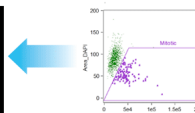
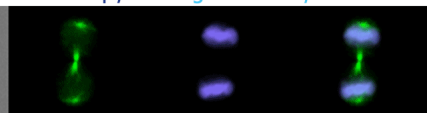
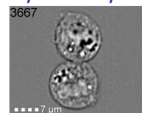




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Unlike in Children with Allergic Asthma, IgE Transcripts from Preschool Children with Atopic Dermatitis Display Signs of Superantigen-Driven Activation

Sebastian Kerzel,^{*,†} Tobias Rogosch,[†] Benjamin Struecker,[†] Rolf F. Maier,[†] Michael Kabesch,^{*} and Michael Zemlin[†]

The IgE repertoire in children with asthma reflects an adaptive B cell response, indicative of Ag-driven selection. However, the same might not apply to atopic dermatitis, which is often the first manifestation of atopy. The objective of our present study was to characterize the IgE repertoire of preschool children with atopic dermatitis with regard to signs of superantigen-like activation, clonal relationship, and indications of Ag selection. Total RNA was isolated from PBMCs of five children with atopic dermatitis. IgE transcripts were amplified, cloned, and sequenced using RT-PCR. We obtained 200 functional IgE sequences, which were compared with 1140 sequences from 11 children with asthma. Whereas variable gene segment of the H Ig chain (V_H) gene usage in asthma reflected germline distribution, IgE transcripts from children with atopic dermatitis displayed a dominance of the otherwise scarcely expressed V_H2 and V_H4 family. Whereas IgE transcripts from children with asthma were highly mutated (7.2%), somatic mutation rate in atopic dermatitis was less than half as high (3.4%). Moreover, the proportion of transcripts that were indicative of Ag selection was reduced to 11% in atopic dermatitis (24% in asthma). In summary, IgE repertoires vary significantly between children with different atopic diseases. Compared with children with asthma, IgE transcripts from preschool children with atopic dermatitis are significantly less mutated, clonally less focused, and less indicative of Ag selection. We consider our data reconcilable with the hypothesis that a superantigen-like activation contributes to the maturation and selection of the IgE repertoire in atopic dermatitis. *The Journal of Immunology*, 2016, 196: 000–000.

Immunoglobulin E is a central effector molecule in allergy. Whether the IgE response in allergic diseases represents 1) a classical B cell response with affinity maturation, 2) a superantigen-like activation, or 3) a polyclonal B-1 cell expansion is still under debate (1). We have shown that IgE transcripts in PBMCs from children with allergic asthma reflect a classical, adaptive B-2 cell response that is indicative of Ag-driven selection (2).

However, the same might not apply to other allergic disease entities, such as atopic dermatitis (eczema). Atopic dermatitis affects up to 30% of children in industrialized countries and often represents the first manifestation of atopy (3). In 81% of the patients with atopic eczema, the skin is colonized with *Staphylococ-*

cus aureus (4). It was hypothesized that *S. aureus* contributes to the pathogenesis in atopic dermatitis, not only by enzymatically increasing skin permeability but also by releasing proinflammatory mediators. One factor of *S. aureus*-driven inflammation might be the superantigenic capacity of enterotoxins that stimulate the cellular immune response in an Ag-independent fashion (5). Besides modulation of T cell differentiation (6), the superantigen-driven inflammation in atopic dermatitis could also influence the B cell response. Thus, unlike in asthma, the IgE repertoire in atopic dermatitis might reflect patterns of superantigen-like activation. Indications for such a superantigen-driven modification of the IgE repertoire could be a biased usage of variable gene segment of the H Ig chain (V_H) genes, a low level of somatic hypermutation, and a reduced clonal restriction of the IgE transcripts.

Although some of these aspects have already been addressed in studies with adult patients [(7–9), reviewed in Refs. 1 and 10], hitherto the molecular characteristics of the IgE response have not been analyzed systematically in children with atopic dermatitis, even though this specific age group is of particular interest for the evolution of the IgE response in atopic dermatitis. The natural history of atopic eczema is currently understood as a multistage process (3), with an initial nonatopic phase of dermatitis during infancy, resulting from genetically determined dysfunction of the epidermal barrier. Together with a skewed Th1/Th2 balance, this initial dermatitis lays the groundwork for allergic sensitization and concomitant IgE production. Infancy and early childhood represent the time frame during which the IgE response is principally coined and potentially modulated by immunologically active mediators, such as bacterial superantigens. Therefore, the objective of our study was to characterize the IgE repertoire of preschool children with atopic dermatitis with regard to somatic hypermutation, clonal relationship, and traces of superantigen-like activation.

