapter). Consequently we expected high serum total-IgE levels on the island. Our sTot-IgE raw data was already published in 2009[186] yet we will have a closer look at the figures now. As a first step, we have to transform our data, as the histogram shows us, that – as expected – sTot-IgE is not normal distributed (see fig. 14).[187] Yet normal distribution is necessary for the accurate description of the data (central tendency and dispersion) and the application of parametric statistical tests.[188] Figure 15 shows that normalization occurs after $\log_{10}$-transformation, i.e. the distribution becomes Gaussian; this shows that sTot-IgE values are log normal distributed.[189]

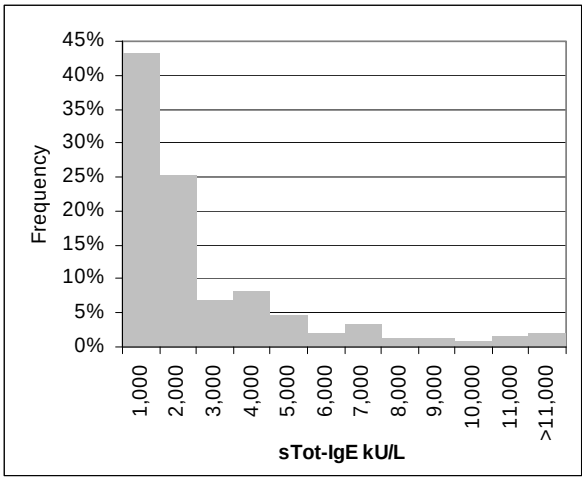


Fig. 14

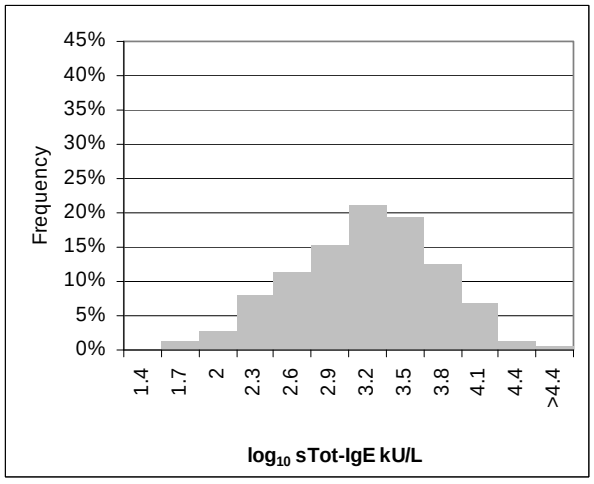


Fig. 15

Distribution of sTot-IgE values in the Karkar population (n=248). Prior to logarithmic conversion the distribution is skewed (fig. 14). Normalization occurs following $\log_{10}$-conversion (fig. 15). The arithmetic mean of the logs is 3.029 kU/l representing a GM of 1,069.0 kU/l.

185 For example Platts-Mills TAE, Lee BW, Arruda LK, Chew FT, 2011: 82 and Winter WE, Hardt NS, Fuhrman S, 2000: 1383.

186 Herbert O, 2009: 34f.

187 Many studies show that (sTot-) IgE is not normally distributed: e.g. Barbee RA, Halonen M, Lebowitz M, Burrows B, 1981: 106-111, van den Biggelaar AH et al., 2000: 1724.

188 In agreement Barbee RA et al.: "Serum IgE levels were converted to $\log_{10}$ for calculations…" (Barbee RA, Halonen M, Lebowitz M, Burrows B, 1981: 108).

189 The fact that normal distribution occurs following logarithmic conversion of IgE values has been described by Barbee RA, Halonen M, Lebowitz M, Burrows B, 1981: 106-111 and Lynch NR, Hagel I, Perez M, Di Prisco MC, Lopez R, Alvarez N, 1993: 406. Bousquet J et al. declared that "All [IgE] data were analyzed after log transformation" (Bousquet J, Chanez P, Chanal I, Michel FB, 1990: 1041).

The log-transformed IgE values are now used to calculate the geometric mean (GM) representing the adequate measurement for central tendency of log normal distributed data.[190] Furthermore, the log-transformed IgE values can be used to calculate the standard deviation on the log scale, necessary for giving correct population confidence intervals [CI] and cutoff points[191]. The geometric mean of sTot-IgE in our sample (n = 248) is high with 1,069.0 kU/l (95% CI: 906.2-1261.2 kU/l).[192] Altogether 96.0% (238/248) (95% CI: 92.7-98.1%)[193] of our participants showed sTot-IgE exceeding 100 kU/l. In other words, 96% had sTot-IgE levels we would consider elevated in "modern western countries".

We investigated the hair lead concentration of the Karkar and found – unexpectedly and against all odds – increased values (see chapter 4.4). We could not determine the origin of the contamination. It has been reported that exposure to lead (even in utero) is positively related to Tot-IgE.[194] Yet on Karkar we consider this effect (if relevant at all) to be negligible in comparison to parasitic infestations. Serum Tot-IgE is mostly described to be low in childhood, highest in youth (10-14 years), decreasing with age.[195] We correlated age and sTot-IgE. As before, we used the $\log_{10}$-transformed sTot-IgE values. Serum Tot-IgE did not show the expected behaviour: there was no change of sTot-IgE levels with increasing age (r^2=0.002, p=0.48) (see fig. 16).

190 For details see chapter 4.5. We calculated the GM using the arithmetic mean of the logs. Both, specific and total IgE values are log normal distributed; however some studies give median and range for IgE values (e.g. Rancé F, Abbal M, Lauwers-Cancès V, 2002: 1029).

191 Olivier J, Johnson WD, Marshall GD, 2008: 333ff.

192 In 1986 Turner KJ was one of the first to measure sTot-IgE levels in the PNG highlands. He reported an even higher sTot-IgE in the South Fore (64.8% of the subjects were hookworm infected) than what we found on Karkar. In the age group 16-20 years (n=49; this age group was most representative for our Karkar data) the GM was 2,460 kU/l (Turner KJ, Dowse GK, Stewart GA, Alpers MP, 1986: 561f). Maybe this finding is due to less anthelmintic treatment in the 1980s.
Generally however, already our 1069.0 kU/l sTot-IgE GM must be considered extremely high. Even asthmatic children in Recife, Brazil, showed "only" a sTot-IgE GM of 591.7 kU/l (Lopes MI, Miranda PJ, Sarinho E, 2006: 206). The sTot-IgE GM of the worm infected (Ascaris l. and/or Trichuris t. prevalence = 74%) general population (children, n=520) in Lambaréné, Gabon, was reported to be 727 kU/l (van den Biggelaar AH et al., 2001: 235). The sTot-IgE GM in "affluent Western societies" is much lower: in a random sample of the Norwegian general population (n=1512) Omenaas E et al. found a GM of only 16 kU/l (Omenaas E et al., 1994: 535) and Barbee RA et al. reported a sTot-IgE GM of just 32.1 kU/l within the general Anglo-white US population (Tucson, Arizona, n=2,743) (Barbee RA, Halonen M, Lebowitz M, Burrows B, 1981: 106-111).

193 Exact binominal confidence interval (Clopper-P-interval).

194 Annesi-Maesano I et al., 2003: 589ff.

195 For example Annesi-Maesano I et al., 2003: 589ff and Winter WE, Hardt NS, Fuhrman S, 2000: 1382. Barbee RA et al. found the highest levels of sTot-IgE among 6- to 14-year-olds; in later life there was a monotonous decrease of sTot-IgE values (Anglo-white US population) (Barbee RA, Halonen M, Lebowitz M, Burrows B, 1981: 106-111). Omenaas E et al. compiled four studies which showed "a fall in [sTot-IgE] levels by increasing age" (Omenaas E et al., 1994: 536).

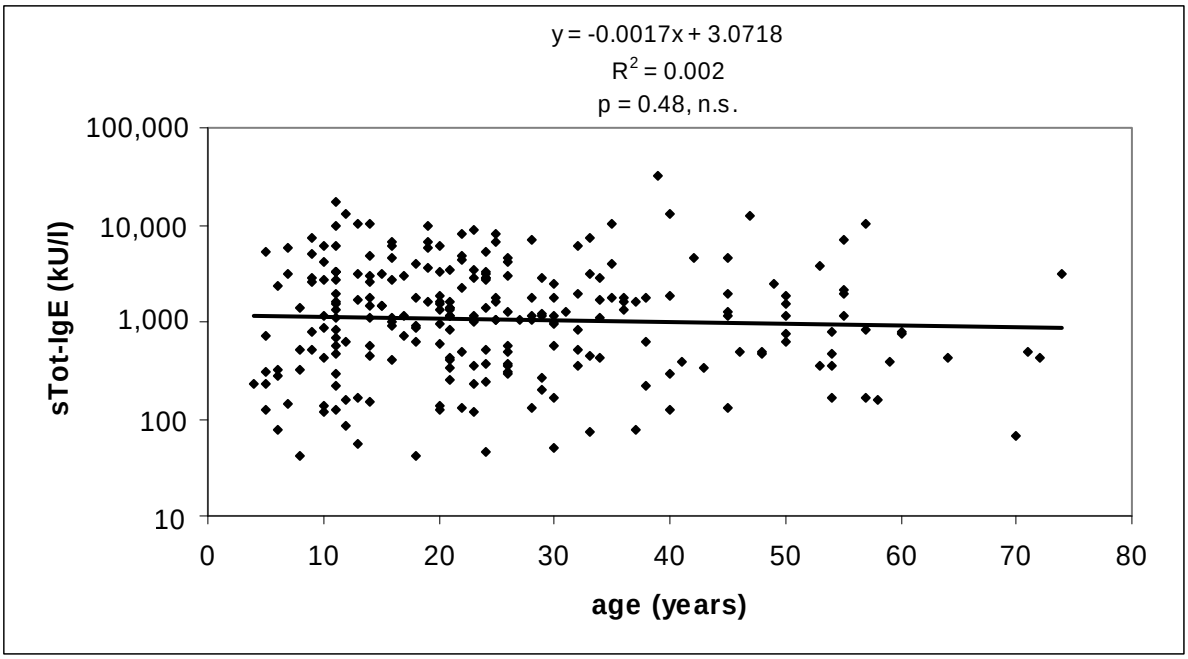


Fig. 16. No correlation of sTot-IgE and age (n=246) (Pearson product moment). Spearman correlation yielded similar results.

Anticipating our later results we may already mention at this point, that this finding may be due to an ongoing, largely age-independent continuous stimulus of the IgE system by helminth infestations.

5.3 Worm infestations explain the high serum total-IgE levels

Ascaris lumbricoides (roundworm; Pidgin *Eskeris*[196]) and Ankylostoma duodenale/Necator americanus (hookworm, Pidgin *Hukwom*[197]) represent the most relevant worms on Karkar. As helminths play a crucial role in our study, we want to give some background information on these parasites. The Pidgin expression for worms in general is *snek b[i]long bel*, the snake(s) of the belly.[198] Despite of the fact, that Ascaris and Ankylostoma are both nematodes[199] they nevertheless show different life circles (including infection modes) and cause different symptoms.

Ascaris lumbricoides (roundworm) is transmitted by oral ingestion (hand-to-mouth) of vital eggs. In the host's small intestine eggs develop into adult worms (after lung passage).[200] The female roundworms emit thousands of eggs per day into the patients' faeces. In the warm, moist soil (toilets are not always available or used) eggs become infective after 3-6 weeks[201] and stick to surfaces, foods, feet and hands.[202] Ingestion closes the infectious circle. Ascaris infection is omnipresent on the island. We found anti Ascaris lumbricoides IgE in 82% of the population.[203] These antibodies indicate a past and/or present infection with roundworms. In contrast to Ascaris, Ankylostoma duodenale (hookworm) can't be avoided by hand hygiene (alone) but mainly by wearing shoes or sandals. Infectious faeces contain Ankylostoma larvae which are able to penetrate the healthy skin. When a person steps on stool contaminated soil (larvae may survive for months in the soil and even proliferate there), the larvae actively transverse all dermal layers and get into the host's blood stream.[204] After a passage into the lung and ascending the trachea, the small worms are swallowed and get to their final destination, the small intestine, where they develop into adult worms. In contrast to Ascaris (living on intestine content) hookworms perforate little vessels and live on the host's blood, thereby often causing anaemia.[205] Edwin Tscharke (see below) explained this in a quite comprehensible way to his Karkar medical assistants: … *hookworm i … stilim blood bilong man… [em] i pulim blut olsem pikinini i pulim susu long mama bilongem … bambai i finisim tru blood bilong man … Nau yu lukim man blut i wait tumas (anemia)*. "Hookworms … steal the blood of the people. They suck blood like babies suck milk from the breast of their

196 Kaiser M, 1990: chapter 21-14 (no pagination).
197 Kaiser M, 1990: chapter 5-9 (no pagination).
198 Not all Karkar are familiar with the Pidgin terminology *Eskeris* and *Huwkom*. Thus they use the umbrella term *snek bilong bel*. However they may distinguish between different kinds of worms in Takia and Waskia language.
199 Gause WC, Urban JF Jr, Stadecker MJ, 2003: 270.
200 Quirmbach G, Müller S, 1988: 84f.
201 Quirmbach G, Müller S, 1988: 84.
202 Food and Drug Administration, U.S. Department of Health and Human Services. Bad Bug Book, Foodborne Pathogenic Microorganisms and Natural Toxins: no pagination.
203 Herbert O, 2009: 24.
204 Quirmbach G, Müller S, 1988: 86.
205 Quirmbach G, Müller S, 1988: 86.

mothers … they will/are able to finish the blood of the people … thus you [will] see that the blood [of the infected person] is very/too white (anaemia)".[206] In 1978 Anderson reported mean haemoglobin in Karkar children of only 9.3%.[207] Often anaemia is aggravated by malaria[208] and during pregnancy or illness the situation may become critical. We have no accurate epidemiological information on the present prevalence of hookworm infection on Karkar, yet results from Gaubin hospital laboratory indicate, the infection is of similar importance compared to roundworm infestation.

Known history of helminth infections and their prevalence on Karkar is short and vague: the first accounts originate from the "medical missionary" E. Tscharke who arrived on Karkar in 1947[209] and the early planters. Tscharke stated in his above mentioned "Medical Handbook" written in his early years on Karkar (published probably in 1952): *Long New Guinea oltogeta man, oltogeta meri i gat hookworm* ("In New Guinea every man and woman suffers from hookworms").[210] And in respect to Ascaris he continues: *Plenti man bilong New Guinea i gat disfela kaind snek long bel bilongem* ("Many people in New Guinea have this kind of snake of the belly in their body").[211] Anderson mentioned in 1978 shortly that "worm infestations (ascaris, hookworm) are common [on Karkar]"[212]. The planter Sir John Middleton who came to Karkar in 1948 remembered: "[After the Second World War] worm infections were endemic." In respect to the current infection prevalence he stated: "But it's not nearly the problem now, than it was then."[213] The planter Paul Goodyear however showed a less optimistic point of view: "Worm infections are still very much a daily part of life … the spread of worms is still very much the same like it was."[214]

Even without knowing the exact development of helmith prevalence rates over the last 100 years, it is probably safe to claim the following: Two different medical systems failed to eradicate parasitic worms on Karkar: the indigenous medical system (basically no treatment effect) and the Western medical system (practically only limited treatment effect). The traditional phytomedicine – while effective in respect to many other illnesses – can not control helminth infestation. This may be deduced from epidemiology (extremely high prevalence on the island) and the statements of the Karkar themselves.

206 Tscharke E, 1952?: 95.

207 Children were younger than 15 years (Anderson HR, 1978: 64). Reference haemoglobin values are 12-17%. In 1973 Cotes JE et al. reported the following mean haemoglobin levels from Kaul Village on Karkar: 11.0% for young men and 9.9% for young women (Cotes JE, Saunders MJ, Adam JE, Anderson HR, Hall AM, 1973: 322f).

208 Cotes JE et al. stated for Karkar: "Anaemia is common on account of malaria and worm infestations" (Cotes JE, Saunders MJ, Adam JE, Anderson HR, Hall AM, 1973: 320).

209 Tscharke E, 1973: 12. The Karkar and also the later doctors in Gaubin Hospital call Tscharke a "legend" (Bertram S, 2012: 47).

210 Tscharke E, 1952?: 93. Tscharke mentions New Guinea as a whole, but his practical experience was basically limited to Karkar Island.

211 Tscharke E, 1952?: 98.

212 (Anderson HR, 1978: 64).

213 Sir John Middleton (*July 1930), interview, October 19th 2009, Karkar Island.

214 Goodyear, Paul James (*April 14th 1965, Gaubin Hospital), interview, October 2009, Karkar Island.

The only study on phytomedicine ever conducted on Karkar (published in 1984) listed over 20 different symptoms and diagnoses (fever, sores, malaria, snake bites etc) which were (and still are) treated with traditional plant medicine on Karkar. Yet none of the mentioned 40 different plant species was considered by the informants to be effective in case of worm infestations.[215] This is in line with our results: we conducted over 350 interviews on the use of phytomedicine on Karkar. Only one informant, Joannah, considered a plant effective for worm infections (papaya seeds).[216] There was just one more piece of information given by Brett Middleton in respect to the husk of betel nut: "… betel nut skin [is] sweet and nice and I am told anecdotally that it is a deworming agent."[217] The Karkar are obviously aware that in the special case of helminth infestation *marasin bilong bus* (bush medicine) is not effective. This shows how robust worms are: they are not only able to resist the host's immune defence but they also withstood the manifold ingested natural substances the Islanders will have tried over thousands of years to rid themselves from theses parasites. Even informants who were generally in favour of traditional phytomedicine like Russel Usel admitted that Western drugs may be the better treatment option in case of worm infestations: *Em i gat sampela ebs i stap, tasol ol i no wokim gut…* ("There exist some plants [to treat worms], they just do not work well …"). He added that Papaya seeds would not help against worms (however he considered them to be effective for malaria) and continued: *Mi ting olsem faktori marasin bilong snek stret em bai rausim* ("I think that factory medicine [i.e. Western drugs] against worms will effectively expel [the parasites from the body]").[218]

Despite of the unquestionable higher effectiveness and decade long availability of biomedical deworming drugs, the prevalence of Ascaris within our study group shows that helminths were not too "impressed" by the introduction of western anthelmintic drugs (as mentioned above eighty two percent (203/248) of our participants showed antibodies against roundworm). The fact that helminth infections are far from being eradicated on Karkar Island is mainly due to the absence of "Western style hygiene"; this leads to continuous re-infection. Hygiene has early been identified as the crucial parameter in the battle against worms, as pointed out by Sir John Middleton[219]: "At the end of the [Second World] War, once again the government sponsored a program: they [orally] used

215 Holdsworth D, 1984: 111 ff.

216 During the interview it became apparent that Joannah was not very experienced in traditional phytomedicine. Generally however, she was very convinced of the effectiveness of phytomedicine: "At home we have a lot of bush medicine. You can use the seeds of papaya for worms … I like the bush medicine so much, because it is a kind of medicine which has already been used by our *tumbuna* [ancestors]." Most biomedical studies consider Papaya (seeds) – despite of a wide range of enzymatic properties – to be at best only marginally effective for worm infections; moreover the biomedical level of evidence of the studies is often low (e.g. Aravind G, Debjit B, Duraivel S, Harish G, 2013: 7-15).

217 Brett Maxwell Salum Middleton (*1962, Madang), interview, October 2009, Karkar Island. On the Island, the husk of betel nut is widely and quite effectively used for oral hygiene instead of a tooth brush. Yet no other of the over 350 informants connected betel and worms. As already in the case of papaya, there exists conflictive data in respect to deworming properties of betel nut, but this is out of the scope of this work.

218 Russell Usel (*1970, Gaubin, Karkar Island), interview, October 19th 2009, Karkar Island.

219 Sir John Middleton (*July 1930), interview, October 19th 2009, Karkar Island.

tetrachloride [tetrachlorethylene] and then a purge [magnesium sulfate][220] to eliminate it [worms], but re-infection always occurred ... The Karkar used pit toilets, but they didn't use them 100% of the time so it never broke the [infection] chain". The "medical missionary" E Tscharke considered "hygiene" the basic problem of health on Karkar. In his already mentioned "Medical handbook for New Guinea's native medical assistants" he made it very clear that *wok hygiene i numbawon wok long New Guinea* ("to address the hygiene problem is the main task in New Guinea").[221] Tscharke was well aware of the high prevalence of helminth infestation and the reinfection problem. As primary prevention he suggested every Karkar house should dispose of a pit toilet and that *hol bilong haus pekpek igo daun moa* ("the hole of the toilet house should go deeper").[222] He did not get tired to repeat that in respect to hookworm it is *Tambu tru fasin bilong pekpek nabaut [ontop long graun long bus]* ("The habit of defecating somewhere in the bush is really forbidden")[223]. However – even today – there are not too many pit toilets on Karkar; the officer in charge of the Gaubin Hospital confirmed this in 2009: "There are [only] some "small houses"[224]. Moreover several informants stated that people believe that the smell in the houses may be unhealthy/contagious. Thus we can confirm one of Tscharke's early observations: *Ol i ting smel bilong pekpek i as bilong sik nau ol i fred long go insait long haus pekpek i gat smel* ("The people think that the smell of excrements is the reason for illness. That's why they fear to use the smelling pit toilets").[225] For decades Tscharke kept repeating the importance of hand hygiene: *Oltaim wasim gud hand. Lukautim kaikai; nogud pekpek igo fas longen* ("You always have to wash your hands well. Be careful with the food; it is bad/dangerous if it gets contaminated with excrements").[226] Interestingly, we may discern an analogous fear of excrements – quite differently motivated – in two different cultures: The "modern Western" culture fears excrements as a vehicle for communicable diseases, the traditional Karkar culture fears excrements as a vehicle for contagious magic.[227]

During seven decades of contact with the Western hygiene concept, ideas of "morally good hygiene"[228] and "dangerous contact", vigorously pushed by Tscharke and his successors, began to sink into the Karkar's minds and to modify their explanatory models

220 The historical biomedical use of magnesium sulfate as a salinic/osmotic purgative and tetrachloroethylene (tetrachloroethene) as an anthelmintic drug for hookworms and Ascaris on Karkar Island was confirmed by Tscharke (Tscharke E, 1952?: 96ff). Meanwhile tetrachloroethylene is not used any more due to possible carcinogenic effects and environmental toxicity.

221 Tscharke E, 1952?: 36.

222 Tscharke E, 1952?: 36.

223 Tscharke E, 1952?: 36.

224 "Es gibt [nur] einige kleine Häuschen" (interview with Christoph Ihle, October 15th 2009, Gaubin Hospital, Karkar Island).

225 Tscharke E, 1952?: 34.

226 Tscharke E, 1952?: 99.

227 For details on contagious magic on Karkar see Herbert O, 2011: 242ff.

228 In his work "Homo hygienicus" Labisch A pointed exactly to this typical "modern Western" construct of a connection between hygiene and moral: „Ein sauberes Leben wurde zum moralisch angemessenen Leben" ("A clean life became a morally appropriate life") (Labisch A, 1989: 321).

of illness. Meanwhile most Karkar know the biological way of transmission of worms. However, "incorporating" this "modern" knowledge into a "traditional" society often generates cultural problems as R. Usel confirmed: "Tscharke told us what we can not do … for example … now I don't like to share the same betel husks, spoons, the same cups [any more]. I do not like to eat together with the others from one plate … doing so is not good because the illness will go from one person to the other. We must avoid the spread of diseases. Thus now, when all eat, I leave the food to the others. Only concerning respect and demonstration of my solidarity with the *lain* (clan) … you will feel that this conduct is not good. It is bad when the *lain* thinks: *Oh, this man does not like our food."*[229]

All in all the efforts of the *wait skins* (white people) to foster hygiene were only partially effective. On the one hand Tabitha Tscharke (wife of Edwin Tscharke) wrote in a private letter (not dated, probably 1965): "When comparing their … personal hygiene with what it was before [after the Second World War], we do feel our efforts have not been in vain."[230] On the other hand Wells described the situation on Karkar in the year 1983 in a less optimistic way: "… they prepare food with unwashed hands that have touched suppurations, excrement …"[231] The observations of the author in the villages and the hospital do not allow any conclusion whether the level of "Western style hygiene" has really risen during the last decades. We may only conclude that a combination of practical reasons (no running water supply to wash hands), traditional believes/customs (reluctance to reject offered food prepared by potentially infected individuals, belief in dangerous smell) and plain thoughtlessness keeps assuring the survival of worms – and thereby, at the same time, allergies may be held at bay.

After this more cultural introduction to worm infestations we will now focus on the immunological implications of these parasites. Many studies indicate that helminth infections increase serum total-IgE (sTot-IgE) levels considerably.[232] Unfortunately it was not possible to perform stool tests to detect eggs in faeces thus we will follow two approaches – one clinical and one serological – to verify the association of worms and sTot-IgE in our study population: Firstly we will compare sTot-IgE levels in people stating that they are currently seeing worms in their stools with those claiming to never have seen parasites in their stool (clinical approach). Secondly we will compare sTot-IgE levels in people who show antibodies against Ascaris with those who are Ascaris antibody free (serological approach).

229 *Tscharke toktok long mipela wanem no ken mekim … kain olsem … nau mi no laik … serim seim beetel shells, spoon, seim cap, kaikai seim plate wantaim ol … dispela em i no gutpela bikos bai pasim sik i go long narapela. Yumi mas avoidim ol sik bai spred around. Tasol nau ol kaikai, em [mi] larim ol kaikai long ol yet. Tasol long pasin bilong respect na soim pasin bilong yu long ol dispela ol lain manmeri … bai yu pilim i no gutpela. Nogut ol lain bai ting olsem: Oh, man em no laikim long kaikai bilong yumi.*

230 Tscharke T, 1965?: Manuscript Section, MS 9067, Box 1, Series 1, Folder 2.

231 Wells M, 1985: 148.

232 For example: Van den Biggelaar AH et al., 2000: 1725; Lynch NR, Hagel I, Perez M, Di Prisco MC, Lopez R, Alvarez N;1993: 404-11.

5.3.1 Serum Total-IgE is higher in people who see "currently worms in stool" than in people who “never have seen worms in stool”

We asked 248 Karkar if/when they noticed worms in their faeces for the last time. The geometric mean (GM) of sTot-IgE in the group stating never to have seen parasites in their stools was lowest with 1021 kU/l (95% CI: 843-1235 kU/l [n = 191]) and in the group admitting to be currently affected by a noticeable infection the GM was highest with 1788 kU/l (95% CI: 935-3422 kU/l [n = 20]).[233] The difference in sTot-IgE (GM difference) between the “never parasites in stool” group and the “currently parasites in stool” group showed a p = 0.077 (t-test). The following three factors are likely to have contributed to this "non significance" on a 0.05 alpha level.

Firstly the number of cases in the "currently worms in stool" group is small (n = 21).[234] Secondly we have to assume a considerable “cultural bias” towards less significance as the group of people stating to never have seen worms in their stools [n = 191] is very likely to include islanders who have actually seen parasites in the faeces (cultural stigma of infestation), yet not wanting to admit it. And thirdly an individual may not be conscious of a current infestation as an infection may go clinically unapparent, depending on the worm species[235] and worm loads. The likelihood of expulsing worms is not high in individuals with low Ascaris burdens[236]. The notion that our “never parasites in stool” group may well include people either not admitting or not knowing that they are currently suffering from an helminth infection is supported by two facts: On the one hand a sTot-IgE GM of 1021 kU/l would be suspiciously high for a worm free group. On the other hand 81.2% (155/191) of the “never worms group” showed antibodies against Ascaris lumbricoides.[237] Despite of the likelihood of a certain degree of “overlap” between the "currently worms in stool" group and the “never worms in stool” group, the “self categorization” of the islanders seems to be “stochastically meaningful”. This is not only shown by the described between group differences in sTot-IgE but also by a significant group difference of anti-Ascaris IgE: the level of anti-Ascaris IgE was significantly lower in the “never have seen worms in stool” group (median 2.32 kU/l) than in the "currently worms in stool" group

233 In the group stating to have seen parasites “in the past” the GM was coherently intermediate with 1228 kU/l (95% CI: 803-1877 kU/l [n = 20]). Yet as the somewhat imprecise “in the past” formulation may have generated a quite inhomogeneous group (one month or ten years ago?) we just compared the clearly defined “never parasites in stool” group and the “currently parasites in stool” group using a standard t-test (instead of an ANOVA for a comparison of three groups).
The groups were generally adjusted for age and sex, yet the effect of adjustment was margial.

234 The small number increases the standard error of differences between two means (SEDM) in the t-Test (Van Emden H, 2008: 58ff).

235 The CDC state that most hookworm infected people show no symptoms (Centers for Disease Control and Prevention. Parasites–Hookworm: no pagination). This holds true for most helminth infestations.

236 “Infection with one or a few Ascaris spp. may be asymptomatic…” (Food and Drug Administration, U.S. Department of Health and Human Services. Bad Bug Book, Foodborne Pathogenic Microorganisms and Natural Toxins: no pagination).

237 The presence of anti-Ascaris IgE does not necessarily indicate a present infection, as antibodies may persist even after a cure, yet it can be excluded that there was never any infection with Ascaris.

(median 6.79 kU/l) (adjusted H = 7.31, p = 0.007, Kruskal-Wallis-test)[238]. Last but not least, literature supports the notion that worm infestations cause high serum Tot-IgE levels. Just to give two examples: Santiago HdaC et al. described elevated sTot-IgE levels in filarial infection[239] and van den Biggelaar reported that long-term treatment of intestinal helminths leads to a significant and monotonous decrease in sTot-IgE levels.[240]

We recapitulate that because of the mentioned twofold bias, i.e. cultural bias and the possible ignorance of infection, our "never worms in stool" group it is very likely to include people suffering from a helminth infestation. These people's high sTot-IgE values increase the "never worms in stool" group GM towards the "currently worms in stool" group GM, thereby decreasing the real inter group difference and thus contributing to a p slightly larger than 0.05. Taking this and the low number of cases in the smaller group into account, we consider the "real" sTot-IgE levels of the "currently worms in stool" group significantly higher than of the "never have seen worms in stool" group – even on a 0.05 alpha level.

5.3.2 Serum Total-IgE is significantly higher in people who show antibodies against Ascaris lumbricoides than in those who are Ascaris antibody free

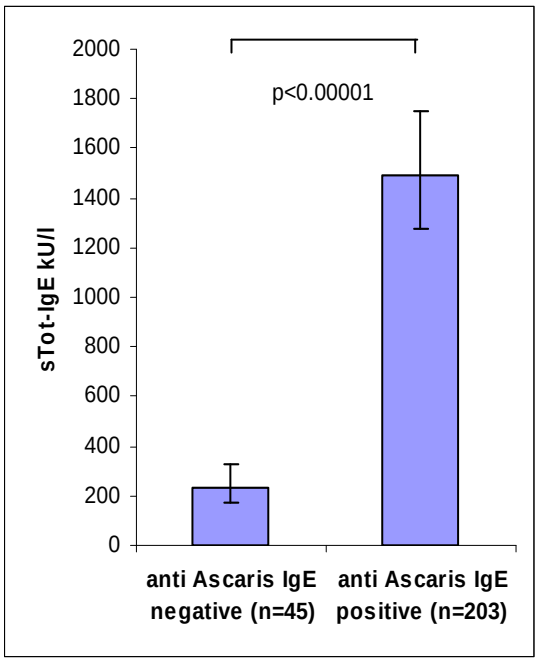
We measured antibodies against Ascaris lumbricoides (roundworm) in 248 participants. Despite of the fact that these antibodies are of the IgE type – just like the other 19 antibodies we measured in our study – they have a different "meaning". Anti Ascaris IgE is not directed against a "dead", per se harmless environmental allergen, but against a parasite. In contrast to "normal" IgE, anti Ascaris IgE is not involved in allergic reactions. It is not clear what the actual function of anti worm IgE is, and whether it conveys effective host protection against the parasites.[241] We cannot answer this question either and qualify anti Ascaris IgE just as a marker for roundworm infection. Figures 17 and 18 both show the highly significant correlation between anti Ascaris IgE and sTot-IgE levels.

238 A t-test or ANOVA could not be performed on $\log_{10}$-transformed data, as too many cero values impeded log-normalization. Prior to the Kruskal Wallis test we proved homogeneity of variance in both groups using the Bartletts test (p = 0.457). The chi^2 approximation was used to calculate p in the Kruskal Wallis test.

239 Santiago HdaC, Ribeiro-Gomes FL, Bennuru S, Nutman TB, 2015: 95.

240 Van den Biggelaar estimated the decline of sTot-IgE to amount to 138 kU/l every 6 months during a 30 months treatment period (Van den Biggelaar AH et al., 2004: 896 f.).

241 Data from a study on the effect of the anti-IgE drug omalizumab (Xolair®, Novartis) indicate, there is no increased likelihood of helminth infection during the time of the "removal" of IgE (total and specific) by the drug. The authors see "some evidence" to the contrary, yet this is due to the application of a statistically most questionable approach (one sided test instead of two sided) (Cruz Filho A, 2007: 197-207).



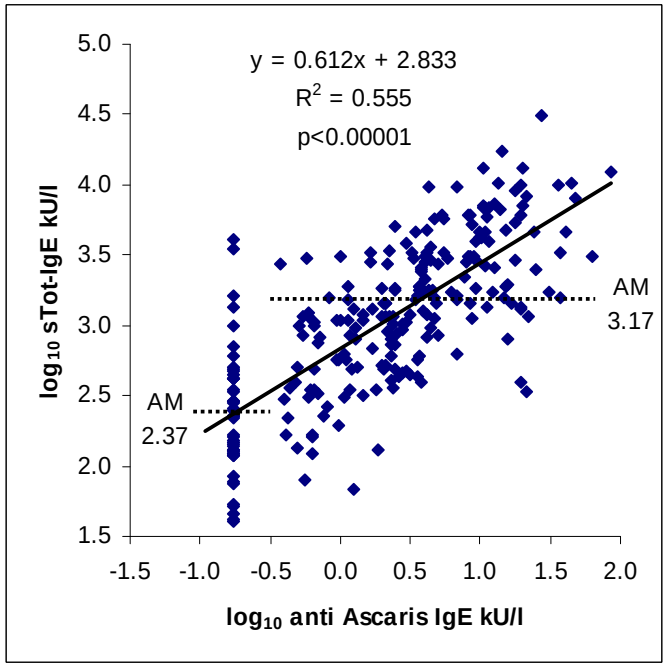


Fig. 17 Fig. 18

Fig. 17. Significantly ($p<0.00001$) higher sTot-IgE levels in anti Ascaris IgE positive than anti Ascaris IgE negative islanders (bar graph): GM 1495 kU/l (95% CI: 1280-1747 kU/l) vs. GM 235 kU/l (95% CI: 171-325 kU/l) (standard t-test). Fig. 18. The same data is displayed in a $\log_{10}$ scale scattergram. There is a significant positive linear correlation between anti Ascaris IgE and sTot-IgE ($R^2 = 0.55$, $p<0.00001$; $n = 248$). The arithmetic means (AMs, two dotted horizontal lines) of the log transformed data (corresponding to the GMs in fig 17) are given for the Ascaris negative group (2.37; line of vertical points on the left) and the Ascaris positive group (3.17; rest of points, cloud).

As presented in fig. 17, anti Ascaris IgE positive Karkar show significantly ($p<0.00001$) higher sTot-IgE levels than anti Ascaris IgE negative islanders: GM 1495 kU/l (95% CI: 1280-1747 kU/l) vs. GM 235 kU/l (95% CI: 171-325 kU/l) (standard t-test[242]). In fig. 18 we display the same data on a $\log_{10}$ scale scattergram. A highly significant positive linear correlation between anti Ascaris IgE and sTot-IgE becomes apparent ($R^2 = 0.55$, $p<0.00001$; $n = 248$). The arithmetic means (AMs, two dotted horizontal lines) of the log transformed data are given for the Ascaris negative sub-group (2.37; line of vertical points on the left)[243] and the Ascaris positive sub-group (3.17; rest of points, cloud). Back transformation of the two arithmetic means gives us the corresponding GMs (original scale) already displayed in fig. 17. The variability of the sTot-IgE values of the Ascaris negative sub-group (visualized left hand in fig. 18 as a

242 Again, the t-test was performed on $\log_{10}$-transformed data and preceded by an F-test ($p = 0.74$) to check for homogeneity of variances.

243 The 45 anti Ascaris IgE negative islanders are placed at -0.76 on the X axis as they were ascribed an anti Ascaris IgE value of 0.175 kU/l on the original scale to allow for $\log_{10}$-transformation. This value of 0.175 kU/l corresponds to -0.76 after $\log_{10}$-transformation.

vertical line of 45 points) is considerably high: the lowest value occurs at 1.61 corresponding to 40.7 kU/l on the normal scale and the highest at 3.61 corresponding to 4060.0 kU/l on the normal scale (range = 4019.3 kU/l). Only eight of the 45 specific Ascaris IgE free individuals (18%) showed sTot-IgE levels under 100 kU/l. The large dispersion embracing many clearly elevated sTot-IgE values and few low sTot-IgE values (i.e. within the "Western normal range") is most likely a consequence of Ascaris not being the only helminth on Karkar Island: Hookworms (Necator americanus) are at least as wide spread and other nematodes like Trichuris trichiura (whipworm) are prevalent as well[244]. For this reason we consider the sTot-IgE levels a better indicator of worm infestation than the exclusive presence of anti Ascaris antibodies.

We summarize our conclusions from the clinical and serological approaches as follows: our findings support the notion that high sTot-IgE levels in a traditional society living in the tropics are a marker of parasitic diseases, especially helminth infestations.[245] Already in 2003 Cooper et al. considered high sTot-IgE an appropriate indicator for worm infections in rural Ecuador[246] and in 2007 Erb called "large amounts of IgE detected in the serum" "the greatest hallmark of a helminth infection".[247] Thus in the following chapters we will use sTot-IgE levels as a marker for immune system stimulation by helminths.

244 Author's communication with personal of Gaubin Hospital laboratory in different years.
A study carried out on 320 people in the village of Kaul on Karkar Island reports 57% of the villagers being infected with Ascaris lumbricoides, 70% with Necator americanus and 21% with Trichuris trichiura (Jones H, 1976: 166ff.).

245 There was no relevant correlation of sTot-IgE levels with the time of the last malaria episode as given by the participants (categorization: malaria symptoms currently, within last 3 months, within last 6 months, within last 12 months, more than 12 months ago and never). The number of suspected current scabies cases (three) was not high enough to draw conclusions concerning the effect of scabies on sTot-IgE (scabies is known to be able to increase sTot-IgE. For example Falk ES, 1981: 167ff).

