Fakultät für Medizin
Zentrum Allergie und Umwelt (ZAUM), Technische Universität München Institut für Allergieforschung, Helmholtz Zentrum München /Lehrstuhl für Molekulare Allergologie

Analysis of IgE-mediated autoreactivity in atopic diseases in children and adults

Tanja Seher

Vollständiger Abdruck der von der Fakultät für Medizin der Technischen Universität München zur Erlangung des akademischen Grades eines
Doctor of Philosophy (Ph.D.)
genehmigten Dissertation.

Betreuer: Univ.-Prof. Dr. Carsten Schmidt-Weber

Vorsitzende: Univ.-Prof. Dr. Agnes Gerlach

Prüfer der Dissertation:
1. Priv.-Doz. Dr. Olaf Groß
2. Prof. Dr. Jan Gutermuth,
   Universität Brüssel, Belgien

Die Dissertation wurde am 15.06. 2015 bei der Fakultät für Medizin der Technischen Universität München eingereicht und durch die Fakultät für Medizin am 02.09.2015 angenommen.

TABLE OF CONTENTS

List of Abbreviations

AA	Acrylamide
AE	Atopic eczema
AID	Activation induced deaminase
ANA	Anti-nuclear antibodies
APC	Antigen presenting cell
APS	Ammonium persulfate
AP1	Activator protein 1
auto-IgE	Autoreactive IgE
BAT	Basophil activation test
Bis-AA	N,N'-Methylene-bisacrylamide
BMI	Body Mass Index
BPE	Bovine pituary extract
BSA	Bovine serum albumine
CCR	C-C chemokine receptor
CD	Cluster of differentiation
CDLE	Chronic discoid lupus erythematosus
CI	Confidence interval
CMF	Cow-milk based non-hydrolyzed infant food
DMEM	Dulbecco´s modified eagle medium
DMSO	Dimethylsulfoxide
DNA	Desoxyribonucleic acid
DTT	Dithiotreitol
EDTA	Ethylendiamintetraacetat
EGF	Epidermal growth factor
eHF-W	Extensive hydrolyzed whey formula
ELISA	Enzyme-linked-immunosorbent assay
Fc	Fragment crystallizable region of Ig
FCS	Fetal calf serum
FcεRI	Fc-epsilon receptor I
FEV1	Forced expiratory volume in one second
FIA	Flourescence immunoassay
FITC	Fluorescein isothiocyanate
FLG	Filaggrin
fMLP	Formyl-methyl-leucyl-phenylalanin
fx5	Allergen mixture of the most frequent food allergens to test in allergy screen
GINI	German Infant Nutritional Intervention study
h	human
HaCaT	Human adult keratinocytes at reduced Calcium concentration and elevated Temperature
HDL	High density lipoprotein
hr	human recombinant
IFNγ	Interferon gamma
Ig	Immunoglobulin
IgE	Immunoglobulin E
IgG	Immunoglobulin G

IL	Interleukin
ISAAC	International Study of Asthma and Allergies in Childhood
LDL	Low density lipoprotein
LISA	Study to investigate the Influence of Life-style related factors on the development of the immune system and allergies in childhood study
LPS	Lipopolysaccharide
MHC	Major histocompatibility complex
mOVA	Ova membrane localized
NAbs	Natural (auto)-antibodies
NaCl	Sodium chloride
NFκB	Nuclear factor kappa B
NHBE	Normal human bronchial epithelial cells
nm	nanometer
MnSOD	Mangan superoxide dismutase
OR	Odds Ratio
p	p-value, significance level is 0.5
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate Buffered Saline
PC	Patients Control
PE	Phycoerythrin
pHF-C	partial-hydrolyzed casein formula
pHF-W	partial-hydrolyzed whey formula
pKc	primary keratinocyte
RAD	Respiratory rate; Accessory muscle use; Decreased breath sounds
RAST	Radio-Allergo-Sorbent Test
RIPA	Radioimmunoprecipitation assay buffer
ROC	Receiver operating characteristic
SCLE	Subacute cutaneous lupus erythematosus
SCORAD	Scoring Atopic Dermatitis
SDS	Sodium-Dodecyl-Sulfate
SFAR	Score for allergic rhinitis
sIgE	specific IgE
SLE	Systemic Lupus Erythematosus
sOVA	soluble ovalbumin
STAT6	Signal transducer and activator of transcription 6
sx1	allergen mixture of the most frequent aeroallergens to test in allergy screen
TBS	Tris-buffered saline
TBS-T	Tris-buffered saline with added Tween®
TCR	T-cell receptor
TEMED	Tetramethylethylendiamine
TGF	Transforming Growth Factor
Th1	T-helper 1
Th2	T-helper 2
TMB	Tetramethylbenzidine
TNF	Tumor Necrosis Factor

1. ABSTRACT

Background: Atopic diseases are IgE-mediated and bear an immunologic reaction towards usually innocuous environmental factors to what healthy subjects are tolerant. Atopic diseases can manifest and pass into chronic forms particularly atopic eczema and allergic asthma. The cause of the manifestation is rather ambiguous; furthermore it is unclear why atopy manifests in some individuals but not others, although the exposure to environmental allergens is similar. One hypothesis is the occurrence of autoreactive IgE that targets primarily tissues and cells of allergic inflammation. Due to tissue damage intracellular proteins are released and recognized by autoreactive IgE antibodies. However, if auto-IgE is cross-reactive to human self-proteins or whether it is produced by neo-synthesis subsequently to autosensitization is not clear so far.

Methods: We established a high-throughput immunoassay in 384-well format to screen auto-IgE in serum. As target protein we used whole cell protein of keratinocytes, bronchial epithelial cells. We investigated adult atopic patients with different skin diseases as atopic eczema or asthma to figure out whether those patients have higher quantity of auto-IgE in serum. A small group of patients with heterogenous age having atopic eczema was analyzed initially, before we investigated children at the age of ten out from the GINI/LISA birth cohorts to reveal whether auto-IgE is generated physiologically and if auto-IgE is associated with atopic diseases in infancy.

Results: We observed two different properties of auto-IgE in two periods of life. During childhood auto-IgE was generated physiologically and there is evidence that auto-IgE is a natural occurring antibody. No association of clinical manifestation of atopy and auto-IgE in children could be assessed. Furthermore, sensitization enhanced the quantity of auto-IgE reactivity and there is evidence that the occurrence of auto-IgE is protective towards eczema. In contrast, adults having atopic eczema showed more frequent and higher auto-IgE reactivity. In asthmatic patients auto-IgE reactivity was significantly associated with the control status of the disease. The less asthma was controlled the higher the auto-IgE reactivity. Healthy adult subjects do not show auto-IgE reactivity.

Conclusion: The study indicates dissimilarities of autoreactive IgE between childhood and adulthood. Whereas auto-IgE seems to represent a natural response that is not necessarily associated with pathology in childhood, the occurrence of auto-IgE is disease associated in adults and one can assume a pathomechanistic effect in adult atopic patients. Based on these new aspects of auto-IgE the present study represents fairly new aspects on autoallergy. One can assume a pathomechanistic effect in adult atopic patients.

2. INTRODUCTION

Atopic diseases are IgE-mediated and bear an immunologic reaction towards usually innocuous environmental substances, against what a healthy system is not reactive but tolerant. In atopy the immunologic reaction is an overreaction leading to typical symptoms of rhino conjunctivitis, asthma or atopic eczema (WÜTHRICH 1989). Particularly allergic asthma and atopic eczema can manifest and pass into chronic forms of atopy. The cause and why only a part of the atopic patients is affected of the chronic manifestation are still unclear. Moreover patients with severe atopic eczema or asthma are difficult or impossible to cure or even to treat and represent an unmet clinical need.

Although atopic eczema and asthma are atopic diseases it is reported, that an exacerbation of chronic and severe forms can occur in the absence of exposure to the initiating allergen. These observations led already in the 1940s to consider autosensitization as a conceivable pathomechanism to cause severe atopic dermatitis (HAMPTON and COOKE 1941). Until today there is no distinct concept of a helpful therapy of the chronic and severe forms of atopy as asthma and atopic eczema. Moreover there is still or rather again ongoing discussion about the possible pathophysiology of autosensitization in atopic eczema (NATTER et al. 1998; VALENTA et al. 1996). To investigate and understand the role of auto-IgE antibodies and its impact on atopic diseases particularly in atopic eczema and allergic asthma we established a procedure to measure auto-IgE reactivity in serum, we analyzed different atopic patient groups and a part of two German birth cohorts to examine its role in healthy and atopic children.

The allergic immune response is an exaggerated, specific defense reaction due to the sensitization towards normally innocuous environmental substances leading to a pathogenic hypersensitivity (RING et al.). The task of the immune system is to identify foreign substances that penetrate into the body and its elimination. Antigen presenting cells (APC) recognize, process and present antigens to T helper cells that are located in the lymph nodes, skin and mucosal tissues with the following expression of T helper 2 response (Th2) cytokines as Interleukin-4 (IL-4), IL-5 and IL-13. Finally, B cells are activated and produce immunoglobulins. Allergy is thought to be driven by a Th2-response and the attendant cytokines rather than Th1 cytokines as IFN-γ (interferon gamma) (MOSMANN and COFFMAN 1989; DEL PRETE et al. 1993). A typical Th2-response leads to the stimulation of B cells to produce IgE which can bind on the FcεRI located mainly on the surface of mast cells, basophils or eosinophils. Entered allergens cross-link the bound IgE on the cell surface and lead to degranulation of the cells (KINET 1999). The degranulation bears the effector function of e.g. mast cells as soluble vasoactive mediators

that are preformed and stored in cytoplasmic granules and released upon cross-linking of IgE molecules (KINET 1999). Biogenic amines with its major agent of histamine, enzymes and proteoglycans (tryptase, chymase) and lipid mediators (e.g. prostaglandins) as well as cytokines and chemokines are released by degranulation causing the acute allergic reaction within few minutes (BERGER et al. 1999; FOKKENS et al. 1992; STONE et al. 2010). Allergic inflammation bears enhanced mucus secretion in the lung, itchiness, pain, bronchoconstriction, vascular permeability vasodilatation and immune cell recruitment (ABBAS et al.). Depending on the type of cells involved in the allergic reaction an immediate type, specified above and a late phase reaction are feasible. The late phase reaction is specified by the integration and activation of allergen-specific, cytokine producing CD4+ T cells accompanied by the influx of eosinophils, neutrophils and macrophages. The combination of immediate and late phase reaction is designated as immediate hypersensitivity that is termed in clinical medicine as allergy or atopy (ABBAS et al.).

The best-known function of IgE is its ability to degranulate mast cells via the high-affinity receptor FcεRI and the subsequent triggering of the immediate-hypersensitivity as described above. Further IgE can facilitate antigen capture by B cells which act as antigen presenting cells than (GUSTAVSSON et al. 2000; HEYMAN et al. 1993) and the regulation of its receptors FcεRI and CD23 (FcεRII)(KISSELGOF and OETTGEN 1998; YAMAGUCHI et al. 1997) and the elimination of helminths (OBATA-NINOMIYA et al. 2013; ROSENBERG et al. 1971; ROSENBERG et al. 1970).

Preceding to the account of the function of IgE some specific processes are required. IgM antibodies that are initially generated of B cells, due to the absence of T helper signals can be switched following an appropriate stimulation. B cells act as APC and present processed antigens in the context of MHC class II on antigen specific T helper cells previously activated by dendritic cells. MHC-peptide complex recognition succeeds via the T cell receptor (TCR) and stimulates B cell response via the induction of CD40 ligand (CD40L) expression on the surface of the T cell that engages the CD40 receptor on the surface of antigen-stimulated B cells. In response to the CD40 engagement NF-κB and AP-1 are activated and translocate into the nucleus stimulating the proliferation of B cells and an increased synthesis of immunoglobulins. Simultaneously the enzyme activator-induced deaminase (AID) is induced, an enzyme that is crucial for isotype switching and somatic mutation. Consequently, in response to the CD40 engagement the process of heavy chain isotype switch is activated.

Beside the mentioned activation of CD40 a second signal is required to induce the production of IgE which is provided by IL-4 and IL-13. The binding of IL-4 or IL-13 on B

cells leads to the activation of Stat6 and the initiation of germline transcription at the Cε locus, but only through the synergistic effect of NF-κB and Stat6 the Iε promoter can be activated (ABBAS et al.; GAUCHAT et al. 1990; DEL PRETE et al. 1988; SHEN and STAVNEZER 1998).

The variety of IgE-mediated or allergic diseases is wide-ranging, to name but a few; seasonal and perennial rhinitis/rhino conjunctivitis, urticaria, asthma bronchiale, contact eczema, atopic eczema, food allergy and finally the anaphylactic shock (RING et al.).

To confirm the diseases as allergic, serum tests for total and specific IgE are performed, as most of the patients have elevated serum IgE levels, skin prick testing or patch testing are the standard diagnostic procedures (HANIFIN, JON M. and RAJKA, GEORG 1980). Specific serum IgE is first tested using a screening of a mixture of different food allergens (fx5 (mixed allergen test); ovalbumin, milk, flour, codfish, peanut, soybean) and aeroallergens (sx1 (mixed allergen test); grass, rye, mugwort, birch, Cladosporium herbarum, house dust mite, cat, dog, mugwort) and are measured by **F**luorochrome-**I**mmuno-**A**ssay (FIA) formerly analyzed by **R**adio-**A**llergo-**S**orbent-**T**est (RAST). In the following RAST will be used in reference to the analysis of specific IgE. These allergen mixtures test allergens with the most frequent sensitization.

There finds more specific tests to define e.g. asthma. By using spirometry as a pulmonary function test assay to define the **F**orced **E**xpiratory **V**olume in one second (FEV1). Atopic eczema is more defined by the **SC**oring **A**topic **D**ermatitis (SCORAD) as there is no defined diagnostic tool for the diagnosis of atopic eczema it is generally diagnosed on physical examination and visual inspection of the skin by a dermatologist.

The prevalence of the three atopic disorders is divergent around the world, without a clear pattern for the change of prevalence over the last years. Phase I and III of the **I**nternational **S**tudy of **A**sthma and **A**llergies in **C**hildhood (ISAAC) was designed to achieve data for trends in prevalence of asthma, rhino conjunctivitis and atopic eczema over time. Most study centers assessed an increasing change for at least one disorder. The increase of prevalence occurred more frequent in the younger age groups of six to seven years old children than in the older age group (13- 14 years). The increase of prevalence was greatest for eczema in the younger group, eczema in the older age group have leveled off or even decreased. For asthma the decrease of prevalence was more common at locations with formerly high prevalence and more study centers report a decrease in asthma symptoms for 13-14 years old children. Anyhow the prevalence for atopic eczema in adolescents in affluent countries leveled off or even decreased, whereas eczema symptoms rose in developing countries (ASHER et al. 2006; FLOHR 2010; WILLIAMS et al. 2008). This

fast change of prevalence over a time period of five to ten years argues that environmental factors are key factors for the change of prevalence of atopic disorders. Nevertheless genetic factors as a cause for atopic eczema are already reported.

Atopic eczema is a chronic or chronically relapsing inflammatory skin disease attended by pruritus that results in scratching, lichenification and prurigo papules (HANIFIN, JON M. and RAJKA, GEORG 1980; LEUNG and BIEBER 2003). Transcriptome analysis of atopic eczema patients revealed in several differentially expressed genes in different cell types as CD4+ T cells, monocytes, eosinophils and in skin lesions, as nicely reviewed from Saito (SAITO 2005). The most established genetic mutation leading to disturbed skin barrier function accounting for an increased risk for eczema is the mutation of the filaggrin (*FLG*) gene, coding for a protein located in the stratum corneum (FLOHR 2010; O'REGAN et al. 2008). Still the *FLG* mutation is not the only factor leading to atopic eczema as on should consider that around 40 % of all carriers of null mutation of the *FLG* allele never show any signs of eczema. On the other side patients with filaggrin-mutation related eczema have more persistent disease and a greater risk of asthma and allergic sensitization, in addition a filaggrin mutation is positively associated with food allergy (FILIPIAK-PITTROFF et al. 2011; HENDERSON et al. 2008). Still, there are more genetic factors that account for atopic eczema as parental atopy is one of the greatest risk factors for children to develop atopy (VON BERG et al. 2012; DOLD et al. 1992). Moreover, several environmental risk factors as diet and nutrition (ELLWOOD et al. 2001; WEILAND et al. 1999), climate (WEILAND et al. 2004), infections (AABY et al. 2000; BACH 2002; FLOHR et al. 2006; MATRICARDI et al. 1997; VON MUTIUS et al. 2000), combustion byproducts (DIAZ-SANCHEZ et al. 1997; DIAZ-SANCHEZ et al. 1996) or pollens (BURR et al. 2003) are proofed or discussed as risk factors. Anyhow, no single genetic or environmental risk factor explains the occurrence of atopy and the related diseases, so as to a multifactorial assortment leads to the disease.

Allergic asthma is the second atopic disease that evolves during childhood although at a later time point as atopic eczema. This phenomenon of atopic progression and manifestation is widely established as the *atopic march*. All three diseases, asthma, rhinitis and atopic eczema are all accompanied by elevated serum IgE levels and peripheral eosinophilia but atopic eczema is often the initial step into the atopic march as the further stages are asthma and allergic rhinitis often associated with food allergy already in early stages of atopy (LEUNG et al. 2004; SPERGEL and PALLER 2003).

The last years the "traditional" course of the atopic march came into discussion as Illi et al. discussed already in 2004 if there is an early asthmatic co-manifestation concurrent with atopic eczema rather than a progressive atopic march (ILLI et al. 2004). Furthermore,

there is an ongoing discussion whether allergic sensitization occurs secondary to an impaired epidermal barrier in eczematous skin. As oral tolerance is induced during infancy due to oral ingestion of different potentially allergenic food, the question rises whether the intrusion of food allergens and aeroallergens onto the inflamed skin and its penetration through the impaired barrier of the eczematous skin leads to allergic sensitization (FLOHR 2010). As atopic eczema is referred to as the cutaneous manifestation of a systemic disorder and occurs as an "entry point" for subsequent allergic diseases these would support the later hypothesis.

Anyhow the prevalence of asthma varies in different areas of the world likewise to atopic eczema. The one-year prevalence varies from 1 % in Indonesia to 30 % in Great Britain (ASHER et al. 2006; FLOHR 2010). Asthma bronchiale is a chronic inflammatory respiratory disease attended by recurrent episodes of wheeze, cough and breathlessness and respiratory obstruction. Asthma goes along with a previous sensitization and IgE response to aeroallergens as e.g. grass- or birch pollen, house-dust mites, cat or dog dander and eosinophilic airway inflammation (DOUWES et al. 2002; GUILBERT et al. 2004; MATRICARDI et al. 2009; SIMPSON et al. 2006). The release of mast cell mediators in succession of IgE cross-linking leads to mucosal edema, mucus production and smooth muscle constriction in the bronchial mucosa and a following inflammatory infiltration (ABBAS et al.; OETTGEN and GEHA 1999). Asthma is a complex and heterogeneous disease difficult to diagnose (DOUWES et al. 2011; FRANKLAND 1959). In 2009 the *„Programm für Nationale Versorgungsleitlinien"* changed within their *„Versorgungsleitlinie Asthma"* the classification for asthma. Before asthma was classified in classes of severity, due to its inappropriate surveillance on patients ingesting therapeutics the graduation was changed and is today compiled on the grade of asthma control (BUNDESÄRZTEKAMMER et al. 2009).

As already discussed in the hygiene hypothesis in 1980s – that suggests a lack of infectious agents during early life (STRACHAN 1989) which would favoring a Th1 prone cell response, a deficiency of Th1 responses in the developing immune system is thought leading to a Th2 prone immune system and increases the risk of allergic sensitization (BACH 2002). A further aspect of atopy is the gut microbiome (ABRAHAMSSON et al. 2012; BISGAARD et al. 2011; SJÖGREN et al. 2009) and Tregs seem to play a crucial role in the process of allergic sensitization (AKDIS et al. 2004; CARAMALHO et al. 2003; GRINDEBACKE et al. 2004).

In addition to the hygienic hypothesis and the non-encountering of molds, bacteria and viruses during early infancy several more factors are associated with allergic sensitization and manifestation and to be positively associated with atopy. The highest risk is parental atopy, the family history for allergy doubles the risk for atopic eczema (VON BERG et al.

2012). As mentioned above the mutation of certain candidate genes still have to become characterized, but some seem to be relevant for the development of atopic diseases (FILIPIAK-PITTROFF et al. 2011; HENDERSON et al. 2008; O'REGAN et al. 2008; RAJE et al. 2014; SHARMA et al. 2015; WHEATLEY et al. 2002). Further, breast feeding or hydrolyzed formula nutrition for infancies is discussed to be preventive against allergic sensitization (VON BERG et al. 2003; GDALEVICH et al. 2001). Environmental factors as traffic-related air-pollution but even indoor and outdoor air pollution e.g. fine particles, ozone are reported to be associated with atopy (JUNG et al. 2012; KÜNZLI et al. 2008; SILVERMAN and ITO 2010; SPIRA-COHEN et al. 2011).

Although some risk factors are known and confirmed but it remains still unclear why atopy develops and chronifies in some individuals but not others, despite similar exposure to allergen (WHEATLEY et al. 2002). For chronic airway inflammation in the case of perennial allergen sensitization and exposure it has been postulated that chronic symptoms result from the late-phase inflammatory response, which occurs many hours after the acute reaction dependent on eosinophils (OETTGEN and GEHA 1999).

The chronic forms of atopic diseases are in fact accompanied with a mixed type of Th1 and Th2 immune response. IFN-γ expressing cells, hence a Th1 response in skin lesions of atopic eczema patients was already shown in 1994 (GREWE et al. 1994; HAMID et al. 1994). Consequently atopic eczema is not a distinctive disease of an IgE-mediated immediate-type hypersensitivity response. Moreover it was reported that acute atopic eczema is associated with increased numbers of infiltrating CD4+ T cells compared with normal skin, suggesting a specialized form of delayed type hypersensitivity (HAMID et al. 1994; LEUNG et al. 1983; WILLEMZE et al. 1983). Acute atopic eczema is genetically different in its expression pattern of interleukins and cytokines as skin from acute atopic eczema contains no significant amount of mRNA of IFN-γ and IL-12. In contrast skin lesions from chronic atopic dermatitis shows lower amount of mRNA of IL-4 and IL-13 but increased numbers of cells expressing mRNA of IL-5, GMCSF, IL-12 and IFN-γ. Activated T cells induce apoptosis of keratinocytes contributing to the spongiosis seen in acute atopic eczema – mediated by IFN-γ derived from T cells which upregulates Fas on keratinocytes by Fas-ligand on T cells (LEUNG and BIEBER 2003). Anyway, new therapeutic strategies including the human monoclonal antibody targeting the IL-4 receptor α lead to rapid improvement of atopic eczema disease activity (BECK et al. 2014; HAMILTON et al. 2014).

In contrast to atopy, autoimmunity is mainly Th1 driven (FOULIS et al. 1991; KALLMANN et al. 1997; VAN DER VEEN et al. 1993; VOSKUHL et al. 1993). For this reason it was established for long that allergy and autoimmunity are mutually exclusive as the immune response is a

Th1 and Th2 polarized response and the acting cytokines are opposing. The main difference between allergy and autoimmunity is the seasonal restricted occurrence of allergic symptoms dependent on the allergen exposure and the generally chronically manifested, often relapsing disease of autoimmunity because the impossibility to be cleared by the immune system. However, a given percentage of atopic patients develop a chronic manifestation of the disease even during absence of the initiating, exogenous allergen which is more disclosed to the disease pattern of autoimmunity. Due to this phenomenon the verification of autoreactive IgE antibodies was provoked already in the beginning of the 20th century. In 1921 Whitfield reported eczema as a manifestation of autosensitivity, as his patients were sensitized towards their own skin (WHITFIELD 1921). Van Leeuwen et al. demonstrated (auto-) sensitivity against human skin dander in atopic patients (LEEUWEN 1926; LEEUWEN et al. 1922). Furthermore this was confirmed in several other reports (HAMPTON and COOKE 1941; KELLER and MARCHIONINI 1926; SIMON 1945). Not before the 1990´s Valenta et al. refreshed the issue of autoallergy via the isolation of cDNAs coding IgE-binding proteins or autoallergens respectively and the subsequent cloning and identification of autoantigens (NATTER et al. 1998; VALENTA et al. 2000; VALENTA et al. 1998). Henceforth, the concept that autosensitization, referred to as autoallergy, could play a pathomechanistic role in atopic eczema was reawakened. The discovery that environmental proteins share structural and immunologic similarities with human proteins scooped this hypothesis ahead. Some proteins found with homology; plant profilins, manganese superoxide dismutase (MnSOD) (mold-derived) and dog serum albumin (CRAMERI et al. 1996; SPITZAUER et al. 1994; VALENTA et al. 2000; VALENTA et al. 1998). Later on in 1996 autoreactive IgE antibodies in sera from atopic eczema patients were verified (VALENTA et al. 1996). Over the intervening years several potential autoallergens were identified, few are selected in Table 1.

Table 1: List of identified autoallergens,gene names and – if applicable the homologous antigen

Autoallergen	Genes	Homologous antigen	Function	Reference
Hom s 1 (tri-snRNP associated protein)	SART-1		poly(A)-RNA-binding	(VALENTA et al. 1998; WHEATLEY et al. 2002; ZELLER et al. 2008)
Hom s 2	a-NAC		DNA-binding, Transcription coactivator activity	(MITTERMANN et al. 2007; MOSSABEB et al. 2002; NATTER et al. 1998; ZELLER et al. 2008)
Hom s 3	BCL7B		Oncogene, actin binding	(NATTER et al. 1998; ZELLER et al. 2008)
Hom s 4 (Ca2+-uptake protein 1, mitochondrial)	MICU1	Cyp c1 Phl p7	Ca^2+-binding, protein hetero-dimerization activity	(AICHBERGER et al. 2005; GLASER et al. 2008; ZELLER et al. 2008)
Hom s 5 (Keratin type II)	KRT6A		Structural constituent of cytoskeleton	(NATTER et al. 1998; ZELLER et al. 2008)
ribosomal protein P2	RPLP2	Asp f8	RNA binding, role on elongation step in protein synthesis	(MAYER et al. 1999; ZELLER et al. 2008)
MnSOD	SOD2	Asp f6 Mala s11 Hev b10	Catalytic activity 2 superoxide+2 $H^+ = O_2+H_2O_2$	(CRAMERI et al. 1996; ZELLER et al. 2008)
Profilin	PFN1/PFN2	Bet v2	Actin binding	(VALENTA et al. 1991; ZELLER et al. 2008)
Thioredoxin	TXRD	Mala s13 Tri a 25 Zea m25 ZmTRXh2	Thioredoxin + $NADP^+$ = Thioredoxin-disulfide + NADPH	(GLASER et al. 2008; LIMACHER et al. 2007;ZELLER et al. 2008)

Autoallergens have been assured in a variety of human cell and tissue types (APPENZELLER et al. 1999; NATTER et al. 1998; SEIBERLER et al. 1999). Still, whether autosensitization and the succeeding recognition of human self-proteins by autoreactive IgE has actually an impact on the pathomechanism, the aggravation and the chronic manifestation of atopic eczema or other IgE-mediated or atopic diseases is not elucidated to date. A further possibility is the existence of auto-IgE as an epiphenomenon.

The proposed mechanism of autoallergy is shown in Figure 1. An initial allergic sensitization and the second contact to the exogenous allergen is supposed to occur and leading to allergic inflammation. Allergic inflammation is triggered including the degranulation of mast cells with the following symptoms and criterion of atopy, mucus secretion, itch, bronchoconstriction, vasodilatation and increased vascular permeability and the recruitment of immune cells leading to a tissue damage (ABBAS et al.). In cases of atopic eczema pruritus is triggered amongst others through the secretion of IL-31 by Th2 cells. Pruritus provokes itchy skin and scratching and a subsequent barrier break through mechanical destruction further supported by microbes (e.g. *Staph. aureus)* and physical or

chemical factors. Hence, the first release of intracellular and membrane self-proteins occur. Allergic inflammation causes tissue damage and the release of self-proteins and a conceivable initial autosensitization simultaneously. The chronic manifestation of atopic eczema for example is accompanied by the participation of Th1 cells perpetuating chronic inflammation via the release of inflammatory cytokines as IFN-γ and TNF-α and subsequent continuing tissue damage. In chronic atopic eczema lesions increased numbers of eosinophils, due to increased expression of IL-5, were reported leading, due to their granule mediators to tissue damage as well. The multifactorial triggered barrier break and tissue damage leads to the release of autoantigens and a subsequent autosensitization and the reaction with the present IgE. Moreover, inflamed and activated epithelium releases the cytokine thymic stromal lymphopoietin (TSLP) and local dendritic cells (DC), namely Langerhans cells, subjected to TSLP will induce further Th2 response and the generation of autoreactive IgE antibodies, those will bind on DC and amplify the inflammatory immune response (AURIEMMA et al. 2013; HAMID et al. 1994; TANG et al. 2012; VALENTA et al. 2009). Further, in healthy subjects Th2 sensitization in the skin is inhibited by regulatory T cells (Treg), however in atopic patients a lack of Treg or the loss of their immunosuppressive function results in the allergic reaction (LESIAK et al. 2012; OU et al. 2004). Actually this fact was left aside in the figure illustrating the proposed mechanism on autoallergy. To sum up, several factors that perpetuate tissue damage and barrier break (exogenous factors, enhanced quantity of eosinophils, inflammatory cytokines) leading to the continuous release of autoantigens that are not eliminable and perpetual present leading to the maintenance of the vicious circle of (auto-)inflammation Figure 1.

The mechanisms which cause autosensitization are not understood but it is supposed that IgE-autoreactivity can be driven by two possible mechanisms; the first mechanism is the new synthesized IgE that is specific to autoallergens bearing autoreactivity, the second mechanism favors IgE cross-reactivity. For the latter the reactive auto-IgE originates from the one that is specific to exogenous allergens and bears cross-reactivity due to structural similar epitopes of self-proteins. Moreover, the combination out of cross-reactivity and neo-synthesized IgE represent a further possibility.

Cross-reactivity of human self-proteins and environmental allergens were already mentioned above and account for several publications in which structural similarities between those were investigated (GIEHL et al. 1994; VALENTA et al. 1991).

In addition, the involvement of autoreactive T cells in atopic eczema is already described. Mossabeb et al. isolated peripheral mononuclear blood cells (PBMC) from patients with atopic eczema and stimulated the cells with Hom s2 (α-NAC) and induced

lymphoproliferative response in a Hom s2 reactive patient (MOSSABEB et al. 2002). Mittermann et al. measured the cytokine response of the α-NAC stimulated PBMC and indicated high amounts of IFN-γ that in succession lead to impairment of keratinocytes and therefore to further barrier disruption of the skin (MITTERMANN et al. 2007). Activated Tcells induce apoptosis of keratinocytes contributing to the spongiosis seen in acute atopic eczema – mediated by IFN-γ derived from Tcells which upregulates Fas on keratinocytes by Fas-ligand on Tcells (LEUNG and BIEBER 2003). One further investigation specified the proliferation to skin homing T cells in vitro in PBMCs derived from atopic eczema patients (HERATIZADEH et al. 2011; HRADETZKY et al. 2014; MITTERMANN et al. 2007). Schmid-Grendelmeier et al. demonstrated PBMC proliferation likewise after stimulation with allergens that were tested as IgE-reactive in the patients before e.g. MnSOD (SCHMID-GRENDELMEIER et al. 2005). These results indicate the involvement of auto-reactive, α-NAC and MnSOD specific T cells respectively.

Although several studies give evidence of the pathomechanistic impact of autoreactive IgE antibodies on the aggravation and manifestation of chronic atopic diseases and the accompanied tissue inflammation the chance to run riot as auto-IgE is merely an epiphenomenon it is still not deprecated.

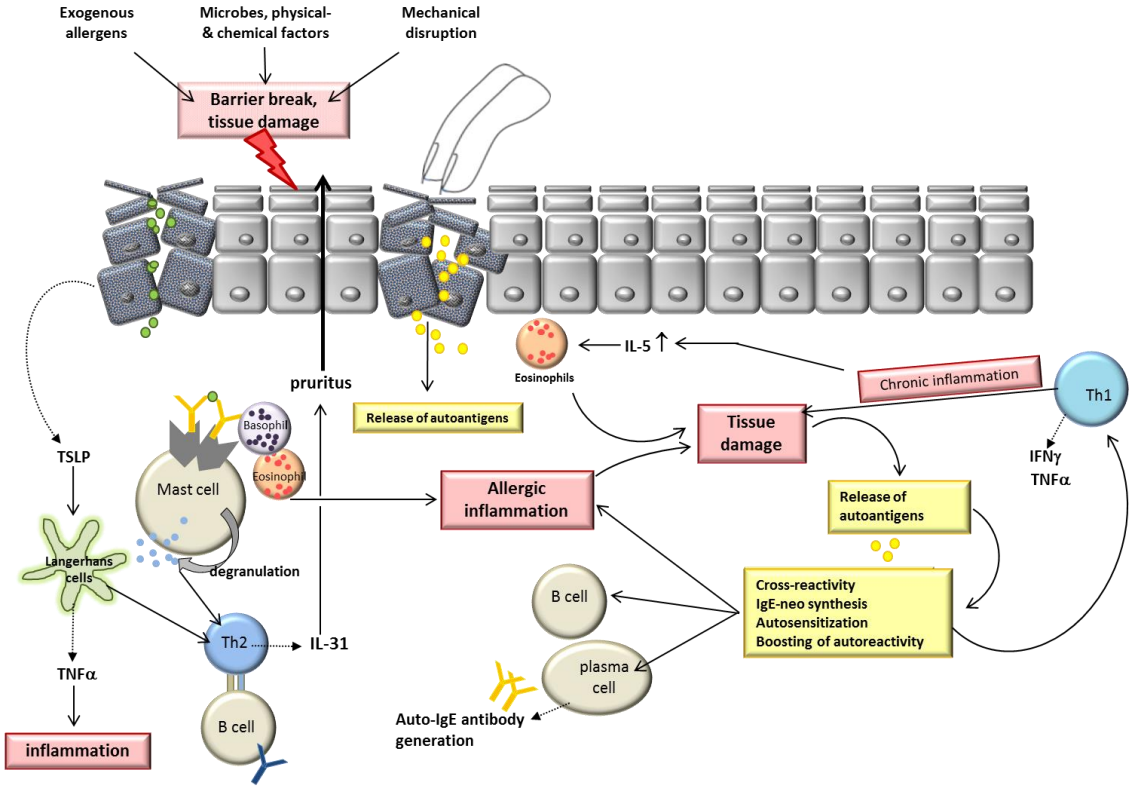


Figure 1: . The proposed mechanism of autoallergy.
To date it is still unclear how and why allergic diseases manifest into chronic forms as atopic eczema or asthma. The proposed mechanism of autoallergy is based on an initial allergic sensitization and the following degranulation of mast cells (eosinophils/basophils) in a second contact to the exogenous allergen *(green circles)*. This process drives an allergic inflammation *(middle red box)* open out into including itch, vasodilatation and immune cell recruitment with a subsequent tissue damage. Further a Th2 response is initiated and IL-31 is secreted that triggers pruritus provoking itchy skin and scratching. This mechanical disruption plus further exogenous factors as e.g. *Staphylococcus aureus* or physical or chemical compounds leading to a boost of barrier break and tissue damage and the first release of autoallergens or self-proteins *(yellow circles)* respectively. Due to the release of autoantigens the immune system is auto-sensitized and a boost of inflammation is executed by cross-reactivity of auto-IgE or even autoallergen specific IgE *(lowermost yellow box)* that is newly synthesized by plasma cells. Ongoing reaction leads to activation of Th1 cells and the secretion of pro-inflammatory cytokines as TNFα and IFNγ causing chronic inflammation. In addition already initialy inflamed and activated epithelial cells release TSLP and activate dendritic cells (Langerhans cells) that are located in the skin that lead in turn to inflammation. As IL-5 is increased in chronic atopic eczema here the activation and survival of eosinophils is enhanced and granule mediators leading to a further tissue damage and an ever and anon release of autoantigens into the interstitium and to the maintenance of the vicious circle of autoallergy and inflammation. **(Auriemma et al. 2013; Hamid et al. 1994; Tang et al. 2012; Valenta et al. 2009)**

3. Materials and Methods

3.1 Human clinical studies

3.1.1 Retrospective analysis of auto-IgE in serum

For the establishing and for the first results of auto-IgE reactivity we quantified sera from the Department of Dermatology and Allergy Biederstein. Subsequently we utilized different study-cohorts retrospectively, which lead to the results that are described within this thesis.

3.1.1.1 Study population of atopic eczema patients from the Dermatology at the Biederstein

We obtained sera that were collected at the Department of Dermatology and Allergy Biederstein. We used n = 105 sera to analyze auto-IgE reactivity retrospectively in this study. All study-participants suffered from atopic eczema. We retrieved data as age, sex, body height and weight, SCORAD, total IgE and data about the actual therapy from each of the patient. Furthermore, we tried to retrieve more medical data for of the patient, however as the medical records were not complete some of the analysis could not be done. Table 2 shows the study population characteristic.

Table 2: Study population characteristic of patients with atopic eczema screened for autoreactive IgE retrospectively.

Screened patient group	n	105
sex (male/female)	n (%)	50 (47.6)/55(52.4)
age	mean (sd)	21.9 (20.3)
BMI	mean (sd)	20.34 (4.9)
total IgE [kU/L]	mean (sd)	2135(6056)
SCORAD	mean (sd)	33.7(16.0)
AE-profile		
Intrinsic AE/ extrinsic AE	n (%)	51(48.6)/54(51.4)

3.1.1.2 Auto-IgE analysis in a birth cohort combined from GINI and LISA birth cohorts

To investigate auto-IgE reactivity and have a possibility to use these as a longitudinal study we combined two ongoing german birth cohorts. The German Infant Nutritional Intervention study (GINI) and the LISA (Influence of Life-style related factors on the development of the immune system and allergies in childhood study) cohort, as prospective birth cohort studies. Aim of the GINI birth cohort is to investigate the

influence of nutritional intervention during infancy as well as air pollution and genetics on allergy development (KOHLBOECK et al. 2012). Between September 1995 and June 1998 a total of 5.991 healthy full-term newborns were recruited in two different regions of Germany; Munich as an urban area and Wesel as a rural area. 3.317 children particitpated in the 10-year follow-up (KOHLBOECK et al. 2012). We used the 10-year follow up sera for auto-IgE analysis. Furthermore we combined our samples from the GINI-study with serum samples of 10-year old children participating the LISA-study a prospective population-based birth cohort study that recruited newborns between 1997 and 1999 in four locations in Germans (Munich, Leipzig, Wesel and Bad Honnef) (SCHMITT et al. 2011)

3.1.1.3 ASTHMATIC PATIENTS FROM THE MEDIZINISCHE KLINIK DES UNIVERSITÄTSKLINIKUMS MAINZ

Another aim was to investigate asthmatic patients as a second group of patients with a different atopic disease as asthma. By courtesy of Prof. Buhl from the Medizinische Klinik from the Clinic from the Universitätsklinikum in Mainz, Germany, we obtained 80 sera of asthmatic patients. 55 of them had allergic asthma and 25 patients non-allergic asthma. The patients were well characterized with data about sex, allergy (yes/no), therapy, FEV1, body-height, body weight, total IgE, smoker (yes/no), asthma control status and grade of asthma severity. We analyzed the sera using the high-throughput immunoassay for their auto-IgE reactivity and a subsequent statistical analysis to investigate the above mentioned factors and its association with auto-IgE reactivity.

3.1.2 PROSPECTIVE CLINICAL STUDY WITH PATIENTS FROM THE BIEDERSTEINER DERMATOLOGY (BIA-STUDY)

Retrospective studies as the utilized study cohort of atopic eczema patients and the asthmatic patient study group and as well the combined birth cohorts GINI/LISA are restricted, due to absent information in the medical records or impurity about the diagnosis for atopic eczema or allergic asthma. A retrospective study is always performed for a study specific set of questions, analyses and hypothesis – to use those studies to find a response to answer further hypothesis can be complex and is often biased. Therefore our aim was to design a prospective study with patients from the Biedersteiner Dermatology.
Our hypothesis was that adult atopic eczema patients have higher auto-IgE reactivity in serum. A further aim was to test those patients during active and relapsing disease for their auto-IgE reactivity in serum, their auto-reactivity in skin prick-to-prick test with autologous skin suspension and the activatability of basophils after incubation with

autologous skin suspension as self-allergen. In addition we aimed to analyze auto-IgE reactivity in other IgE-mediated diseases and different skin diseases.

We started to recruit patients in summer 2012. We included atopic eczema patients that came into the clinic for the first time of diagnosis and without any kind of former therapies.

Furthermore we recruited different skin diseases as Psoriasis, bullous Pemphigoid, Dermatomyositis, Cutaneous Lupus Erythematosus, Systemic Lupus Erythematosus, Pemphigus vulgaris, Urticaria and healthy controls.

Due to the restrictive inclusion of first diagnosis and no former therapy we could not recruit as many patients as we planned and the study is still ongoing.

We characterized the patients beside their auto-IgE reactivity, for SCORAD, complete blood count, RAST (fx5, sx1), total IgE, SFAR-score (Score For Allergic Rhinitis) (OLOGE et al. 2013), RAD-score (Respiratory rate; Accessory muscle use; Decreased breath sounds)(ARNOLD et al. 2011), Desmoglein antibodies, anti-DNA antibodies and ANAs. Further we obtained a very detailed questionnaire of each of the patient. We obtained information about body height, body weight, age, allergen exposition (e.g. pet owning), family history of atopy, skin diversification, infectious diseases and vaccination, gastrointestinal disorders or pain, autoimmune diseases, thyroid gland disorders, lifestyle habits and medication/therapy.

3.1.2.1 SKIN PRICK TESTING

Patients that were positive tested for auto-IgE reactivity in serum were recruited again to conduct a prick-to-prick test. Therefore we took a biopsy of a small skin area from the patient and mashed the sample with a sonicator directly in ice cold PBS on ice until we reached a homogeneous skin suspension. We took biopsies of healthy skin containing the dermis and epidermis, in that the keratinocytes reside. If fat tissue was taken with the biopsy it was removed completely before mashing the sample. The homogeneous suspension of the skin biopsy was pricked into the upper layer of healthy skin areas in the forearm and was incubated 20 min. Histamine was used as a positive control and all patients reacted adequately. As a negative control we used NaCl and none of the patients showed a reaction against NaCl. A prick test gives evidences about a Type-I-hypersensitivity, which is IgE-mediated by doing a prick-to-prick test with autologous skin proteins we determined a possible allergic reaction towards self-proteins.

3.1.2.2 BASOPHIL ACTIVATION TEST

As a second method to test the type-I-hypersensitivity we conducted the basophil-granulocyte activation test (BAT). The reaction is alike the skin-prick test but independent from the intake of antihistamine. In addition the assay catches the biological active IgE bound on the cell membrane of basophils. A serologic determination includes the detached, biological non-active IgE only. Adding a potential allergen to basophils leads to cross-linking of IgE-molecules bound on the cell surface, leading to degranulation of the cell which is characterized by up regulation of CD63 which is analyzed in the BAT.

We used the Flow Cast® Kit (Bühlmann, Switzerland) for the measuring of BAT. Instead of available allergens we used the homologous allogenic HaCaT-protein suspension that was also used for the immunoassay.

As there is no recommendation for the concentration of proteins others than the purchased ones we titrated the potential allergen as described in the results (4.5.4.).

The procedure of the BAT was done as the manufacturer´s instruction pretend to conduct the assay. In brief, we thawed the allogenic proteins shortly before use and diluted the proteins in supplied stimulation buffer to final concentrations of 5 µg/ml, 2.5 µg/ml, 1.25 µg/ml, 625 ng/ml and 312.5 ng/ml. We retrieved EDTA blood from the patients and processed it as soon as possible. The following samples and controls were prepared as the following:

PB - Patient basis or negative control (50 µl stimulation buffer)

PC1a – stimulation control 1a containing anti-FcεRI (50µl of stimulation control anti-FcεRI)

PC1b – stimulation control 1b containing anti-FcεRI and RIPA-buffer 1:560

PC2 – stimulation control 2 containing fMLP (50 µl of fMLP)

A1 –A5 allergen titration (50 µl of the diluted allergen)

Afterwards 100 µl of stimulation buffer into each sample was added and 50 µl of the whole blood EDTA sample and mixed gently.

20 µl of staining-reagent – mix of anti-CD63 (FITC) and anti-CCR3 (PE) was added to each sample and mixed gently before incubation for 15 min at 37°C in a water bath. In a second step cells are lysed, therefor 2 ml of lysing reagent were added and incubated for 5 -10 min at 18 -28°C. The samples were centrifuged for 5 min at 500 x g and the supernatant was decanted and the pellet using 300 µl washing buffer resuspended, carefully vortexed and measured using a flow cytometer (488 nm Argon-laser).

To analyze the samples we gated first the $CCR3^{pos}$ cells including the entire basophil population. A second step is the gating and calculation of the amount of CD63 positive cells defining the activated basophil population.

The negative control (patient basis) defines the background of activated basophils and is commonly below 5 %. The first stimulation control anti-FcεRI mimics the cross-link of two IgEs bound on the cell surface caused by allergen binding. The fMLP (formyl-methionyl-leucyl-phenylalanin, unspecific cell activator) is a tripeptide and causes basophil activation on a non-immunologic way. At least one of the samples has to show an activation level of more than ten percent.

3.1.3 CELL CULTURE/ CELL BIOLOGICAL METHODS

To analyze the auto-IgE reactivity towards different cell-/tissue types whole cell protein was extracted from various cell types itemized in 5.1.1. and [Table 3].

3.1.4 CELL LINES AND PRIMARY CELLS

As a surrogate for human epithelium various primary epithelial cells or epithelial cell lines obtained from human bronchia, keratinocytes and the small intestine were cultivated for whole protein extraction to use as target protein in the auto-IgE Immunoassay. Furthermore we obtained peripheral mononuclear blood cells (PBMC) to test auto-IgE specificity and reactivity towards non-epithelial proteins. In order to test IgE auto-reactivity towards skin proteins the HaCaT (*H*uman *a*dult low *C*alcium high *T*emperature*)* cell line as well as primary keratinocytes (pKc) from patients with atopic eczema (pKc-AE) and healthy subjects (pKc-non-AE) were used. To test the reactivity towards lung epithelium primary normal bronchial/tracheal epithelial cells (NHBE) were used as well as human fetal epithelial primary cells from the small intestine (FHs74 int). All cells were selected as non-tumorigenic, non-malignant cells [Table 3].

Table 3: Cells utilized as a surrogate for different human epithelial to test IgE-autoreactivity in an immunoassay.

Cell	Description	Citation	Origin
HaCaT (cell line)	***H***uman ***a***dult low ***Ca***lcium high ***T***emperature, keratinocytes, adherent	Boukamp et al. 1988	Back skin 62-yr-old male patient (Boukamp et al., 1988)
pKc AE	Primary keratinocytes from AE-patients, adherent, non-tumorigenic	-	Suction blisters on forearm, ZAUM. Munich
pKc healthy	Primary keratinocytes from healthy subjects, adherent, non-tumorigenic		
NHBE	Normal human bronchial/tracheal epithelial cells, adherent, non-tumorigenic,	Lechner et al. 1983, 1984	LONZA, Basel, Switzerland CC-2540
FHs74 int	Human fetal small intestinal epithelial cells, adherent, non-tumorigenic[2,3]	Owens et al. 1976, Smith 1979	ATCC, Manassas, Vancouver, USA, CCL-241
PBMC	Peripheral blood mononuclear cells	-	Different healthy donors obtained from ZAUM/IAF

3.1.5 CELL CULTIVATION AND PASSAGING

All cells were cultivated in standard cell culture flasks each in specific medium [Table 4] at 37°C, 5 % CO_2 in a humidified incubator. Medium was changed every 2 - 4 days depending on cell type, growth and condition of the cell. Cell splitting was conducted when a confluence of 70 – 80 % was reached. After washing the cell monolayer with PBS, the appropriate amount of TrypLE™ was added to disperse cells. After 5 – 12 min, depending on the cell type, the reaction was stopped by adding 10 % FCS in PBS to the cells. Cells were washed by centrifugation at 301 x g, 22°C for 8 min. The supernatant was discharged, the pellet resuspended and fresh medium was added immediately to the cells. According to the split ratio (1:2 to 1:50, depending on the cell type) [Table 4] the required amount of cells were transferred into a cell culture flask.