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The sequences presented in this article have been submitted to GenBank (<http://www.ncbi.nlm.nih.gov/genbank>) under accession numbers HM116098–HM116226 and KM209339–KM209361.

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The online version of this article contains supplemental material.

Abbreviations used in this article: FWR, framework region; J_H, joining gene segment of the H Ig chain; V_H, variable gene segment of the H Ig chain.

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Materials and Methods

Patients

In this analysis, we included five preschool children with atopic dermatitis who were recruited from the outpatient clinic of the University Children's Hospital Marburg. Atopic dermatitis was diagnosed according to standardized criteria (11). Transcripts from these patients were compared with transcripts from 11 previously published patients with allergic asthma (2). Asthmatic children with active atopic dermatitis as secondary diagnosis were excluded from the analysis. This exclusion of patients with double diagnosis explains the minor differences in the asthma group, compared with our previous publication (2). Detailed patient characteristics are summarized in Table I. After informed consent, 1.2 ml blood was collected subsequent to a routinely performed blood withdrawal. No additional punctures were performed. The study was approved by the Ethics Committee of the Philipps-University Marburg.

Isolation of total RNA and RT-PCR

After lysis of erythrocytes, leukocytes were recovered by density centrifugation. Total RNA was isolated using the QIAamp RNA Blood Mini-Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. RNA was obtained by reverse transcription into cDNA according to standard protocols with SuperScript III (Invitrogen, Karlsruhe, Germany).

Amplification of IgE transcripts by seminested PCR

Amplification of human IgE transcripts was performed as previously described (2). In brief, a combination of primers for each V_H gene family (IGHV1-IGHV7) was used together with an antisense primer specific for the first exon of the ϵ C region (Ce reverse primer 1). In a second step, PCR products were reamplified using the same V_H -specific forward primers together with an inner antisense primer specific for the first exon of the ϵ C region (Ce reverse primer 2). PCR amplifications were carried out in a total volume of 25 μ l containing 1.5 mM $MgCl_2$, 0.2 mM of each forward and reverse primer, and 1 μ l RNA eluate or primary amplificate, using the standard Platinum *Taq* polymerase (Invitrogen). The following program was performed on a thermocycler (SensoQuest, Göttingen, Germany): 5 min at 94°C; 30 cycles with 30 s at 94°C, 50 s at 60°C, and 90 s at 72°C; 10 min at 72°C. GAPDH gene products were amplified as positive control. For primer sequences, see Kerzel et al. (2) and Rogosch et al. (12). PCR products were purified by gel electrophoresis. DNA with the corresponding length (400 bp) was extracted using the QIAquick gel extraction kit (Qiagen).

Quantitative IgM PCR to control for different amplification efficiency of V_H primers

Using the identical forward primers (IGHV1-IGHV7) as in our study experiments together with an IgM-specific antisense primer (5'-ACG GGG

AAT TCT CAC AGG AGA C-3') (13) and the identical PCR conditions, we amplified IgM-specific cDNA in a quantitative PCR on an iQ5 Real Time PCR Detector System (Bio-Rad, Munich, Germany) using SYBR Green (SsoAdvanced SYBR Green Supermix; Bio-Rad) to detect dsDNA.

Cloning of PCR products

PCR products were cloned into *Escherichia coli* using standard protocols according to the manufacturer's instructions (TOPO-TA cloning kit; Invitrogen).

Sequence analysis

Randomly selected cloned PCR products were sequenced by GATC biotech (Konstanz, Germany) with a capillary sequencer (Applied Biosystems, Darmstadt, Germany). Gene segments were aligned to germline sequence using the ImMunoGeneTics database with the program HighV-QUEST (14). A minimum of six nonmutated nucleotides with at least two nonmutated nucleotides at each end was required to identify a diversity (D) gene (15). We defined the CDR-H3 as those residues between the conserved cysteine (C104) of framework region (FWR)-H3 and the conserved tryptophan (W118) of FWR-H4. To assess Ag selection, we used the algorithms of Lossos et al. (16) and Chang and Casali (17) as previously described by Dahlke et al. (18) and by our own group (2). We calculated the selection strength for CDR and for FWR using the BASELINE software tool (19, 20). The software tool is provided online by Yale University (<http://selection.med.yale.edu/baseline/>). Sequences with <2% mutation rate were excluded from the analysis of Ag-driven selection strength. Sequence analyses were performed using the Ig analysis tool (IgAT) (21). For consideration as clonally related, clones must: 1) use the same V_H gene, 2) have a highly homologous CDR-H3 (i.e., <10% difference in nucleotide sequence), and 3) have an identical length of CDR-H3.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 5.0 (GraphPad, La Jolla, CA) and SPSS (Chicago, IL). Normality distribution was assessed with a Kolmogorov-Smirnov test. Differences between populations were determined by unpaired two-tailed Student *t* test for normally distributed data or Mann-Whitney *U* test for nonnormally distributed data, respectively. For categorical data, a χ^2 test with post hoc analysis was applied as described by Collis et al. (22). In column graphs, means are given with SEM; in scattergrams, the bars represent the median ($\pm$ interquartile range). Correlation between two parameters was assessed using Pearson product-moment correlation coefficients.