246 Cooper PJ, Chico ME, Rodrigues LC, Ordonez M, Strachan D, Griffin GE, Nutman TB, 2003: 995ff.

247 Erb KJ, 2007: 1170.

5.4 Differences between RAST and SPT within the Karkar population

To understand, why we may call the pattern of allergic sensitization on Karkar "unusual", we first have to recapitulate that there are two different ways of measuring allergic sensitization: The first way is to look at the level of specific IgE (kU/l or RAST-Classes) in the blood serum as already described in detail in chapter 2.3.2. The second way is to perform Skin Prick Tests (SPT)[248]. Despite the fact that SPT could be regarded as just another means of detecting allergic sensitization, the test yields different information. Whereas the detection of specific IgE (i.e. positive RAST)[249] "only" tells us, the body recognizes the respective substance, builds antibodies (IgE) and could thus theoretically react in an allergic way, a positive SPT is several steps "nearer" to a clinically relevant reaction[250]: the contact of the allergen (SPT solution) with the skin (dermis) actually leads to a measurable allergic inflammation (wheal). This implies that the whole "atopic pathway" must have been successfully activated upon allergen contact: we can conclude that there exist a sufficient number of "adequately" sensitized dermal mast cells, bearing the correct amount and composition of specific IgE on its surfaces. Moreover we deduce, that the allergen must have been able to crosslink the specific IgEs without relevant inhibitory interference that the entire process of degranulation worked well and that – as a final step – the surrounding tissue reacted upon histamine release. As Witteman AM et al. rightfully remarked: "… IgE antibody levels and skin test results are not interchangeable as an indicator of the degree of allergic sensitization."[251]

Despite of the higher physiological complexity involved in a SPT reaction, SPT is nevertheless more sensitive in detecting atopy than "simple" RAST.[252] We can only guess why this is the case. One possibility would be that because of the high affinity of the FcεRI IgE receptors on tissue mast cells, not enough specific IgE is left in the serum to be detected by RAST. Another possibility would be that differences in the binding-mechanism are responsible for the discrepancies in sensitivity: In RAST there is just ONE binding involved (one arm of the Y-shaped IgE binds to the solid phase test allergen), whereas in SPT at least TWO bindings occur (two or more arms of different[253] IgE on the mast cell surface bind to one test allergen; moreover the whole IgE-allergen cluster is stabilised/fixated on the effector cell surface via FcεRI). Thus maybe the unphysiological

248 The basics have already been described in chapter 4.2.

249 In RAST measurements "specific IgE from the patient's serum binds to the allergen embedded in the [ImmunoCAP] polymer" (Siles RI, Hsieh FH, 2011: 586).

250 Winter WE, Hardt NS, Fuhrman S, 2000: 1383: "… skin testing more closely relates to clinical disease than RAST testing …"
Siles states: "… skin testing correlates better with nasal allergen challenge (the gold standard) than blood testing… " (Siles RI, Hsieh FH, 2011: 589).

251 Witteman AM et al., 1996: 16.

252 "When the test results [for allergic sensitization] were discordant, the skin test was usually positive and RAST negative" (Haahtela T, Jaakonmäki I, 1981: 251 ff). "The sensitivity of blood allergy testing [RAST] is 25% to 30% lower than that of skin testing" (Siles RI, Hsieh FH, 2011: 587).

253 I.e. the same antigen specificity yet different sub-specificity (clonality) as laid down in chapter 2.3.4.

association of IgE (antibody) and allergen (antigen) based on one binding in RAST is not strong enough to yield a positive RAST (no association at all or dissociation of the low affinity IgE during washing procedures of RAST testing). In contrast, in SPT the physiological multivalent binding of two or more mast cell bound IgEs and allergen could lead to complexes sufficiently stable to avoid antibody-antigen dissociation and to result in mast cell degranulation – even if the IgE clones involved in the clustering are low-affinity. Such a constellation would be in line with recent findings that the real relevance of low-affinity IgE in the causation of atopic symptoms has been underestimated.[254] This probable "underestimation of low-affinity IgE" will represent the backbone of our "change theory" of allergogenesis laid down in chapter 6.3.

The following fig. 19 gives the most concise information on allergic sensitization currently available for the entire Madang Province. It incorporates results of eleven additional Skin Prick Tests performed on 129 Karkar in 2002[255] in our pre-existing Karkar atopy data base. Skin Prick Tests results for a certain allergen are displayed directly after the corresponding RAST results, provided both tests were performed. Taking the generally higher sensitivity of SPT into account it becomes clear, why we may call the pattern of allergic sensitization on Karkar "unusual".

254 Handlogten MW, Kiziltepe T, Serezani AP, Kaplan MH, Bilgicer B, 2013: 789ff.

255 Raw data pre-published in Herbert O, 2009. Eight subjects had to be excluded: they were proposed as study participants by an Aid Post Orderly thus they don't represent an unselected population.

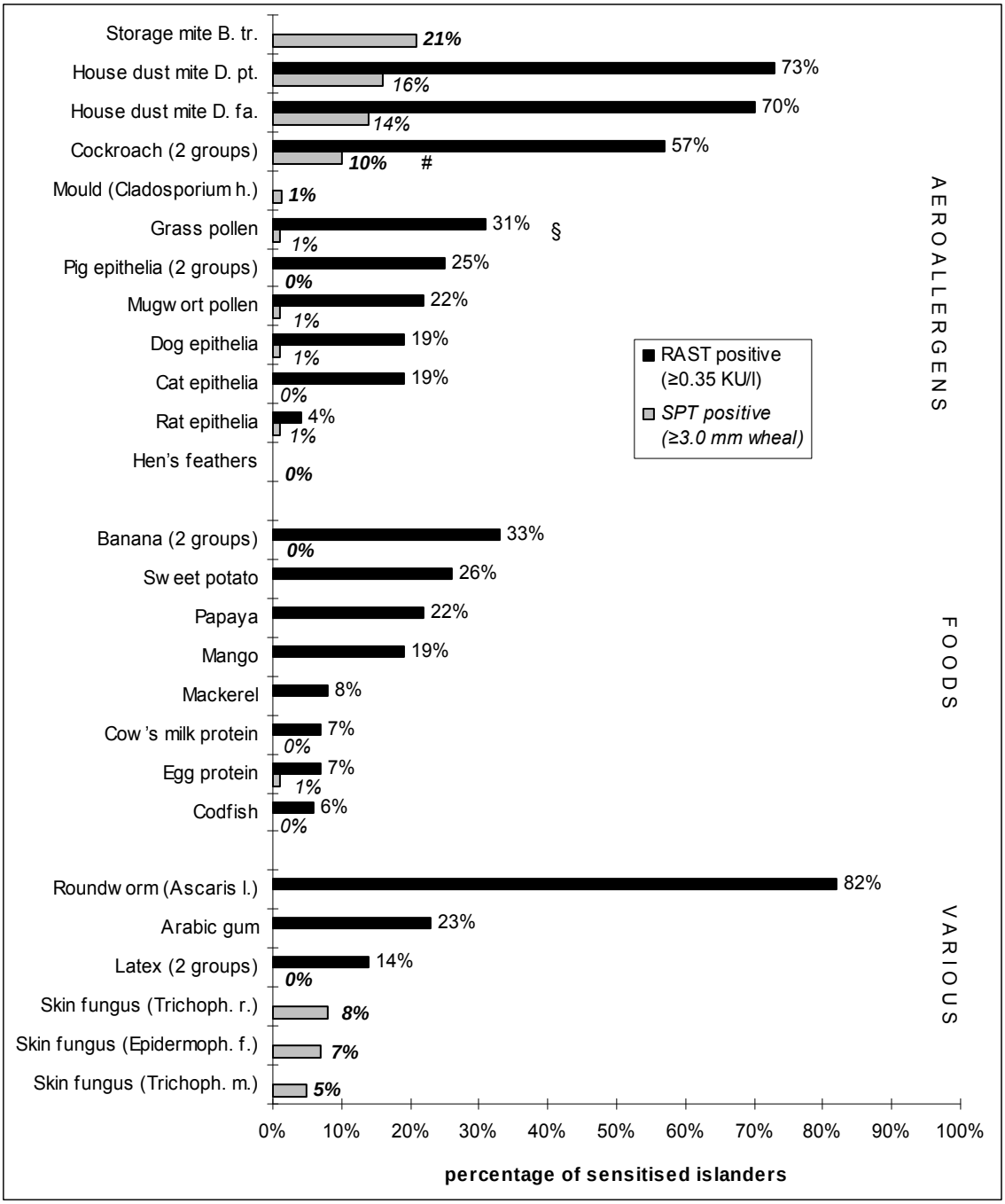


Fig. 19. Dissociation of RAST and SPT: high prevalence of in-vitro sensitization (RAST) and low prevalence of in-vivo sensitization (SPT). RAST was considered positive at specific IgE levels ≥0.35 kU/l and SPT at wheal diameters ≥3 mm. Case numbers for RAST (year 1996) n = 248, SPT (year 1996) n = 243 and SPT (year 2002) n = 129. Abbreviations, meaning of printing types and symbols (hash/paragraph) are explained in footnote number 256 on the following page.

Despite of the higher sensitivity of SPT in respect to the detection of atopy, there is – quite on the contrary – a marked discrepancy between an extremely high prevalence of serological RAST sensitization (in vitro) and low prevalence of "clinical" SPT reactivity (in vivo).[256] This "unusual" constellation is seen with aeroallergens as well as foods (and for latex in the "various" group).

A direct comparison RAST vs. SPT was possible (same year and group) within the group of aeroallergens for the house dust mite Dermatophagoides pteronyssinus (D. pt.) (73% vs 16%)[257] and for the house dust mite Dermatophagoides farinae (D. fa.) (70% vs 14%). Compared to Western countries, a 14% (D. fa.) and 16% (D. pt.)[258] prevalence of positive SPT to house dust mite (HDM) is low: A random sample on adolescents in southeast Finland showed around 38% positive SPT for house dust.[259] In a large convenient sample of the unselected Belgian population the corresponding level was 25.9%.[260] The prevalence in Finland and Belgium would probably be even higher if the environmental conditions were as "mite friendly" as in Papua New Guinea (tropical climate, extremely high humidity etc).On Karkar, the discrepancy in grass pollen sensitization was most pronounced: The RAST on timothy-grass pollen (Phleum pratense) alone yielded already 31% sensitization[261], whereas the SPT for grass pollen mixture[262]

256 Explanations of fig. 19:
Abbreviations: B. tr = Blomia tropicalis, D. pt./fa = Dermatophagoides pteronyssinus/farinae, Cladosporium h. = herbarum, Ascaris l. = lumbricoides, Trichoph. r. = Trichophyton rubrum, Epidermoph. f. = Epidermophyton floccosum, Trichoph. m. = Trichophyton mentagrophytes.
Printing types: percentages RAST non-italic, percentages SPT italic (year 1996 italic non-bold, year 2002 italic bold).
Symbols: # Blattella germanica SPT was positive in 12% of the subjects when using the SPT solution produced by Allergopharma, and in 8% when using the solution by Stallergene. The mean of 10% is displayed. § RAST was performed on timothy grass (Phleum pratense) exclusively, SPT on grass pollen mixture. Despite of the general cross-reactivity between grass species, discrepancies between RAST and SPT could have been even larger if a timothy grass SPT had yielded 0% of sensitization.

257 Van den Biggelaar AH et al. reported a similar discrepancy between high prevalence of positive D. pt. RAST and low prevalence of positive D. pt. SPT in Gabon: D. pt. RAST was positive in 47% of the cases (positivity arbitrary defined as ≥ 1kU/l), D. pt. SPT only in 11% (van den Biggelaar AH et al., 2001: 235).

258 As early as 1986, Turner KJ et al. reported a very similar prevalence of SPT sensitization in children and adolescents in the PNG highlands (South Fore): 13.5% (n=192) showed a positive SPT to D. pt. extracts (yet a wheal diameter of 2 mm was already recorded as positive) (Turner KJ, Dowse GK, Stewart GA, Alpers MP, 1986: 561).

259 The percentage of positive SPT reactions against house dust was 40% for boys and 36% for girls (rather small n=137); wheals ≥3 mm were considered positive. Remarkably mite RAST was only positive in 19% of the subjects (Haahtela T, Jaakonmäki I, 1981: 253 f). The fact that HDM mite RAST is enormously increased in the Karkar population is underlined by a comparison with a random sample of the Norwegian general population (n= 1512): On Karkar prevalence of positive D. fa. RAST was 70%, in Norway only 3.9% (Omenaas E et al., 1994: 534, fig. 2; notably, in the Norwegian study specific IgE was measured with Phadebas RAST, which is less sensitive than our ImmunoCAP system; thus the "real" prevalence of positive D. fa. RAST in the Norwegian population may be slightly higher).

260 Blomme K et al., 2013: 201. As Blomme et al. rightly point out: "Few studies report SPT prevalence in an unselected population-based sample."

261 Positive timothy-grass RAST reactivity is probably due to cross-reactions between different grass pollen species. Timothy-grass as a representative of the grass subfamily Pooideae is not common in the tropics. Yet certain allergens like grass allergen group 1, a major grass pollen allergen, are produced by every grass

showed just 1% of positive reactions; within the Belgian population the prevalence of positive grass SPT was 25.9%.[263] Mugwort pollen (Artemisia vulgaris)[264] as a representative of weeds was positive in 22% of the RAST yet only in 1% of the SPT. Dog and cat epithelia showed similar discrepancies between RAST and SPT: Dog RAST was positive in 19%, dog SPT in 1%.[265] The values for cat were 19% RAST and 0% SPT respectively. The above mentioned Finnish study found a similar RAST sensitization against cat (20%), yet 27% of the girls and 32% of the boys were SPT positive for cat epithelium.[266] The smallest difference of all comparisons within our Karkar data was seen for rat epithelia: RAST was positive in 4%, SPT in 1% of the cases. As in 1996 only cockroach RAST (Blattella germanica)[267] yet no SPT was done, we performed Blattella germanica SPT in 2002, using two different solutions: 12% of the 129 study subjects were positive when using the cockroach SPT solution produced by Allergopharma, yet only 8% using the Stallergene solution. The mean of 10% cockroach SPT sensitization is much lower than the 57%[268] cockroach RAST sensitization. An additional comparison was made possible by performing pig epithelia SPT in 2002. We had found positive pig RAST in 25% of the subjects, whereas SPT yielded 0% positive results. Altogether we have performed nine aeroallergen RAST-SPT comparisons (seven comparisons within the same group, two between groups). Prevalence of in vitro RAST sensitization was generally much higher than prevalence of in vivo SPT sensitization: the smallest discrepancy was found for rat (4% vs. 1%) and the largest discrepancies for grass pollen (31% vs. 1%) and pig epithelia (25% vs. 0%). Within the group of foods a direct comparison of the percentage of positive RAST vs. SPT was possible for the following three allergens. Cow's milk[269] 7% vs 0%, egg protein[270] 7% vs. 1% and Codfish[271] 6% vs. 0%. To

species. Furthermore there exists cross-reactivity between Pooideae and Panicoideae, a grass subfamily growing in tropical environments (Kleine-Tebbe J, Davies J, 2014: 22ff).
As already shown for HDM, also timothy grass RAST is enormously increased in the Karkar population in comparison with the above mentioned Norwegian study: On Karkar prevalence of positive timothy RAST was 31%, in Norway only 5% (Omenaas E et al., 1994: 534, fig. 2; again, "real" Norwegian prevalence may be slightly higher due to the smaller sensitivity of Phadebas RAST).

262 The mixture consisted of the following six Pooideae: Holcus, Dactylis glomerata, Lolium perenne, Phleum pratense, Poa pratensis and Festuca pratensis (SPT solution by Allergopharma).

263 Blomme K et al., 2013: 200.

264 Artemisia forms part of the compositae family. It is a "widely spread" allergenically relevant weed, occurring in temperate regions (e.g. Great Britain) but also in subtropical and tropical regions (Italy, India) (Mohapatra S, Lockey R, Polo F, 2004: 208). Artemisia vulgaris is also reported to grow in Indonesia (Arundina I, 2009: no pagination).

265 The above mentioned PNG study of Turner reported a prevalence of positive dog SPT in 2% of the children and adolescents (Turner KJ, Dowse GK, Stewart GA, Alpers MP, 1986: 561).

266 Haahtela T, Jaakonmäki I, 1981: 253 f.

267 To clarify on the author's own behalf: despite of its name, Blattella "germanica" is a cosmopolitan insect.

268 Prevalence of positive RAST against B. germanica was only 21.4% in non asthmatic children in Recife, Brazil (Lopes MI, Miranda PJ, Sarinho E, 2006: 206).

269 To the author's knowledge, there are water buffalos yet no cows on Karkar. Cow's milk is most commonly seen in the form of milk powder. Mothers bottle-feed their babies if breast feeding is not enough.

270 Chicken are roaming freely through the villages and gardens.

compare banana RAST with SPT we had to perform SPT in the year 2002. Banana RAST was positive in 33%, SPT in 0% of the subjects. The four food RAST-SPT comparisons reflect the pattern found in the nine aeroallergen comparisons: again RAST sensitization is clearly higher than SPT sensitization. Within the "various" group RAST vs. SPT sensitization was compared only for latex (different groups). For the other allergens we alternatively measured either RAST or SPT values. Latex (Hevea brasiliensis) RAST was positive in 14%[272] and SPT in 0% of the subjects.

Recapitulation: Generally we would have expected a much better concordance between skin and blood testing.[273] RAST (in vitro) sensitization is positive in a strikingly higher percentage of the average Karkar population than SPT (in vivo) sensitization. This was true for 14 different allergens: nine aeroallergens, four food allergens and latex. This may be called an "unusual" constellation; SPT is normally more sensitive concerning the detection of allergic sensitization than RAST.

5.5 The relationship between sTot-IgE and specific IgE

In chapter 5.3 we concluded, that sTot-IgE levels can be seen as a marker for immune system stimulation by helminths. Now we want to investigate whether there is a relationship between sTot-IgE (triggered by worms) and specific IgE levels. Therefore we performed a correlation of sTot-IgE and specific IgE. In this case, the specific IgE consists of the pooled 19 specific IgE values in order to give a general impression of the behaviour of specific IgE with increasing sTot-IgE. Ascaris lumbricoides IgE was excluded as – in contrast to the other 19 IgE types – it does not represent sensitization to an allergen.[274] After summing up the measured specific kU/l of the 19 allergens, we performed a $\log_{10}$-

271 Codfish is not living in PNG waters, yet cross-reactivity between fish species is not rare. Considerable IgE cross-reactivity (proved with RAST inhibition) occurs especially between codfish and mackerel (Hansen TK, Bindslev-Jensen C, Skov PS, Poulsen LK, 1997: 190ff). Mackerel on the other hand is probably the most important food fish on Karkar. Due to overpopulation and overfishing in costal areas, tinned mackerel has become one of the main protein sources on Karkar. "Besta Mackerel" is the by far most common brand on the island, produced by a Malaysian-owned company based in Lae, PNG. RAST against mackerel was positive in 8% of the population.

272 There are two different possible reasons for latex RAST sensitization in the absence of rubber trees: 1. Primary sensitization against latex: latex gloves have been used on Karkar in the long history of Western medicine during many procedures like births, tube ligations, tuberculosis treatment, drainage of abscesses, trauma treatment etc. Contact was possible either as staff (likely only in one case, Gaubin hospital worker) or more regularly as patient (most people on Karkar have been treated in the western health system). 2. Cross-reactivity with food antigens (e.g. latex-banana cross-reactivity). Cross-reactivity with local plant saps can not be excluded either.

273 Siles remarks: "… recent studies using modern technologies demonstrate reasonable concordance (67%) between skin testing and blood testing (specifically, ImmunoCAP)" (Siles RI, Hsieh FH, 2011: 589).

274 The 19 pooled RAST were: House dust mite D. pt., house dust mite D. fa., cockroach, timothy grass, pig epithelium, mugwort, dog epithelium, cat epithelium, rat epithelium, banana, sweet potato, papaya, mango, mackerel, cow's milk protein, egg protein, codfish, arabic gum and latex.

transformation on the pooled specific IgE value. This $\log_{10}$ pooled specific IgE value was compared with the proband's $\log_{10}$ sTot-Ige value. Results are shown in fig. 20.

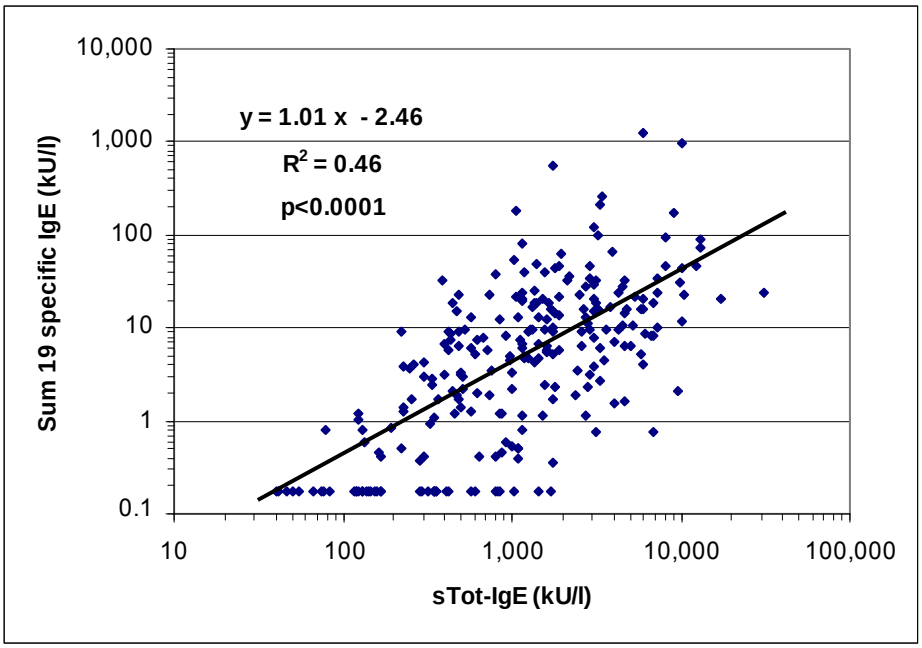


Fig. 20. Significant high correlation ($p<0.0001$) between sTot-IgE and the sum of 19 specific IgE values.

There is a significant high positive correlation ($p<0.0001$) between increasing sTot-IgE levels and an increasing sum of the 19 specific IgE values. Probably, worms do not only increase sTot-IgE but they seem to exert a similar "boost effect" on specific IgE. This notion is supported by the association of the people's anamnestic worm status and their pooled 19 specific IgE values: Subjects claiming to have never seen parasites in their stools showed the lowest pooled geometric mean (GM) for specific IgE with 3.78 kU/l, subjects reporting an earlier infection showed a corresponding value of 4.85 kU/l and subjects admitting to be currently affected by a noticeable infection showed the highest GM with 6.21 kU/l. However this was only a non-significant tendency ($p = 0.24$; Kruskal-Wallis test). The possible reasons why the association may not be significant have already been discussed in detail in chapter 5.3.2 (the argumentation had been in respect to the association of clinical worm status and sTot-IgE, yet the same arguments apply to the association of clinical worm status and pooled specific IgE): in particular cultural bias and ignorance of infection. A twofold/parallel boost of sTot-IgE AND specific IgEs could constitute the reason for the "unusually" high prevalence of RAST sensitization on Karkar.

Only few studies considered the possibility that worms may increase specific IgE (as well). Santiago HdaC et al. remark that the observation that Ascaris lumbricoides drives allergen-specific IgE, published in two studies (one in 1998 and one in 2013), "has drawn

little attention so far".[275] In their own study – published in 2015 – they confirmed increased specific IgE levels in a helminth-infested population in Brazil: Subjects infected with tissue invasive filaria (nematode)[276] showed increased cockroach and HDM IgE levels. In 1993 Lynch et al. proposed that – according to their data collected from children in a Venezuelan slum – helminths are actually able to enhance "… the [specific IgE] response against allergens that are continually present in the environment".[277] In 2001 van den Biggelaar reported significantly more mite sensitization (RAST) in worm infested children than in their non infected counterparts in Gabon.[278] As cockroach and HDM allergens share molecular structures ("high levels of homology") with helminth proteins, Santiago HdaC et al. concluded that cross-reactivity between the worm proteins and environmental (cockroach, HDM) proteins may be responsible for the increased specific IgE levels in infected subjects. Their conclusion was strengthened by the fact that IgE against environmental antigens with low levels of worm homology (e.g. timothy grass) was not increased in filarial infected subjects.[279] What does this mean for our Karkar data? In order to examine whether the increase of specific IgE with increasing sTot-IgE only/predominantly affects allergens with helminth homologs we compare the sensitization pattern of cockroach (example for high level of homologies with worms; fig. 21) with the pattern of timothy grass (example for low level of homologies with worms; fig. 22). Admittedly we see – as we already expected – more sensitizations against cockroach than against timothy grass, yet the overall pattern of increasing prevalence and increasing level of sensitization with increasing sTot-IgE levels is basically the same for both antigens. Thus within our Karkar data, cross-reactivity between worms and environmental antigens does not seem to play a major role. A similar conclusion was drawn by van den Biggelaar in a similar setting in Gabon: "… cross-reactivity between house dust mite and helminths … is not likely".[280]

275 Santiago HdaC, Ribeiro-Gomes FL, Bennuru S, Nutman TB, 2015: 98.

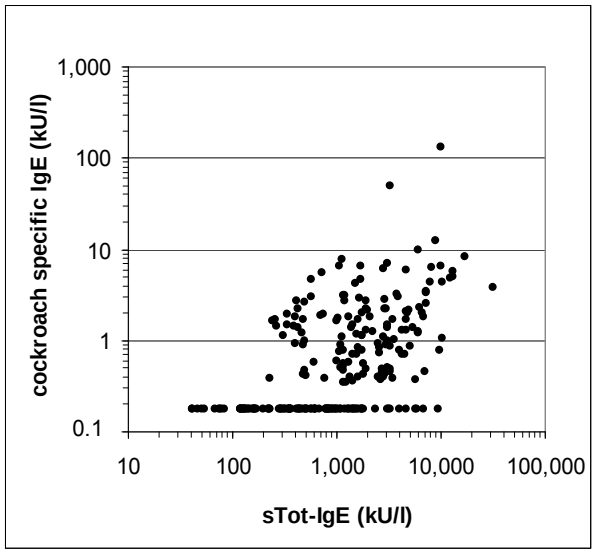
276 Filariae are nematode worms, just like the worms prevalent on Karkar (Ascaris lumbricoides, hookworm and whipworm).

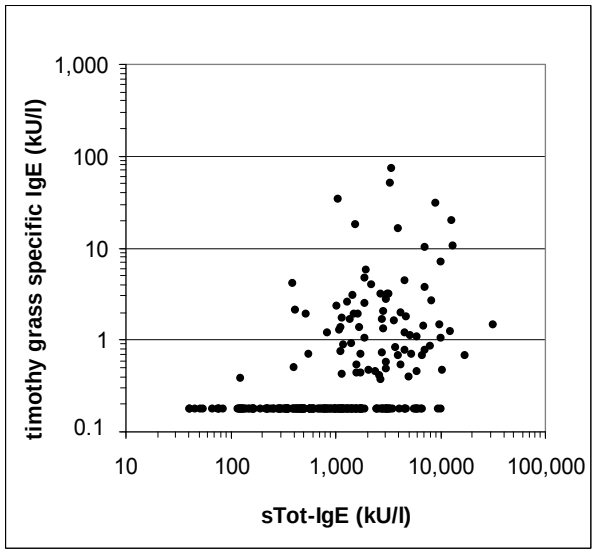
277 Lynch NR, Hagel I, Perez M, Di Prisco MC, Lopez R, Alvarez N, 1993: 409. Unexpectedly, according to Lynch et al. "low level contact with helminths" seemed to be the most adequate trigger for specific IgE.

278 The respective helminths were Schistosoma haematobium and/or filariae (Van den Biggelaar AH et al., 2001: 237).

279 Santiago HdaC, Ribeiro-Gomes FL, Bennuru S, Nutman TB, 2015: 93 ff.

280 Van den Biggelaar AH et al., 2001: 237.





Figures 21 and 22. Analogue significant positive correlation ($p<0.0001$; 246 df) of sTot-IgE and cockroach specific IgE (fig. 21; $\rho=0.54$) and of sTot-IgE and timothy grass specific IgE (fig. 22; $\rho=0.52$) (similar results for Person correlation on log values). In contrast to fig. 21 (cockroach: high level of homologies with worms), it would not be possible to explain the pattern in fig. 22 (timothy grass: low level of antigen homologies with worms) with cross-reactivity to helminth antigens.

As sTot-IgE in the former correlations supposedly represents the effect of polyclonal B cell stimulation by different worm species prevalent on Karkar (roundworm, hookworm, whipworm) we additionally performed a correlation exclusively between specific anti Ascaris IgE and cockroach IgE (suspected cross-reactivity) as well as specific anti Ascaris IgE and timothy grass IgE (cross-reactivity not suspected). Both Spearman correlations using Ascaris IgE confirmed what we found for sTot-IgE: with increasing Ascaris sensitization there was a significant increase in cockroach sensitization ($\rho=0.50$) but also in timothy grass sensitization ($\rho=0.47$). To give a more illustrative idea of the behaviour of cockroach and timothy IgE in relation to Ascaris we calculated the percentage of subjects with positive cockroach and timothy sensitization at different anti Ascaris IgE levels. Results are displayed in fig. 23. Cross-reactivity between anti Ascaris IgE and environmental antigens can not explain the generally increased specific IgE levels within the Karkar population: With increasing anti Ascaris IgE antibodies, there is a parallel increase in the prevalence of IgE antibodies against environmental antigens with relevant (cockroach) and without relevant (timothy grass) structural worm homologies. Thus our Karkar data does not confirm the conclusions of Santiago HdaC et al. about cross-reactivity. Interestingly, Santiago HdaC et al. conceded that in their mouse model worm infection "induced [specific] IgE [also] to allergens … without parasite homologs".[281]

281 Mice were experimentally infected with the mouse nematode Heligmosomoides polygyrus (rodent roundworm). The increase of specific IgE levels was larger for allergens with homologs however. (Santiago HdaC, Ribeiro-Gomes FL, Bennuru S, Nutman TB, 2015: 96f).

They deduce – exactly in line with our thoughts – that this effect (which they can not explain with molecular homology and cross-reactivity) "is associated with the induction of polyclonal IgE by worm infections …"[282]

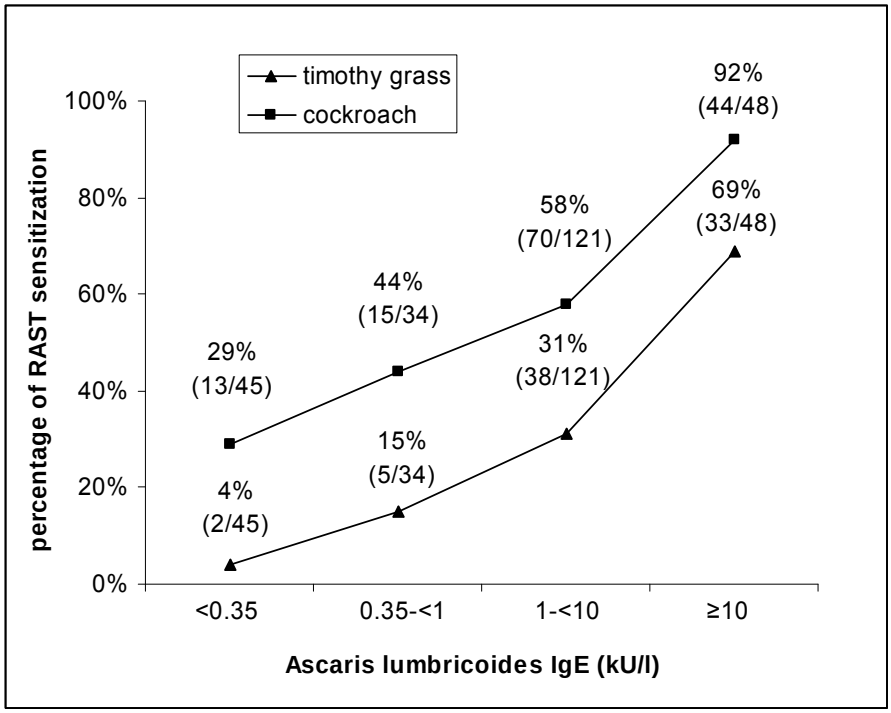


Fig. 23. Parallel increase of sensitization prevalence against environmental allergens with relevant (cockroach) and without relevant (timothy grass) molecular worm homologies with increasing levels of anti Ascaris IgE.

Conclusion: Despite the fact, that allergy prevalence is low on Karkar, 84% (208/248) of the Islanders were RAST sensitized to environmental proteins/antigens[283]. The respective in vitro sensitization level worldwide is "only" up to a maximum of 40% of the population.[284] This unusually high level of sensitization to aeroallergens and foods can not be explained by cross-reactivity between anti worm/Ascaris IgE and environmental antigens. It seems rather a consequence of a general induction of polyclonal IgE by helminth infestations. Here we have to understand "polyclonal" in its etymologically correct sense: Worms seem to trigger IgE production by "many clones"; a part of the clones produces "non-specific" IgE and another part "specific" IgE directed against various environmental antigens ("dual boost effect").

282 Santiago HdaC, Ribeiro-Gomes FL, Bennuru S, Nutman TB, 2015: 98.

283 Altogether 19 environmental proteins (Ascaris lumbricoides was excluded).

284 Pawankar R, Canonica GW, Holgate ST, Lockey RF, 2011: 16. Of course, the level of sensitization against environmental proteins/antigens depends largely on the number of measured proteins/antigens.

Before investigating, whether there is a similar positive correlation between sTot-IgE and pooled SPT values in the following chapter, we will give an overview over the percentaged weight of the different IgE subgroups in our data base. In short, an average of 99.4% of sTot-IgE is of unknown specificity ("non"-specific IgE), 0.4% are allergen specific IgE (pooled 19 allergen IgE values) and the remaining 0.2% are worm specific IgE (Ascaris lumbricoides).[285] In other words, despite of testing the large number of 20 IgE specificities, "non"-specific IgE remains a "big black box" representing 99.4% of our sTot-IgE.[286] The GM of sTot-IgE is 1,069.0 kU/l[287] (100.0%), the GM of 19 pooled allergen IgE is 4.1 kU/l[288] (0.4%) and the GM of one worm IgE (Ascaris lumbricoides) is 2.1 kU/l[289] (0.2%). This means, one parasite species alone is able to generate an average IgE response (GM) equivalent to more than half of the pooled allergen IgE response: a single worm species induces 2.1 kU/l of specific IgE (GM) whereas a broad array of important allergens (nineteen aero- and food-allergens) induces "only" 4.1 kU/l of pooled specific IgE (GM).[290] As on Karkar hookworms are at least as important as roundworms, and as other worm species are prevalent as well, we presume that on the island worms alone generate a similar or probably even higher IgE response than the total of the most important allergens together. In addition to the fact that worms boost sTot-IgE and (presumably) at the same moment specific IgE, this clear dominance of worm IgE within the "specific IgE subgroup" underlines the pre-eminent effect of worm infestation on the overall IgE system.

5.6 The relationship between sTot-IgE and Skin Prick Tests

Having found a significant correlation between sTot-IgE and specific IgE, we want to investigate whether there is a similar relationship between sTot-IgE and SPT reactions. As already done for specific IgE, we pooled ten SPT results[291] of each individual in order to give a general impression of the behaviour of skin prick test reactivity with increasing

285 The percentages were calculated in respect to the geometric means (GM) of the different IgE subgroups. The exact group GMs are given down below.

286 Platts-Mills seems euphemistic when he states that in helminth infected traditional tropical villages the "detailed specificity of the [sTot-] IgE is not known" (Platts-Mills TAE, Lee BW, Arruda LK, Chew FT, 2011: 82). It may be more accurate to admit that we know pretty little about the overall specificities.

287 95% confidence limit of values: 79.2 kU/l; 14,436.5 kU/l.

288 95% confidence limit of values: 0.1 kU/l; 201.5 kU/l.

289 95% confidence limit of values: 0.1 kU/l; 49.8 kU/l.

290 A similar predominance of anti Ascaris lumbricoides IgE in comparison to anti allergen IgE was described by Arruda LK et al. within a group (n=107) of allergic (asthma and/or rhinitis) children in Natal, Brazil: in this parasite-endemic area, IgE to Ascaris lumbricoides accounted for 0.38% of sTot-IgE. However IgE to mite accounted for only 0.08% and cockroach for only 0.04% of sTot-IgE (Arruda LK, Camara AA, Ferriani VP, Sales VS, Santos AB, 2009: 98).

291 The ten pooled SPT results were: House dust mite Dermatophagoides pteronyssinus, House dust mite Dermatophagoides farinae, Grass pollen mixture (composition see footnote 262), mugwort pollen, dog epithelia, cat epithelia, rat epithelia, egg, cow's milk and codfish.

sTot-IgE levels. After summing up the measured wheal diameters of the ten allergens, we correlated the resulting sum with the proband's $\log_{10}$ sTot-Ige value. Results are shown in fig. 24.

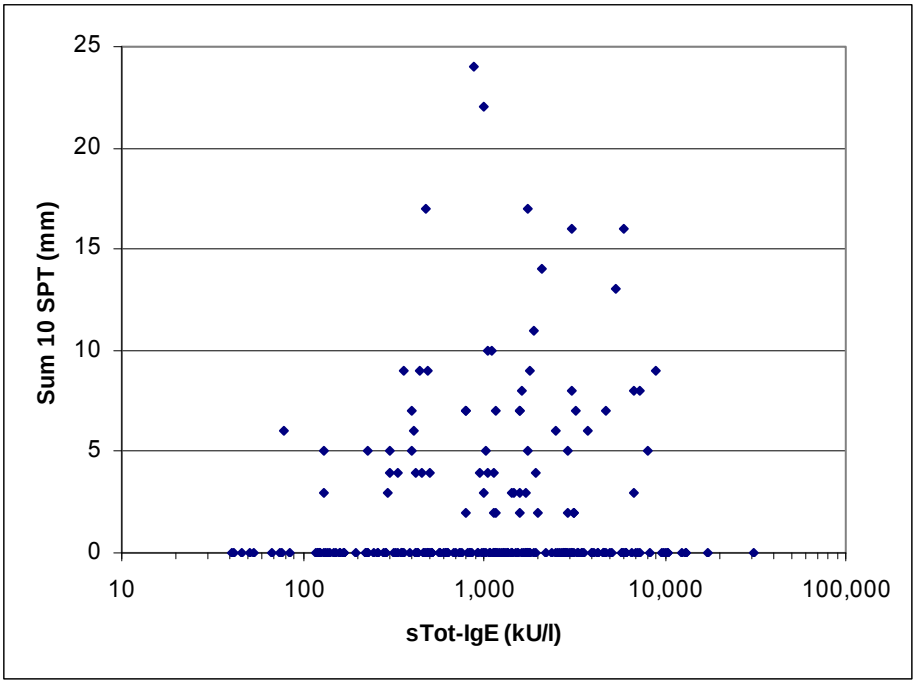


Fig. 24. Negligible, non significant correlation (ρ=0.06; p=0.36; 246df) between sTot-IgE and the sum of ten skin prick tests (pooled wheal diameters in mm).