Table 4: Respective media to cultivate named primary cells and cell lines.

Cell type	Medium	Split ratio	Company
HaCaT cell line	Keratinocyte serum-free medium (KCSFM) with L-Glutamine 50 µg/ ml bovine pituitary extract (BPE) 5 ng/ ml epidermal growth factor (EGF) 100 U/ml Penicillin	1:50	Gibco 17005-075
Primary keratinocytes (pKc)	DermaLife® Basal Medium L-Glutamine Apo-Transferrin rh TGF-α rh Insulin Hydrocortisone Epinephrine Extract-P	1:2 – 1:3	Cell Systems LL-0007
NHBE cells	Bronchial Epithelial Basal Medium BPE Hydrocortisone hEGF Epinephrine Insulin Trijodthyronine Transferrin Gentamicin Retinoic Acid	1:3 – 1:4	Lonza CC-3170
FHs74 int	DMEM GlutaMax, pyruvate 4,5 g/l glucose 10% FCS 10 µg/ml hr Insulin 30 ng/ml hr EGF	1:2 – 1:3	Gibco 31966 PAA Laboratories A15-102 Invitrogen 12585-014 Sigma E9644

3.1.6 Isolation of Peripheral mononuclear blood cells (PBMC)

Whole blood was drawn from peripheral vein with heparinized syringe and mixed 1:2 with PBS buffer. 10 ml of Ficoll were filled in 50 ml tubes and slowly covered with the diluted blood and centrifuged at 400 x g for 25 min at 21°C without using centrifuge brake. After removing the upper layer of plasma, the interphase of PBMC was aspirated and washed twice in PBS/EDTA [5mM] centrifuged at 301 x g at 21°C for 10 min.

3.1.7 Freezing and thawing cells

To save a cell stock by freezing in liquid nitrogen, cells were grown and detached as described in 5.1.2. Cells were resuspended in ice-cold cell-transition medium consisting of 10 % DMSO, 10 % Cell-specific culture medium and 80 % FCS and cell count is determined and finally adjusted to the desired cell count of 1.5 to 3.5 x 10^6 cells/ ml depending on cell number and type. To adapt cells to lower temperature slowly we kept cells for about 10 min on ice before they were first transferred into – 40°C storage overnight and to – 80°C on the following day overnight again. Finally cells are stored at – 196°C in liquid nitrogen.
To thaw cells they were transferred from – 196°C directly into tepid water bath and thawed shortly prior the volume is defrosted. To devoid any risk of toxicity of DMSO, cells were immediately diluted in a culture flask with preheated cell-specific culture medium and incubated as described above.

3.1.8 IFN-γ stimulation of cells

To mimic in vivo conditions in time of inflammation as e.g. in skin of atopic eczema we stimulated cells with Interferon gamma (IFN-γ) (Grewe et al. 1994). To investigate the most suitable confluence of cells, stimulation time and concentration of IFN-γ we performed test series for all conditions and combinations of those three parameters for each cell type as shown in Table 5. Since we could not stimulate FHs74 int cells using IFN-γ we tested further conditions using LPS (concentration LPS). To evaluate comparative conditions we tested NHBE and HaCaT cells additionally.

Table 5: All tested conditions to ascertain the best stimulation of each cell named in table 2 by IFN-γ and LPS

Tested Parameter	Tested conditions
Cell confluence	10%, 20%, 30%, 50%, 60%, 70%, 80%, 90%, 100%, >100%
Time of stimulation	3h, 6h, 12h, 24h, 48h, 72h
IFN-γ concentration	10 µg/ml, 20µg/ml
LPS concentration	3 ng/ml, 5 ng/ml, 10 ng/ml
Cell types tested	NHBE, HaCaT, FHs74 int

3.2 PROTEIN BIOCHEMISTRY METHODS

To assume identical initial conditions for every screen and each plate of the auto-IgE screen we generated a large scale of the required protein extracts. Consequently, each assay was performed with the same lot of the generated protein pool. Therefore the comparability of all assays is given. Each screen was run with two plates of HaCaT derived proteins and two plates of NHBE derived proteins and consisted of four plates. The coating of four to six plates, which is the number of plates that were screened in one week, of each protein (HaCaT and NHBE) was done on a given day to avoid as much deviation as possible on the first step of the assay.

3.2.1 WHOLE CELL PROTEIN EXTRACTION AND DENATURATION

The optimal condition to extract proteins from cells is a confluence of about 70 – 80 %. Cells grown to this optimum were rinsed twice with ice-cold PBS before adding the appropriate amount of RIPA-Buffer [Table 6] that was dependent on the size of the cell culture flask (0.5, 1.0 or 1.5 ml). The cells were scraped with a cell-scraper to exert sheer-stress on the cells in beside the mechanical separation. The cell suspension was pipette up and down 5-10 times to form a homogenous lysate and put into a 1.5 ml centrifuge tube. The next 2 min we incubated the sample on ice. Followed of 2 cycles of a 1 min sonification and resting 5 min on ice. The centrifugation step was 21.100 x g for 25 – 30 min at 4°C. The supernatant was aliquoted without the pelleted debris and immediately frozen at -80°C. One aliquot was taken for protein determination. All performed steps were done on ice. Furthermore one aliquot of the supernatant of the cultured cells was taken to perform an ELISA of e.g. IP10 to assure the stimulation of the cells.

Table 6: Composition of the RIPA-buffer used for protein extraction.

RIPA Buffer	
Substance	Final concentration/volume
0.5 M Tris, pH 7.2	50 mM
1.5 M NaCl	150 mM
Nonidet (NP40)	1 % (v/v)
Na-Deoxycholat [10 %]	0.5 % (v/v)
SDS [10 %]	0.05 % (v/v)
Dithiotreitol (DTT)	1mM
Protease inhibitor (Thermo fisher)	10 µl according to manufacturer´s protocol
EDTA (Thermo fisher)	10 µl according to manufacturer´s protocol
ddH_2O	
Adjust to pH 7.0 with 1 M HCl	

3.2.2 Protein determination by BCA-Assay

The BCA-Assay was performed according to the manufacturer´s protocol and is given shortly. Bovine serum albumin (BSA) is used as a standard and was diluted from 2000 µg/ml to 15 µg/ml in Reagent A supplied by the manufacturer. Samples were diluted 1:2, 1:5 and 1:10 in Reagent A. 25 µl of each standard (that was done in duplicate) and each sample, run in duplicate or triplicate was added into a 96-well plate and 200 µl of "working reagent", supplied from the manufacturer was applied. After shaking the plate for 30 seconds it was incubated for 30 min at 37°C. Before measuring the plate was cooled down to room temperature and finally measured at 562 nm.

3.2.3 Protein denaturation

To have the same conditions in Western blot analysis and the immunoassay we decided to denature the proteins for both assays. In addition we performed assays with native proteins to investigate if there is a different reactivity pattern. However, this is no subject of this thesis and therefore not described as a method or as an obtained result. To denature the proteins the samples were boiled for 10 minutes at 96°C in a thermomixer (Eppendorf, Germany).

3.2.4 SDS-GEL ELECTROPHORESIS/ PROTEIN SEPARATION AND COOMASSIE GEL STAINING

For gel electrophoresis of proteins we used SDS (sodium-dodecyl sulfate) gels. We poured three different gels, the base gel was just to seal the glassware and the second gel was the separation gel to separate proteins due to their size and a stacking gel to collect the proteins before separation. The compositions of the single gels are listed in Table **7**. All components were poured together, besides TEMED (N,N,N',N'-Tetramethylethyldiamin) and APS (Ammonium persulfate), both components were added immediately before pouring the gel to avoid an early polarization. First the bottom gel was poured into a tray that stabilizes the glass plates. To have a standardized gel thickness we put a spacer in between the two glass plates, the gel thickness was 1mm. The separation gel was poured next; to evade bubbles on the gel surface we added a modicum of isopropanol onto the gel surface. The gel run at 27 to 30 mA until the proteins reached the separation gel. The separation was run at 35 – 40 mA until the proteins reached the area shortly before the base gel. After gel running the gel was removed carefully from the glas plates and stained in Coomassie-staining solution over night at 4°C [Table 8].

The next morning we destained the excess staining solution from the gel by heating 3 times at 750 W for 5 min in distilled water in the microwave. A sponge to absorb the excess of staining solution was put into the distilled water to accelerate the destaining procedure. Afterwards the gel was cooled down to room temperature and incubated in gel drying solution for at least 30 min shaking [Table 8].

Table 7: Composition of the three different gels used for SDS gel-electrophoresis.
First we poured a base gel to seal the glassware, the separation gel was poured with 12 % to separate the proteins, the concentration of the stacking gel was dependent on the proteins 4 to 5 %. All compounds were assorted besides TEMED and APS, both components were added immediately before pouring the gel to avoid an early polarization. The gels were poured between two glass plates, by using a spacer the space between the glass plates was 1 mm. To avoid bubbles separation gel was covered with a modicum of isopropanol that was added on the gel surface and washed out before pouring the stacking gel.

Base gel		Separation gel 12 %		Stacking-gel 4 %		Running buffer (10 x)	
	[ml]		[ml]		[ml]		[ml]
30 % Acrylamide solution	6.7	26 % AA/ 0.7 % bisAA	7.83	26 % AA/0.7 % bisAA	1.54	Glycine	144 g
2 % N,N'-methylene-bis-Acrylamide	1.1	Tris 1,875 M pH 8,8	3.4	Tris 0,6 M pH 6,8	2.5	Tris-base	30.3 g
10 % SDS	0.1	SDS [10 %]	0.17	SDS [10 %]	0.1	SDS [10 %]	100 ml
Tris 1,875 M pH 8,8	2.0	ddH_2O	5.41	ddH_2O	5.8	q.a. 1000 ml ddH_2O, pH 8.8	
Ammonium persulfate	0.05	Ammonium persulfate	0.17	APS	0.05		
TEMED	0.025	TEMED	0.017	TEMED	0.01		
final volume	10	final volume	17	final volume	10		

Table 8: Composition of the Coomassie gel staining solution and the Coomassie gel drying solution.

Coomassie staining solution 500 ml		
	final concentration	volume
Coomassie Brilliant Blue	0.1 %	0.5 g
Methanol p.a.	50 %	250 ml
Acetic acid	10 %	50 ml

Coomassie gel drying solution 1000 ml		
	final concentration	volume
Glycerol	3 %	30 ml
Methanol p.a.	30 %	300 ml
ddH_2O		670 ml

3.3 IMMUNOLOGICAL METHODS

3.3.1 AUTO-IGE HIGH-THROUGHPUT IMMUNOASSAY

To detect autoreactive IgE in serum we established the here described immunoassay. The procedure given below is the finalized protocol for the high-throughput immunoassay performed in a 384-well Maxisorp® plate (nunc, Denmark). Figure 2 depicts in brief the procedure of the high-throughput immunoassay. Whole cell protein extracted from HaCaT-, NHBE and FHs74 int cells served as antigens and were coated on the solid phase of 384-well Maxisorp® plates. Before coating the proteins were denatured by boiling at 96°C for 10 min. The protein amount for coating was calculated for each protein one by one. We coated 1 µg protein per well in 20 µl of the special coating buffer and incubated the plate over night at 4°C.

After washing the plate three times in washing buffer using the plate washer (Tecan, Maennedorf, Switzerland) we blocked each well with 1 % BSA in TBS to inhibit unspecific binding in the ongoing procedure. Plates were washed 3 times in TBS-Tween® (TBS-T), plates that were not used directly were stored with 50 µl/ well TBS and closed with Bar Seal® (nunc, Denmark) and stored at 4°C until usage. Sera for auto-IgE analysis were diluted 1:2 with TBS-T in 96-well plates and transferred onto 384-well plates in triplicates (20 µl of the diluted sample in each well) and incubated for two hours at 22°C, shaking at 300 rpm on a thermomixer (Eppendorf, Germany). After washing again 3 times with TBS-T 20 µl of the 1:800 diluted secondary anti-human IgE antibody, specific towards the ε-chain (Sigma-Aldrich, Missouri, USA) was given into each well and incubated for two hours at 20°C, shaking at 300 rpm on a thermomixer (Eppendorf, Germany). The following washing step was increased and we washed the plate eight times with TBS-T to eliminate the entire unbound secondary antibody and went on with the chromogen development using a 1-step Ultra-Tetramethylbenzidine (TMB) (Thermo Fisher Scientific, Massachusetts, USA). We used 20 µl of TMB in each well for chromogen development and stopped the reaction with 10 µl of 2M H_2SO_4 when the negative control started to turn

slightly blue, which was about after 10 minutes. The whole plate was shaken and measured at 450 nm and 700 nm as reference wavelength (epoch Reader, BioTek).

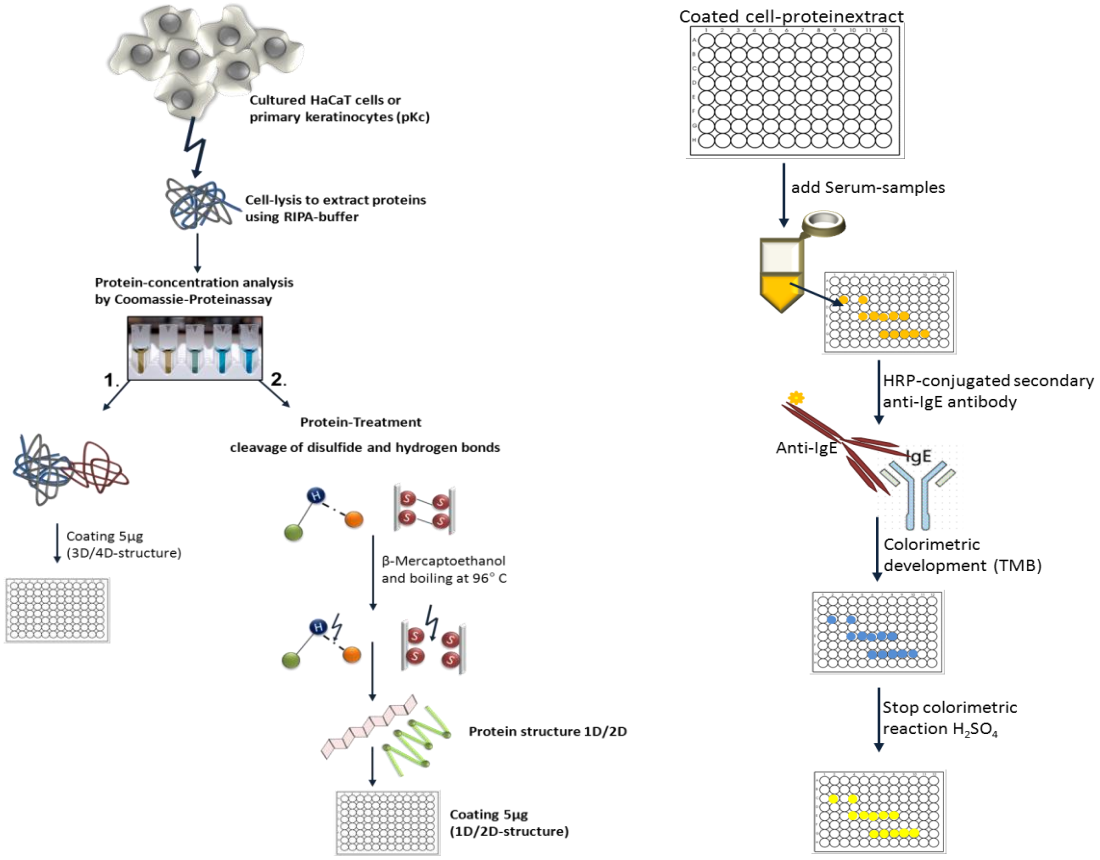


Figure 2: Procedure of the high-throughput immunoassay
To detect autoreactive IgE in serum. Cells were cultivated up to a confluence of 70-80 %, lysed with RIPA buffer, denatured and coated on the solid phase of a 384-well Maxisorp® plate. The protein is now linearized in 1D/2D structure. To detect the bound autoreactive IgE a secondary anti-human IgE antibody, specific to the ε-chain, was added and the chromogen development was initiated by adding a 1-step ultra TMB and stopped with 2M H_2SO_4. The plate was measured at 450 nm and 720 nm as reference wavelength. The second possibility not to linearize or denature the proteins was established but not used for the results presented in the current study.

3.3.2 CUT-OFF AND REACTIVITY CALCULATION FOR SAMPLES ANALYZED WITH THE HIGH-THROUGHPUT IMMUNOASSAY

To determine a discrimination threshold in a binomial classification that determines if a patient has a certain disease or not commonly a ROC curve is determined, in which the sensitivity (true positive rate) and specificity (wrong positive rate) of the assay is calculated to use for a novel parameter.

As there is no disease defined if a patient shows IgE autoreactivity there was no possibility to use a ROC curve for the determination of a threshold that discriminates patients as diseased and not diseased.

To calculate the height and the dimension of the auto-IgE reactivity in serum we defined a cut-off that was more multi-staged for children as children showed a higher quantity of IgE-reactivity as adults, due to the physiologically occurrence of auto-IgE in serum.

As shown in the following figure we determined the cut-off by the calculated mean of six non-reactive samples and added a threefold standard deviation of the non-reactive samples for adult samples [Figure 3].

The reactivity-factor of the sample was adjusted by dividing the OD of the sample with the calculated cut-off.

As we had to verify the cut-off for children of the GINI/LISA birth cohort we added two further levels of reactivity. For that we used a 2. cut-off, calculating with a 4.5-fold standard-deviation and a 3. cut-off calculating with a 6-fold standard deviation. Again the reactivity for each sample was calculated by dividing the sample OD by the calculated cut-off. A sample was evaluated as reactive or positive when the final calculated factor was equal or higher than one.

Another possibility to define this cut-off is the following when the factor is ≥1:

1. cut-off = reactive sample
2. cut-off = high reactive sample
3. cut-off = very high reactive sample

The analysis of the *"Factor of reactivity"* is very similar when compared to the calculation of the 95-percentile as used for the definition of a reference range used in laboratory praxis.

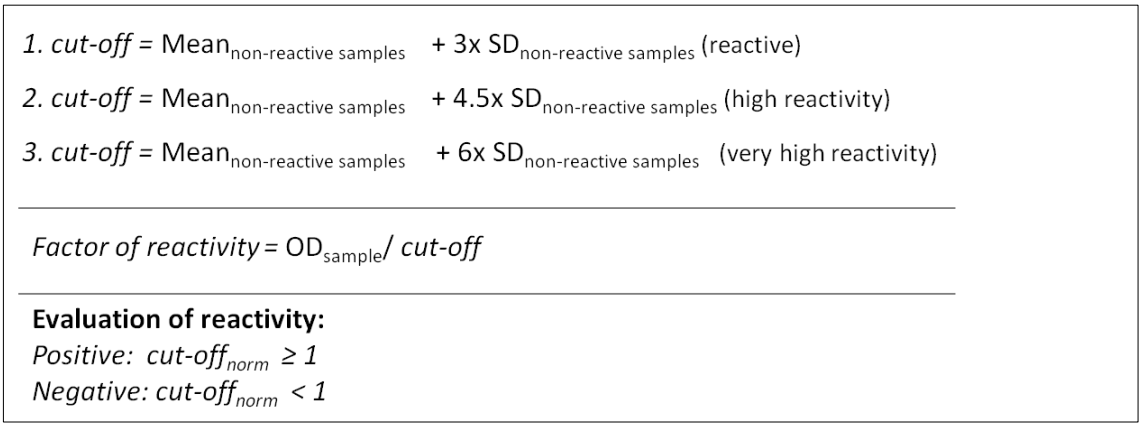


Figure 3: Cut-off calculation for the evaluation of the auto-IgE immunoassay.
The cut-off was calculated by the mean of simultaneously analyzed non-reactive controls. The mean and the standard deviation of six non-reactive samples were calculated. To expand the graduation of the reactivity of children, as the quantity of reactive samples was much higher and much more frequent as in adult patient-groups we established three increasing cut-offs. For the first cut-off (1. cut-off) the mean of the non-reactive controls was sum up with the 3-fold standard deviation. Finally, the OD of the sample (OD_{sample}) was divided by the 2.- or the 3. cut-off and was calculated with 4.5-fold and 6-fold standard deviation respectively. A final calculated factor equal or higher than 1 was evaluated as a positive and reactive sample. Adult samples were calculated using only the 1 cut-off.

3.4 Mouse studies

3.4.1 Purification of OVA-specific IgE from mouse serum

From the crude serum we obtained for IgE enrichment we took about 20 µl for IgE and IgG ELISA to determine the total IgE, total IgG and OVA-specific IgE in the source material.
Prior using the melon gel® we exchanged the buffer using the protein desalting spin column (Zeba® column), (Pierce/ Thermo Scientific, Rockford, USA). After preparing the Zeba® columns with 300 µl of purification buffer from the Melon gel® Kit we applied 100 µl of the serum on the resin and centrifuged at 1500 x g for 2 min to collect the buffer exchanged sample. In the next step we used the melon gel®, (Pierce/ Thermo Scientific, Rockford, USA), to remove albumin. After equilibration and adding the slurry and preparing the slurry with purification buffer we applied 100 µl of the buffer exchanged serum and incubated it for 5 min at room temperature with end-over mixing. The sample was then centrifuged at 5500 x g for 1 min to collect the purified antibody. For the use of a protein G column and to purify the IgE from IgG the buffer had to be exchanged again using a Zeba column®. After preparing the protein G column we applied 400 µl of the purified serum sample incubated for 10 min at room temperature with end-over mixing. The IgE eluate was obtained by centrifuging at 5000 x g for 1min. A sample was taken for IgE (total and OVA-specific) and total IgG was taken. The resin was washed two times and the IgG fraction after adding of neutralization and elution buffer eluted and collected. We obtained three IgG fractions each was analyzed for IgE by ELISA to proof whether IgE was gone with the IgG fraction and to assure to re-obtain all IgG antibodies in this fraction. The procedure was optimized to the needs of our application, on the whole the procedure was done as given in the manufacturer´s instruction to use the Zeba column®, the melon gel® and the protein G column.

3.4.2 ELISA

To obtain OVA-specific IgE, to use as a self-antigen in experimental mouse models we had to enrich IgE from serum from OVA-sensitized BALB/c to inject this into C57BL/6 mice. The first sample to test in ELISA was taken before we started the enrichment for IgE from the crude serum. The following steps were the removal of albumin by using a melon gel® (Pierce/ Thermo Scientific, Rockford, USA) and the last step the removal of IgG antibodies using a Protein G column (Pierce/ Thermo Scientific, Rockford, USA). The second sample to test in ELISA was the IgE-eluate obtained from the Protein G column; we performed a total-IgE, an OVA-specific IgE ELISA and an IgG ELISA out from it. A final step was to eluate the IgG from the Protein G column; again we took a sample to measure in ELISA to verify

how much IgE is in the IgG fraction. IgA and IgM were not depleted due to naturally low concentration in serum.

The dilution of the different samples used in the different (total-IgE, OVA-specific IgE and total IgG) ELISA is shown in Table 9.

Table 9: Sample dilution obtained from the IgE enrichment to determine total-IgE, OVA-specific IgE and total IgG in ELISA.

	Dilution			
	IgG total	**IgE total**	**OVA-IgE**	
Serum	1:5000 1:10.000	1:10 1:100	1:10 1:100	**all samples in duplicate**
IgE Eluate	1:10 1:100	1:10 1:100	1:10 1:100	
IgG fraction I-III	1:1000 1:10.000	undiluted	undiluted	**triplicates**

The total IgE ELISA was coated with 0.5 µg/well of the coating antibody (sheep, anti-mouse IgE, monoclonal) diluted in coating buffer [Table 10] and incubated 2 h at room temperature. The plate was washed three times in washing buffer and afterwards blocked for 2 h at room temperature in 300 µl blocking buffer [Table 10]. For the next step standard (Purified mouse IgE) and samples were transferred into the wells of 96 well plates, each 50 µl. The standard-row was diluted from 250 ng/ml to 0.98 ng/ml. Again the samples were incubated for 2 h at room temperature and afterwards again washed three times in washing buffer. In the next step 50 µl of the detection antibody (1 µg/ml), (Biotin-conjugated rat anti-mouse IgE) was given into the wells and incubated again 2 h at room temperature. After the incubation of the detection antibody we washed the plate 8 times using the washing buffer. 50 µl streptavidin-peroxidase, Calbiochem (1 µg/ml) was added into each well and incubated 30 min at room temperature. And finally we added the substrate and incubated the chromogen development in the dark. The reaction was stopped using 2M H_2SO_4 25 µl per well.

Table 10: Composition of all buffers and substances used in the total-IgE -, OVA-specific IgE- and total-IgG ELISA.

Coating buffer	4.2 g NaHCO3
	1.78 g Na_2CO_3
	q.s. to 500 ml aqua dest adjust pH to 9.5

10x Tris (500mM)	60.55 g Trizma® Base
	q.s. to 1 l in aqua dest, adjust pH to 7.4 using 5MHCl

Washing buffer	1x Tris (50 mM)
	100 ml 10x Tris
	900 ml aqua dest
	Adjust pH using 5M HCl to 7.4
	0.5 ml Tween

Blocking buffer	1x Tris (50 mM)/ 1 % BSA
	400 ml 1x Tris
	4 g BSA, Fraction V

Substrate	5445 µl substrate buffer
	2.55 µl cold H_2O_2
	55 µl dissolved TMB
	50 µl/ well

Substrate buffer (Galati buffer)	8.41 g Citric Monohydrate
	q.s. to 200 ml aqua dest adjust pH to 3.95 using 4M KOH

TMB	24 mg TMB
	Dissolve in 500 µl Ethanol absolute and 500 µl DMSO

For the ELISA of OVA-specific IgE we used 100 µg in 100 µl per well of the coating antibody (Ovalbumin from chicken egg-white, grade V) diluted in coating buffer [Table 10] and incubated 2 h at room temperature. The plate was washed three times in washing buffer and afterwards blocked for 2 h at room temperature in 300 µl blocking buffer [Table 10]. For the next step the standard (mouse anti-ovalbumin IgE) and samples were transferred into the wells of 96 well plates, each 50 µl. The standard-row was diluted from 1000 ng/ml to 3.9 ng/ml. Again the samples were incubated for 2 h at room temperature and afterwards again washed three times in washing buffer [Table 10]. Next 50 µl of the detection antibody (1.25 µg/ml), (Biotin anti-mouse IgE) was added into the wells and incubated 2 h at room temperature. After the incubation of the detection antibody we washed the plate 3 times using the washing buffer. 50 µl streptavidin-peroxidase (Calbiochem), (1 µg/ml) was added into each well and incubated 30 min at room temperature. Finally we added the substrate and incubated the chromogen development in the dark. The reaction was stopped using 2M H_2SO_4 25 µl per well [Table 10]. To

determine the total-IgG in serum and the eluates we used the Mouse IgG Quantitation Kit (Bethyl Laboratories). The procedure was done as given in the instruction manual. In brief 50 µl of the coating antibody, goat anti-mouse IgG affinity purified (10 µg/ml) was added and incubated the coating for 1 h at room temperature. After washing the plate with washing buffer [Table 10] we blocked the plate using blocking buffer (for 30 minutes at room temperature and washed again 3 times using the washing buffer [Table 10]. For the next incubation step we added the standard and samples into the wells, 50 µl each. The standard row consisted of concentrations from 500 ng/ml to 7.8 ng/ml, the incubation time was 1 hour at room temperature. The next washing step was repeated five times. The next step was adding 50 µl/well of the detection antibody (10 ng/ml) and incubated 1 hour at room temperature. The plate was again washed five times, before adding the substrate [Table 10] and incubated the chromogen development in the dark. The reaction was stopped using 2M H_2SO_4 25 µl per well [Table 10].

3.5 Statistical analysis

Statistical analyses were done mainly with R (R Development Core Team 2009), partly with GraphPad Prism 6.05 (GraphPad Software La Jolla, CA, USA).
For metric data normality was tested using a QQ-plot or using a Shapiro-Wilk test to assess the normal or Gaussian distribution respectively of the values. If Gaussian distribution was given appropriate tests were as linear or logistic regression was performed. Binominal data were tested using a QQ-plot (R3.1.2) only.

An initial analysis to test differences between categorical variables was analyzed using Pearson's´ chi-squared test (χ^2-test). If a difference was given by a significant χ^2-test we performed continuing analyses.

Associations between auto-IgE reactivity and several different factors were analyzed using multiple logistic regression models adjusted for sex, study, city, age, BMI, parental education, and parental atopy. The results were presented in odds ratios (OR) with 95 % confidence intervals (CI).
Receiver operating characteristic (ROC) curve analysis was used to evaluate the value of auto-IgE reactivity to predict atopic eczema in children.

3.6 CHEMICALS AND BUFFERS

chemical/ substance	product number	company
1-Step Ultra TMB-ELISA	34029	Pierce / Thermo Fisher
Acrylamide 4x solution 40 % (w/v)	10677.01	Serva
Amphotericin B	A2942	Sigma-Aldrich
Anti-Human IgE (ε-chain specific)–Peroxidase antibody produced in goat (2nd Antibody)	A9667	Sigma-Aldrich
APS; Ammonium persulfate	13375.01	Serva
BCA assay Kit	23227	Pierce / Thermo Fisher
BEGM BulletKit	CC-3170	Lonza
biotin anti-mouse IgE	553419	BD
Biotin-conjugated rat anti-mouse IgE	553419	BD
N,N'-Methylene bisacrylamide 2x Solution 2 % (w/v)	29197.01	Serva
BSA (Albumin Fraction V)	8076	Carl Roth, Karlsruhe
BSA (Albumin Fraction V)	A-2153	Sigma-Aldrich
Citric Monohydrate	c-1909	Sigma-Aldrich
DermaLife® Basal Medium	LL-0007	Cell Systems
Di-Sodiumcarbonate	s-7795	Sigma-Aldrich
DL-Dithiothreitol	43815	Sigma-Aldrich
DMSO	109678	Merck, Darmstadt
DMSO	D2650	Sigma-Aldrich
DPBS, calcium, magnesium	14040091	Life Technologies, Carlsbad, CA
DPBS, no calcium, no magnesium	14190094	Life Technologies, Carlsbad, CA
EDTA	15575-038	Life Technologies, Carlsbad, CA
EGF, human recombinant	E9644	Sigma-Aldrich, Steinheim
Ethanol absolute	100983	Merck, Darmstadt
Fetal calf serum	A15-102,	PAA Laboratories, Pasching, Austria
Ficoll		
goat anti-mouse IgG affinity purified	A90-131A	Bethyl Laboratories
Halt Protease & Phosphatase Inhibitor Cocktail	78440	Pierce / Thermo Fisher
Hydrogen peroxide solution, 30 %	216763	Sigma-Aldrich, Steinheim
IFN-γ, human recombinant	300-02	PeproTech
Insulin, human recombinant	12585-014	Life Technologies, Carlsbad, CA
Keratinocyte serum-free medium (KCSFM) with L-Glutamine	17005-075	Gibco / Life Technology
KOH	1.050.210.250	Merck, Darmstadt
L-glutamine	25030	Life Technologies, Carlsbad, CA
Melon Gel IgG spin purification Kit	45206	Pierce
mouse anti-ovalbumin IgE	BZL05775	Biozol
Mouse IgG ELISA Quantitation Kit	E90-131	Bethyl Laboratories
Sodiumhydrogencarbonate	1.063.290.500	Merck, Darmstadt
NHBE; Bronchial Epithelial Cells w/RA, BEGM, cryo	CC-2540	Lonza
Nonidet™ P 40 Substitute (NP-40)	74385	Sigma-Aldrich, Steinheim
Ovalbumin from chicken egg-white, grade V	A5503	Sigma-Aldrich

chemical/ substance	product number	company
Polyvinylpyrrolidon 40.000 mol average	PVP-40	Sigma-Aldrich
Potassium chloride (KCl)	104933	Merck, Darmstadt
Potassium dihydrogen phosphate (KH2PO4)	104873	Merck, Darmstadt
purified mouse IgE	557079	BD
sheep, anti-mouse IgE, monoclonal	PC284	The binding site
Sodium carbonate (Na_2CO_3)	S7795	Sigma-Aldrich, Steinheim
Sodium chloride (NaCl)	S9888	Sigma-Aldrich, Steinheim
Sodium deoxycholate monohydrate	D5670	Sigma-Aldrich, Steinheim
Sodium dodecyl sulfate solution (SDS), 10 %	L4522	Sigma-Aldrich, Steinheim
Sodium hydrogen carbonate ($NaHCO_3$)	106329	Merck, Darmstadt
Sodium phosphate dibasic	S9763	Sigma-Aldrich, Steinheim
Sodium phosphate monobasic monohydrate (NaH_2PO_4)	S9638	Sigma-Aldrich, Steinheim
Sodium pyruvate	11360	Life Technologies, Carlsbad, CA
ß-mercaptoethanol	M6250	Sigma-Aldrich, Steinheim
Streptavidin-Peroxidase	189733	Calbiochem
Sulfuric acid (H_2SO_4)	100731	Merck, Darmstadt
Teba Desalt Spin Columns	89883	Pierce
TEMED; N,N,N',N'-Tetramethylendiamin	35925.01	Serva
TMB	87748	Sigma-Aldrich, Steinheim
TMB	87748	Fluka
TMB, 1-Step Ultra TMB-ELISA Substrate	34028	Thermo Fisher Scientific, Waltham, MA
Trizma® base	T1503	Sigma-Aldrich, Steinheim
Trypan blue 0.5 %	295833	Boehringer Mannheim
Trypsin/EDTA	35400-027	Life Technologies, Carlsbad, CA
TWEEN® 20	P7949	Sigma-Aldrich, Steinheim
TWEEN® 20	P7949	Sigma-Aldrich
Zeba Spin Desalting Columns, 7K MWCO, 0.5 ml	89882	Pierce

3.7 CONSUMABLES, LABORATORY INSTRUMENTS AND LABORATORY DEVICES

Instrumentation/materials Laboratory instrumentation	**Model/ product number**	**Manufacturer**
1 ml pipette	604181	Greiner bio-one
10 ml pipette	607180	Greiner bio-one
15 ml tubes	62554502	Sarstedt
2 ml pipette	710180	Greiner bio-one
25 ml pipette	760180	Greiner bio-one
384 well maxisorp® plate	464718	nunc
5 ml pipette	606180	Greiner bio-one
6 well plates	140685	nunc
96 well plate flat-bottom	655101	Greiner
96 well plate round-bottom	651101	Greiner
96-well flat-bottom microplate, MaxiSorp™	439454	nunc
Bar Seal	44636	nunc
cell culture flask 175 cm^2	353118	Becton Dickinson
cell culture flask 25 cm^2	136196	nunc
cell culture flask 75 cm^2	353136	Becton Dickinson
cell scraper	disposable cells scraper	Corning
cell scraper	polyethylene cells lifter	Sarstedt
Centrifuge	Megafuge 1.OR	Heraeus
Centrifuge	Biofuge pico	Heraeus
Centrifuge	Heraeus Fresco 21	Thermo Scientific
Cryotubes	375418	nunc
electronic pipette 10 - 100 µl	Xplorer® plus	Eppendorf AG
Electrophoresis chamber		PR-Tech
ELISA-Reader	Epoch	Bio Tek
Freezer (-20°C)	Gs2503-11	Liebherr
Fridge (4°C)	SN-ST 26164 4	Liebherr
Incubator	IR-Sensor	Sanyo
laminar flow	Herasafe HS 15	Thermo Fisher Scientific
Liquid nitrogen storage tank	Locator 6 PLUS	Thermo Fisher Scientific
Multichannel pipette, adjustable vol.	Multipette plus	Eppendorf AG
Multichannel pipette, adjustable vol.	Transferpipette-12	BRAND GMBH + CO KG
Multipette® plus	-	Eppendorf AG
Neubauer hemacytometer	-	Glaswarenfabrik, Karl Hecht GmbH&Co KG
Optical microscope	Axiovert 4CFL	Zeiss
pH-Meter	CG 841	Schott
pipett tips, ART ®, filtered	2140 (10 µl)	Promega
pipett tips, ART ®, filtered	2065E (100 µl)	Promega
pipett tips, ART ®, filtered	2079E (1000 µl)	Promega
pipett tips, ART ®, filtered	2149P (20 µl)	Promega
pipett tips, ART ®, filtered	2069 (200 µl)	Promega
pipett tips, epT.I.P.S	0030 000.854 (0.5-20 µl)	Eppendorf AG
pipett tips, epT.I.P.S	0030 000.870 (2-200 µl)	Eppendorf AG
pipett tips, epT.I.P.S	0030 000.897 (20-300 µl)	Eppendorf AG
pipett tips, epT.I.P.S	0030 000.919 (50-1000 µl)	Eppendorf AG
Pipette controller	Accu Jet	BRAND GMBH + CO KG
Pipettes, Reference, adjustable volume	Reference	Eppendorf AG
Plate washer	Hydrospeed	Tecan

Instrumentation/ materials Laboratory instrumentation	Model/ product number	Manufacturer
Precision balance	PB303-S/FACT deltarange	Mettler Toledo
Reagent and centrifuge tube, 15 ml	62554502	Sarstedt
Reagent and centrifuge tube, 50 ml	62547254	Sarstedt
Research pro	electronic air displacement pipette	Eppendorf AG
Safe lock tubes 0.5 ml	0030 121.023	Eppendorf AG
Safe lock tubes 1.5 ml	0030 120. 086	Eppendorf AG
Safe lock tubes 2 ml	0030 120.094	Eppendorf AG
Semi-Dry Western Blot chamber		PR-Tech
Sterile bench	HS15	Hereaus
Sterile filter unit, Millex-GP, 0.22 µm	SLGP033RS	Millipore
Taumelroller	707661 RM5.40	Karl Hecht GmbH&CoKG
Thermomixer	Thermomixer comfort	Eppendorf AG
Tubes, safe-lock 0.5 ml	0030 121.023	Eppendorf, Hamburg
Tubes, safe-lock 1.5 ml	0030 120.086	Eppendorf, Hamburg
Tubes, safe-lock 2.0 ml	0030 120.094	Eppendorf, Hamburg
Ultrasonic bath	Sonorex R52	BANDELIN electronic GmbH & Co. KG
Varioclav Dampfsterilisator	400 E	H+P Labortechnik AG
Vortex mixer	IKA MS 1 minishaker	Sigma-Aldrich
Vortex mixer	MINI-Vortex	Kisker Biotech
Vortexer	MS1 minishaker	IKA®-Werke GmbH & CO. KG
Water purification system	MilliQ1, EASYpure UV	Milipore
Waterbath	1083	GFL Gesellschaft für Labortechnik GmbH

4. Results

4.1. Establishing of a high-throughput immunoassay for the detection of autoreactive IgE in human serum

To screen large cohorts and sample sizes using the minimal amount of sample volume we established a screen to detect autoreactive IgE in 384 well format.

4.1.1. Extension of 96-well plate immunoassay to a high-throughput assay in 384 well plate format

The Immunoassay was first established on 96-well format and later on extended to a 384-well format to reach a higher sensitivity and a high-throughput system, that allows screening large birth cohorts in short time periods. In addition, the sample volume applied in each well could be reduced till 10 µl serum, from 25 µl in 96 well-format. For that we tested two different plate surfaces (Maxisorp™ and Immobilizer™ Amino) whereas the surface of Maxisorp™ revealed in specifiable results of reactive and non-reactive samples compared to Immobilizer™ Amino. Furthermore, we titrated the amount of protein coated to the surface in concentrations of 0.25, 1 and 5 µg/well, whereat we revealed lower IgE-binding when 5 µg of protein per well was bound. Finally, as there were only minor differences between 0.25 µg and 1 µg, we decided to apply 1 µg of protein to enhance the variability of proteins in each well (Beck 2012).

Moreover, we had to optimize the assay in 384-well format to conduct a stable and feasible system. As the blank was fairly elevated with buffers and settings used in 96-well format, the 384-well format had to be optimized with entirely new settings. Since we could not claim samples as right or false positive or negative for auto-IgE reactivity, as there is no definition for disease, we used in all upcoming steps of establishment samples with known reactivity we assessed before, as assay controls. The availability of samples without any reactivity (non-reactive samples, non-reactive controls (nC)) and others with known reactivity (reactive controls (react. Ctrl)) were utilized.

To coat the proteins in 384-well format we used Dithiotreitol (DTT), which we did not use in 96-well. Therefore we reassessed whether DTT is the background causing component. As the testing with and without DTT revealed no difference in background (blank), but better results in low positive samples we kept coating with DTT (data not shown). In addition we obtained far better consistence of the values when the plate was shaking at 300 rpm in each incubation step during the entire assay, compared to stationary incubation (data not shown). To save sample volume of valuable samples, we tried to

reduce the applied sample volume till 10 µl in total without gaining satisfactory results, so we remained using 20 µl of total sample volume (1:2 dilution).

The blocking strategy is one of the possible factors to diminish the assay background; nevertheless it is a fine line between deprivation of rarely occurring auto-antigenic epitopes and blocking unspecific binding showing up in the extent of background. Three blocking strategies were tested to reveal the most suitable one for the present assay. First we compared 1 % and 2 % of bovine-serum albumin (BSA) blocking each for 2h at room temperature (RT). Simultaneously we compared sample dilution using PBS and PBS-T, as PBS was the buffer utilized in 96-well format. Figure 4 depicts the results of the two concentrations of BSA and the two ways of sample dilution, each combined. Resulting in higher optical density (OD) when blocking was performed using 1 % BSA (light and dark brown bars) compared to 2 % BSA blocking (grey bars). Furthermore, we revealed slightly higher values when using 0.05 % Tween® in the sample dilution buffer (PBS), particularly in low reactive samples or higher dilution respectively. For the third type of blocking we tried using the non-protein component Polyvinylpyrrolidon (PVP) to obtain more consistent results but lower background signals (HAYCOCK 1993). Figure 5 depicts the IgE-reactivity after 1 % BSA or 1 % PVP blocking. Again, we used a dilution row of one reactive sample, measured each in triplicate to assess the specificity and sensitivity of the changed assay condition. The blue bars (1 % BSA) show a preferable decreasing auto-IgE reactivity following the sample dilution, in contrast values obtained from wells that were blocked with PVP, do not show a dilution row accompanied with decreasing OD. Finally, we determined blocking with 1 % BSA as the most suitable approach for the present auto-IgE immunoassay.

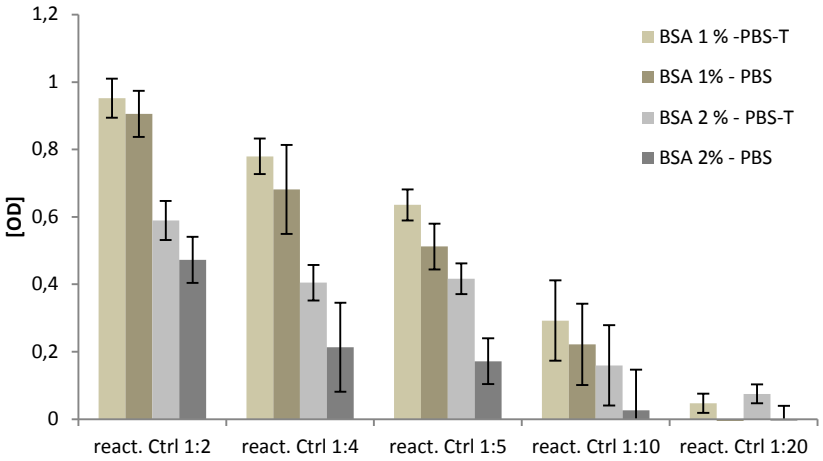


Figure 4: Blocking with 1% of BSA and using Tween® for sample dilution is more efficient for the detection of auto-IgE reactivity in serum.
A dilution row of one reactive sample was analyzed using a blocking of 1% (beige bars) or 2% (grey bars) BSA. 2% BSA repressed target epitopes of autoreactive IgE that reveals in less abundant results compared to the blocking with only 1% BSA. Slightly enhanced detection of auto-IgE reactivity we obtained when Tween® was used for sample dilution particularly in samples with marginal reactivity. Data are given ± SD.

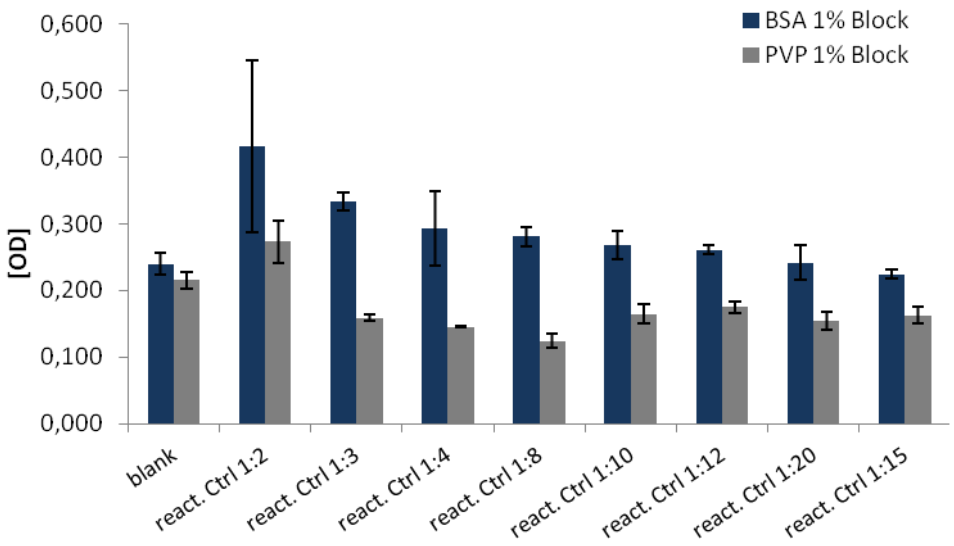


Figure 5: Samples blocked with Polyvinylpyrrolidon (PVP) are not evaluable.
The dilution row of the reactive sample shows no decreasing reactivity when blocked with 1% PVP [grey bars] as it is when the blocking is conducted with 1% BSA [blue bars]. As the background in PVP-blocked plates is as high as the highest sample (reactive control 1:2) a sample evaluation is not possible. Data are given ± SD.

Despite the optimization of blocking, the background remained quite high referring to unspecific binding of e.g. the secondary antibody (2nd antibody). To further optimize conditions for less unspecific binding of the 2nd antibody we tried to modify the buffer system from phosphate base (PBS) to Tris(hydroxymethyl)-aminomethan buffered saline (TBS) containing Tween® (TBS-T). Along with a titration of the 2nd antibody [Figure 6 [A] 1:500, [B] 1:800, [C] 1:1000] we could reduce the background by using TBS buffer, whereupon the utilization of PBS revealed in higher values of background in 1:500 and 1:800 dilution. Moreover the range to discriminate reactive and non-reactive samples is insufficient broad [Figure 6 A, B] when PBS is used. Applying less concentrated 2nd antibody (1:1000 diluted) revealed in less unspecific binding but no better discrimination feasibility of the samples [C]. However, using TBS buffer disclosed lower unspecific binding when diluting 2nd antibody 1:500 or 1:800 [A, B] and enhanced feasibility to discriminate reactive and non-reactive samples [A, B, C]. A higher OD was reached after incubation with 1:1000 diluted 2nd antibody at which the background OD increased in TBS conditions likewise the PBS one. Still remaining the suboptimal decline of values of the sample diluted serial in PBS.