Table I. Patient characteristics

Patient	Age (y)	Sex	Total IgE (kU/l)	Allergic Sensitization	Functional Sequences	Unique Sequences	Clonotypes
Atopic dermatitis							
AD-1	1	M	750	HE, PN, HN, CM, soy, DD, CD, CowD	45	36	27
AD-2	2	M	7902	Ce, HDM, CD, DD, HE, CM, cod, WF, PN, soy, HN, Car, Ap, GP, Bi, Hz, MW	33	32	17
AD-3	4	M	2883	Hz, HN, DD, CD, GP, RP, Ap, Car, Se	37	32	28
AD-4	1	F	19,100	CM, WF, Hz, HN, GP, RP	40	33	30
AD-5	2	M	433	HDM, PN, HD, CM, HE, HN	45	23	9
					200	156	111
Allergic asthma							
AA-1	5	F	311	HDM, GP, RP, Be, DD	161	61	6
AA-2	14	F	375	Bi, Al, Hz, Be,	129	46	10
AA-3	8	M	94	HDM, GP, RP, DD	117	39	5
AA-4	5	M	184	GP, RP, MW, Bi, Al, Hz, CD, Rab, CH, Alt, AF	114	42	14
AA-5	3	M	393	GP, RP	82	30	14
AA-6	13	M	2257	HDM, GP, RP, MW, Bi, Al, Hz, CD, DD	87	32	5
AA-7	7	M	671	HDM, GP, RP, Bi, Be, CD, Ho	81	30	7
AA-8	6	M	6931	HDM, GP, CD	74	38	18
AA-9	12	M	1054	HDM, GP, RP, Bi, Rab	134	43	7
AA-10	13	M	941	GP, RP, CD, Rab	84	28	4
AA-11	16	M	2945	GP, RP, RW, CD, DD, Ho, Rab, CH	77	31	10
					1140	420	100

AA, allergic asthma; AD, atopic dermatitis; AF, *Aspergillus fumigatus*; Al, alder; Alt, *Alternaria alternata*; Ap, apple; Be, beech; Bi, birch; Car, carrot; CD, cat dander; Ce, celery; CH, *Cladosporium herbarum*; CM, cow's milk; CowD, cow dander; DD, dog dander; GP, grass pollen; HDM, house dust mite; HE, hen's egg; HN, hazelnut; Ho, horse; Hz, hazel; MW, mugwort; PN, peanut; Rab, rabbit; RP, rye pollen; RW, ribwort; WF, wheat flour.