In contrast to the behaviour of specific IgE reactivity, there is no increase of SPT reactivity with increasing sTot-IgE levels. The vast majority of subjects showed no positive skin prick tests: 74% (183/248) of the Karkar population did not react to any of the ten tested allergens – irrespective of the sTot-IgE level. Worms do not seem to increase SPT reactivity, on the contrary: despite of a considerable increase of specific IgE levels with increasing sTot-Ige, in vivo SPT does not reflect the higher sensitization as assessed in vitro by RAST.

5.7 Exclusion of some explanations for the differences between RAST and SPT

5.7.1 Increased RAST due to non-specific binding?

A quite plausible explanation for increasing specific IgE measurements with increasing sTot-IgE levels would be non-specific IgE binding, generating false-positive RAST results at high sTot-IgE levels. The non-specific binding question may seem to constitute a less interesting biochemical issue, best dumped in the material and methods section. Yet far from being an analytical minor point, non-specific binding represents a crucial concern – not only for this work, but also for allergy testing in large parts of the world. Therefore,

we will give a short introduction on non-specific binding before interpreting our results. Non-specific binding means, that the congruence between the steric conformation of antigen (more precisely: epitope of antigen) and antibody (more precisely: antigen-binding site of antibody), a basic prerequisite for an association of the two molecule structures under normal circumstances, is not essential any more. As a consequence, antibodies from the sTot-IgE fraction (not specific for a certain allergen) could bind to solid phase RAST allergens or to the carrier material, causing false-positive results. For a long time, markedly high concentrations of sTot-IgE have been suspected of generating non-specific binding.[292] Nevertheless it is still contested if non-specific binding really occurs in subjects with high sTot-IgE levels, and – if so – which level of sTot-IgE may cause which level of false-positive RAST. The importance of the question concerning non-specific binding is immense, not least for pharmaceutical companies offering IgE assays: up to now, allergy tests are mostly performed in Western affluent countries, where sTot-IgE levels are comparatively low. As soon as there is a rise in allergies and purchasing power in "third world" countries, the demand for RAST assays will explode in many parts of the world. Thus the somewhat "exotic" problem of non-specific binding in the presence of highly elevated sTot-IgE will become a matter of primary importance. Literature is most inconsistent quantifying the influence of elevated sTot-Ige on specific IgE. On extreme states, there is a general ~0.1-0.2% non-specific binding over 1,000 kU/l sTot-IgE.[293] One could argue that this was a result based on old assays of the 1980s which are not representative any more, yet in 1997 Jensen reported unspecific binding starting at levels of 500 kU/l sTot-IgE, reaching "false" RAST class 2 at 1,000 kU/l.[294] In 1981 Falk considered the possibility that "some Class 1 results represent non-specific reactions due to a raised IgE level."[295] The scientific director of the manufacturer of the RAST assays used in our study did not want to exclude the existence of non specific binding but considered it a theoretical and marginal problem at "very high total IgE" levels: … *I agree that with very high total IgE we may sometimes see some low level specific IgE results that are really "non specific" binding in the assay, i.e increased background.*[296] Mayo Clinic Medical Laboratories consider false-positive specific IgE results possible over 2,500 kU/l sTot-IgE[297]. The other extreme states, there is no interference[298] at all. For

292 For example: Platts-Mills T, Chapman M, Tovey E, 1981: 291ff.

293 Platts-Mills T, Chapman M, Tovey E, 1981: 292.

294 Jensen-Jarolim E, Vogel M, Zavazal V, Stadler BM, 1997: 847. The reason why we consider these results based on myeloma IgE not to be representative for non-specific binding in general is laid down later in this chapter.

295 Falk had examined a group of 120 scabies patients with "high" sTot-IgE levels (Falk ES, 1981: 172). Yet the "high" sTot-IgE levels were generally under 1,000 kU/l (two exceptions). In comparison to worm infected societies this represents only a moderate sTot-IgE increase.

296 Personal e-mail communication with Phadia scientific Director Dr. Anita Kober, Uppsala, Sweden, April 20th 2012.

297 Mayo Clinic Medical Laboratories: IgE (no pagination).

298 From the technical point of view, no interference means, that it is possible to wash away all IgE with insufficient affinity (i.e. non-specific IgE) during the RAST measurements processes.

example Bousquet's group could not detect any interference even at 20,000 kU/l sTot-IgE (CAP)[299] and van den Biggelaar AH et al. showed in a worm infested population with high sTot-IgE and high mite IgE levels that non-specific binding was "unlikely" as mite RAST could be "fully inhibited" by pre-incubation with mite extract.[300] In the light of this longstanding dispute over non-specific binding, it becomes apparent, why our data from Karkar provide a rare opportunity to investigate the correlation of elevated sTot-IgE and specific IgE. On the one hand, sTot-IgE levels are extremely high in a considerable percentage of our study population: 96% (238/248) of the general Karkar population showed tot-IgE levels, we would consider elevated in modern western societies (i.e. >100 kU/l), in 57% (141/248) of the cases levels were over 1,000 kU/l, in 31% (78/248) over 2,000 kU/l and in 12% (30/248) even over 5,000 kU/l. On the other hand, we dispose of specific IgE levels of a large number of allergens: nineteen specific IgE levels were measured in every of the 248 subjects. This allows to investigate the behaviour of 4,712 pairs of values (sTot-IgE/specific IgE), a quite unique opportunity.

Looking back at fig. 20 already gives a first impression. On the one hand ("pro non-specific binding") there is a significant correlation between sTot-IgE and the pooled specific IgE (sum of 19 allergens). Moreover – according to the regression equation – a tenfold increase in sTot-IgE results in an average tenfold increase in pooled specific IgE. On the other hand ("contra non-specific binding") variation of pooled specific IgE at given sTot-IgE levels is considerably high and there are three subjects who show a pooled specific IgE of cero (indicating all 19 specific allergens are negative), despite sTot-IgE levels over 1,000 kU/l. These three subjects are represented by the three dots on the far right at the horizontal bottom line at y = 0.175 kU/l[301]. Another subject whose specific IgE levels are obviously not influenced in any considerable way by non-specific binding is the proband who shows a sTot-IgE value around 7,000 kU/l in conjunction with a pooled specific IgE lower than 1 kU/l[302]. In summary we can already deduce from the data displayed in fig. 20 that there is at least no general non-specific binding over 1,000 kU/l sTot-IgE as in this case pooled specific IgE values of cero would be impossible. Yet in order to get a more accurate idea of the behaviour of specific IgE with increasing sTot-IgE we will now break down the pooled specific IgE values into the single specific IgE values for the 19 allergens (see fig. 25).

299 Bousquet J et al. tested the behaviour of specific IgE against six allergens (measured with the Pharmacia CAP system – the system used in our study) in the presence of high sTot-IgE. The six allergens included D. pt. and cat (Bousquet J, Chanez P, Chanal I, Michel FB, 1990: 1041).

300 Van den Biggelaar AH et al., 2001: 237. Unfortunately more information is not available as van den Biggelaar's RAST inhibition studies have not been published in detail.

301 The y value of 0.175 kU/l is just representing cero for later log transformation.

302 A pooled specific IgE level under 1 kU/l means, that in the group of 19 specific IgEs there can be only one RAST class 2 or two RAST class 1. Thus despite of around 7,000 kU/l sTot-IgE there are 17 or 18 specific IgE values which are negative.

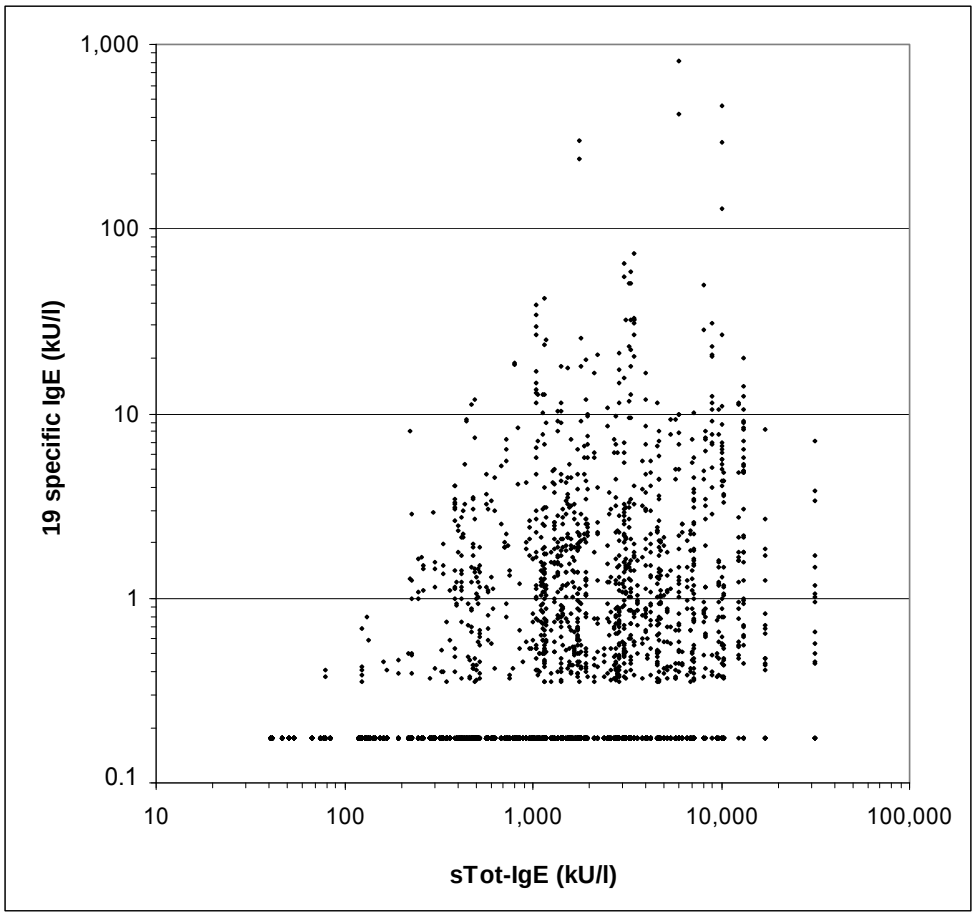


Fig. 25. Increasing number and levels of specific sensitization with increasing sTot-IgE levels: 4,712 pairs of values (19 allergens x 248 subjects) on $\log_{10}$ scale.

We have 248 subjects each at a different sTot-IgE level.[303] That means each subject is represented by a vertical line of points at the individual's corresponding sTot-IgE level (x-axis) and each of these points represents one of the 19 specific IgE values. If a subject is sensitized against all 19 allergens, the vertical line consists of 19 points (maximum), if there are less than 19 points in a vertical line, the missing points are in most cases cero values (overlap of points at y = 0.175 kU/l, representing cero).[304] If there is just "one" point at y = 0.175 kU/l (no vertical line of points at all), all the 19 specific IgE measurements were negative, and the "one" point represents an overlap of 19 points.
Despite highly elevated sTot-IgE levels within the general Karkar population, there are more cons than pros in respect to non-specific binding:

303 Only in nine cases the sTot-IgE level difference to the neighbouring sTot-IgE was smaller than 1 kU/l. Unfortunately, due to limitations of scale, the values of individual subjects ore often not discernable in fig. 25 – even if the distance to the next sTot-IgE level was much larger.

304 Theoretically a subject could show a sensitization with exactly the same specific IgE level for different allergens, or IgE levels so near to each other that they are not distinguishable. Yet this is very unlikely. The general overlap of points occurs at specific IgE levels of cero.

We can exclude the possibility that high sTot-IgE levels lead to general non-specific binding affecting all allergen specificities. Reasons: We have already mentioned the idea that there might be a general (irrespective of the kind of allergen) non-specific binding in RAST measurements equivalent to e.g. 0.1% of the corresponding sTot-IgE level.[305] If this were true all the 19 different allergens of our study should experience a similar increase in specific reactivity as a roughly linear function (log scale) of increasing sTot-IgE. Adding a "false positive value" to every specific IgE measurement would "lift" all specific IgE levels to or over the "0.1% threshold". This threshold lies at specific IgE levels which are equivalent to 0.1% of the sTot-IgE. For example, if a subject had a sTot-IgE of 10,000 kU/l, non-specific binding would increase each specific IgE measurement by 10 kU/l (i.e. 0.1% of sTot-IgE). Consequently we would not encounter any of the 19 specific IgE values under 10 kU/l. In fig. 26 we displayed the 0.1% threshold for our data: the light grey area (including the dark) holds only data points which show specific IgE levels that are lower than 0.1% of the respective sTot-IgE thus the whole area should be devoid of points. Even if the "general" non-specific binding were only 0.01% of the sTot-IgE level, all the data points in the dark grey area should not exist. A similar view would be that there is a general non-specific binding corresponding to one RAST class (plus 0.7 kU/l) or even two RAST classes (plus 3.5 kU/l). It is said, that this may occur at sTot-IgE levels over 1000 kU/l (sometimes higher sTot-IgE are named). In fig. 26 the areas of RAST class 1 and 2 associated with sTot-IgE levels over 1000 kU/l are marked by rectangles. In analogy to what was said above for fixed percentages, we would see no RAST class cero, one or even two if sTot-IgE is over 1000 kU/l. This is exactly a pattern Jensen-Jarolim et al. described, when they investigated "real" non-specific binding: Myeloma IgE bound non-specifically to all tested allergens, thus no RAST class cero or one were observed, yet only RAST class two.[306] The high "physiological" sTot-IgE on Karkar definitely behaves differently. We think that only "abnormal, non-physiological" malign myeloma IgE is "omnipotent" in the sense of being able to bind to any steric structure. Biologically this makes sense: an immunoglobulin erroneously recognizing "everything" would be quite dangerous; not least in respect to autoimmune disease. Therefore even if we do not know much about the multiple specificities of our huge sTot-IgE fraction, we can exclude that sTot-IgE in a worm infested society is (totally) unspecific.

305 Similar to the view of Platts-Mills T, Chapman M, Tovey E, 1981: 292.

306 Jensen-Jarolim E, Vogel M, Zavazal V, Stadler BM, 1997: 847. Raw data for six indoor and outdoor allergens are presented in fig. 1 of the mentioned study. In table 2 there is a mistake for "molds", which should say RAST class two and not one. Just patient "VL" was slightly under RAST class two in respect to one allergen (grass pollen).

One reason why we may see values in the "contested non-specific binding areas" (in the "percent triangles" or "RAST rectangles") – despite the existence of non-specific binding – could be that different allergens experience different degrees of non-specific binding. It would be possible, that only certain allergen groups are affected by non-specific binding (consequence of their particular steric structure) whereas others remain uninfluenced even at very elevated sTot-IgE levels.[307] For example: proband number 214 showed a sTot-IgE of 5,760 kU/l. In this subject several allergens like e.g. house dust mite were class 0 (not influenced by non-specific binding), dog epithelia was class 1 (possibly influenced by non-specific binding) and cow's milk class 3 (probably to high to be explained exclusively by non-specific binding). Thus this proband's values may suggest that house dust mite is not prone to non-specific binding whereas dog epithelia and cow's milk might be. Yet proband number 163 with a very similar sTot-IgE of 6,005 kU/l showed a reverse pattern: dog epithelia and cow's milk class 0 but house dust mite class 6. We examined our data, whether this example is representative. Indeed, we could not find any "dualism" in our allergens: there wasn't one group of allergens experiencing a more or less uniform continuous increase of specific IgEs with increasing sTot-IgE and at the same time absence of RAST class 0 (pattern for existing non-specific binding) and another group with no increase of specific IgE with increasing sTot-IgE and at the same time occurrence of RAST class 0 (pattern for absence of non-specific binding). On the contrary: of course there is an all over increase in specific IgE with increasing sTot-IgE levels, but it is impossible to predict the single specific IgE values of a certain subject. The only common feature shared by all probands with higher sTot-IgE is that in each individual, some specific IgE stay cero or low, whereas others get high or very high. To underline that "resistance against non-specific binding" is not only a special property of a particular group of allergens we could show that all our specific allergens could stay RAST negative despite of highly increased sTot-IgE levels. Subsequently we will give the highest sTot-IgE level associated with a RAST negative allergen in our study group. No specific IgE was found at sTot-IgE level of 31,400 kU/l (!) for rat epithelia, cow's milk, codfish, mango and arabic gum, at sTot-IgE level of 17,225 kU/l for latex, at sTot-IgE level of 13,030 kU/l for egg, at sTot-IgE level of 10,345 kU/l for mackerel and papaya, at sTot-IgE level of 10,260 kU/l for cat epithelium, dog epithelium, mugwort, sweet potato and banana, at sTot-IgE level of 10,020 kU/l for timothy grass, at sTot-IgE level of 9,760 kU/l for pig epithelium, at sTot-IgE level of 9,555 kU/l for cockroach,[308] at sTot-IgE level of

307 In such a scenario we could conclude that the carrier material does not play a relevant role in non-specific binding, as the carrier is the same for the different allergens. The question would rather be whether the preference to bind to certain antigen groups should still be labelled "non-specific binding behaviour".

308 In Recife, Brazil, Lopes MI et al. did not measure any negative cockroach RAST when sTot-IgE was higher than 2,500 kU/l. Thus they concluded that a "loss of specificity" of the RAST essays might be the reason (Lopes MI, Miranda PJ, Sarinho E, 2006: 204 ff).

6,810 kU/l for both house dust mite species[309] and at sTot-IgE level of 4,860 kU/l for roundworm. Obviously even at extremely high sTot-IgE values all allergens can stay unaffected by non-specific binding; at least in a certain proportion of the population. Up to now we can summarize our interpretation of the behaviour of specific IgE levels at very high sTot-IgE levels as follows: there is no general non-specific binding to all allergens and all allergen specificities can stay unaffected by very high sTot-IgE levels.

There is one more option how high sTot-IgE could increase specific IgE levels in a way that results in a pattern like the one displayed in fig. 25: It may be possible that it depends on the individual's sTot-IgE composition/repertoire whether an interaction between high sTot-IgE and certain specific allergens takes place. Yet we have to be careful not to confound non-specific binding and cross-reactivity[310]: If, for example, an individual disposes of IgE molecules within her/his sTot-IgE pool, which "happen to fit" to a banana epitope of a solid phase bound banana allergen (due to casual steric congruence) but not to the other allergens, this would be a case of cross-reactivity. The resulting elevated RAST exclusively for banana represents no "false" measurement. In short: non-specific binding is not reflected in increased SPT reactivity as it constitutes a false positive RAST measurement; cross-reactivity however is not a false positive RAST measurement but a real, specific antigen-antibody interaction. As such, it is reflected in positive SPT measurements, because cross-reacting IgE clones to a certain allergen lead to mast cell degranulation just like not cross-reacting specific IgE. Consequently, if a lot of the high specific IgE values were caused by cross-reactivity, we would not see such a low prevalence of positive SPT. On the contrary: SPT reactivity would increase with increasing sTot-IgE and specific IgE levels. This is not the case within our Karkar data.

309 Bousquet J et al. could prove the existence of negative D. pt. RAST (CAP) only at a sTot-IgE level of 1,452 kU/l. (Bousquet J, Chanez P, Chanal I, Michel FB, 1990: 1041). Thus we could demonstrate that even sTot-IgE 4.96 times higher than this did not influence specific IgE measurements in D. pt. mite.

310 Cross-reactivity will be discussed more in detail in chapter 6.3.

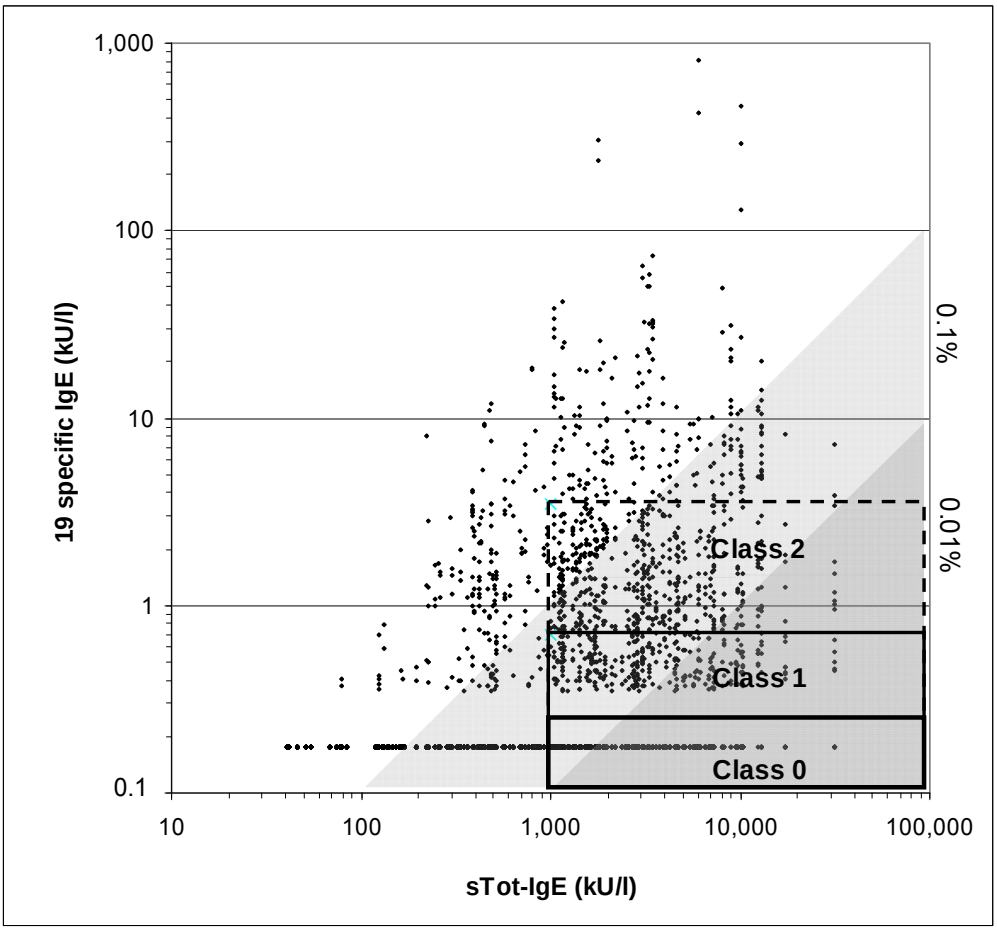


Fig. 26. Areas of contested non-specific binding. Correlation of sTot-IgE and 19 specific IgE values in connection with areas of contested non-specific binding marked as a) grey triangles (light grey 0.1% and dark grey 0.01% non-specific binding area) and b) rectangles (non-specific binding in area of RAST class 1 and 2).

All in all, we are quite confident, that non-specific binding does not play a major role in our data base for several other reasons.

1. Data displayed in fig. 25 was kindly evaluated by the manufacturer (medical director Germany, Austria, Switzerland professor Johannes Huss-Marp and scientific director Dr. Anita Kober)[311]. They shared our more general assumptions against non-specific binding: … *this is certainly not only background/non specific, since you can find high total IgE with no or very low specific IgE results. This is a kind of proof of specificity*.[312]

2. Non-specific binding is generally said to occur at sTot-IgE levels over 1,000 kU/l (or even higher). Yet looking at fig. 25 reveals that within our data the most pronounced increase in number and levels of specific sensitization occurs under and not over 1,100 kU/l sTot-IgE. Over 1,100 kU/l sTot-IgE number and levels of sensitization remain

311 Associate professor Johannes Huss-Marp, Phadia, Freiburg, Germany; Dr. Anita Kober, Phadia, Uppsala, Sweden.

312 Dr. Anita Kober, personal e-mail communication April 20th 2012.

stable, despite of a further immense increase of sTot-IgE to a maximum of 31.400 kU/l. This clearly constitutes a pattern we would not expect as a consequence of non-specific binding.

3. Another argument against non-specific binding is the pattern of specific sensitization displayed in fig. 19. If prevalence of sensitization largely followed chance (i.e. non-specific binding), we should NOT see a sensitization pattern precisely reflecting the people's environment (curiously enough the sensitization pattern is NOT depicting the allergenic potency/biologic activity of the allergens within the people's environment!)[313]. There is a relationship between the (postulated) level of allergen exposure on Karkar Island and RAST sensitization. For example we see in the group of aeroallergens: High exposure to house dust mites (humid and warm environment; 70-73% sensitization), intermediate exposure to grass pollen (worldwide allergen; 31% sensitization) and little exposure to cat (very few cats, not in the huts; [still] 19% sensitization). This reflection of the Karkar's environment in aeroallergen sensitization was confirmed in the group of food allergens: High exposure to banana and sweet potato (staple diet; 26-33% sensitization), intermediate exposure to papaya and mango (regularly consumed; 19-22% sensitization) and little exposure to cow's milk and codfish (rarely/"never" consumed; [still] 6-7% sensitization). We will get back to this immunologic "mirroring of the environment" in chapter 6.2.

4. Last but not least, especially food antigens were suspected to be more prone to non-specific binding than aeroallergens. To compare the behaviour of eight anti-food IgE with nine anti-aerogen IgE, fig. 27 shows the correlation of both groups with sTot-IgE.

313 We will try to explain this finding in chapter 6.2. Generally, the biologic activity of allergens (potency) seems to be determined by the "… number, [affinity] and location of the IgE-binding epitopes on the allergen molecule …" (Witteman AM et al., 1996: 17). We just had to add "affinity".

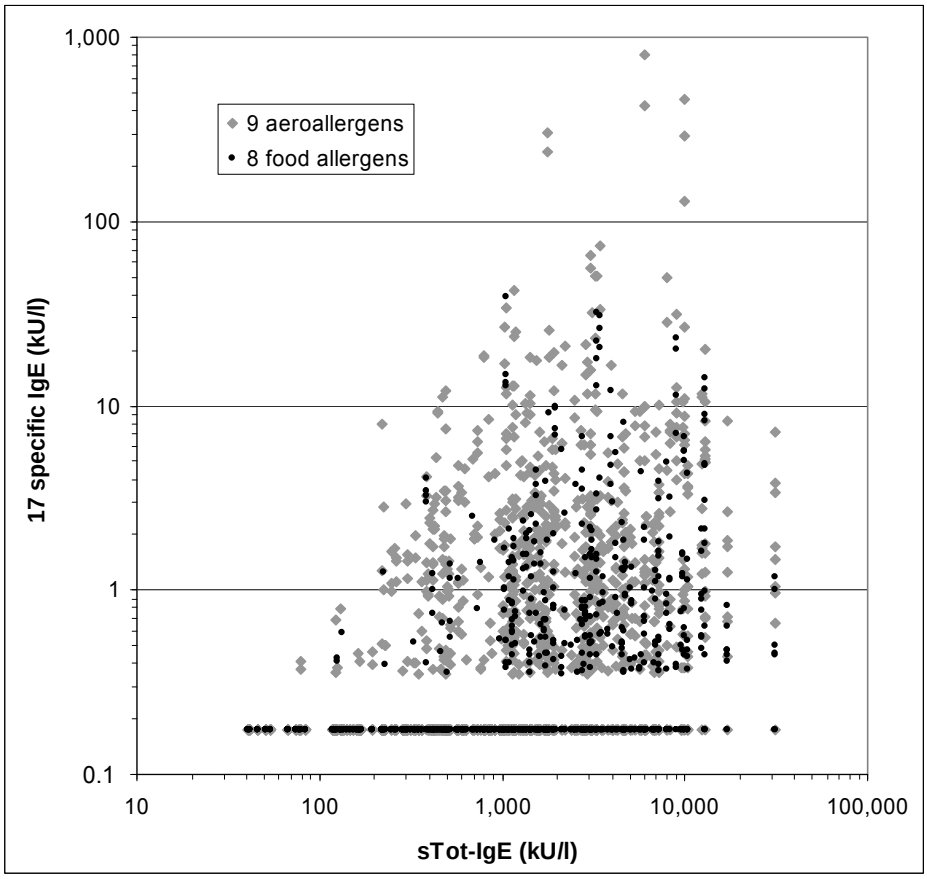


Fig. 27. Comparison of the behaviour of food- vs. aeroallergen-IgE in the presence of increasing sTot-IgE levels.

The pattern for both groups is principally the same. There is a slightly larger boost in aeroallergen IgE than in food allergen IgE with increasing sTot-IgE.

Conclusion: Increase in specific IgE with increasing sTot-IgE is "real", not due to non-specific binding. As sTot-IgE increases people show a higher number of sensitizations AND at the same time higher levels of sensitization. As already discussed in chapter 5.5 worms seem not only to boost sTot-IgE but also a large variety of specific IgE – irrespective of the corresponding allergenic potency. Scientific Director Dr. Anita Kober working for Phadia, Uppsala, Sweden (manufacturer of the RAST assays which have been used in this study) kindly evaluated the data displayed in fig. 25. Dr. Kober shared our notion of a parallel increase of sTot-IgE and specific IgE: *It seems as if the IgE immune system is switched on and produces antigen specific responses to a number of substances.*[314] A worm boost affecting parts of the specific IgE repertoire could explain the "unusually" high prevalence of RAST sensitization on Karkar. Notably it is something like "common knowledge" that worms increase sTot-IgE (which is nothing but a mix of IgE with innumerable specificities and levels of affinity), but few seem to bother to ask,

314 Personal e-mail communication April 20th 2012.

whether the allergologically important specificities are increased as well – why should only these be excluded? Maybe researchers want to avoid the following "paradox": if worms increase allergological sensitization, why don't they increase the prevalence of atopic diseases? We will address this question in chapter 5.8.

5.7.2 Low SPT due to increased tissue histamine tolerance?

As the difference between high RAST and low SPT prevalence basically results from a "true" increased RAST sensitization prevalence at higher sTot-IgE levels (probably due to a "worm boost"), the following question arises: Why is this increased RAST sensitization prevalence not reflected in an equivalent increase in SPT prevalence? Maybe some factor associated with high sTot-IgE levels decreases SPT reactivity, thereby balancing the increased RAST sensitization. Theoretically some helminth induced regulatory substance could decrease histamine reactivity, i.e. induce an elevated tissue histamine tolerance/resilience. To answer this question we correlated sTot-IgE levels with the in vivo skin reactivity to histamine (see fig. 28).[315]

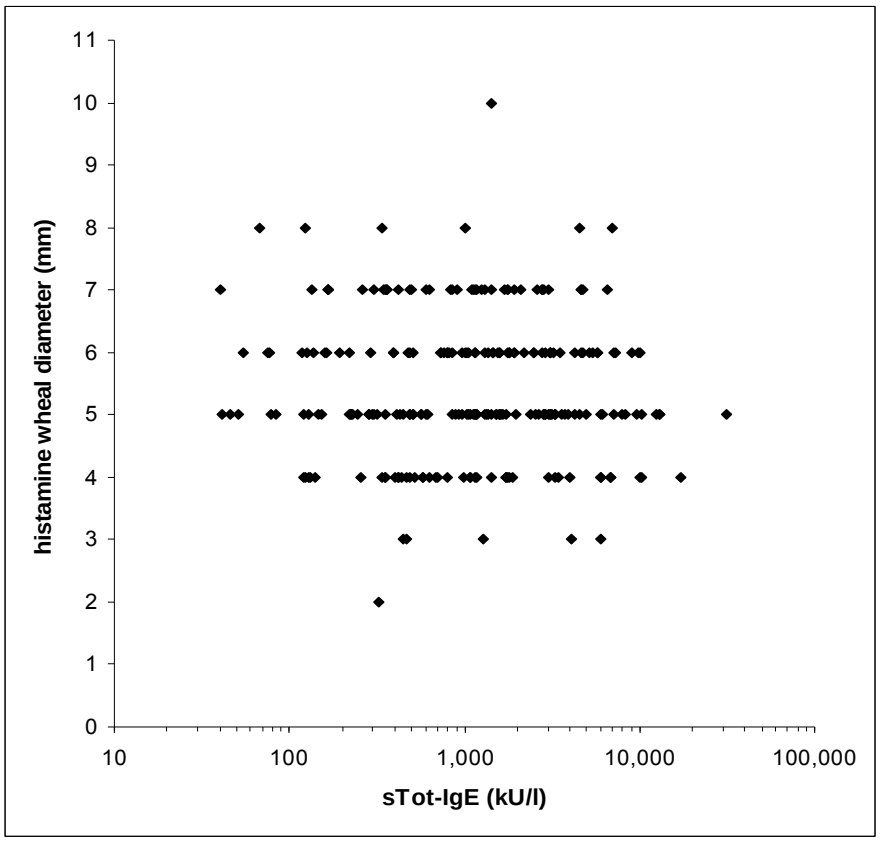


Fig. 28. No decrease in SPT histamine reactivity with increasing sTot-IgE levels (n = 247) (Pearson product moment correlation r^2=0.001; p=0.60).

315 Histamine SPT reactivity is normally just used as a positive control during skin prick testing.

As histamine reactivity is not decreasing with increasing sTot-IgE levels, other factors must be responsible for the unchanged low SPT reactivity (despite of increasing RAST reactivities at higher sTot-IgE levels). Notably the "classic" reasons for false negative respectively decreased SPT reactivity have been excluded: No SPT influencing drugs like antihistamines, glucocorticoids or antidepressants have been used by the study population. Furthermore the dark pigmented skin of most Karkar admittedly renders interpretation of SPT more difficult. Yet this holds true especially in respect to the reading of the SPT erythema.[316] The more relevant correct evaluation of the histamine wheal diameter was not impeded: even in black skin, the reading of the SPT wheal was unproblematic under the good tropical natural light conditions, provided the lower forearm was held in the correct (flat) angle. The arithmetic mean of the histamine wheal on Karkar was 5.44 mm (s = 1.15 mm), median 5 mm. This is in line with the histamine wheal median found in a worm infested population in Lambaréné, Gabon (also 5 mm).[317] As we expected based on our results, deworming with associated decrease of sTot-IgE had no effect on the histamine wheal size within the Lambaréné children.[318]

Conclusion: Up to this point we can not explain, why the – probably worm triggered – high RAST sensitization prevalence on Karkar (which is obviously not due to non-specific binding) is not reflected in a similar SPT prevalence. As there is no increased histamine resilience with increasing sTot-IgE levels, it seems, that in many cases there is no histamine release from mast cells upon antigen contact – despite of the presence of specific IgE. We try to explain this pattern focusing on the house dust mite allergen in the following chapter.

316 Other studies performed in similar settings encountered the same problem: "Erythema was not used in the interpretation of skin tests because of the difficulty to record this in deeply pigmented skins" (Van den Biggelaar AH et al., 2001: 233).

317 Van den Biggelaar AH et al., 2004: 896.

318 „No differences in wheal sizes to the positive histamine control were found between the control [no deworming] and treatment [deworming] groups during the follow-up period [30 months] of the study" (Van den Biggelaar AH et al., 2004: 893).

5.8 The key to the differences between RAST and SPT: The Specific Activity

In order to investigate the relationship between sTot-IgE, specific IgE and SPT we choose house dust mite (species: D. pt and D. fa.) for two different reasons: Firstly, house dust mite (HDM) is the most important allergen on Karkar Island (see chapter 5.1). Secondly, HDM is the only allergen which created positive SPT reactions in a considerable number of subjects;[319] and a sufficiently high number of SPT reactive individuals is necessary to identify relationship patterns with parameters like sTot-IgE and specific activity (SA)[320] (the relationship between cockroach SPT and cockroach SA could not be investigated, as data came from different groups).

To begin with, both HDM species (D. pt. and D. fa.) show the expected sensitization pattern already suggested by the general behaviour of IgE antibodies and SPT in the presence of increasing sTot-IgE levels (see fig. 20 for pooled specific IgE and fig. 24 for pooled SPT): increasing sTot-IgE is associated with a significant increase in mite RAST (for D. pt. $\rho=0.56$ and for D. fa. $\rho=0.53$; each 246df; $p<0.0001$) but unchanged mite SPT. Van den Biggelaar reported a very similar association between sTot-IgE and D. pt. specific IgE within a group of 520 schoolchildren in Lambaréné, Gabon ($\rho=0.494$; $p<0.0001$; see fig. 39).[321] Unfortunately this highly significant correlation was not discussed in van den Biggelaar's work – we will speculate about the possible reasons later in this chapter. Within our Karkar data we found the following pattern: with increasing sTot-IgE there is an increasing probability of a) positive HDM RAST and b) high HDM sensitization levels in vitro (see fig. 29). In the RAST context we have to remember two points. Firstly, as indicated in chapters 5.5 and 5.7.1, worms seem to boost sTot-IgE AND specific IgE. Secondly, high specific IgE levels are normally associated with increased SPT reactivity and clinical allergy. Considering these points, it may (theoretically) even be plausible, that worm infestation increases (!) allergy.

319 An analogous approach was chosen by van den Biggelaar AH et al. in a similar setting in Gabon: "Since most skin test responses were to mite and only very few to other allergens tested, we concentrated on mite reactivity" (van den Biggelaar AH et al., 2001: 235).