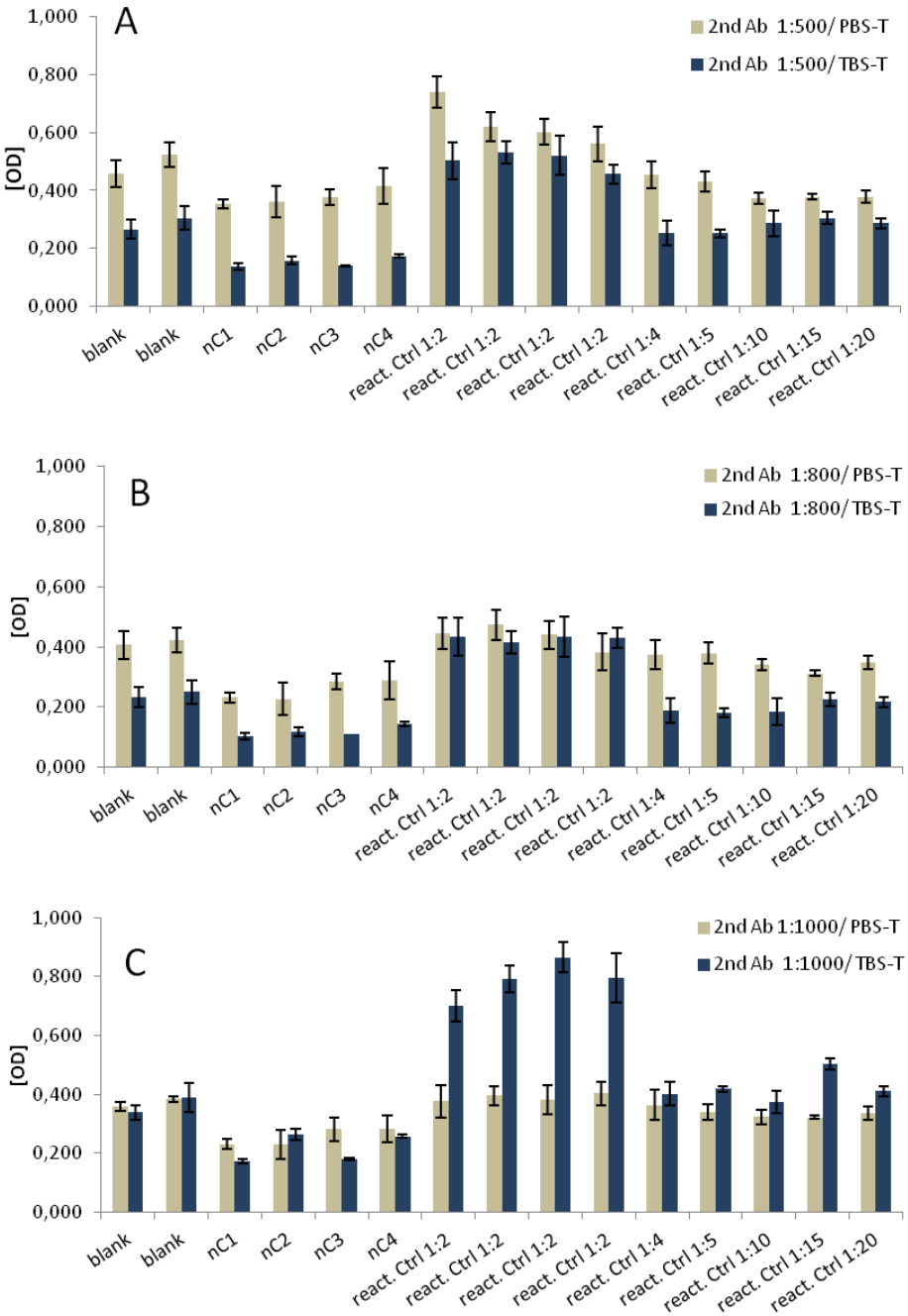


Figure 6: Tris-based buffer lowered the background and increased the dynamic range of the assay.
The antibody dilution of 1:500 [A] and 1:800 [B] showed less background when TBS-T buffer (blue bars) is used compared to PBS-T (beige bars). The discrimination of reactive (react. Ctrl) and non-reactive samples (nC) is much better when TBS-T was used as the range of OD is wider between TBS-T samples, here the dynamic range is increased. The 1:1000 dilution of the 2nd antibody [C] shows a higher background for both buffer systems but still a higher dynamic range for TBS-T assay. Data are given ± SD.

In a further step we compared Tris-based buffer with pH of 7.4 as it was used up to the present assay and pH 9.6. In Figure 7 dissimilarities of using pH 9.6 [A, C] and pH 7.4 [B, D] for analysis of auto-IgE reactivity are shown. Actually the higher pH of 9.6 revealed a better distinction of values analyzing the dilution row compared to pH 7.4 where the dilution row is indeterminable. Moreover, measuring a reactive sample four times (n = 8-12 each) shows more consistency when pH 9.6 is used. According to these results we changed the buffer system to Tris-based with a pH of 9.6.

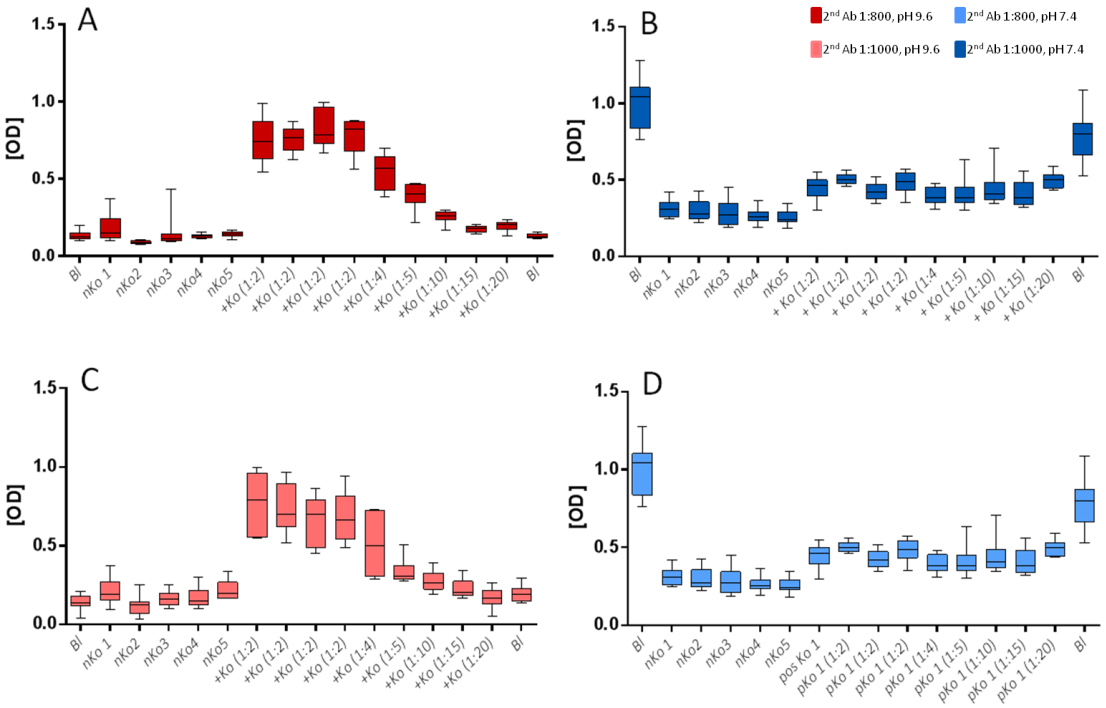


Figure 7: A higher pH of 9.6 reveals in more consistent results and higher sensitivity.
Four conditions with Tris buffer of pH 9.6 **[A, C]** and 7.4 **[B, D]** each with titration of the secondary antibody (1:800 **[A, B]**, 1:1000 **[C, D]** were tested using similar test samples. Five different non-reactive controls (nC1 - nC5) were used. In addition we tested one reactive sample in a dilution row, in which the 1:2 dilution was measured several times (each boxplot n = 8-12) to obtain the consistence of the analysis. **A** and **C** (pH 9.6) show the same median for the 1:2 dilution of the reactive sample (+ctrl (1:2)) and the decreasing median for the dilution row. Whereas using a pH 7.4 [B, D] does not reveal the required dilution row of the reactive control. Thus the pH 9.6 was utilized from now on. Data are given in a boxplot, showing the median, upper and lower quartile.

Commonly in ELISA blocking buffer is used to dilute samples, containing proper amounts of BSA and Tween®. At the risk of blocking the rarely occurring auto-antigenic epitopes we tested the use of TBS-T containing 1 % BSA for sample dilution. As expected BSA used for sample dilution, when autoreactive antibodies are supposed to be detected is too strong for rarely occurring target proteins [Figure 8].

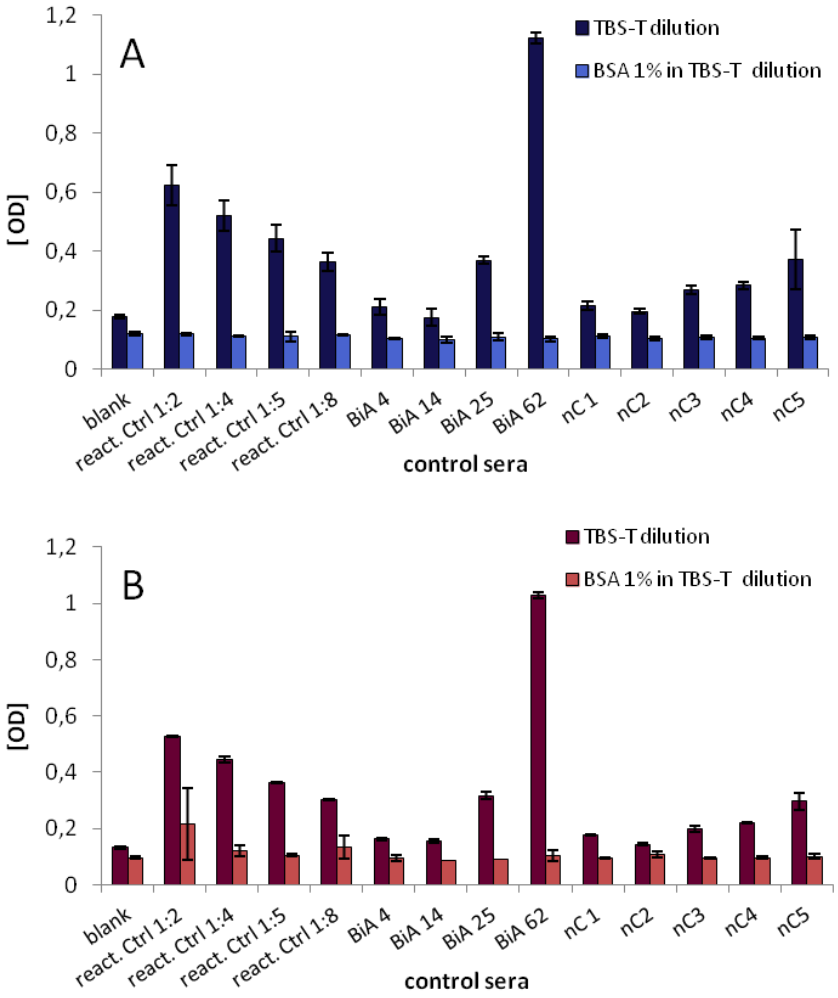


Figure 8: The sample dilution with 1% BSA in the dilution buffer blocks the accessibility of target proteins for autoreactive IgE.
The detection of auto-IgE reactivity was compared in different samples (react. Ctrl: auto-IgE reactive sample, BiA-samples, nC1 – nC5: non-autoreactive samples) diluted with and without 1 % BSA. **[A]** OD of IgE-autoreactivity towards HaCaT cell derived proteins. **[B]** OD of IgE-autoreactivity towards NHBE derived proteins. A and B show significant differences of using 1 % BSA in sample dilution buffer (light red, light blue), as the BSA containing buffer revealed in no differentiability of positive or negative samples. Compared to samples diluted with sample-dilution buffer without BSA (dark red, dark blue bars) Data are shown ± SD.

In a last step we tested a second anti-IgE secondary antibody (Novus biological) in addition to the anti-IgE used hitherto (Sigma-Aldrich) and a second 384-well plate (Immunosorp®, Brand) that binds proteins with the same principle as the Maxisorp® (nunc) plate does. We tested the four combinations illustrated in Figure 9, each antibody in each plate using different non-reactive (nC1 – nC12) and reactive samples (react. Ctrl) additionally a dilution row (1:2 to 1:20) of the last. Figure 9A depicts the Maxisorp® plate in combination with the antibody from Sigma-Aldrich (brownish bars) and with the new tested antibody from Novus biological (blue bars) both diluted 1:800. The same combination depicts [B] with a dilution of 1:1000 of both 2nd antibodies. The second plate (Immunosorp, Brand), tested with both antibodies (Sigma-Aldrich; red bars, Novus biological; grey bars) is shown in [C] with a dilution of 1:800 and [D] 1:1000. The utmost discrepancy was obtained for the dynamic range of the assay when testing the different combinations of antibody and plates. Therefore the dynamic range is higher when Maxisorp®-plate and the antibody from Sigma-Aldrich are combined, as if the same plate with the antibody from Novus biological as shown in [A] is used. Here the range between the non-reactive (nC) samples and the reactive (react. Ctrl) is much broader within the brownish bars (antibody, Sigma-Aldrich 1:800) compared to the blue bars (antibody, Novus biological 1:800). The same result is reflected in [C] in the Immunosorp® plate (Brand). Hence the combination of the 2nd antibody from Sigma-Aldrich and the Maxisorp® plate from nunc yields in the higher dynamic range for this Immunoassay, hence a better discrimination of the difference of reactive samples is given. In [B] and [D] the higher dilution (1:1000) of both antibodies confirms the latter results, moreover the same combination of plate and antibody reveals higher consistency when the same samples (react. Ctrl 1:2) are measured four times (each n = 3) as illustrated in [B].

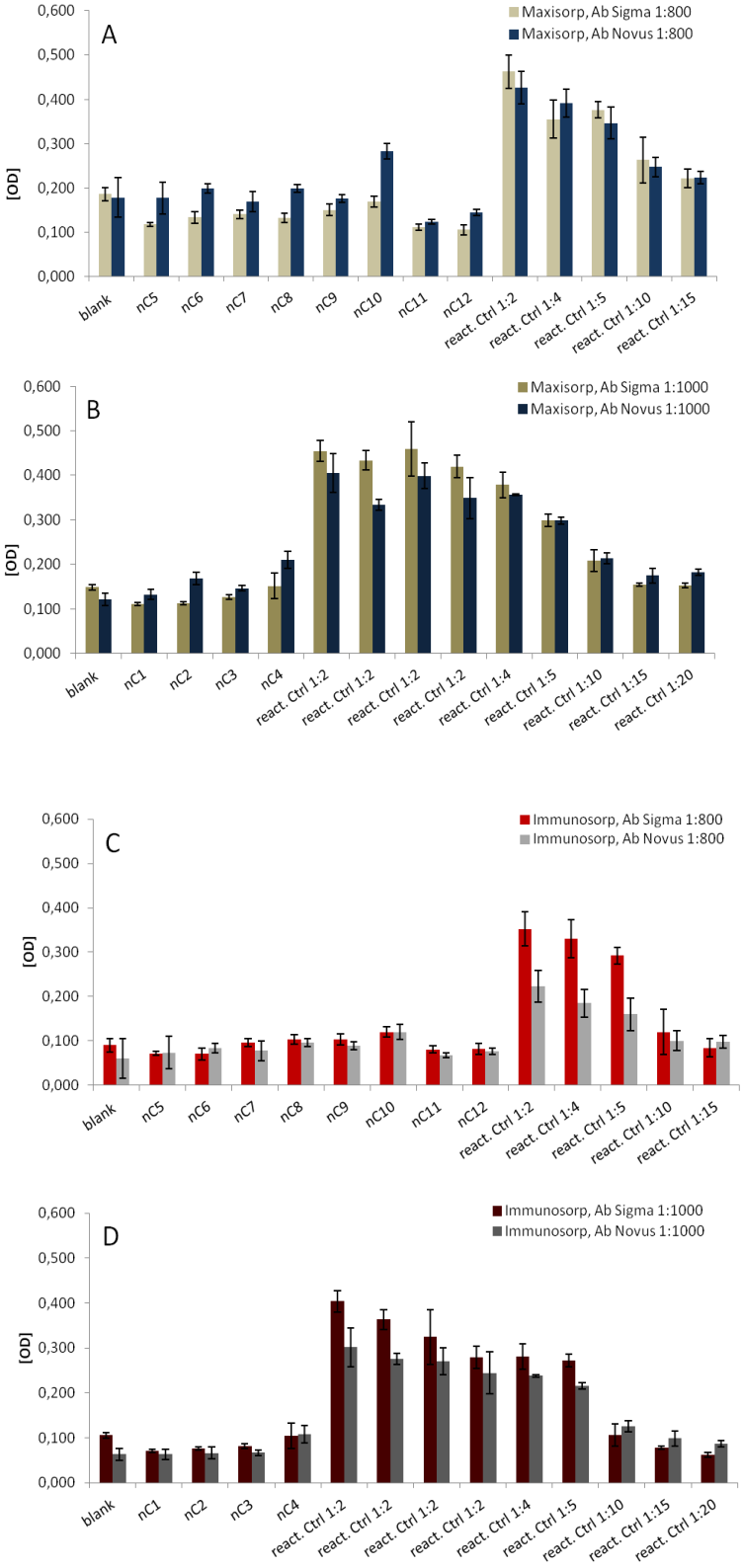


Figure 9: The combination of Maxisorp and the anti-IgE antibody from Sigma-Aldrich is the most adequate combination for the present system to detect auto-IgE.

[A], [B]: compare Maxisorp® plate, (nunc) and two different anti-IgE antibodies (Sigma Aldrich, Novus biological) and two dilutions (1:800/1:1000). Non-reactive samples (nc1-nc4) showed less reactivity, but reached a higher dynamic range by using the anti-IgE antibody from Sigma-Aldrich, compared to the anti-IgE from Novus biological. Compared to the higher dilution of 1:1000 of both antibodies **[B], [D]** the antibody from Sigma-Aldrich revealed in more consistent results when a sample dilution of 1:2 was measured several times (each n = 3). The combination of both antibodies and the Immunosorp® plate **[C], [D]** (brand, Germany) revealed in less background and a broader dynamic range using the Sigma-Aldrich Ab. The higher dilution of 1:1000 **[D]** revealed in less consistent results for the Sigma Antibody and a non-detectable dilution row for the Ab from Novus biological. Data are given ±SD.

4.2. Characteristic of atopic eczema patients tested retrospectively for IgE-autoreactivity in serum towards skin-derived proteins

Sera from 107 patients which had been diagnosed for intrinsic or extrinsic atopic eczema were collected at the Department of Dermatology and Allergy at Biederstein. Out of these, 105 were screened for their auto-IgE reactivity in serum and are characterized in Table 11.

All required informations for the subsequent analysis were anonymously retrieved by the inspection of medical records, i.e. total IgE serum level, RAST-data, SCORAD and age. For several parameters as duration of atopic eczema (AE) or other atopic diseases (e.g. allergic asthma, rhino conjunctivitis, hay fever) medical records were not sufficiently informatory, so as to no complete analysis of those parameters could be conducted.

This study-group was analyzed retrospectively for their auto-IgE reactivity towards proteins extracted from primary keratinocytes of healthy subjects and atopic eczema patients. None of the tested patients showed a significant difference between the two cell types (data not shown), so that all results show the mean of their reactivity towards the two primary cell types.

Table 11: Study population characteristics for atopic eczema patients (n=105).

Screened patient group	n	105
sex (male/ female)	n (%)	50 (47.6)/ 55(52.4)
age	mean (sd)	21.9 (20.3)
BMI	mean (sd)	20.34 (4.9)
total IgE [kU/L]	mean (sd)	2135(6056)
SCORAD	mean (sd)	33.7(16.0)
AE-profile		
Intrinsic AE/ extrinsic AE	n (%)	51 (48.6)/ 54 (51.4)

4.2.1. IgE autoreactivity is not associated with the main symptoms of disease of atopy or atopic eczema

Sera of AE-patients and healthy control subjects (n = 33) were tested for auto-IgE against protein extracts of primary keratinocytes (pKc). All healthy, non-reactive assay controls were shown to lack auto-IgE reactivity against these proteins givin a mean reactivity-factor of 0.5 (reactivity ≥1 is classified as positive or IgE autoreactive respectively).
We performed a correlation analysis by linear regression for the main criteria of diagnosis of atopic diseases as AE to figure out any relations between auto-IgE reactivity and clinical parameters.

4.2.1.1. Serum level of total IgE is not correlated with the occurrence or level of IgE-autoreactivity

Figure 10 depicts the correlation of auto-IgE reactivity and serum level of total IgE in 105 patients, without any correlation between the two parameters (p = 0.96, CI = 95 %). We confirmed this outcome for different classes of age (0 - 5 y, 6 -12 y, 13 – 18 y and adults), but no sub-analysis showed any correlation at any age in this study population.

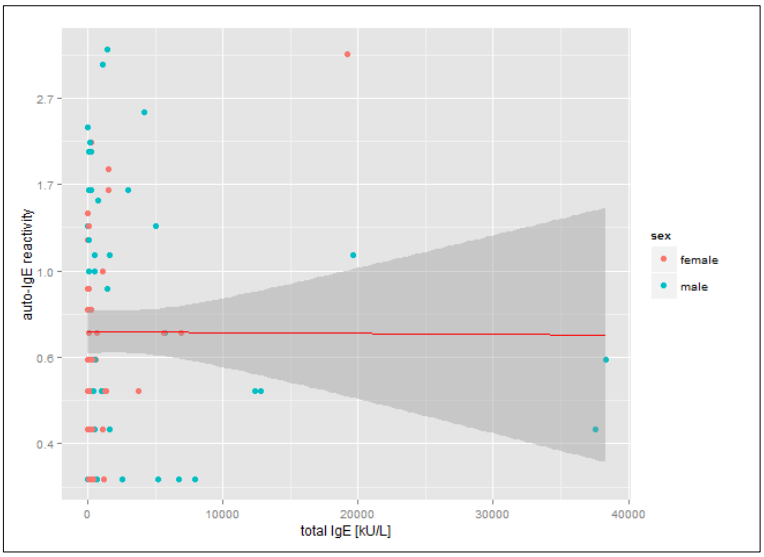


Figure 10: No correlation between auto-IgE-reactivity and total IgE.
105 AE-patients were analyzed for the correlation of the amount of total-IgE in serum and their auto-IgE reactivity towards proteins of primary keratinocytes. No correlation in this study group for auto-IgE reactivity towards skin-proteins and total IgE in serum was observed. The x-axis shows the amount of total IgE analyzed in serum and the y-axis the auto-IgE reactivity calculated by the normalized cut-off. Patients showing a reactivity ≥ 1 are classified as positive/reactive. p=0.96, Confidence Interval 95 %.

4.2.1.2. The extent of SCORAD in AE patients is not associated with auto-IgE reactivity

In a second analysis we performed a correlation analysis for SCORAD (**Sco**ring **A**topic **D**ermatitis) and auto-IgE reactivity for all patients and for classified age-groups. Figure 11 depicts the linear regression for all patients showing no interrelation between the two parameters. As there was no difference for any group of age (0 - 5 y, 6 -12y, 13 – 18y and adults), data of those sub-analyses are not shown here.

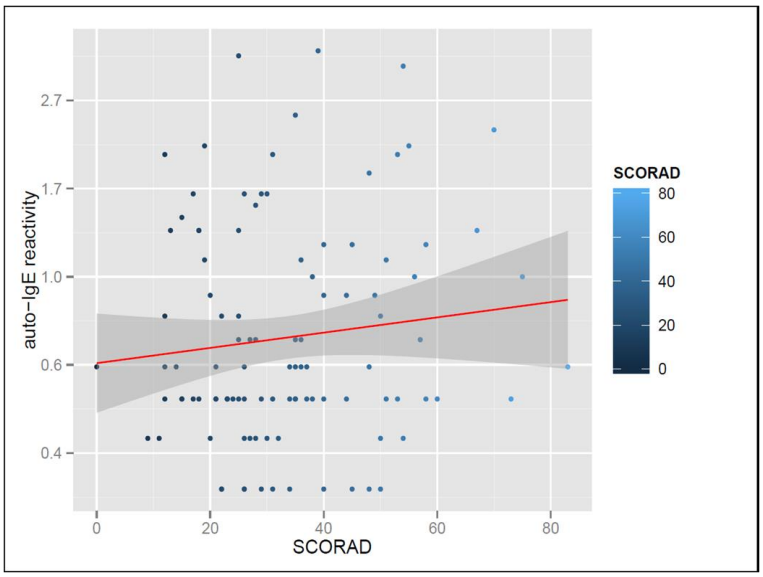


Figure 11: Linear regression analysis for auto-IgE-reactivity and SCORAD.
105 AE-patients were analyzed for the correlation of the SCORAD auto-IgE levels towards proteins of primary keratinocytes. There finds no correlation in this study group for auto-IgE reactivity towards skin-proteins and the extent of SCORAD. The x-axis shows the SCORAD, the y-axis the auto-IgE reactivity calculated by the normalized cut-off. Patients showing a reactivity ≥ 1 are classified as positive/reactive. Data are shown with confidence interval 95 %. Adj. R^2= 0.003, p=0.26.

4.2.2. AUTO-IGE REACTIVITY IS NOT ASSOCIATED TO THE BODY MASS INDEX

Given that there is ongoing discussion on the link of obesity and allergy we tested this relation by linear regression of the body-mass index (BMI) in our study group likewise. As shown in Figure 12 we could not find a significant interrelation between auto-IgE reactivity towards skin-proteins and the BMI in our study population. In addition we analyzed whether there is a difference between patients showing a positive auto-IgE (factor ≥1) reactivity against skin and those who do not show auto-IgE reactivity (factor <1) (data not shown) and again we analyzed the different age-classes (0 - 5 y, 6 -12 y, 13 – 18 y and adults), again all sub-analyses revealed in the same result of no correlation between the two factors (data not shown).

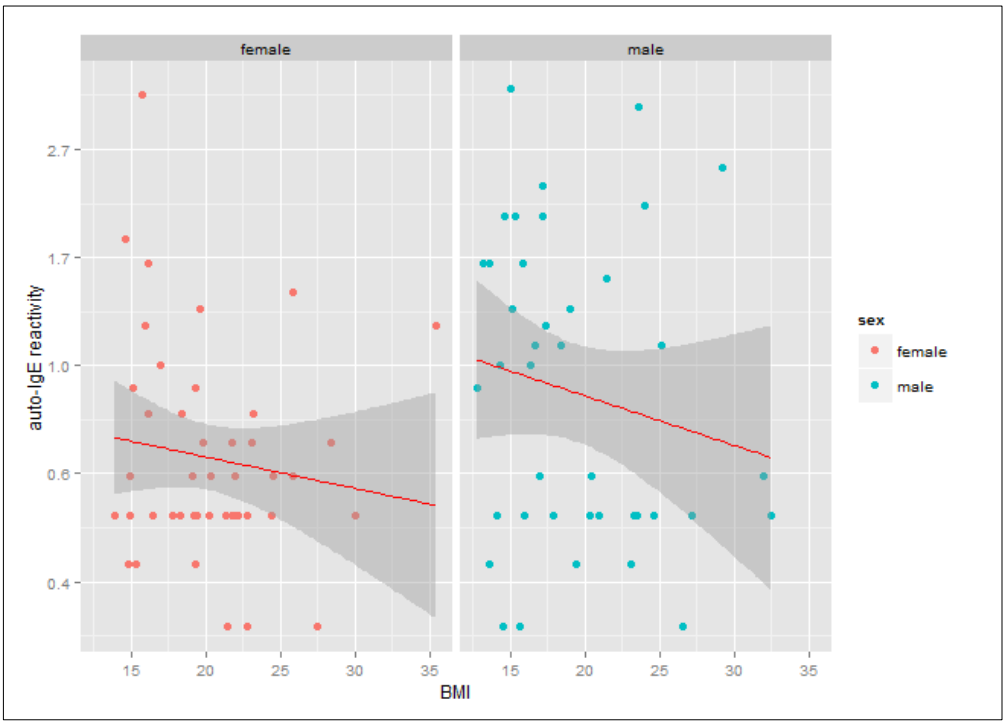


Figure 12: No correlation between auto-IgE-reactivity and BMI.
105 AE-patients were analyzed for the correlation of their BMI and the auto-IgE reactivity towards proteins of primary keratinocytes in a linear regression model. In this study group there is no correlation for auto-IgE reactivity towards skin-proteins and the BMI whether for females (f) (adj. R^2=0.02, p=0.161) nor for males (m) (adj. R^2=0.02, p=0.175) The x-axis shows the calculated BMI and the y-axis the auto-IgE reactivity calculated by the normalized cut-off. Patients showing a reactivity ≥ 1 are classified as positive/reactive. Data are shown with confidence interval 95 %.

4.2.3. IgE-AUTOIMMUNITY REVEALS NO GENDER SPECIFICITY

Since long the effects of certain hormones, especially estrogens, in females are discussed to play an active role in allergic predisposition and allergic disease in women, we focused one part of our analysis on the gender differences in auto-IgE occurrence and reactivity. Figure 13 depicts a boxplot for all male and female auto-IgE reactivity towards skin proteins and shows no substantial difference in both groups. In addition we conducted the analysis for males and females classified for age (0 - 5 y, 6 -12 y, 13 – 18 y and adults) without any gender difference in the subgroups (data not shown).

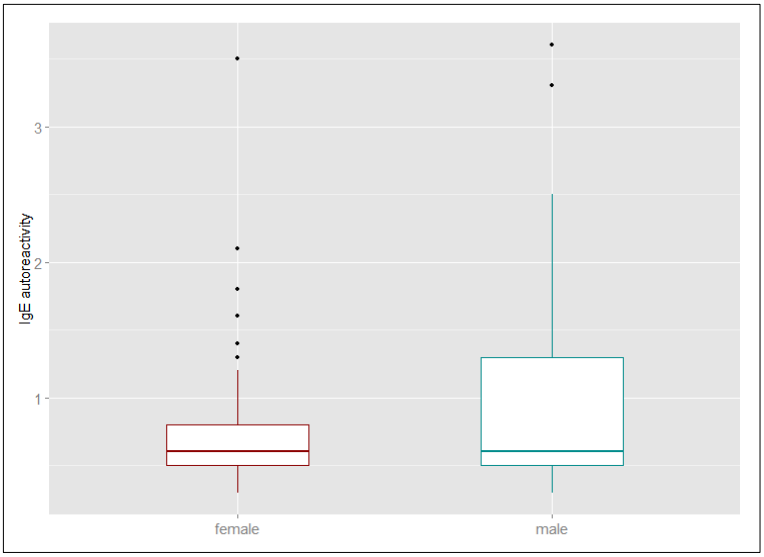


Figure 13: Boxplots representing the IgE-autoreactivity against proteins from primary keratinocytes in males and females depict no gender specific difference.
The y-axis shows the auto-IgE reactivity calculated by the normalized cut-off. Patients showing a reactivity ≥ 1 are categorized as positive/reactive without any difference between males and females p=0.157.

4.2.4. There are no evidences for different auto-IgE reactivity in atopic eczema with high or low total IgE in serum

Moreover we assessed the difference of IgE autoimmunity in atopic eczema with high (extrinsic) and low (intirinsic) total serum IgE. The cut-off for total IgE in serum to classify intrinsic AE was set at <125 kU/L, we did not evaluate the presence of specific IgE for environmental and food allergens, due to absent information in the medical records. Although there are differences in IgE reactivity against skin proteins, mainly in the age group of 6 – 12 years (p=0.03) the evaluation of this analysis is limited due to the low number of patients in each single group [

Table 12].

Since there is no consistent classification for the distinction of both types of atopic eczema, the analysis was additionally conducted for several cut-offs for total serum IgE mentioned in the literature (100 kU/L, 150 kU/L, 200 kU/L). There was no difference between the analysis of 100 kU/L and 125 kU/L, the other two analyses for 150 and 200 kU/L revealed very similar results, but no significant difference in the age group 6 – 12 years. In conclusion we could not confirm any differences for auto-IgE autoimmunity between intrinsic and extrinsic AE.

Table 12: Numbers of patients in each group for the analysis of IgE-autoimmunity in intrinsic and extrinsic AE.

Age class	[n]	Intrinsic AE [n]	extrinsic AE [n]
0 – 5 years	12	4	8
6 – 12 years	12	9	3
13 – 18 years	16	11	5
adults	65	27	38

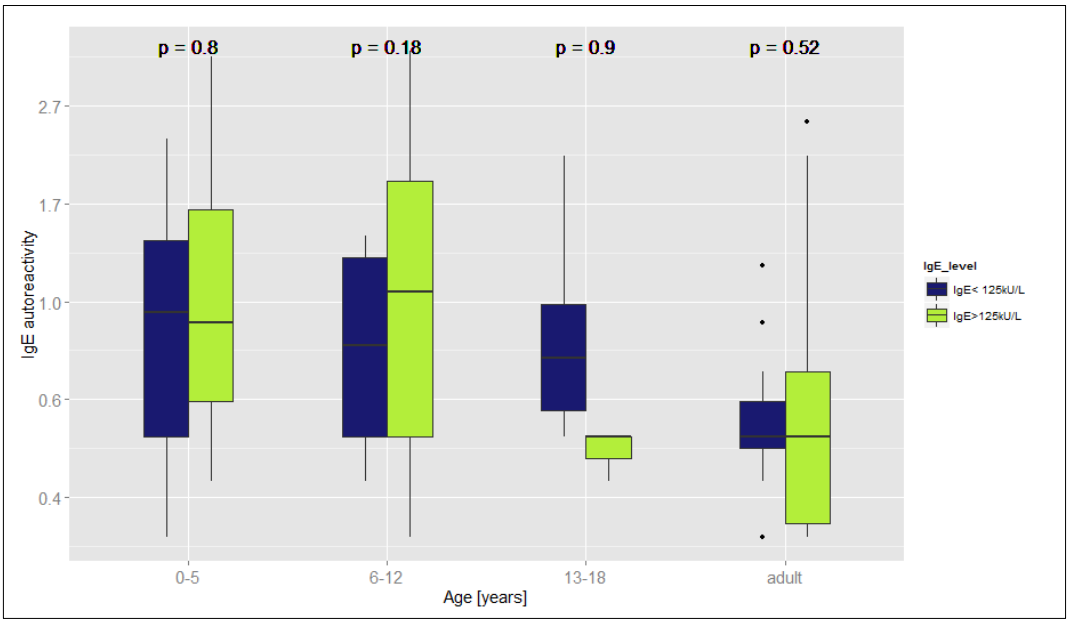


Figure 14: IgE auto-reactivity shows no difference between patients having lower levels (<125 kU/L) and higher levels (>125kU/L) of total serum IgE .
No differences could be observed for AE with high and low levels of total serum IgE. 0-5 yrs: n=21, 6-12 yrs: n=27, 13-18 yrs: n=11, adults: n=46.

We could confirm all results for the mentioned diagnostic and clinical parameters by screening the GINI/LISA birth cohorts [Table 14]. Hence, there is no correlation of auto-IgE and the total amount of serum IgE, neither for SCORAD nor BMI; furthermore any gender difference could be proven in both screened birth cohorts.

Due to absent information of the duration of atopic eczema in numerous medical records, it was not possible to conduct a significant analysis on the impact or relation of auto-IgE and the duration of disease.

4.2.5. QUANTITY AND EXTENT OF AUTO-IGE REACTIVITY IN CHILDREN TOWARDS SKIN IS HIGHLY PRONOUNCED COMPARED TO ADULTS

The study group of AE-patients comprised a wide distribution of age (10 month to 81 years) for which reason we classified all subjects into four age categories (0 – 5 y, 6 – 12 y, 13 – 18 y and adults) to analyze their auto-IgE reactivity towards skin derived proteins. Figure 15 depicts the percentage of reactive (cut-off ≥1) subjects in each age group. Unexpectedly we revealed a rather high percentage of reactive young children compared to adults.

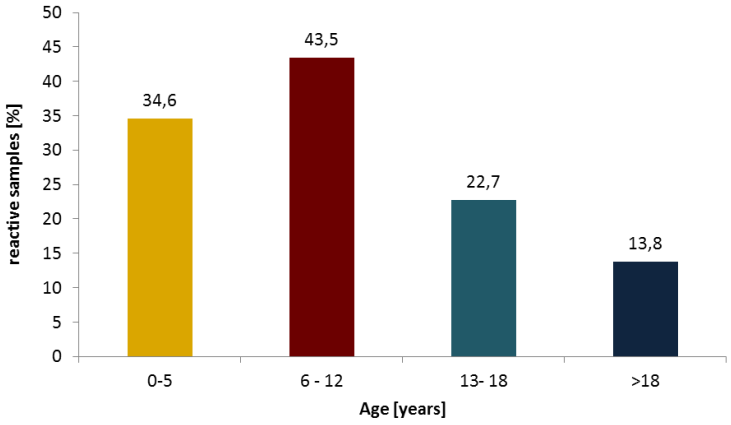


Figure 15: Auto-IgE reactivity against skin derived proteins in children shows higher quantity and reactivity compared to adults. Patients were classified for age, the percentage of reactive subjects (cut-off ≥1) are depicted in the bargraph. The higher extent of IgE-autoreactivity against skin derived proteins is shown, that is higher in children compared to adults.

Moreover, we conducted a correlation analysis of age and IgE-reactivity by linear regression analysis as shown in Figure 16. We obtained a significant correlation $R^2 = 0.113$, p=0.00027 for both sexes that implies less and decreasing auto-IgE reactivity in adults [Figure 16A]. In addition we investigated the same analysis stratified for sex to investigate a potential difference in males and females as shown in Figure 16B. As the distribution of the data differs between the two groups we restricted the analysis until an age of 50. The decrease with age is slightly different between males (adj.R^2=0.082, p=0.032) and females (adj.R^2=0.087, p=0.022). The analysis for both sexes until an age of 50 years analog to Figure 16A shows only slight differences (adj.R^2=0.102, p=0.00098) to the analysis until 80 years.

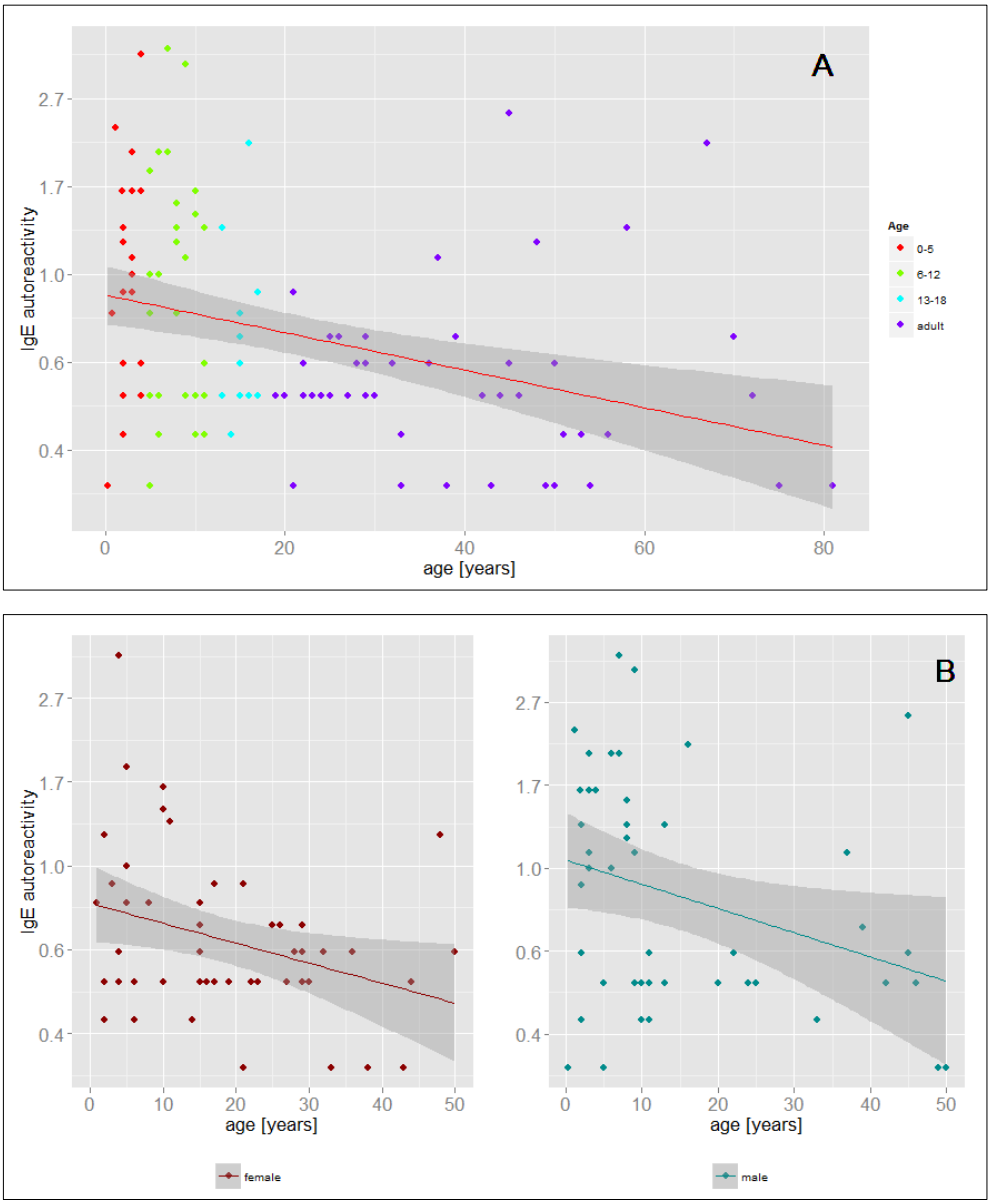


Figure 16: Auto-IgE reactivity decreases significantly with increasing age, as well for males and females.
Atopic eczema patients were taken for the correlation analysis by linear regression. **[A]** shows the regression for all patients 0 – 81 yrs (adj. R^2=0.113 p=0.0003). Whereas in **[B]** the analysis was created for males (R^2=0.082, p=0.032) and females (R^2=0.087, p=0.022) separately. Whereat auto-IgE reactivity of ≥1 is classified as positively self-reactive. Data are shown with CI= 95%.

4.3. CHARACTERIZATION AND ANALYSIS OF SERA OF BIRTH COHORTS GINI/LISA

To investigate and approve the higher percentage and extent of IgE-autoreactivity in children, as described in 0, in more detail we investigated 2861 children out of the two German birth cohorts GINI (***G**erman **I**nfant **N**utritional **I**ntervention birth cohort*) and LISA (*Influence of **L**ifestyle-related Factors on the **I**mmune **S**ystem and Development of **A**llergies in Childhood*). Table 13 itemizes the characteristic of the mixed study population that was screened for IgE-autoreactivity in serum towards skin (extracted from HaCaT cell line) and lung/ bronchia derived cell proteins (NHBE cells). The results of the GINI and LISA birth cohorts were done in cooperation with Dr. Elisabeth Thiering and Dr. JoachimHeinrich from the Institute for epidemiology I from the Helmholtz Zentrum.

Table 13: Study population characteristic of children from the combined GINI and LISA cohort screened for auto-reactive IgE in serum.

Screened GINI/ LISA cohort (10 yrs.)	n	2861
sex (male/ female)	n (%)	1459 (51)/ 1402 (49)
Bad Honnef	n (%)	150 (5.2)
Leipzig	n (%)	284 (9.9)
Munich	n (%)	1575 (55.1)
Wesel	n (%)	852 (29.8)
Age	mean (sd)	10.2 (0.2)
BMI	mean (sd)	17.4 (2.5)
RAST inhalants	n (%)	1128 (39.5)
RAST food	n (%)	531 (18.6)
Diagnosis		
asthma	n (%)	109 (3.9)
hay fever	n (%)	247 (9.0)
eczema	n (%)	138 (5.0)

4.3.1. CONFIRMATION IN SAMPLES OF THE GINI/LISA BIRTH COHORTS THAT DIAGNOSTIC AND CLINICAL PARAMETERS DO NOT CORRELATE WITH IGE-AUTOREACTIVITY

To confirm the non-relation of auto-IgE and common clinical and diagnostic parameters specific for allergy and atopy verified in the AE-patient group (also refer to chapter: 4.2.1.1 to 4.2.4), we analyzed these parameters likewise for the GINI/LISA sera. As itemized in Table 14 there is hardly any difference of the level of total serum IgE in auto-reactive and non-reactive children. Shown is the percentage of auto-reactive and non-reactive children for auto-IgE reactive against skin and lung derived proteins classified for the three set cut-offs. Regarding the increasing cut-off for skin autoreactivity, children showing very high

autoreactivity (3. cut-off) have significantly higher total IgE (p = 0.039), whereas this could not be shown for less skin-autoreactivity nor for lung-reactivity. Neither the BMI nor SCORAD showed any relation to auto-IgE reactivity. Furthermore we confirmed that no gender difference in IgE-autoreactivity can be observed or between atopic eczema patients with lower total serum IgE (<125 kU/L, intrinsic AE) and high total serum IgE levels (>125 kU/L, extrinsic AE).

Table 14: Total IgE level, sex or BMI showed no relation to auto-IgE reactivity towards skin and lung derived proteins.
Listed are the percentages of auto-reactive and non-reactive children for each parameter. Since there is no significant difference, we conclude no relation for auto-IgE and any of the listed parameters for children in the tested cohorts.

		IgE-autoreactivity skin [reactive/ non-reactive]			IgE-autoreactivity lung [reactive/ non-reactive]		
	n	1. cut-off	2. cut-off	3. cut-off	1. cut-off	2. cut-off	3. cut-off
total IgE [%]	625	47.0/49.3 p=0.340	30.1/33.4 p=0.121	**19.8/23.7** **p=0.039**	45.1/47.5 p=0.294	29.8/31.2 p=0.540	20.2/22.4 p=0.252
Total IgE [OR(95 % CI)]	625	1.12 (0.93-1.36) p=0.228	1.20 (0.98-1.46) p=0.077	1.25 (0.99-1.56) p=0.055	1.08 (0.90-1.31) p=0.408	1.04 (0.85-0.28) p=0.688	1.15 (0.92-145) p=0.216
sex (female/male) [%]	2861	46.8/48.1 p=0.502	30.0/31.5 p=0.408	19.3/21.9 p=0.085	45.6/45.5 p=0.971	29.9/30.3 p=0.843	20.1/21.2 p=0.511
BMI [OR(95 % CI)]	2861	1.00 (0.97-1.04) p=0.802	1.01 (0.98-1.05) p=0.433	1.02 (0.98-1.06) p=0.312	1.05 (1.01-1.08) p=0.007	1.01 (0.98-1.05) p=0.395	1.02 (0.98-1.06) p=0.363

4.3.2. Reduced auto-IgE reactivity in clinical relevant atopic disease compared to individuals with sensitization only

Preceding analyses of IgE autoreactivity in patients sensitized for perennial, seasonal or food allergens indicated distinct auto-IgE reactivity patterns (Beck 2012). At the 10-year follow up of the two birth cohorts GINI/ LISA, data for specific IgE were collected. The analyzed specific IgE (sIgE) levels include the most common food allergens (fx5) and inhalant allergens (sx1) and were measured by RAST/FIA. We first calculated percentages of sensitized children that show auto-IgE reactivity towards skin and lung. The first row of Table 15 depicts the percentage for children with and without food sensitization, the second row children with and without sensitization for aeroallergens, each stratified for the increasing cut-off. Children who are sensitized to food-allergens show statistically significant higher quantity in their IgE auto-reactivity towards lung and skin that stays

consistent with increasing cut-off, whereas children with sensitization for inhalant allergens show IgE-autoreactivity towards lung derived proteins exclusively.

Table 15: Percentage of children with auto-IgE stratified for the different cut-off, with food or inhalant allergen sensitization.
Shown is the percentage of non-sensitized and sensitized children (no/yes) for food-allergens (fx5) and inhalant allergens (sx1) and the calculated p-value. Total number of children who were analyzed for fx5 and sx1 are given (n).