Results

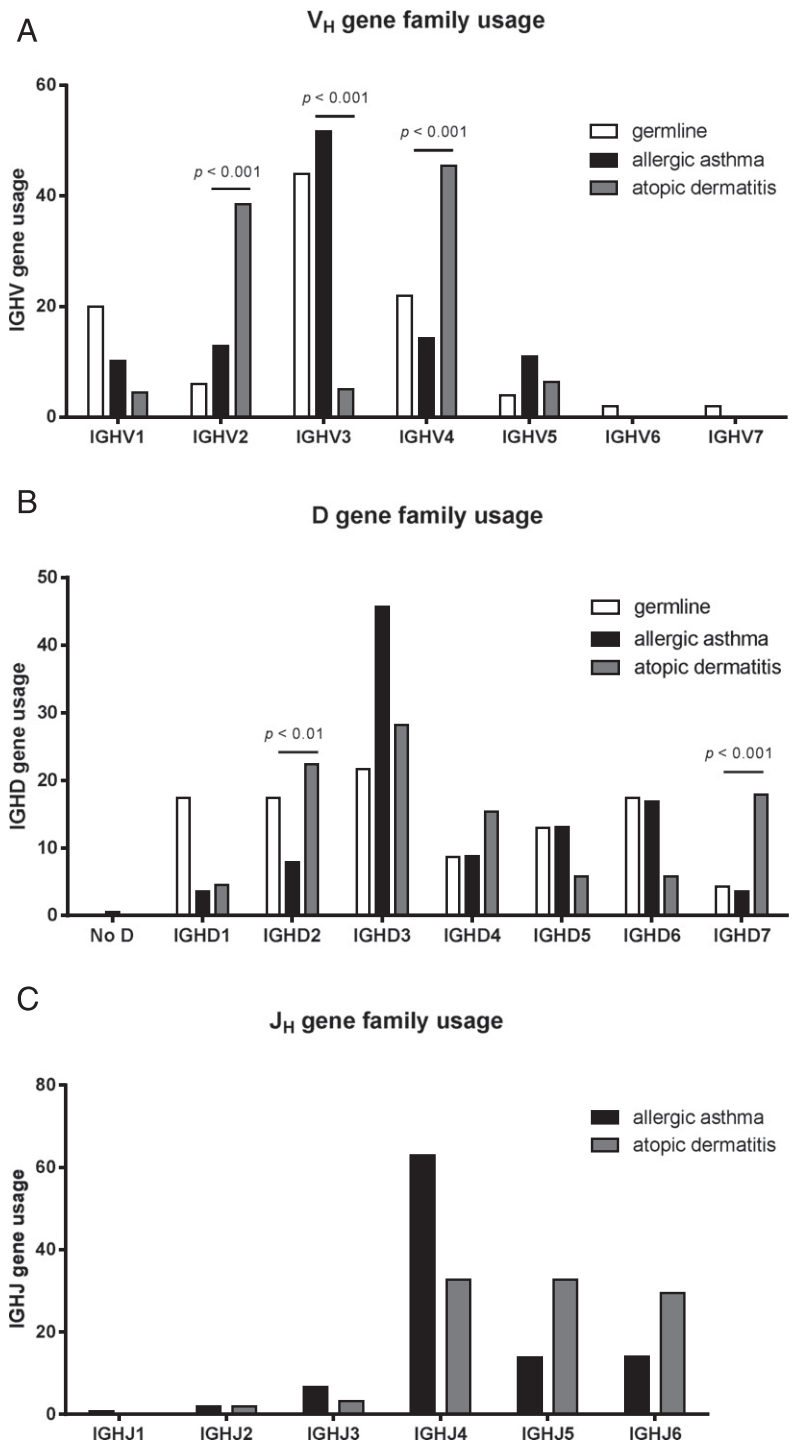
To achieve a reliable database for statistical analysis, we aimed to gain ~100 clonotypes per group and at least 20 unique sequences per patient. This demand could be met with 5 patients in the atopic dermatitis group and with 11 patients in the asthma group, respectively (Table I). In total, we gained 200 functional sequences of IgE transcripts from children with atopic dermatitis, of which 156 sequences were unique (GenBank accession no. HM116098-HM116226 and KM209339-KM209361; www.ncbi.nlm.nih.gov/genbank). For the asthma group, we analyzed 1140 functional IgE sequences (420 unique). The IgE sequences of children with asthma have been published in a preceding work of our group, in which we compared the IgE response in allergic

children with the primary immune response (IgM) (2). In contrast with our previous article, an active atopic dermatitis as secondary diagnosis was an exclusion criterion for the asthma group in this analysis. Moreover, updated classification criteria for the Ig sequence analysis explain slight differences in absolute sequence numbers and assignments by the ImMunoGeneTics database. All analyzed sequences in this article were IgE-encoding transcripts, and products encoding other isotypes were not obtained.

Pattern of VDJ gene family usage shows significant V_H bias in atopic dermatitis

The V_H family frequency distribution in IgE transcripts from children with allergic asthma did not differ significantly from the statistically

FIGURE 1. Usage of IGHV (A), D gene family (IGHD) (B), and J_H gene family (IGHJ) (C) genes in IgE transcripts from children with allergic asthma and children with atopic dermatitis. Although the V_H gene family distribution in asthma reflected germline complexity, we found a significantly biased pattern for IgE transcripts from children with atopic dermatitis. Only 3% of these transcripts used members of the V_H3 gene family, which is the largest V_H gene family on germline level and by far the dominating in IgE sequences from children with asthma ($p < 0.001$). On the other hand, we found a dominance of otherwise scarcely used V_H2 and V_H4 family members in atopic dermatitis ($p < 0.001$, compared with asthma for both V_H families). Apart from a more frequent usage of IGHD2 genes ($p < 0.01$) and IGHD7 genes ($p < 0.001$) in IgE transcripts from children with atopic dermatitis, the overall D and J_H gene usage did not differ significantly between asthma and atopic dermatitis. IgE transcripts of asthma patients are from Kerzel et al. (2).



expected germline complexity, rendering the V_H3 family by far the dominating V_H gene family (52% of all IgE sequences from asthmatic children) (Fig. 1A). However, in IgE transcripts from children with atopic dermatitis, the pattern of V_H gene family usage was fundamentally different: whereas members of the V_H3 gene family were used by a mere 3% of all sequences ($p < 0.001$, compared with asthma, χ^2 test with post hoc analysis), we found a dominance of V_H2 and V_H4 family members. These otherwise scarcely expressed V_H families accounted for 40 (V_H2) and 46% (V_H4) of all IgE transcripts from children with atopic eczema ($p < 0.001$, compared with asthma for both V_H families, respectively, χ^2 test with post hoc analysis). Whereas a wide spectrum of different V_H genes was used by IgE sequences from asthmatics, we found a clustering of V_H gene usage in IgE transcripts from children with atopic dermatitis (Fig. 2): the significant shift in V_H usage pattern was due to an overexpression of the $V2-5$ gene and of almost all V_H4 family genes.