320 IgE antibody-specific activity (SA) is defined as allergen-specific IgE to sTot-IgE ratio, see chapter 2.3.3.

321 Van den Biggelaar AH et al., 2000: 1725, figure 1.

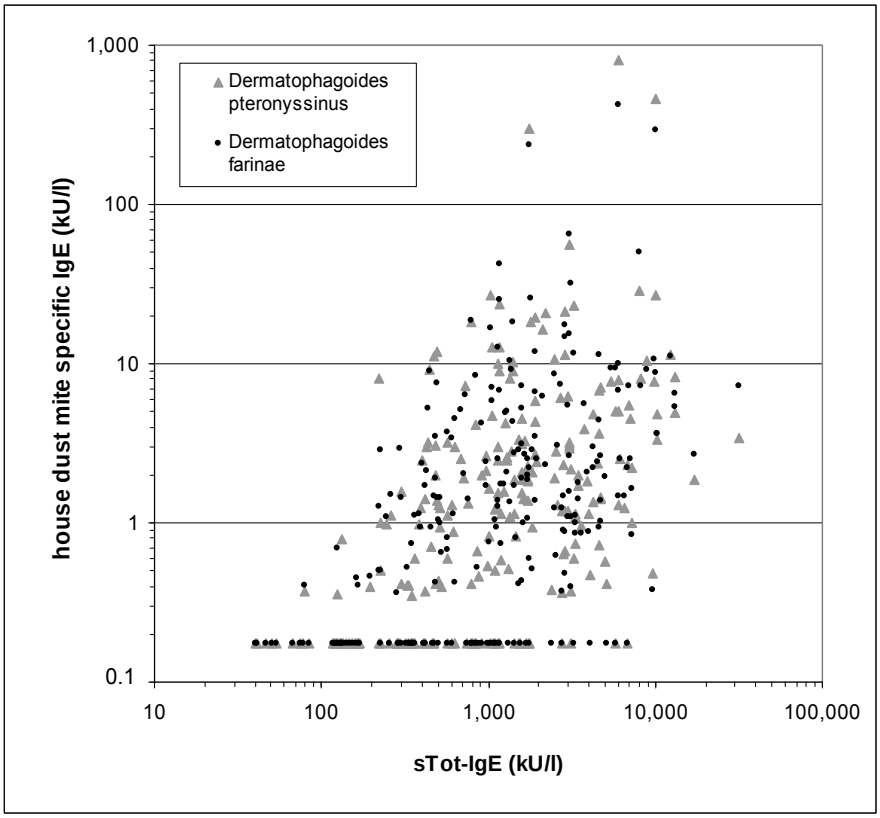


Fig. 29. Significant correlation ($p<0.0001$) of sTot-IgE and house dust mite specific IgE (for D. pt $\rho=0.56$ and for D. fa. $\rho=0.53$): increasing probability and level of house dust mite sensitization (in vitro) with increasing sTot-IgE levels.

Increasing sTot-IgE is not only associated with significantly increasing mite RAST but also with unchanged mite SPT (for D. pt. $\rho=0.066$; $p=0.30$ and for D. fa. $\rho=0.075$; $p=0.24$; each 242df). Also in this respect the data from Lambaréné coincided with our data.[322] Within the Karkar data we found the following pattern: with increasing sTot-IgE there is neither increasing probability of a) positive house dust mite SPT nor b) high level of house dust mite SPT reactivity in vivo (see fig. 30).[323] In contrast to the house dust mite RAST results, the vast majority of both house dust mite SPT results stay negative even at clearly increased sTot-IgE levels of over 1,000 kU/l. All in all only 21.6% (38/176) of the islanders with positive D. pt. in vitro/RAST sensitization showed a positive D. pt. in vivo/SPT reaction. The corresponding percentage for D. fa. was 19.4% (33/170). The inverse constellation – i.e. negative RAST in the presence of positive SPT – was negligibly rare: 2.9% (2/68) for D. pt. and 1.4% (1/74) for D. fa.

322 Van den Biggelaar found no association between sTot-IgE ("polyclonal IgE antibodies") and D. pt. SPT (van den Biggelaar AH et al., 2000: 1725).

323 There might arise the visual impression that there is a tendency for higher SPT reactions at very high sTot-IgE levels. Yet this is mainly due to two D. pt. data points on the far upper right. In a bulk of 244 x 2 data points, this is statistically irrelevant.

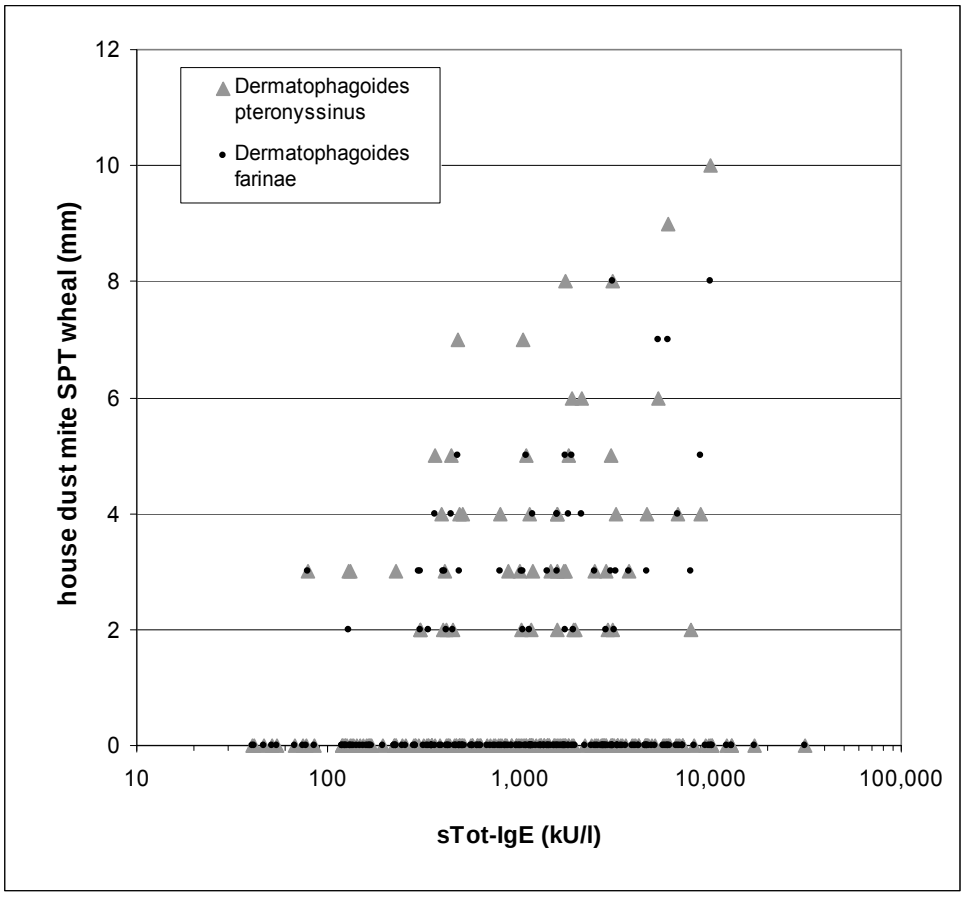


Fig. 30. Negligible, non significant correlation of sTot-IgE and house dust mite SPT (for D. pt. ρ=0.066; p=0.30 and for D. fa. ρ=0.075; p=0.24; each 242df).

As specific IgE increases with sTot-IgE, in the next step we want to examine the behaviour of house dust mite specific activity with increasing sTot-IgE. We display the two house dust mite specific activities in separate figures: fig. 31 for Dermatophagoides pteronyssinus and fig. 32 for Dermatophagoides farinae.

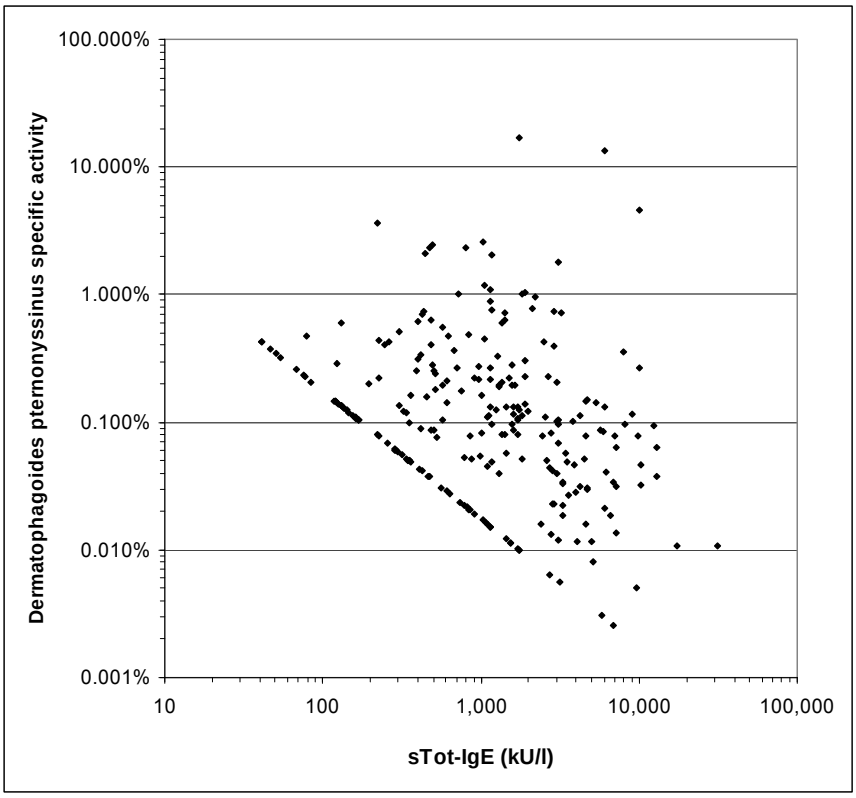


Fig. 31. Moderate negative correlation of sTot-IgE and D. pt. SA (ρ= -0.53; 178df; p<0.0001).

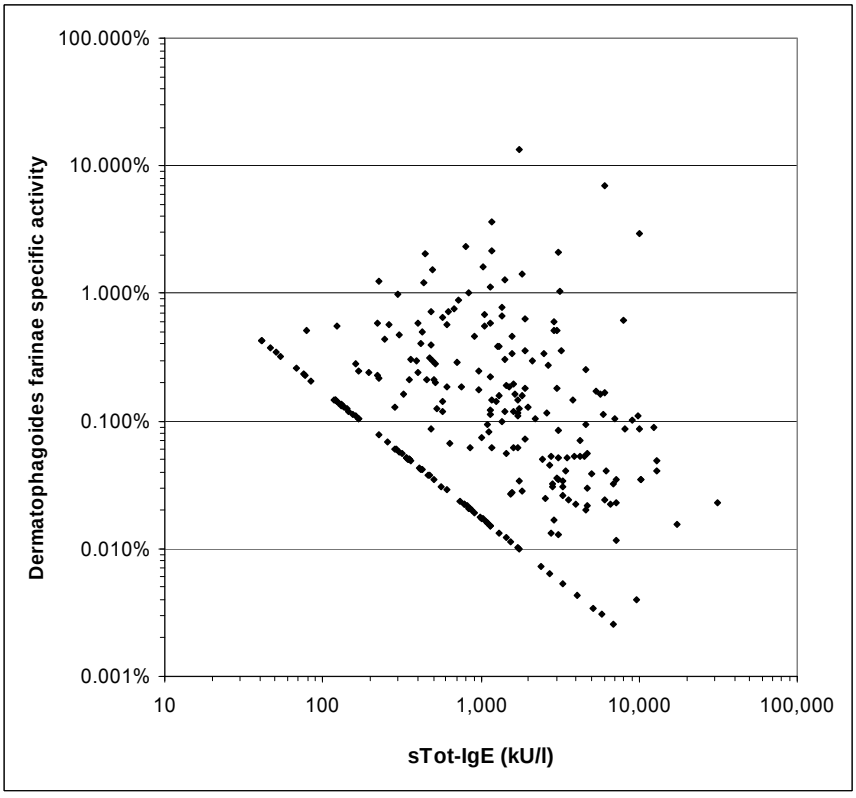


Fig. 32. Moderate negative correlation of sTot-IgE and D. fa. SA (ρ= -0.56; 172df; p<0.0001).

The two figures – the first for Dermatophagoides pteronyssinus (D. pt.) and the second for Dermatophagoides farinae (D. fa.) – display a very similar pattern: the negative correlation between the two variables[324] is significant but only moderate (ρ= -0.53 for D. pt. and ρ= -0.56 for D. fa.).[325] Yet already at a first glance we see that with increasing sTot-IgE there is no overall increase of house dust mite (HDM) specific activity (SA) – despite of the positive association of sTot-IgE and specific IgE. Quite on the contrary: rho is negative (for D. pt. as well as D. fa.). To start with, we want to give some clarification about the interpretation of the two figures. The values are displayed on $\log_{10}$ scales. Specific activities i.e. the ratio house dust mite specific IgE to sTot-IgE are given as percentages. The decreasing line of points (slope -1) represents the house dust mite negative islanders (RAST class 0).[326] They were ascribed a constant value of 0.175 kU/l (half the detection limit) to allow for $\log_{10}$ transformation.[327] A point at sTot-IgE 1,000 kU/l and HDM SA 0.1% means that the respective HDM specific IgE amounts to 1 kU/l (i.e. RAST class 2), the same SA at sTot-IgE 10,000 kU/l is reached with 10 kU/l of HDM specific IgE (i.e. RAST class 3). In short: if we move on virtual horizontal lines to the right, the ratio of HDM IgE to sTot-IgE (=SA) always stays equal, if we move diagonally up, HDM specific IgE increases proportionally more than sTot-IgE and if we move diagonally down, HDM specific IgE increases proportionally less than sTot-IgE. At this stage we may summarize: as shown before (see fig. 29) with increasing sTot-IgE there was an increase (probably worm boost) of specific IgE. In contrast, looking at figs. 31 and 32 we see that up to 1,000 kU/l sTot-IgE mite SA may increase or decrease with increasing sTot-IgE. Over 1,000 kU/l sTot-IgE mite SA tends to decrease. Generally (independently of our data) higher specific IgE leads to higher SPT probability; thus due to the association between sTot-IgE and mite specific IgE within the Karkar data we should expect increasing positive mite SPT (probability and level) with increasing sTot-IgE. Yet this was NOT the case (see fig. 30). Possibly the ratio specific IgE to sTot-IgE (specific activity) is more relevant to SPT reactivity than the specific IgE alone. This possible "(SPT) protective effect" of high sTot-IgE levels could explain why we see an increased prevalence of positive HDM RAST but an unchanged prevalence of positive HDM SPT at high sTot-IgE levels. In a next step we want to examine the suspected relevance of SA for SPT reactivity. Therefore we will reuse the figures 31 and 32, yet displaying additionally the SPT reactivity of the corresponding HDM specific activities. In the subsequent figures 33

324 The decreasing linear line of points representing the mite RAST negative subjects does not have to be taken into account (in both figures) as we attributed an arbitrary constant for log presentation.

325 The exact values were: Spearman correlation between sTot-IgE and D. pt. SA ρ= -0.53; 178df; p<0.0001. We performed a Pearson product moment correlation as well: the correlation coefficient was slightly smaller with r=-0.46 (equal df and p; r^2=0.21). This is due to the fact, that despite of log transformation there are some outliers which decrease parametric correlation. The corresponding values for D. fa. are ρ= -0.56; 172df; p<0.0001. The Pearson correlation coefficient was again slightly smaller with r=-0.49 (equal df and p; r^2=0.24) for the reason given above.

326 In fig. 31 the decreasing line of points consists of 68 D. pt. IgE negative subjects (27.4% of 248) and in fig. 32 the decreasing line of points consists of 74 D. fa. IgE negative subjects (29.8% of 248).

327 Why we ascribed this constant is explained in addendum fig. 49.

and 34 we added a SPT legend with the following meaning: “minus” representing no/irrelevant SPT reaction (wheal diameter < 3 mm), “small circle” representing a noticeable SPT reaction (wheal diameter = 3-4 mm) and “large circle” representing a strong SPT reaction (wheal diameter > 4 mm). This form of simultaneously depicting many of the crucial variables of type one allergies – i.e. sTot-IgE, (allergen) specific activity (including the hidden factor specific IgE), and degree of SPT reactivity – in one figure has not been used before. Moreover we added an additional y-axis which indicates the estimated percentage of effector cell degranulation at the given SA levels. These estimated degranulation percentages are based on Christensen’s in vitro experiments already presented in chapter 2.3.3. We are fully aware that the results were found in in-vitro experiments with differences to in-vivo conditions: Effector cells in vitro were basophils instead of mast cells[328] and degranulation was stimulated with the recombinant single major HDM allergen (D. pt. 2) and not with a physiological mixture of HDM allergens. Yet all parameters of the experiment were kept as “near to the biological reality” as possible. Thus in the author’s opinion Christensen’s “elegant” model is currently the most precise and most representative for the complex conditions prevailing in natural/physiological IgE repertoires.

For both house dust mite species there is a clear correlation of higher SA with increased probability of positive SPT and increased level of SPT reactivity. Strong SPT reactions (wheal diameter > 4 mm) only occur over a HDM SA of 0.1%[329]. Smaller SPT reactions (wheal diameter = 3-4 mm) were possible under 0.1% SA, yet cases were few.

328 As Stone et al. point out: “Basophils share many features with mast cells …” (Stone KD, Prussin C, Metcalfe DD, 2010: S77).

329 Exception: one subject showed a strong SPT reaction to D. fa. slightly below the 0.1% threshold at 0.0944% (sTot-IgE 1,101.5 kU/l).

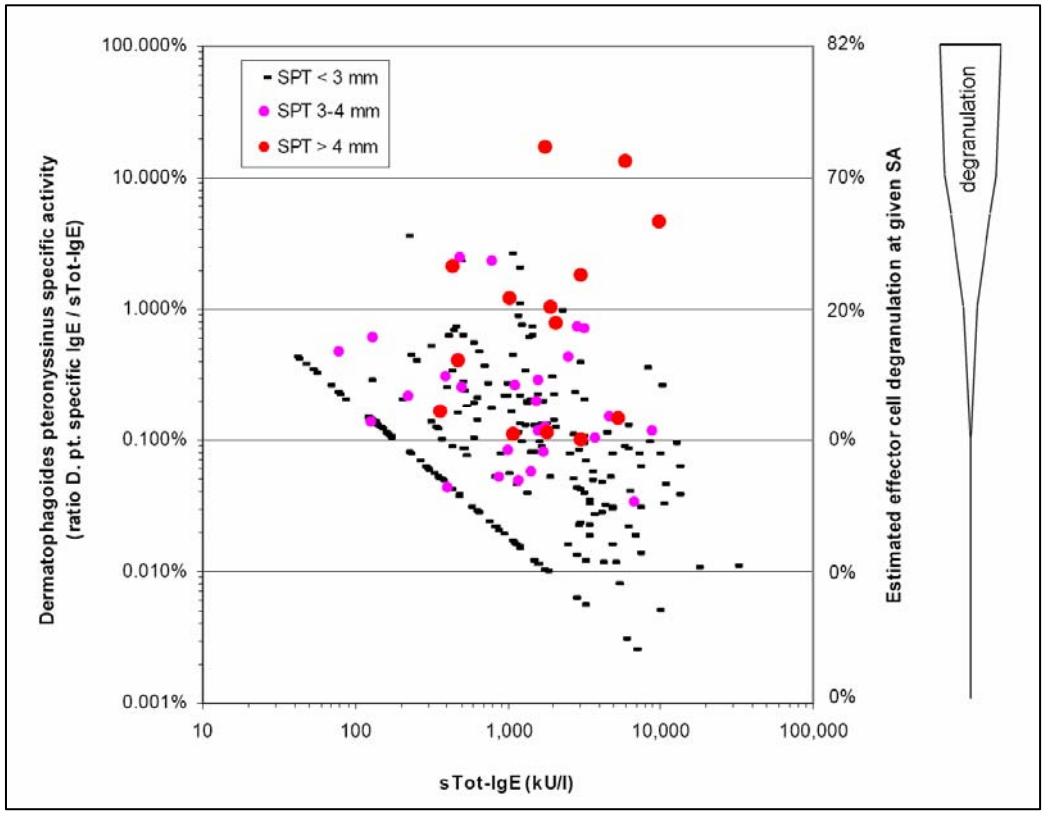


Fig. 33. Correlation of sTot-IgE and D. pt. SA (including degranulation) with associated SPT reaction.

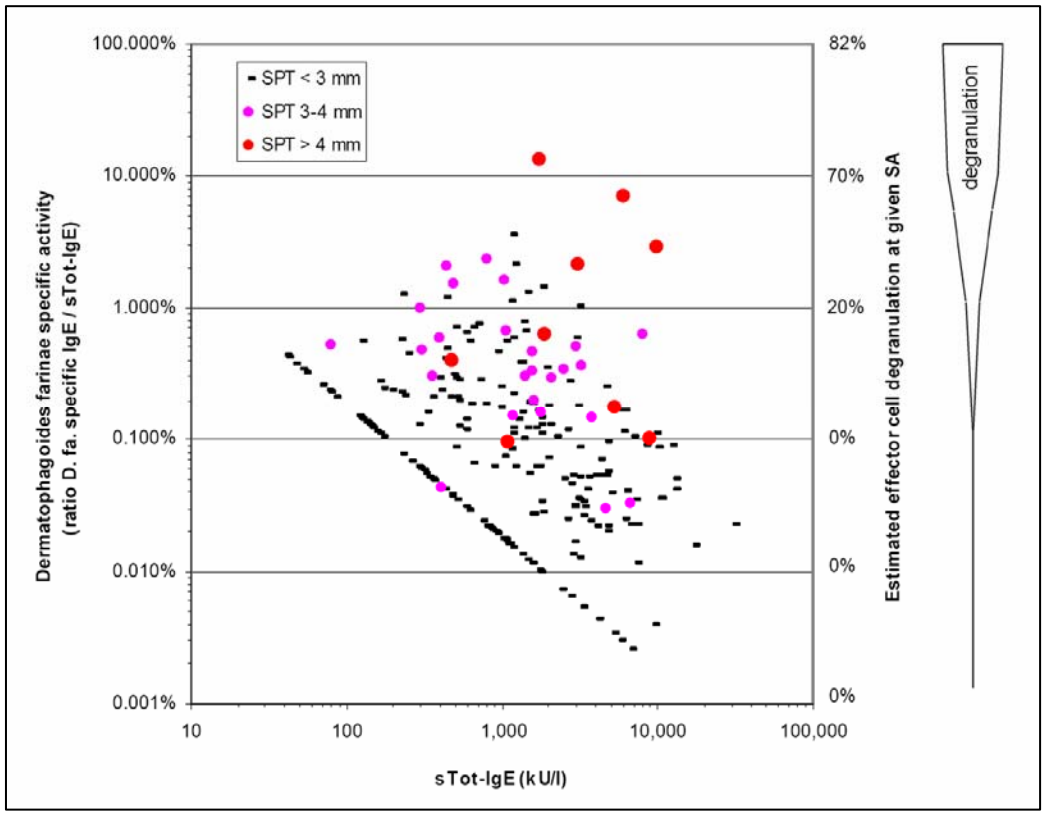


Fig. 34. Correlation of sTot-IgE and D. fa. SA (including degranulation) with associated SPT reaction.

The following table 2 pools strong and medium SPT reactions as "positive" SPT reactions and gives the percentage of positive SPT at five different levels of SA for both HDM species.

Table 2. SPT reactivity for D. pt. and D. fa. at five different levels of HDM specific activity. Higher HDM SA is clearly associated with higher probability of positive SPT reactions.

HDM specific activity (SA)[330]	positive SPT D. pt.		positive SPT D. fa.	
SA 0.001-0.01%	0/8	0%	0/9	0%
SA 0.01-0.1%	7/106	7%	4/103	4%
SA 0.1-1%	24/115	21%	22/116	19%
SA 1-10%	7/13	54%	7/15	47%
SA 10-100%	2/2	100%	1/1	100%

As the five SA bins are quite large, we will perform a logistic regression analysis for D. pt. (fig. 35) and D. fa. (fig. 36). This allows a much more precise description of the relationship between HDM specific activity (independent variable) and the probability of positive HDM SPT reaction (dependent variable). Figures 35 and 36 show that the probability of a positive SPT at a SA of 0.1% is only 12.4% for D. pt. and 8.1% for D. fa. The respective values at a SA of 0.01% were even smaller with 2.9% for D. pt. and 1.1% for D. fa. Finally, predicted positive SPT at SA of 0.001 is negligible: 0.6% for D. pt. and 0.1% for D. fa. Furthermore the logistic model suggests that when HDM SA is over around 1.6% there is an over 50% chance that respective SPT result be will positive.[331] In other words: the biggest effect of a given change in HDM SA on SPT reactivity is around 1.6 % HDM SA: The slope of the curve is highest at 1.8% SA for D. pt. and at 1.4% SA for D. fa. This is fully in line with Christensen's et al. in-vitro results on effector cell degranulation: they also found that the effect of changes in SA on "allergological reactivity" was strongest when changes were made in the interval between one and five percent SA. At 1% SA in-vitro degranulation was still only 20%, yet at 5% SA degranulation was already 53%.[332] Clinical studies consider a similar SA range to be crucial: Johansson SG et al. regarded (implicitly) the interval between 1% and 4% SA as most relevant in respect to clinical reactivity: they defined a (cat) SA <1% as "low" and a (cat) SA >3.8% as "high".[333]

330 There were no values precisely on the dividing SA threshold levels (0.01%, 0.1%, 1% and 10%).

331 The exact values with confidence intervals (CI) are: at 1.78% D. pt. SA the 95% CI for a positive D. pt. SPT is 34% - 66% and at 1.40% D. fa. SA the 95% CI for a positive D. fa. SPT is 33% - 65%.

332 Christensen LH et al., 2008: 302.

333 Subsequently they showed in their double-blind placebo controlled study that effector cell sensitivity was significantly lower in the "low" SA allergic group than in the "high" SA allergic group after 16 weeks of omalizumab treatment (Johansson SG et al., 2009: 1472-77).

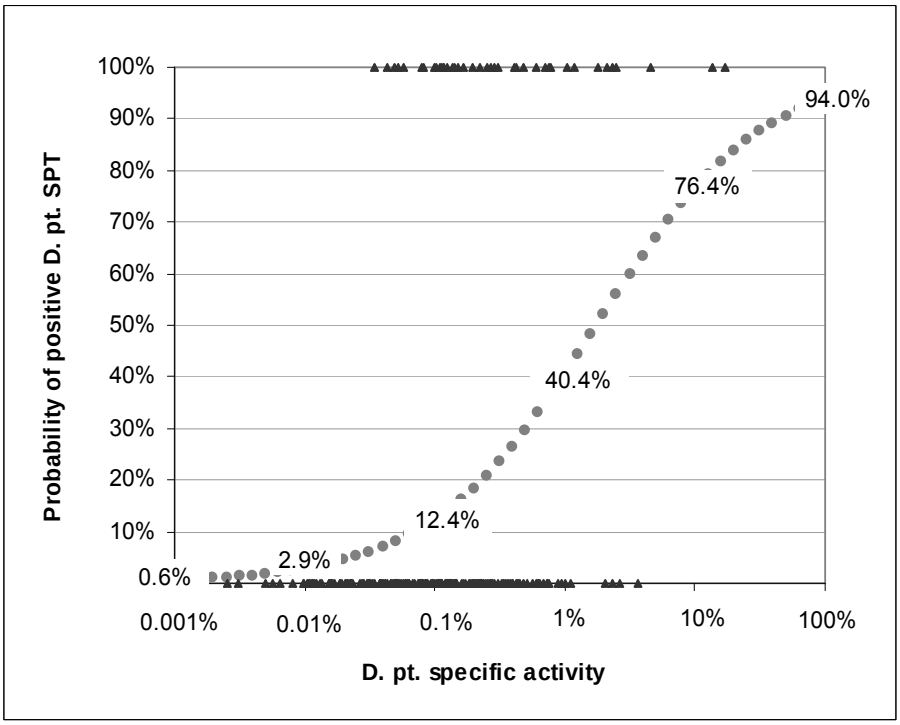


Fig. 35. Variation of the estimated proportions of positive D. pt. SPT results according to D. pt. specific activity. Black triangles at the top of the plot represent positive SPT, at the base negative SPT. Simple logistic regression; overall model fit χ^2=28.33; p<0.00001.

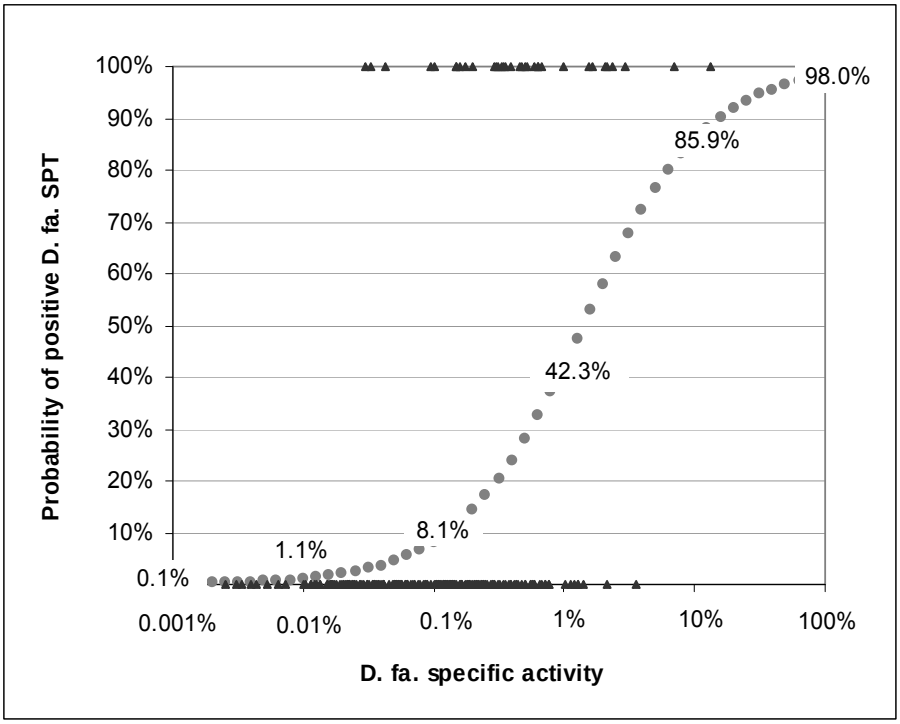


Fig. 36. Variation of the estimated proportions of positive D. fa. SPT results according to D. fa. specific activity. Black triangles at the top of the plot represent positive SPT, at the base negative SPT. Simple logistic regression; overall model fit χ^2=40.33; p<0.00001.

Reason for the decreasing probability of positive SPT reactions with decreasing SA levels may be blocking/saturation of the high affinity IgE receptors on mast cells (and basophils)[334]: helminth induced polyclonal ("non"-specific) IgE seems to outcompete allergen-specific IgE for FcεRI binding on effector cells. The resulting low density of specific IgE is insufficient to allow for the required level of bridging between specific IgE molecules. Thus effector cell degranulation is impeded. This is an old[335] and contested theory as it may seem too simple and mechanistic in times of rapidly increasing knowledge about the highly complex regulatory cytokine interactions. We fully agree with Erb who stated in 2007: "Currently this explanation [the blocking hypothesis] is no longer in vogue not because it has been experimentally … disproved but … simply because explaining the decreased allergic responses [in worm infected individuals] … by the induction of Treg [regulatory T] cells is more "trendy" because numerous researchers are investigating this … mechanism in great detail."[336] In our opinion, one major "problem" of the different cytokine/regulatory theories/concepts is that the interactions are of such a complexity[337] that the clinical effects of the single cytokines are (currently) difficult to evaluate and consequently study results are often contradictory: not only that the different cytokines interact in highly interwoven networks; moreover the effect of a certain cytokine varies depending on the kind of tissue, kind of producing cell and time of production. The blocking/saturation theory however is comparatively "unspectacular". Already in 1967 Stanworth et al. reported that myeloma "IgND" inhibited the sensitizing effects of intradermal injection of "allergic serum".[338] In more "modern" terminology: Preliminary injection of high concentrations of non-specific (myeloma) IgE into the skin of a non-allergic test person impeded subsequent passive transferral of type I hypersensitivity by injection of serum from an allergic donor containing specific IgE ("allergic serum").[339] In 1976 Godfrey RC et al. published in "Nature" that lung fragments became resistant to sensitization with grass pollen IgE when they had first been exposed to serum with high Tot-IgE.[340] In 1993 Lynch et al. described a greatly increased SPT reactivity to house dust after anthelmintic treatment of children in a Venezuelan slum. This finding was mainly attributed to "decreased mast cell saturation [with polyclonal IgE]".[341] In 1996 Witteman AM et al. reported a "significant inhibitory effect of [high] total serum IgE [on skin test

334 Another explication would be lower levels of specific IgE. Yet we will see that we find lower SPT prevalence even within the same RAST class, when sTot-IgE is higher (i.e. SA is lower).

335 Mitre E et al. called the IgE-blocking hypothesis "the most longstanding" explanation for the decreased rates of allergy in helminth infected subjects (Mitre E, Norwood S, Nutman TB, 2005: 4106).

336 Erb KJ, 2007: 1171.

337 Summarizing results on the regulation of T helper type 2 cell immunity McKenzie confirms: "These results emphasise the complexity of the cytokine network …" (McKenzie AN, 2000: 143).

338 Stanworth DR, Humphrey JH, Bennich H, Johansson SG, 1967: 332.

339 The inhibited kind of passive sensitization is called the "Prausnitz-Küstner reaction".

340 Godfrey RC, Gradidge CF, 1976: 484-6.

341 Lynch NR, Hagel I, Perez M, Di Prisco MC, Lopez R, Alvarez N, 1993: 409.

sensitivity]" in Dutch allergic patients.[342] They considered it a "likely explanation" that there exists "competition of the irrelevant IgE with specific IgE for binding to the IgE receptors on basophils and mast cells."[343] Results quite similar to the Dutch ones were reported in 2003 from Ecuador: Cooper PJ et al. were able to "demonstrate a negative association between very high levels of total IgE and skin test reactivity" in nematode infected school-age children. According to Cooper PJ et al., this finding may be due to "saturation of … FcεRI receptors on mast cells and basophils by IgE of multiple specificities"[344]. In 2010 Hamilton RG et al. concluded that "irrelevant IgE can dilute out or swamp the surface receptors [on effector cells]"[345] and one year later Platts-Mills formulated more cautiously that "at least in some cases, IgE induced by parasites can actually block allergic responses".[346] The recent molecular biologically based study by Christensen et al. showed that there was indeed no in vitro degranulation at SA levels lower than 0.1% (see figure 2 in chapter 2.3.3). Despite of this longstanding history of reports suggestive of blocking, the (IgE-) blocking hypothesis as such remains contested.[347] Investigating the competitive inhibition theory in vivo is generally very difficult as most present populations do not show sTot-IgE levels high enough, to reach "obvious blocking thresholds" (one exception is van den Biggelaar`s Lambaréné study, which will be discussed in detail later). Our data support the notion that this kind of "trivial" mast cell inhibition really exists. Positive mite SPT reactions under what we will call the "obvious inhibition threshold" (i.e. SA under 0.1%) were rare: 6.1% (7/114) for D. pt. and 3.6% (4/112) for D. fa. Even – and more importantly in respect to the blocking hypothesis – looking at people with the same RAST class (i.e. with the same level of in vitro sensitization), the probability of positive SPT reactions was lower, when the SA was under 0.1% (i.e. when sTot-IgE was higher). This pattern – highly suggestive of IgE blocking – was found for D. pt. and D. fa. The results are displayed in fig. 37 (for D. pt.) and in fig. 38 (for D. fa.). Yet before discussing both figures in detail, we want to have a look at a study, which is relevant in respect to our assumed 0.1% blocking threshold in a more general way.

Fully in line with our epidemiological calculations (blocking threshold 0.1% SA) and Christensen's above mentioned in vitro results (blocking threshold 0.1% SA, publication

342 "With similar levels of specific IgE, the amount of allergen that is required for a positive skin test result may differ by as much as a factor of 100 between patients" (Witteman AM et al., 1996: 16ff).

343 Witteman AM et al., 1996: 23.

344 Cooper PJ, Chico ME, Rodrigues LC, Ordonez M, Strachan D, Griffin GE, Nutman TB, 2003: 999.

345 Hamilton RG, MacGlashan DW Jr, Saini SS, 2010: 283.

346 Platts-Mills considers it possible that "at least in some cases, IgE induced by parasites can actually block allergic responses" (Platts-Mills TAE, Lee BW, Arruda LK, Chew FT, 2011: 82).

347 "Epidemiologic and experimental data obtained since then [1967], however, have provided mixed support for this [blocking] hypothesis" (Mitre E, Norwood S, Nutman TB, 2005: 4106). Notably, one of the two studies cited by Mitre et al. and arguing against the blocking hypothesis is van den Biggelaar's work published in the year 2000 (Van den Biggelaar AH et al., 2000: 1723-27). Why in the author's opinion the latter study supports the blocking hypothesis – against van den Biggelaar's intention – is laid down in detail later in this chapter.

year 2008) is Mitre's suppression level at 0.2% SA and blocking threshold of 0.1% SA (in vitro experiments, publication year 2005).[348] Strikingly enough (and despite of his in vitro results) Mitre rejects the clinical existence of IgE blocking as already expressed in the title of his work: "Saturation[349] of immunoglobulin E (IgE) binding sites by polyclonal IgE does not explain the protective effect of helminth infections against atopy."[350] This obvious discrepancy requires a closer look at Mitre's data. To start with, Mitre's in vitro experiments pointed to an over 50% decreased histamine release at 0.2% SA[351] and the existence of a blocking threshold of 0.1% SA[352]. Mitre states that these low SA levels were not reached in neither of two in-vivo experiments within his filaria infected study group. In the first experiment, the lowest SA (filaria) was 0.3%.[353] In the second experiment, the "lowest" SA (D. pt.) was 1.2% (the highest SA was 6.3%).[354] Mitre considered the range between 1.2% and 6.3% to represent low specific activity[355] – an evaluation we do not share.[356]

Mitre's conclusion that the IgE blocking found in vitro is clinically irrelevant[357] appears to be ill-founded. Firstly we consider Mitre's generalisation in respect to different populations questionable: As the 0.2% suppression level and the 0.1% SA inhibition threshold were never reached in his small filaria infected Maryland sample (n = 13), Mitre concludes that the mentioned levels would generally rarely be reached in worm infested societies.[358] This is definitely not true for our Papua New Guinea population: anticipating our later results, we may already mention at this stage, that 64.1% (771/1203) of the positive RASTs on Karkar resulted in SAs under 0.1% (see fig. 43). Secondly Mitre

348 Mitre E et al. presented specific activities in the "reverse form" i.e. as the ratio polyclonal IgE to specific IgE (Mitre E, Norwood S, Nutman TB, 2005: 4108, table 1).

349 Mitre uses the terms "saturation" and "blocking" in a synonymous way.

350 Mitre E, Norwood S, Nutman TB, 2005: 4106-11.

351 "Quantities of released histamine could be decreased to less than half of maximal histamine release, but only when [effector] cells were sensitized with polyclonal to specific IgE ratios of greater than 500:1" (ibid., 4109).