Sensitization	n	IgE autoreactivity skin			IgE autoreactivity lung		
		1. cut-off	2. cut-off	3. cut-off	1. cut-off	2. cut-off	3. cut-off
food allergen (fx5) (no/yes) [%]	2328	45.1/58.2 p<0.001	28.0/43.1 p<0.001	18.3/31.1 p<0.001	43.3/55.7 p<0.001	27.7/40.7 p<0.001	18.3/30.9 p<0.001
inhalant allergen (sx1) (no/yes) [%]	1731	46.4/49.1 p=0.175	29.5/32.8 p=0.069	19.8/22.0 p=0.164	43.9/48.2 p=0.026	28.5/32.5 p=0.025	19.3/22.8 p=0.028

In order to overcome the impact of potential confounders a logistic regression was performed and adjusted for sex, study, city, age, BMI, parental education and parental atopy as shown in the two forest plots Figure 17 A (skin) and Figure 17B (lung). Figure 17 shows the association of skin autoreactivity and sensitization to inhalant allergens (upper part) and food allergens (lower part). The calculated OR is depicted as diamond with the according 95 % confidence interval. The dotted line indicates the OR = 1.0 that represent no association and therefore no higher/ lower risk to develop auto-IgE reactivity or sensitization. Children that are sensitized towards aeroallergens show slightly higher but not significant OR for autoreactivity. Whereas the sensitization to food allergens shows a significant higher auto-IgE reactivity, the risk increases with rising cut-off, suggesting an increasing association between sensitization and increasing auto-IgE reactivity *(OR(95 % CI) 1.cut-off: 1.86(1.52-2.27), p>0.001; 2.cut-off: 2.09(1.7-2.57), p<0.001; 3.cut-off: 2.14(1.71-2.68)p<0.001),*

The relationship between auto-IgE reactivity towards lung and sensitization to aeroallergens (upper part) and food allergens (lower part) is depicted in Figure 19. Actually both groups of allergens show an association that is again rising with increasing autoreactivity. A sensitization equal to inhalant and food allergens shows an increase in auto-IgE reactivity towards lung derived proteins, interesting here is that inhalant allergens have an effect on lung autoreactivity exclusively. *(OR(95 % CI) food sensitization: 1.cut-off: 1.74(1.42-2.12), p>0.001; 2.cut-off: 1.90(1.55-2.33), p<0.001; 3.cut-off: 2.18(1.75-2.73)p<0.001)*

Inhalant allergen sensitization: *1.cut-off: 1.18(1.00-1.38), p<0.005; 2.cut-off: 1.21(1.02-1.44), p<0.005; 3.cut-off: 1.26(1.04-1.53)p<0.005).*

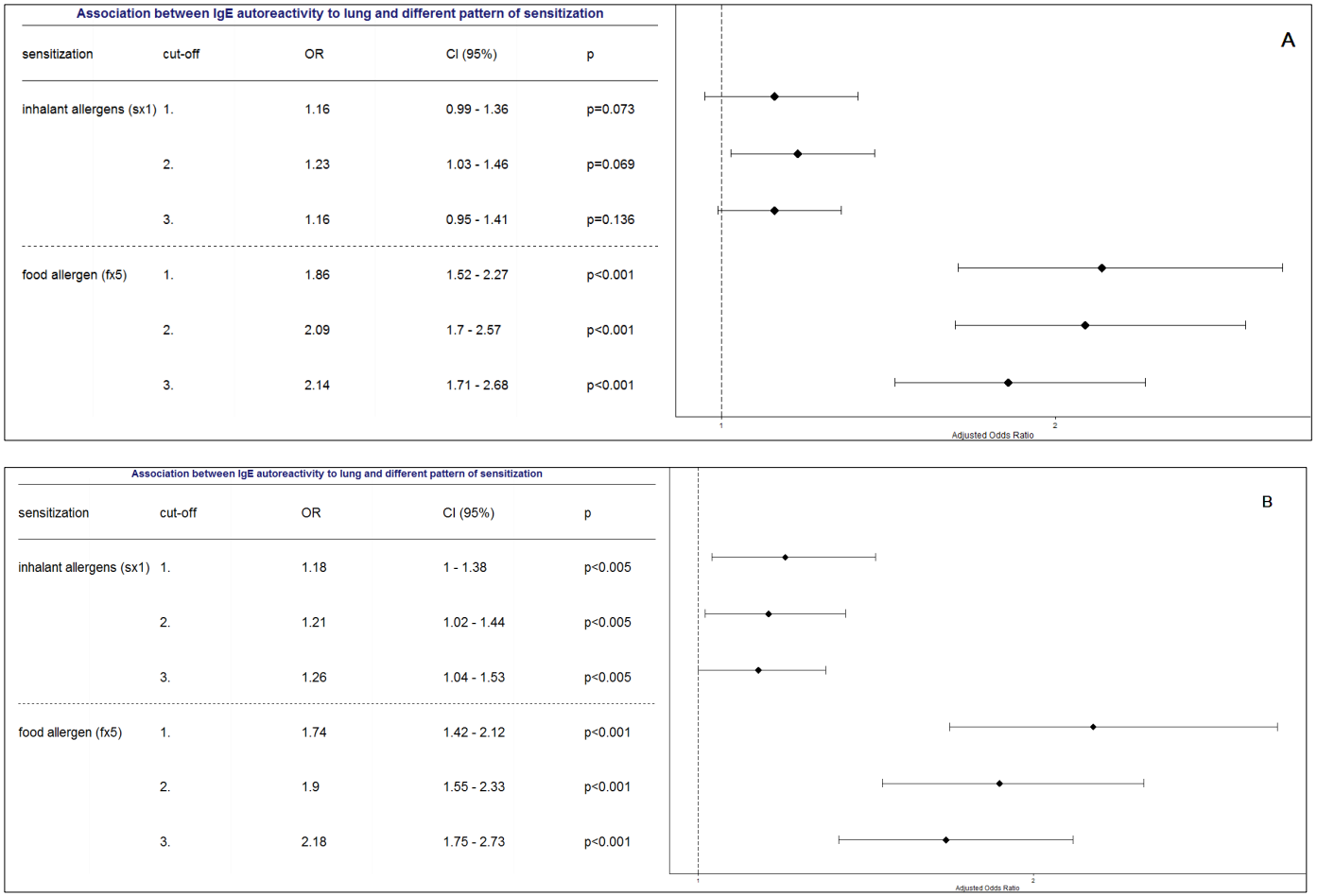


Figure 17: Sensitization towards aeroallergens predisposes lung autoreactivity only, whereas sensitization towards food allergens predisposes lung and skin autoreactivity.
The forest plot shows the results of a logistic regression model. The odds ratio (OR) is depicted as a black diamond and the according 95 % confidence intervall (CI) for skin **[A]** and lung **[B]** IgE-autoreactivity. The model was adjusted for sex, study, city, age, BMI, parental education, parental atopy.
A sensitization towards inhalant allergens shows a positive association both for skin **[A, upper forest plot]** and lung **[B, lower forest plot]** but only the association to lung autoreactivity is significant (p<0.005). In contrast a sensitization towards food allergens is positively associated with IgE-autoreactivity towards akin **[A]** and lung **[B].** Children sensitized towards inhalant allergens n = 1731 and food allergens n = 2328.

Furthermore we conducted continuative analyses for each single allergen enclosed in fx5 and sx1 as shown in Figure 18 and Figure 19. In order to point out a dose-response of the association between higher auto-IgE reactivity (id est increasing cut-off) and the sensitization for the single allergens, each image illustrates the OR and the attendant 95 % CI using a forest plot. Each single allergen is named on the left, the cut-offs are one below the other. Again we performed a logistic regression and adjusted the model for sex, study, city, age, BMI, parental education and parental atopy as potential confounder. A dose-response is defined as the OR rises with increasing cut-offs as the "risk" of autoreactivity gets higher with sensitization. This observation is even more distinct when the p-value decreases with the rising OR.

Figure 18 illustrates the analyses for each single allergen contained in the allergen-mixture of fx5 (ovalbumin, milk protein, flour, codfish, peanut, soybea) and the combination of those (fx5). Each allergen and its three cut-offs is shown in one color, as the diamond depicts the OR and the bars the 95 % CI. A sensitization towards single allergens is analyzed only if the patient showed a positive reactivity in the test towards the allergen mix sx1 or fx5. Anyhow, a positive test reaction towards those mixed allergens is possible both if a sensitization towards single allergens exists and when the patient shows a multiple sensitization towards several allergens out from the mixture. In case of a positive sx1 or fx5 testing the serum was analyzed for its reactivity towards each single allergen. Figure 18 shows the association of auto-IgE reactivity towards skin [Figure 18a] and lung [Figure 18b] and the sensitization towards food allergens. Further the analysis of the test of sensitization in the combined mixture of all food allergens (fx5; ovalbumin, milk protein, codfish, flour, peanut, and soybean). As shown already before there is an association between the sensitization to food (fx5 -Figure 17) and auto-IgE reactivity to skin and lung. A positive test reaction to fx5 shows a significant dose-response as the OR rises significantly with the height of auto-IgE reactivity as well for skin- *(OR (95 %CI); 1. cut-off 1.86(1.52-2.27), p<0.001, 2. cut-off 2.09 (1.70-2.57), p<0.001; 3. cut-off 2.14(1.71-2.68), p<0.001)* and lung reactivity *(OR (95 %CI); 1. cut-off 1.74(1.42-2.12), p<0.001, 2. cut-off 1.90 (1.55-2.33), p<0.001; 3. cut-off 2.18(1.75-2.73), p<0.001)*. For skin autoreactivity the sensitization to codfish shows a very strong and significant association between the 2. and 3. cut-off and a dose-response as the OR rises with increasing cut-off. Even for lung reactivity we calculated a very high association with codfish sensitization, the 2. cut-off shows a relation that is not significant; therefore there is no significant dose-response.

Codfish/ skin: *(OR (95 %CI); 1. cut-off 2.44(0.91-6.51), p=0.075, 2. cut-off 3.69 (1.43-9.52), p=0.007; 3. cut-off 3.74 (1.39-8.68), p=0.008)* and lung *(OR (95 %CI); 1. cut-off 3.48(1.24-6.51), p=0.018, 2. cut-off 2.09 (0.84-5.20), p=0.114; 3. cut-off 3.61(1.45-9.01), p=0.006)*

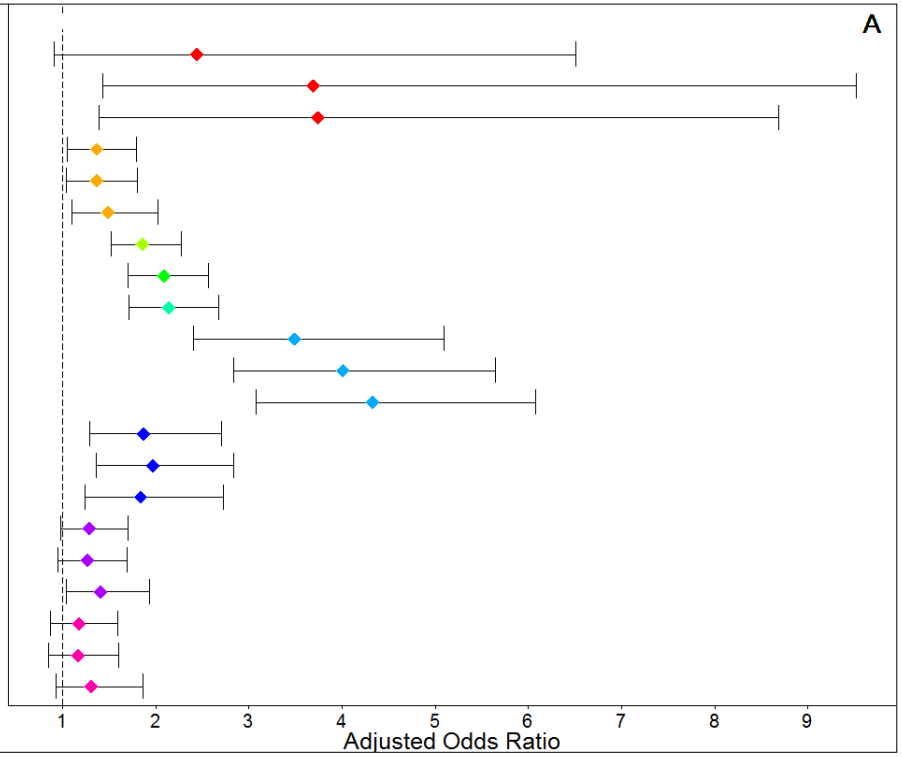
Moreover, a strong association and dose-response relation was observed for sensitization to milk proteins in both skin and lung IgE-autoreactivity. Milk protein/ skin: *(OR (95 %CI); 1. cut-off: 3.49(2.40-5.09), p<0.001, 2. cut-off: 4.01 (2.84-5.65), p<0.001; 3. cut-off: 4.33(3.08-6.08), p<0.001)* andlung *(OR (95 %CI); 1. cut-off: 4.43(3.00-6.54), p<0.001, 2. cut-off: 4.72 (3.33-6.68), p<0.001; 3. cut-off: 4.92(3.51-6.90), p<0.001).*

A sensitization to ovalbumin (OVA) shows a dose-response for skin reactive children as the OR rises significantly from cut-off 1 (reactive children) to cut-off 2 (high reactive children), however the OR for cut-off 3 stagnates on the level of the first. There is no dose-response for lung autoreactivity as the OR rises not over the three cut-offs. Anyhow there is a strong association between OVA-sensitization and auto-IgE reactivity for both cell types; skin *(OR (95 %CI); 1. cut-off: 1.87(1.29-2.71), p<0.001, 2. cut-off: 1.97 (1.36-2.84), p<0.001; 3. cut-off: 1.84(1.24-2.73), p<0.001)* and ung *(OR (95 %CI); 1. cut-off: 1.82 (1.26-2.63), p=0.002, 2. cut- off: 1.61(1.11-2.34), p=0.011; 3. cut- off: 1.82(1.82-2.71), p=0.003).*

For flour finds a dose-response from the 2. cut-off (high reactivity) to the 3. cut-off (very high reactivity), for the first two cut-offs the risk is the same *OR: 1.37 (skin (OR (95 %CI); 1. cut- off: 1.37(1.05-1.79), p=0.020, 2. cut- off: 1.37 (1.04-1.80), p=0.026; 3. cut-off: 1.49(1.10-2.02), p=0.009))*. The same for lung reactivity the OR rises with rising cut-offs but shows only for very high reactive children a significant association, hence there finds no significant dose-response for a sensitization to flour and auto-IgE reactivity in lung. The sensitization to peanut and soybean shows no significant dose-response, and no significant association to auto-IgE reactivity [Figure 18]

Dose-response analysis of auto-IgE reactivity to skin and single food allergens

allergen / protein	patients	cut-off	OR	CI (95%)	p
codfish	(99.3)/20 (0.7)	1	2.44	0.91 - 6.51	p=0,075
		2	3.69	1.43 - 9.52	p=0,007
		3	3.74	1.39 - 8.68	p=0,008
flour	(90.5)/272 (9.5)	1	1.37	1.05 - 1.79	p=0,020
		2	1.37	1.04 - 1.8	p=0,026
		3	1.49	1.1 - 2.02	p=0,009
fx5	(81.4)/531 (18.6)	1	1.86	1.52 - 2.27	p<0,001
		2	2.09	1.7 - 2.57	p<0,001
		3	2.14	1.71 - 2.68	p<0,001
milk protein	(94.5)/158 (5.5)	1	3.49	2.4 - 5.09	p<0,001
		2	4.01	2.84 - 5.65	p<0,001
		3	4.33	3.08 - 6.08	p<0,001
ovalbumin	(95.3)/134 (4.7)	1	1.87	1.29 - 2.71	p<0,001
		2	1.97	1.36 - 2.84	p<0,001
		3	1.84	1.24 - 2.73	p<0,001
peanut	(91.0)/257 (9.0)	1	1.29	0.98 - 1.7	p=0,067
		2	1.27	0.95 - 1.69	p=0,102
		3	1.41	1.04 - 1.93	p=0,029
soybean	(92.9)/203 (7.1)	1	1.18	0.87 - 1.59	p=0,287
		2	1.17	0.85 - 1.6	p=0,342
		3	1.31	0.93 - 1.86	p=0,124



Dose-response analysis of auto-IgE reactivity to lung and single food allergens

allergen / protein	patients	cut-off	OR	CI (95%)	p
codfish	(99.3)/20 (0.7)	1	3.48	1.24 - 9.76	p=0,018
		2	2.09	0.84 - 5.2	p=0114
		3	3.61	1.45 - 9.01	p=0,006
flour	(90.5)/272 (9.5)	1	1.08	0.83 - 1.41	p=0,577
		2	1.29	0.98 - 1.7	p=0,073
		3	1.55	1.15 - 2.1	p=0,004
fx5	(81.4)/531 (18.6)	1	1.74	1.42 - 2.12	p<0,001
		2	1.9	1.55 - 2.33	p<0,001
		3	2.18	1.75 - 2.73	p<0,001
milk protein	(94.5)/158 (5.5)	1	4.43	3 - 6.54	p<0,001
		2	4.72	3.33 - 6.68	p<0,001
		3	4.92	3.51 - 6.9	p<0,001
ovalbumin	(95.3)/134 (4.7)	1	1.82	1.26 - 2.63	p=0,002
		2	1.61	1.11 - 2.34	p=0,011
		3	1.82	1.82 - 2.71	p=0,003
peanut	(91.0)/257 (9.0)	1	0.99	0.75 - 1.3	p=0,951
		2	1.16	0.87 - 1.54	p=0,362
		3	1.37	1 - 1.87	p=0,051
soybean	(92.9)/203 (7.1)	1	0.96	0.71 - 1.3	p=0,810
		2	1.12	0.81 - 1.54	p=0,499
		3	1.34	0.95 - 1.9	p=0,099

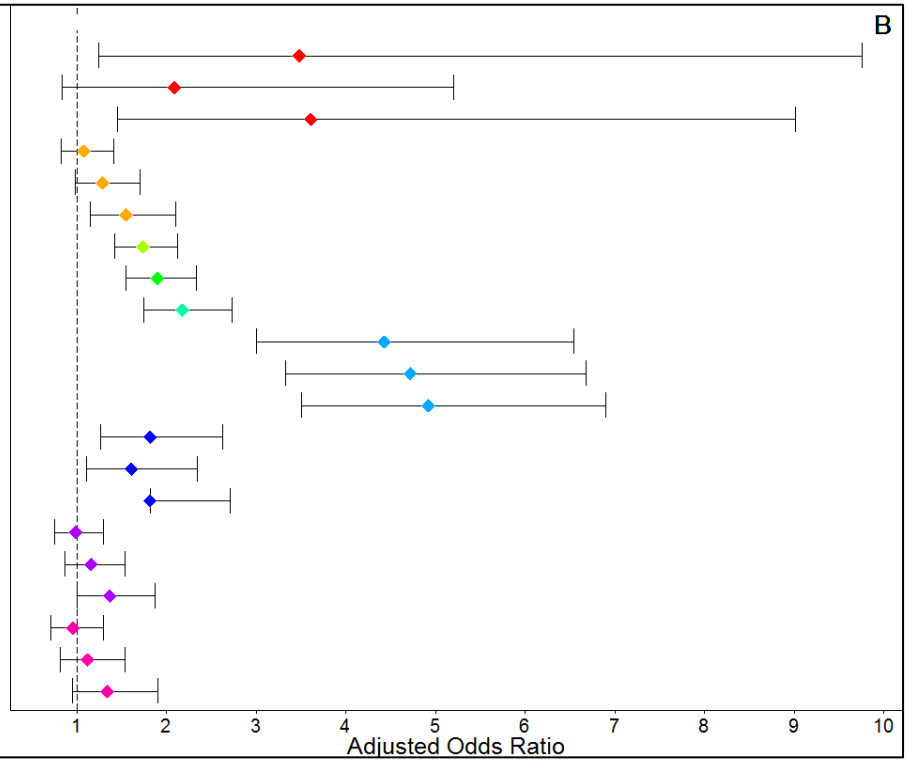


Figure 18: Single food allergens provoke a dose-response in auto-IgE reactivity against skin [A] and lung [B].
The forest plot shows the results of a logistic regression model. The OR is depicted as colored diamonds with the according 95 % CI. Each allergen and the three according cut-offs are depicted in one color. The model was adjusted for sex, study, city, age, BMI, parental education, parental atopy. Fx5 is the mixture of all analyzed food allergens and was tested likewise. Positive fx5 reactivity shows a positive significant dose-response as the OR rises with increasing cut-off for skin and lung. A sensitization towards codfish reveals in a high dose-response in skin (that is significant for the 2nd and 3rd cut-off). A sensitization towards milk proteins reveals an even higher dose-response and association to auto-IgE reactivity that is significant for both skin and lung. A sensitization to ovalbumin is as well significantly associated with a lower dose-response as the OR rises with the 1. and 2. cut-off, the 3.cut-off however stagnates in the skin analysis. There is no dose-response for lung autoreactivity but a significant association. Likewise for a sensitization to flour as the OR rises from 2. to 3. cut-off in skin analysis and in lung analysis only the „very high reactive" children show a positive association.

The design of Figure 19 (**A: skin reactivity, B: lung reactivity**) is the same as described for

Figure 18 but analyzed for aeroallergens and the mixture of aeroallergens (sx1; grass pollen, rye pollen, mugwort pollen, birch pollen, Cladosporium herbarum (C. herbarum), House dust mite (HDM, Derp1), cat, dog and mugwort pollen). Each allergen with the three cut-offs of auto-IgE reactivity is shown in one color, as the diamond depicts the OR and the bars the 95 % CI. Figure 19A shows the association on lung autoreactivity, m mon skin autoreactivity.

The analysis of reactivity against the allergen mixture of aeroallergens (sx1, next to last row, orange diamonds) actually depicts a dose-response for autoreactivity towards lung and sx1 sensitization as the OR rises slightly and the p-value decreases with increasing auto-IgE reactivity *(OR(95 % CI); 1. cut-off 1.18(1.00-1.38), p=0.045, 2. cut-off 1.21 (1.02-1.44), p=0.029; 3. cut-off 1.26(1.04-2.53), p=0.019)* [

Figure 19A]. The same analysis for skin autoreactivity reveals no dose response as the association between the 2. cut-off of auto-IgE reactivity and sx1-testing is significant only (p= 0.02) and the OR is not rising with increasing auto-IgE reactivity [

Figure 19A, orange diamonds]. Further we see a dose-response of patients sensitized against house-dust mite (HDM) as the OR rises with increasing auto-IgE reactivity (*OR(95 % CI); 1. cut- off: 1.2(0.99-1.45), p=0.06, 2. cut- off: 1.25 (1.02-1.52), p=0.03; 3. cut- off: 1.31(1.05-1.64), p=0.016)*, [

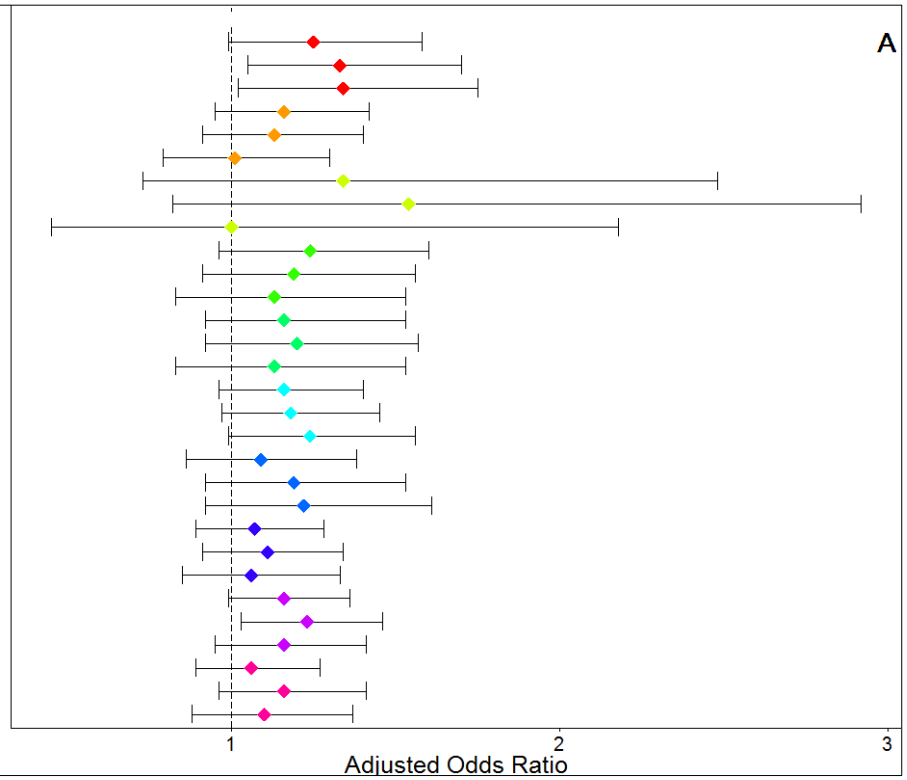
Figure 19A, light green diamonds]. The analysis for HDM sensitization and skin reactivity reveals a dose-response that is not significant [

Figure 19A], light green diamonds]. Any other of the analyzed single allergen shows neither a positive dose-response nor any significant association for IgE autoreactivity against lung.

Figure 19B depicts further a minor dose-response for skin autoreactivity and mugwort sensitization (first row, violet diamonds), *(OR(95 % CI); 1. cut-off 1.25(0.99-1.58), p=0.056, 2. cut-off 1.33 (1.05-1.70), p=0.019; 3. cut-off 1.34(1.02-1.75), p=0.032)* [Figure 19B]. No other single allergen sensitization shows a dose-response with skin autoreactivity.

Dose-response analysis of auto-IgE reactivity to skin and single allergens

allergen / protein	patients	cut-off	OR	CI (95%)	p
ambrosia	(86.7)/378 (13.3)	1	1.25	0.99 - 1.58	0.056
		2	1.33	1.05 - 1.7	0.019
		3	1.34	1.02 - 1.75	0.032
birch	(82.2)/508 (17.8)	1	1.16	0.95 - 1.42	0.149
		2	1.13	0.91 - 1.4	0.278
		3	1.01	0.79 - 1.3	0.91
C. herbarum	(98.3)/48 (1.7)	1	1.34	0.73 - 2.48	0.344
		2	1.54	0.82 - 2.92	0.181
		3	1	0.45 - 2.18	0.992
cat dander	(89.8)/291 (10.2)	1	1.24	0.96 - 1.6	0.105
		2	1.19	0.91 - 1.56	0.206
		3	1.13	0.83 - 1.53	0.448
dog dander	(89.7)/293 (10.3)	1	1.16	0.92 - 1.53	0.187
		2	1.2	0.92 - 1.57	0.175
		3	1.13	0.83 - 1.53	0.439
HDM (Derp1)	(78.0)/630 (22.0)	1	1.16	0.96 - 1.4	0.124
		2	1.18	0.97 - 1.45	0.101
		3	1.24	0.99 - 1.56	0.057
mugwort	(87.7)/352 (12.3)	1	1.09	0.86 - 1.38	0.497
		2	1.19	0.92 - 1.53	0.177
		3	1.22	0.92 - 1.61	0.171
rye	(75.9)/688 (24.1)	1	1.07	0.89 - 1.28	0.479
		2	1.11	0.91 - 1.34	0.314
		3	1.06	0.85 - 1.33	0.602
sx1	(60.5)/1128 (39.5)	1	1.16	0.99 - 1.36	0.073
		2	1.23	1.03 - 1.46	0.02
		3	1.16	0.95 - 1.41	0.136
timothy grass	(74.3)/734 (25.7)	1	1.06	0.89 - 1.27	0.514
		2	1.16	0.96 - 1.41	0.118
		3	1.1	0.88 - 1.37	0.392



Dose-response analysis of auto-IgE reactivity to lung and single allergens

allergen / protein	patients	cut-off	OR	CI (95%)	p
ambrosia	(86.7)/378 (13.3)	1	1.02	0.81 - 1.29	0.834
		2	1.23	0.97 - 1.57	0.089
		3	1.36	1.04 - 1.78	0.023
birch	(82.2)/508 (17.8)	1	1.02	0.83 - 1.25	0.877
		2	1.01	0.81 - 1.25	0.953
		3	0.99	0.77 - 1.27	0.918
C. herbarum	(98.3)/48 (1.7)	1	1.25	0.68 - 2.3	0.477
		2	1.09	0.56 - 2.11	0.803
		3	0.98	0.45 - 2.14	0.955
cat dander	(89.8)/291 (10.2)	1	1.14	0.88 - 1.47	0.331
		2	1.1	0.84 - 1.44	0.494
		3	1.03	0.76 - 1.41	0.844
dog dander	(89.7)/293 (10.3)	1	1.01	0.78 - 1.3	0.956
		2	1.11	0.85 - 1.45	0.456
		3	1.11	0.82 - 1.51	0.491
HDM (Derp1)	(78.0)/630 (22.0)	1	1.2	0.99 - 1.45	0.06
		2	1.25	1.02 - 1.52	0.03
		3	1.31	1.05 - 1.64	0.016
mugwort	(87.7)/352 (12.3)	1	0.97	0.77 - 1.24	0.828
		2	1.19	0.92 - 1.52	0.184
		3	1.32	1 - 1.73	0.052
rye	(75.9)/688 (24.1)	1	0.99	0.83 - 1.19	0.933
		2	1.04	0.86 - 1.27	0.677
		3	1.11	0.89 - 1.38	0.361
sx1	(60.5)/1128 (39.5)	1	1.18	1 - 1.38	0.045
		2	1.21	1.02 - 1.44	0.029
		3	1.26	1.04 - 2.53	0.019
timothy grass	(74.3)/734 (25.7)	1	1.04	0.87 - 1.24	0.662
		2	1.11	0.92 - 1.35	0.27
		3	1.17	0.94 - 1.45	0.149

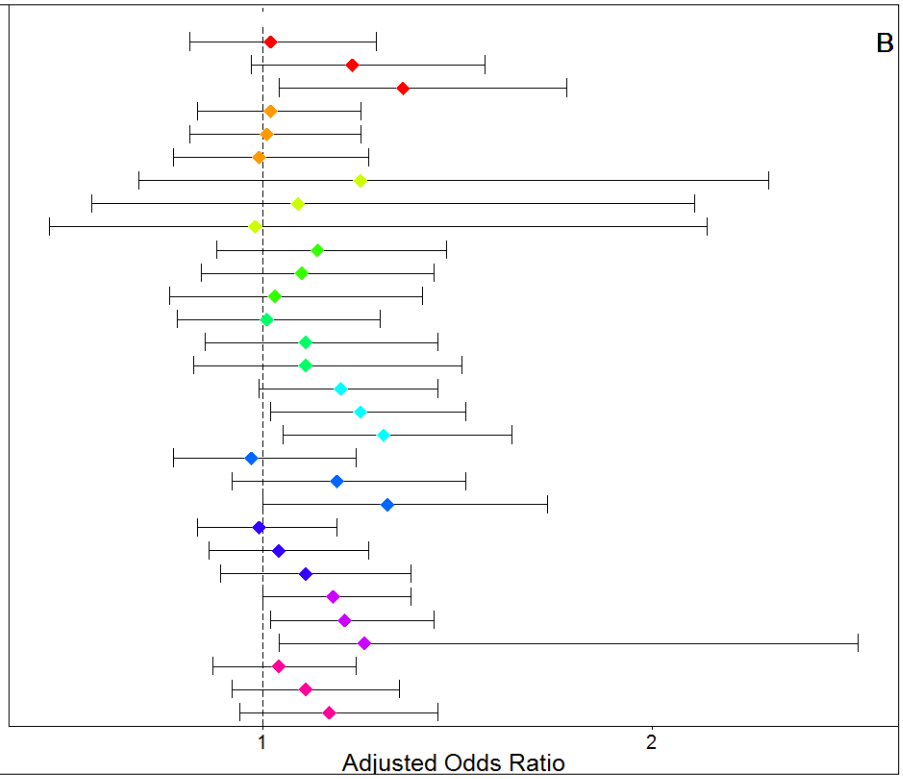


Figure 19: Sensitization to house-dust mite provoke a dose-response in auto-IgE reactivity against lung autoreactivity.
The forest plot shows the results of a logistic regression model testing the association between **skin-[A]** and **lung [B]** autoreactivity and the sensitization to aeroallergens. The OR is depicted as colored diamonds with the according 95 % CI. Each allergen and the three according cut-offs are depicted in one color. The model was adjusted for sex, study, city, age, BMI, parental education, parental atopy. Sx1, as the mixture of all tested aeroallergens shows slight dose-response in the lung analysis as the OR rises with increasing reactivity or cut-off respectively. This effect cannot be shown for skin-autoreactivity. There is only one allergen leading to a dose-response by oneself. A sensitization to house-dust mite (HDM) leads to increasing OR with the rising cut-off and a decreasing p-value that could not be shown for skin. Any other aeroallergen sensitization does not lead to significant association or dose-response whether in skin nor in lung.

Furthermore we investigated whether any kind of clinical manifestation of atopy is associated with a higher of IgE-autoreactivity towards skin or lung cell proteins. Against our expectations an adjusted logistic regression model revealed no association of IgE-reactivity and clinical manifestations of atopic disease (allergic asthma, allergic rhinoconjunctivitis, atopic eczema, or IgE-mediated food allergy). Data on children´s health and atopy were collected by using repetitive parent-completed questionnaires every six months during the first two years of age and later at age 4, 6 and 10 years.

The upper part of Table 16 and Table 17 display all medical diagnosed atopy in the last 12 months at age 10. Column three shows the number (n) and percentage (%) of children who were diagnosed with the listed atopy form or not (no/ yes). The following columns illustrate the OR and the 95 % confidence interval calculated for each cut-off and the accompanied strength of reactivity.

The lower part of each table illustrates a more detailed analysis on atopic diagnosis (asthma, eczema, hay fever/rhinitis and food allergy) as we determined the frequency of a given "Yes" in the questionnaires for each single child and diagnosis over ten years of data collection.

Here we excluded data sets of children when data more than four times were not applicable (n.a.). The combination of *"No"* diagnosed atopy and of 1-4 times *"n.a."* data were analyzed separately, likewise as 1times, 2-3 times and ≥4 times the answer *"Yes"* for diagnosed atopy.

Table 16 shows the association between auto-IgE reactivity towards skin cell proteins and the clinical manifestation of atopy. The general analysis revealed no association between auto-IgE reactivity and asthma or food allergy. For eczema we investigated a significant lower chance to develop very high autoreactivity when eczema was answered with one time "Yes" and with greater or equal four times "Yes" for a reactivity that exceeds the lowest cut-off. A significant higher risk was shown in one case (2-3x "Yes") for hay fever/ rhinitis. Anyhow, there is no observable consistent pattern of an association of both parameters.

Likewise we investigated the association for auto-IgE reactivity towards lung derived proteins [Table 17]. As in Table 16, isolated models for eczema show a significantly reduced risk to develop autoreactivity against lung and a higher risk for hay fever/rhinitis for IgE-autoreactivity. Nevertheless, there is no consistent association between autoreactivity and the clinical manifestation of atopy identifiable. Additionally we analyzed any other disease that was given as free text in the questionnaires without any association to autoreactivity (data not shown).

Table 16: The clinical manifestation of atopic diseases in children is not associated with auto-IgE reactivity against skin derived proteins.

The upper part of the table shows the OR(95 % CI) of increasing auto-IgE reactivity in children with (atopic) symptoms as allergic asthma, hayfever, atopic eczema or food allergy or non-IgE mediated diseases including obstructive bronchitis, bronchitis and pneumonia. We did not find any association between the selected symptoms and auto-IgE reactivity. The lower part is more detailed as we categorized for the frequency of a given yes for asthma, eczema, hayfever/rhinitis and food allergy. But even with this more detailed categorization we could not find any association of any of the analyzed symptom and auto-IgE reactivity.

Adjusted logistic regression for auto-IgE reactivity towards skin					
medical diagnosis last 12 month	**diagnosis**	**n(%)**	**1. cut-off [OR(95% CI)]**	**2. cut-off [OR(95% CI)]**	**3. cut-off [OR(95% CI)]**
food allergy	no/yes	2686(97.1)/81 (2.9)	0.89(0.56-1.43) p=0.640	0.85(0.51-1.43) p=0.544	0.65(0.34-1.25) p=0.199
obstructive bronchitis	no/yes	2437(95.3)/119 (4.7)	1.02(0.69-1.51) p=0.919	1.06(0.70-1.60) p=0.789	1.15(0.73-1.82) p=0.551
asthma	no/yes	2671(96.1)/109 (3.9)	0.90(0.60-1.34) p=0.599	0.86(0.55-1.33) p=0.495	0.57(0.32-1.01) p=0.052
hayfever	no/yes	2511(91.0)/247 (9.0)	1.09(0.83-1.44) p=0.543	1.17(0.87-1.57) p=0.296	0.99(0.70-1.40) p=0.959
rhinitis	no/yes	2649(95.9)/113 (4.1)	1.17(0.79-1.73) p=0.446	1.08(0.71-1.65) p=0.707	1.07(0.66-1.72) p=0.795
eczema	no/yes	2628(95.0)/138 (5.0)	0.90(0.62-1.29) p=0.552	0.92(0.62-1.38) p=0.698	0.98(0.62-1.53) p=0.916
Bronchitis	no/yes	2377(87.0)/355 (13)	1.05(0.83-1.33) p=0.683	1.03(0.80-1.32) p=0.838	0.98(0.73-1.30) p=0.880
Pneumonia	no/yes	2738(98.1)/53 (1.9)	0.63(0.35-1.12) p=0.115	0.98(0.54-1.80) p=0.952	0.91(0.45-1.83) p=0.787
medical diagnosis ever	**survey reply on diagnosis**	**n(%)**	**1. cut-off [OR(95% CI)]**	**2. cut-off [OR(95% CI)]**	**3. cut-off [OR(95% CI)]**
asthma	no/1-4 n.a.	2210(77.7)/401 (14.1)	0.84(0.65-1.07) p=0.160	0.76(0.58-1.01) p=0.058	0.86(0.63-1.17) p=0.333
	1x yes	93 (3.3)	0.79(0.51-1.23) p=0.297	0.76(0.47-1.24) p=0.270	0.68(0.38-1.21) p=0.190
	2-3x yes	68 (2.4)	1.22(0.73-2.04) p=0.445	1.27(0.74-2.16) p=0.387	1.42(0.80-2.53) p=0.227
	≥4x yes	74 (2.6)	1.00(0.61-1.63) p=0.985	1.23(0.73-2.05) p=0.439	0.73(0.38-1.40) p=0.342
Eczema	no/1-4 n.a.	1681 (59.1)/ 316 (11.1)	0.88(0.66-1.16) p=0.362	0.75(0.55-1.03) p=0.075	0.76(0.53-1.10) p=0.145
	1x yes	329 (11.6)	0.82(0.64-1.05) p=0.108	0.79(0.60-1.04) p=0.090	**0.71(0.52-0.98) p=0.039**
	2-3x yes	276 (9.7)	0.82(0.63-1.08) p=0.161	0.84(0.63-1.13) p=0.260	0.81(0.57-1.14) p=0.218
	≥4x yes	240 (8.4)	**0.72(0.54-0.97) p=0.029**	0.79(0.58-1.09) p=0.146	0.75(0.52-1.09) p=0.130
Hayfever/ rhinitis	no/1-4 n.a.	1944 (69.0)/348 (12.4)	0.87(0.66-1.13) p=0.292	0.92(0.69-1.24) p=0.598	0.94(0.67-1.32) p=0.720
	1x yes	169 (6.0)	0.91(0.65-1.27) p=0.591	0.97(0.67-1.39) p=0.859	0.77(0.50-1.20) p=0.245
	2-3x yes	167 (5.9	1.20(0.86-1.68) p=0.286	1.34(0.94-1.89) p=0.103	**1.49(1.03-2.17) p=0.037**
	≥4x yes	188 (6.7)	1.16(0.84-1.60) p=0.368	1.35(0.96-1.89) p=0.083	1.18(0.80-1.73) p=0.401
Food allergy	no/1-4 n.a.	1954 (68.8)/385 (13.6)	0.82(0.63-1.05) p=0.122	0.79(0.60-1.05) p=0.106	0.76(0.55-1.06) p=0.107
	≥1x yes	502 (17.7)	0.87(0.71-1.07) p=0.189	0.95(0.76-1.19) p=0.665	0.90(0.69-1.16) p=0.403

Table 17: The clinical manifestation of atopy in children is not associated with auto-IgE reactivity against lung derived proteins.

As in table 12, the upper part of the table shows the OR(95 % CI) of the increasing auto-IgE reactivity in children with (atopic) symptoms allergic asthma, hayfever, atopic eczema or food allergy or non-IgE mediated diseases including obstructive bronchitis, bronchitis and pneumonia. We did not find any association between the selected symptoms and auto-IgE reactivity. The lower part is more detailed as we categorized for the frequency of a given yes for asthma, eczema, hayfever/rhinitis and food allergy. For children whose parents answered 2-3x with "Yes, hayfever/rhinitis was diagnosed" we calculated higher auto-IgE reactivity or a higher chance to develop auto-IgE or vice versa. But there are no evidences for eczema or asthma.

Adjusted logistic regression for auto-IgE reactivity towards lung					
medical diagnosis last 12 month	**diagnosis**	**n(%)**	**1-fold cut-off [OR(95% CI)]**	**1.5-fold cut-off [OR(95% CI)]**	**2-fold cut-off [OR(95% CI)]**
food allergy	no/yes	2686 (97.1)/81 (2.9)	0.64(0.40-1.04) p=0.070	0.61(0.35-1.07) p=0.085	0.58(0.30-1.14) p=0.114
obstructive bronchitis	no/yes	2437 (95.3)/119 (4.7)	0.90(0.61-1.34) p=0.607	0.72(0.46-1.13) p=0.159	0.78(0.47-1.30) p=0.348
asthma	no/yes	2671 (96.1)/109 (3.9)	0.77(0.52-1.16) p=0.217	0.64(0.40-1.03) p=0.069	0.63(0.36-1.10) p=0.103
hayfever	no/yes	2511 (91.0)/247 (9.0)	0.89(0.67-1.17) p=0.408	0.91(0.67-1.23) p=0.522	0.81(0.57-1.17) p=0.261
rhinitis	no/yes	2649 (95.9)/113 (4.1)	1.00(0.68-1.49) p=0.985	1.08(0.71-1.65) p=0.714	1.07(0.67-1.73) p=0.771
eczema	no/yes	2628 (95.0)/138 (5.0)	0.75(0.52-1.08) p=0.122	0.86(0.57-1.28) p=0.455	0.93(0.59-1.46) p=0.751
Bronchitis	no/yes	2377 (87.0)/355 (13.0)	1.02(0.80-1.29) p=0.876	0.94(0.72-1.21) p=0.617	0.85(0.63-1.14) p=0.280
Pneumonia	no/yes	2738 (98.1)/53 (1.9)	0.74(0.41-1.31) p=0.300	0.68(0.35-1.31) p=0.246	0.69(0.32-1.49) p=0.346
medical diagnosis ever	**survey reply on diagnosis**	**n(%)**	**1-fold cut-off [OR(95% CI)]**	**1.5-fold cut-off [OR(95% CI)]**	**2-fold cut-off [OR(95% CI)]**
asthma	no/1-4 n.a.	2210 (77.7)/401 (14.1)	0.88(0.69-1.13) p=0.320	0.90(0.69-1.18) p=0.458	0.83(0.61-1.14) p=0.250
	1x yes	93 (3.3)	0.88(0.57-1.36) p=0.555	0.85(0.52-1.37) p=0.495	0.71(0.40-1.25) p=0.231
	2-3x yes	68 (2.4)	1.15(0.69-1.92) p=0.590	1.18(0.69-2.03) p=0.539	1.09(0.59-2.00) p=0.789
	≥4x yes	74 (2.6)	1.09(0.67-1.78) p=0.737	0.83(0.48-1.44) p=0.506	0.80(0.43-1.52) p=0.501
Eczema	no/1-4 n.a.	1681 (59.1)/ 316 (11.1)	0.92(0.69-1.22) p=0.556	0.80(0.58-1.09) p=0.161	0.73(0.51-1.05) p=0.093
	1x yes	329 (11.6)	**0.78(0.61-1.00) p=0.049**	0.86(0.65-1.12) p=0.253	0.83(0.61-1.12) p=0.219
	2-3x yes	276 (9.7)	0.85(0.65-1.12) p=0.254	0.83(0.61-1.11) p=0.209	**0.68(0.47-0.97) p=0.032**
	≥4x yes	240 (8.4)	**0.70(0.53-0.94) p=0.017**	0.84(0.61-1.14) p=0.263	0.82(0.57-1.17) p=0.273
Hayfever/ rhinitis	no/1-4 n.a.	1944 (69.0)/348 (12.4)	0.95(0.73-1.24) p=0.703	0.92(0.68-1.23) p=0.576	0.97(0.70-1.35) p=0.854
	1x yes	169 (6.0)	0.89(0.64-1.24) p=0.499	0.78(0.53-1.13) p=0.189	0.78(0.50-1.20) p=0.250
	2-3x yes	167 (5.9	**1.45(1.04-2.03) p=0.030**	**1.64(1.17-2.31) p=0.004**	1.20(0.81-1.78) p=0.352
	≥4x yes	188 (6.7)	0.97(0.70-1.34) p=0.861	0.98(0.69-1.38) p=0.891	1.08(0.73-1.59) p=0.695
Food allergy	no/1-4 n.a.	1954 (68.8)/385 (13.6)	0.92(0.71-1.18) p=0.499	0.81(0.61-1.08) p=0.150	0.77(0.56-1.06) p=0.113
	≥1x yes	502 (17.7)	0.84(0.68-1.04) p=0.103	0.89(0.71-1.12) p=0.334	0.90(0.70-1.17) p=0.443

4.3.3. Parental atopy is negatively associated with the auto-IgE reactivity of their children

In addition we analyzed the impact of parental atopy on their children´s autoreactivity. The analysis was performed for parental "atopy", "asthma", "hay fever" and "eczema" [Table 18] for autoreactivity towards lung and skin [Table 19, Table 20]. Table 19 shows the percentage of autoreactive children of parents with atopy, the p-values were calculated by a χ^2-test. To investigate whether there is an association, due to the significance of the χ^2 we performed again an adjusted logistic regression [Table 20]. Parental atopy revealed a significant lower OR for skin-autoreactivity *(OR(95 % CI) 1.cut-off: 0.85(0.72-1.00), 2.cut-off: 0.83(0.69-0.99), 3.cut-off: 0.81(0.66-0.99)* and for lung autoreactivity *(OR(95 % CI) 1.cut-off: 0.82(0.70-0.97), 2.cut-off: 0.77(0.65-0.93), 3.cut-off: 0.82(0.67-1.00).* In a more detailed analysis according to disease subclasses, children whose parents were food sensitized had an increased risk to develop IgE reactivity towards skin *(OR(95 % CI) 1.cut-off: 1.71(1.38-2.13), 2.cut-off: 1.91(1.52-2.39), 3.cut-off: 2.2(1.72-2.81)* and lung *(OR(95 % CI) 1.cut-off: 1.88(1.51-2.34), 2.cut-off: 2.16(1.72-2.70), 3.cut-off: 2.25(1.75-2.88).* Parental sensitization to inhalant allergens revealed no association to auto-IgE reactivity.

Table 18: Number and percentage of children whose parents suffer from atopy or atopic symptoms as asthma, hayfever or eczema.

medical diagnosis		n (%)
parental atopy	yes	1667 (59.8)
parental asthma	yes	453 (16.1)
parental hayfever	yes	1464 (52.9)
parental eczema	yes	489 (17.4)

Table 19: Percentage of autoreactive children whose parents are atopic.

We performed a χ^2-test to test the hypothesis whether children of atopic parents show higher auto-IgE. Actually, children whose parents indicate suffering from atopy show a significant χ^2-test, p=0.028/ p=0.012 (first row), further parental hayfever results in a significant calculation.

In a second step we investigated whether there finds an association due to the significance of the χ^2-test as shown in Table 20.

target organ	medical diagnosis	1. cut-off [%]	2. cut-off [%]	3. cut-off [%]
Skin	parental atopy [no/yes]	**50.1/45.8 p=0.028**	**33.7/29.1 p=0.012**	22.0/19.6 p=0.145
	parental asthma [no/yes]	48.1/44.8 p=0.219	31.4/28.3 p=0.210	21.2/17.4 p=0.078
	parental hay fever [no/yes]	49.3/45.9 p=0.076	**32.9/29.2 p=0.039**	20.8/20.4 p=0.787
	parental eczema [no/yes]	48.1/45.2 p=0.271	31.5/28.6 p=0.239	21.3/17.8 p=0.093
Lung	parental atopy [no/yes]	46.5/44.9 p=0.414	31.6/29.2 p=0.191	22.1/19.7 p=0.152
	parental asthma [no/yes]	46.1/43.0 p=0.246	30.9/26.3 p=0.054	21.3/17.7 p=0.090
	parental hayfever [no/yes]	45.9/45.2 p=0.748	30.7/29.8 p=0.677	21.2/20.4 p=0.605
	parental eczema [no/yes]	45.7/46.2 p=0.881	30.7/28.0 p=0.266	21.3/18.2 p=0.145

.