Extensive PCR amplification procedures, as used in this work, are by nature prone to amplification biases. To control for different amplification efficiency of the V_H family specific primers (IGHV1-IGHV7), we performed quantitative PCR of IgM transcripts. As demonstrated by Brezinschek et al. (23), the distribution of V_H families in the productive IgM repertoire reflects the naturally occurring and fixed germline frequency of V_H genes, rendering the largest V_H family (IGHV3), the most frequent in the productive repertoire, followed by IGHV1 and IGHV4. The quantitative IgM PCR showed a strong correlation ($R^2 = 0.817$) between the cycle thresholds of the different V_H forward primers and the germline frequency of the corresponding V_H family (Supplemental Fig. 1). The order of appearance of the different V_H families was identical to the frequency of germline genes in the corresponding gene family. For both V_H gene families that were overrepresented in our study (IGHV2 and IGHV4), the primers displayed an amplification efficiency that corresponded to the stochastically predicted frequency. Hence we

conclude that the observed V_H bias is not introduced by the PCR process itself.

Unlike variable gene segment usage, the D and joining gene segment of the H Ig chain (J_H) gene family usage was similar between asthma and atopic dermatitis (Fig. 1B, 1C). The only noticeable difference was an overexpression of genes of the D_H2 and D_H7 family in transcripts from the atopic eczema group ($p < 0.01$ and $p < 0.001$, respectively, χ^2 test with post hoc analysis). However, D gene usage of IgE from patients with atopic dermatitis did not differ significantly from germline frequency. In all atopic dermatitis sequences and in all but two IgE sequences from children with asthma, a D gene segment could be aligned.

Same CDR-H3 length of IgE transcripts in both groups

Although a wide range of CDR-H3 length was covered by the analyzed IgE sequences (Fig. 3A), frequency distribution and mean CDR-3 length did not differ significantly between the two groups. Fig. 3B shows the internal CDR-H3 structure, allowing for a detailed assessment of relative contributions of V_HDJ_H germline sequence, exonucleolytic nibbling, as well as N- and P-nucleotide addition to the CDR-H3. Although the total CDR-H3 length was almost identical in transcripts from asthma patients (48 ± 13 nt, mean $\pm$ SD) and from atopic dermatitis patients (47 ± 9 nt), the J_H -segment was significantly shorter in favor of a significantly longer D-segment in IgE transcripts from asthma patients ($p < 0.001$ for both differences, U test).

IgE sequences in atopic dermatitis are less mutated than in allergic asthma

IgE transcripts from children with asthma are highly mutated (2). The mean somatic mutation rate of IgE sequences from asthmatic children in this study was 72 (per 1000 nt) (Fig. 4). In contrast, the mutation rate of IgE transcripts from children with atopic dermatitis was less than half as high with 34 (per 1000 nucleotides) ($p < 0.0001$, compared with asthma group, U test). Although only 3 (of 420) unique IgE sequences from asthma patients displayed <10 mutations/1000 nt (corresponding to 0.71% of all asthma sequences), a fourth of all IgE transcripts (24%) from children with atopic dermatitis belonged to this quasi unmutated group of sequences.

To quantify the *Taq* polymerase error, we calculated the *Taq* error rate within the C region of the IgH sequences, which was included in the amplicates. The *Taq* error rate was 1.6 (per 1000 nucleotides). In consideration of the observed somatic mutation rate, it is unlikely that the *Taq* error rate significantly influenced the downstream analyses.