352 "[We] … were able to completely extinguish these [histamine] responses with polyclonal to antigen-specific IgE ratios of greater than 1,000:1" (ibid., 4110).

353 The filaria SA range was between 7.1% and 0.3%. Mitre et al. could not detect an association of decreasing filaria SA and decreasing histamine release when basophils were stimulated with filaria antigens (Brugia malayi). However the total case number was very small (n=13) and only six subjects showed a filaria SA < 1% (Mitre E, Norwood S, Nutman TB, 2005: 4108, table 1).

354 Ibid., fig. 2.

355 As before, (D. pt.) SA is presented in the reverse form: "… we also demonstrated that high polyclonal to antigen-specific IgE ratios [between 16:1 and 86:1] do not inhibit … histamine release …" (Ibid., 4110).

356 Understandably, Mitre et al. could not detect IgE blocking in this range. As in the first experiment, there was no association of decreasing D. pt. SA and decreasing histamine release when basophils were stimulated with D. pt. antigens. In the second experiment only four different SAs were compared (Ibid., 4108).

357 "… while saturation of IgE binding sites by high ratios of polyclonal to specific IgE is physically possible in vitro, this phenomenon likely occurs only rarely in helminth-infected patients (Mitre E, Norwood S, Nutman TB, 2005: 4108, table 1).

358 "While the percentage of helminth-infected patients that fit these criteria [SA<0.2%] is not known, it is probably low…" (Ibid., 4110).

argues that even if SA fell under 0.2% in vivo we may not find a suppressive effect, as the in vitro results may not be applicable in vivo. This consideration is based on the assumption that up-regulation of the number of FcεRI on effector cells with increasing sTot-IgE levels renders saturation of the receptors difficult.[359] Mitre et al. could indeed confirm such receptor up-regulation in worm infected subjects. Yet the correlation between sTot-IgE (log_{10} scale) and IgE receptor number (normal scale) was not linear and Mitre et al.'s interpretation of the Spearman correlation seems dubious as they claimed that there was "no evidence of a plateau in the intensity of IgE staining".[360] However – looking at Mitre's scattergram reprinted in fig. 50 (addendum) – there actually seems to exist a plateau in the group with higher sTot-IgE levels (>1.000 ng/ml). Moreover we do not consider a plateau – i.e. the cessation/ending of up-regulation – a conditio sine qua non for the existence IgE blocking as competitive inhibition depends rather on ratios than on absolute numbers.[361] Thus we can not follow Mitre's argumentation why the in vitro found thresholds should not be valid in vivo. All in all Mitre argues against the IgE blocking hypothesis, but his data actually support it. Besides his own work, Mitre mentions two further studies which reject the blocking hypothesis. One is a study published 2000 by van den Biggelaar in "The Lancet"[362] and discussed in detail later in this chapter. We think that in Mitre's and Biggelaar's works the presented data could be interpreted in a different way.

Getting back to our Karkar data, we now want to discuss figures 37 and 38, which are highly suggestive of IgE blocking. Subjects with the same in vitro mite sensitization level/RAST class had a lower probability of positive mite SPT reactions when mite SA was under 0.1%. Subjects without in vitro mite sensitization (RAST class 0) developed – as expected – rarely positive in vivo mite reactions (SPT): of the RAST negative islanders only 3% (2/68) showed a reactive SPT for D. pt and 1% (1/74) for D. fa.[363] On the other hand, very highly in vitro sensitized subjects (mite RAST classes 5 and 6) always developed positive skin reactions (yet only 4 cases exist for D. pt. and 4 cases for D. fa.). A differentiation between over and under 0.1% mite SA was not possible for RAST class 4 as sTot-IgE levels are not even high enough on Karkar to push SA under 0.1%, thus all subjects with RAST class 4 had SA over 0.1% (SPT reactivity in this group was 40% for D. pt. and 29% for D. fa.). The large majority of in vitro mite sensitized islanders fall in the RAST classes one, two and three: 92% (162/176) of D. pt. sensitised subjects were in these groups and 94% (159/170) of D. fa. sensitised subjects.

359 Ibid., 4110.

360 Ibid., 4109. The intensity of IgE staining reflects the number of IgE bound to the cell surface.

361 However, the assumed existence of lateral shift of FcεRI on effector cells may give a certain importance to the absolute number of cell bound receptors.

362 Van den Biggelaar AH et al., 2000: 1723-27.

363 There is no differentiation between higher and lower SA within RAST class 0 as SA is always cero. In respect to SA, the diagonally descending line of points in fig. 33 and fig. 34 is an artefact due to the attribution of 0.175 kU/l specific IgE for log representation.

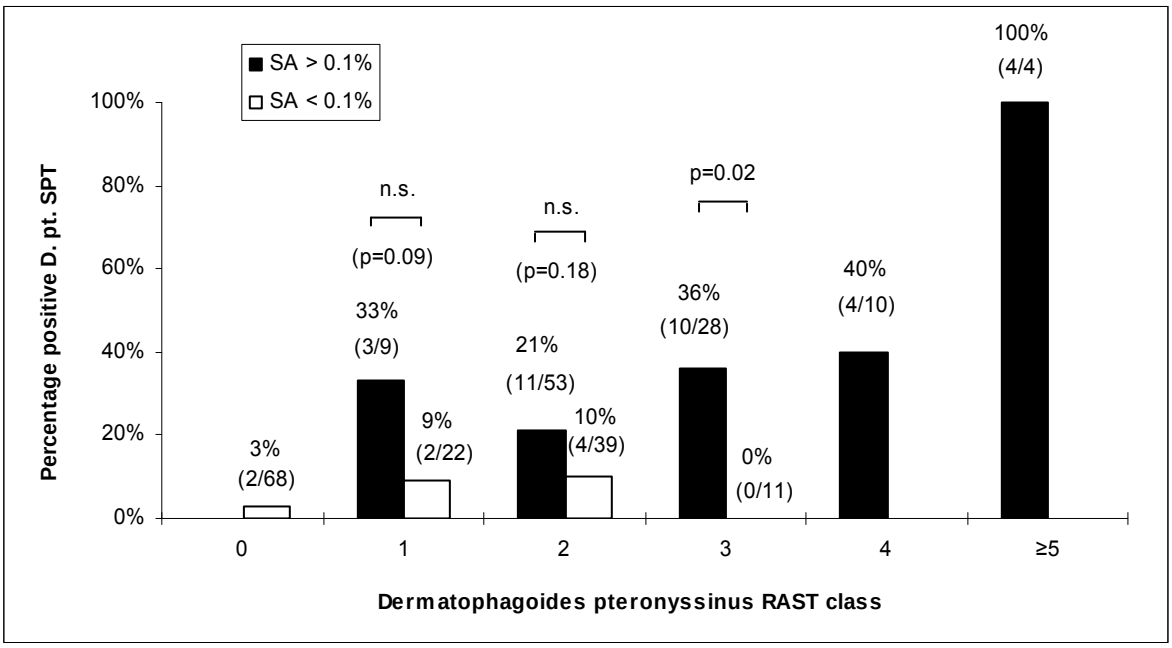


Fig. 37. Reduced D. pt. SPT reactivity at SA levels under 0.1%. Subjects with identical D. pt. RAST class showed decreased SPT reactions when D. pt. SA was under 0.1%. The difference in comparison to SA over 0.1% was significant for RAST class 3 (Fisher exact test), a confirming trend was observed for classes one and two.

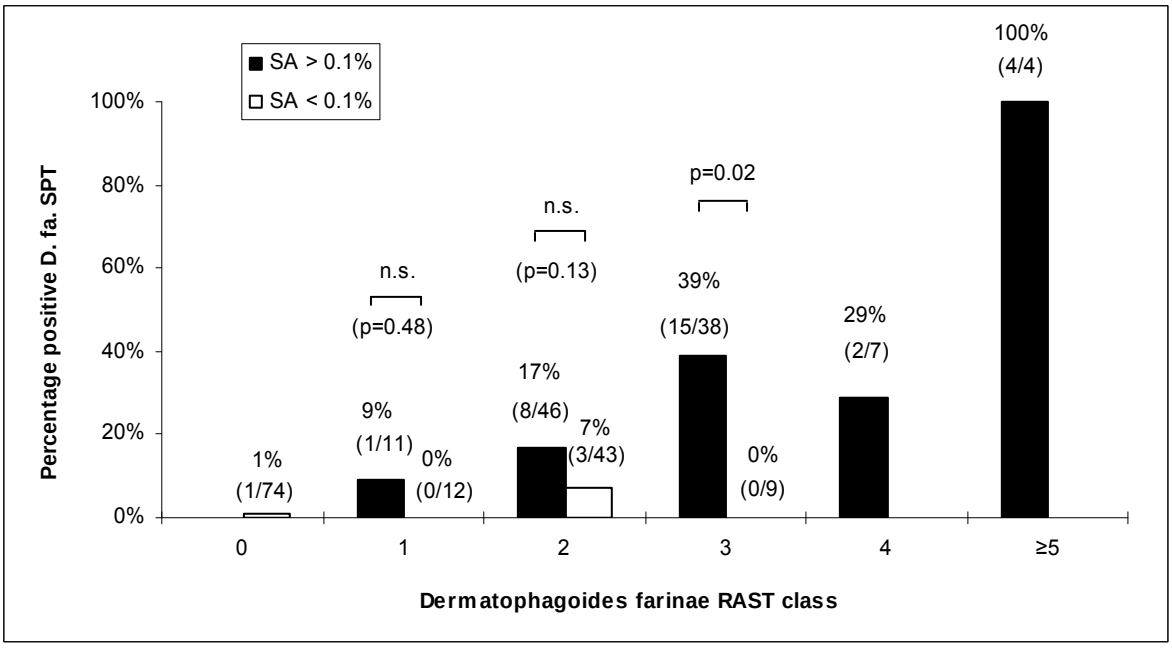


Fig. 38. Reduced D. fa. SPT reactivity at SA levels under 0.1%. Subjects with identical D. fa. RAST class showed decreased SPT reactions when D. fa. SA was under 0.1%. The difference in comparison to SA over 0.1% was significant for RAST class 3 (Fisher exact test), a confirming trend was observed for classes one and two.

The interpretation of RAST classes one to three depends on SA as lower SA is associated with lower SPT reactivity. People with mite RAST class 3 showed a significantly decreased (p=0.02 Fisher exact test) percentage of positive mite SPT if mite SA was under 0.1%: a positive SPT reactivity to D. pt. extract was found in 36% of the islanders with SA over 0.1% yet in 0% of the islanders with SA under 0.1% (values for D. fa. 39% vs 0% respectively, p=0.02). The trend for RAST classes two and one (D. pt and D. fa.) confirmed our findings of RAST class 3.[364] If SA was lower than 0.1% the prevalence of positive mite SPT was always less than half in comparison to SA levels over 0.1% (for detailed values see figures 37 and 38). The pattern that the differences between SA<0.1% and SA>0.1% only reached the level of significance (p<0.05) in RAST class three (for both HDM species) whereas classes one and two only showed a congruent/confirming tendency seems plausible: With increasing RAST class, i.e. increasing levels of specific IgE, "normally" (i.e. in the case of SA>0.1%) the prevalence of positive SPT reactions increases. As in the case of SA<0.1% prevalence of positive SPT stays low with increasing RAST class (basically unchanged) the between-groups discrepancy becomes more pronounced with increasing RAST class[365] – and thus statistically significant even within our comparatively small study group. The previous findings are an indication that high sTot-IgE levels can decrease skin reactivity to allergens.

Yet as increased ("protective") sTot-IgE levels are associated with disproportionately increased ("harmful") specific IgE levels in some individuals[366] there is statistically no decrease of positive SPT prevalence with increasing sTot-IgE levels. The possibility that worm infections may trigger protective yet at the same time "harmful" effects, may be the reason, why – despite of the existence of IgE receptor blocking –different studies yielded different results in respect to possible allergy protective effects of worm infections.

364 Yet the differences between high and low SA did not reach significance. For comparison within RAST class two the chi square test was applied, for comparison within RAST class one the Fisher exact test. The exact p values are displayed in fig. 37 and 38.

365 We found the same pattern in van den Biggelaar's Lambaréné data: the SPT differences between D. pt. SA<0.1% and D. pt. SA>0.1% were not significant within RAST class 1 and reached significance already in class 2 (mainly due to larger case numbers). For details see fig. 41 and corresponding text.

366 As already shown in chapter 5.7.1 despite of high sTot-IgE, specific IgE may stay negative in some individuals or increase disproportionately in others.

The possibility that worm infestations represent a double-edged sword in respect to allergies seems not to be a "popular" point of view. This may be explained by a research and publication bias: mostly towards protective effects of worms, sometimes towards "harmful" ones[367] and – to our knowledge – never to ambivalent/conflictive[368] ones. Helmby's article aiming to offer an overview over available literature on the relationship between worms and allergies bears the title: "Helminths and our immune system: Friend or foe?" The "clear" answer was that it is impossible to answer the question;[369] we just know for sure that worms "modulate, alter, suppress, manipulate" our immune system.[370] Helmby shares our suspicions concerning a publication bias ("… negative data is, unfortunately, in general not considered very attractive from a publication point of view"); she is most certainly right when she states: "… it is easy to get the impression that helminth infections will always, in one way or another, affect the outcome of any concurrent immune response".[371]

In the context of contested "protective" effects of sTot-IgE (blocking theory) and the neglect to consider the possibility of (partial) negative effects of worms we want to have a closer look at the study published by van den Biggelaar et al. in "The Lancet" in 2000[372], which attracted a lot of attention. This epidemiological survey is one of very few investigating similar parameters (mite RAST, mite SPT, sTot-IgE, worm infection, PEFR)

367 A work by Santiago HdaC et al. mentions ten studies in favour of decreased allergy prevalence in helminth-infected subjects (when compared to helminth-uninfected controls) and six studies suggesting that helminths actually drive allergies (Santiago HdaC, Ribeiro-Gomes FL, Bennuru S, Nutman TB, 2015: 93).

368 Here we refer to simultaneous opposing influences of worms on the immune system. Notably there exist mouse model based studies suggesting e.g. that acute helminth infections enhance allergies and chronic infections protect from allergies (McConchie BW et al., 2006: 6632). Smits et al. point to the possibility that "heavy parasite burden may induce immune suppression, whereas mild infections may promote allergic disease" (Smits HH, Everts B, Hartgers FC, Yazdanbakhsh M, 2010: 4)

369 "… it is currently difficult to draw any firm conclusions from these studies other than more field studies are certainly needed" (Helmby H, 2009: 122). "In summary, it is probably safe to conclude that based on the current human and experimental literature it is not possible to firmly establish whether helminth infections really do protect against allergic disease" (Helmby H, 2009: 123). "… we … have generated more questions than we have answered in this field" (Helmby H, 2009: 125).
Other reviews draw the same conclusion. Cooper et al. state: "… there is little consensus on whether the association [between geohelminth infections and allergy] is causal and if so, whether geohelminth infections may increase or decrease the risk of allergy" (Cooper PJ, Barreto ML, Rodrigues LC, 2006: 203). Von Hertzen et al. conclude: „Irrespective of the abundant literature of the inverse association between exposure to microorganisms/helminths and the occurrence of atopic diseases, no definite conclusion of the causality of this association are yet warranted" (Von Hertzen LC, Haahtela T, 2004: 133). Santiago HdaC remarks: "The effects of helminth infection al allergy have been widely investigated with widely varying conclusions" (Santiago HdaC, Ribeiro-Gomes FL, Bennuru S, Nutman TB, 2015: 97). Smits et al. state: "… studies involving intestinal helminth infections did not yield a general consensus regarding protection against different forms of allergic disease" (Smits HH, Everts B, Hartgers FC, Yazdanbakhsh M, 2010: 4).

370 Helmby H, 2009: 121 ff. Smits et al. mentioned an "altered programming of the immune system" (Smits HH, Everts B, Hartgers FC, Yazdanbakhsh M, 2010: 3).

371 Helmby H, 2009: 125.

372 Van den Biggelaar AH et al., 2000: 1723-27. Unfortunately the time of data collection is not mentioned, yet data from the same study group/data collection have been used for another publication (van den Biggelaar AH et al., 2001: 232). Thus the year of data collection has obviously been 1999.

in a "similar setting": Tropical environment with similar temperature[373] (Lambaréné, Gabon), high parasite burden (nematodes but in contrast to Karkar also schistosoma[374]) and low prevalence of atopic diseases. The main finding was that schistosomiasis appeared to suppress atopy in African children[375] yet concerning the underlying mechanism van den Biggelaar argues specifically *against* suppression of atopy by high sTot-IgE levels and instead *in favour of* suppression of atopy by the anti-inflammatory cytokine interleukin-10. We think there is unexplained and conflicting information in the Lambaréné data.

We want to start with what we called "the neglect to consider the possibility of (partial/minor) negative effects of worms". Van den Biggelaar states "Helminths … stimulate a strong amplification of polyclonal *non*-specific IgE". She describes a highly significant positive correlation (ρ=0.494; $p<0.0001$) between sTot-IgE and D. pt. specific IgE (just as we found it on Karkar; the Lambaréné values reprinted in fig. 39 were very similar to the Karkar values displayed in fig. 29)[376] and underlines in the discussion that it would be "clear that high concentrations of IgE are produced to D. pteronyssinus antigen in the [highly worm infected] study population". Despite of this positive correlation of sTot-IgE and specific IgE helminths were considered to be responsible *only* for the "good" allergologically unsuspicious non-specific IgE amplification, yet not for the parallel "bad" allergologically suspicious specific IgE boost (which should be associated with increased SPT reactivity).[377] If worms really boost the IgE system in an unspecific way, why should this unspecific boost specifically exclude the allergologically relevant specificities? This would be a paradox. The question what – if not worms – may have actually caused the undeniable specific IgE boost is not asked in van den Biggelaar's work. Maybe the main statement of the study (worms suppress atopy; in respect to allergies they only have a "good" side and not a "bad" one) should not be challenged. One year later, van den Biggelaar published another article – obviously based on the same data – where she actually specifies: "Mite sensitization was found to be the highest in children infected with schistosomes and/or filariae …"[378] Her data displayed in fig. 1 of the latter work supports the existence of an unspecific boost of the "entire" (total and specific) IgE system by worms (filariae): we see a parallel increase of worm prevalence, sTot-IgE GM and mite

373 „Temperature oscillates around 27°C …" (van den Biggelaar AH et al., 2001: 232).

374 Schistosoma are parasitic flatworms.

375 Schistosoma infected individuals had a lower prevalence of positive SPT to D. pt. than uninfected individuals.

376 Van den Biggelaar only correlated IgE directed against one mite species (D. pt.) and sTot-IgE. We could confirm the highly significant association pattern for two mite species (D. pt. and D. fa.).

377 As van den Biggelaar admits: "… higher concentrations of these antibodies [D. pt. IgE] were associated with a [significantly] higher risk of skin reactivity to mite allergen preparation" (van den Biggelaar AH et al., 2000: 1725).

378 Van den Biggelaar AH et al., 2001: 231.

specific IgE GM.[379] We do not want to deny an overall allergy protective effect of worms (e.g. the increase of sTot-IgE seems more important than the increase of specific IgE in a part of the population and other "protective worm factors" may exist as well); we just think, we should not turn a blind eye to the possibility of negative effects of worms in allergy and consider that the IgE mechanisms may be double-edged.

The second point we want to discuss in respect to van den Biggelaar's work concerns the question whether mast cell blockage exists in the presence of elevated sTot-IgE levels within worm infested societies: We argued in favour of "protective" effects of sTot-IgE (nematode induced), van den Biggelaar however rejected an atopy suppressive effect of sTot-IgE and argued in favour of "protective" effects of interleukin-10 (schistosoma induced). Van den Biggelaar may have started to think about SPT suppression – just as we did – because of the obvious discrepancies between RAST and SPT. She found a high prevalence of RAST sensitization against D. pt. which was not reflected by a high prevalence of D. pt. SPT reactivity: About 60%[380] of the children showed positive RAST to D. pt. yet positive SPT prevalence for this allergen was only 11%. This constellation is similar to the one we found on Karkar Island (73% positive D. pt. RAST and 16% positive D. pt. SPT).

We think schistosoma induced interleukin-10 (Il-10) is not the only cause (it may not be even the main cause) for atopy (SPT) suppression for two reasons. Firstly Schistosoma infection is not endemic on Karkar.[381] We do not know whether Ascaris and/or hookworm infections generate a similar Il-10 pattern; and we do not know whether this pattern is capable of suppressing SPT reactivity on Karkar Island. Secondly we doubt that the schistosoma-interleukin-10 effect (alone) may be strong enough to create such a huge RAST – SPT difference in Lambaréné. On the one hand only 40% of the population were schistosoma positive.[382] Thus schistosoma-Il-10 "protection" (SPT suppression) should be absent in the majority (60%) of the population. On the other hand the following fact backs the impression of an insufficient schistosoma-Il-10 effect: despite of an odds ratio (OR) of 0.32 for a positive SPT in the schistosoma positive group, positive SPT prevalence was still considerably low in the schistosoma negative group: 5% of the schistosoma positive group showed a positive SPT to D. pt., in the schistosoma negative group positive SPT was still only 15%. Yet a discrepancy of 60% RAST sensitization within the population and only 15% SPT sensitization (in the absence of schistosoma!) is still a finding one may consider worthwhile explaining.

379 Van den Biggelaar AH et al., 2001: 234, fig. 1 (a) in conjunction with (c). However, we are not too confident that the given significance levels of the increases are correct, as the necessary adjustments for multiple comparisons do not seem to have been performed.

380 Van den Biggelaar only gave the exact percentage of individuals with D. pt. specific IgE >1 kU/l (Van den Biggelaar AH et al., 2000: 1725). Thus low in vitro sensitization represented by RAST class 1 and lower parts of RAST class 2 was excluded (class 2 = 0.7-<3.5 kU/l). Based on her raw data displayed in figure 1 (re-printed as fig. 39 in this work) we estimated the percentage of individuals with a positive D. pt. RAST (>0.3 kU/l) to be about 60%.

381 World Health Organization. Status of schistosomiasis endemic countries: 2015: no pagination.

382 Percentage calculated based on the raw data given in table 1 (Van den Biggelaar AH et al., 2000: 1724).

But if atopy/SPT suppression in Lambaréné is not mainly due to schistosoma-interleukin-10, what may be the (additional) protective factors? Which SPT suppressive factor(s) affect the whole Lambaréné population including the schistosoma negative reference group? Naturally we thought of nematodes and sTot-IgE: especially as according to van den Biggelaar 74% of the children were infected with nematodes.[383] In contrast to 40% schistosoma prevalence, 74% nematode prevalence seems sufficiently high to generate a strong effect within the whole Lambaréné study group. Furthermore sTot-IgE distribution was very similar to Karkar; thus we thought that the high sTot-IgE levels in Lambaréné may well "neutralize"[384] the high specific IgE levels (if SA is low) just as on Karkar. This could also explain why the prevalence of positive D. pt. SPT in the schistosoma negative group was only 15% despite of 60% D. pt. RAST sensitization. Yet van den Biggelaar underlines: "Rigorous statistical analysis here showed that the concentrations of polyclonal IgE [sTot-IgE] did not [!] influence the risk of skin reactivity to mite extract"[385]. To confirm this statement she points to "tables 1 and 3, figure 1". In short: in our opinion values of table one are inapplicable in this context as a necessary statistical adjustment has not been performed. Table three is based on a small non-representative subgroup with odds ratios indicating that the mentioned adjustment would have been indeed necessary. And finally figure 1 shows that if an adjustment had been made there would have been a negative influence of sTot-IgE on mite SPT – just like on Karkar. Subsequently we will explain our mentioned points of critique more in detail; especially why in this case adjustment for specific IgE is so important.

Van den Biggelaar detected a significant correlation between sTot-IgE and specific IgE (see fig. 39). She intends to interpret the association of two variables: SPT and sTot-IgE. Knowing that sTot-IgE is significantly correlated with specific IgE and that specific IgE is the most important factor influencing the corresponding SPT, the only possible consequence is to adjust for specific IgE when investigating the association between SPT reactivity and sTot-IgE. Yet this adjustment was done only in a small subgroup (n=132) of the entire study population (n=520). Already at this point it is not comprehensible why controlling for the essential confounder was not done for the entire study group. Controlling for specific IgE would have been possible in the big group as well: As fig. 39 shows, the three relevant variables were available for all individuals of the big group and not only for the individuals of the subgroup.

The need for controlling for specific IgE in the big group becomes even more obvious when looking at the results of the calculations in the small group: Firstly the small group does not seem to be representative for the big group and secondly the odds ratios in the small group underline the importance of adjustment in the big group. We think that the

383 Percentage based on a random group of 66 children; detection of Ascaris lumbricoides and/or Trichuris trichiura eggs in faeces (Van den Biggelaar AH et al., 2000: 1724; the same value/result given in van den Biggelaar AH et al., 2001: 232).

384 Details later in this chapter.

385 Van den Biggelaar AH et al., 2000: 1726. This statement is correct only as long as we do not control for the most important confounder: mite specific IgE.

relevant values within the small subgroup are probably not representative for the big group for the following reasons: The median sTot-IgE in mite SPT positive children (n=58) in the whole study (=big group) was "only" 767 kU/l, the median sTot-IgE in mite SPT positive children (n=30) in the subgroup was 1,976 kU/l. This is a quite unusual confounder discrepancy between entire group and subgroup. Furthermore, we have to consider the significant positive correlation between sTot-IgE and mite specific IgE: As the big group shows a much lower median sTot-IgE in the SPT positive children than the subgroup (767 kU/l vs. 1976 kU/l) we may expect an analogue relationship concerning mite specific IgE. Yet the contrary is the case: median mite specific IgE in the SPT positive children in the big group was 2.28 kU/l but median mite specific IgE in the SPT positive children in the subgroup was only 2.19 kU/l. The mentioned two arguments indicate that the subgroup may not be representative for the whole group. Another argument which points to the necessity to control for mite specific IgE in the big group is the considerable effect which is caused by adjusting for mite specific IgE in the subgroup. According to the subgroup data (before adjustment), higher sTot-IgE seemed to increase (!) the risk of positive mite SPT (OR 1.79) (non-significant tendency). Yet after including D. pt. specific IgE (and two other immunological factors[386]) in a multivariate model sTot-IgE "suddenly" (as we expected) appeared to decrease the risk of positive mite SPT (OR 0.82).[387] Notably this is only a non-significant tendency, but an OR of 0.82 is already much less contradictory in respect to our results than an OR of 1.79. The considerable fall of the OR after controlling for mite specific IgE underlines the importance to take the positive association of specific IgE with sTot-IgE into account, and to control for "the confounder" mite specific IgE also in the big group. Another reason to perform the adjustment in the big group is the higher statistical power in larger groups: a OR which is not significant in the subgroup may be significant in the entire group.

All in all we think that the result "no association between polyclonal IgE antibodies and mite skin-test results was found" is a consequence of the omission of control for mite specific IgE in the total group. Instead adjustment for mite specific IgE was performed in a small subgroup. Probably due to non representativeness of the relevant values in the subgroup and the small number of cases, no significant correlation was found even after controlling for specific IgE in the subgroup. We doubt that the result "no association of sTot-IgE and SPT even after controlling for mite specific IgE" can be applied to the entire study group. To test our suspicion that the apparent absence of SPT suppression by high sTot-IgE may be due to the omission of controlling for the most important confounder (specific IgE), we had to fall back on data displayed in an already mentioned figure in van den Biggelaar's work. Much of the information relevant for our task is displayed in this figure: it correlates sTot-IgE with D. pt. specific IgE and differentiates between positive and negative D. pt. SPT (see fig. 39).

386 The other factors were interleukin-5 and interleukin-10 (van den Biggelaar AH et al., 2000: 1726).
387 Van den Biggelaar AH et al., 2000: 1726.

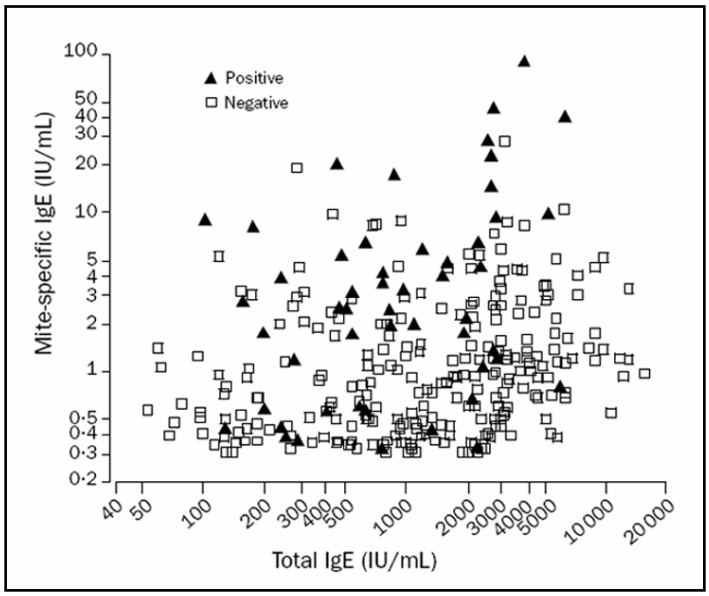


Fig. 39. Raw data from Lambaréné, as published in "The Lancet" in 2000: Significant correlation (ρ=0.494; $p<0.0001$) between sTot-IgE and D. pt. specific IgE (negative RAST values are not displayed).

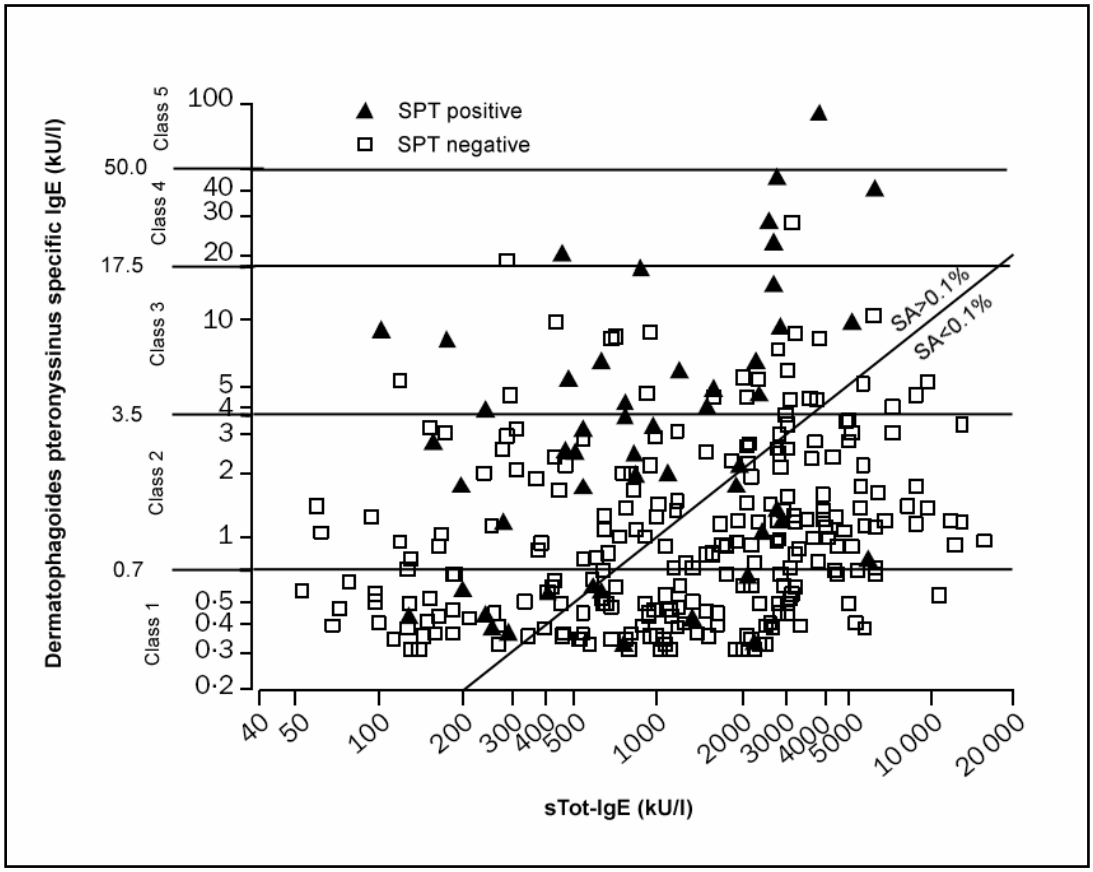


Fig. 40. Magnification of fig. 39 with additional classifying information added by the author: The diagonal line divides data points representing D. pt. SA>0.1% (upper left; n=142) and D. pt. SA<0.1% (lower right; n=150). Horizontal lines divide RAST classes. Within RAST class 2 significantly lower percentage of positive D. pt. SPT if D. pt. SA<0.1% (p=0.014). Confirming tendency for RAST classes 1 and 3.

As we do not dispose of the exact values of the data points we could not control for mite specific IgE using a multivariate model. Thus we had to go a more tedious way and "controlled" for mite specific IgE by grouping all study participants into their corresponding RAST classes.[388] From the practitioner's point of view this is not really a disadvantage, as RAST classes (and not kU/l) are used in daily allergological practice to specify levels of specific IgE. As a starting point we magnified van den Biggelaar's original figure (see fig. 40)[389] and added four horizontal lines representing the borders of the RAST classes. We also added the values of the RAST class dividing lines (0.7; 3.5; 17.5 and 50.0 kU/l) on the logarithmic scale and labelled the RAST classes one to five. The essential step is to place the diagonal line, which divides data points representing individuals with D. pt. SA>0.1% (upper left) and D. pt. SA<0.1% (lower right). We chose a mite SA of 0.1% as a dividing line as we have learned from our Karkar data that SPT suppression seems to get intense under a specific activity of 0.1%. At a first glance the number of cases with SA>0.1% seems bigger than the number of cases with SA<0.1%, but it's the other way round: only 48.6% (142/292) of the D. pt. sensitized children showed a mite SA>0.1% but 51.4% (150/292) a mite SA<0.1%. Already at this stage it is possible to discern a visual pattern: within the different RAST classes, more SPT positive subjects seem to lie on the left side (SA>0.1%) of the dividing diagonal.[390] Exactly as within our Karkar data there is no SA<0.1% over RAST class 3; in other words: not even the extremely elevated sTot-IgE levels are able to push SA under 0.1% if mite specific IgE is over 17.5 kU/l. The next step was to count the classified data points[391] and to calculate the percentages of mite SPT positive individuals in the separate RAST classes for SA>0.1% vs. SA<0.1%. Subsequently we checked for significance of differences between lower and higher SA in each RAST class (chi-square or Fisher exact test). In fig. 41 the results are presented in the same way as already done in fig. 37 (D. pt.) and fig. 38 (D. fa.) for our Karkar data.

388 Children with D. pt. RAST class 0 are not displayed in the figure.

389 We performed some minor changes to adapt formal presentation to our study: the x-axis was labelled "Total IgE (IU/mL) which is identical to "sTot-IgE (kU/l)"; the y-axis was labelled "Mite-specific IgE (IU/mL)" which is identical to (more precisely) "Dermatophagoides pteronyssinus specific IgE (kU/l)".

390 Just one relevant data point could not be grouped: one subject with a sTot-IgE of about 600 kU/l and a D. pt. specific IgE of about 0.6 kU/l lay exactly on our 0.1% SA dividing line. Thus this value had to be excluded from the calculations.

391 In order to count the data points correctly, we magnified the figure much more than displayed in fig. 40. As already mentioned, one data point could not be grouped visually. Of course, we can not exclude that data points may have been located exactly at the same place or so near to each other, that a visual differentiation was not possible even at maximum magnification. Yet due to a cross check of van den Biggelaar's total numbers with our total counted numbers we can be confident, that the overall result is not confounded in any significant way.

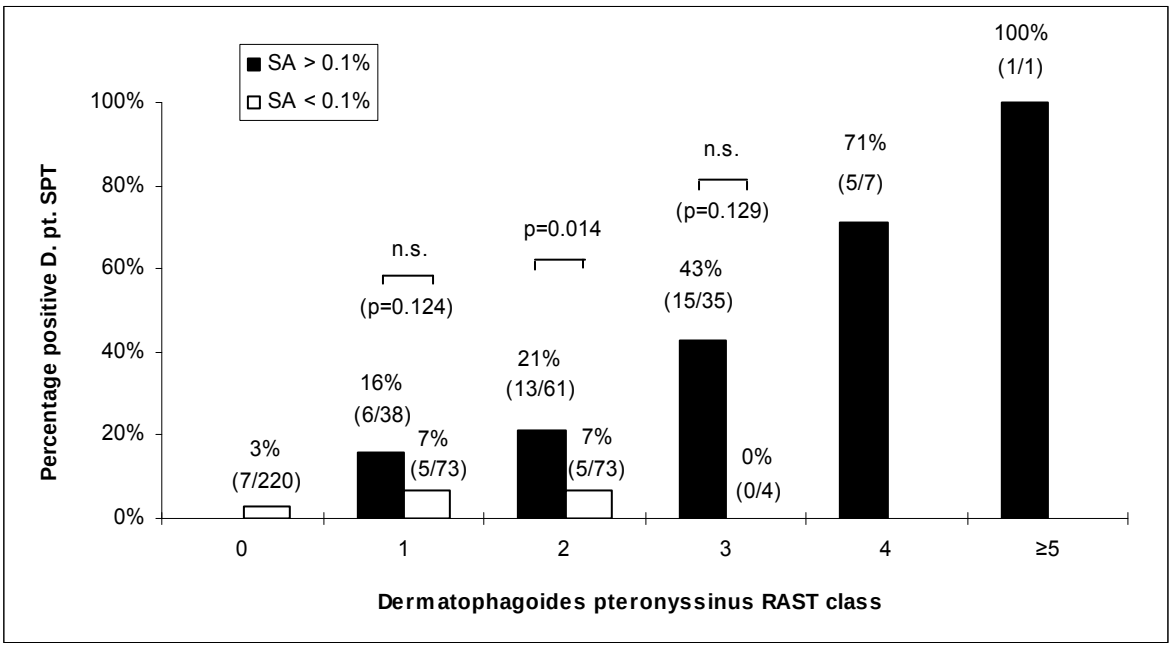


Fig. 41. Lambaréné data: reduced D. pt. SPT reactivity at SA levels under 0.1%. Subjects with identical D. pt. RAST class showed decreased SPT reactions when D. pt. SA was under 0.1%. The difference in comparison to SA over 0.1% was significant for RAST class 2 (chi square test), a confirming trend was observed for classes one and three.