Table 20: Parental atopy is negatively associated with auto-IgE reactivity towards skin and lung in their children.

Children whose parents indicate being atopic show lower auto-IgE reactivity as the OR, calculated by a logistic regression of an adjusted model (adjusted for: sex, study, city, age, BMI, parental education) is significantly reduced (OR=0.81- 0.85, first row). On the contrary, children whose parents are food sensitized (fx5) show a significant higher OR (skin: 1.71-2.2, lung: 1.88-2.25), as a parental food sensitization is positively associated with IgE autoreactivity in their children. However, there is no association when parents are sensitized towards inhalant allergens (sx1).

logistic regression adjusted model				
target organ	**medical diagnosis**	**1. cut-off [OR(95 % CI)]**	**2. cut-off [OR(95 % CI)]**	**3. cut-off [OR(95 % CI)]**
Skin	parental atopy	**0.85(0.72-1.00) p=0.055**	**0.83(0.69-0.99) p=0.044**	**0.81(0.66-0.99) p=0.04**
	Food sensitization (Fx5)	**1.71(1.38-2.13) p<0.001**	**1.91(1.52-2.39) p<0.001**	**2.2(1.72-2.81) p<0.001**
	Inhalant sensitized (sx1)	1.05(0.88-1.25) P=0.57	1.04(0.83-1.21) p=0.97	0.98(0.79-1.22) p=0.88
Lung	parental atopy	**0.82(0.7-0.97) p=0.02**	**0.77(0.65-0.93) p=0.005**	**0.82(0.67-1.0) p=0.005**
	Food sensitization (fx5)	**1.88(1.51-2.34) p<0.001**	**2.16(1.72-2.70) p<0.001**	**2.25(1.75-2.88) p<0.001**
	Inhalant sensitized (sx1)	1.0(0.84-1.19) p=0.97	0.99(0.82-1.20) p=0.95	0.89(0.71-1.11) p=0.286

4.3.4. Infantile generation of auto-IgE antibodies occurs physiologically in healthy children

The analysis of the occurrence of auto-IgE in our age stratified study population revealed a high quantity and extent of auto-IgE antibodies in young children, raising the question if this enormous appearance of autoreactive IgE is physiologic. To analyze a physiologic occurrence of auto-IgE a healthy population is needed, that was valid in the GINI/LISA cohort. For the use in our study we combined sera from the two birth cohorts GINI and LISA.

To calculate whether healthy children generate autoreactive-IgE we performed a calculation of the percentage of reactive and non-reactive children for skin- and lung-reactivity for each cut-off [Figure 20, Figure 21]. In both figures each row is a new calculation we subtracted all children whose parents ever referred to asthma (2nd row), eczema (3rd row), hayfever/rhinitis (4th row) and food allergy (5th row) successively from all screened children (n = 2861), each done for the calculated cut-offs (1. cut-off (1-fold), 2. cut-off (1.5-fold), 3. cut-off (2-fold) each in one column). The first row shows all children (healthy and diseased) and the difference of reactive and non-reactive subjects. The first column shows the lowest cut-off, hence the lowest auto-IgE reactivity in children. There is no difference between the number of autoreactive and non-autoreactive children, certainly with the rising IgE autoreactivity, hence the rising cut-off, the number of reactive children decreases.

The last row shows the remaining healthy children exclusively; here never any disease was mentioned in the questionnaires. The comparison of the percentage of healthy children showing autoreactivity (49.5 %, last row) with the percentage of all children (47.5 %, first row) reveals no difference; hence the occurrence of autoreactivity towards skin [Figure 20] and lung [Figure 21] is comparable in healthy and diseased children. Expectedly the proportion of reactive children declines with higher cut-offs as we increased the threshold with those.

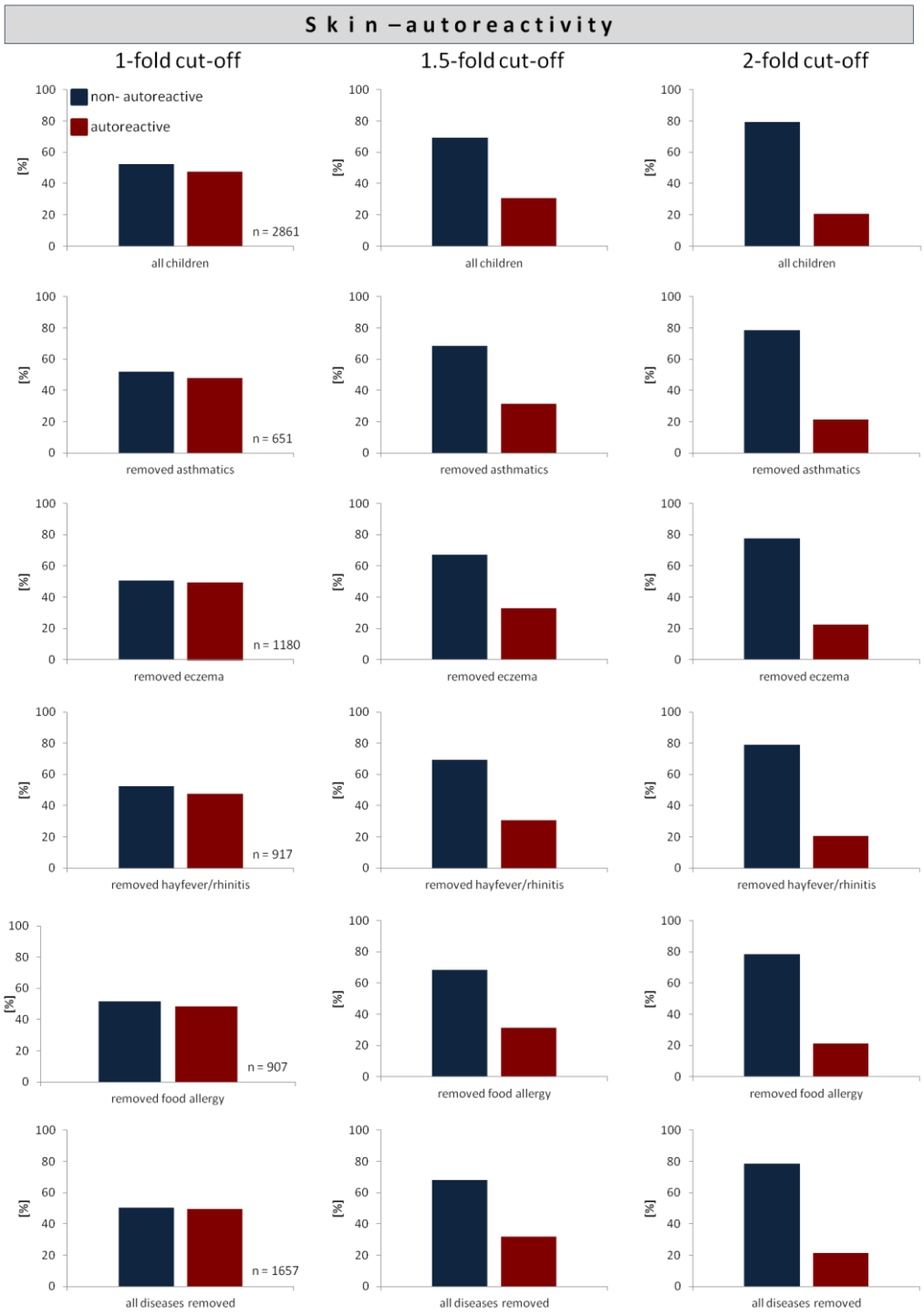


Figure 20:Auto-IgE is generated physiologically in healthy children.
We performed a calculation of the percentage of skin reactive and non-reactive children for each of the calculated cut-off, that is represented in columns in the figure. The first row displays all children analyzed for auto-IgE (healthy and diseased). In each of the following rows we subtracted children with asthma (2nd row), eczema (3rd row), hayfever/rhinitis (4th row) and food allergy (5th row). The last row contains only healthy children and its auto-IgE reactivity towards skin dervied proteins. The comparison of reactive healthy children 49.5 % (last row) and the percentage of all children (47.5%, first row) reveals no difference, suggesting the occurrence of auto-IgE towards skin is comparable in healthy and diseased children. As expected the proportion of reactive children declines with the rising cut-off, as we increased the threshold with those.

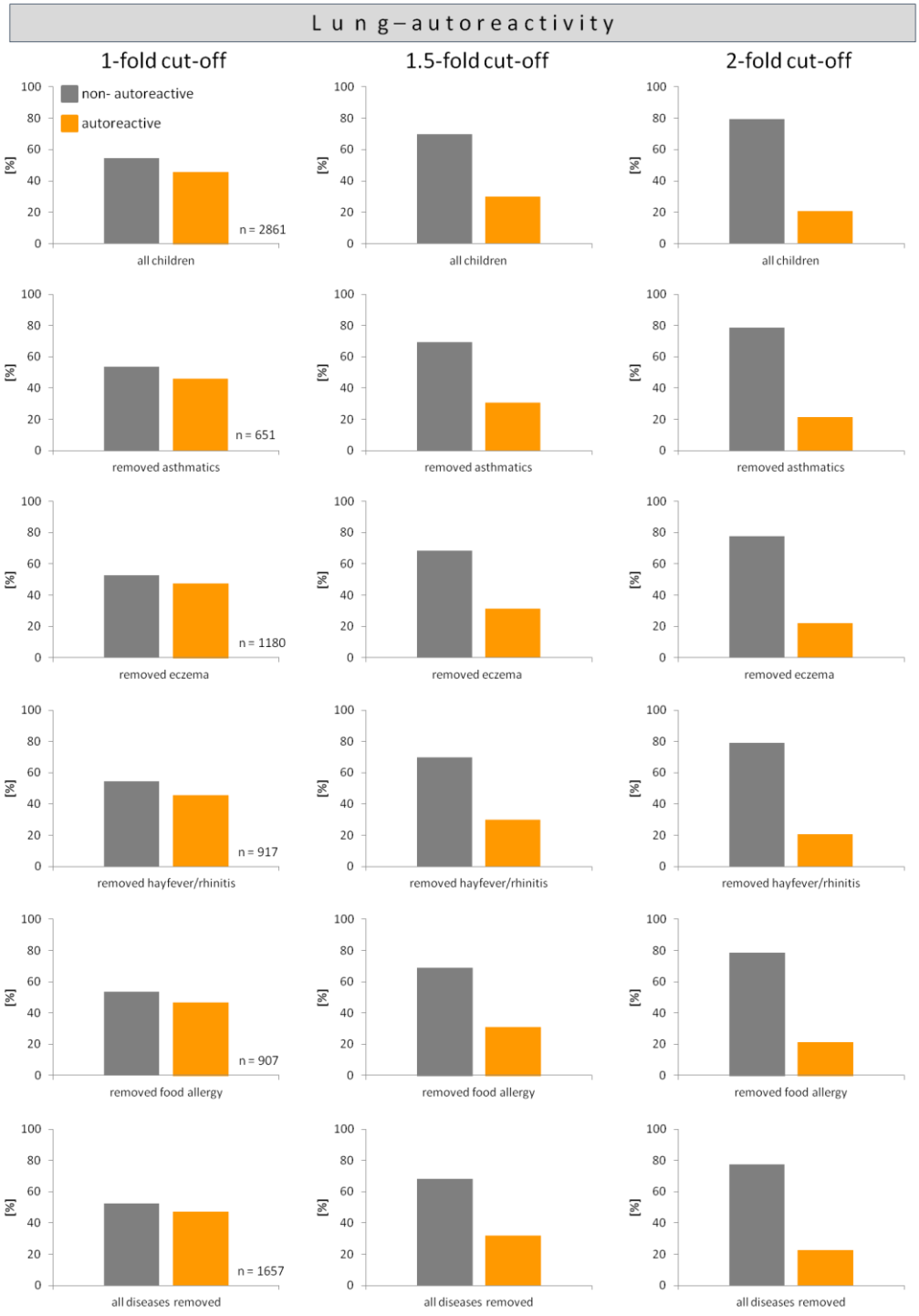


Figure 21: Auto-IgE is generated physiologically in healthy children.
We performed a calculation of the percentage of lung reactive and non-reactive children for each of the calculated cut-off, that is represented in columns in the figure. The first row displays all children analyzed for auto-IgE (healthy and diseased). In each of the following rows we subtracted children with asthma (2nd row), eczema (3rd row), hayfever/rhinitis (4th row) and food allergy (5th row). The last row contains only healthy children and its auto-IgE reactivity towards skin derived proteins. The comparison of reactive healthy children 49.5 % (last row) and the percentage of all children (47.5%, first row) reveals no difference, suggesting the occurrence of auto-IgE towards skin is comparable in healthy and diseased children. As expected the proportion of reactive children declines with the rising cut-off, as we increased the threshold with those.

4.3.5. Evidences for a protective role of autoreactive IgE in early childhood

Our results show that autoreactive IgE in children is generated physiologically. Furthermore there are results that indicate less auto-IgE reactivity in children having eczema. To verify and confirm these finding we calculated these data in a more detailed and stringent statistic model.

First we calculated whether eczema and asthma, each stratified for allergic (extrinsic) and non-allergic (intrinsic) by the amount of total IgE (≥ 180 kU/L), have an effect on IgE autoreactivity. The reference groups were non-asthmatics for the asthma effect and patients that never have had eczema for the eczema effect. The calculation revealed a protective effect on non-allergic eczema that would imply that children having intrinsic eczema have lower auto-IgE reactivity towards skin and lung (data not shown).

But due to the results of the effect of the different sensitization patterns (inhalant sensitization (sx1) and food sensitization (fx5)) as given above, we did further calculations with data that were stratified on sx1 and fx5 (threshold ≥ 0.35 kU/L).

4.3.5.1. A sensitization without clinical symptoms of eczema is attended by a higher auto-IgE reactivity

For this calculation the reference group contained healthy children only, which do not show any sensitization (sens -) and never had atopic eczema (eczema-).

Further groups were stratified as the following:

Sensitized children having eczema	(sens+ eczema +)
Sensitized children without having eczema	(sens+ eczema –)
Children without sensitization but having eczema	(sens– eczema +)

The effect of a sensitization on auto-IgE reactivity (1. /2. and 3. cut–off) was calculated in a logistic regression model that was adjusted for age, sex, BMI, parental education, study center and study.

The following forest plots [Figure 22 skin autoreactivity, Figure 23 lung autoreactivity] shows each in the upper group, which comprises children that are sensitized and have eczema (sens+ eczema+) that there is no effect on the auto-IgE reactivity which is not higher in those children and the OR remains around one (*OR(CI 95 %) skin: 1. cut-off: 1.06(0.84-1.33), p=0.613, 2. cut-off: 1.18(0.92-1.51), p=0.187, 3. cut-off: 1.05(0.78-1.40), p=0.762* and lung: *1. cut-off: 1.03(0.82-1.30), p=0.775, 2. cut-off: 1.20(0.93-1.53), p=0.156, 3. cut-off: 1.05(0.78-1.40), p=0.762).*

In contrast the middle part of the plot – showing the OR and the 95 % confidence interval of auto-IgE reactivity towards skin [Figure 22] and lung [Figure 23] indicates children who are sensitized without having atopic eczema (sens+ eczema-), have significantly higher auto-IgE reactivity. The ORs show a significant positive association between auto-IgE reactivity and the sensitization without eczema *(OR(CI 95 %) skin: 1. cut-off: 1.48(1.22-1.79), p<0.001, 2. cut-off: 1.63(1.33-1.99), p<0.001, 3. cut-off: 1.67(1.33-2.09), p<0.001* and lung: *1. cut-off: 1.58(1.30-1.91), p< 0.001, 2. cut-off: 1.48(1.21-1.82), p<0.001, 3. cut-off: 1.67(1.33-2.09), p<0.001)*. Actually the OR rises with the increasing cut-off for skin reactivity, which implies an increasing association between the rising auto-IgE reactivity and the impact of sensitization.

Finally the data suggest that a sensitization in children results in higher auto-IgE reactivity towards skin and lung.

In turn, children having eczema, but are not sensitized (sens– eczema+) show less auto-IgE reactivity, hence there is a negative association between eczema without sensitization and auto-IgE reactivity, but this effect is not significant *(OR(CI 95 %) skin: 1. cut-off: 0.85(0.67-1.08), p=0.188, 2. cut-off: 0.89(0.68-1.17), p=0.396, 3. cut-off: 0.92(0.68-1.26), p=0.623* and lung: *1. cut-off: 0.88(0.69-1.12), p=0.289, 2. cut-off: 0.86(0.66-1.13), p=0.279, 3. cut-off: 0.92(0.68-1.26), p=0.623)*.

Impact of a sensitization and eczema on IgE autoreactivity to skin

effect	cut-off	OR	CI (95%)	p
sens+ eczema+	1.	1.06	0.84 - 1.33	0.613
	2.	1.18	0.92 - 1.51	0.187
	3.	1.05	0.78 - 1.4	0.762
sens+ eczema-	1.	1.48	1.22 - 1.79	<0.001
	2.	1.63	1.33 - 1.99	<0.001
	3.	1.67	1.33 - 2.09	<0.001
sens- eczema+	1.	0.85	0.67 - 1.08	0.188
	2.	0.89	0.68 - 1.17	0.396
	3.	0.92	0.68 - 1.26	0.623

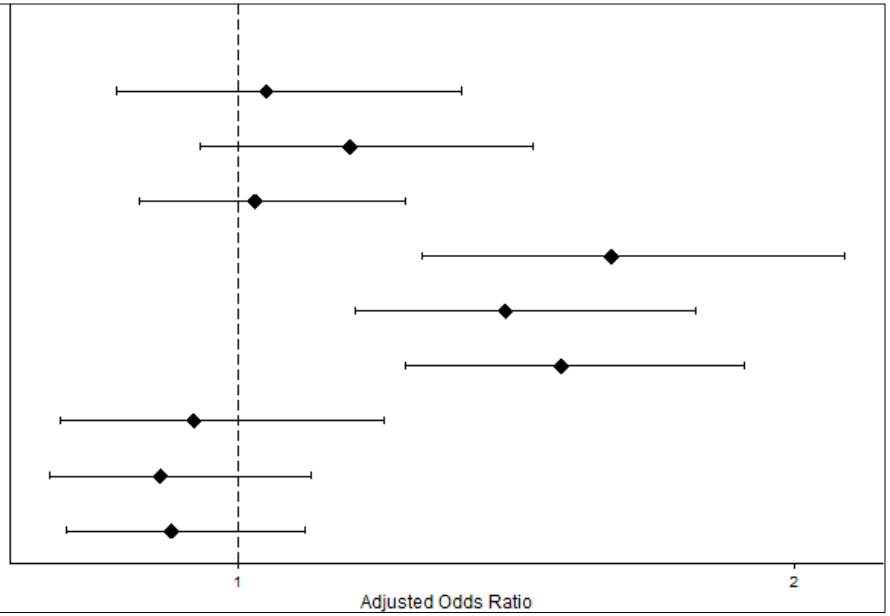


Figure 22: Sensitization only results in higher auto-IgE reactivity towards skin in children of the GINI/LISA cohort.
To investigate whether auto-IgE is associated with the occurrence of eczema or sensitization we performed a logistic regression model that is shown in the forest plot. As reference group children without sensitization and without eczema was taken (Reference group: sens- eczema-). We investigated the effect of sensitization with or without eczema (sens+ eczema+, sens+ eczema-) and eczema without sensitization (sens- eczema+). The first plot (sens+ eczema+) shows no effect on IgE-autoreactivity, as the OR oscillates around one. In contrast, auto-IgE is significantly positive associated with sensitization (sens+ eczema-) the second effect group. Here the OR is significant and much higher than one. Children without sensitization but with eczema show a decrease in auto-IgE reactivity as the OR is below one, but this effect is not significant. Finally we can predicate that a sensitization in children results in higher auto-IgE reactivity towards skin.

Impact of a sensitization and eczema on IgE autoreactivity to lung

effect	cut-off	OR	CI (95%)	p
sens+ eczema+	1.	1.03	0.82 - 1.3	0.775
	2.	1.2	0.93 - 1.53	0.156
	3.	1.05	0.78 - 1.4	0.762
sens+ eczema-	1.	1.58	1.3 - 1.91	0.001
	2.	1.48	1.21 - 1.82	<0.001
	3.	1.67	1.33 - 2.09	<0.001
sens- eczema+	1.	0.88	0.69 - 1.12	0.289
	2.	0.86	0.66 - 1.13	0.279
	3.	0.92	0.68 - 1.26	0.623

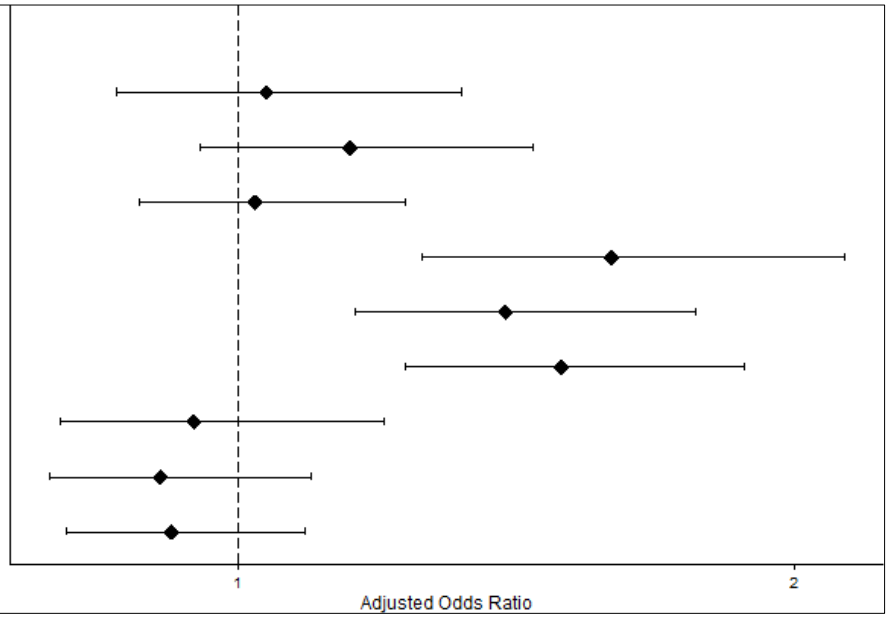


Figure 23: Sensitization only results in higher auto-IgE reactivity towards lung in children of the GINI/LISA cohort.
To investigate whether auto-IgE towards lung derived proteins is associated with the occurrence of eczema or sensitization we performed a logistic regression model that is shown in the forest plot. As reference group children without sensitization and without eczema was taken (Reference group: sens- eczema-). We investigated the effect of sensitization with or without eczema (sens+ eczema+, sens+ eczema-) and eczema without sensitization (sens- eczema+). The first plot (sens+ eczema+) shows no effect on IgE-autoreactivity, as the OR oscillates around one. In contrast, auto-IgE is significantly positive associated with sensitization (sens+ eczema-) the second effect group. Here the OR is significant and much higher than one. Children without sensitization but with eczema show a decrease in auto-IgE reactivity as the OR is below one, but this effect is not significant. Finally we can predicate that a sensitization in children results in higher auto-IgE reactivity towards lung.

4.3.5.2. THE OCCURENCE OF AUTO-IGE REACTIVITY HAS AN PROTECTIVE EFFECT ON ECZEMA

One further analysis was done to confirm the upper results and extend the calculation with an additional stratification.

Further we assessed whether there is a relation of children having eczema without sensitization referred to as intrinsic atopic eczema. Children having atopic eczema without sensitization show lower OR oscillating between 0.74 – 0.90 but without statistical significance. We stratified next children having eczema and are sensitized to both aeroallergens and food allergens referred to as extrinsic eczema. The reference group was set to children that are sensitized without having eczema. The same analysis we accomplished for asthmatic children.

In brief:

1. **eczema without sensitization** (Reference group: sens- eczema-) and
2. **eczema with sensitization** to aeroallergens *and* food allergens (Reference group: sens+ eczema -)

3. **asthma without sensitization** (Reference group: sens- asthma-) and
4. **asthma with sensitization** to aeroallergens *and* food allergens (Reference group: sens+ asthma -)

The following forest plot Figure 24 shows the effect of an increasing auto-IgE reactivity defined by the different cut-offs on extrinsic atopic eczema [A, B] and allergic asthma [C, D]. The OR is depicted as a black diamond and the appropriate CI is given with the black lines. The OR of one is indicated as a black dotted vertical line. Figure 24A, B depicts the analysis of children having atopic eczema combined with sensitization. In Figure 24A the OR is given for skin-autoreactivity and in Figure 24B for lung autoreactivity. As the OR is less than one for skin reactivity there is a negative association between auto-IgE reactivity and food and aeroallergen sensitization, hence the chance to develop high auto-IgE reactivity towards skin and having extrinsic eczema is 30 % less compared to children that are sensitized without having eczema.

Skin auto-IgE reactivity associated with having extrinsic eczema: *OR (95 %CI) 1. Cut-off: 0.74(0.58-0.94), p=0.015; 2. Cut-off: 0.74(0.57-0.96), p=0.023; 3. cut-off: 0.65 (0.48-0.87), p=0.004*.

As the p-value decreases with increasing cut-off it can be suggested that the chance to develop eczema decreases with increasing auto-IgE reactivity. There finds an exclusive negative association between lung reactivity and extrinsic eczema, as a lower auto-IgE reactivity (1.cut-off) shows a significantly lower chance to develop auto-reactivity towards lung and having extrinsic atopic eczema.

Lung auto-IgE reactivity associated with having extrinsic eczema: *OR (95 %CI) 1. cut-off: 0.68(0.53-0.87), p=0.002; 2. cut-off: 0.85(0.66-1.10), p=0.212; 3. cut-off: 0.79 (0.59-1.06), p=0.115*.

Figure 24C, D show the association between auto-IgE reactivity towards skin [Figure 24C] and lung [Figure 24D] in allergic asthmatic children. Actually there finds no significant association as the OR oscillates around one.

Skin auto-IgE reactivity associated with having allergic asthma: *OR (95 %CI) 1. cut-off: 0.86(0.61-1.22), p=0.399; 2. cut-off: 1.00 (0.70-1.43), p=0.999; 3. cut-off: 0.90 (0.60-1.35), p=0.601*.

Lung auto-IgE reactivity associated with having allergic asthma: *OR (95 %CI) 1. cut-off: 1.01(0.72-1.43), p=0.939; 2. cut-off: 0.96(0.67-1.38), p=0.834; 3. cut-off: 0.79 (0.52-1.20), p=0.272.*

Finally we can say, if children are not sensitized, there is no effect between atopic eczema or allergic asthma on the auto-IgE reactivity. In contrast children having eczema show significantly less auto-IgE reactivity, if they are sensitized against inhalant and food allergens.

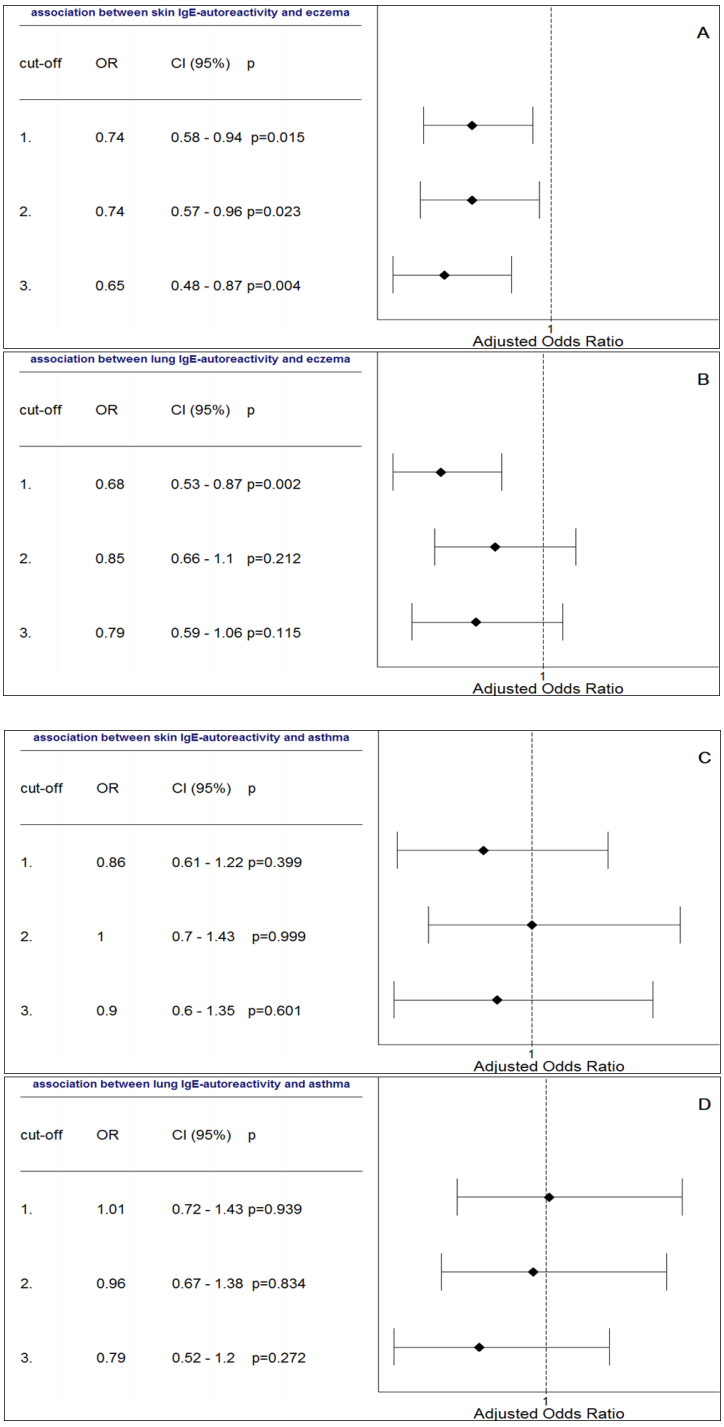


Figure 24: Children having eczema in combination with a sensitization to food or aeroallergens show negatively associated auto-IgE reactivity towards skin.
The figures show the OR (black diamond) and the appropriate 95 % CI, an OR of one is indicated with the black vertical dotted line. **[A]** shows the OR of auto-IgE reactivity towards skin and the association to eczema combined with sensitization to food and aeroallergens. As the OR is less than one, there finds a negative association between eczema and auto-IgE ($p<0.05$). In contrast the auto-IgE reactivity against lung **[B]** is only affected in children showing lower auto-IgE reactivity to lung (1. Cut-off reactivity), a higher auto-IgE reactivity towards lung is not significantly associated with auto-IgE reactivity. Further we analyzed whether there finds a relation between auto-IgE reactivity against skin **[C]** and lung **[D]** and allergic asthma. But there is no significant association between those two factors.

4.3.5.3. THE DETERMINATION OF CHILDHOOD IGE AUTOREACTIVITY HAS NO PREDICTIVE EFFECT FOR THE DEVELOPMENT OF ATOPIC ECZEMA

Due to these last results, we investigated, whether auto-IgE has a predictive value for eczema by using a ROC (Receiver operating characteristic) analysis [Figure 25]. We calculated two models one with sensitization one without sensitization and adjusted for sex, parental education, parental atopy, study center, study, age, BMI and the interaction of fx5 and sx1 and lung reactivity.

Model with sensitization:

Eczema ~sex + parental education + parental atopy + study center + study +age + BMI+ fx5*lung3SD +sx1*lung 3SD

Model (without) sensitization

Eczema ~sex + parental education + parental atopy + study center + study + age + BMI + fx5 + sx1

The analysis was performed each for skin and lung and for each cut-off. Exemplary analyses of the calculation on the three cut-offs on skin are shown in Figure 25.
As there is no difference between of the AUC of the ROC analysis there is no predictive value of the auto-IgE on eczema.

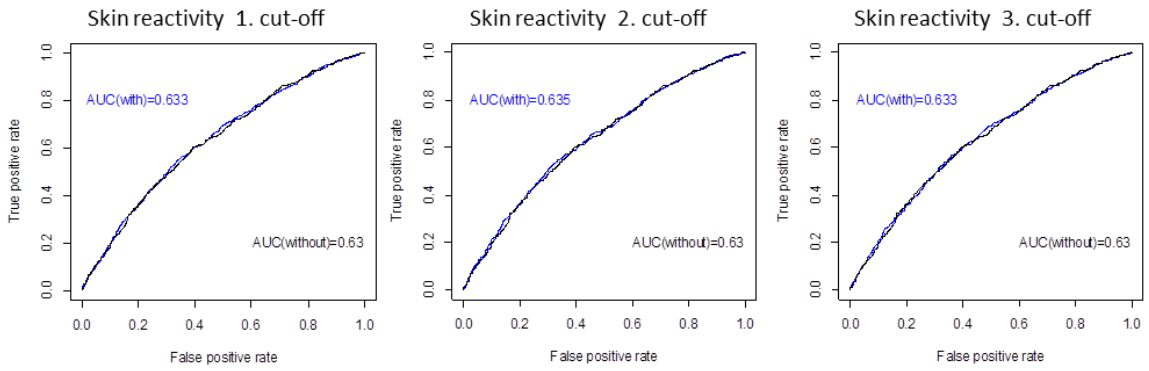


Figure 25: Analysis of auto-IgE reactivity has no predicitive value on the occurrence of atopic eczema.
There is no difference of the calculated area under the curve (AUC) when two different models are used. We compared the AUC of the occurrence of eczema from two models – with and without sensitization. As there is no difference in the calculated AUC there is no predictive value of the analysis of auto-IgE reactivity on the occurrence of eczema. The calculation was done for skin and lung and for each cut-off, but revealed in no difference. The here shown graph is exemplary for all calculations. Here the ROC AUC is shown for skin-reactivity and the rising cut-off. In each ROC curve the AUC with sensitization (AUC(with)) and without sensitization (AUC(without)) shows the same value of about 0.63.

4.3.6. Aspects on early nutrition and lipid metabolism on auto-IgE

4.3.6.1. Hypoallergenic feeding by hydrolyzed baby food or breast feeding has no impact on auto-IgE reactivity at age of ten

The data of the randomized, double-blind intervention study arm of the GINI cohort were utilized to analyze the impact of the preventive effect of breast feeding or hydrolyzed infant formulas to prevent IgE-autoreactivity.

The intervention study consisted of four different intervention arms (cow-milk based non-hydrolyzed infant food (CMF), partial-hydrolyzed whey formula (pHF-W), extensive hydrolyzed whey formula (eHF-W) and partial hydrolyzed casein formula (pHF-C). Furthermore we stratified the participants by "per protocol", by definition and the "Intention to treat". The per protocol group, by definition used hydrolyzed infant formulas congenitally, whereas the intention to treat group combined breast and formula feeding, as all women were introduced to fulfill breast feeding until the fourth month of infant life.

Table 21 and Table 22 illustrate the association between differently used infant formula nutrition and auto-IgE reactivity to skin [Table 21] and lung [Table 22] calculated by a logistic regression adjusted to city, age, sex, BMI, parental education and atopy. We could not identify any association between hypoallergenic formula feeding and IgE-autoreactivity, neither without adjusting any confounder (data not shown) nor after adjusting the models for potential confounder named above. To exclude breast feeding as a potential confounder we excluded women who breast fed in the intention to treat group, but still no association could be identified here.

Table 21: Breast feeding or hypoallergenic formula nutrition has no impact on IgE autoreactivity in serum in the GINI/LISA study.

Four different intervention arms (cow-milk based non-hydrolyzed infant food (CMF), partial-hydrolyzed whey formula (pHF-W), extensive hydrolyzed whey formula (eHF-W) and partial hydrolyzed casein formula (pHF-C)) were investigated on their impact on auto-IgE reactivity at age of ten. But there finds no association between the early nutrition of infants and the later auto-IgE reactivity towards skin.

auto-IgE reactivity towards skin					
study group	**infant formula**	**n(%)**	**1-fold cut-off**	**1.5-fold cut-off**	**2-fold cut-off**
Per protocol	CMF	110 (25.6)	ref	ref	ref
	pHF-W	105 (24.4)	0.81(0.45-1.44) p=0.468	1.13(0.59-2.16) p=0.710	1.23(0.61-2.51) p=0.562
	eHF-W	118 (27.4)	0.63(0.37-1.10) p=0.106	0.84(0.45-1.58) p=0.599	0.88(0.44-1.78) p=0.723
	eHF-C	97 (22.6)	0.64(0.35-1.17) p=0.145	1.05(0.53-2.05) p=0.895	0.99(0.46-2.09) p=0.969
	all-not CMF	320 (74.4)	0.69(0.43-1.09) p=0.112	0.99(0.59-1.66) p=0.961	1.02(0.57-1.82) p=0.955
Intention to treat	CMF	221 (23.5)	ref	ref	ref
	pHF-W	239 (25.59	0.89(0.60-1.31) p=0.543	1.02(0.66-1.57) p=0.921	1.04(0.64-1.68) p=0.881
	eHF-W	245 (26.1)	0.84(0.57-1.23) p=0.359	0.84(0.55-1.29) p=0.432	0.88(0.54-1.41) p=0.587
	eHF-C	234 (24.9)	0.84(0.57-1.24) p=0.382	1.13(0.74-1.74) p=0.569	0.94(0.57-1.53) p=0.796
	all-not CMF	718 (76.5)	0.85(0.62-1.17) p=0.327	0.99(0.69-1.40) p=0.945	0.95(0.64-1.40) p=0.784
Intention to treat - breast feeding excluded	CMF	117 (24.7)	ref	ref	ref
	pHF-W	120 (25.4)	0.75(0.44-1.30) p=0.308	1.17(0.65-2.12) p=0.604	1.24(0.65-2.36) p=0.514
	eHF-W	124 (26.2)	0.59(0.34-1.01) p=0.053	0.78(0.42-1.42) p=0.409	0.79(0.40-1.52) p=0.475
	eHF-C	112 (23.7)	0.61(0.34-1.07) p=0.086	0.88(0.47-1.65) p=0.691	0.83(0.41-1.67) p=0.601
	all-not CMF	356 (75.3)	0.65(0.41-1.01) p=0.054	0.93(0.57-1.52) p=0.770	0.94(0.55-1.61) p=0.820

Table 22: Breast feeding or hypoallergenic formula nutrition has no impact on IgE autoreactivity towards lung derived proteins in serum in the GINI/LISA study.

Four different intervention arms (cow-milk based non-hydrolyzed infant food (CMF), partial-hydrolyzed whey formula (pHF-W), extensive hydrolyzed whey formula (eHF-W) and partial hydrolyzed casein formula (pHF-C)) were investigated on their impact on auto-IgE reactivity at age of ten. But there finds no association between the early nutrition of infants and the later auto-IgE reactivity towards lung.

auto-IgE reactivity towards lung					
study group	**infant formula**	**n(%)**	**1-fold cut-off**	**1.5-fold cut-off**	**2-fold cut-off**
Per protocol	CMF	110 (25.6)	ref	ref	ref
	pHF-W	105 (24.4)	1.11(0.62-1.98) p=0.721	1.29(0.69-2.41) p=0.417	1.10(0.56-2.18) p=0.784
	eHF-W	118 (27.4)	0.74(0.43-1.28) p=0.278	0.86(0.47-1.59) p=0.634	0.69(0.35-1.37) p=0.287
	eHF-C	97 (22.6)	0.69(0.38-1.26) p=0.230	1.05(0.55-2.02) p=0.880	0.85(0.41-1.77) p=0.668
	all-not CMF	320 (74.4)	0.83(0.52-1.31) p=0.421	1.04(0.63-1.73) p=0.871	0.86(0.49-1.49) p=0.584
Intention to treat	CMF	221 (23.5)	ref	ref	ref
	pHF-W	239 (25.59	1.00(0.68-1.48) p=0.999	1.19(0.78-1.80) p=0.418	1.14(0.72-1.81) p=0.572
	eHF-W	245 (26.1)	0.94(0.64-1.38) p=0.765	0.95(0.63-1.44) p=0.806	0.74(0.46-1.19) p=0.208
	eHF-C	234 (24.9)	0.83(0.56-1.23) p=0.344	1.11(0.73-1.70) p=0.622	0.85(0.52-1.38) p=0.506
	all-not CMF	718 (76.5)	0.92(0.67-1.27) p=0.618	1.08(0.76-1.52) p=0.680	0.90(0.61-1.31) p=0.573
Intention to treat - breast feeding excluded	CMF	117 (24.7)	ref	ref	ref
	pHF-W	120 (25.4)	0.99(0.57-1.71) p=0.963	1.12(0.63-1.99) p=0.695	1.09(0.58-2.06) p=0.781
	eHF-W	124 (26.2)	0.73(0.43-1.25) p=0.251	0.75(0.42-1.34) p=0.327	0.60(0.31-1.17) p=0.133
	eHF-C	112 (23.7)	0.67(0.38-1.19) p=0.173	0.96(0.53-1.76) p=0.898	0.77(0.39-1.53) p=0.459
	all-not CMF	356 (75.3)	0.79(0.51-1.23) p=0.300	0.93(0.58-1.49) p=0.756	0.80(0.48-1.36) p=0.412

4.3.6.2. CHILDREN WITH HIGH SERUM LEVELS OF HIGH-DENSITY LIPOPROTEIN SHOW LOWER AUTO-IGE REACTIVITY

To investigate associations of auto-IgE reactivity and the level of high-density lipoprotein (HDL) and low-density lipoprotein (LDL) we classified the serum level of both parameters in tertiles [
Table 23]. The odds ratio (OR) was calculated with a logistic regression for each tertile and the different cut-offs of auto-IgE reactivity for lung (left side) and skin (right side). Figure 26 shows the cut-off as different reactivity in rows for lung and skin, the OR on the y-axis and the 2. and 3. tertile of HDL on the x-axis. Interestingly, the OR decreases with increasing levels of HDL as well for reactivity towards lung and skin, p-values are given for the calculated OR of both 2. and 3. tertile. A lower OR indicates a lesser auto-IgE reactivity in the case of a higher level of HDL. That means the higher the self-reactivity towards skin and lung derived proteins the lower the OR. In contrast there is no association between the serum level of LDL and auto-IgE reactivity (data not shown).

Table 23: Allocation of the tertiles and number of patients for the investigation of the association of HDL and LDL and auto-IgE reactivity.

Parameter	Tertile	mmol/L	n
HDL	1.	< 1.14	922
	2.	1.14 - 1.37	966
	3.	> 1.37	970
LDL	1.	0.24 - 1.86	944
	2.	1.87 - 2.36	954
	3.	2.37 - 8.28	960

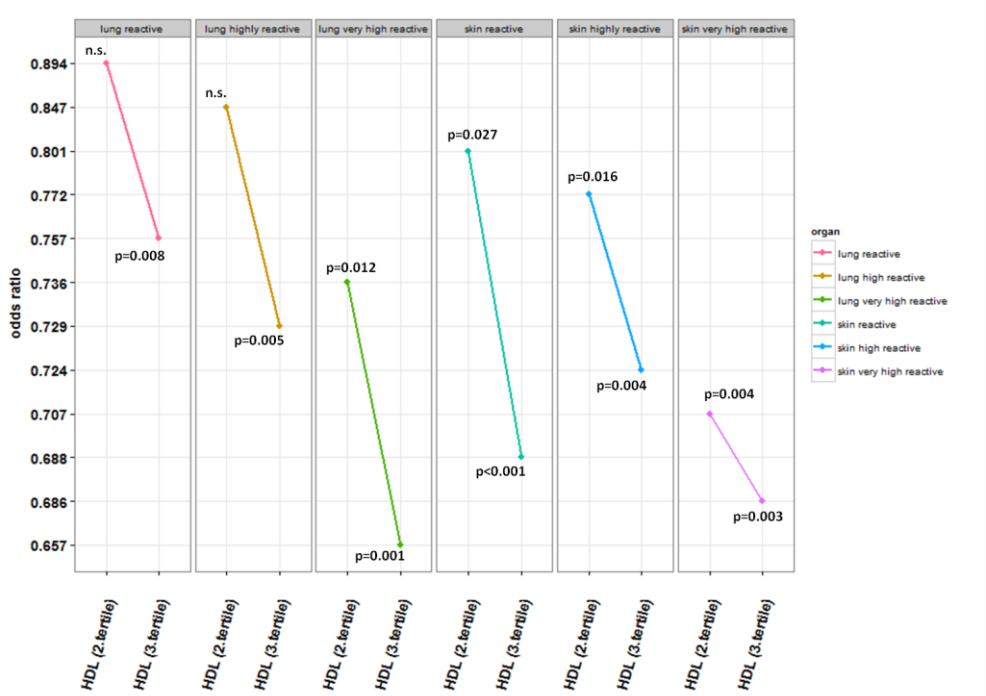


Figure 26: Higher HDL serum-levels are associated with lower auto-IgE reactivity.
The calculated OR is given for the lower value (2. tertile, 1.14 – 1.37 mmol/L) and the higher value (3. tertile, > 1.37 mmol/L) of HDL and the respective calculated p-value. The Graph illustrates the decrease of odds ratios with rising HDL-levels for each height of auto-IgE reactivity, indicating a lower risk to develop auto-IgE reactivity against lung and skin when high levels of HDL are present. Values are given for each cut-off as different reactivity (1. cut-off = reactive, 2. cut-off = highly reactive, 3. cut-off = very high reactive).

4.3.7. Environmental factors reveal not to have influence on auto-IgE reactivity

Allergy is caused by a multifactorial interaction of genetics, the immune system and environmental factors. Some of the latter can be influenced and therefore modify the risk of getting allergic or atopic. To investigate whether we have an impact of influencing environmental factors on auto-IgE reactivity we tested several factors that are known to have an impact on allergy or atopy. Air pollution as a discussed factor in urban areas did not have an influence on the occurrence on the tested autoreactivity to lung and skin (data not shown). However, the association between auto-IgE reactivity to skin, but not lung and the life in Munich is positively associated for the two first cut-offs whether there is no significant association in children living in Wesel, Leipzig or Bad Honnef [Figure 22]. Association to lung reactivity reveals only single significant values as the higher risk (OR 1.3, p = 0.04) emerges for children from the GINI study living in Wesel [Figure 22] whereas children participating the LISA study living in Leipzig revealed a lower chance to develop auto-IgE reactivity, showing a significant lower risk for the second cut-off (OR 0.57. p =

0.003) [Figure 22] but there finds no upcoming pattern for the risk living in a certain area and IgE self-reactivity.