Proportion of IgE transcripts with clustering of replacement mutations in CDR is half as high in atopic eczema than in asthma

We determined the distribution pattern of different types of mutations along the Ig V region. As previously reported (2), the statistical frequency of replacement mutations into the CDR was calculated with the method of Lossos et al. (16), and confidence limits for the ratio of replacement mutations in the CDR to the number of total mutations in the V region, as described by Dahlke et al. (18), were determined with the binomial distribution method of Chang and Casali (17). Because this methodological approach cannot determine Ag selection in its proper sense (24), we prefer to speak of sequences with significant clustering of replacement mutations in the CDRs as indicative of Ag selection rather than Ag-selected. The shaded areas in Fig. 5 indicate the 95% confidence limit for the statistically random accumulation of replacement mutations in the CDRs. An allocation of a sequence above this confidence limit indicates an enhanced clustering of replacement mutations in the

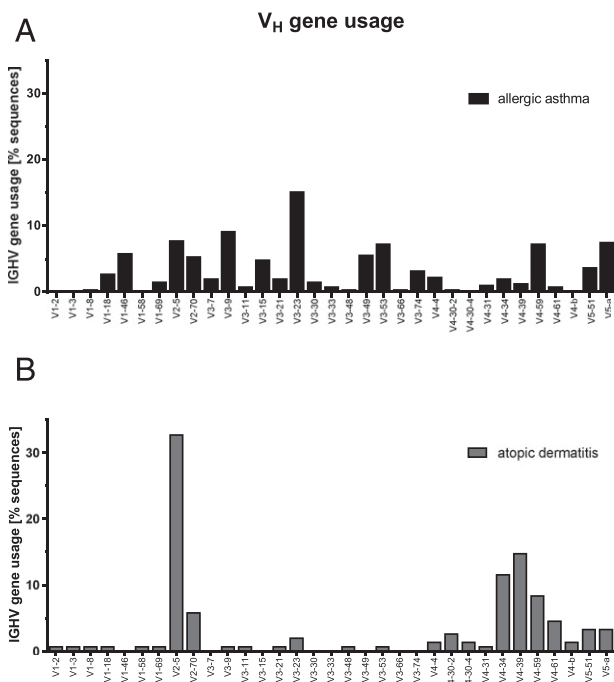
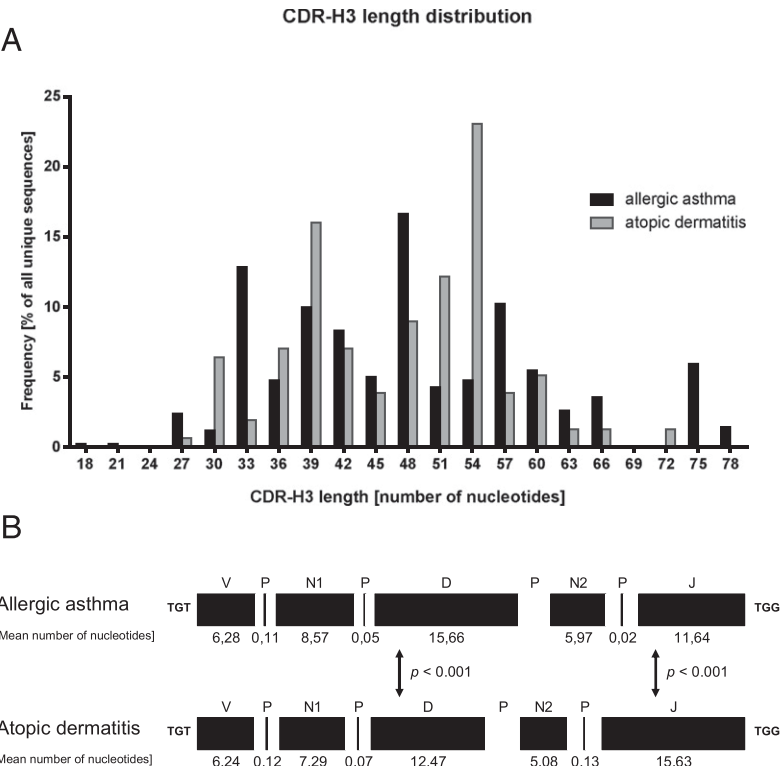


FIGURE 2. Usage pattern of single V_H genes. Whereas a wide spectrum of different V_H genes was used by IgE sequences from asthmatics (A), we found an overexpression of the $V2-5$ gene and of almost all genes of the V_H4 family in atopic dermatitis (B). IgE transcripts of asthma patients are from Kerzel et al. (2).

FIGURE 3. CDR-H3 length distribution and composition. The IgE transcripts displayed a broad range of CDR-H3 length distribution that did not differ between asthma and atopic dermatitis (**A**). Although the total CDR-H3 length was almost identical between the two study groups, an assessment of the relative contribution to the CDR-H3 (**B**) revealed that the J_H-segment was significantly shorter in favor of a longer D-segment in IgE transcripts from asthma patients ($p < 0.001$ for both differences, U test). IgE transcripts of asthma patients are from Kerzel et al. (2).



CDRs. The statistical chance of such clustering by mere random mutation is $<5\%$. A data point above the confidence limit was considered indicative of a possible Ag-driven selection. Although 24.2% of all unique transcripts from children with asthma showed significant clustering of replacement mutations to the CDRs (Fig. 5A), this proportion was only 10.9% in atopic dermatitis (Fig. 5B) ($p < 0.001$, χ^2 test).