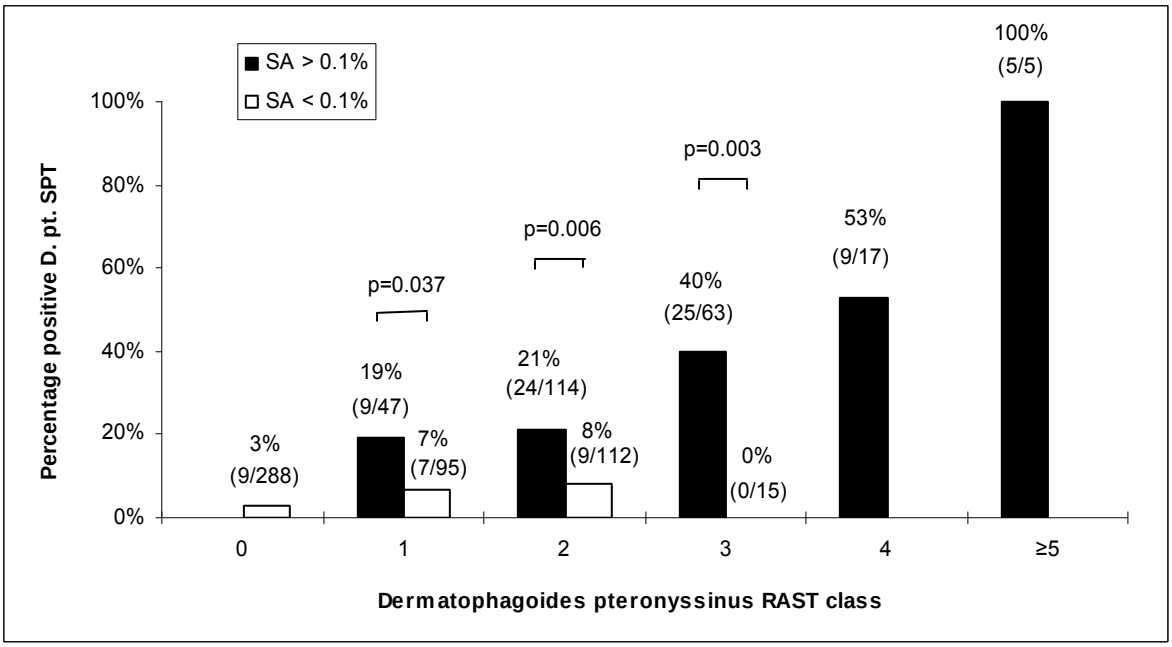


Fig. 42. Lambaréné data pooled with Karkar data: significantly reduced D. pt. SPT reactivity at D. pt. SA levels under 0.1% in all relevant RAST classes (chi square tests). The strength of significance of the between group differences increases from RAST class 1 (p=0.037) to class 2 (p=0.006) and finally to class 3 (p=0.003).

Most of the Lambaréné in vitro mite sensitized children (46% [134/292]) were in RAST class 2. Within RAST class 2 we find a significantly lower prevalence of positive D. pt. SPT if D. pt. SA<0.1% (p=0.014; χ^2 test).[392] Tendency was identical for RAST classes 1 and 3 with almost identical p values (p=0.124 for RAST class 1 and p=0.129 for RAST class 3; in both cases Fisher exact test). Obviously the pronounced between group difference in RAST class 3 did not reach significance only due to the small number of cases (four) in the "SA<0.1% group". As mentioned before, RAST classes over 3 were always associated with a SA>0.1%. Three percent of the subjects without specific IgE against D. pt (RAST class 0)[393] developed – nevertheless – a positive SPT reaction to D. pt. extract.[394]

Comparing the effect of mite specific activity on mite SPT reactivity in Africa and Papua New Guinea reveals striking parallels: If SA is over 0.1% we see an almost linear increase of SPT reactivity with increasing RAST classes in both populations; this is what we expected in the presence of increasing specific IgE.[395] Yet if SA is under 0.1% there is no (!) increase of SPT reactivity with increasing RAST classes. In other words: we see a dissociation of RAST and SPT. Within the RAST classes one, two and three SPT reactivity was always considerably lower if SA was under 0.1% (subjects with RAST class 3 never reacted in the SPT if SA was under 0.1%, neither in Africa nor in PNG). On Karkar the differences reached significance at RAST class 3 (for both, D. pt. and D. fa.) and in Lambaréné already at RAST class 2.[396] On Karkar and in Lambaréné specific activity is relevant to interpret RAST classes one to three, RAST classes four to six however are always associated with a SA>0.1%. This means that SA is relevant in OVER 90% of the mite sensitized population: In PNG in 92% (162/176) for D. pt., in 94% (159/170) for D. fa. and in Africa in 97% (284/292) for D. pt. These values show that interpretation of positive in vitro sensitization (RAST) without knowing the specific activity is impossible in heavily worm infested populations. In respect to the striking parallels between both populations we may add that even the percentage of D. pt. positive

392 Despite of very similar positive SPT prevalences within D. pt. RAST class 2 in PNG (also 21% for SA>0.1% and 10% for SA<0.1%; see fig. 37) only the between group difference in Lambaréné reached significance at a 0.05% level. The non significance on Karkar is basically due to the lower case number: Karkar RAST class 2 comprised only 92 subjects, Lambaréné RAST class 2 comprised 134 subjects.

393 Logically, RAST class 0 is always associated with a SA<0.1%. More precisely: SA will be cero, of course.

394 The values for RAST class 0 were not given by van den Biggelaar. Yet from table 1 (p. 1724) in the original work we could calculate that in the whole study group (n=520) the number of SPT negative subjects was 462, the number of SPT positive subjects was 58. From our figure 40 we know that RAST classes 1-6 comprised 242 SPT negative subjects and 51 SPT positive subjects. The remaining 7 SPT positive and 220 SPT negative subjects must constitute RAST class 0. Consequently the prevalence of positive SPT in RAST class 0 is 7/220 = 3%.

395 There were only two exceptions within the Karkar data, probably due to very small case numbers: D. pt. RAST class 1 (3/9 yields a too high percentage) and D. fa. RAST class 4 (2/7 yields a too low percentage). Within the Lambaréné data the increase was strictly monotonous as expected.

396 As already mentioned before, the probability of significant differences in positive SPT prevalence between SA<0.1% and SA>0.1% increases from RAST class one to three.

SPT reactivity in the absence of specific D. pt. IgE (which per se constitutes a certain paradox) was exactly the same in Lambaréné and Karkar: three percent.

To support our statement that SA must not be ignored, in a final step we pooled the Lambaréné and Karkar data for D. pt. This seems legitimate because both studies are – notwithstanding the geographical distance – quite homogenous as described before. Despite the fact that the Lambaréné study was exclusively performed on children, the distribution of sTot-IgE, D. pt. specific IgE and SPT was very similar to our Karkar data. The same definitions were applied for positive/negative SPT reactivity and RAST results are given in the same units. Figure 42 shows the pooled Lambaréné and Karkar data for D. pt. All the effects already described above become clearer: the plausible, almost linear increase of SPT reactivity with increasing RAST if SA is over 0.1% and the absence of this increase if SA is under 0.1% (dissociation of RAST and SPT). The prevalence of positive D. pt. SPT is now significantly lower at SA<0.1% for RAST class one ($p=0.037$), two ($p=0.006$) and three ($p=0.003$). The decreasing p values with increasing RAST class (one to three) reflect the increasing strength of significance of the between group differences within the respective RAST classes. This is due to the fact that positive SPT prevalence keeps increasing in the SA>0.1% groups, whereas it stays basically stable in the SA<0.1% groups. In respect to the pooled data, knowing the SA is relevant in 95.3% (446/468) of the RAST sensitized individuals (in only 4.7% [22/468] SA is "irrelevant": RAST classes four to six).

Summing up our critique of van den Biggelaar's study we may conclude: Looking at her Gabon data, the statement that (non-adjusted) sTot-IgE was not associated with SPT reactivity has not to be questioned (we found the same result in PNG). Yet this does NOT exclude the "old" (FcεRI) mast cell block theory which van den Biggelaar wanted to prove wrong. She states: "Some researchers have argued that the polyclonal nature of this [sTot-IgE] antibody may result in blocking allergic hypersensitivity by … saturating IgE receptors on mast cells …"[397] As the announced "rigorous statistical analysis" (i.e. adjustment for specific IgE) – which would have been necessary to reject the inhibition theory – was only done for a small subgroup, it was possible to overlook the opposing effect of the significantly associated variables sTot-IgE and specific IgE on SPT reactivity in the entire group. All in all the Lancet study (just as Mitre's work before) intended to reject the old blocking theory but actually supports it.

In 2001, exactly one year after her Lancet article, van den Biggelaar published another work in the "International Archives of Allergy and Immunology"[398] obviously based on the same Gabon data (520 Lambaréné school children).[399] It becomes apparent that van

397 Van den Biggelaar AH et al., 2000: 1726.

398 Title: „The prevalence of parasite infestation and house dust mite sensitization in Gabonese schoolchildren" (Van den Biggelaar AH et al., 2001: 231-38).

399 For several reasons we are most confident that the data base of both studies is actually identical: Not only the place of the study (Lambaréné) and number of participants coincide (520 schoolchildren) but also diverse descriptive statistical parameters. For example prevalence of mite specific IgE > 1 kU/l (47%),

den Biggelaar is well aware of the fact that mite specific IgE is a most important confounder when evaluating the effect of sTot-IgE on mite SPT.[400] The necessary adjustment for specific IgE is "finally" made for the entire study group and a significant (p=0.041) negative effect of sTot-IgE on SPT is found (OR=0.50).[401] Yet again, a statistical step is undertaken, which leads to the rejection of the blocking hypothesis: Van den Biggelaar defines specific (mite) IgE levels under 1 kU/l as negative.[402] This is a rather elevated arbitrary threshold.[403] Then she restricts the adjustment for specific IgE (the multiple analysis) to the "mite-sensitized" subgroup, i.e. – according to her definition – to individuals with mite specific IgE over 1 kU/l. She argues that in this subgroup we would find the children "being at risk of a positive skin test"[404]. However it is not clear, why she considers subjects with measurable mite IgE levels between 0.3 kU/l (her detection limit) and 1.0 kU/l (i.e. RAST class 1 and lower parts of class 2) not sensitized and generally not at risk of a positive mite SPT. Admittedly the risk for a positive mite SPT is lower at lower RAST sensitization levels, yet why we should turn a blind eye to possible blocking effects of sTot-IgE on RAST classes one and parts of two is neither explained nor evident. The dubious data base restriction to a considerably smaller subgroup with n = 246 (47.3% of 520)[405] entailed a twofold consequence: On the one hand the negative effect of sTot-IgE on SPT became even stronger (!) (OR in the entire group was 0.50, now in the restricted group OR becomes 0.45). Yet on the other hand p was pushed slightly over the 5% limit – simply due to the reduced case number: p increased from originally p=0.041 in the entire group to p=0.071 in the restricted subgroup.[406] Thus even if we intend not to question van den Biggelaar's statistical steps but accept her data restriction, we may still be 92.9% confident that the strong negative association between sTot-IgE and positive SPT is not due to chance. Even this "attenuated" message should not be ignored as vigorously as van den Biggelaar does when

prevalence of positive mite SPT (11%) and detection of Ascaris lumbricoides and/or Trichuris trichiura eggs in faeces (74%) (Van den Biggelaar AH et al., 2001: 231-38).

400 „In the univariate model total IgE … [was] not associated with [mite] skin test reactivity. However, as total IgE and mite-specific IgE are positively correlated, the effect of total IgE on skin test reactivity was studied in a multiple model … adjusting for the positive correlation between total and mite-specific IgE" (Van den Biggelaar AH et al., 2001: 235).

401 „The result showed a negative association between total IgE and skin test reactivity [OR = 0.50; 95% confidence interval (CI) = 0.26-0.97; p = 0.041]" (Van den Biggelaar AH et al., 2001: 235).

402 „Subjects were considered sensitized when concentrations of specific IgE of more than 1.0 IU/ml [1.0 kU/l] were measured" (Van den Biggelaar AH et al., 2001: 233).

403 Nowadays the detection limit for specific IgE is 0.1 kU/l (Hamilton RG, MacGlashan DW Jr, Saini SS, 2010: 275). Admittedly specific IgE values in the range between 0.1 and 1.0 kU/l are not always relevant, yet RAST classes 1 and 2 should not be ignored in general.

404 Van den Biggelaar AH et al., 2001: 235.

405 According to van den Biggelaar's raw data published one year earlier (in 2000) in "the Lancet" and reprinted in fig. 39 of this work, we estimate that 111 individuals showed RAST class 1 (0.3-0.7 kU/l) and further 30 individuals showed lower RAST class 2 (0.7-1 kU/l). Thus about 141 mite RAST sensitized individuals were excluded.

406 Van den Biggelaar shortly states: "The effect of total IgE [on SPT reactivity within the restricted group] was no longer significant …" (Van den Biggelaar AH et al., 2001: 237).

she concludes: "… total IgE does not play a prominent role in the strong negative association between helminth infections and skin test reactivity, as indicated by multiple logistic regression. Other factors … have to be considered."[407]

Getting back to our Karkar data and to figures 33 and 34 we see that the lowest SA still allowing a positive SPT reaction was 0.034% for D. pt. (sTot-IgE 6820 kU/l; SPT reaction 4 mm) and 0.029% for D. fa. (sTot-IgE 4695 kU/l; SPT reaction 3 mm). Using these numbers as a point of reference we may well declare a SA of 0.05% and below a "safe blocking area". If we intended to develop a perfect blocking molecule (e.g. something like a gene technologically designed IgE Fc fragment with equal or higher FcεRI[408] affinity than regular IgE), this designed antibody should not display any specificity/binding activity neither to human structures nor to any allergen. Provided this "protective" molecule shall not only decrease mast cell releasibility (probably already happening at SA levels smaller 1%) but induce a total inhibition, the required dose of the molecule would be quite elevated: we should be able to decrease a patient's clinically relevant SA to 0.05%. In order to "block" an allergic subject with e.g. RAST class 4, which corresponds to 17.5-50 kU/l of specific IgE, we would need 2,000 times more "non-specific molecules" i.e. an equivalent to 35,000 to 100,000 kU/l of non-specific IgE.

If worms really increase the "allergy risk factor" specific IgE – as suggested by our data – the reason why this increased risk factor (i.e. positive RAST) does not manifest itself as increased SPT reactivity seems to be that worms cause a parallel increase of the "allergy protective factor" sTot-IgE. The parallel increase of sTot-IgE (mostly) "neutralizes" the effect of higher specific IgE. This equalisation is (mostly) expressed in the form of a comparatively low SA despite of high specific IgE levels. The word "mostly" refers to the fact, that correlation between sTot-IgE and mite SA is not too strong (ρ= -0.53 for D. pt. and ρ= -0.56 for D. fa.). This means that at a given level of sTot-IgE there is a large variability of SA (e.g. at 1,000 kU/l sTot-IgE there is a 52-fold difference between the lowest [0.05%] and highest [2.6%] D. pt. SA).[409] Even at sTot-IgE levels higher than 1,000 kU/l there are islanders, who are able to mount a mite SA over 0.1%[410]. Between 0.1% and 1% SA the degranulation of mast cells still seems supressed, but not as massively as under 0.1% SA. In other words: in the presence of high environmental mite antigen levels (climate/lifestyle) additional triggering of mite IgE production by worm infestation may lead to a specific IgE boost comparatively larger than the background sTot-IgE boost in some individuals. The resulting SA is high enough to allow histamine release even at sTot-IgE levels over 1,000 kU/l and in few extreme cases even over 5,000 kU/l. To clarify this situation we want to repeat the example given above. A Karkar showing RAST class 4 against HDM (17.5-50 kU/l) would need a sTot-IgE of 17,500 to 50,000 kU/l to bring HDM SA down to 0.1% – the level where total blocking seems to

407 Ibid.
408 High affinity IgE receptor.
409 See figure 33.
410 A few were actually able to mount a mite SA over 1%.

start. Yet these are sTot-IgE levels, which are not even reached by the heavily worm infected Karkar population (just one person had a sTot-IgE over 17,500 kU/l: proband S.S. with 31,400 kU/l). In other words: it is not possible to exert a general “full” inhibition/blocking on RAST class 4; not even on Karkar island.

As shown before, allergens which are less important on Karkar than mites also seem to experience a boost by worms, yet this boost only leads to comparatively low in vitro sensitization levels. Logically, these lower RAST classes of less important allergens are more affected by the high background sTot-IgE levels: the resulting SA levels are lower than those of mites and much more often lying under the “strong inhibition/blocking threshold” of 0.1% SA.

Of course, we would not expect high SPT sensitisation at low RAST classes anyway, yet the virtual absence of positive SPT reactions to all allergens except mite and cockroach[411] could be due to a simple fact: On Karkar, almost all islander's SA levels lie under the "noticeable" suppression threshold of 1% SA (and in many cases even under the strong inhibition/blocking threshold of 0.1% SA). Figure 43 shows the SA levels of 248 Karkar for 19 allergens (4,712 pairs of sTot-IgE : specific IgE).[412]

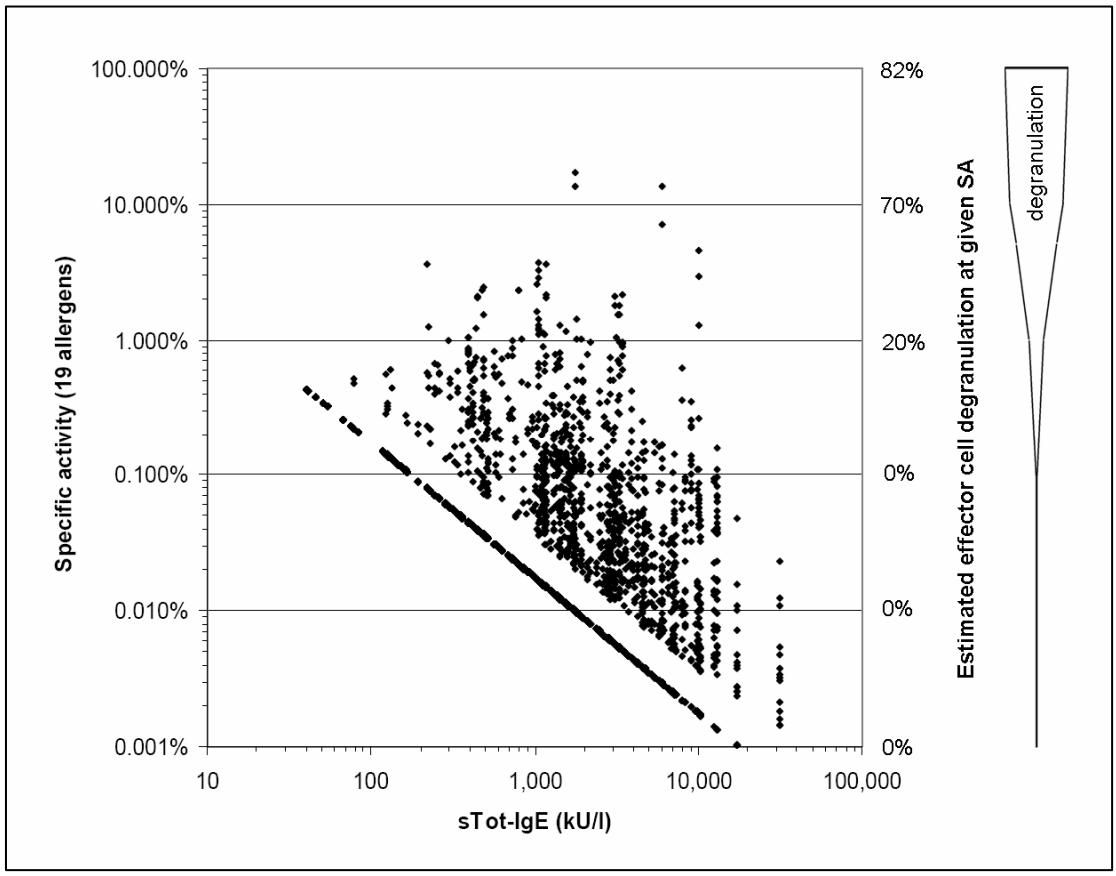


Fig. 43. Correlation of sTot-IgE and specific activities of 19 allergens (including HDM) in 248 Karkar. Altogether 64.1% (771/1203) of the SA (based on positive RAST) lie under the assumed strong inhibition threshold of 0.1% SA. SA levels over 1% are scarce (as before, estimated degranulation is based on Christensen's work).

As expected, the overall specific activities are lower for the 19 allergens than for HDM: more points lie under the 0.1% threshold when we compare fig. 43 with figs. 31 and 32 (SA of D. pt and D. fa.). This visual impression is confirmed by the following numbers:

411 See fig. 19. Positive SPT prevalence was only between 0% and 1% for all allergens except mites and cockroach. SPT to skin funguses was not taken into account as we do not know the corresponding RAST values.

412 Including both mite species (D. pt. and D. fa.) yet excluding Ascaris lumbricoides.

"Only" 38% (134/354) of the mite SA lie under 0.1% SA whereas 75% (637/849) of the remaining (clinically less important) SA lie under the 0.1% threshold.[413]

Figure 43 brings us back to our initial "paradox": How is it possible that a society like the Karkars' with a Th2 dominated immune system and multiple RAST sensitizations shows little SPT reactivity and few allergies? There seem to exist two different patterns of Th2 (IgE) dominated response: one "allergy pattern" resulting in high SA ("modern" Western societies, often over 1% SA) and one "worm pattern" resulting in low SA (traditional societies, on Karkar almost always under 1% SA an often even under 0.1% SA). Looking at fig. 43 we have seen that the Karkar's specific activities almost never get as high as to the "vulnerable" SA region between one and five percent – the SA region where degranulation and SPT reactivity seem to "explode" (see in particular chapter 2.3.3, fig. 2). In order to support our assumption of the existence of two different Th2 (IgE) dominated patterns, we will now compare two populations, which probably represent the most pronounced antipoles in respect to atopic disease: the Papua New Guinea general population (our sample; fig. 44) with a United States allergic group (Hamilton RG, MacGlashan DW Jr, Saini SS, 2010: 273-284; fig. 45).

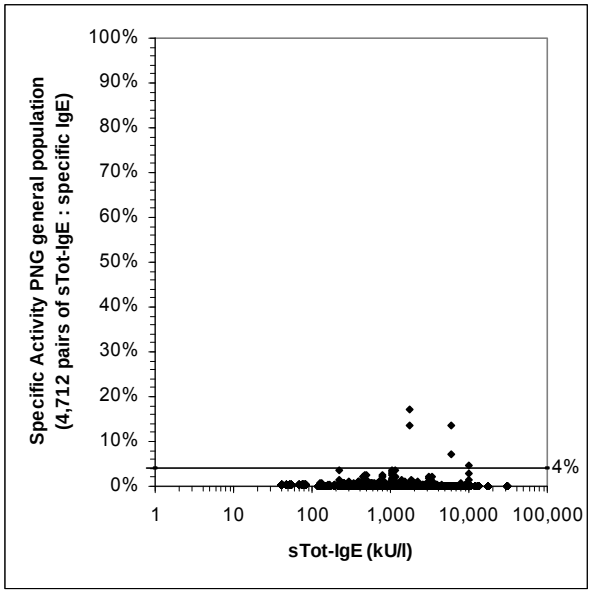


Fig. 44

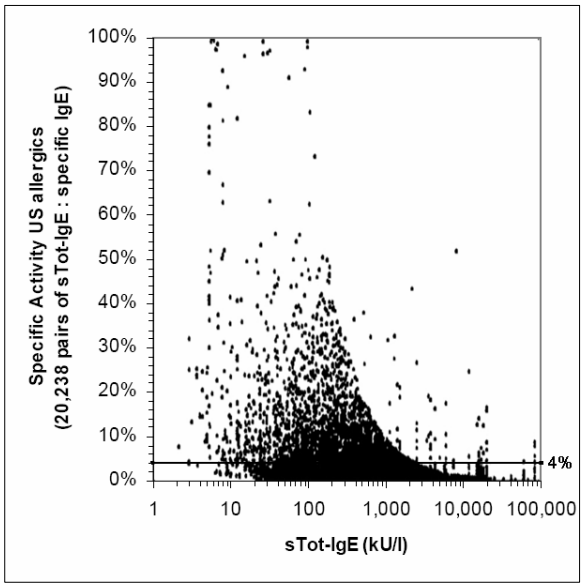


Fig. 45

Figs. 44 and 45. Comparison of specific activities (multiple different allergens) in the PNG general population with the US allergic population. Fig. 44. Correlation of sTot-IgE (log scale) and specific activities (normal scale) on Karkar Island (4,712 pairs of sTot-IgE : specific IgE). Fig. 45. Correlation of sTot-IgE (log scale) and specific activities (normal scale) within a group of US allergics (20,238 pairs of sTot-IgE : specific IgE).[414]

413 Only specific activities based on positive RAST were taken into account as the position of the specific activities based on negative RAST (diagonally descending line of points) is arbitrary.

414 Adapted from Hamilton RG, MacGlashan DW Jr, Saini SS, 2010: 280.

Before we compare the specific activities in the two – not only different, but opposing – groups (Papua New Guinea general population vs. United States allergic group) we shall clarify the following points. It was not possible to compare the SA of Karkar non-allergics with Karkar allergics as the low prevalence[415] of atopic diseases on the island did not allow us to sample enough allergic probands for a statistically meaningful comparison. Thus we chose to compare SA of two inhomogeneous groups (Karkar general population vs. US allergic group). However doing so, we have to bear in mind that we can not draw clear conclusions whether SA group differences are due to "nationality" (different genetics and/or environmental conditions in PNG vs US) or "health status" (non-allergic vs. allergic). For the direct comparison PNG-US, the SA values which have already been given on a log scale (y-axis) in fig. 43 are now displayed on a normal scale. Hamilton RG et al. suggested a 4% threshold to differentiate between low and high specific activities.[416] As this is in accordance with Christensen's in-vitro[417] and our epidemiological PNG results – we had calculated that the biggest effect of a given change in SA on SPT/clinical reactivity lies between one and five percent (see figs. 35 and 36) – the 4% threshold is displayed in both figures. Even without disposing of the US raw data the difference PNG-US is visible at a first glance: within the Karkar general population, only five out of 4,712 specific activities (0.1%) exceed the 4% SA threshold;[418] however within the US allergic group many SAs exceed the 4% threshold.

Displaying the specific activities (y-axis) as log transformed values (as already shown in fig. 43) allows a much more detailed insight into the correlation between sTot-IgE and SA. Thus for the subsequent precise comparison of the PNG general population with the US allergic population, we used log values on the x- and y-axis. In a first step we modified the original US scattergram by adapting the x- and y-axis to the more meaningful proportion of 1:1. This means, if we shift on the x-axis the distance to the right which represents a tenfold increase in sTot-IgE, shifting the same distance up on the y-axis represents a tenfold increase in specific activity. In a second step we exactly adjusted the PNG and US scales. Subsequently we faded the Karkar specific activities (black) over on top of the US specific activities (grey). The result is shown in fig. 46. As discussed above, fig. 46 is only a graphical representation of the SA distribution in the two opposing groups. We just want to point to the comparatively higher SA levels in the US allergic group – refraining from statements in respect to causality.

415 The prevalence of allergic diseases on Karkar is only 4.4% according to the author's earlier results. We estimate that only approximately 3.000 allergics live (undiagnosed) on Karkar Island (4.4% out of 70.000).

416 Hamilton RG, MacGlashan DW Jr, Saini SS, 2010: 280. Hamiltion et al. chose the 4% SA threshold due to the results of a study on the efficacy of anti-IgE therapy with Omalizumab (Ibid., 276).

417 Christensen LH et al., 2008: 302.

418 In all of the five instances, where a SA over 4% was reached, house dust mite was the relevant allergen and in all of the 5 cases the corresponding SPT reaction was strongly positive (5, 7, 8, 9 and 10 mm wheals). This is in accordance with our conclusion that mites represent the clinically most relevant allergen on Karkar Island (see chapter 5.1).

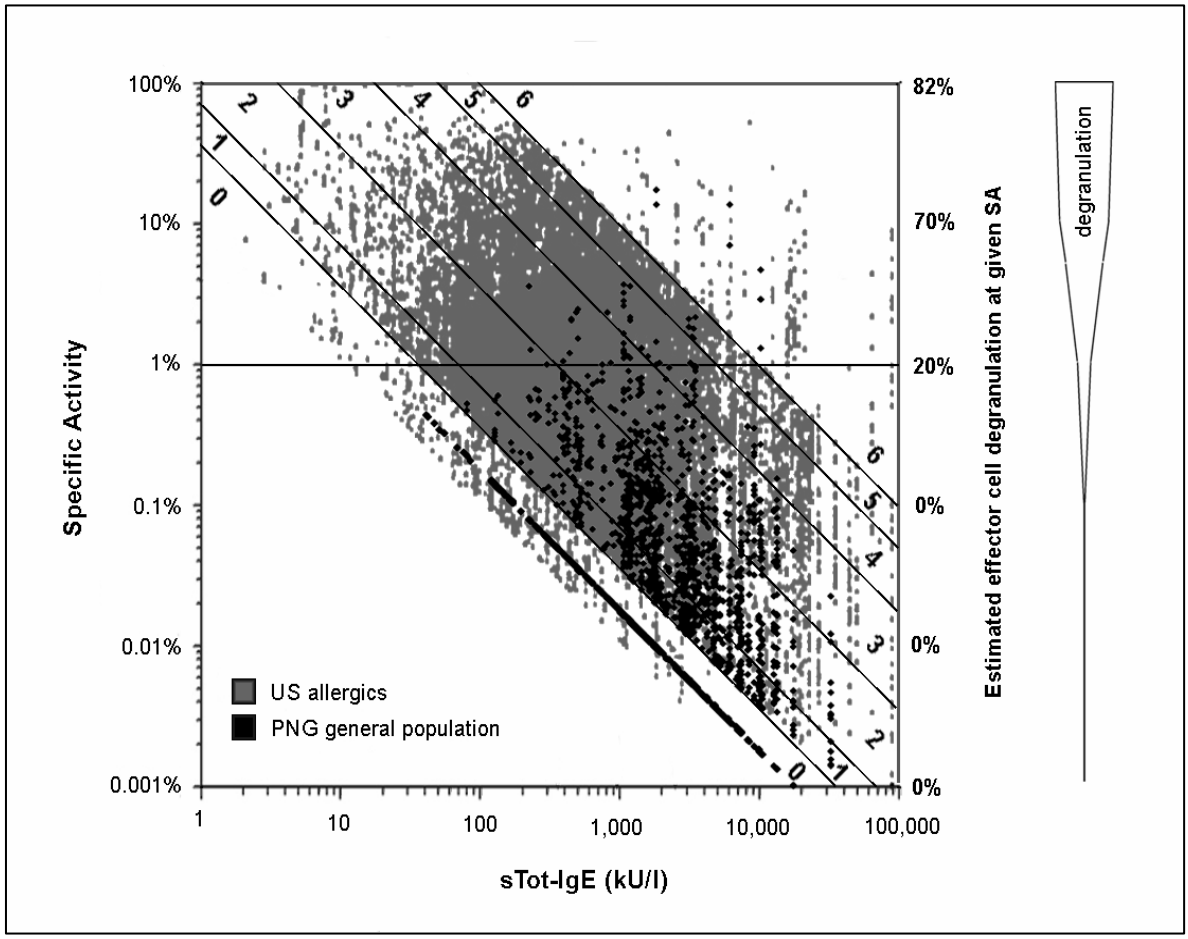


Fig. 46. Comparison of specific activities in two opposing populations. PNG specific activities (Karkar general population, black) are faded over on top of US specific activities (US allergic group, grey). The horizontal line at 1% SA divides high SAs (associated with increased effector cell degranulation) and low SAs (associated with suppressed effector cell degranulation). The estimated degranulation percentages (at given SA levels) on the additional (right) y-axis are based on Christensen's results.[419] Diagonally descending lines separate the different RAST classes which are labelled from 1 to 6. Within the same RAST classes (representing equal levels of sensitization) the PNG SAs are lower than the US SAs. In contrast to US SAs, PNG SAs rarely exceed the 1% SA threshold.

Only 1% (48/4,712) of the Karkars' SAs exceeded the 1% SA threshold (in 69% [33/48] of the cases where SA of 1% was reached, the relevant allergen was HDM).[420] In contrast, the SAs of US allergics regularly exceeded the 1% SA threshold: even without disposing of the exact US numbers, based on the scattergram we estimate that around 50% of the

419 Christensen's in-vitro experiments have already been presented in chapter 2.3.3.

420 This supports our notion that mites represent the clinically most important allergen on the island. More precisely: we calculated SAs for 19 different aero- and food-allergens (Ascaris was excluded). The exact numbers for the different allergens exceeding the 1% SA threshold are: 17 x D. fa., 16 x D. pt., 5 x timothy grass, 2 x cockroach, 2 x papaya, 2 x gummi arabicum, 1 x mugwort, 1 x sweet potato, 1 x mango and 1 x banana. The remaining nine allergens never reached the 1% SA threshold.

SAs of US allergic subjects exceed the 1% SA threshold. In other words: every second US sensitization leads to a SA high enough to allow a considerable effector cell degranulation upon allergen challenge. In order to underline the fact that the same levels of specific sensitization – i.e. equal RAST classes – have different "allergological meaning" (i.e. different probability of degranulation and clinical reaction), we added diagonally descending lines, separating the RAST classes. The RAST classes are labelled from 0 to 6.[421] We will give a short example of how to read and interpret the scattergram in relation to the RAST classes. For all points on the scattergram the specific IgE can be easily calculated. For example: if we look at the point representing 1% SA (y-axis) at 35 kU/l sTot-IgE (x-axis), we know that 1% of the 35 kU/l sTot-IgE is specific IgE. This means specific IgE is 0.35 kU/l. And 0.35 kU/l specific IgE is on the threshold between RAST Class 0 and 1. Starting from this point, there are two ("extreme") ways of reaching the border to RAST class 2: either going vertically up to 2% SA (2% of 35 kU/l sTot-IgE is 0.7 kU/l specific IgE = border to RAST class 2) or going horizontally right to 70 kU/l sTot-IgE (1% of 70 kU/l sTot-IgE is again 0.7 kU/l specific IgE = border to RAST class 2). Why may we talk about different "allergological meaning" of RAST classes depending on SA? We have seen within the Karkar population that in the same RAST class there was a higher probability of positive SPT reactivity at higher SA (see figs. 37 and 38). Yet all in all, the Karkar are a "non-allergic" society. If we compare for example the Karkar with RAST class two with the US allergics with RAST class two, we see, that the Karkar do not exceed the SA threshold of 1% (one exception). Thus the Karkar show a "protected" RAST class two, whereas the US allergics show in about 50% of the cases an "unprotected/risk" RAST class two. We may remark that strikingly within the US allergic population there seem to be a lot of subjects with RAST class 5, yet RAST class 6 is scarce. This is obviously an artefact as many RAST assays only measure up to a level of 100 kU/l of specific IgE – which happens to represent the border between RAST classes 5 and 6. However, the omission of RAST class 6 values within the US data leads to an inappropriate overestimation of the strength of negative correlation of sTot-IgE and SA.

Especially in traditional societies, where parasite infections boost the (specific and total) IgE system, the detection of specific IgE alone is not an appropriate value to assess allergic sensitization: the physiological effect of specific IgE may be suppressed or even blocked in the presence of high sTot-IgE levels. The consequence is a "clinically irrelevant (yet "real") serological allergic sensitization". Generally it is possible to use in-vitro assays for specific IgE under these circumstances, as our data indicates that major non-specific binding is most unlikely despite of extremely high sTot-IgE levels. Yet sTot-IgE should always be measured as well to allow for the calculation of SA, which is a more appropriate variable to assess the relevance of in-vitro sensitization. However, even in "affluent Western medical systems" sTot-IgE concentration is "rarely measured clinically,

421 We shortly recapitulate the six RAST classes: Class 0 = <0.35 kU/l, class 1 = 0.35-<0.7 kU/l, class 2 = 0.7-<3.5 kU/l, class 3 = 3.5-<17.5 kU/l, class 4 = 17.5-<50 kU/l, class 5 = 50.0-<100.0 kU/l, class 6 = >100 kU/l.

partially because of cost … Thus, the specific IgE to total IgE ratio [SA] is almost never computed …"[422]. All in all it becomes more and more clear, that SA is an important marker, which influences "the translation of IgE response into clinically evident allergic symptoms"[423]. This notion is supported by the fact that exactly those allergens elicit the "highest percentage of sensitized individuals with IgE specific activities >4% [which cause] the most severe allergic reactions worldwide": hymenoptera[424] venom (54%) and peanut (33%).[425] Hamilton RG and Saito H consider specific activity of such an eminent importance that they predict that in future laboratories "might elect to provide an allergen-specific IgE antibody result to clinicians … [also] as a specific IgE/total IgE ratio"[426].

In short: within traditional societies in the tropics RAST on its own seems to be even less appropriate for allergy screening than in industrialized Western societies.[427] On Karkar there are very few allergic individuals, yet – based on a limited case number – there was a much better correlation of positive SPT (in vivo sensitization) and allergic symptoms than of positive RAST (in vitro sensitization) and allergic symptoms. Taking the plausibility of mast cell inhibition into account, this is a quite comprehensible finding. Even if the ratio specific IgE to sTot-IgE (i.e. the SA) is calculated, in the author's opinion there is still no advantage of using SA instead of directly measuring mast cell releasability by means of SPT under "normal" clinical/diagnostic circumstances.[428] Only if performing SPT is not possible (risk of false negative results due to medicaments, possibility of anaphylactic reactions, extensive eczema, non-compliance of small children, nobody available who is able to perform an SPT etc.)[429] using the more expensive[430] and laboratory dependent SA may be an alternative to predict histamine release/clinical reactivity.

To conclude this chapter on specific activity we may recapitulate our result as follows. On the one hand SA explains our RAST SPT discrepancies we have not been able to account for previously. The decreasing SPT reactivity with decreasing SA is strongly suggestive of inhibitory mechanisms (competitive inhibition of FcεRI in the presence of higher sTot-IgE levels). This is confirmed by the fact, that prevalence of positive mite

422 This statement was made in respect to the United States in 2010 (Hamilton RG and Williams PB, 2010: 36).

423 Hamilton RG, MacGlashan DW Jr, Saini SS, 2010: 273.

424 Basically wasps and bees.

425 Hamilton RG and Williams PB, 2010: 38.

426 Hamilton RG, Saito H, 2008: 306.

427 In Western societies RAST screening also faces the problem of "clinically meaningless in vitro sensitizations", yet to a lesser extent.