Table 24: Children attending the GINI study and live in Munich show a positive association between autoreactivity towards skin but not lung.
We analyzed whether the area where the children live has an impact on IgE autoreactivity utilizing a logistic regression model. Children that attend the GINI study and live in Munich have higher auto-IgE reactivity and show auto-IgE reactivity more frequent. Living in Wesel also shows a higher chance to develop auto-IgE reactivity towards lung. In contrast children participating the LISA study and live in Leipzig or Bad Honnef have a lower risk to develop IgE autoreactivity, however this is not significant beside the 2. cut-off in lung reactivity.

study city	study	autoreactivity towards SKIN			autoreactivity towards lung		
		1-fold cut-off [OR(95% CI)]	1.5-fold cut-off [OR(95% CI)]	2-fold cut-off [OR(95% CI)]	1-fold cut-off [OR(95% CI)]	1.5-fold cut-off [OR(95% CI)]	2-fold cut-off [OR(95% CI)]
Munich	GINI	**1.59(1.26-2.006)** **p<0.001**	**1.49(1.16-1.92)** **p=0.002**	1.33(0.99-1.78) p=0.058	1.18(0.93-1.48) p=0.168	0.99(0.77-1.27) p=0.93	1.2(0.89-1.60) p=0.233
Wesel	GINI	1.13(0.88-1.46) p=0.348	1.22(0.92.1.62) p=0.16	1.31(0.95-1.8) p=0.1	**1.3(1.01-1.68)** **p=0.04**	1.04(0.79-1.36) p=0.78	1.35(0.98-1.85) p=0.07
Wesel	LISA	1.22(0.77-1.93) p=0.39	1.5(0.92-2.45) p=0.102	1.5(0.86-2.6) p=0.15	1.47(0.93-2.31) p=0.1	1.01(0.2-1.65) p=0.96	1.37(0.78-2.38) p=0.27
Leipzig	LISA	0.98(0.71-1.36) p=0.92	1.03(0.71-1.48) p=0.89	0.99(0.65-1.51) p=0.95	0.79(0.57-1.09) p=0.15	**0.57(0.39-.0.83)** **p=0.003**	0.79(0.51-1.23) p=0.3
Bad Honnef	LISA	0.83(0.54-1.25) p=0.37	1.0(0.63-1.59) p=0.99	1.04(0.62-1.77) p=0.88	0.81(0.54-1.22) p=0.31	0.81(0.52-1.28) p=0.37	1.29(0.78-2.13) p=0.32

Furthermore we investigated whether parental education or the early visit of day nursery have an impact on the occurrence of auto-IgE. But neither parental education [Table 25] nor the early visit of day nursery (data not shown) had any impact on the occurrence or the extent of auto-IgE reactivity. In addition we calculated by a linear regression model the impact of pets on the autoreactivity. 2045 children never lived together with an animal at home. 351 (12.7 %) children had a cat and 281 (10.2 %) a dog. 89 (3.2 %) had both a cat and a dog. We could not find any association for auto-IgE-reactivity and keeping a dog or both a cat and a dog. Certainly, children who ever had a pet showed a negative association between the ownership of a pet and the very high IgE-reactivity (3. cut-off) here the OR(95 % CI) for skin reactivity is *0.79(0.65-0.97) p=0.021* and for lung *0.77(0.63-0.94) p=0.01* [Table 26].

Table 25: There is no effect of parental education on auto-IgE reactivity.
We performed a linear regression model to calculate a possible association but there is no significant difference in auto-IgE reactivity when the parents are more or less educated.

	autoreactivity towards skin			autoreactivity towards lung		
parental education	1. cut-off [OR(95 % CI)]	2. cut-off [OR(95 % CI)]	3. cut-off [OR(95 % CI)]	1. cut-off [OR(95 % CI)]	2. cut-off [OR(95 % CI)]	3. cut-off [OR(95 % CI)]
medium (10 yrs. School)	1.04 (0.72-1.5) p=0.83	0.99 (0.67-1.46) p=0.95	0.94 (0.61-1.46) p=0.79	1.09 (0.93-2.39) p=0.66	1.38 (0.76-1.56) p=0.11	1.5 (0.94-2.39) p=0.09
high (12 yrs. School)	1.03 (0.73-1.45) p=0.88	1.01 (0.6-1.47) p=0.95	0.97 (0.64-1.47) p=0.87	0.97 (0.69-1.37) p=0.87	1.05 (0.72-1.53) p=0.82	1.28 (0.81-2.0) p=0.29

Table 26: Children who ever have had a pet show lower auto-IgE reactivity.
Children who ever have had a pet show lower auto-IgE reactivity towards skin and lung derived proteins, statistical significance is given for children showing very high IgE autoreactivity (3.cut-off >1) only.

	autoreactivity towards skin			autoreactivity towards lung		
	1. cut-off [OR(95% CI)]	2. cut-off [OR(95 % CI)]	3. cut-off [OR(95 % CI)]	1. cut-off [OR(95 % CI)]	2. cut-off [OR(95 % CI)]	3. cut-off [OR(95 % CI)]
ever have had an animal	0.93 (0.79-1.09) p=0.39	0.86 (0.72-1.02) p=0.08	**0.79 (0.65-0.97) p=0.021**	0.87 (0.74-1.02) p=0.08	0.86 (0.73-1.03) p=0.1	**0.77 (0.63-0.94) p=0.01**

4.4 Studies in auto-IgE reactivity on adult asthmatic and skin disease patients

4.4.1 Adult asthmatic patients with allergic asthma show IgE-autoimmunity towards lung proteins

Allergic asthma also called extrinsic asthma is associated with allergic sensitization to inhalant allergens such as those from house dust mites and is an IgE mediated chronic relapsing disease with airway constriction. Another type of asthma is categorized as intrinsic or non-allergic asthma, as no allergic sensitization could be confirmed, the asthma is triggered from intrinsic factors. Data on IgE-autoreactivity asthma is scarce; therefore we investigated a total of 80 asthmatic patients for their auto-IgE reactivity towards lung and skin. 25 patients were intrinsic (non allergic) and 55 patients extrinsic (allergic) asthmatics.

4.4.2 STUDY POPULATION CHARACTERISTIC OF ASTHMATIC PATIENTS

We investigated 55 patients with allergic asthma, as an allergic sensitization was diagnosed and 25 patients with non-allergic asthma retrospectively. The sera were kindly provided from Prof. Buhl from the Medizinische Klinik and Poliklinik of the clinic of the university Mainz. The patients were characterized for their severity (obsolete classification) and their asthma control status (current classification of asthma severity) [Table 27]. Further the patients were characterized for age, sex, lung function, total IgE, BMI and smoking.

Table 27: Study population characteristic of asthmatic patients, we investigated retrospectively.

Asthmatic patients	n	80
(Intrinsic) non-allergic asthma	n	25
(extrinsic) allergic asthma	n	55
Class of severity (1 - 4)		
Class 1	n (%)	5 (6.25)
Class 2	n (%)	15 (18.8)
Class 3	n (%)	24 (30.0)
Class 4	n (%)	36 (45.0)
Class of asthma control (1 – 3)		
Class 1 (controlled asthma)	n (%)	17 (21.5)
Class 2 (partly controlled asthma)	n (%)	28 (35.4)
Class 3 (uncontrolled asthma)	n (%)	34 (43.0)
Classification of the California Asthma Public Health Initiative (CAPHI). Based on NAEPP EPR-3 (National Asthma Education and Prevention Programm, Expert Panel Report 3) recommendations for classification of asthma severity and control.		

4.4.3 More patients with allergic asthma show IgE-autoreactivity towards lung- and skin derived proteins

Figure 27 shows the IgE-reactivity towards skin and lung derived proteins of non-allergic asthmatic and allergic asthmatic patients. Reactivity equal or higher than one is classified as autoreactive as distinguished by the red dotted line. As expected, due to the level of total IgE, (total IgE non-allergic asthmatics: median: 32.4 kU/L, mean: 98.3 kU/L, allergic asthmatics: median: 120 kU/L, mean: 547 kU/L) patients with allergic asthma show higher frequency of IgE-autoreactivity against proteins from human skin (21.8 %) and lung (25.5 %) than patients with non-allergic asthma (12 % lung and skin) [Figure 27]. Similar to atopic eczema patients most patients do not show IgE autoreactivity towards the tested proteins in both asthma groups.

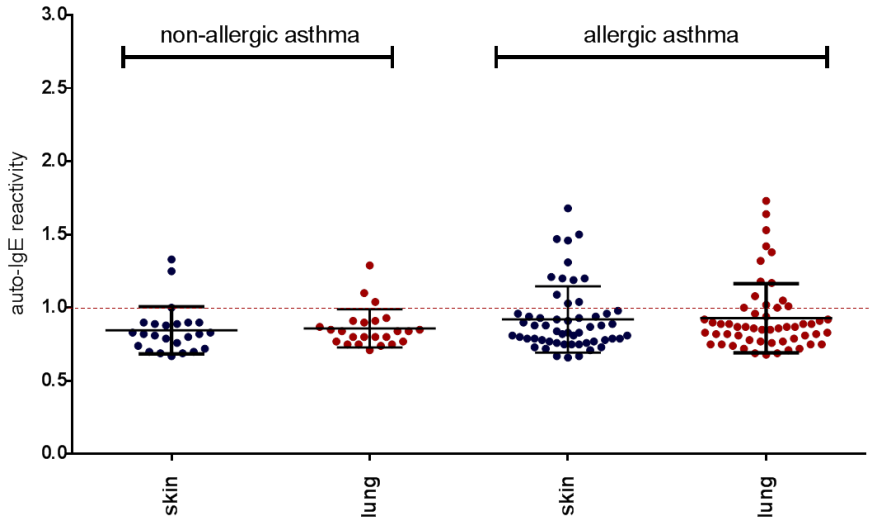


Figure 27: Patients with allergic asthma show higher frequency of auto-IgE reactivity towards skin and lung than non-allergic asthmatics.
On the left side the graph shows IgE-autoreactivity of asthmatic patients without allergic sensitization. Three patients out of 25 (12 %) show autoreactivity against skin and lung in this group. Whereas 21.8 %/25.5 % of patients with allergic asthma show auto-IgE reactivity towards skin/lung. Nevertheless most of the patients do not show IgE-reactivity. Autoreactivity ≥ 1 are rationed as positive/reactive as distinguished by the red dotted line.

4.4.4 Class of asthma control is significantly correlated with auto-IgE reactivity

The control status of all asthmatic patients was classified into three classes. Class 1 is a controlled asthma, class 2 a partly controlled asthma and class 3 an uncontrolled asthma. First we performed a correlation analysis for auto-IgE reactivity towards skin and lung and the given control level of each asthma patient stratified for non-allergic and allergic asthma. Figure 28 shows the differences in the correlation for non-allergic (A, B) and allergic asthmatics (C, D) each for skin and lung derived proteins. Whereas patients without allergic sensitization do not show a significant correlation with their auto-IgE reactivity against skin (A) or lung (B), allergic asthmatics do show a significant ($p=0.03$) slight positive correlation $R^2=0.09$). The worse the asthma is controlled the higher the auto-IgE reactivity against skin (C) and lung (D). We calculated the effect for allergy and the rising control status in a generalized linear model. To compare allergic asthmatic patients with non-allergic asthmatics we calculated the mean of auto-IgE reactivity of both groups. Patients with allergic asthma show higher auto-IgE reactivity by the factor of 0.77.

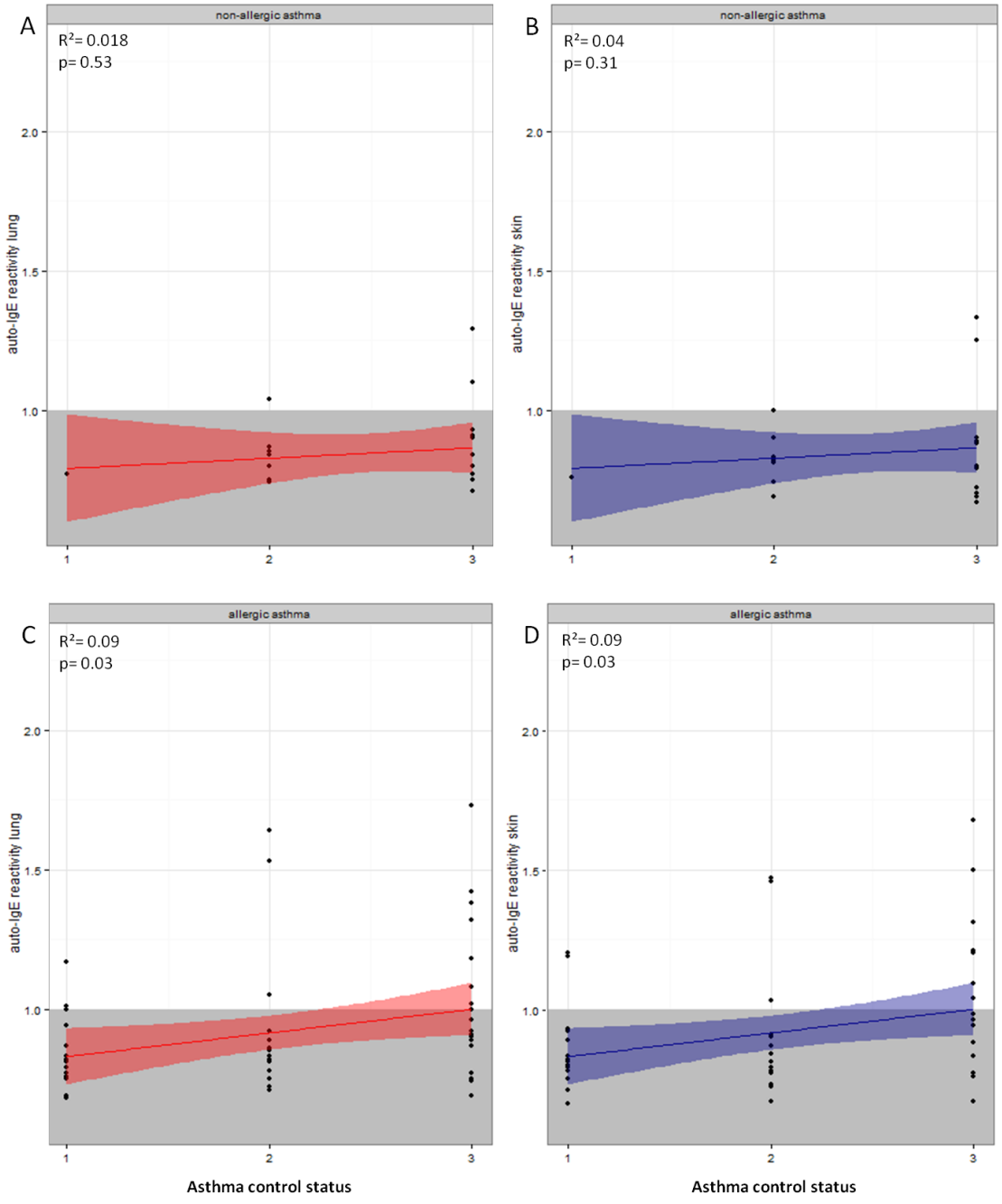


Figure 28: Patients with allergic asthma show higher auto-IgE reactivity the worse the asthma is controlled.
Allergic asthmatics show a significant positive association of autoreactivity towards skin and lung derived proteins and the asthma control status, whereas non-allergic asthmatics do not show these parallel. In [A] the correlation of IgE-autoreactivity (y-axis) and the asthma control status (x-axis) against lung proteins of non-allergic asthmatics are shown (R^2= 0.018, n.s.), in [B] the same group of patients and their reactivity towards skin proteins (R^2= 0.04, n.s.). Patients with allergic asthma do show a significant, slightly positive correlation between their auto-IgE reactivity towards lung (R^2=0.09, p= 0.03) [C] and skin (R^2=0.09, p= 0.03) [D]. Data are given with 95% confidence interval.

4.4.5 Increasing auto-IgE reactivity is accompanied with slightly decreased FEV1

As all asthmatic patients were further surveyed for age, sex, BMI, oral/inhalative corticosteroid therapy and smoking as well as analyzed for serum total IgE, nitric oxygen (NO) and their forced expiration in one second (FEV1), we analyzed all parameters statistically on their influence on auto-IgE reactivity.

We used a linear model adjusted for age and sex to show the significant effect of FEV1 ($p<0.05$) as with increasing autoreactivity towards skin or lung the FEV1 decreased slightly. No other parameter showed any significance with IgE autoreactivity.

4.5 Study population of Biedersteiner Autoallergy study (BiA-study)

In our ongoing and still not finalized prospective BiA-study we recruited and analyzed until now 181 adult patients with different skin diseases to investigate the influence of auto-IgE in autoimmunity and other IgE-mediated diseases.

4.5.1 Patients with atopic eczema and dermatomyositis represent the two patient groups with high levels of auto-IgE in our prospective clinical study

In Figure 29 auto-IgE reactivity of BiA patients is shown. We analyzed n = 46 patients with different skin diseases for auto-IgE reactivity in serum. Atopic eczema patients *(red bullets)* do show a high frequency of auto-reactivity of IgE towards proteins derived from skin, lung and PBMCs. Unexpected is the auto-reactivity of patients with Dermatomyositis *(light green bullets)*, but rather expectable the auto-IgE reactivity of patients with systemic lupus erythematosus *(SLE, blue bullets)*. Two patients with pemphigus vulgaris (PV, magenta bullets) show low auto-IgE reactivity towards PBMC and lung derived proteins or against PBMC proteins only. Anyhow, patients with Bullous Pemphigoid (BP, yellow bullets), cutaneous Lupus *(cut. LE, turquoise bullets)*, Psoriasis *(green bullets)* and Urticaria *(grey bullets)* did not show IgE-autoreactivity against the tested allogenic proteins. Control patients are healthy individuals who got a treatment in the clinic of dermatology due to harmless diagnoses as Dermatitis solaris or nevi. None of the healthy individuals showed any autoreactivity towards the tested proteins.

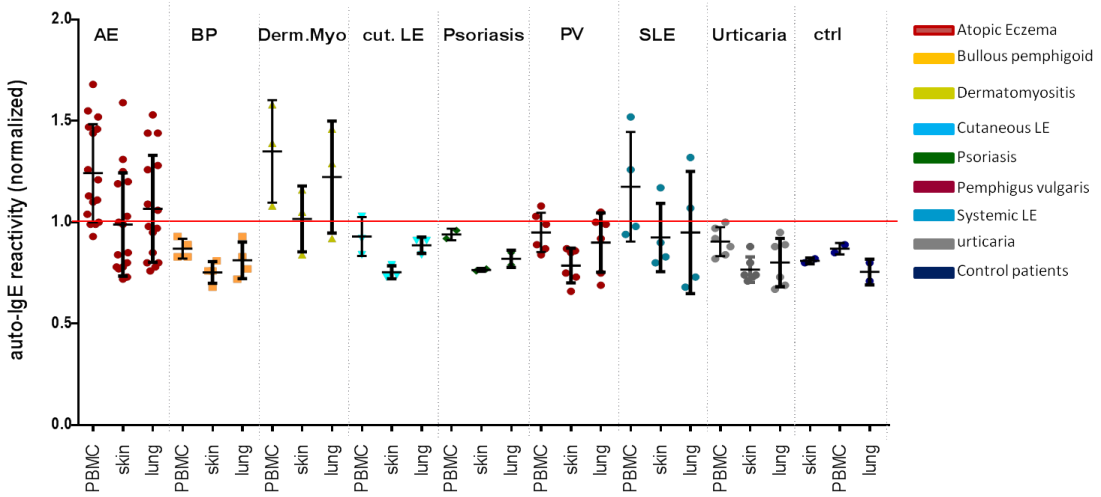


Figure 29: Atopic eczema patients do show autoreactivity more frequent than patients suffering from different skin diseases.
We analyzed patients (n = 46) from the prospective BiA-study for their autoreactivity in serum. Depicted is the factor as calculated for the 1. cut-off. A reactivity ≥1 is rationed as reactive or positive. Atopic eczema (AE, red bullets) patients show the highest frequency of auto-IgE reactivity compared to other skin diseases. Bullous pemphigoid (BP, yellow bullets), cutaneous Lupus erythematosus (cut. LE, turquoise bullets), psoriasis (green bulltes) and urticaria (grey bullets) patients do not show any auto-IgE reactivity towads PBMC-, skin- or lung- derived proteins. Patients with Dermatomyositis (light green bullets) and few patients with SLE (blue bullets) and Pemphigus vulgaris (PV, magenta bullets) do show auto-IgE reactivity. Control (ctrl, dark blue bullets) patients are not auto-IgE reactive. Single data are shown as bullets, further the median is given.

4.5.2 Auto-IgE is not specific towards epithelial tissue protein but is reactive towards proteins from PBMCs

To investigate whether auto-IgE reactivity is exclusive to epithelial proteins or whether there is a variation of reactivity we tested 168 patients for their auto-IgE specificity against epithelial proteins derived from keratinocytes (HaCaT cells) and primary bronchia epithelial cells (NHBE cells) and non-epithelial proteins derived from primary peripheral mononuclear blood cells (PBMC) from four genetically unrelated subjects. Figure 30 depicts the auto-IgE reactivity against the three different tested sets of proteins from 168 patients. The autoreactivity here is not restricted towards epithelial proteins derived from skin or lung, but shows reactivity towards non-epithelial derived proteins from PBMC as well.

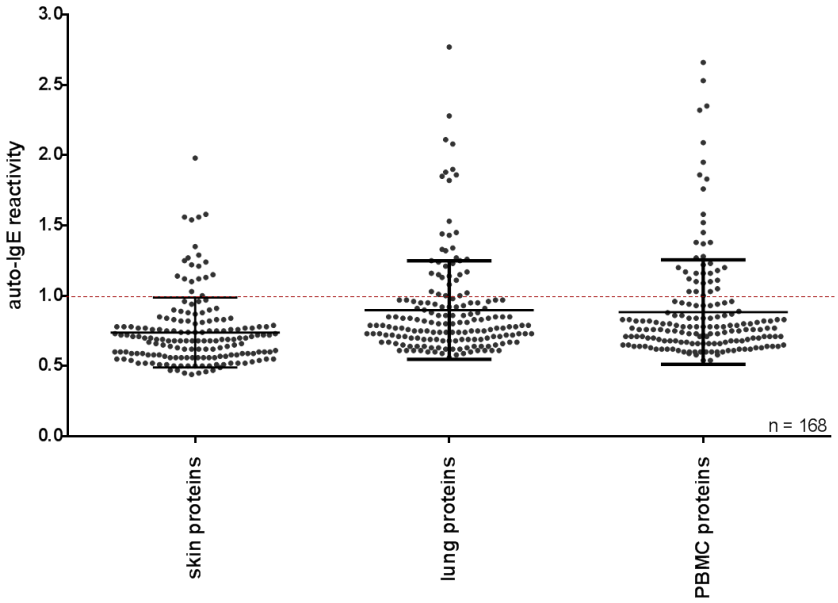


Figure 30: Auto-IgE reactivity shows no specificity towards epithelial proteins derived from skin or lung, but is reactive towards non-epithelial proteins derived from PBMC as well.
We tested n = 168 patients against the three differently derived set of proteins, whereas skin proteins derived from keratinocytes (HaCaT cells), lung proteins derived from primary NHBE cells and proteins from PBMC were extracted from primary PBMC.

4.5.3 AUTOLOGOUS SKIN PRICK TEST REVEALS INCONSISTENT REACTIVITY

To test an immediate hypersensitivity reaction or type I reaction in patients with serological auto-IgE reactivity we performed a skin-prick test using a solution from autologous skin. After testing each patient for their IgE-autoreactivity using the immunoassay we recruited those patients again for skin-prick test and Basophil-activation test (BAT) (6.4.2.4.). The results for the skin prick test revealed in inconsistent results for the few tested patients by now. However, we tested only patients with different skin diseases each (Atopic eczema, subacute cutaneous lupus erythematosus (SCLE), chronic discoid Lupus erythematosus CDLE, systemic LE, Psoriasis, SLE combined with Sharp-Syndrome). No patient did show a positive skin reaction. However, the required positive (histamine) and negative (NaCl) control were appropriate (data not shown). As the study is still ongoing we planned prick-testing with more atopic eczema patients.

4.5.4 MISSING BASOPHIL ACTIVATION AFTER EXPOSURE WITH ALLOGENIC KERATINOCYTE PROTEINS

As a second method to assess an immediate type response we analyzed the quantitative basophil activation by flow cytometry using a basophil activation test (BAT). We performed the BAT on patients that were positively tested for serological IgE-

autoreactivity by immunoassay. In total we tested seven patients with afore mentioned skin diseases (4.5.3). The BAT was accomplished within two hours after blood draining according to the manufacturer´s protocol. Instead of purchasable allergen we used the same allogenic protein extracts from activated HaCaT cells as we used for the immunoassay. For each patient sample the keratinocyte protein concentration was titrated (5 µg/ml, 2.5 µg/ml, 1.25 µg/ml, 625 ng/ml, 312.5 ng/ml) to ensure the correct protein amount for basophil activation for each patient. However, we could not assess basophil activation in any sample. In a second step we prolonged the allergen/basophil incubation up to 30 min (15 min according to protocol) without any enhancement of basophil activation. Furthermore we ruled out the RIPA buffer, used for the protein extraction as a disturbing factor. Therefore we added the same concentration of RIPA buffer we used in all samples, by adding the protein extracts to each control sample, without any change of the results of the controls. As we could not assess any activation of basophils after incubation with allogenic keratinocyte protein data are not shown here.

4.6 Establishment of OVA specific IgE enrichment from serum from sensitized BALB/c mice

For the establishment of a murine model of passive auto-sensitization using chicken ovalbumin (OVA) as a model self-antigen we established the enrichment of OVA-specific IgE from murine serum. We used remaining serum from sensitized BALB/c mice from prior sensitization experiments. sOVAtg and mOVAtg mice, both on C57BL/6J background, are intended to be passively vaccinated with OVA-specific IgE generated in BALB/c wild type mice. The here established method to enrich IgE molecules in serum by the combination of albumin and twofold IgG extraction was not available before. We optimized the combination of all used resins and buffers to yield the highest amount of IgE and the lowest amount of Albumin and IgG in the final eluate. The serum had to be extracted from Albumin first, to obtain the accurate salt concentration and pH of the solution we exchanged the buffer prior albumin extraction using Zeba®Spin Columns (Pierce) to avoid the dilution of the sample. In a second step we extracted the murine serum albumin by using a commercial available resin (Melon Gel®, Pierce), the buffer exchanged serum was given onto the resin, permitted to flow through after a short incubation. Figure 31, a SDS gel coomassie stained shows the albumin content in serum prior to extraction (left lane) and after albumin extraction (right lane) at 69 kDa. In the last step our aim was to extract IgG from the serum by binding on Protein G. As the salt

concentration after albumin extraction contained to few sodium ions and to avoid a dilution by adding sodium phosphate the buffer was exchanged again (Zeba® Spin Columns) by using the appropriate binding buffer used in the Protein G column. We optimized the removal of IgG molecules from serum by running the serum twice over a Protein G column. The IgG fraction was eluted out from the column to check the amount of total and OVA-specific IgE lost in this fraction. Each Protein G column was eluted three times, thus we obtained three IgG fractions in total. Figure 32 depicts the concentration of OVA-specific IgE [A] and of total IgG [B] in the serum pool prior treatment, the IgE eluate after enrichment and in the IgG-fraction eluted from one to four columns. The IgE-eluate contains 4260 ng/ml, whereas the IgG fraction contains no OVA-specific IgE, as there was no loss of OVA-specific IgE Figure 32A. The concentration of IgG is depicted in Figure 32B again in the serum pool prior enrichment, the IgE-eluate and the IgG fractions out from four elutions. The first elution of IgG (column1 and 2) contain the highest amount of IgG as the last to eluate (column 3 and 4) contain much less of the IgG. The last column of Figure 32B (dark green) shows the total amount of the concentration of all IgG fractions giving the entire recovery of IgG.

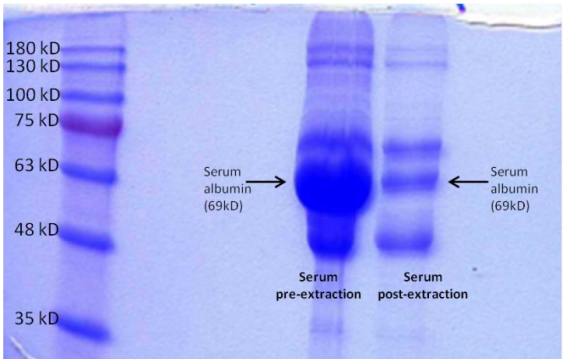


Figure 31: Murine serum albumin was minimized after albumin extraction using Melon gel® resin.
Serum proteins prior (left) and after (right) extraction were run on a 12% SDS gel and stained with Coommassie. After extraction the sample shows highly reduced albumin content (69 kDa), compared to the native serum sample prior to extraction.

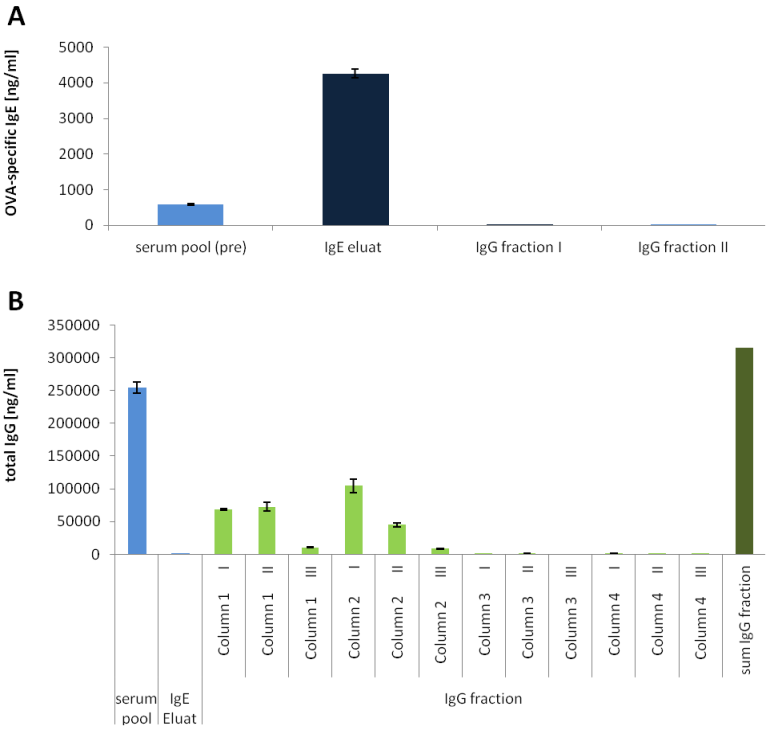


Figure 32: The IgE eluate is free of IgG after extraction by protein G.
[A]: OVA-specific IgE is shown in the first bar in the native serum before IgE-enrichment. The blue bar shows the enhancement of IgE in the eluate. All IgG fractions arer free of IgE. **[B]:** The chart shows the IgG content of the serum prior IgG extraction (light blue bar) and the IgG content in the IgE-eluate after extraction(2nd bar). The IgG eluate fractions contain the extracted IgG (light green bars). The first eluate depicted in column 1 and 2 and contain the highest amount of IgG. To remove the entire amount of IgG from the IgE eluate a second run on protein G columns was performed, shown as the eluate of column 3 and 4, with much less IgG content. To ensure the entire recovery of IgG the last bar (dark green) shows the calculated sum up of all fractions of IgG. Data are given ±SD.

5. DISCUSSION

The initial aim of this study was to assess whether auto-IgE has a pathomechanistic impact on the manifestation and aggravation of atopic eczema and allergic asthma. In the course of the study and the analysis of different patient groups and cohorts we extended the hypothesis if auto-IgE is physiologically generated during childhood and does auto-IgE protect from developing atopic eczema.
Based on new findings of the physiologic role of autoreactive IgE during childhood and their protective potential towards eczema, the present study shows an unpredicted new aspect on the evaluation of autoreactive IgE that has previously predominantly been described in adult atopic eczema patients.

The cause of atopic eczema, the most common inflammatory skin disease manifesting early in life (ILLI et al. 2004), and the cause of the frequent progression into allergic asthma (STRACHAN et al. 1996; WÜTHRICH 1999) is not fully understood. The answers to these questions are of particular clinical and scientific interest.
The majority of atopic eczema patients are sensitized against exogenous allergens with increased serum levels of total and specific IgE also referred as to extrinsic or allergic atopic eczema (ILLI et al. 2004). Still, there is no convincing evidence that allergic sensitization is a substantial feature of childhood eczema (JOHANSSON et al. 2001). Moreover, it is not well understood why acute allergic symptoms can turn into chronic disease, either atopic eczema or chronic asthma, but does not affect all patients (ILLI et al. 2004). One hypothesis is that IgE antibodies directed against the body´s own proteins aggravate acute allergic disease into chronic disease (AICHBERGER et al. 2005; ALTRICHTER et al. 2008; VALENTA et al. 2000; VALENTA et al. 1991).
The possibility to screen 2861 children at the age of ten combinig the two birth cohorts GINI and LISA was unique and yielded new aspects on autoallergy in early life. These birth cohorts allowed analyzing healthy and atopic children side by side and the investigation of longitudinal issues. Based on the decline of auto-IgE with age and the high incidence of auto-IgE found in a small group of young children with atopic eczema in this study, we hypothesized the physiologic generation of auto-IgE. To prove this hypothesis we made use of the GINI and LISA birth cohorts.

The analysis of the occurrence of auto-IgE in healthy and diseased children, both out of the two birth cohorts, side by side, confirmed that about half of the children of each group generate auto-reactive IgE antibodies. The finding that healthy and diseased children

show an IgE auto-reactivity to the same extent of about 50 %, suggests a physiologic formation of self-reactive IgE antibodies during childhood.

Antibodies that are auto-reactive are known to be present in healthy individuals already during childhood (MADI et al. 2012), and are, beside auto-antibodies, referred to as natural auto-antibodies (NAbs) (ACKERMANN-SCHOPF et al. 1974; DIGHIERO et al. 1986; WARDEMANN et al. 2003).

According to the clonal selection theory from Burnet in 1959, auto-reactive lymphocytes are deleted to assure self-tolerance (BURNET 1959). In the early 1960s, Boyden questioned this and claimed natural auto-reactivity particularly towards skin antigens (BOYDEN 1964). Today, NAbs are investigated profoundly and their existence has been approved. To date, most NAbs are known to derive from the IgM class. They develop in the absence of germinal centers, are germline gene encoded respectively (BACHARIER and GUILBERT 2012; CASALI and SCHETTINO 1996; HARINDRANATH et al. 1993; MOTHES et al. 2005). However, Avrameas et al. described the majority of NAbs belong to the IgG class (AVRAMEAS 1991). NAbs are spontaneously produced by innate-like B cells (KEARNEY 2005) and, as known so far, are poly-reactive with moderate affinity Due to their broad reactivity and their potential to bind commensal and pathogenic bacteria (CAPOLUNGHI et al. 2008; ZHOU et al. 2007) or microbial components in the intestine and airways (CAPOLUNGHI et al. 2008), it was suggested that they play a major role in the first line of defense (ARANBURU et al. 2010) towards infections even if the cell has never been exposed to the reactive antigen before (ARANBURU et al. 2010; CAPOLUNGHI et al. 2008; LONGO et al. 2009).

Furthermore, a tumor cell defense for natural IgM antibodies was described, as these NAbs induce apoptosis in tumor cells (HENSEL et al. 2001; HENSEL et al. 1999; POHLE et al. 2004; VOLLMERS et al. 1989).

Since IgM antibodies are generated without a class switch recombination, a natural auto-reactivity seems more obvious or easier to develop, than in other classes of immunoglobulins. Natural antibodies of the IgA and IgG class are known as natural polyreactive IgG and IgA and NAbs were detected in healthy humans in the early eighties (HENSEL et al. 2001; HENSEL et al. 1999; VOLLMERS et al. 1989) with a broad range of specificities. Examples of IgG-autoreactive antigens are senescent red blood cells (LUTZ 2012), several intracellular proteins such as actin, myosin, DNA or cytochrome C, membrane components as β2- microglobulin and cytokines (IL-1α, interferon, TNF) or hormones (thyroglobulin, insulin) (AVRAMEAS 1991). So far, only a few of the listed proteins are proven auto-allergens that bind to auto-IgE. Khalaf et al. reported the natural

occurrence of IgE antibodies against peptide hormones in man and showed that about 15 % of the tested atopic patients were IgE-reactive towards insulin (KHALAF et al. 1989). IgE-reactivity against thyroglobulin has been shown in one out of 20 patients in a study conducted in patients with Hashimoto´s diseases and urticaria (CONCHA et al. 2004). There might be a link between actin and profilin auto-reactivity. Profilin is a well- known allergen, first identified in 1977 in non-muscle cells and later on as an allergen in birch pollen (Bet v2) (CARLSSON et al. 1977; VALENTA et al. 1992). Valenta et al. described cross-reactivity of Bet v2 to human profilin suggesting that profilin acts as an IgE-autoallergen (VALENTA et al. 1991). As profilin binds actin there might be an interference of these two auto-allergens disclosing an epitope to IgG and IgE. Profilin is an actin binding protein regulating actin polymerization in the cytoskeleton and the addition of actin monomers to preformed actin filaments (REICHSTEIN and KORN 1979; VALENTA et al. 1992).

Natural anti-FcεRI and anti-IgE/IgG antibodies were found in patients with chronic urticaria (FIEBIGER et al. 1995), in patients with asthma (SMITH et al. 1995) and in autoimmune disorders (FIEBIGER et al. 1998; GRUBER et al. 1988) but were not detected in patients with atopic eczema or healthy subjects (FIEBIGER et al. 1998; FIEBIGER et al. 1995).
In contrast, natural antibodies of the IgE class were described in mice (MCCOY et al. 2006). and a few studies explored natural IgE antibodies in humans. Ackermann-Schopf et al. attempted to detect antibodies without a defined specificity by immune fluorescence in tissue obtained from adults but without success (ACKERMANN-SCHOPF et al. 1974). Another study aimed to detect natural IgE antibodies in the placenta, but no natural antibody of the IgE class was found. Concurrently, they analyzed the maternal plasma for natural IgE antibodies with defined specificity towards phosphorylcholine (PC), a carbohydrate that is present on the surface of apoptotic cells. Low levels of anti-PC IgE in plasma were quantified by ELISA (JOERINK et al. 2009). Mc Coy et al. confirmed the spontaneous generation of natural IgE in serum of wild type germ-free mice, which were elevated in T-cell deficient mice (MCCOY et al. 2006). The study indicates that the pathway involved in the induction of spontaneous class switch of natural IgE is less dependent on conventional T cell help, as compared with allergic or parasite induced IgE generation. To confirm their results, they indicate that the baseline IgE was not associated with subclinical infections (MCCOY et al. 2006).

The issue whether auto-reactive IgE antibodies are cross-reacting with endogenous proteins produced during allergic reaction against exogenous allergens was already

discussed earlier for other NAbs (AVRAMEAS 1991). The second conceivable theory is the neo-synthesis of auto-reactive IgE against self-proteins.

Considering that even newborn mice and humans (LUTZ 2012; UNDERWOOD et al. 1985), as well as germ-free, nude and antigen-free mice (PEREIRA et al. 1986; UNDERWOOD et al. 1985) synthesize NAbs and the physiologic generation in children as shown in the present study, the latter theory seems more likely. Beside the release of proteins acting as auto-allergens, another hypothesis is that proteins such as the identified Hom's auto-allergens escape central tolerance mechanisms due to their localization within the cell resulting in an autoimmune response (VALENTA et al. 2009). However, the hypothesis of cross reactivity should not be completely disregarded as highly conserved epitopes are likely to be epitopes for NAbs e.g. keratin, laminin and actin (AVRAMEAS 1991).

To date, according to the literature, the rate of natural antibodies is unknown, but it seems reasonable to assume that, physiologically generated auto-IgE as a natural auto-antibody should be more than 50 % in healthy children. To evaluate this rate of reactivity it is important to mention that up to 50 % of children with eczema do not show detectable amounts of specific IgE. This rate is perhaps even higher based on overview studies as evaluated in systemic reviews (FLOHR et al. 2004; WILLIAMS and FLOHR 2006). Even if atopic eczema is clearly associated with the sensitization to environmental allergens, specific IgE seems not to be an important causative factor or discriminator for the diagnosis of atopic eczema.

Evidence from these overview studies show that patients with more severe eczema are also more likely to be sensitized and therefore explaining the higher sensitization rates in studies performed in hospitals compared to community studies (FLOHR et al. 2004). The hospital cases of atopic eczema tend to be more severe than cases in community settings (EMERSON et al. 1998), leading to a biased interpretation of clinical studies.

Furthermore, the association between sensitization and eczema is weaker in countries with a low income compared to countries with a high income suggesting risk factors that are linked to "western lifestyle" (WILLIAMS and FLOHR 2006).

In conclusion, whether the classification of atopic eczema in intrinsic (non-allergic) and extrinsic (allergic) is still accurate or if the two types are not two distinct forms of atopic eczema is not clear. Whether the intrinsic form is a transitional phase of the IgE associated at least in infancy is also not clear (BIEBER 2010).

For the analysis of the results of the GINI/LISA study, we used the categorization as determined for extrinsic atopic eczema, as the parents were questioned for the diagnosis

of atopic eczema. There is evidence that if intrinsic atopic eczema is a transitional form or at least not a complete distinct type of atopic eczema, we might have misdiagnosed a few children due to erroneous diagnostic and categorization tools.

Furthermore, it has to be added, that the assertion given in the questionnaires was not verified. If we consider allergic or atopic eczema as a distinct form and the questionnaire asked whether a physician diagnosed atopic eczema or asthma or rhinitis and the parent check marked atopic eczema as the diagnosis, the diagnosis was not verified. Subsequently we also do not know whether atopic eczema was diagnosed using the major criteria including specific IgE or a positive skin prick test (HANIFIN and LOBITZ 1977) or if the diagnosis was done using visual examination of the skin only.

In 1995, Mouthon et al. showed considerable differences in the reactivity of natural IgG antibodies between healthy individuals (MOUTHON et al. 1995). They reported a higher heterogeneity of auto-IgG reactivity between individuals towards proteins derived from liver, kidney and muscle and less heterogeneity of auto-IgG reactivity towards brain, thymus and stomach (MOUTHON et al. 1995). In a previous study we did on IgE auto-reactivity, we could show that there is no significant difference in individuals between skin, lung and gut IgE auto-reactivity but an unexpected high reactivity against liver derived proteins.
Since we utilized the tumor cell line HepG2, this effect is likely due to its carcinoma nature, with a repertoire of protein expression highly modified in these mutated cells (BECK 2012). However, different reactivity against various tissues or cell types amongst the investigated individuals was not analyzed and this has to be done in further experiments.

Differences between IgG from whole serum and purified IgG have been shown with higher rates of reactivity for purified IgG (MOUTHON et al. 1995). Here, IgM antibodies in serum are considered to control IgG auto-reactivity by inhibiting the binding of auto-reactive IgG to self-antigens (ADIB et al. 1990; HUREZ et al. 1993). In our assay we used whole serum IgE, which could not exclude the possibility of a higher or more frequent reactivity or sensitivity when compared to purified IgE.

Madi et al. analyzed NAbs of IgM and IgG class in newborns and adults. Besides that a healthy immune system possess auto-reactivity to a selected set of particular self-molecules, they believe that primary auto-antibody generation begins already during early

life in the uterus (MADI et al. 2012). The authors suggested that self-molecules serve as an immunologic stimulus inducing IgM and IgA auto-antibodies during that early stage of life. A correlation analysis of auto-reactive IgG auto-antibodies in newborns with their mothers yielded a high degree of correlation as one would expect as IgG can cross the placenta (MADI et al. 2012). However, the correlation was weak between each of the mothers and each of the infants, suggesting an individual repertoire of NAb of IgG class (MADI et al. 2012).

In contrast, an insignificant correlation between the mother's and their newborn's IgM was found but a strong correlation between all newborns, indicating the importance of natural IgM (MADI et al. 2012), indicating that children already show a more "mature" set of NAbs compared to newborns (MADI et al. 2012). As a result, children already bear a large repertoire of NAbs, although not all epitopes of auto-reactive IgE are present in the utilized protein pool we used in our assay. As we used a protein pool with a defined amount of total protein, another possibility is that the concentration of single target proteins could be in the pico or femto molar range and might therefore be too low for the few auto-reactive IgE antibodies in serum in addition to a low concentration of epitopes or auto-IgE antibodies.

Corrective would be the identification of the specificity of auto-IgE to offer the target epitopes in higher concentration.

Previous purification of IgE, as mentioned above, would offer the target epitopes in higher concentration and corrective this issue. In addition, the assay offered optimized conditions for several but not all contained proteins in the used protein pool and therefore we lost epitopes due to the pH conditions, the protein linearization or the assay procedures.

The only study that investigated auto-reactive IgE in infants was performed by Mothes et al. in 1995. The objective of this study was to investigate whether IgE autoimmunity could play a role in the development of atopic eczema already during childhood.

Sera from 120 infants were investigated for the presence of auto-reactive IgE by Western Blot. The study showed that auto-reactive IgE is already produced in young children, 2-6 years of age and the formation of IgE auto-reactivity during early childhood has an impact on the pathogenesis of atopic eczema later in adulthood (MOTHES et al. 2005). However, the lack of using control sera from healthy children in this study led the physiologic generation of auto-IgE in children undetected. Moreover, they evaluated the clinical manifestation of skin lesions in atopic eczema retrospectively by surveying patients for the period from early infancy to 20 years of age. The given information of the onset of

atopic eczema in early childhood and its association with the appearance of auto-IgE in adulthood confirmed a significantly earlier onset and manifestation of atopic eczema in patients with auto-IgE reactivity. But due to the long time period from childhood to adulthood and the gaps in correctly remembering from the symptoms as a child, the outcome seems a bit biased.

Based on the effect of age on the immune system in humans, one could argue the reduced amount of auto-IgE is caused by the age-depending decrease in antibody-producing B cells and a lower amount of switched immunoglobulin isotypes (BLOMBERG and FRASCA 2013; BOYD et al. 2013; SCHOLZ et al. 2013). On the other hand, there are findings that an inappropriate immune activation leading to an increased level of auto-antibodies in serum with age resulting in an increase in inflammatory mediators (BOYD et al. 2013). These findings are in agreement with our results in asthmatic adults where disease activity is highly associated with presence of auto-IgE reactivity.

The present study shows a decline of auto-IgE with age. At present, there are no data that auto-IgE or its properties increase with age in humans although, Mc Coy et al. showed an increase in natural IgE with age in mice (MCCOY et al. 2006).

In one of our previous analyses of the GINI/LISA cohort it was noticeable that fewer children with eczema showed IgE auto-reactivity. To determine whether auto-IgE antibodies could have a protective effect on the development of childhood eczema, we corroborated this hypothesis statistically. Children, sensitized towards food allergens, diagnosed by a positive RAST/FIA using the allergen mixture of food allergens (fx5) and aeroallergens (sx1) with the presence of eczema, showed a negative association between auto-IgE reactivity and eczema. These children showed less auto-IgE reactivity, consequently we can confirm a positive effect between eczema and auto-IgE, and that sensitization lead to higher auto-IgE reactivity.
We investigated whether auto-IgE can be used as a predictive factor for eczema, using a ROC-analysis. Our data revealed that auto-IgE has no predictive value so far.
For future applications, it might be useful to determine the specificity and possible targets of auto-IgE.
However, the risk of early sensitization seems also a risk factor for the entire course of atopic diseases. The Melbourne Atopic Cohort Study (MACS) found that infants who developed eczema within the first six months of life were at increased risk of new

sensitization later in life and that eczema is a predictor of sensitization. However, sensitization to food at an age of six months was associated with an increased risk of developing eczema (LOWE et al. 2007).
All detailed data about eczema and asthma or other diseases examined in the GINI/LISA cohorts relied on information the parents provided on their children in questionnaires but eczema or asthma was not directly diagnosed by a physician. We do not know and cannot retrace whether the diagnosis of atopic eczema was made using the major criteria diagnostics of the analysis of specific IgE or a positive skin prick test results (HANIFIN and LOBITZ 1977) or if the diagnoses were made by visual examination of the skin only.
This impreciseness in the field of diagnosis is one of the biggest disadvantages in large birth cohort studies.

A putative protective role of auto- IgE in atopic eczema based on the occurrence of auto-IgE is new, but known for years for the role of NAb of the IgM class (AVRAMEAS 1991; COHEN 1992; LUTZ 2012). However, it is more complex to predicate the function of these globulins in a specific disease as there were few options to test NAbs in adequate study groups to investigate their function in specific diseases. This has to be confirmed in a larger numbers of patients.

The diagnosis of asthma in pre-school children is complex due to the lack of objective lung function measurements and a defined set of biomarkers as Bacharier et al. discussed in his review (BACHARIER and GUILBERT 2012). At the age of ten, children are definitely school children and the diagnosis of asthma is easy to make.
Due to the fact that we included asthma diagnosis throughout the whole study, it is possible that we did not include some asthmatic patients with onset early in life due to the complicated diagnosis.
Several children suffering from eczema in early childhood develop asthma in later stages of atopy (BERGMANN et al. 1998; RHODES et al. 2001; SPERGEL and PALLER 2003) or develop asthma during puberty or later (RHODES et al. 2001), requiring a second screening at an age of 15 years. Blood samples of children from the GINI/LISA at that age are available and should be screened for the presence of auto-IgE to have the longitudinal effect of its occurrence. The asthma rate rises to eight per cent at the age of 15 in the GINI/LISA birth cohort (oral consultation with Dr. J. Heinrich). Therewith we would have the possibility to expose a potential association of IgE auto-reactivity with asthma.