We calculated the selection strength for CDR and for FWR using the BASELINE software tool (19, 20). IgE transcripts from children with allergic asthma showed significant negative selection strength for FWRs and for CDRs (Supplemental Fig. 2). In the

atopic dermatitis group, no positive CDR selection strength was detected for any V_H family but IGHV2.

The IgE repertoire in atopic dermatitis is clonally less restricted than in asthma

Whereas the 156 unique IgE sequences from children with atopic dermatitis (corresponding to 200 functional sequences) pertained to 111 different clonotypes, the picture was strikingly different for IgE transcripts from asthmatic children. The IgE repertoire in asthma was clonally restricted to a mere 100 different clonotypes of 420 unique sequences (corresponding to 1140 functional sequences).

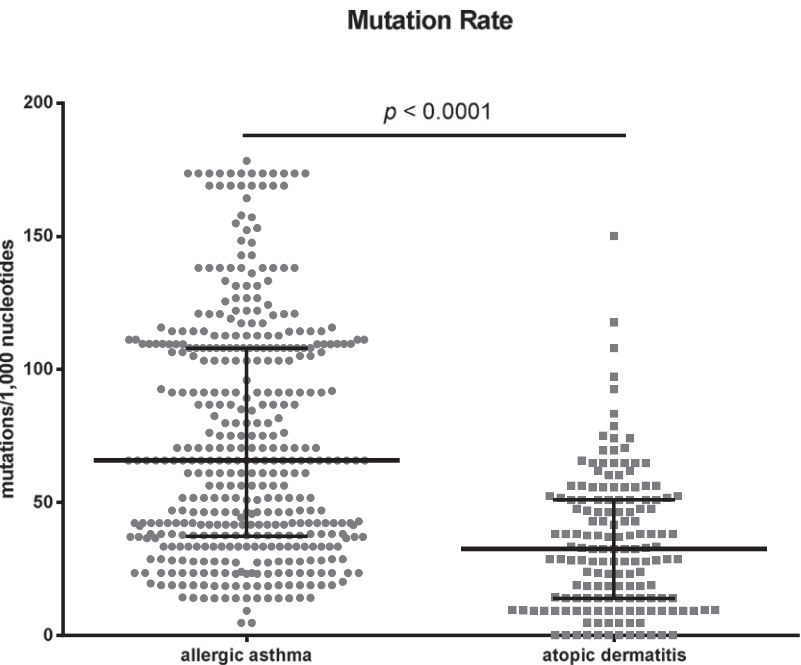
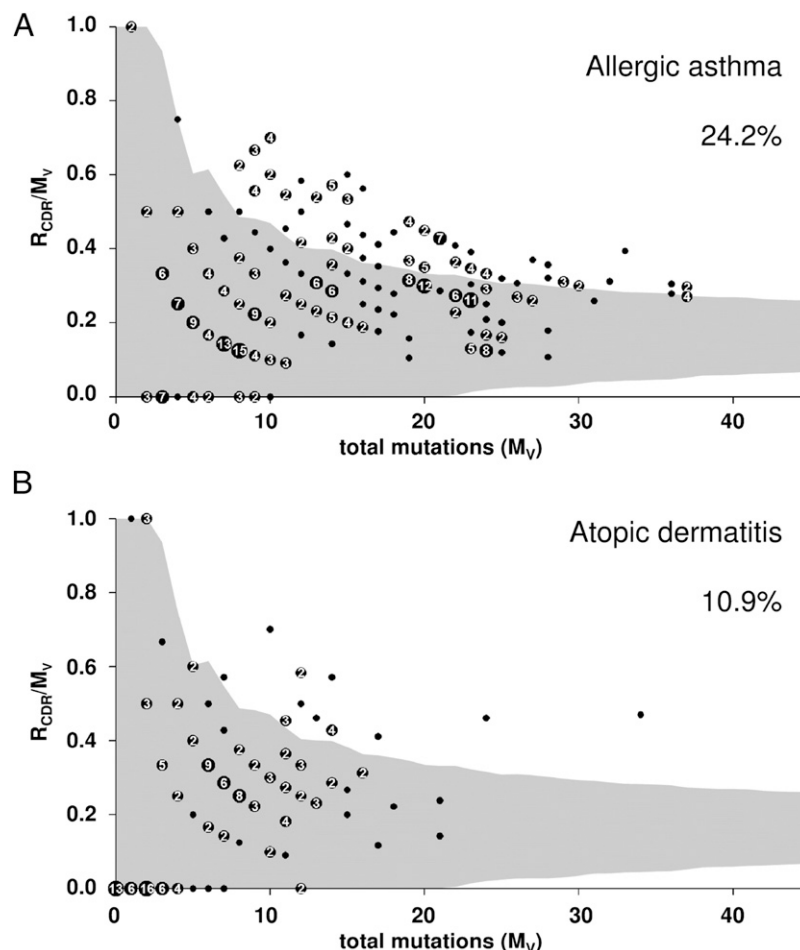