428 This is in line with the statement of Hamilton RG and Saito H: "The bioassay (skin test) is powerful in its ability to integrate all these parameters [IgE concentration, clonality, affinity and specific activity] to provide a biologically relevant IgE response as a visually measured wheal …" (Hamilton RG, Saito H, 2008: 305).

429 Similar points have been listed by Siles (Siles RI, Hsieh FH, 2011: 586).

430 Especially if we test for "a large battery of allergens" the costs of RAST are high (Winter WE, Hardt NS, Fuhrman S, 2000: 1383).

SPT is lower within the same RAST class, if mite SA is under 0.1%.[431] On the other hand we could not show a general decreasing prevalence of positive SPT reactivity with increasing sTot-IgE values. Yet this does not need to lead us to question the protective effect low specific activities. It just points to the fact that strength of correlation between (probably worm triggered) sTot-IgE and SA was not very high: With increasing sTot-IgE in some individuals sTot-IgE increased quicker than specific IgE (consequence: falling SA) yet in others specific IgE (probably in part worm triggered) increased quicker than sTot-IgE (consequence: increasing SA). Some subjects produce specific IgE at levels which are not entirely blocked by the parallel existence of high sTot-IgE. Thus we can't give a "convenient" answer like "the higher the sTot-IgE, the lower the SPT reactivity". Obviously the effects of worms on the IgE system can not be characterised by such a simple formula; we need to consider the individual SA for each subject.

May it be possible that the allergological benefit caused by worms (increased sTot-IgE) is to a large extent "consumed" to balance a parallel allergologcical harm caused by worms (increased specific IgE)? We do not think that this "worst case" scenario is "true". It seems more likely that worms and the high sTot-IgE levels really protect the Karkar from allergies: the prevalence of atopic diseases and positive SPT reactions is undeniably low on the island and the "overall SA" is generally low as well. As s-Tot-IgE is extremely high on Karkar (96.0% of our test persons showed a sTot-IgE exceeding the "Western normal range" of 100 kU/l and the sTot-IgE GM is 1,069.0 kU/l) it is comprehensible that over 93%[432] of the subjects showed a HDM SA lower than 1%. Even if strong mast cell inhibition seems to occur under 0.1% SA, SA levels less than 1% already appear to exert a SPT/degranulation protective effect. As shown by Christensen LH et al. the most noticeable increase of effector cell degranulation occurs between 1% and 10% SA: degranulation at 1% SA was 20% (still comparatively low), at 5% SA degranulation was already 53% and at 10% SA degranulation reached 70%.[433] The notion that SPT suppressive effects may not require extremely low SA or immensely increased sTot-IgE is supported by results published by Witteman AM et al. They demonstrated a skin test inhibitory effect of increasing sTot-IgE already at sTot-IgE levels lower than 1,000 kU/l.[434]

Admittedly, the "protection by low SA" may only represent one of many factors explaining the low prevalence of atopic diseases on Karkar in comparison to the "modern

431 According to Erb, one of the currently (year 2007) most important "unanswered questions" in respect to helminth infestation is: "… is the hypothesis correct that the large amounts of polyclonal IgE induced by the helminths can inhibit allergen … induced degranulation of mast cells *in vivo*?" (Erb KJ, 2007: 1172). Our Karkar data suggest that this hypothesis is actually correct.

432 The exact values are: 93.9% (229/244) of the subjects showed a D. pt. SA lower than 1% and 93.4% (228/244) of the subjects showed a D. fa. SA lower than 1%.

433 Christensen LH et al., 2008: 302.

434 In a group of allergic subjects 88% (38/43) showed sTot-IgE levels lower than 1,000 kU/l; nevertheless a skin test suppressive effect of increasing sTot-IgE could already be seen in this sTot-IgE range (Witteman AM et al., 1996: 22). The percentage of subjects with sTot-IgE levels less than 1,000 kU/l was calculated based on raw data displayed in fig. 3.

Western world". The existence of additional worm triggered protective pathways like reduced activation of dendritic cells[435], alteration of T and B regulatory cell function, production of cytokines or parasite-specific proteins (like ES-62)[436] capable of down-regulating allergic response can not be excluded.[437] Smits et al. are probably right when they suggest that there may exist "multiple" mechanisms by which worms "dampen allergic responses" and that the real challenge is to "find out which part of the regulatory network provides the strongest … inhibitory response."[438]

Furthermore, we have to bear in mind, that SA does not take two further quite important properties of the IgE repertoire into account: affinity and – in particular – clonality. Whereas sTot-IgE and specific IgE levels are often measured in allergological (Western) practice and specific activity may easily be calculated from the latter two parameters, affinity and clonality are different: Currently, affinity and clonality are not determined in allergological day to day work – thus they (still) represent hidden[439], yet immunologically most relevant variables. Consequently Hamilton RG and Saito H consider our present serologic assessment of IgE "a rather crude measure" which is still "in its infancy"[440] In the next chapter we will try to explain the differences in allergy prevalence between traditional and affluent "modern" societies developing a theory which is largely based on clonality: We think that "modern Western IgE repertoires" are often characterized not only by low specific activity but also by high clonality (and partially low affinity); this pattern could explain the increase of allergies in affluent societies.

435 Suppression of mucosal allergic inflammation mediated by Ascaris suum products in a mouse model (McConchie BW et al., 2006: 6632 ff). However, this mechanism would not be consistent with our increased specific IgE levels in worm infected subjects as dendritic cells play an important role (antigen presentation) in the initial process of IgE production.

436 Melendez AJ et al., 2007: 1375ff.

437 We agree with Helmby that the available facts suggest that "… a complex network of several factors [is] involved in helminth-induced immunomodulation" (Helmby H, 2009: 123). Her conclusion is doubtlessly right: "The wide, and sometimes completely contradictory range of [study] results … clearly suggest that the impact of helminth co-infections on the immune response is far more complicated than we would hope and it is obvious that we are in fact rather limited in our knowledge regarding the interactions between these parasites and the immune system" (Ibid., 125).

438 Smits HH, Everts B, Hartgers FC, Yazdanbakhsh M, 2010: 6.

439 Hamilton RG et al. considered specific activity the "least studied humoral immune response parameter" (Hamilton RG, MacGlashan DW Jr, Saini SS, 2010: 277). Christensen LH et al. called affinity and clonality the "more subtle and less accessible parameters" of the IgE repertoire (Christensen LH et al., 2008: 298). They conclude: "… although allergic patients' sera might present similar titers of allergen-specific IgE, they might differ with regard to heir ability to mediate effector cell degranulation in the presence of allergen" (Ibid., 303).
In respect to the limitations of RAST measurements Hamilton RG and Williams PB state that there rose a "critique of current IgE antibody assays that provides [sic] … no information on … affinity, clonality …" (Hamilton RG and Williams PB, 2010: 36).

440 Hamilton RG, Saito H, 2008: 305.

6 Why are allergies on the rise? The "change theory", a step beyond the jungle hypothesis

6.1 Background 1: Worms and IgE boost. Cui bono?

In chapter 5.5 we described, that worm infestations seem to be associated with a "dual" IgE boost, affecting specific and "non" specific IgE. Generally "the IgE system" is understood to represent a main defence system of the host against worms.[441] But maybe, at this point, we have to differentiate between "parasite versus host interests" to answer the question put forth by Erb: "Currently it is unclear whether the regulatory responses [associated with helminth infections] have a greater benefit for the host … or the parasite …"[442] Asking the old question "cui bono?"[443] may not only help to reveal the author of a crime but also the "author" of a biological observation (when the actual pathways are hidden). In respect to worms and elevated IgE levels we can state: High specific anti-ascaris IgE probably helps the host: Specific anti worm IgE binds to the parasite; subsequently eosinophils bind to the worm-bound specific IgE and degranulate toxic products against the parasite[444]. As in the case of any other immunoglobulin type (IgG, IgA, IgM and IgD) the specific recognition of "the enemy" is crucial for the host to mount an effective immune defence. Thus it is likely, that the production of anti-ascaris IgE is triggered by the human immune system. But what about IgE with other specificities? In other words: what about the approximately 99.8% of IgE within our population sample?[445] There are at least no obvious advantages of increased "non" specific IgE (or increased anti allergen IgE) for the host. Yet in the author's opinion there is an advantage for the parasite: the possibility to become invisible (or at least less visible) to the host's immune system by generating a huge confounding IgE background. Thus in contrast to anti worm IgE, sTot-IgE may be triggered by the parasite.[446] Subsequently we will explain this possibility more in detail.

As discussed in chapter 5.8 our data indicates that competitive inhibition of the high affinity IgE receptor FcεRI on mast cells entails reduced mast cell histamine releasability. This means, if the ratio of allergen specific IgE to sTot-IgE (i.e. the allergen SA) is very small the bridging of two or more allergen specific IgE molecules is less likely to occur. Consequently there is less histamine release from mast cells and less allergic

441 Gounni AS et al., 1994: 183 f.

442 Erb KJ, 2007: 1171.

443 Latin, "who is the beneficiary" of a certain observation (translation by the author).

444 Winter WE, Hardt NS, Fuhrman S, 2000: 1382. Granules of eosinophils contain e.g. major basic protein and eosinophil cationic protein; both substances show "in vitro activity against parasites, including helminths" (Stone KD, Prussin C, Metcalfe DD, 2010: S78).

445 See chapter 5.5.

446 Erb shares this view and remarks: "… how helminths induce the production of these large amounts of polyclonal IgE is not known" (Erb KJ, 2007: 1171).

inflammation. Yet worms would probably not bother mitigating their host's allergic symptoms by increasing sTot-IgE. There may be a less "altruistic" reason: recent studies show, that mast cells and basophils[447] can play an important role in the protection against parasites.[448] A decreased worm (in our study Ascaris) specific activity may save the parasite from e.g. histamine effects (like activation of smooth muscle with increased helminth expulsion from the gut) and possibly also from the release of eosinophile chemotactic factors (see below).[449] As mast cells do not only contain histamine, but a large variety of mediators, the parasite could try to rid itself from other "host defensive substances" as well. All in all, Urb and Sheppard consider mast cells to "increase host defence locally".[450] Furthermore we know that high affinity IgE receptors are not only found on mast cells and basophils but also on eosinophil granulocytes.[451] Eosinophils are major effector cells in immune defence against worms as their granules store for example the toxic eosinophile peroxidase. Results published in 1994 by Gounni et al. in "Nature" indicate that FcεRI is not only expressed on eosinophils, but is also "involved in eosinophile degranulation" and thus plays "a major part in immune defence against parasites"[452]. As competitive inhibition of FcεRI is likely to occur also on eosinophils, we may expect reduced eosinophil peroxidase release. In other words, if the ratio of anti worm specific IgE to sTot-IgE (i.e. the worm/Ascaris SA) is very small, IgE dependent eosinophile activation is less likely to occur. Consequently we would encounter less eosinophile peroxidase release and less cytotoxicity against parasites. Thus lowering worm SA by increasing sTot-IgE may be a countermeasure induced by the parasite to render helminth specific IgE (induced by the host's immune system) less effective.[453] This is what we meant when we previously talked about a parasite becoming "less visible" to the host's immune system: Befogging the IgE system by generating a huge confounding background of sTot-IgE could constitute one more effective "trick" of worms to evade human immune defence. If this theory were true, it would underline the old notion that helminths are "masters of hiding" (see e.g. molecular mimicry of schistosoma)[454]. Hiding

447 „… IgE-mediated activation of basophils and the release of basophil-derived IL-4/IL-13 are critical steps in protective immunity against helminths" (Schwartz C et al., 2014: E5169ff). Stone et al. remark: "… basophils … are thought to play a role in host defence, particularly against parasites" (Stone KD, Prussin C, Metcalfe DD, 2010: S77).

448 "… one of the most important [anti worm] defence mechanisms [of the host is] mast cell and basophil degranulation …" (Erb KJ, 2007: 1172).

449 Urb M, Sheppard DC, 2012: 1f.

450 Urb M, Sheppard DC, 2012: 2.

451 Gounni AS et al., 1994: 183 f.

452 Gounni AS et al., 1994: 183 f.

453 A similar view is held by Erb: "The induction of polyclonal IgE by the helminth may … protect the parasite from the host defence by … inhibiting the binding of helminth-specific IgE to the mast cells or basophils. This would also explain why the helminths induce the production of large amounts of non-specific IgE in the first place …" (Erb KJ, 2007: 1171).

454 The fact that the worm Schistosoma mansoni is able to express host antigens on its surface in order to cloak itself and evade the host's detection and immune response was already described in 1971 (Clegg JA, Smithers SR, Terry RJ, 1971: 653f.).

from the host's immune system is biologically a more sophisticated strategy than causing "exaggerated" suppression of the host's immune system: It would not be in the parasite's "interest" to endanger the host, rendering him more susceptible to other infections. Hiding from specific anti worm/Ascaris IgE in an unspecific IgE background would be a newly described form of immunological disguise. Figure 47 shows the correlation of anti Ascaris IgE and anti Ascaris specific activity within the Karkar population.

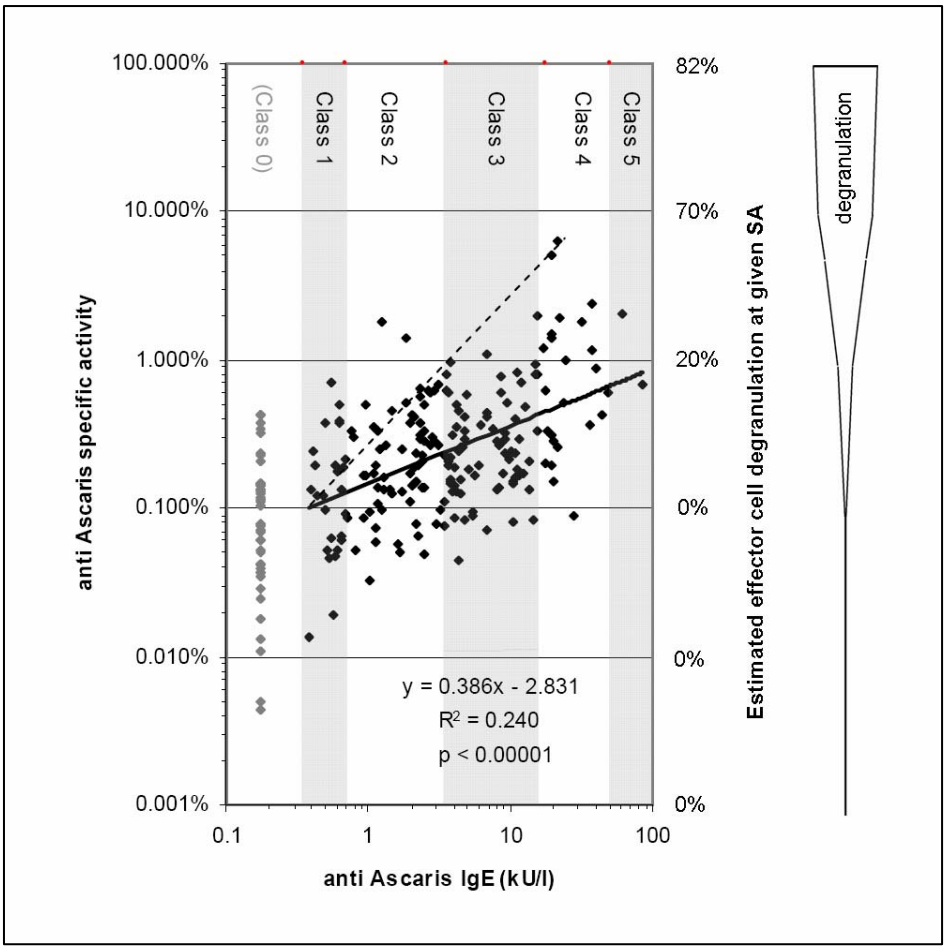


Fig. 47. Only modest increase of anti Ascaris specific activity with increasing anti Ascaris IgE. The level of anti Ascaris IgE is expressed in kU/l on the x-axis. The corresponding Ascaris RAST classes are named in the upper area of the figure (class 0 to 5) and marked as differently shaded vertical bars. The second y-axis and bar show the estimated mast cell degranulation at given specific activities.[455] Without the "protection" of high sTot-IgE, most Karkar would exhibit an anti Ascaris SA over 1%. Thus the expected effect of anti worm IgE would be considerably higher.

455 Estimated degranulation levels according to Christensen et al. as already described in chapter 2.3.3.

Figure 47 shows that the unspecific boost of the IgE system lowers worm SA. The figure has to be interpreted[456] as follows: A tenfold increase of anti Ascaris IgE (x-axis) would lead to a tenfold increase of Ascaris specific activity (y-axis) IF the sTot-IgE level were kept unchanged. The resulting slope of the regression line would be 1. Yet the actual slope of our line is only 0.386. This means, that the increase of anti Ascaris IgE is paralleled by an increase of sTot-IgE.[457] This keeps the ratio anti Ascaris IgE to sTot-IgE (the anti Ascaris SA) quite low. Because of the high sTot-IgE levels within the population, only 6.9% of the Ascaris positive islanders (14/203) showed anti Ascaris specific activity over 1%. And between 1% SA and 10% SA is the range (according to in vitro experiments), where effector cell degranulation experiences the most rapid increase (from 20% to 70%, see fig. 2). However, 93.1% of the Ascaris infected Karkar stayed under the 1% threshold, i.e. in an SA area, where degranulation seems partially inhibited. This is remarkable as the anti Ascaris sensitization levels are quite high: 51.7% of the Ascaris positive Islanders (105/203) showed the higher RAST classes three, four or five.[458] If sTot-IgE levels were "only" in the hundreds, the Ascaris specific activities would be about ten times higher – thus the RAST classes 3 to 5 would easily exceed the 1% SA threshold. Even the most extreme point on the far right, representing the Islander with the highest anti Ascaris IgE (84.4 kU/l, RAST class 5) does not traverse the 1% SA threshold (actually 0.68%) as the associated sTot-IgE is 12,350.0 kU/l. The estimated degranulation level at 0.1% anti Ascaris SA (0% degranulation, i.e. blocking threshold according to Christensen) has been confirmed for worm SA (filaria) by Mitre et al.: "… histamine release could be completely abrogated …" at filaria SA lower than 0.1% when effector cells were stimulated with filaria (Burgia malayi) antigen.[459] The notion that worms "hide" in an elevated sTot-IgE background is supported by the results from Lynch et al.: The anthelmintic treatment of children in a slum area of Caracas, Venezuela, resulted in a decrease of sTot-IgE (i.e. decreased "hiding background") combined with an increase of Ascaris lumbricoides SPT reactivity (i.e. increased clinical recognition of worm antigens).[460]

456 Anti Ascaris IgE negative subjects were attributed a specific Ascaris IgE level of 0.175 kU/l for logarithmic representation. This may seem to be a high value, yet we chose it due to the distribution of the measured levels. The anti Ascaris IgE negative subjects (45/248=18.1%) are represented by the vertical line of grey points within the RAST class 0 bar. As the calculated specific activities of RAST negative subjects are based on an arbitrary/attributed value they were not included in the regression analysis.

457 Yet the increase of sTot-IgE is not equivalent to the increase in anti Ascaris IgE: if a tenfold increase of anti Ascaris IgE were paralleled by exactly a tenfold increase of sTot-IgE, anti Ascaris specific activity would remain unchanged.

458 Interestingly, in his review article "Helminths, allergic disorders and IgE-mediated immune responses: where do we stand?" (published in 2007) Erb considered the question whether „the induction of polyclonal IgE [is] associated with the inhibition of helminth specific IgE generation" one of the major "unanswered questions" (Erb KJ, 2007: 1172). Our Karkar data clearly show that production of high levels of polyclonal IgE does NOT inhibit/suppress the production of helminth specific (anti Ascaris) IgE.

459 Mitre E, Norwood S, Nutman TB, 2005: 4109. The 0.1% filaria SA blocking threshold is based on in vitro basophile sensitization experiments.

460 Lynch NR, Hagel I, Perez M, Di Prisco MC, Lopez R, Alvarez N, 1993: 404-11. Admittedly – and unexpectedly – Ascaris lumbricoides specific IgE also increased after worm therapy, yet not to the same extent as sTot-IgE decreased (ibid., 407f).

We recapitulate: It seems that an unspecific polyclonal worm induced boost of sTot-IgE lowers worm SA. A reduced anti Ascaris SA may render the host's specific anti worm IgE defence less effective (less degranulation of eosinophiles, mast cells and basophils). Obviously Erb thought along the same lines when he assumed that the association of "very little parasite-specific IgE" and "large amounts of polyclonal … [IgE]" (i.e. a small parasite SA) could be the reason for a limited anthelmintic effectiveness of the overall IgE system.[461] However, instead of focussing on misleading (or even wrong) quantities like "little" and "large", it may be more appropriate to refer directly to the ratio of both variables (SA): on Karkar, the "parasite-specific IgE" (i.e. the anti Ascaris IgE) is definitely NOT "a little" but if anything "a lot"[462]. Yet as the amount of polyclonal IgE is not only "large" but rather "extremely large", it remains true, that the SA is low. That the IgE system could indeed be less effective than thought is supported for example by the two following arguments. Firstly, in a certain way, worms seem to be the winners of the millions of years of co-evolution[463] of helminths and human beings. Harnett remarks: "There are many reports of nematodes surviving in humans for more than a decade … a somewhat remarkable feat given the [general] effectiveness of the human immune system."[464] Pearce concludes: "Helminth nematodes are incredibly successful pathogens …"[465] And as Weinstock et al. rightly point out: "Many worms seem invincible to human defense".[466] All this may be currently forgotten in "modern Western societies", but without the effective anthelmintic treatments worms would still affect virtually 100% of the populations in most of the countries of the world[467] – despite of the existence of an IgE system.[468] This quasi-failure of the IgE system has also been brought up by Erb: "Interestingly, … there is little evidence suggesting that IgE plays a significant role in host defence against helminths."[469] Admittedly worms normally don't kill humans, but this should not be interpreted as a "weakness" of helminths: it is just in the own interest of any parasite not to kill the host. Secondly the incipient use of the recently developed monoclonal IgE antibody omalizumab (Xolair®)[470] in worm infested societies indicates that "knocking out" the entire IgE system does not entail an obvious aggravation of helminth infections. The manufacturer advises to use Xolair® only with caution (special

461 Erb KJ, 2007: 1171.

462 As mentioned above, 51.7% of the Ascaris positive Islanders (105/203) showed the higher RAST classes three, four or five.

463 Weinstock JV, Elliott DE, 2009: 128.

464 Harnett W, Harnett MM, 2008: 392.

465 Pearce EJ, 2007: 1288.

466 Weinstock JV, Elliott DE, 2009: 128.

467 Weinstock et al. point out that the colonization of humans with helminths was "nearly universal until the early 20th century" (Weinstock JV, Elliott DE, 2009: 128).

468 Helmby states: "Despite the successful generation of the Th2 response [incorporating IgE], worms are often able to survive in the [immunocompetent] host for long periods of time" (Helmby H, 2009: 122).

469 Erb KJ, 2007: 1170f.

470 Omalizumab reduces the circulating free (specific and "non"-specific) IgE by 90% (Hamilton RG and Williams PB, 2010: 36) and was approved by the U.S.-FDA in 2003 (treatment of severe IgE-mediated asthma).

consideration of each single case) if there is "a high risk of worm infection".[471] Yet this suggestion seems to be based on comprehensible but mainly theoretical safety considerations. Between 2001 and 2004 Cruz AA et al. conducted a study in Brazilian allergic patients at high risk of helminth infection receiving omalizumab.[472] They reported "some evidence" for a "modest increase" in helminth infection incidence during a 52-week omalizumab treatment period. Yet they were applying a one-sided test. Using a more appropriate two sided test would have yielded p=0.27 (according to our calculation based on the provided raw data).[473] Thus the difference between the omalizumab group (50% infection) and placebo group (41% infection) is not big enough to reject H0. Cruz et al. admit that there was no increased maximal helminth infection intensity, no shorter time to first infection and no inferior response to anthelmintic treatment within the omalizumab group. Thus they consider omalizumab "safe and well tolerated" in a population at high risk of helminth infections; a result we would not expect "knocking out" an extremely effective major anti worm defence mechanism.[474] Maybe omalizumab only switches off a defence system which has already been rendered partially ineffective by worms – certainly good news for worldwide sales and distribution of the extremely high priced pharmaceutical product omalizumab.

Conclusion: Maybe worms found a way to successfully mitigate the effects of specific IgE: "Intending" to mitigate anti worm specific IgE by competitive inhibition, helminths may have "inadvertently" also mitigated anti allergen specific IgE, a quite welcome side effect for the host. As shown previously, specific activity against HDM is generally low within the Karkar population – probably due to worm infestations. This could convey a certain protection against allergic reactions and partially explain the low prevalence of atopic diseases on Karkar. On the other hand, there was no clear decrease of HDM SPT reactivity with increasing sTot-IgE levels as some individuals were able to mount high HDM SA despite of extremely high sTot-IgE levels. Thus additional factors – apart from the disappearance of worm infections – are likely to be contributing to the rise of allergies in affluent "modern" societies as well.

6.2 Background 2: Do polyclonal worm boosts reveal broad scale sampling of environmental allergens?

The RAST sensitization pattern of the Karkar Islanders seems to be in a way contradictory: On the one hand we see a "specific" image of the environmental allergens; on the other hand there seems to exist an "unspecific" worm boost. In this chapter we want

471 Rote Liste Service GmbH, 2014 : 659.

472 Cruz AA et al., 2007: 197-207.

473 Even after adjusting for study visit, infection status, gender and age the difference was not significant (p=0.07 for a two-sided test).

474 Wu calls IgE a "first-line defence against parasites such as helminths…" (Wu LC, Zarrin AA, 2014: 247).

to explain what we mean with this "contradiction" and to give a possible explanation for our findings. To clarify what we mean when we talk about the RAST pattern being a "specific" image of environmental allergens we have to look again at fig. 19: Sensitization against e.g. storage and house dust mite, cockroach, pig epithelia, banana, sweet potato, papaya and mango is definitely a pattern we would not expect in Moscow, Sydney or Berlin; it's definitely a "tropical immunological footprint". A substantial proportion of the subjects developed IgE antibodies against the environmental allergens (e.g. 73% against D. pt.). IgE antibody formation does not seem to be necessarily depending on the allergic potency of the corresponding allergen (e.g. 33% sensitization against banana)[475]. To show what we mean when we talk about the existence of an "unspecific" worm boost we have to go back to chapter 5.7.1 where we described that it is impossible to predict the single specific IgE values of a certain subject. At high specific IgE levels, one subject seems to experience e.g. a banana boost (no anti dog IgE), whereas another subject seems to experience a dog boost (no anti banana IgE) – even if sTot-IgE is very similar. The triggered RAST sensitization may not be paralleled by increased SPT reactivity, as specific activities stay stable due to increased sTot-IgE levels.

Putting both observations together, we could explain the sensitization pattern of the Karkar as follows: The IgE system generates a "specific" immunological image of the environment. This shows that the immune system actually recognizes more substances than we currently think. However, in the absence of helminths this thorough sampling may go unnoticed, i.e. it may not necessarily lead to specific IgE levels high enough to be measured. Helminth infections may generate an "unspecific" boost of specific IgE which is able to make the pre-existing immunological image "visible" (measurable). The boost is "unspecific" as it is affecting only a certain proportion of the pre-existing immunological image i.e. in every subject IgEs with different specificities may be boosted. Theoretically, worms may not only boost a pre-existing sensitization (activation of pre-existing allergen specific B-cells/plasma cells), but initiate the whole sensitization process. Yet the latter option seems unlikely as it would require the parasite's ability to switch on a whole array of additional pathways e.g: phagocytosis of antigens, processing, presentation etc. Thus it is more likely that there is generally a quite extensive sampling of environmental antigens and worms only boost/lift these pre-existing "invisible" low level sensitizations over the detection threshold. Our considerations are in agreement with Lynch et al. who concluded that worms are able to enhance "… the [specific IgE] response against allergens that are continually present in the environment".[476] The possibility that actually "more" of our environment is sampled/recognised than we think is supported by molecular biological findings: Immunoblotting showed for example that the immune (IgE) systems of more than a quarter of people with no clinical codfish allergy nevertheless recognized codfish

475 Also see chapter 5.7.1.
476 Lynch NR, Hagel I, Perez M, Di Prisco MC, Lopez R, Alvarez N, 1993: 409.

proteins.[477] Furthermore, broad food panels (RAST) often show clinically non-relevant (IgE) sensitizations – yet these sensitizations are "real" and not "false-positive" as often claimed.[478] The immune system's high activity in sampling "innocent" antigens is also reflected by the fact that (allergologically irrelevant) immunoglobulin G (IgG) directed against well tolerated foods is often found.[479]

6.3 The "change theory" of allergogenesis

If our considerations were true, that there is a general sampling of a multitude of environmental antigens (including allergologically "unsuspicious" substances) in traditional tropical village societies, there is no reason why this should not hold true for modern Western societies as well. If an immunological imaging of the environment resulted in a "low level" production of corresponding specific IgE, we just may not be aware of this polyclonal specific IgE background for several reasons: Firstly and quite trivially we may not consider a certain environmental antigen "allergologically important" thus we would not measure it. Secondly, if we measured the "unsuspected" environmental antigen (in the absence of worm infestation with IgE boost), the level could be under the detection limit of 0.35 kU/l (recently 0.1 kU/l)[480]. Thirdly the affinity of IgE to certain environmental antigens could be too low to be detected in RAST (only monovalent binding; see chapter 5.4) yet enough to play a relevant role in allergogenesis under physiological conditions during clustering on mast cells (polyvalent binding).[481] Perhaps what we call "non" specific IgE represents nothing but the "hidden" immunological footprint of the environment: the sum of theoretically "measurable" (yet actually not measured) specific IgE (possibly even over the detection limit) and a large number of "non measurable" IgE specificities (under the detection limit).[482] In other words: the author is of the opinion that the human immune system does not "invent" IgE antibodies against "non existing antigens" ("non" specific IgE).[483] It seems more likely that it samples a large variety of existing environmental substances and produces a large variety of specific IgE possibly at low levels and maybe with low affinities.

477 This result was just an incidental finding in Hansen's work (dealt with in a single sentence), yet we think it is quite informative (Hansen TK, Bindslev-Jensen C, Skov PS, Poulsen LK, 1997: 191). It may be added that subjects who were codfish allergic recognized not only one but up to 17 codfish proteins.

478 "Broad food panels have been shown to have false-positive rates higher than 50% – i.e. in more than half of [the] cases, positive results have no clinical relevance" (Siles RI, Hsieh FH, 2011: 588).

479 Siles RI, Hsieh FH, 2011: 591.

480 Hamilton RG, MacGlashan DW Jr, Saini SS, 2010: 275.

481 This would be in line with the recent biomolecular findings of Handlogten et al. (Handlogten MW, Kiziltepe T, Serezani AP, Kaplan MH, Bilgicer B, 2013: 789ff).

482 "Non measurable" refers to the single RAST measurements: for each IgE specificity the detection limit is not reached, yet the sum of specificities is measurable as sTot-IgE.

483 Real non-specific IgE exists maybe only in non-immunocompetent systems: IgE produced by myeloma cells bears features of "real non-specificity" (see chapter 5.7.1).

Even if sensitizations are very low (in respect to concentration and affinity of specific IgE), this does not imply that this low level sensitization is generally clinically irrelevant, as it may play an important role in cross-reactivity. Cross-reactivity was already mentioned in chapter 2.3.2: If different environmental antigens "share" certain antigenic determinants (resemblance in the protein fold)[484], IgE antibodies with the same specificity may be able to bind to both antigens i.e. they are cross-reacting. Phylogenetically related proteins/molecules (i.e. proteins sharing a common evolutionary origin and thus displaying a "high degree of homology in the primary structure"[485]) and sometimes even unrelated (non-homologous) proteins/molecules can share antigenic determinants.[486] Recently it became apparent that cross-reactivity is more common and thus more important "than thought previously".[487] We will give an example for the possible effects of cross-reactivity: the "borad sampling" of environmental antigens may lead to low level IgE production against a certain environmental antigen A and simultaneously against antigen B which happens to share peptides with sequence homologies (cross-reactivity). This cross-reacting low level IgE may not lead to a reaction against environmental antigen A (if no further epitopes of A are recognized), but to a clinical reaction against antigen B (if further epitopes of B are already recognized).[488] Thus the cross-reacting IgE (originally directed against environmental antigen A) increases clonality (the number of recognized epitopes) in respect to antigen B. As already described in chapter 2.3.4, higher IgE clonality increases effector cell sensitivity.[489] Thus an allergic reaction upon contact with antigen B becomes more likely. Recent studies show that low concentrations of specific IgE can nevertheless lead to considerable histamine release under certain circumstances. A 95:5 ratio of two IgE clonalities can still induce 93% of the maximum in-vitro degranulation (occurring at equimolarity, i.e. at a 50:50 ratio of both clones)[490] and even a 99:1 ratio astonishingly still triggered 46% of the maximum degranulation.[491]

484 Sometimes not proteins but sugars are the reason for cross-reactivity (Aalberse RC, Akkerdaas J, van Ree R, 2001: 478).

485 Aalberse RC, Akkerdaas J, van Ree R, 2001: 478.

486 Cross-reactivity has been described for many allergens. Mohapatra et al. pointed out the importance of cross-reactivity in respect to grass pollen allergy (Mohapatra S, Lockey R, Polo F, 2004: 200). Santiago showed cross-reactivity between worm antigens and environmental antigens (Santiago HdaC, Ribeiro-Gomes FL, Bennuru S, Nutman TB, 2015: 93ff). Aalberse et al. mentioned cross-reactivity between "distantly related organisms", cross-reactivity which was sometimes "unexpected" like ragweed/banana, birch/apple and latex/banana/shrimp (Aalberse RC, Akkerdaas J, van Ree R, 2001: 478). There would be countless further examples as our knowledge about cross-reactivity is continually increasing.

487 Santiago HdaC, Ribeiro-Gomes FL, Bennuru S, Nutman TB, 2015: 94.

488 See chapter 2.3.4 on clonality.

489 Christensen et al., 301ff.

490 "Equimolar concentrations of individual allergen-specific [nonoverlapping] IgEs result in the highest maximal degranulation level" (Christensen LH et al., 2008: 301).

491 Percentages were calculated by the author from the raw data given by Christensen in fig. 5. (Christensen LH et al., 2008: 303). We chose values at a stimulation level of 1 ng/ml rDer p2, as higher recombinant allergen levels seemed to yield unreliable results: either due to artefacts or due to over-saturation (unphysiologically high allergen concentrations may block IgE bridging by "oversaturation" of effector cell bound IgE with allergen).

Subsequently we want to apply these in-vitro results to theoretical example: if an allergen B is recognized by specific IgE x with a concentration of 3.5 kU/l (low RAST class 3), the existence of a cross-reacting IgE y (equal affinity) with a concentration of only 0.175 kU/l[492] (which is under the current detection limit of 0.35 kU/l!) would still induce 93% of the maximum histamine release (which would have occurred at equimolarity, i.e. 3.5 kU/l of IgE x and 3.5 kU/l of IgE y). Thus extremely small and hence (at present) undetectable concentrations of IgE may well play an important role in cross-reactivity. Even if the specific IgE does not "fit" well on antigen B (i.e. it displays low affinity to B), histamine release can still be substantial: If an allergen epitope is recognized by high affinity IgE, recognition of a further epitope by low affinity IgE is enough to induce high effector cell reactivity upon allergen contact.[493] As already mentioned in chapter 2.3.5, Christensen LH et al. showed that the combination of a high with a low affinity IgE clone (directed against a recombinant HDM allergen) triggered virtually the same maximal degranulation in-vitro as two high affinity IgE clones;[494] only sensitivity was slightly diminished when one of the high affinity clones was replaced by a low affinity clone.[495] Such a constellation is remarkable – this was also emphasised by Christensen et al. Remarkable, not least when considering the facts that the affinity of the replacing low affinity clone was much lower than the original high affinity clone[496] and that there was an "approximately 1000-fold difference in affinity" between the affinities within the "high affinity – low affinity" combination.[497] Fully in agreement with our thoughts is Christensen's conclusion: "… the presence of low-affinity allergen-specific IgEs in a patient's serum should not be neglected. A prominent role of low-affinity IgE antibodies for allergic symptoms could very well be in the context of allergenic cross-reactivity…"[498]

In respect to cross-reactivity we may conclude: even if the cross-reacting IgE shows low concentration and low affinity to an antigen (antigen B in our previous example), the additional "low concentration low affinity" clonality may increase mast cell sensitivity (upon contact with B) in a significant way. All in all, the allergological importance of

492 0.175 kU/l is 5% of 3.5 kU/l.

493 Recently Handlogten et al. could demonstrate that "low-affinity epitopes have a major role in eliciting an allergic response when presented in combination with high-affinity epitopes" (Handlogten MW, Kiziltepe T, Serezani AP, Kaplan MH, Bilgicer B, 2013: 789ff).

494 "Interestingly, we found that the presence of only a single [IgE] antibody of high affinity in the allergen specific IgE repertoire is required for the recruitment of an IgE of even very low affinity into productive FcεRI/IgE/allergen complexes" (Christensen LH et al., 2008: 303). They specified: "All rIgE affinity combinations resulted in similar maximal basophil degranulation levels …, except for the combination of 2 low-affinity antibodies" (Ibid, 301 and fig. 6 p 303).

495 "… when a low-affinity rIgE clone was combined with a high-affinity rIgE clone … this … combination showed a basophil sensitivity that was only 2- to 5-fold lower than that of 2 high-affinity rIgE clones" (Christensen LH et al., 2008: 301 and fig. 6 p 303).

496 High affinity clone "H12" showed an affinity of K_D 1.1 nM and low affinity clone "H7:H12" only K_D 30.0 nM (raw data extracted from Christensen LH et al., 2008: 302 fig. 2).