Numerous factors are considered driving factors for the manifestation of atopy. As breast feeding seems to have a protective effect on the development of atopic eczema (VON BERG et al. 2003; GDALEVICH et al. 2001; KRAMER MS et al. 2001) and asthma (DOGARU et al. 2014), but data from the literature are controversial (LAUBEREAU et al. 2004; NWARU et al. 2013). Parental atopy (VON BERG et al. 2012; HEINRICH et al. 2012), genetic susceptibility (FILIPIAK-PITTROFF et al. 2011; HEINRICH et al. 2012; HENDERSON et al. 2008; O'REGAN et al. 2008; PATERNOSTER et al. 2012; RAJE et al. 2014; SHARMA et al. 2015; WHEATLEY et al. 2002), several environmental factors such as outdoor and indoor air-pollution, ozone or fine air-borne particles (CRAMER et al. 2007; HEINRICH et al. 2012; JUNG et al. 2012; KÜNZLI et al. 2008; SILVERMAN and ITO 2010; SPIRA-COHEN et al. 2011) or hygiene (hygiene hypothesis) might all play a role (STRACHAN 1989). No single factor might be accountable as the only risk factor in the development of atopy. It is more likely that multiple factors are to be blamed in atopic eczema, asthma or rhino conjunctivitis.
Moreover, risk factors are also likely to change over time for areas and countries as a consequence of demographic factors including family size, hygiene and allergen exposure (WILLIAMS et al. 2008), and all these environmental factors might have a different impact on the development of atopy in different areas of the world (FLOHR et al. 2004).

As auto-IgE is believed as a triggering factor for atopic diseases, provoking its manifestation or aggravation we expected an association with atopy driving factors mentioned above. But rarely a parameter we analyzed is associated with the occurrence of auto-IgE. Neither breast feeding nor the feeding of formula with different levels of hydrolyzed proteins affect auto-IgE reactivity nor the early day nursery of children.
Further we could show few associations between the area where these children live and auto-IgE reactivity. As children who live in Munich have a higher chance to develop auto-IgE reactivity towards skin but not against the lung. In contrast children living in more rural areas as Bad Honnef showed no association to the occurrence of auto-IgE. All these related factors display an inconsistent pattern, render it is impossible to predicate a putative risk to develop auto-IgE when living in certain areas.
Parental atopy showed a significantly higher risk for their offspring to develop auto-IgE when the parents were sensitized to food allergens. This is recurrent, as children show the same phenomenon.

However, taking the entirety of the results there is a lack of association. As auto-IgE in

children does not show any association with the clinical outcome of atopy it seems likely that there is no association between factors that trigger atopy. In contrast we showed evidence for a protective role of auto-IgE towards atopic eczema in children.

This is different for adults with atopic eczema as shown for patients recruited for the BiA study as well for adult asthmatic patients we investigated. To date we can predicate a higher incidence of IgE auto-reactivity for atopic eczema patients compared to patient with other inflammatory skin diseases such as urticaria, psoriasis, bullous pemphigoid, we investigated in the BiA-study. Several previous studies already have shown an association of auto-IgE reactivity and atopic eczema (KORTEKANGAS-SAVOLAINEN et al. 2004; NATTER et al. 1998; SCHMID-GRENDELMEIER et al. 2005).

A highly significant correlation between IgE auto-reactivity and the control status of adult allergic asthmatic patients was found. Patients with non-allergic asthma showed no significant correlation with respect to auto-IgE reactivity.
Non-allergic asthma is independent of IgE it is possible that the IgE antibodies present in the serum of those patients are not sufficient to react with the offered antigens or epitopes.
However, we discovered atopic patients with consistently lower IgE levels showing auto-IgE reactivity in the immunoassay.
The auto-reactivity in allergic asthmatic and atopic eczema patients was proof for proteins derived from skin and lung, suggesting ubiquitous expressed proteins as a target for the auto-reactive IgE antibody.
This has already been shown for several constitutive expressed proteins such as MnSOD (CRAMERI et al. 1996; FLÜCKIGER et al. 2002), ribosomal phosphoprotein type 2 (p2) (MAYER et al. 1999), profilin (VALENTA et al. 1991), thioredoxin, Hom s1(VALENTA et al. 1998), Hom s2 (MITTERMANN et al. 2007), Hom s3 (VALENTA et al. 2000), Hom s5 (VALENTA et al. 2000).

To date, there is little known about the impact of auto-reactive IgE antibodies on asthma.
The assumption of the same effect of auto-IgE on inflammatory lung and on inflamed skin of atopic eczema patients both with an impaired barrier function (DE BOER et al. 2008; XIAO et al. 2011) seems obvious, anyhow no study for human beings is available to date.
Bünder et al. showed in 2004 that in a murine model of bronchial asthma, the sensitization with a potential self-antigen induced an allergic immune response. The authors hypothesized that a cross-sensitization to self-protein represents a pathomechanism for

the continuation to chronic forms of allergy (BÜNDER et al. 2004). The impact of IgE auto-reactivity on airway inflammation and asthma remains still unknown. Thus our study is the first investigation of IgE auto-reactivity in asthmatic patients to date.

We were able to identify similarities between adults and children, confirming that factors like sex, BMI and total-IgE have no influence on auto-IgE reactivity. Our analyses in both, adults and children, did not reveal any correlation regarding these factors. A few studies revealed the same outcome for sex (ALTRICHTER et al. 2008; SCHMID-GRENDELMEIER et al. 2005; ZELLER et al. 2009).

In our investigation, we revealed the contrary for parameters, confirmed of being associated with IgE auto-reactivity. To date, a positive correlation between auto-IgE reactivity and the total amount of IgE in serum has been shown numerous times (ALTRICHTER et al. 2008; SCHMID-GRENDELMEIER et al. 2005; VALENTA et al. 1996; ZELLER et al. 2009). Valenta et al. showed no analysis of the mentioned regression, but referred to a list of collected data only. Nevertheless, the authors stated that these two parameters are not always related in certain patients they investigated. The authors concluded that a high amount of total IgE is not an absolute prerequisite for the occurrence of IgE reactivity, which is in agreement with our observation (VALENTA et al. 1996).

None of our study populations revealed a correlation between the total and the self-specific IgE antibodies. Another factor that was frequently indicated as being associated with the occurrence of auto-IgE and its reactivity, is the severity of disease in atopic eczema patients as assessed by SCORAD (Scoring Atopic Dermatitis) (HIGASHI et al. 2009; KINACIYAN et al. 2002; MOTHES et al. 2005; NATTER et al. 1998; SCHMID-GRENDELMEIER et al. 2005; SZAKOS et al. 2004; ZELLER et al. 2009). Only two studies stated a statistical significance (SCHMID-GRENDELMEIER et al. 2005; SZAKOS et al. 2004), while Mothes et al., Higashi et al. and Zeller et al. reported an insignificant association between IgE auto-reactivity and an increased disease severity. Regarding SCORAD as measure for disease severity, one has to consider the 2-sided variability, the patient and the medical personal. From patients' side sleeplessness and pruritus are individually perceived differently resulting in variable, individualized SCORAD. From the medical personal side, assessments between physicians might be different as well, yielding to biased SCORAD results.

Methodological variations including the selection of patients, diagnostics, and different laboratories are all factors to be considered leading to different results of the same parameters investigated. As a result, direct comparison across different studies is not

always beneficial. Molecular factors or better still a molecular profile specific for the dimension and severity of atopic eczema would be more reliable because less subjective (HAMID et al. 1996; HAMID et al. 1994; SAITO 2005) Presently, we do not know how to replace diagnostics by a medical examiner.

Sensitization data for children who participate in the GINI or LISA study are publicized. All children were tested for their sensitization to aeroallergens (mixed allergen test sx1; grass, rye, mugwort, birch, Cladosporium herbarum, house dust mite, cat, dog) and food allergens (mixed allergen test fx5; ovalbumin, milk, flour, codfish, peanut, soybean) by RAST/FIA.

The result was unexpected, as the self-reactivity was different for the two sensitization patterns against aeroallergens and food allergens. Children who are sensitized against food did show significant auto-IgE reactivity towards skin and lung. In contrast, children who are sensitized against aeroallergens, showed significant auto-IgE reactivity exclusively towards lung derived proteins.
A subsequent analysis showed, that some allergens that are significantly associated with auto-IgE reactivity in a dose-dependent manner, leading to increasing auto-IgE reactivity.

Sensitization to specific allergens led to a more frequent “high” (cut-off 2 reactivity) and “very high” (cut-off 3 reactivity) auto-reactivity. As we calculated the odds ratios it was not possible to determine the risk factor or dependent variable.
We were able to show that sensitization drives auto-IgE reactivity, which is one presumable possibility for the risk to develop higher auto-IgE reactivity towards lung and skin derived proteins depending on the sensitization pattern.
Furthermore, we have shown that there is a homology in the protein sequence between the allergens driving a dose response in auto-reactivity and any of the known autoallergens (Hom s). The major allergen of codfish (Gad m1) is parvalbumin, a calcium binding protein, with a protein sequence similar (http://www.uniprot.org/blast) to the sequence of homolog proteins such as Hom s4, also a calcium binding protein.
Thus far, none of the major tested allergens (sx1/ fx5) revealed sequence homology with any of the known Hom s auto-allergens, except for conserved proteins like MnSOD. It might be possible that smaller sequences than the calcium-binding site or other epitopes show a minor sequence homology.
So far the entire outcome, mainly in adults, showed the same reactivity towards proteins

from different origin, suggesting ubiquitous expressed proteins as target for auto-IgE.

In this case it seems that the lung is the only target organ for IgE auto-reactivity, when a sensitization towards inhalant allergens exists. Suggesting a sensitization through the respiratory tract encounters the lung more exclusively including tissue damage and the externalizing of target tissue proteins.
It is a point of discussion whether spreading of target self-proteins are distributed from the skin, as this is the first point of contact with mechanical tissue damage combined with (chronic) inflammation.

There is evidence that asthma is associated with food allergy, linking sensitization to food with respiratory symptoms (GILLMAN and DOUGLASS 2010; VAN DEN OORD and SHEIKH 2009), and speculation to whether a mutation of the filaggrin gene is a predisposing factor for allergic sensitization and its progression to allergic disease (VAN DEN OORD and SHEIKH 2009; VENKATARAMAN et al. 2014).

Another aspect that needs to be considered is sensitization towards inhaled house dust mite allergens (Der p1, Der p2) as besides mugwort, these allergens demonstrate a significant risk for IgE auto-reactivity. Der p1 and Der f1 are known allergens with protease activity (THOMAS et al. 2007). Der p1 cleaves intercellular epithelial tight junctions, allowing allergens penetration from the airway lumen to submucosal structures with antigen-presenting cells (GREGORY and LLOYD 2011; WAN et al. 1999). This tissue damage leads to an impaired epithelial barrier function which is potentially one of the thriving factors for poorly controlled asthma (BHURE et al. 2009; TURI et al. 2011).
The impact of a single allergen like Der p1 has to be investigated in more detail as we have a significant risk of self-reactivity after sensitization with Der p1 and Der p2 and the release of self-proteins with a potential risk for self-reactivity by IgE. A final judgment about the difference of self-reactivity and sensitization is not feasible yet.

To investigate whether the auto-IgE reactivity varies among different cell types or whether it is exclusively reserved for proteins derived from epithelial cells, we analyzed various patients of the BiA study for their auto-reactivity against proteins extracted from PBMCs received from four genetically unrelated donors. These serum samples varied in their intensity and reactivity to the different protein extracts from skin, lung or PBMCs but showed a consistent pattern of reactivity for all analyzed samples.

Our statement that the reactivity of auto-reactive IgE is not specific for epithelial proteins is also supported by observations of Valenta et al. in 1996. Valenta et al. investigated protein extracts from different cells including PBMCs using immunoblotting. Atopic eczema patients showed reactivity towards proteins from several cell types including PBMCs (VALENTA et al. 1996). Seiberler et al. investigated protein extracts from different tissues and allergic effector cells and could show that auto-IgE target proteins are scattered even among tissues that are no target for atopic manifestation (SEIBERLER et al. 1999).

Along with some of our previously results (BECK 2012) Seiberler et al. showed auto-reactivity towards liver proteins, in agreement with our results of IgE auto-reactivity towards lung proteins, although Seiberler et al. utilized a different tissue source, as they used biopsies to extract the proteins (SEIBERLER et al. 1999). Target proteins of auto-IgE are not restricted to epithelial cells and seem to be distributed over several organs and cell types. Intracellular proteins that are potentially auto-antigens are released into the circulation or tissue environment due to tissue damage that is triggered by (chronic) inflammation or mechanical irritation like scratching (Figure 1).

The distribution of the target proteins leads to the immobilization on effector as well as inducer cells of atopy via specific bound IgE and IgE receptors (FcεRI, CD23) (SEIBERLER et al. 1999) (Figure 1). In addition one has to consider, that FcεRI bearing cells (e.g. basophils, eosinophils) are isolated within PBMC fraction. FcεRI is the high affinity receptor for IgE, as we extracted the whole protein extract FcεRI is potentially part of it. We cannot exclude that some IgE antibodies were bound to its high affinity receptor although this might only be few percentage of the measured reactivity due to the low numbers of these cells.

If auto-IgE is a natural auto-antibody with known properties, the characteristic of the polyspecificity and polyreactivity could explain the non-specificity of the verified IgE auto-antibodies to epithelial protein. NAbs are polyreactive but this does not mean lack of specificity. Each natural autoantibody includes its own distinct set of epitopic specificities and is therefore unique (LACROIX-DESMAZES et al. 1998; TRENYNCK and AVRAMEAS 1986). NAb show a broad range of affinity as known for the past decades (AVRAMEAS 1991; LACROIX-DESMAZES et al. 1998) and this might explain why different subjects show a different reactivity to auto-IgE.

As lifestyle and nutrition are indirect factors, we analyzed the serum *high density lipoprotein cholesterol* (HDL) and the presence of auto-IgE reactivity using a logistic regression analysis. The results from these analyses revealed in an inverse correlation between HDL and auto-IgE reactivity the higher the serum HDL, the lower the auto-IgE reactivity.

It is known that IgE plays a role in coronary heart diseases and there is evidence that high level of serum IgE is a prognostic factor for myocardial infarction and cardiac death in dyslipidemia and positive correlated with the severity of cardiovascular disease (KOVANEN et al. 1998; WANG et al. 2011). In contrast, subjects with low HDL are at greater risk to develop coronary heart disease (TAN 1980). In most of our categorized groups, the total IgE decreased with an increasing HDL as the auto-IgE did (data not shown).
Lower IgE levels imply a lower risk for CVD as a higher HDL does, and this observation is in agreement with the findings by Kovanen et al. Why these patients have a lower IgE auto-reactivity remains unknown. A more detailed and sophisticated analysis is necessary to elucidate a possible association. There is evidence that overweight is associated with asthma (PLATTS-MILLS et al. 2000), overweight is associated with less exercise and bad eating habits as the HDL does and most overweight or obese subjects have a very low circulating HDL levels. A new aspect on auto-reactivity research would investigate if life style and nutrition have an impact on auto-IgE reactivity.

The BiA study was conducted using study internal healthy controls and is therefore different to all other studies presented. For each of the other studies, we used control patients from the Department of Dermatology, Biederstein Hospital as well as study participants recruited at different locations or at different times (year/ month). However, we had no negative controls that were study-internal controls in other studies. The healthy control subjects used in the BiA study were patients that came to the Department of Dermatology at the Biederstein Hospital due to non-immunologic health issues such as epidermal nevus or sunburn.
The BiA study represents atopic eczema patients as the only group with highly reactive auto-IgE against all proteins from three different cell types, PBMCs, skin, and lung. IgE auto-reactivity in atopic eczema patients has been confirmed in several studies during the last decades (KORTEKANGAS-SAVOLAINEN et al. 2004; NATTER et al. 1998; SCHMID-GRENDELMEIER et al. 2005). In 1996 twenty atopic eczema patients were analyzed for their

autoreactivity by immunoblotting, whereat 70 % were tested as autoreactive against different human proteins (VALENTA et al. 1996). In 1999 eight atopic eczema patients were tested against their self-reactivity towards sub-cellular fragments and again proteins from different tissues in which all tested atopic eczema patients show autoreactivity (SEIBERLER et al. 1999).). To distinguish the repertoire of IgE-binding target antigens that are associated with atopic eczema, Zeller et al. analyzed 71 atopic eczema patients. A positive reaction against a variable pattern of IgE auto-reactivity towards one or more tested self-antigens was shown in 71.8 % (ZELLER et al. 2009). Our result suggests a considerable difference between the IgE self-reactivity in children and adults. In children, we could not find any association between auto-IgE reactivity and symptoms or manifestation of atopic eczema as compared to adults.

At a glance, in children we could not verify a difference of the occurrence and the height of reactivity of auto-IgE antibodies between healthy and diseased children, neither atopic nor autoimmune or any other disease.

Children having atopic eczema or allergic asthma do not show a higher incidence on the generation or the occurrence of auto-IgE than healthy ones. Rather we identified a significant protective effect of auto-IgE in preventing atopic eczema. However, with regard to a small sample size, the calculated ROC was statistically not significant.

We could demonstrate an association between atopic eczema and auto-IgE in adult patients as patients with atopic eczema is the group with the highest incidence of IgE self-reactivity in the BiA study. Likewise, we showed a significant association between auto-IgE reactivity and allergic asthma as these patients have a higher rate of auto-IgE reactivity towards lung and skin.

In a small group of adolescents, we showed a decrease in auto-IgE, however, the sample size was too small, and it seems intriguing to continue screening samples of 15-years old from the GINI/LISA cohort.

A second disease emerging from the BiA study was the Dermatomyositis, an idiopathic inflammatory ischemic myopathy. This disease is considered to result from a complement-mediated injury without knowing how this pathway is activated (KISSEL et al. 1986). Beside several auto-antibodies, the involvement of CD4 T cells (Th1, Th17), dendritic cells and mainly B cells is known. The role of auto-antibodies in the pathogenesis of muscle damage as well as the entire cause and onset of this disease remains unknown (ARAHATA and ENGEL 1984; LUO and MASTAGLIA). In consideration of this insufficient knowledge of

Dermatomyositis it is hard to predicate evidences about the auto-IgE occurrence. Moreover, Dermatomyositis can classified in different subgroups based on molecular analysis and categorization, which was not done in our survey (DALAKAS and HOHLFELD 2003; GREENBERG et al. 2002; LUO and MASTAGLIA).

A second small group of patients, suffering from SLE, showed higher auto-IgE reactivity. The detection of auto-reactive IgE antibodies was already confirmed by Charles et al. and Dema et al. as they show auto-reactive IgE antibodies in mice (CHARLES et al. 2010) and men (CHARLES et al. 2010; DEMA et al. 2014) towards dsDNA (CHARLES et al. 2010; DEMA et al. 2014) and several other auto-antigens (DEMA et al. 2014).
As both of these studies showed, (CHARLES et al. 2010; DEMA et al. 2014). gE is involved in circulating immune complexes. Systemic lupus erythematosus is characterized by hyperactive cells including B cells resulting in polyclonal B cell activation and increased numbers of plasma cells. Systemic lupus erythematosus subsequently is characterized by an increased production of autoantibodies and the presence of various immunoglobulins. The role of the different immunoglobulin isotypes in the onset and progression of SLE remains unclear (DEMA et al. 2014). Dema et al. showed a link between auto-IgE directed against seven auto-antigens and disease activity in 65 % of SLE patients. In patients with active disease, this was raised up to 83 % further suggesting that IgE may be a reasonable predictor of SLE (CHARLES et al. 2010; CHARLES and RIVERA 2011; DEMA et al. 2014).

Several IgM NABs are related to autoimmune diseases and SLE is associated with IgM NAbs towards Hep-2 although several other NAb do not show any clear disease association (WARDEMANN et al. 2003).
In contrast, Valenta et al. and Seiberler et al. showed any auto-IgE reactivity in SLE patients. They studied ten female SLE patients with a wide range of total IgE, but the reactivity might have been hampered by to the treatment with corticosteroids and immune suppressants (VALENTA et al. 1996). Seiberler et al. studied only two SLE patients without clinical symptoms. According to Dema et al. the incidence of auto-IgE rises with disease activity (DEMA et al. 2014), which might the restricting factor here.

The non-reactivity of healthy subjects (SEIBERLER et al. 1999; VALENTA et al. 1996) or patients with urticaria (VALENTA et al. 1996) and psoriasis (ZELLER et al. 2009) is similar to our results. One patient with Bullous pemphigoid showed auto-IgE reactivity towards proteins derived from PBMCs and lung, but not from skin, but the rest of Bullous

pemphigoid patients does not show any self-reactivity, in accordance with findings from Valenta et al (VALENTA et al. 1996).
Autoreactive patients out from the BiA study were recruited a second time to test self-reactivity by skin prick testing. A biopsy from healthy skin was used as a prick to prick test of autologous skin protein suspension. None of the patients showed a distinct and highly positive prick test. Certainly, most of the tested patients were not atopic eczema patients but suffered from different skin diseases. The few tested atopic eczema patients were in remission at the time of the prick test. For futures studies, we should concentrate on these particular atopic eczema patients.

Moreover we performed a basophile activation test, for each patient where we used the same protein solution to activate basophiles as we used for the immunoassay. However, we could not activate basophils in any of the patients. Concerning the prick test, we have to focus on atopic eczema patients as we expect a possible activation in these patients. In addition, the concentration of target protein in our protein suspension has to be enriched, as the concentration of the target proteins might have been too low.

In summary, we have shown that auto-reactive IgE is a naturally occurring antibody in children. Auto-IgE is associated with atopic eczema and the severity of asthma in adults suggesting a pathomechanism of auto-IgE in adults. If auto-IgE has a similar effect in children is still unclear, since auto-IgE is produced physiologically. Preliminary results in a small group of adolescents suggest a decline of auto-IgE starting at the age of thirteen years.

The two distinct properties of a self-reacting antibody in two different stages of life leads to speculations which individuals keep auto-IgE antibodies until adulthood and which will loose them or is this a recurrent phenomenon in adults?
Since self-reactivity is physiologic and needs to be distinguished from autoimmunity (WARDEMANN et al. 2003), it would be interesting for future studies to elucidate when the physiologic self-reactivity turns into pathologic IgE auto-reactivity.
Auto-reactivity decreases with each step of B cell development although is not eliminated (WARDEMANN et al. 2003) it might be mandatory to test whether auto-IgE antibodies are germline encoded.

Various NAbs are not related to particular disease as we know of to date and many NAbs might have a potential for diagnostic or predictive value (LESLIE et al. 2001).
Further future research should focus on naturally generated self-reactive antibodies in different diseases in human which are associated with high serum IgE, for example the Wiskott-Aldrich Syndrome (WAS).
The Wiskott-Aldrich Syndrome is characterized by immune deficiencies including lymphopenia with T and B cells depletion in the thymus and a impaired delayed type hypersensitivity but is strongly associated with high serum IgE levels. Patients with WAS have hardly any T or B cell but highly elevated IgE and IgA levels in serum from age six to eight years of age and eczema is a frequent diagnosed (OCHS 2001).

Another disease which warrants further analysis of serum auto-IgE reactivity, is Churg-Strauss Syndrome (CSS) also known as eosinophilic granulomatosis with polyanngiitis. Similar to WAS, CSS patients show elevated serum IgE levels as well as elevated numbers of eosinophils in blood and tissue, most commonly in the lung. In patients with CSS, asthma and vasculitis are concurrent symptoms (EUSTACE et al. 1999). The asthma is frequently severe and associated with eosinophilia typically seen in bronchial asthma and most of these patients have no family history of atopy (EUSTACE et al. 1999). Therefore, both diseases would be good candidates to investigate if IgE has an auto-reactive character

To further understand the mechanism of auto-IgE reactivity in adults and its association in atopic eczema and allergic asthma, an experimental mouse model of passive sensitization needs to be established. To further address the question if auto-IgE manifests or aggravates atopic eczema or asthma, The intention of this animal model is to passively sensitize mice by transferring syngeneic IgE specific to ovalbumin into animals that express membrane-bound (mOVA) or soluble ovalbumin (sOVA) as a model self-antigen under the control of the K14-promotor (SHIBAKI et al. 2004). Our intention was to accomplish OVA sensitization in C57BL/6 wild type mice, since mOVA and sOVA transgenic mice are based on the C57BL/6 background knowledge. C57BL/6 mice can be immunized against OVA but this mouse strain is considered to generate only small amounts of IgE (HERZ et al. 1998; ZHANG et al. 1997) as it has been shown in a former study (BECK 2012). As the IgE concentrations were not sufficient for passive immunization, we decided to isolate OVA-specific IgE from sera of BALB/c mice that were sensitized in our laboratory for a previous study. To reduce any reaction due to the two different mouse strains' background and the concurrent presence of OVA-specific IgG

antibodies, we extracted albumin and the entire IgG compound. A pilot study was performed but the analysis was not completed when this dissertation was written.

6. List of figures

7. LIST OF TABLES

8. REFERENCES

AABY, P., S. O. SHAHEEN, C. B. HEYES, A. GOUDIABY, A. J. HALL, A. W. SHIELL, H. JENSEN and A. MARCHANT (2000): Early BCG vaccination and reduction in atopy in Guinea-Bissau. In: Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology 30 (5): 644–650.

ABBAS, A. K., A. H. LICHTMAN and S. PILLAI): Cellular and Molecular Immunology. Internet: https://studentconsult.inkling.com/store/book/cellular-and-molecular-immunology-abbas-7th/ (23.03.2015).

ABRAHAMSSON, T. R., H. E. JAKOBSSON, A. F. ANDERSSON, B. BJÖRKSTÉN, L. ENGSTRAND and M. C. JENMALM (2012): Low diversity of the gut microbiota in infants with atopic eczema. In: The Journal of Allergy and Clinical Immunology 129 (2): 434–440, 440.e1–2.

ACKERMANN-SCHOPF, C., R. ACKERMANN, P. I. TERASAKI and J. LEVY (1974): Natural and Acquired Epidermal Autoantibodies in Man. In: The Journal of Immunology 112 (6): 2063–2067.

ADIB, M., J. RAGIMBEAU, S. AVRAMEAS and T. TERNYNCK (1990): IgG autoantibody activity in normal mouse serum is controlled by IgM. In: The Journal of Immunology 145 (11): 3807–3813.

AICHBERGER, K. J., I. MITTERMANN, R. REININGER, S. SEIBERLER, I. SWOBODA, S. SPITZAUER, T. KOPP, G. STINGL, W. R. SPERR, P. VALENT, A. REPA, B. BOHLE, D. KRAFT and R. VALENTA (2005): Hom S 4, an IgE-Reactive Autoantigen Belonging to a New Subfamily of Calcium-Binding Proteins, Can Induce Th Cell Type 1-Mediated Autoreactivity. In: The Journal of Immunology 175 (2): 1286–1294.

AKDIS, M., J. VERHAGEN, A. TAYLOR, F. KARAMLOO, C. KARAGIANNIDIS, R. CRAMERI, S. THUNBERG, G. DENIZ, R. VALENTA, H. FIEBIG, C. KEGEL, R. DISCH, C. B. SCHMIDT-WEBER, K. BLASER and C. A. AKDIS (2004): Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. In: The Journal of Experimental Medicine 199 (11): 1567–1575.

ALTRICHTER, S., E. KRIEHUBER, J. MOSER, R. VALENTA, T. KOPP and G. STINGL (2008): Serum IgE Autoantibodies Target Keratinocytes in Patients with Atopic Dermatitis. In: J Invest Dermatol 128 (9): 2232–2239.

APPENZELLER, U., C. MEYER, G. MENZ, K. BLASER and R. CRAMERI (1999): IgE-mediated reactions to autoantigens in allergic diseases. In: International Archives of Allergy and Immunology 118 (2-4): 193–196.

ARAHATA, K. and A. G. ENGEL (1984): Monoclonal antibody analysis of mononuclear cells in myopathies. I: Quantitation of subsets according to diagnosis and sites of accumulation and demonstration and counts of muscle fibers invaded by T cells. In: Annals of Neurology 16 (2): 193–208.

ARANBURU, A., S. CECCARELLI, E. GIORDA, R. LASORELLA, G. BALLATORE and R. CARSETTI (2010): TLR Ligation Triggers Somatic Hypermutation in Transitional B Cells Inducing the Generation of IgM Memory B Cells. In: The Journal of Immunology 185 (12): 7293–7301.

ARNOLD, D. H., T. GEBRETSADIK, T. J. ABRAMO, K. G. MOONS, J. R. SHELLER and T. V. HARTERT (2011): The RAD score: a simple acute asthma severity score compares favorably to more complex scores. In: Annals of Allergy, Asthma & Immunology: Official Publication of the American College of Allergy, Asthma, & Immunology 107 (1): 22–28.

ASHER, M. I., S. MONTEFORT, B. BJÖRKSTÉN, C. K. W. LAI, D. P. STRACHAN, S. K. WEILAND, H. WILLIAMS and ISAAC PHASE THREE STUDY GROUP (2006): Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. In: Lancet 368 (9537): 733–743.

AURIEMMA, M., G. VIANALE, P. AMERIO and M. REALE (2013): Cytokines and T cells in atopic dermatitis. In: European Cytokine Network 24 (1): 37–44.

AVRAMEAS, S. (1991): Natural autoantibodies: from 'horror autotoxicus' to 'gnothi seauton'. In: Immunology Today 12 (5): 154–159.

BACHARIER, L. B. and T. W. GUILBERT (2012): Diagnosis and management of early asthma in preschool-aged children. In: Journal of Allergy and Clinical Immunology 130 (2): 287–296.

BACH, J.-F. (2002): The effect of infections on susceptibility to autoimmune and allergic diseases. In: The New England Journal of Medicine 347 (12): 911–920.

BECK, H. (2012): IgE-autoreactivity towards intestinal, airway and skin epithelial cells in sera from atopic individuals.

BECK, L. A., D. THAÇI, J. D. HAMILTON, N. M. GRAHAM, T. BIEBER, R. ROCKLIN, J. E. MING, H. REN, R. KAO, E. SIMPSON, M. ARDELEANU, S. P. WEINSTEIN, G. PIROZZI, E. GUTTMAN-YASSKY, M. SUÁREZ-FARIÑAS, M. D. HAGER, N. STAHL, G. D. YANCOPOULOS and A. R. RADIN (2014): Dupilumab Treatment in Adults with Moderate-to-Severe Atopic Dermatitis. In: New England Journal of Medicine 371 (2): 130–139.

VON BERG, A., B. FILIPIAK-PITTROFF, U. KRÄMER, E. LINK, J. HEINRICH, S. KOLETZKO, A. GRÜBL, U. HOFFMANN, C. BECKMANN, D. REINHARDT, C. P. BAUER, H.-E. WICHMANN and D. BERDEL (2012): The German Infant Nutritional Intervention Study (GINI) for the preventive effect of hydrolysed infant formulas in infants at high risk for allergic diseases. Design and selected results. In: Allergologie 35 (1): 32–43.

VON BERG, A., S. KOLETZKO, A. GRÜBL, B. FILIPIAK-PITTROFF, H.-E. WICHMANN, C. P. BAUER, D. REINHARDT, D. BERDEL and GERMAN INFANT NUTRITIONAL INTERVENTION STUDY GROUP (2003): The effect of hydrolyzed cow's milk formula for allergy prevention in the first year of life: the German Infant Nutritional Intervention Study, a randomized double-blind trial. In: The Journal of Allergy and Clinical Immunology 111 (3): 533–540.

BERGER, COMPTON, MOLIMARD, WALLS, N'GUYEN, MARTHAN and TUNON-DE-LARA (1999): Mast cell tryptase as a mediator of hyperresponsiveness in human isolated bronchi. In: Clinical & Experimental Allergy 29 (6): 804–812.

BERGMANN, EDENHARTER, BERGMANN, FORSTER, BAUER, WAHN, ZEPP and WAHN (1998): Atopic dermatitis in early infancy predicts allergic airway disease at 5 years. In: Clinical & Experimental Allergy 28 (8): 965–970.

BHURE, U. N., S. U. BHURE, B. M. BHATT, S. MISTRY, S. J. PEDNEKAR, V. V. CHARI, S. A. DESAI, J. M. JOSHI and A. J. PAIDHUNGAT (2009): Lung epithelial permeability and inhaled furosemide: added dimensions in asthmatics. In: Annals of Nuclear Medicine 23 (6): 549–557.

BIEBER, T. (2010): Atopic dermatitis. In: Annals of Dermatology 22 (2): 125–137.

BISGAARD, H., N. LI, K. BONNELYKKE, B. L. K. CHAWES, T. SKOV, G. PALUDAN-MÜLLER, J. STOKHOLM, B. SMITH and K. A. KROGFELT (2011): Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age. In: The Journal of Allergy and Clinical Immunology 128 (3): 646–652.e1–5.

BLOMBERG, B. B. and D. FRASCA (2013): Age effects on mouse and human B cells. In: Immunologic Research 57 (1-3): 354–360.

DE BOER, W. I., H. S. SHARMA, S. M. I. BAELEMANS, H. C. HOOGSTEDEN, B. N. LAMBRECHT and G. J. BRAUNSTAHL (2008): Altered expression of epithelial junctional proteins in atopic asthma: possible role in inflammation. In: Canadian Journal of Physiology and Pharmacology 86 (3): 105–112.

BOYDEN, S. (1964): AUTOIMMUNITY AND INFLAMMATION. In: Nature 201: 200–201.

BOYD, S. D., Y. LIU, C. WANG, V. MARTIN and D. K. DUNN-WALTERS (2013): Human lymphocyte repertoires in ageing. In: Current Opinion in Immunology 25 (4): 511–515.

BÜNDER, R., I. MITTERMANN, U. HERZ, M. FOCKE, M. WEGMANN, R. VALENTA and H. RENZ (2004): Induction of autoallergy with an environmental allergen mimicking a self protein in a murine model of experimental allergic asthma. In: The Journal of Allergy and Clinical Immunology 114 (2): 422–428.

BUNDESÄRZTEKAMMER, KASSENÄRZTLICHE VEREINIGUNG und ARBEITSGEMEINSCHAFT DER WISSENSCHAFTLICHEN MEDIZINISCHEN FACHGESELLSCHAFTEN (2009): Nationale VersorgungsLeitlinie Asthma. Internet: http://www.leitlinien.de/nvl/asthma/mdb/downloads/nvl/asthma/asthma-2aufl-vers5-lang.pdf.

BURNET, F. M. (Frank M. (1959): The clonal selection theory of acquired immunity. Internet: http://archive.org/details/clonalselectiont00burn (23.10.2014).

BURR, M. L., J. C. EMBERLIN, R. TREU, S. CHENG, N. E. PEARCE and ISAAC PHASE ONE STUDY GROUP (2003): Pollen counts in relation to the prevalence of allergic rhinoconjunctivitis, asthma and atopic eczema in the International Study of Asthma and Allergies in Childhood (ISAAC). In: Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology 33 (12): 1675–1680.

CAPOLUNGHI, F., S. CASCIOLI, E. GIORDA, M. M. ROSADO, A. PLEBANI, C. AURITI, G. SEGANTI, R. ZUNTINI, S. FERRARI, M. CAGLIUSO, I. QUINTI and R. CARSETTI (2008): CpG Drives Human Transitional B Cells to Terminal Differentiation and Production of Natural Antibodies. In: The Journal of Immunology 180 (2): 800–808.

CARAMALHO, I., T. LOPES-CARVALHO, D. OSTLER, S. ZELENAY, M. HAURY and J. DEMENGEOT (2003): Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide. In: The Journal of Experimental Medicine 197 (4): 403–411.

CARLSSON, L., L. E. NYSTRÖM, I. SUNDKVIST, F. MARKEY and U. LINDBERG (1977): Actin polymerizability is influenced by profilin, a low molecular weight protein in non-muscle cells. In: Journal of Molecular Biology 115 (3): 465–483.

CASALI, P. and E. W. SCHETTINO (1996): Structure and function of natural antibodies. In: Current topics in microbiology and immunology 210: 167–179.

CHARLES, N., D. HARDWICK, E. DAUGAS, G. G. ILLEI and J. RIVERA (2010): BASOPHILS AND THE T HELPER 2 ENVIRONMENT CAN PROMOTE THE DEVELOPMENT OF LUPUS NEPHRITIS. In: Nature medicine 16 (6): 701–707.

CHARLES, N. and J. RIVERA (2011): Basophils and Autoreactive IgE in the Pathogenesis of Systemic Lupus Erythematosus. In: Current allergy and asthma reports 11 (5): 378–387.

COHEN, I. R. (1992): The cognitive principle challenges clonal selection. In: Immunology Today 13 (11): 441–444.

CONCHA, L. B., C. C. CHANG, A. M. SZEMA, R. J. DATTWYLER and H. E. CARLSON (2004): IgE Antithyroid Antibodies in Patients with Hashimoto's Disease and Chronic Urticaria. In: Allergy and Asthma Proceedings 25 (5): 293–296.

CRAMER, C., U. RANFT, J. RING, M. M&OUML;HRENSCHLAGER, H. BEHRENDT, H. OPPERMANN, M. WILHELM and U. KR&AUML;MER (2007): Allergic Sensitization and Disease in Mother-Child Pairs from Germany: Role of Early Childhood Environment. In: International Archives of Allergy and Immunology 143 (4): 282–289.

CRAMERI, R., A. FAITH, S. HEMMANN, R. JAUSSI, C. ISMAIL, G. MENZ and K. BLASER (1996): Humoral and cell-mediated autoimmunity in allergy to Aspergillus fumigatus. In: The Journal of Experimental Medicine 184 (1): 265–270.

DALAKAS, M. C. and R. HOHLFELD (2003): Polymyositis and dermatomyositis. In: The Lancet 362 (9388): 971–982.

DEMA, B., C. PELLEFIGUES, S. HASNI, N. GAULT, C. JIANG, T. K. RICKS, M. M. BONELLI, J. SCHEFFEL, K. SACRÉ, M. JABLONSKI, D. GOBERT, T. PAPO, E. DAUGAS, G. ILLEI, N. CHARLES and J. RIVERA (2014): Autoreactive IgE Is Prevalent in Systemic Lupus Erythematosus and Is Associated with Increased Disease Activity and Nephritis. In: PLoS ONE 9 (2). Internet: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3938730/ (05.12.2014).

DIAZ-SANCHEZ, D., A. TSIEN, A. CASILLAS, A. R. DOTSON and A. SAXON (1996): Enhanced nasal cytokine production in human beings after in vivo challenge with diesel exhaust particles. In: The Journal of Allergy and Clinical Immunology 98 (1): 114–123.

DIAZ-SANCHEZ, D., A. TSIEN, J. FLEMING and A. SAXON (1997): Combined diesel exhaust particulate and ragweed allergen challenge markedly enhances human in vivo nasal ragweed-specific IgE and skews cytokine production to a T helper cell 2-type pattern. In: Journal of Immunology (Baltimore, Md.: 1950) 158 (5): 2406–2413.

DIGHIERO, G., P. LYMBERI, B. GUILBERT, T. TERNYNCK and S. AVRAMEAS (1986): Natural Autoantibodies Constitute a Substantial Part of Normal Circulating Immunoglobulins. In: Annals of the New York Academy of Sciences 475 (1): 135–145.

DOGARU, C. M., D. NYFFENEGGER, A. M. PESCATORE, B. D. SPYCHER and C. E. KUEHNI (2014): Breastfeeding and Childhood Asthma: Systematic Review and Meta-Analysis. In: American Journal of Epidemiology 179 (10): 1153–1167.

DOLD, S., M. WJST, E. VON MUTIUS, P. REITMEIR and E. STIEPEL (1992): Genetic risk for asthma, allergic rhinitis, and atopic dermatitis. In: Archives of Disease in Childhood 67 (8): 1018–1022.

DOUWES, J., C. BROOKS, C. VAN DALEN and N. PEARCE (2011): Importance of allergy in asthma: an epidemiologic perspective. In: Current Allergy and Asthma Reports 11 (5): 434–444.

DOUWES, J., P. GIBSON, J. PEKKANEN and N. PEARCE (2002): Non-eosinophilic asthma: importance and possible mechanisms. In: Thorax 57 (7): 643–648.

ELLWOOD, P., M. I. ASHER, B. BJÖRKSTÉN, M. BURR, N. PEARCE and C. F. ROBERTSON (2001): Diet and asthma, allergic rhinoconjunctivitis and atopic eczema symptom prevalence: an ecological analysis of the International Study of Asthma and Allergies in Childhood (ISAAC) data. ISAAC Phase One Study Group. In: The European Respiratory Journal 17 (3): 436–443.

EMERSON, WILLIAMS and ALLEN (1998): Severity distribution of atopic dermatitis in the community and its relationship to secondary referral. In: British Journal of Dermatology 139 (1): 73–76.

EUSTACE, J. A., T. NADASDY and M. CHOI (1999): Disease of the month. The Churg Strauss Syndrome. In: Journal of the American Society of Nephrology: JASN 10 (9): 2048–2055.

FIEBIGER, E., F. HAMMERSCHMID, G. STINGL and D. MAURER (1998): Anti-FcepsilonRIalpha autoantibodies in autoimmune-mediated disorders. Identification of a structure-function relationship. In: Journal of Clinical Investigation 101 (1): 243–251.

FIEBIGER, E., D. MAURER, H. HOLUB, B. REININGER, G. HARTMANN, M. WOISETSCHLÄGER, J. P. KINET and G. STINGL (1995): Serum IgG autoantibodies directed against the alpha chain of Fc epsilon RI: a selective marker and pathogenetic factor for a distinct subset of chronic urticaria patients? In: Journal of Clinical Investigation 96: 2606–2612.

FILIPIAK-PITTROFF, B., C. SCHNOPP, D. BERDEL, A. NAUMANN, S. SEDLMEIER, A. ONKEN, E. RODRIGUEZ, R. FÖLSTER-HOLST, H. BAURECHT, M. OLLERT, J. RING, C. CRAMER, A. VON BERG, C. P. BAUER, O. HERBARTH, I. LEHMANN, B. SCHAAF, S. KOLETZKO, H.-E. WICHMANN, J. HEINRICH, S. WEIDINGER and GINIPLUS AND LISAPLUS STUDY GROUPS (2011): Predictive value of food sensitization and filaggrin mutations in children with eczema. In: Journal of Allergy and Clinical Immunology 128 (6): 1235–1241.e5.

FLOHR, C. (2010): What can we learn about eczema from the International Study of Asthma and Allergies in Childhood (ISAAC)? In: ALLERGOLOGIE 33 (6).

FLOHR, C., S. G. O. JOHANSSON, C.-F. WAHLGREN and H. WILLIAMS (2004): How atopic is atopic dermatitis? In: Journal of Allergy and Clinical Immunology 114 (1): 150–158.

FLOHR, C., L. N. TUYEN, S. LEWIS, R. QUINNELL, T. T. MINH, H. T. LIEM, J. CAMPBELL, D. PRITCHARD, T. T. HIEN, J. FARRAR, H. WILLIAMS and J. BRITTON (2006): Poor sanitation and helminth infection protect against skin sensitization in Vietnamese children: A cross-sectional study. In: The Journal of Allergy and Clinical Immunology 118 (6): 1305–1311.

FLÜCKIGER, S., P. R. E. MITTL, L. SCAPOZZA, H. FIJTEN, G. FOLKERS, M. G. GRÜTTER, K. BLASER and R. CRAMERI (2002): Comparison of the Crystal Structures of the Human Manganese Superoxide Dismutase and the Homologous Aspergillus fumigatus Allergen at 2-Å Resolution. In: The Journal of Immunology 168 (3): 1267–1272.

FOKKENS, W. J., T. GODTHELP, A. F. HOLM, H. BLOM, P. G. MULDER, T. M. VROOM and E. RIJNTJES (1992): Dynamics of mast cells in the nasal mucosa of patients with allergic rhinitis and non-allergic controls: a biopsy study. In: Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology 22 (7): 701–710.

FOULIS, A. K., M. MCGILL and M. A. FARQUHARSON (1991): Insulitis in type 1 (insulin-dependent) diabetes mellitus in man--macrophages, lymphocytes, and interferon-gamma containing cells. In: The Journal of Pathology 165 (2): 97–103.

FRANKLAND, A. W. (1959): The Place of Allergy in Asthma. In: Postgraduate Medical Journal 35 (406): 451–456.

GAUCHAT, J. F., D. A. LEBMAN, R. L. COFFMAN, H. GASCAN and J. E. DE VRIES (1990): Structure and expression of germline epsilon transcripts in human B cells induced by interleukin 4 to switch to IgE production. In: The Journal of Experimental Medicine 172 (2): 463–473.

GDALEVICH, M., D. MIMOUNI, M. DAVID and M. MIMOUNI (2001): Breast-feeding and the onset of atopic dermatitis in childhood: A systematic review and meta-analysis of prospective studies. In: Journal of the American Academy of Dermatology 45 (4): 520–527.

GIEHL, K., R. VALENTA, M. ROTHKEGEL, M. RONSIEK, H.-G. MANNHERZ and B. M. JOCKUSCH (1994): Interaction of Plant Profilin with Mammalian Actin. In: European Journal of Biochemistry 226 (2): 681–689.

GILLMAN, A. and J. A. DOUGLASS (2010): What do asthmatics have to fear from food and additive allergy? In: Clinical & Experimental Allergy 40 (9): 1295–1302.

GLASER, A. G., G. MENZ, A. I. KIRSCH, S. ZELLER, R. CRAMERI and C. RHYNER (2008): Auto- and cross-reactivity to thioredoxin allergens in allergic bronchopulmonary aspergillosis. In: Allergy 63 (12): 1617–1623.

GREENBERG, S. A., D. SANOUDOU, J. N. HASLETT, I. S. KOHANE, L. M. KUNKEL, A. H. BEGGS and A. A. AMATO (2002): Molecular profiles of inflammatory myopathies. In: Neurology 59 (8): 1170–1182.

GREGORY, L. G. and C. M. LLOYD (2011): Orchestrating house dust mite-associated allergy in the lung. In: Trends in Immunology 32 (9): 402–411.

GREWE, M., K. GYUFKO, E. SCHÖPF and J. KRUTMANN (1994): Lesional expression of interferon-gamma in atopic eczema. In: Lancet 343 (8888): 25–26.

GRINDEBACKE, H., K. WING, A.-C. ANDERSSON, E. SURI-PAYER, S. RAK and A. RUDIN (2004): Defective suppression of Th2 cytokines by CD4CD25 regulatory T cells in birch allergics during birch pollen season. In: Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology 34 (9): 1364–1372.

GRUBER, B. L., L. D. KAUFMAN, M. J. MARCHESE, W. ROTH and A. P. KAPLAN (1988): Anti-ige autoantibodies in systemic lupus erythematosus. In: Arthritis & Rheumatism 31 (8): 1000–1006.

GUILBERT, T. W., W. J. MORGAN, R. S. ZEIGER, L. B. BACHARIER, S. J. BOEHMER, M. KRAWIEC, G. LARSEN, R. F. LEMANSKE, A. LIU, D. T. MAUGER, C. SORKNESS, S. J. SZEFLER, R. C. STRUNK, L. M. TAUSSIG and F. D. MARTINEZ (2004): Atopic characteristics of children with recurrent wheezing at high risk for the development of childhood asthma. In: The Journal of Allergy and Clinical Immunology 114 (6): 1282–1287.

GUSTAVSSON, S., S. WERNERSSON and B. HEYMAN (2000): Restoration of the antibody response to IgE/antigen complexes in CD23-deficient mice by CD23+ spleen or bone marrow cells. In: Journal of Immunology (Baltimore, Md.: 1950) 164 (8): 3990–3995.

HAMID, Q., M. BOGUNIEWICZ and D. Y. LEUNG (1994): Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. In: The Journal of Clinical Investigation 94 (2): 870–876.

HAMID, Q., T. NASEER, E. M. MINSHALL, Y. L. SONG, M. BOGUNIEWICZ and D. Y. M. LEUNG (1996): In vivo expression of IL-12 and IL-13 in atopic dermatitis. In: Journal of Allergy and Clinical Immunology 98 (1): 225–231.

HAMILTON, J. D., M. SUÁREZ-FARIÑAS, N. DHINGRA, I. CARDINALE, X. LI, A. KOSTIC, J. E. MING, A. R. RADIN, J. G. KRUEGER, N. GRAHAM, G. D. YANCOPOULOS, G. PIROZZI and E. GUTTMAN-YASSKY (2014): Dupilumab improves the molecular signature in skin of patients with moderate-to-severe atopic dermatitis. In: Journal of Allergy and Clinical Immunology 134 (6): 1293–1300.

HAMPTON, S. F. and R. A. COOKE (1941): The sensitivity of man to human dander, with particular reference to eczema (allergic dermatitis). In: Journal of Allergy 13 (1): 63–76.

HANIFIN, J. M. and W. C. LOBITZ (1977): Newer concepts of atopic dermatitis. In: Archives of Dermatology 113 (5): 663–670.

HANIFIN, JON M. and RAJKA, GEORG (1980): Diagnostic features of atopic dermatitis. In: Acta dermato venerologica 92 (suppl.): 44–47.

HARINDRANATH, N., H. IKEMATSU, A. L. NOTKINS and P. CASALI (1993): Structure of the VH and VL segments of polyreactive and monoreactive human natural antibodies to HIV-1 and Escherichia coli β-galactosidase. In: International Immunology 5 (12): 1523–1533.

HAYCOCK, J. W. (1993): Polyvinylpyrrolidone as a Blocking Agent in Immunochemical Studies. In: Analytical Biochemistry 208 (2): 397–399.

HEINRICH, J., C. BRÜSKE, U. HOFFMANN, M. SCHNAPPINGER, B. SCHAAF, A. VON BERG, D. BERDEL, U. KRÄMER, I. LEHMANN, O. HERBARTH, M. BORTE, A. GRÜBL, C. P. BAUER, C. BECKMANN, BEHRENDT, HEIDRUN, J. RING and S. KOLETZKO (2012): GINIplus and LISAplus. Design and selected results of two German birth cohorts about natural course of atopic diseases and its determinants. In: Allergologie 35 (1): 20–31.

HENDERSON, J., K. NORTHSTONE, S. P. LEE, H. LIAO, Y. ZHAO, M. PEMBREY, S. MUKHOPADHYAY, G. D. SMITH, C. N. A. PALMER, W. H. I. MCLEAN and A. D. IRVINE (2008): The burden of disease associated with filaggrin mutations: a population-based, longitudinal birth cohort study. In: The Journal of Allergy and Clinical Immunology 121 (4): 872–877.e9.

HENSEL, F., R. HERMANN, S. BRÄNDLEIN, V. KRENN, B. SCHMAUSSER, S. GEIS, H. K. MÜLLER-HERMELINK and H. P. VOLLMERS (2001): Regulation of the New Coexpressed CD55 (Decay-Accelerating Factor)

Receptor on Stomach Carcinoma Cells Involved in Antibody SC-1–Induced Apoptosis. In: Laboratory Investigation 81 (11): 1553–1563.

HENSEL, F., R. HERMANN, C. SCHUBERT, N. ABÉ, K. SCHMIDT, A. FRANKE, A. SHEVCHENKO, M. MANN, H. K. MÜLLER-HERMELINK and H. P. VOLLMERS (1999): Characterization of Glycosylphosphatidylinositol-linked Molecule CD55/Decay-accelerating Factor as the Receptor for Antibody SC-1-induced Apoptosis. In: Cancer Research 59 (20): 5299–5306.

HERATIZADEH, A., I. MITTERMANN, H. BALAJI, K. WICHMANN, M. NIEBUHR, R. VALENTA and T. WERFEL (2011): The role of T-cell reactivity towards the autoantigen α-NAC in atopic dermatitis. In: British Journal of Dermatology 164 (2): 316–324.

HERZ, U., A. BRAUN, R. RÜCKERT and H. RENZ (1998): Various immunological phenotypes are associated with increased airway responsiveness. In: Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology 28 (5): 625–634.

HEYMAN, B., L. TIANMIN and S. GUSTAVSSON (1993): In vivo enhancement of the specific antibody response via the low-affinity receptor for IgE. In: European Journal of Immunology 23 (7): 1739–1742.

HIGASHI, N., Y. NIIMI, M. AOKI and S. KAWANA (2009): Clinical Features of Antinuclear Antibody-positive Patients with Atopic Dermatitis. In: Journal of Nippon Medical School 76 (6): 300–307.

HRADETZKY, S., L. M. ROESNER, H. BALAJI, A. HERATIZADEH, I. MITTERMANN, R. VALENTA and T. WERFEL (2014): Cytokine effects induced by the human autoallergen α-NAC. In: The Journal of Investigative Dermatology 134 (6): 1570–1578.

HUREZ, V., S.-V. KAVERI and M. D. KAZATCHKINE (1993): Expression and control of the natural autoreactive IgG repertoire in normal human serum. In: European Journal of Immunology 23 (4): 783–789.

ILLI, S., E. VON MUTIUS, S. LAU, R. NICKEL, C. GRÜBER, B. NIGGEMANN, U. WAHN and THE MULTICENTER ALLERGY STUDY GROUP (2004): The natural course of atopic dermatitis from birth to age 7 years and the association with asthma. In: Journal of Allergy and Clinical Immunology 113 (5): 925–931.

JOERINK, M., E. RINDSJÖ, F. STENIUS, J. ALM, G. LILJA, H. GRÖNLAND and A. SCHEYNIUS (2009): Evidence for allergen-specific IgE of maternal origin in human placenta. In: Allergy 64 (6): 905–912.

JOHANSSON, S. G. O., J. o'. HOURIHANE, J. BOUSQUET, C. BRUIJNZEEL-KOOMEN, S. DREBORG, T. HAAHTELA, M. L. KOWALSKI, N. MYGIND, J. RING, P. VAN CAUWENBERGE, M. VAN HAGE-HAMSTEN and B. WÜTHRICH (2001): A revised nomenclature for allergy: An EAACI position statement from the EAACI nomenclature task force. In: Allergy 56 (9): 813–824.

JUNG, K. H., S.-I. HSU, B. YAN, K. MOORS, S. N. CHILLRUD, J. ROSS, S. WANG, M. S. PERZANOWSKI, P. L. KINNEY, R. M. WHYATT, F. P. PERERA and R. L. MILLER (2012): Childhood exposure to fine particulate matter and black carbon and the development of new wheeze between ages 5 and 7 in an urban prospective cohort. In: Environment International 45: 44–50.

KALLMANN, B. A., M. HÜTHER, M. TUBES, J. FELDKAMP, J. BERTRAMS, F. A. GRIES, E. F. LAMPETER and H. KOLB (1997): Systemic Bias of Cytokine Production Toward Cell-Mediated Immune Regulation in IDDM and Toward Humoral Immunity in Graves' Disease. In: Diabetes 46 (2): 237–243.

KEARNEY, J. F. (2005): Innate-like B cells. In: Springer Seminars in Immunopathology 26 (4): 377–383.

KELLER, P. and A. MARCHIONINI (1926): Beitrag zu den Beziehungen von Asthma und Ekzem. In: Archiv für Dermatologie und Syphilis 150 (1): 41–46.

KHALAF, A.-N., L. KERP, B. SCHLIEBS, M. JÄGLE and K.-G. PETERSEN (1989): Natural occurrence of IgE antibodies to peptide hormones in man. In: European Journal of Clinical Investigation 19 (5): 464–465.

KINACIYAN, T., S. NATTER, D. KRAFT, G. STINGL and R. VALENTA (2002): IgE autoantibodies monitored in a patient with atopic dermatitis under cyclosporin A treatment reflect tissue damage. In: Journal of Allergy and Clinical Immunology 109 (4): 717–719.

KINET, J.-P. (1999): THE HIGH-AFFINITY IgE RECEPTOR (FcεRI): From Physiology to Pathology. In: Annual Review of Immunology 17 (1): 931–972.

KISSELGOF, A. B. and H. C. OETTGEN (1998): The expression of murine B cell CD23, in vivo, is regulated by its ligand, IgE. In: International Immunology 10 (9): 1377–1384.

KISSEL, J. T., J. R. MENDELL and K. W. RAMMOHAN (1986): Microvascular Deposition of Complement Membrane Attack Complex in Dermatomyositis. In: New England Journal of Medicine 314 (6): 329–334.

KOHLBOECK, G., S. SAUSENTHALER, M. STANDL, S. KOLETZKO, C.-P. BAUER, A. VON BERG, D. BERDEL, U. KRÄMER, B. SCHAAF, I. LEHMANN, O. HERBARTH, J. HEINRICH and GINI-PLUS AND LISA-PLUS STUDY GROUPS (2012): Food intake, diet quality and behavioral problems in children: results from the GINI-plus/LISA-plus studies. In: Annals of Nutrition & Metabolism 60 (4): 247–256.

KORTEKANGAS-SAVOLAINEN, O., S. PELTONEN, K. PUMMI, K. KALIMO and J. SAVOLAINEN (2004): IgE-binding components of cultured human keratinocytes in atopic eczema/dermatitis syndrome and their crossreactivity with Malassezia furfur. In: Allergy 59 (2): 168–173.

KOVANEN, P. T., M. MÄNTTÄRI, T. PALOSUO, V. MANNINEN and K. AHO (1998): Prediction of myocardial infarction in dyslipidemic men by elevated levels of immunoglobulin classes A, E, and G, but not M. In: Archives of Internal Medicine 158 (13): 1434–1439.

KRAMER MS, CHALMERS B, HODNETT ED and ET AL (2001): Promotion of breastfeeding intervention trial (probit): A randomized trial in the republic of belarus. In: JAMA 285 (4): 413–420.

KÜNZLI, N., L. PEREZ, F. LURMANN, A. HRICKO, B. PENFOLD and R. MCCONNELL (2008): An Attributable Risk Model for Exposures Assumed to Cause Both Chronic Disease and its Exacerbations: In: Epidemiology 19 (2): 179–185.

LACROIX-DESMAZES, S., S. V. KAVERI, L. MOUTHON, A. AYOUBA, E. MALANCHÈRE, A. COUTINHO and M. D. KAZATCHKINE (1998): Self-reactive antibodies (natural autoantibodies) in healthy individuals. In: Journal of Immunological Methods 216 (1–2): 117–137.

LAUBEREAU, B., I. BROCKOW, A. ZIRNGIBL, S. KOLETZKO, A. GRUEBL, A. VON BERG, B. FILIPIAK-PITTROFF, D. BERDEL, C. PETER BAUER, D. REINHARDT, J. HEINRICH, H.-E. WICHMANN and FOR THE GINI STUDY GROUP (2004): Effect of breast-feeding on the development of atopic dermatitis during the first 3 years of life—results from the GINI-birth cohort study. In: The Journal of Pediatrics 144 (5): 602–607.

LEEUWEN, P. D. W. S. van (1926): über die Bedeutung von Hautreaktionen für Diagnose und Therapie Allergischer Krankheiten. In: Klinische Wochenschrift 5 (33): 1525–1527.

LEEUWEN, W. S. van, Z. BIEN and H. VAREKAMP (1922): Zur Diagnose der Ueberempfindlichkeitskrankheiten. In: Münch. med. Wschr. 69: 1690.

LESIAK, A., P. SMOLEWSKI, D. SOBOLEWSKA-SZTYCHNY, A. SYSA-JEDRZEJOWSKA and J. NARBUTT (2012): The role of T-regulatory cells and Toll-like receptors 2 and 4 in atopic dermatitis. In: Scandinavian Journal of Immunology 76 (4): 405–410.

LESLIE, D., P. LIPSKY and A. L. NOTKINS (2001): Autoantibodies as predictors of disease. In: Journal of Clinical Investigation 108 (10): 1417–1422.

LEUNG, D. Y., A. K. BHAN, E. E. SCHNEEBERGER and R. S. GEHA (1983): Characterization of the mononuclear cell infiltrate in atopic dermatitis using monoclonal antibodies. In: The Journal of Allergy and Clinical Immunology 71 (1 Pt 1): 47–56.

LEUNG, D. Y. and T. BIEBER (2003): Atopic dermatitis. In: The Lancet 361 (9352): 151–160.

LEUNG, D. Y. M., M. BOGUNIEWICZ, M. D. HOWELL, I. NOMURA and Q. A. HAMID (2004): New insights into atopic dermatitis. In: Journal of Clinical Investigation 113 (5): 651–657.

LIMACHER, A., A. G. GLASER, C. MEIER, P. SCHMID-GRENDELMEIER, S. ZELLER, L. SCAPOZZA and R. CRAMERI (2007): Cross-Reactivity and 1.4-Å Crystal Structure of Malassezia sympodialis Thioredoxin (Mala s 13), a Member of a New Pan-Allergen Family. In: The Journal of Immunology 178 (1): 389–396.

LONGO, N. S., P. L. LUGAR, S. YAVUZ, W. ZHANG, P. H. L. KRIJGER, D. E. RUSS, D. D. JIMA, S. S. DAVE, A. C. GRAMMER and P. E. LIPSKY (2009): Analysis of somatic hypermutation in X-linked hyper-IgM syndrome shows specific deficiencies in mutational targeting. In: Blood 113 (16): 3706–3715.

LOWE, A. J., M. J. ABRAMSON, C. S. HOSKING, J. B. CARLIN, C. M. BENNETT, S. C. DHARMAGE and D. J. HILL (2007): The temporal sequence of allergic sensitization and onset of infantile eczema. In: Clinical & Experimental Allergy 37 (4): 536–542.

LUO, Y.-B. and F. L. MASTAGLIA: Dermatomyositis, polymyositis and immune-mediated necrotising myopathies. In: Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. Internet: http://www.sciencedirect.com/science/article/pii/S0925443914001628 (05.12.2014).

LUTZ, H. U. (2012): Naturally Occurring Autoantibodies in Mediating Clearance of Senescent Red Blood Cells. In: LUTZ, H. U. (Hrsg.) (2012): Naturally Occurring Antibodies (NAbs), Advances in Experimental Medicine and Biology. 76–90. Internet: http://link.springer.com/chapter/10.1007/978-1-4614-3461-0_6 (30.10.2014).

MADI, A., S. BRANSBURG-ZABARY, D. Y. KENETT, E. BEN-JACOB and I. R. COHEN (2012): The natural autoantibody repertoire in newborns and adults: a current overview. In: Advances in Experimental Medicine and Biology 750: 198–212.

MATRICARDI, P. M., A. BOCKELBRINK, T. KEIL, C. GRÜBER, B. NIGGEMANN, E. HAMELMANN, U. WAHN and S. LAU (2009): Dynamic evolution of serum immunoglobulin E to airborne allergens throughout childhood: results from the Multi-Centre Allergy Study birth cohort. In: Clinical & Experimental Allergy 39 (10): 1551–1557.

MATRICARDI, P. M., F. ROSMINI, L. FERRIGNO, R. NISINI, M. RAPICETTA, P. CHIONNE, T. STROFFOLINI, P. PASQUINI and R. D'AMELIO (1997): Cross sectional retrospective study of prevalence of atopy among Italian military students with antibodies against hepatitis A virus. In: BMJ (Clinical research ed.) 314 (7086): 999–1003.

MAYER, C., U. APPENZELLER, H. SEELBACH, G. ACHATZ, H. OBERKOFLER, M. BREITENBACH, K. BLASER and R. CRAMERI (1999): Humoral and Cell-mediated Autoimmune Reactions to Human Acidic Ribosomal P2 Protein in Individuals Sensitized to Aspergillus fumigatus P2 Protein. In: The Journal of Experimental Medicine 189 (9): 1507–1512.

MCCOY, K. D., N. L. HARRIS, P. DIENER, S. HATAK, B. ODERMATT, L. HANGARTNER, B. M. SENN, B. J. MARSLAND, M. B. GEUKING, H. HENGARTNER, A. J. S. MACPHERSON and R. M. ZINKERNAGEL (2006): Natural IgE Production in the Absence of MHC Class II Cognate Help. In: Immunity 24 (3): 329–339.

MITTERMANN, I., R. REININGER, M. ZIMMERMANN, K. GANGL, J. REISINGER, K. J. AICHBERGER, E. K. GREISENEGGER, V. NIEDERBERGER, J. SEIPELT, B. BOHLE, T. KOPP, C. A. AKDIS, S. SPITZAUER, P. VALENT and R. VALENTA (2007): The IgE-Reactive Autoantigen Hom s 2 Induces Damage of Respiratory Epithelial Cells and Keratinocytes via Induction of IFN-[gamma]. In: J Invest Dermatol 128 (6): 1451–1459.

MOSMANN, T. R. and R. L. COFFMAN (1989): TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. In: Annual Review of Immunology 7: 145–173.

MOSSABEB, R., S. SEIBERLER, I. MITTERMANN, R. REININGER, S. SPITZAUER, S. NATTER, P. VERDINO, W. KELLER, D. KRAFT and R. VALENTA (2002): Characterization of a novel isoform of alpha-nascent polypeptide-

associated complex as IgE-defined autoantigen. In: The Journal of Investigative Dermatology 119 (4): 820–829.

MOTHES, N., B. NIGGEMANN, C. JENNECK, T. HAGEMANN, S. WEIDINGER, T. BIEBER, R. VALENTA and N. NOVAK (2005): The cradle of IgE autoreactivity in atopic eczema lies in early infancy. In: Journal of Allergy and Clinical Immunology 116 (3): 706–709.

MOUTHON, L., M. HAURY, S. LACROIX-DESMAZES, C. BARREAU, A. COUTINHO and M. D. KAZATCHKINE (1995): Analysis of the normal human IgG antibody repertoire. Evidence that IgG autoantibodies of healthy adults recognize a limited and conserved set of protein antigens in homologous tissues. In: The Journal of Immunology 154 (11): 5769–5778.

VON MUTIUS, E., N. PEARCE, R. BEASLEY, S. CHENG, O. VON EHRENSTEIN, B. BJÖRKSTÉN and S. WEILAND (2000): International patterns of tuberculosis and the prevalence of symptoms of asthma, rhinitis, and eczema. In: Thorax 55 (6): 449–453.

NATTER, S., S. SEIBERLER, P. HUFNAGL, B. R. BINDER, A. M. HIRSCHL, J. RING, D. ABECK, T. SCHMIDT, P. VALENT and R. VALENTA (1998): Isolation of cDNA clones coding for IgE autoantigens with serum IgE from atopic dermatitis patients. In: The FASEB Journal 12 (14): 1559–1569.

NWARU, B. I., H.-M. TAKKINEN, O. NIEMELÄ, M. KAILA, M. ERKKOLA, S. AHONEN, A.-M. HAAPALA, M. G. KENWARD, J. PEKKANEN, R. LAHESMAA, J. KERE, O. SIMELL, R. VEIJOLA, J. ILONEN, H. HYÖTY, M. KNIP and S. M. VIRTANEN (2013): Timing of infant feeding in relation to childhood asthma and allergic diseases. In: Journal of Allergy and Clinical Immunology 131 (1): 78–86.

SART1 - U4/U6.U5 tri-snRNP-associated protein 1 - Homo sapiens (Human). Internet: http://www.uniprot.org/uniprot/O43290 (25.03.2015).

NACA - Nascent polypeptide-associated complex subunit alpha - Homo sapiens (Human). Internet: http://www.uniprot.org/uniprot/Q13765 (25.03.2015).

BCL7B - B-cell CLL/lymphoma 7 protein family member B - Homo sapiens (Human). Internet: http://www.uniprot.org/uniprot/Q9BQE9 (25.03.2015).

MICU1 - Calcium uptake protein 1, mitochondrial - Homo sapiens (Human). Internet: http://www.uniprot.org/uniprot/Q9BPX6 (25.03.2015).

KRT6A - Keratin, type II cytoskeletal 6A - Homo sapiens (Human). Internet: http://www.uniprot.org/uniprot/P02538 (25.03.2015).

RPLP2 - 60S acidic ribosomal protein P2 - Homo sapiens (Human). Internet: http://www.uniprot.org/uniprot/P05387 (25.03.2015).

SOD2 - Superoxide dismutase [Mn], mitochondrial precursor - Homo sapiens (Human). Internet: http://www.uniprot.org/uniprot/P04179 (25.03.2015).

PFN1 - Profilin-1 - Homo sapiens (Human). Internet: http://www.uniprot.org/uniprot/P07737

TXNRD3 - Thioredoxin reductase 3 - Homo sapiens (Human). Internet: http://www.uniprot.org/uniprot/Q86VQ6 (25.03.2015).

OBATA-NINOMIYA, K., K. ISHIWATA, H. TSUTSUI, Y. NEI, S. YOSHIKAWA, Y. KAWANO, Y. MINEGISHI, N. OHTA, N. WATANABE, H. KANUKA and H. KARASUYAMA (2013): The skin is an important bulwark of acquired immunity against intestinal helminths. In: The Journal of Experimental Medicine 210 (12): 2583–2595.

OCHS, H. D. (2001): The Wiskott-Aldrich syndrome. In: Clinical Reviews in Allergy & Immunology 20 (1): 61–86.

OETTGEN, H. C. and R. S. GEHA (1999): IgE in asthma and atopy: cellular and molecular connections. In: The Journal of Clinical Investigation 104 (7): 829–835.

OLOGE, F. E., S. O. ADEBOLA, A. D. DUNMADE, K. A. ADENIJI and B. A. OYEJOLA (2013): Symptom score for allergic rhinitis. In: Otolaryngology--Head and Neck Surgery: Official Journal of American Academy of Otolaryngology-Head and Neck Surgery 148 (4): 557–563.

VAN DEN OORD, R. A. H. M. and A. SHEIKH (2009): Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: systematic review and meta-analysis. In: BMJ 339 (jul08 4): b2433–b2433.

O'REGAN, G. M., A. SANDILANDS, W. H. I. MCLEAN and A. D. IRVINE (2008): Filaggrin in atopic dermatitis. In: Journal of Allergy and Clinical Immunology 122 (4): 689–693.

OU, L.-S., E. GOLEVA, C. HALL and D. Y. M. LEUNG (2004): T regulatory cells in atopic dermatitis and subversion of their activity by superantigens. In: The Journal of Allergy and Clinical Immunology 113 (4): 756–763.

PATERNOSTER, L., M. STANDL, C.-M. CHEN, A. RAMASAMY, K. BØNNELYKKE, L. DUIJTS, M. A. FERREIRA, A. C. ALVES, J. P. THYSSEN, E. ALBRECHT, H. BAURECHT, B. FEENSTRA, P. M. A. SLEIMAN, P. HYSI, N. M. WARRINGTON, I. CURJURIC, R. MYHRE, J. A. CURTIN, M. M. GROEN-BLOKHUIS, M. KERKHOF, A. SÄÄF, A. FRANKE, D. ELLINGHAUS, R. FÖLSTER-HOLST, E. DERMITZAKIS, S. B. MONTGOMERY, H. PROKISCH, K. HEIM, A.-L. HARTIKAINEN, A. POUTA, J. PEKKANEN, A. I. F. BLAKEMORE, J. L. BUXTON, M. KAAKINEN, D. L. DUFFY, P. A. MADDEN, A. C. HEATH, G. W. MONTGOMERY, P. J. THOMPSON, M. C. MATHESON, P. LE SOUËF, AUSTRALIAN ASTHMA GENETICS CONSORTIUM (AAGC), B. S. POURCAIN, G. D. SMITH, J. HENDERSON, J. P. KEMP, N. J. TIMPSON, P. DELOUKAS, S. M. RING, H.-E. WICHMANN, M. MÜLLER-NURASYID, N. NOVAK, N. KLOPP, E. RODRÍGUEZ, W. MCARDLE, A. LINNEBERG, T. MENNÉ, E. A. NOHR, A. HOFMAN, A. G. UITTERLINDEN, C. M. VAN DUIJN, F. RIVADENEIRA, J. C. DE JONGSTE, R. J. P. VAN DER VALK, M. WJST, R. JOGI, F. GELLER, H. A. BOYD, J. C. MURRAY, C. KIM, F. MENTCH, M. MARCH, M. MANGINO, T. D. SPECTOR, V. BATAILLE, C. E. PENNELL, P. G. HOLT, P. SLY, C. M. T. TIESLER, E. THIERING, T. ILLIG, M. IMBODEN, W. NYSTAD, A. SIMPSON, J.-J. HOTTENGA, D. POSTMA, G. H. KOPPELMAN, H. A. SMIT, C. SÖDERHÄLL, B. CHAWES, E. KREINER-MØLLER, H. BISGAARD, E. MELÉN, D. I. BOOMSMA, A. CUSTOVIC, B. JACOBSSON, N. M. PROBST-HENSCH, L. J. PALMER, D. GLASS, H. HAKONARSON, M. MELBYE, D. L. JARVIS, V. W. V. JADDOE, C. GIEGER, THE GENETICS OF OVERWEIGHT YOUNG ADULTS (GOYA) CONSORTIUM, D. P. STRACHAN, N. G. MARTIN, M.-R. JARVELIN, J. HEINRICH, D. M. EVANS and the Ea. G. & L. E. (EAGLE) CONSORTIUM (2012): Meta-analysis of genome-wide association studies identifies three new risk loci for atopic dermatitis. In: Nature Genetics 44 (2): 187–192.

PEREIRA, P., L. FORNI, E.-L. LARSSON, M. COOPER, C. HEUSSER and A. COUTINHO (1986): Autonomous activation of B and T cells in antigen-free mice. In: European Journal of Immunology 16 (6): 685–688.

PLATTS-MILLS, T. A., G. RAKES and P. W. HEYMANN (2000): The relevance of allergen exposure to the development of asthma in childhood. In: The Journal of Allergy and Clinical Immunology 105 (2 Pt 2): S503–508.

POHLE, T., S. BRÄNDLEIN, N. RUOFF, H. K. MÜLLER-HERMELINK and H. P. VOLLMERS (2004): Lipoptosis Tumor-Specific Cell Death by Antibody-Induced Intracellular Lipid Accumulation. In: Cancer Research 64 (11): 3900–3906.

DEL PRETE, G., E. MAGGI, P. PARRONCHI, I. CHRÉTIEN, A. TIRI, D. MACCHIA, M. RICCI, J. BANCHEREAU, J. DE VRIES and S. ROMAGNANI (1988): IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. In: Journal of Immunology (Baltimore, Md.: 1950) 140 (12): 4193–4198.

DEL PRETE, G. F., M. DE CARLI, M. M. D'ELIOS, P. MAESTRELLI, M. RICCI, L. FABBRI and S. ROMAGNANI (1993): Allergen exposure induces the activation of allergen-specific Th2 cells in the airway mucosa of patients with allergic respiratory disorders. In: European Journal of Immunology 23 (7): 1445–1449.

RAJE, N., C. A. VYHLIDAL, H. DAI and B. L. JONES (2014): Genetic variation within the histamine pathway among patients with asthma – a pilot study. In: Journal of Asthma: 1–10.

R DEVELOPMENT CORE TEAM (2009): R: A Language and Environment for Statistical Computing. In: R Foundation for Statistical Computing Vienna, Austria.

REICHSTEIN, E. and E. D. KORN (1979): Acanthamoeba profilin. A protein of low molecular weight from Acanpthamoeba castellanii that inhibits actin nucleation. In: Journal of Biological Chemistry 254 (13): 6174–6179.

RHODES, H. L., R. SPORIK, P. THOMAS, S. T. HOLGATE and J. J. COGSWELL (2001): Early life risk factors for adult asthma: A birth cohort study of subjects at risk. In: Journal of Allergy and Clinical Immunology 108 (5): 720–725.

RING, J., H. BEHRENDT, D. BERDEL, K.-C. BERGMANN, T. DIEPGEN, W. DORSCH, T. FUCHS, M. KABESCH, U. KRÄMER, W. LEUPOLD, R. MERGET, H. F. MERK, D. NOWAK, B. PRZYBILLA, W. REBIEN, D. REINHARDT, H. RENZ, R. RICHTER, C. BACHERT, F. RUEFF, J. SALOGA, G. SCHULTZE-WERNINGHAUS, J. C. SIMON, D. VIELUF, U. WAHN, A. WALLRAFEN, J. WENNING, T. WERFEL and T. ZUBERBIER: Allergie in Deutschland. Weißbuch, Band 2. München.

ROSENBERG, E. B., S. H. POLMAR and G. E. WHALEN (1971): Increased Circulating IgE in Trichinosis. In: Annals of Internal Medicine 75 (4): 575–578.

ROSENBERG, E. B., G. E. WHALEN, H. BENNICH and S. G. O. JOHANSSON (1970): Increased Circulating IgE in a New Parasitic Disease — Human Intestinal Capillariasis. In: New England Journal of Medicine 283 (21): 1148–1149.

SAITO, H. (2005): Much atopy about the skin: genome-wide molecular analysis of atopic eczema. In: International Archives of Allergy and Immunology 137 (4): 319–325.

SCHMID-GRENDELMEIER, P., S. FLÜCKIGER, R. DISCH, A. TRAUTMANN, B. WÜTHRICH, K. BLASER, A. SCHEYNIUS and R. CRAMERI (2005): IgE-mediated and T cell–mediated autoimmunity against manganese superoxide dismutase in atopic dermatitis. In: Journal of Allergy and Clinical Immunology 115 (5): 1068–1075.

SCHMITT, J., C.-M. CHEN, C. APFELBACHER, M. ROMANOS, I. LEHMANN, O. HERBARTH, B. SCHAAF, U. KRAEMER, A. VON BERG, H.-E. WICHMANN, J. HEINRICH and THE LISA-PLUS STUDY GROUP (2011): Infant eczema, infant sleeping problems, and mental health at 10 years of age: the prospective birth cohort study LISAplus. In: Allergy 66 (3): 404–411.

SCHOLZ, J. L., A. DIAZ, R. L. RILEY, M. P. CANCRO and D. FRASCA (2013): A comparative review of aging and B cell function in mice and humans. In: Current Opinion in Immunology 25 (4): 504–510.

SEIBERLER, S., A. BUGAJSKA-SCHRETTER, P. HUFNAGL, B. R. BINDER, J. ST&OUML;CKL, S. SPITZAUER, P. VALENT and R. VALENTA (1999): Characterization of IgE Reactive Autoantigens in Atopic Dermatitis. In: International Archives of Allergy and Immunology 120 (2): 108–116.

SHARMA, A., J. MENCHE, C. C. HUANG, T. ORT, X. ZHOU, M. KITSAK, N. SAHNI, D. THIBAULT, L. VOUNG, F. GUO, S. D. GHIASSIAN, N. GULBAHCE, F. BARIBAUD, J. TOCKER, R. DOBRIN, E. BARNATHAN, H. LIU, R. A. PANETTIERI, K. G. TANTISIRA, W. QIU, B. A. RABY, E. K. SILVERMAN, M. VIDAL, S. T. WEISS and A.-L. BARABÁSI (2015): A disease module in the interactome explains disease heterogeneity, drug response and captures novel pathways and genes in Asthma. In: Human Molecular Genetics: ddv001.

SHEN, C. H. and J. STAVNEZER (1998): Interaction of stat6 and NF-kappaB: direct association and synergistic activation of interleukin-4-induced transcription. In: Molecular and Cellular Biology 18 (6): 3395–3404.

SHIBAKI, A., A. SATO, J. C. VOGEL, F. MIYAGAWA and S. I. KATZ (2004): Induction of GVHD-like skin disease by passively transferred CD8(+) T-cell receptor transgenic T cells into keratin 14-ovalbumin transgenic mice. In: The Journal of Investigative Dermatology 123 (1): 109–115.

SILVERMAN, R. A. and K. ITO (2010): Age-related association of fine particles and ozone with severe acute asthma in New York City. In: Journal of Allergy and Clinical Immunology 125 (2): 367–373.e5.

SIMON, F. A. (1945): CUTANEOUS REACTIONS OF PERSONS WITH ATOPIC ECZEMA TO HUMAN DANDERRESULTS OF PATCH TESTS ON SCARIFIED SKIN. In: Arch Derm Syphilol 51 (6): 402–404.

SIMPSON, J. L., R. SCOTT, M. J. BOYLE and P. G. GIBSON (2006): Inflammatory subtypes in asthma: assessment and identification using induced sputum. In: Respirology (Carlton, Vic.) 11 (1): 54–61.

SJÖGREN, Y. M., M. C. JENMALM, M. F. BÖTTCHER, B. BJÖRKSTÉN and E. SVERREMARK-EKSTRÖM (2009): Altered early infant gut microbiota in children developing allergy up to 5 years of age. In: Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology 39 (4): 518–526.

SMITH, S. J., A. GALVIN, I. HALL and F. SHAKIB (1995): Circulating human IgG autoanti-IgE antibodies in asthma patients block the binding of IgE to its high affinity receptor. In: Clinical Molecular Pathology 48 (3): M148–M152.

SPERGEL, J. M. and A. S. PALLER (2003): Atopic dermatitis and the atopic march. In: The Journal of Allergy and Clinical Immunology 112 (6 Suppl): S118–127.

SPIRA-COHEN, A., L. C. CHEN, M. KENDALL, R. LALL and G. D. THURSTON (2011): Personal Exposures to Traffic-Related Air Pollution and Acute Respiratory Health among Bronx Schoolchildren with Asthma. In: Environmental Health Perspectives 119 (4): 559–565.

SPITZAUER, S., C. SCHWEIGER, W. R. SPERR, B. PANDJAITAN, P. VALENT, S. MÜHL, C. EBNER, O. SCHEINER, D. KRAFT and H. RUMPOLD (1994): Molecular characterization of dog albumin as a cross-reactive allergen. In: The Journal of Allergy and Clinical Immunology 93 (3): 614–627.

STONE, K. D., C. PRUSSIN and D. D. METCALFE (2010): IgE, mast cells, basophils, and eosinophils. In: Journal of Allergy and Clinical Immunology 125 (2, Supplement 2): S73–S80.

STRACHAN, D. P. (1989): Hay fever, hygiene, and household size. In: BMJ : British Medical Journal 299 (6710): 1259–1260.

STRACHAN, D. P., B. K. BUTLAND and H. R. ANDERSON (1996): Incidence and prognosis of asthma and wheezing illness from early childhood to age 33 in a national British cohort. In: BMJ : British Medical Journal 312 (7040): 1195–1199.

SZAKOS, E., G. LAKOS, M. ALEKSZA, E. GYIMESI, G. PÁLL, B. FODOR, J. HUNYADI, E. SÓLYOM and S. SIPKA (2004): Association between the occurrence of the anticardiolipin IgM and mite allergen-specific IgE antibodies in children with extrinsic type of atopic eczema/dermatitis syndrome. In: Allergy 59 (2): 164–167.

TANG, T. S., T. BIEBER and H. C. WILLIAMS (2012): Does “autoreactivity” play a role in atopic dermatitis? In: Journal of Allergy and Clinical Immunology 129 (5): 1209–1215.e2.

TAN, M. H. (1980): HDL-cholesterol: the negative risk factor for coronary heart disease. In: Annals of the Academy of Medicine, Singapore 9 (4): 491–495.

THOMAS, W. R., T. K. HEINRICH, W.-A. SMITH and B. J. HALES (2007): Pyroglyphid house dust mite allergens. In: Protein and Peptide Letters 14 (10): 943–953.

TRENYNCK, T. and S. AVRAMEAS (1986): Murine Natural Monoclonal Autoantibodies: A Study of their Polyspecificities and their Affinities. In: Immunological Reviews 94 (1): 99–112.

TURI, G. J., R. ELLIS, J. N. WATTIE, N. R. LABIRIS and M. D. INMAN (2011): The effects of inhaled house dust mite on airway barrier function and sensitivity to inhaled methacholine in mice. In: American Journal of Physiology. Lung Cellular and Molecular Physiology 300 (2): L185–190.

UNDERWOOD, J. R., J. S. PEDERSEN, P. J. CHALMERS and B. H. TOH (1985): Hybrids from normal, germ free, nude and neonatal mice produce monoclonal autoantibodies to eight different intracellular structures. In: Clinical and Experimental Immunology 60 (2): 417–426.

VALENTA, R., M. DUCHENE, C. EBNER, P. VALENT, C. SILLABER, P. DEVILLER, F. FERREIRA, M. TEJKL, H. EDELMANN and D. KRAFT (1992): Profilins constitute a novel family of functional plant pan-allergens. In: The Journal of Experimental Medicine 175 (2): 377–385.

VALENTA, R., M. DUCHÊNE, K. PETTENBURGER, C. SILLABER, P. VALENT, P. BETTELHEIM, M. BREITENBACH, H. RUMPOLD, D. KRAFT and O. SCHEINER (1991): Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. In: Science (New York, N.Y.) 253 (5019): 557–560.

VALENTA, R., D. MAURER, R. STEINER, S. SEIBERLER, W. R. SPERR, P. VALENT, S. SPITZAUER, S. KAPIOTIS, J. SMOLEN and G. STINGL (1996): Immunoglobulin E Response to Human Proteins in Atopic Patients. In: J Investig Dermatol 107 (2): 203–208.

VALENTA, R., I. MITTERMANN, T. WERFEL, H. GARN and H. RENZ (2009): Linking allergy to autoimmune disease. In: Trends in Immunology 30 (3): 109–116.

VALENTA, R., S. NATTER, S. SEIBERLER, S. WICHLAS, D. MAURER, M. HESS, M. PAVELKA, M. GROTE, F. FERREIRA, Z. SZEPFALUSI, P. VALENT and G. STINGL (1998): Molecular Characterization of an Autoallergen, Hom s 1, Identified by Serum IgE from Atopic Dermatitis Patients1. In: Journal of Investigative Dermatology 111 (6): 1178–1183.

VALENTA, R., S. SEIBERLER, S. NATTER, V. MAHLER, R. MOSSABEB, J. RING and G. STINGL (2000): Autoallergy: A pathogenetic factor in atopic dermatitis? In: Journal of Allergy and Clinical Immunology 105 (3): 432–437.

VAN DER VEEN, R. C., J. A. KAPP and J. L. TROTTER (1993): Fine-specificity differences in the recognition of an encephalitogenic peptide by T helper 1 and 2 cells. In: Journal of Neuroimmunology 48 (2): 221–226.

VENKATARAMAN, D., N. SOTO-RAMÍREZ, R. J. KURUKULAARATCHY, J. W. HOLLOWAY, W. KARMAUS, S. L. EWART, S. H. ARSHAD and M. ERLEWYN-LAJEUNESSE (2014): Filaggrin loss-of-function mutations are associated with food allergy in childhood and adolescence. In: The Journal of Allergy and Clinical Immunology 134 (4): 876–882.e4.

VOLLMERS, H. P., R. O'CONNOR, J. MÜLLER, T. KIRCHNER and H. K. MÜLLER-HERMELINK (1989): SC-1, a Functional Human Monoclonal Antibody against Autologous Stomach Carcinoma Cells. In: Cancer Research 49 (9): 2471–2476.

VOSKUHL, R. R., R. MARTIN, C. BERGMAN, M. DALAL, N. H. RUDDLE and H. F. MCFARLAND (1993): T Helper 1 (TH1) Functional Phenotype of Human Myelin Basic Protein-Specific T Lymphocytes. In: Autoimmunity 15 (2): 137–143.

WANG, J., X. CHENG, M.-X. XIANG, M. ALANNE-KINNUNEN, J.-A. WANG, H. CHEN, A. HE, X. SUN, Y. LIN, T.-T. TANG, X. TU, S. SJÖBERG, G. K. SUKHOVA, Y.-H. LIAO, D. H. CONRAD, L. YU, T. KAWAKAMI, P. T. KOVANEN, P. LIBBY and G.-P. SHI (2011): IgE stimulates human and mouse arterial cell apoptosis and cytokine expression and promotes atherogenesis in Apoe–/– mice. In: The Journal of Clinical Investigation 121 (9): 3564–3577.

WAN, H., H. L. WINTON, C. SOELLER, E. R. TOVEY, D. C. GRUENERT, P. J. THOMPSON, G. A. STEWART, G. W. TAYLOR, D. R. GARROD, M. B. CANNELL and C. ROBINSON (1999): Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. In: The Journal of Clinical Investigation 104 (1): 123–133.

WARDEMANN, H., S. YURASOV, A. SCHAEFER, J. W. YOUNG, E. MEFFRE and M. C. NUSSENZWEIG (2003): Predominant Autoantibody Production by Early Human B Cell Precursors. In: Science 301 (5638): 1374–1377.

WEILAND, S., A. HUSING, D. STRACHAN, P. RZEHAK and N. PEARCE (2004): Climate and the prevalence of symptoms of asthma, allergic rhinitis, and atopic eczema in children. In: Occupational and Environmental Medicine 61 (7): 609–615.

WEILAND, S. K., E. VON MUTIUS, A. HÜSING and M. I. ASHER (1999): Intake of trans fatty acids and prevalence of childhood asthma and allergies in Europe. ISAAC Steering Committee. In: Lancet 353 (9169): 2040–2041.

WHEATLEY, A. P., D. J. BOLLAND, J. E. HEWITT, J. C. DEWAR and I. P. HALL (2002): Identification of the autoantigen SART-1 as a candidate gene for the development of atopy. In: Human Molecular Genetics 11 (18): 2143 –2146.

WHITFIELD, A. (1921): Lumleian Lectures ON SOME POINTS IN THE ÆTIOLOGY OF SKIN DISEASES. In: The Lancet 198 (5107): 122–127.

WILLEMZE, R., C. B. DE GRAAFF-REITSMA, J. CNOSSEN, W. A. VAN VLOTEN and C. J. MEIJER (1983): Characterization of T-cell subpopulations in skin and peripheral blood of patients with cutaneous T-cell lymphomas and benign inflammatory dermatoses. In: The Journal of Investigative Dermatology 80 (1): 60–66.

WILLIAMS, H. and C. FLOHR (2006): How epidemiology has challenged 3 prevailing concepts about atopic dermatitis. In: Journal of Allergy and Clinical Immunology 118 (1): 209–213.

WILLIAMS, H., A. STEWART, E. VON MUTIUS, W. COOKSON and H. R. ANDERSON (2008): Is eczema really on the increase worldwide? In: Journal of Allergy and Clinical Immunology 121 (4): 947–954.e15.

WÜTHRICH, B. (1989): Epidemiology of the allergic diseases: are they really on the increase? In: International Archives of Allergy and Applied Immunology 90 Suppl 1: 3–10.

WÜTHRICH, B. (1999): Clinical aspects, epidemiology, and prognosis of atopic dermatitis. In: Annals of Allergy, Asthma & Immunology 83 (5): 464–470.

XIAO, C., S. M. PUDDICOMBE, S. FIELD, J. HAYWOOD, V. BROUGHTON-HEAD, I. PUXEDDU, H. M. HAITCHI, E. VERNON-WILSON, D. SAMMUT, N. BEDKE, C. CREMIN, J. SONES, R. DJUKANOVIĆ, P. H. HOWARTH, J. E. COLLINS, S. T. HOLGATE, P. MONK and D. E. DAVIES (2011): Defective epithelial barrier function in asthma. In: Journal of Allergy and Clinical Immunology 128 (3): 549–556.e12.

YAMAGUCHI, M., C. S. LANTZ, H. C. OETTGEN, I. M. KATONA, T. FLEMING, I. MIYAJIMA, J. P. KINET and S. J. GALLI (1997): IgE enhances mouse mast cell Fc(epsilon)RI expression in vitro and in vivo: evidence for a novel amplification mechanism in IgE-dependent reactions. In: The Journal of Experimental Medicine 185 (4): 663–672.

ZELLER, S., A. G. GLASER, M. VILHELMSSON, C. RHYNER and R. CRAMERI (2008): Immunoglobulin-E-mediated reactivity to self antigens: a controversial issue. In: International Archives of Allergy and Immunology 145 (2): 87–93.

ZELLER, S., C. RHYNER, N. MEYER, P. SCHMID-GRENDELMEIER, C. A. AKDIS and R. CRAMERI (2009): Exploring the repertoire of IgE-binding self-antigens associated with atopic eczema. In: The Journal of Allergy and Clinical Immunology 124 (2): 278–285, 285.e1–7.

ZHANG, Y., W. J. LAMM, R. K. ALBERT, E. Y. CHI, W. R. HENDERSON and D. B. LEWIS (1997): Influence of the route of allergen administration and genetic background on the murine allergic pulmonary response. In: American Journal of Respiratory and Critical Care Medicine 155 (2): 661–669.

ZHOU, Z.-H., Y. ZHANG, Y.-F. HU, L. M. WAHL, J. O. CISAR and A. L. NOTKINS (2007): The Broad Antibacterial Activity of the Natural Antibody Repertoire Is Due to Polyreactive Antibodies. In: Cell Host & Microbe 1 (1): 51–61.

9. PUBLICATIONS

Tanja Seher M.Sc., Elisabeth Thiering PhD, Majdah Al Azemi MD, PhD, Joachim Heinrich PhD , Carsten B. Schmidt-Weber PhD , Coleen Kivlahan MD, Jan Gutermuth MD, PhD and Human M. Fatemi MD, PhD
Is parental consanguinity associated with reduced ovarian reserve?
RBM online 2015 in press

10. DECLARATION

With this I declare that the presented work ,title **"Analysis of IgE-mediated autoreactivity in atopic diseases in children and adults"** was written by myself and that I did not use other resources than those indicated. I did not undertake an unsuccessful graduation attempt and this thesis was not submitted to another institution before.

11. ACKNOWLEDGEMENT

Ich bedanke mich bei allen Kollegen und Freunden, sowie meiner Familie für all die Hilfe und Unterstützung, die ich die letzten Jahre erfahren habe und durch die diese Arbeit ermöglicht wurde.

Für die Möglichkeit meine Doktorarbeit am ZAUM bzw. am Institut für Allergieforschung anfertigen zu können danke ich Prof. Carsten Schmidt-Weber aufrichtig.

Bei Prof. Jan Gutermuth möchte ich mich ganz besonders für die Unterstützung, für all die geführten und hilfreichen Diskussionen, die Motivation und den Enthusiasmus der letzten Jahre bedanken.

Dr. Stefan Haak möchte ich dafür danken, dass er das Projekt mit übernommen hat und mit vielen Ideen und großer Unterstützung vorangetrieben hat.
Bei Dr. Olaf Groß möchte ich mich dafür bedanken, dass er am Ende der Dissertation dem Thesis Committee noch beigetreten ist.
Prof. Susanne Krauss-Etschmann möchte ich für die Zeit im Thesis Committee danken.

Für die große Hilfe bei der Epidemiologischen Auswertung der Daten der Geburtskohorten und für die Möglichkeit diese Proben untersuchen zu können, möchte ich herzlich bei Dr. Elisabeth Thiering und Dr. Joachim Heinrich bedanken.

Weiteres Probenmaterial wurden mir von Prof. Claudia Traidl-Hoffmann, Prof. Knut Brockow, Prof. Markus Ollert, und Prof. Buhl zur Verfügung gestellt. Frau Erika Arnold, Dr. Teresa Jäger haben mich bei der Probengewinnnung für die BiA-Studie unterstützt, bei allen möchte ich mich dafür herzlich bedanken.

Großer Dank gilt den Technischen Assistenten und Laboranten unseres Instituts.
An Alexandra Seisenberger geht ein riesiges und herzliches Dankeschön für die Zusammenarbeit beim auto-IgE Screening und ihrer Perfektion und Kompetenz im Labor.
Johanna Grosch, Benjamin Schnautz und Katja Haslauer danke ich zutiefst für die gute Zusammenarbeit und deren unendliche Unterstützung im Labor während der gesamten letzten Jahre.

Bei Dr. Maria Wimmer, Dr. Francesca Allessandrini und Dr. Viviana Marzaioli möchte ich mich für die vielen Diskussionen und Ideen bedanken, die zum Erfolg des Prohektes beigetragen haben.

Ein besonders großer Dank geht an meine Familie. Bei meinem Freund Martin möchte ich mich für all seine große Unterstützung während der letzten Jahre bedanken.
An Elli geht ein großer Dank u.a. wegen der Betreuung unseres Sohnes Emil, diese hat mir immer einige Stunden kinderfreie und dafür schreib- und lesereiche Zeit geschenkt, wodurch die Arbeit letztendlich überhaupt fertig geworden ist.