497 Ibid., 303.

498 Ibid., 304.

sTot-IgE – or more precisely of the so called "non-specific IgE"[499] – as a source of cross-reactivity is possibly largely underestimated. The conceptual base of our "change theory" of allergogenesis is cross-reactivity. If very small or actually undetectable levels of cross-reacting IgE – even with low affinity – can result in clinical reactions, this could well explain the increase in allergies in affluent "modern" Western societies. Yet why should cross-reactivity – which also exists in traditional societies – explain the increased prevalence of allergies in affluent societies? In the author's opinion, cross-reactivity became much more important in the West as the major feature of a "modern" lifestyle is "change". And change is the base of increased antigen diversity (from the traditional societies' point of view we could say that continuity is the base of limited antigen diversity). Immunological sampling of this increased antigen diversity may lead to increased IgE diversity within the sTot-IgE background. This comparatively higher "new/modern" IgE diversity in Western societies could allow more cross-reactivity with the "old/traditional" allergens (pollen, animal epithelia, mites, fungi etc) than in traditional societies. Thus the introduction of "new" substances may increase the allergy prevalence against "old" substances. On the other hand, the "opposite direction" of cross-reactivity is possible as well: Pre-existing sensitization against "old" substances may lead to severe hypersensitivity reactions against "new" substances; even at first contact: Chung et al. described that pre-existing IgE antibodies against an animal oligosaccharide (galactose-α-1,3-galactose) lead to allergic reactions against a new biologic drug (cetuximab) at first exposure.[500] The introduction of only one new food, Kiwi, illustrates how the options for "new IgE" explode: More than twelve allergens have been detected in Kiwi by now and an average allergen disposes of around seven epitopes,[501] thus theoretically 84 "new IgE (sub-) specificities" could be generated sampling this "new environmental substance". Notably only one or a few of these "new" clones could trigger mast cell degranulation if there is cross-reaction with any "old" allergen; even if the cross-reacting "new" IgE molecules are few and show just low-affinity to the "old" allergen.[502] The entire "latex-fruit syndrome" is an illustrative example for the increase of allergies because of the introduction of "new" antigens and subsequent cross-reactivity: About 50% of latex allergic subjects show associated hypersensitivity to fruits like: avocado, kiwi, tomato, potato, peach, banana, capsicum and chestnut.[503] Neither latex (13 known allergens Hev b

499 Not measured but calculated as sTot-IgE minus the sum of all IgEs with known specificity.

500 Chung et al., 2008: 1109ff. Of course allergic reactions may also be directed against newly introduced antigens without the existence of cross-reactivity: especially if the "new" allergen shows immunodominant epitopes. Pollen of Ambrosia artemisiifolia (ragweed) currently invading Germany would be an example for this constellation.

501 Handlogten states: "two to twelve epitopes … are recognized [in typical allergens]" (Handlogten MW, Kiziltepe T, Serezani AP, Kaplan MH, Bilgicer B, 2013: 789).

502 This is analogue to what Handlogten formulated in a negative way: "[Our] results provided further evidence that … inhibition of only a few low-binding epitopes may be sufficient to prevent mast cell degranulation … (Handlogten MW, Kiziltepe T, Serezani AP, Kaplan MH, Bilgicer B, 2013: 794).

503 Wagner et al. also state that "… it is not always clear whether latex sensitization precedes or follows the onset of food allergy" (Wagner S, Breiteneder H, 2002: 935-40).

1-13)[504] nor (sub)tropical fruits are native to most Western countries. The number of epitope homologies and possible "new" interactions seems to be immense.[505] Aalberse et al. are certainly right when they summarise: "The effect of polyclonality on overall cross-reactivity is a matter of statistics: the more diverse the antibody repertoire, the more likely it is to cause some cross-reactivity …"[506] Differences in the number of clones directed against a certain allergen (clonality) could not only help to explain differences in allergy prevalence between "traditional" and "modern" societies; differences in clonality may also explain why there are no reliable specific IgE threshold values predicting the likelihood of clinical allergy (higher allergological reactivity if a certain specific IgE level/concentration is composed from a higher number of clones).

All in all, on Karkar a positive RAST may be clinically not very relevant for two possible reasons: firstly specific IgE directed against a certain allergen may consist of few (worm triggered) clones and secondly the specific activity in respect to that allergen is low because of high (worm triggered) sTot-IgE levels. In "modern" societies however a positive RAST is clinically much more relevant as clonality has probably increased due to increased antigen diversity ("explosion" of options for cross-reactivity) and the increase of SA in the absence of worm infections.

The search for the reasons for the increase of allergies in Western societies is often focussed on single factors of "modern" lifestyle: Is there a rise in allergies because of new foods, less children, more environmental pollution, shorter breast feeding, climate change, less infections, more vaccination etc.?[507] Yet we have to step back to get a broader view of our affluent modern lifestyle in order to discern that maybe the only common link between all these inhomogeneous factors is – abstractly speaking – change. Mutius' results point in the same direction: she found a significantly lower prevalence of hay fever, atopy and bronchial hyperresponsiveness in children living in Bavarian "old-fashioned" (probably mostly farm-) homes, where coal or wood was used for heating.[508] Braun-Fahrländer et al. reported less atopy and allergic rhinitis in Swiss farmer's children[509], Riedler et al. got similar results in Austria[510], Kilpeläinen et al. in Finland[511] and Ernst et al. in Canada.[512] Maybe "traditional coal and wood heating" or "living on a farm" is – just as "traditional tropical village society" – an indicator for the absence of exaggerated change. Maybe

504 Siles RI, Hsieh FH, 2011: 590.

505 One could argue that the Karkar came into contact with latex as well and banana is a staple diet. Yet all in all probability depends on the number of options: on the island there is no avocado, chestnut, kiwi, peach, tomato, capsicum and potato "available" for cross-reactions.

506 Aalberse RC, Akkerdaas J, van Ree R, 2001: 485.

507 Anderson pointed out in 2005, that the current abounding theories about the epidemiology of asthma are dissatisfying. He concludes: "Any advance in our understanding of trends is likely to depend on the development of new theories of causation…" (Anderson HR, 2005: 1038).

508 Mutius E, Illi S, Nicolai T, Martinez D, 1996: 1448ff.

509 Braun-Fahrländer C et al., 1999: 28ff.

510 Riedler J, Eder W, Oberfeld G, Schreuer M, 2000: 194ff.

511 Kilpeläinen M, Terho EO, Helenius H, Koskenvuo M, 2000: 201ff.

512 Ernst P, Cormier Y, 2000: 1563ff.

subjects living in “rural old-fashioned” houses/farms keep consuming their own apples and potatoes at home instead of kiwis and shrimps in fancy restaurants, they may keep spending their holiday at home with their “old antigens” instead of in Thailand with “new antigens”, they may be affected less by environmental changes (pollution) etc. Change is the “hidden connecting factor” in many more studies and theories: Alm et al. reported a lower prevalence of atopy in children of Swedish families with an anthroposophic lifestyle.[513] An anthroposophic lifestyle may be described as more traditional, not following every new development within the surrounding “unsteady” consumer society. Indeed the authors could show that anthroposophs used less “new” medicaments (antibiotics), went on to consume fermented vegetables (containing lactobacilli) etc. All in all they may expose themselves less to “change”. Tedesci found that 84.3% (n=222) of allergic immigrants to affluent Europe (Milan) declared to have been healthy before their arrival in northern Italy.[514] This situation is associated with two kinds of change: there is probably an increased “within country” change/diversity in Italy in comparison to the immigrants home countries, but there is also the “between countries” change/diversity: maybe after originally coping with the recognition of the “home antigens” adding the “antigens of the country of immigration” means too much diversity for the immune system. The concept of sampling increased change/diversity during life may have unpleasant consequences: On the one hand, nowadays people seem to develop allergies even later in adulthood – possibly a consequence of reaching a cumulative change threshold. On the other hand, there might be no way back once you get an allergy. The author doubts that the allergic immigrants’ allergies would disappear if they went back to their “low change/diversity” home countries once their immune systems have been confounded sampling/memorising a “too big” antigen diversity – even if they reacquire a worm infestation (competitive inhibition of mast cells). In Heraclitus’ words: “No man ever steps in the same river twice.” The principle of “futile antigen reduction” once an exaggerated antigen exposition has occurred may not only hold true for migration: abandoning a “modern affluent” lifestyle and reverting to a “traditional” one may not be a very promising therapeutic concept – at least in respect to allergies. The fact that immigrants develop allergies after their arrival in affluent countries brings us to the “hygiene hypothesis” of allergogenesis. We agree with von Hertzen and Haahtela that the hygiene hypothesis is “widely considered as the most plausible working hypothesis to explain both the temporal changes and the regional differences in asthma and atopy prevalence”.[515] The original idea of the hygiene hypothesis (Strachan 1989) was that lack of childhood exposure to “dirt” (more precisely infectious agents: microbes, parasites)

513 Alm JS et al., 1999: 1485ff.

514 Tedeschi A, Barcella M, Dal Bo GA, Miadonna A, 2003: 449ff.
Kalyoncu et al. had similar results: they found increasing allergy in immigrants after they had arrived in Sweden (Kalyoncu A, Stalenheim G, 1992: 277ff). In respect to immigration, D'Amato and Rottem concluded more generally: “The protection conferred by the past rural environment, does not apply for the new environment…” (D'Amato G, Rottem M, 2011: 98).

515 Von Hertzen LC, Haahtela T, 2004: 126.

increases allergy prevalence in later life.[516] Immigrants should thus not develop allergies if their childhood priming of the immune system had been permanent. Sustaining the "original" hygiene hypothesis[517] may be possible, if the protection was only transient. This would be in agreement with our theory of competitive inhibition of mast cells: as soon as the worm triggered high sTot-IgE level falls to "normal" after anthelmintic treatment in Western countries, the protection originally conferred by low mast cell sensitivity would disappear. Yet the whole hygiene hypothesis could largely represent just a "change hypothesis": traditional longstanding infections may be an indicator for stability (absence of western medicine and affluent life-style) but hygiene/cleanliness an indicator for modernity, change and antigen diversity. Even looking directly and exclusively at infections, change may be a relevant factor: prevalence and morbidity of infections is undoubtedly higher on Karkar than in modern societies, yet the same is not necessarily true for the variability of infections: From the immunological point of view, the Karkars' infections are (partially "were") reliable/permanent old enemies, often not self-limiting and in many instances staying untreated and thus (at least immunologically) "active" for years: e.g. malaria, yaws[518], TB[519], worms[520], Helicobacter pylori[521] and hepatitis[522]. In contrast, the Westerners' infections are unreliable/changeable new enemies: e.g. many – often asymptomatic – infections, partially acquired during travelling like rhinoviruses, coronaviruses, (non-polio) enteroviruses, every year a different form of "imported" influenza and norovirus; furthermore less prevalent but still an indicator for volatility of

516 Strachan DP, 1989: 1225ff.

517 The „original" hygiene hypothesis has been extended considerably as pointed out by von Hertzen and Haahtela: meanwhile the hygiene hypothesis incorporates 1. exposure to microbial pathogens, 2. exposure to lipopolysaccharides (bacterial components), 3. exposure to gastrointestinal commensals, 4. exposure to farm/country environment and pets, and 5. exposure to helminths (Von Hertzen LC, Haahtela T, 2004: 126). Revising the abundant epidemiological literature in detail is out of the scope of this study. Yet, as Schaub et al. rightfully remark, the original hygiene hypothesis has undergone numerous modifications and "… a truly unifying concept has not yet emerged …" (Schaub B, Lauener R, von Mutius E, 2006: 969).

518 Due to antibiotic eradication projects Yaws is not very common any more (Herbert O, personal observation).

519 The inhibition of atopic disorder by Mycobacterium tuberculosis was suggested by Shirakawa et al. in 1997. Japanese schoolchildren who developed a positive tuberculin response showed a lower incidence of asthma and lower sTot-IgE levels than children with negative test results (Shirakawa T, Enomoto T, Shimazu S, Hopkin JM, 1997: 77ff). The authors suggested a "modification of immune profiles" as cytokine profiles were biased toward TH1.

520 As Helmby rightfully remarks: "Helminth infections … are notoriously chronic in nature, with some species of worms surviving within their host for many years, in some cases even decades" (Helmby H, 2009: 121).

521 Helicobacter pylori – currently mostly known for its association with peptic ulceration – has been colonising the stomach of humans "since at least Paleolithic times". In developing countries its prevalence is estimated to exceed 80% in adults. Yet in "developed" countries Helicobacter pylori prevalence is "rapidly decreasing", probably due to higher hygiene and repeated use of antibiotics (Blaser MJ, Chen Y, Reibman J, 2008: 561). After revising most of the available literature, Blaser et al. conclude that the "loss of this ancient, dominant and persistent member of the normal biota" is associated with childhood asthma, allergic rhinitis and atopy (Blaser MJ, Chen Y, Reibman J, 2008: 565).

522 Matricardi et al. showed an inverse association between hepatitis A and atopy at an Italian Air force school (Matricardi PM et al.: 2000: 412ff).

infections: middle east respiratory syndrome coronavirus, Zika virus, tick-borne encephalitis virus, etc. In the light of increasing population density (also in many Western countries), increasing concentration of population in cities and increasing travelling the author foresees a further increase in the diversity of infectious antigens. Even if many of the "volatile" infections (self-limiting or quickly treated by biomedicine/antibiotics) in the West do not cause (serious) symptoms, they may nevertheless add antigenic variability, "sampling work" and confusion to the immune system. Maybe the highly variable, volatile, short lived infections in Western countries convey less allergy protection than the permanent, long lived traditional infections (in the earlier chapters we have suggested allergy protective effects of increased sTot-IgE levels triggered by chronic helminth infestations). Our assumption that "volatile" infections convey reduced allergy protection is supported by the fact that the results concerning the allergy protective effect of common childhood infections[523] are inconsistent. Von Hertzen and Haahtela revised four relevant studies: two confirmed a protective effect of common infections, two rejected it.[524] The increased use of antibiotics in Western societies is often mentioned as well in the context of the hygiene hypothesis. In terms of our change theory antibiotics could be considered "terminators of continuity" in two respects. On the one hand antibiotics end or shorten the (originally) permanent, long lived traditional bacterial and helminth infections. On the other hand they bring "discontinuity" to the human gut microflora, e.g. by killing lactobacilli and the above mentioned Helicobacter pylori and consequently enabling re-colonisation with other/new (strains of) biota (antigenic change).[525] All in all, the idea that Western people develop allergies, just because their immune system is "bored" (and thus turns against itself) may be too simplistic. We think that the "modern" immune system is not "insufficiently stimulated"[526] (lack of long-standing infections) but "differently stimulated" (volatile infections/increased antigen variability).

The original "hygiene hypothesis" observation that children with more siblings had less allergies in adulthood may also be explained with "change": maybe large families had less access to change/antigen diversity for economic reasons: less (air) travelling, less exotic food, less contact to the innumerable new antigens of an insatiable consumer market society etc.[527] Mutius compiled an extensive list of studies that show an inverse relationship of number of siblings and type I allergy/atopy.[528] She points out, that most

523 Colds and common respiratory infections.

524 Von Hertzen LC, Haahtela T: 2004: 129.

525 Von Hertzen and Haahtela point out that most studies they revised (six out of eight) showed a positive association between the use of antibiotics during the first two years of life and the subsequent development of atopic diseases (Von Hertzen LC, Haahtela T: 2004: 130).

526 This view was held by Smits et al.: "Reduced infections due to … decreased exposure to microorganisms ... may lead to insufficient stimulation of the immune system" (Smits HH, Everts B, Hartgers FC, Yazdanbakhsh M, 2010: 3).

527 Von Hertzen and Haahtela note that – according to a review of eight studies – it was not possible to show an inverse relationship between early attendance to day care and atopic conditions. This contrasts earlier assumptions (Von Hertzen LC, Haahtela T, 2004: 126).

528 Mutius referred to 14 different studies (Mutius E, 2000: 13).

studies differentiating between the influences of older vs. younger siblings found a stronger "protective effect" of older siblings[529]. Yet her suggested explanations for this observation[530] do not correspond with our line of thought: Maybe we should consider a typical feature of mammalian breeding to be the reason for allergy protection by older siblings: humans tend to offer "the best" to their offspring. "The best" in our post-modern[531] times is "as much as possible from all the available options" (consume, travel etc). The only way of limiting this "over-affluent" care for the young may be "economic competition between the young". The consequence would be that the first sibling gets a "larger blast of (antigen) variability" than the second, where "less change/variability" is affordable as resources have to be shared. On Karkar, the post-modern principle "as much as possible from all the available options" does not really (allergologically) "endanger" the offspring. On the one hand, "theoretically available consume options" are rather limited. On the other hand, the practically existing economic resources (i.e. the key to antigen diversity) have to be shared in the large families. Our 224 informants had a mean number of 4.8 siblings (95% CI 4.5-5.1) and only 3% were the only child. Admittedly, the actual number of siblings may have been overestimated to a certain degree, as traditionally deceased siblings are still counted. This shows not only the respect for the dead, but also the bond between what we call "this and the other world" – or more precisely, the conceptual lack of separation of both realms.

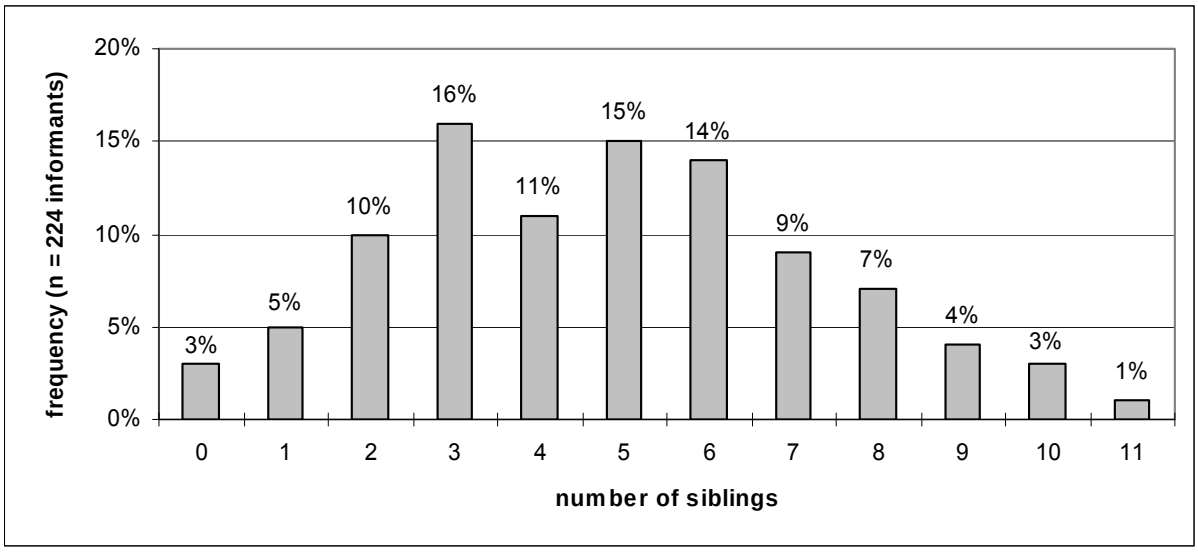


Fig. 48. Frequency distribution of the number of siblings of 224 Karkar Islanders. The mean number of siblings was 4.8 (95% CI 4.5-5.1).

529 Mutius E, 2000: 13. Von Hertzen and Haahtela indicate that the „mechanisms behind the sibling effect are not wholly understood" (Von Hertzen LC, Haahtela T, 2004: 126).

530 Change of fetomaternal immune response with each pregnancy (Mutius E, 2000: 13).

531 For more information on post-modernity see following paragraph.

Higher socioeconomic status has been considered a risk factor for allergy by many authors. The gradient between the affluent and poorer countries provided evidence for this assumption. Yet differences within societies may be even more meaningful, as discrepancies are not likely to be (partially) explained by genetic confounders. The history of allergy already starts with patients of high socioeconomic status: pharaoh Menes from old Egypt, followed by Roman Emperors Augustus and Claudius, and finally Seneca[532]. At the beginning of the twentieth century hay fever was "considered to be a rare disease largely confined to the educated classes of the Western world"[533]. And more recently, "high social class" was shown to be associated with a higher prevalence of (probably often atopic) childhood eczema in the United Kingdom[534] and with increased prevalence of positive SPT in Britain[535]. In the author's opinion, higher socioeconomic status just represents increased accessibility to change and (antigen) diversity. Gupta reported differences in childhood food allergy prevalence in the United States: the prevalence was significantly higher in urban centres than in rural areas.[536] Brabäck L and Kälvesten L described urban living as a risk factor for atopic sensitization in Sweden.[537] Even in the PNG highlands significantly increased allergic sensitization (notably SPT, not RAST!) has been reported in urban compared to rural children as early as 1986.[538] It would be comprehensible that change and antigen diversity is more pronounced in the cities than in the country. Mutius et al. found less current asthma and hay fever in East German children after the reunification than in West German children;[539] this East-West gradient may be explained by comparatively higher change and antigen diversity in West Germany in 1989.[540] Von Hertzen and Haahtela considered "affluence" to be the factor behind the many aspects of modern Western lifestyle,[541] yet we think change and (antigen) diversity are the factors behind "affluence".

Undoubtedly change is getting quicker and quicker, affecting more and more aspects of our "psychological" (social) and "somatic" (biochemical) life. In the author's opinion there are some similar effects of change on mind and body. To begin with, we will cover the psychological and social effects of change. Change is so crucial to our Western present day lifestyle, that sociology declares us living in the "post-modern" age: It is "change" which largely generates the multitude of different new options constituting post-

532 The list of famous subjects who might have been suffering from allergies was compiled by Ring (Ring J, 2014: 11). One may argue, if it had not been people of high socioeconomic status, we would probably have never been informed about their allergies.

533 Jackson M, 2006: 7.

534 Williams HC, Strachan DP, Hay RJ, 1994: 1132ff.

535 Strachan DP, Harkins LS, Johnston ID, Anderson HR, 1997: 6ff.

536 Gupta RS et al., 2012: 856ff.

537 Brabäck L, Kälvesten L, 1991: 14ff.

538 The relevant allergens were D. pt., Aspergillus fumigatus (mould) and dog epithelia (Turner KJ, Dowse GK, Stewart GA, Alpers MP, 1986: 558ff).

539 Mutius E, Martinez F, Fritzsch C, Nicolai T, Roell G, Thiemann H, 1994: 358ff.

540 Genetically the two groups were similar.

541 Von Hertzen LC, Haahtela T, 2004: 124-137.

modernity. Due to an enormously increased diversity of options, nothing seems sure any more yet everything seems possible. This situation is termed contingency**.** From a Western perspective this is certainly true, but it has to be noted, that from a social anthropological point of view, declaring a global post-modern epoch reveals certain characteristics of ethnocentric thinking: a large proportion of the world population in disadvantaged countries is definitely not drowning in a multitude of speedily increasing options. For the privileged, the positive side of change and diversity of options has many aspects: they can choose between different kinds of birth, different school training, different languages, different transport, different places to work and live, different foods, different sexual orientation, different religions and even different ways of burial. The negative side of change and diversity of options affects many areas (including the "health of poor old planet earth") but here we will focus – as a further proof of human egocentrism – exclusively on the health of homo "sapiens sapiens": Change and diversity generates such a magnitude and complexity of information and possibilities that the human being is often unable to "digest" the overwhelming input and to discriminate between the important and unimportant. This "psychological confusion" contributes to the development of the attention deficit hyperactivity disorder (ADHD). Another example for negative health effects of "change and diversity" is occupational burnout: a continuous need for quick decisions in an over demanding working environment leads to mental pressure. The psychological stress of choosing the right option out of a multitude of possibilities may be an important version in the development of occupational burnout.

Now we want to present the somatic effects of change in respect to allergogenesis and draw parallels to psychology. Change and diversity is not only affecting the psychological but also the somatic health of the human being. The Western (post-)modern affluent lifestyle is shaped by omnipresent change which leads to an immensely increased antigen diversity. The chemical industry continuously develops new substances/antigens: pharmaceutical drugs, food additives, preserving agents, cosmetics, synthetic materials, pesticides and herbicides etc. Our food tends to be more and more processed and we do not know whether we thereby generate new antigenic determinants[542] (which could also increase cross-reactivity with other proteins)[543]. Environmental pollution may alter "old" allergens, possibly generating allergen forms with higher allergenic potency. Diesel exhaust particles have been shown to absorb major grass pollen antigens thereby forming "new" complexes and increasing allergen concentration in polluted air.[544] Import delivers "international allergens" on our doorsteps: not too long ago, shrimp, kiwi and mango were rarely consumed in Germany. Visiting an Italian, Thai, Mexican, Indian, Chinese, Vietnamese, Japanese restaurant is not considered too exotic any more. Antigens may

542 As we have already pointed out in chapter 2.3.5, the change of just one amino acid can theoretically turn the allergological status of a protein from "cero/not recognized" into "allergen with high affinity".

543 On the other hand processing of food may also decrease its antigenic potency: e.g. heating of apple juice can alter epitopes in a way, they are not recognized by apple allergic subjects any more.

544 The absorbed major grass pollen allergen was Lol p 1 (Knox RB, 1997: 246ff).

even visit us in the form of intentionally or unintentionally introduced plants: Non-native plants are used for food production or decoration and seeds from highly allergenic weeds have crossed borders hidden in bird- and cropseed (Ambrosia artemisiifolia). Even climate change seems to increase antigen diversity as new plants colonise formerly unsuitable regions.[545] And if some antigens do not find their way to us, we just visit them: the range of travelling started with visiting (antigenically similar) neighbouring villages a century ago and culminates in nowadays intercontinental air trips. There are innumerable possibilities of new antigen contacts when travelling, some are obvious others easily overlooked: travellers may even change their symbiotic bacteria on the skin and in the gastrointestinal system. Many forms of change and increased antigen diversity would not be possible without one key feature of modernity: "mobility". Various of the above given examples are associated with mobility: Exhaust fumes from cars, trucks and planes increase carbon dioxide (thus climate change, invasion of new plants) and produces other forms of air pollution (thus possibly change the antigenic structure of allergens). Without mobility, "new" antigens would not visit us and we would not visit them.

Increased cross-reactivity may be seen as a consequence of an immune system confused by immensely increased environmental antigen diversity. The immune system is overwhelmed by the large number of antigens (psychological analogue: immense information input) and looses the ability to distinguish between the different antigens. Recognition of a certain (irrelevant) environmental antigen (psychological analogue: "the unimportant") may "erroneously" lead to a "better" recognition of a (relevant) environmental antigen which – a priori and per se – exhibits epitopes with major immunogenic properties (psychological analogue: "the important"). Increasing the number of recognized epitopes of a relevant environmental antigen (i.e. adding clonalities) may be the final step to create (or worsen) allergic symptoms. In short, the "somatic confusion" of an increasingly overstrained immune system "mixing up antigen identities" may contribute to the rise of allergies. Cum grano salis we could call allergies "immunological attention deficit hyperactivity disorder (ADHD)": the "somatic" counterpart of the "psychological" ADHD. Maybe in the same way as extreme change (quick and ubiquitous) and diversity confounds our brains (consequence: ADHD, burn out) it also confounds our bodies/immune systems (consequence: type one allergies, maybe even autoimmune diseases). A further feature suggesting parallels between ADHD, occupational burnout, allergies and autoimmune diseases is their similar epidemiology: all these diseases experienced an increase in the (post-)modern times of change and diversity. Admittedly autoimmune diseases are not IgE dominated pathologies, yet the principle of the possibility of cross-reactivity applies to all processes where the immune system has to specifically recognize an antigen structure. In this context it is understandable that autoimmune diseases are often triggered by a harmless cold virus or an ordinary

545 Spread of Ambrosia artemisiifolia (ragweed) in Europe is not only fostered by anthropogenic seed dispersal but also by anthropogenic climate change (Hamaoui-Laguel L, 2015: 1ff.)

gastrointestinal infection.[546] This underlines that infections are not generally allergy protective[547] – especially the unreliable/changeable/short lived ones. One environmental factor which has been blamed to increase e.g. the prevalence of multiple sclerosis (MS) is the lack of natural sunlight. Yet MS prevalence in Spain and Iceland is similar.[548] We think that the existing worldwide overall MS north-south gradient is to a larger extent another manifestation of change/diversity: Antigen "stability" near the equator and antigen "chaos" in northern "modern" societies. As an excessive simplification one could state: Change and diversity lead to "psychological confusion" (ADHD) as well as "somatic confusion" (allergy); and the parallels seem to encompass also the form of treatment of both ailments: "Psychological confusion" is treated with "psychological continuity" ("the discovery of slowness", "Entschleunigung" ["deceleration"], walling-off change and diversity by yoga, stays in cloisters etc). "Somatic (allergological) confusion" is treated with continuous and repeated administration of the same antigen (immunotherapy/hyposensitization).[549]

Change theory of allergy would probably be quite unpopular within (post-)modern affluent populations. It should be much more convenient to scratch "continuity" from a Karkar hut or an old farmhouse wall and develop an injection against allergies. We don't like to abstain from a largely beloved (post-)modern life-style with all its options and possibilities being told: Do what you did and you get what you got. Unfortunately this holds true for most modern non communicable diseases. Even the Karkar would probably willingly accept hay fever or even allergic asthma and atopic dermatitis if accompanied by Western food, medicine, plane tickets, cars and Western housing.

6.4 Conclusion: The connecting links between Western lifestyle and allergies

A major question underlying the section on allergies was: Why is there obviously a positive correlation of Western lifestyle and allergies? Our data point to two possible explanations:

1. There may be a causal relationship between helminth infections within traditional societies and low prevalence of atopic diseases: worm induced increased sTot-IgE may inhibit binding of allergen specific IgE on mast cells and basophils, thereby reducing release of histamine and other pro-inflammatory mediators. In other words: helminth infestation seems to "stabilize" allergen specific activity and may even decrease it.

546 Immune cells do "… not only detect viral antigens, but also cross-react with the body's own molecules" (Doyle C, 2016: 34).

547 We refer to allergies as we have to bear in mind that autoimmune diseases are just variations of allergies where the immune system turns against "harmless self proteins" (autoimmune disease) instead of "harmless environmental proteins" (type I allergy).

548 Browne P et al., 2014: 1022 ff.

549 Remarkably, the other three forms of allergic disease management (pharmacotherapy, allergen avoidance and omalizumab) do not seem to "get to the roots of the problem" in a way immunotherapy does.

2. There may be just an association (yet no causality) between helminth infestations within traditional societies and low prevalence of atopic diseases: worms may represent an indicator of "lifestyle stability", in other words a marker for the absence of change. Change itself may be the causal factor for increased prevalence of allergies in affluent "modern" Western societies, as change is the reason for increased antigen diversity. Increased antigen diversity may potentiate the possibilities for IgE cross-reactivity. And increased cross-reactivity (i.e. increased clonality) allows more IgE bridging on mast cells thereby facilitating the release of histamine and other pro-inflammatory mediators responsible for allergic symptoms. If our considerations were true, why would we consider the "change theory" a step beyond the "jungle/bush hypothesis"? "Jungle" and "bush" may just be associated with low prevalence of allergies. The term "jungle/bush hypothesis" does not tell us what actually protects the people in the jungle/bush from allergies: it could be the lack of change, the continuity of lifestyle and the stability antigen exposure. Thus the "jungle hypothesis" (association) would be the "lack of change hypothesis" (causation) of allergy protection.

Addendum

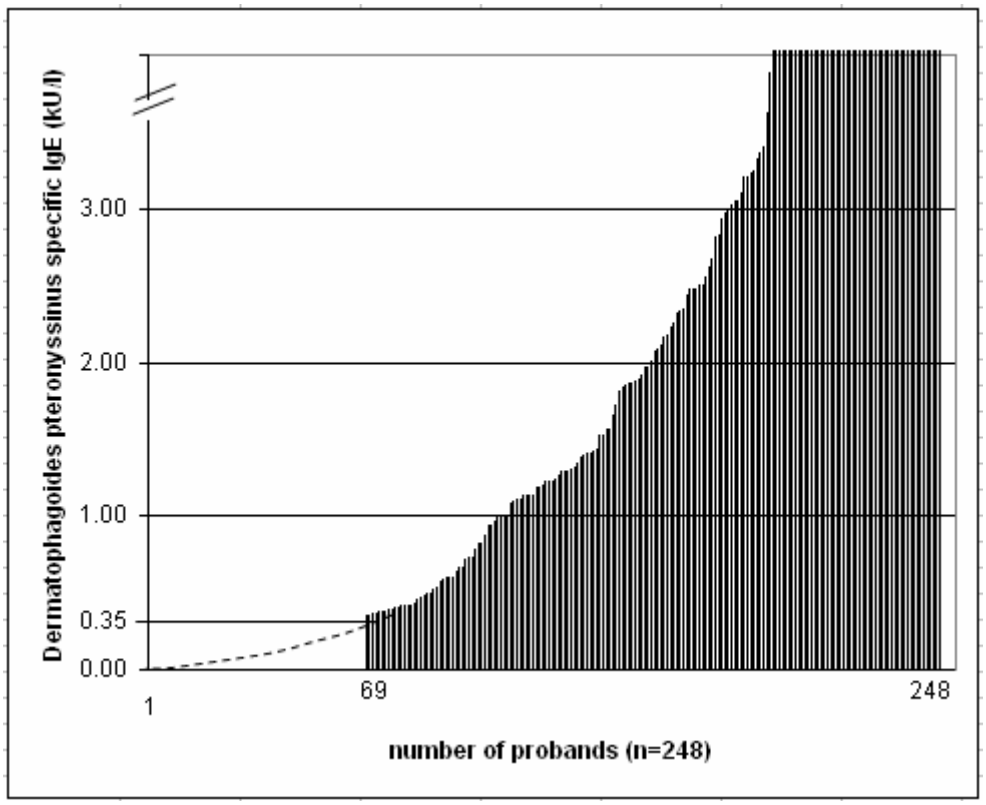


Fig. 49. Karkar islanders with undetectable specific IgE were ascribed the value 0.175 kU/l. As an example D. pt. levels of 248 Karkar are shown. The lowest horizontal line indicates the RAST detection limit of 0.35 kU/l. The author considers 0.175 kU/l a biologically appropriate constant for "specific IgE cero values" (i.e. IgE values too small to be detected) in the context of log transformation: The behaviour of IgE directed against pteronyssinus house dust mite allows an estimation of the curve progression (dotted line) below the detection level. It is not likely that specific IgE abruptly falls to cero when levels are smaller than 0.35 kU/l. The ascribed value of 0.175 kU/l corresponds to -0.76 after $\log_{10}$-transformation. This is in line with van den Biggelaar's approach: in 2001 she worked with a lower D. pt. detection limit of 0.3 kU/l and assigned a value of 0.15 kU/l to negative samples (van den Biggelaar AH et al., 2001: 233); in 2004 she worked with a lower D. pt. detection limit of 0.1 kU/l and assigned a value of 0.05 kU/l to negative samples (van den Biggelaar AH et al., 2004: 894). Thus in our and van den Biggelaar's studies 50% of the value of the specific IgE detection limit was chosen as an estimate for RAST negative measurements. Already 20 years prior to van den Biggelaar's earlier study, Barbee RA et al. ascribed 0.05 kU/l to sTot-IgE negative subjects (detection limit 0.1 kU/l) in order to allow for sTot-IgE log transformation (Barbee RA, Halonen M, Lebowitz M, Burrows B, 1981: 108).

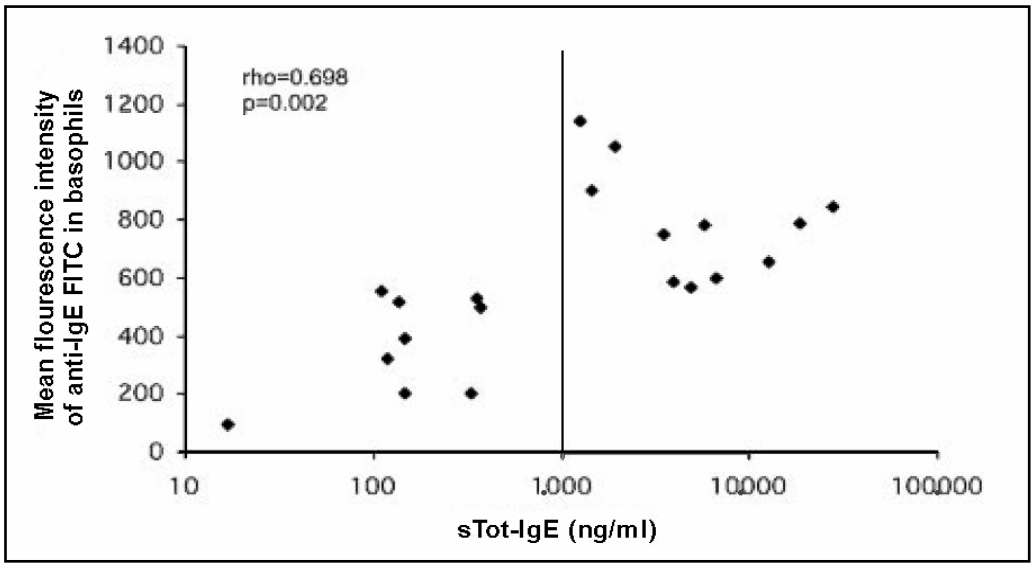


Fig. 50. Raw data from 20 filaria-infected patients in Bethesda, Maryland, US. No evidence of a further increase of mast cell bound IgE over an arbitrary threshold of 1.000 ng/ml sTot-IgE (vertical line added by the author) (Mitre E, Norwood S, Nutman TB, 2005: 4109, fig. 4 B).

Table 3. Composition and properties of the study population.

	Karkar general population [approx. 70.000 total population] [550]			Karkar allergics [approx. 3.000] [551]
Sample type	Convenient sample A	Convenient sample B	Total sample A + B[552]	Deliberately selected allergics[553]
Number of subjects	248	129[554]	282[555]	28
Year of data collection	1997	2002	1997, 2002	2002, 2009[556]
Age years (median)	23	29	24[557]	42
% female	53.2%	50.4%	52.5%	42.9%

550 See chapter 4.1.

551 According to the author's earlier results, 4.4% of the Karkar suffer from allergic diseases. Thus we estimate that only approximately 3.000 allergics live on Karkar Island (4.4% out of 70.000) (see chapter 1).

552 The individuals forming our total sample of the Karkar general population lived in 11 different villages (location of the villages see fig. 6, chapter 4.1).

553 We deliberately selected 28 subjects pre-diagnosed as allergics at Gaubin Hospital, Karkar Island.

554 Only 34 out of the 129 probands of the year 2002 were "new", the remaining 95 were not a convenient sample sensu stricto as they were re-examined subjects from convenient sample A (year 1997).

555 The total sample A + B comprises only 282 different subjects of the general Karkar population, as 95 out of the 129 subjects of year 2002 were re-examined probands from convenient sample A.

556 It was possible to re-evaluate and confirm the allergic status of 25 out of the 28 allergic subjects in the year 2009 (see chapter 5.1).

557 Concerning the 95 re-examined probands we used their age at first contact to calculate the median.

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