FIGURE 4. Somatic mutation rate of IgE transcripts. IgE transcripts from children with allergic asthma displayed a high somatic mutation rate that was comparable with other secondary Ab repertoires. In contrast, the mutation rate of IgE sequences from children with atopic dermatitis was less than half as high ($p < 0.0001$, U test). IgE transcripts of asthma patients are from Kerzel et al. (2).

FIGURE 5. Clustering of replacement mutations to CDRs. To determine possible indications for Ag selection, we analyzed the distribution pattern of mutations along the Ig V_H region. The graph shows the ratio of replacement mutations in CDR-1 and CDR-2 (R_{CDR}) to the total number of mutations in the V region (M_V). The gray shaded area indicates the 95% confidence limits for random mutations. A data point outside this area represents an IgE transcript that has a high proportion of replacement mutation inside the CDRs. The probability that such a clustering has occurred by random mutation is <0.05 . A transcript outside these confidence limits was considered indicative of significant Ag selection. The numbers inside the data points show the frequency of a certain sequence. Although 24.2% of all unique transcripts from children with asthma were indicative of Ag selection (**A**), this proportion was reduced to 10.9% in atopic dermatitis (**B**) ($p < 0.001$, χ^2 test). IgE transcripts of asthma patients are from Kerzel et al. (2).



Accordingly, the clonal diversity (defined as ratio $n[\text{clonotypes}]/n[\text{unique sequences}]$) was 0.25 ± 0.04 in transcripts from asthma patients, but 0.69 ± 0.10 in the atopic dermatitis group ($p < 0.001$, t test) (Fig. 6).

Discussion

The IgE response in children with allergic asthma represents an oligoclonal B cell expansion and follows the traditional adaptive B-2 cell pathway, arguing against a superantigen-like activation (2). This previous finding is in accordance with several other recent publications (reviewed in Ref. 10) that also found that human IgE repertoires are oligoclonal. However, this finding does not apply to all atopic disease entities. In this study, we analyzed IgE transcripts from preschool children with atopic dermatitis, which often represents the first manifestation of atopy. The main finding is that IgE repertoires vary significantly between groups of children with different atopic phenotypes.

Our approach implied a different age distribution in the two study groups. Hence we cannot exclude that part of the observations from the direct comparison of the two study groups were influenced by the lower mean age in the atopic dermatitis group. Particularly, this might be an issue for the mutation analyses. However, reanalyzing our data within the respective groups did not reveal a significant correlation between somatic mutation rate and age or between CDR-H3 length and age (Pearson product-moment correlation). Because the number of individuals in our atopic dermatitis group is relatively small ($n = 5$), we are cautious to draw a general conclusion in this regard. Further studies based on our recent results will be necessary to directly compare the IgE response between different age groups of children but with

the same atopic disease. A second limitation of our study is that we characterized the IgE repertoire in the circulation of these children (PBMC). Whether this systemic B cell repertoire differs from the local IgE response in the inflamed tissue is currently unknown.

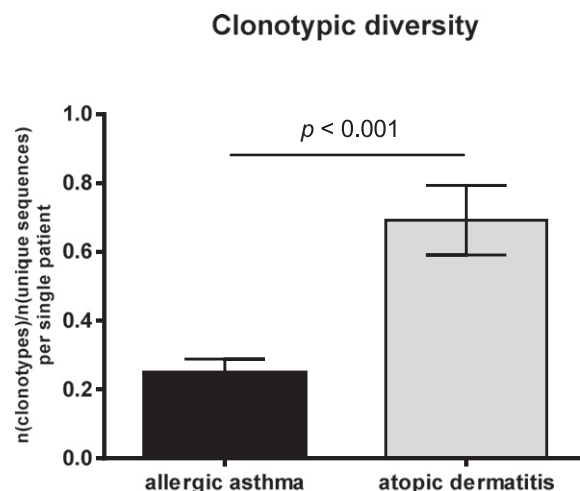


FIGURE 6. Clonotypic diversity of IgE transcripts. Shown is the clonal diversity of IgE sequences, defined as ratio $n[\text{clonotypes}]/n[\text{unique sequences}]$. Although the IgE repertoire in allergic asthma was clonally restricted to a diversity of 0.25, transcripts from children with atopic eczema displayed a significant higher clonal diversity of 0.69 ($p < 0.001$, t test). IgE transcripts of asthma patients are from Kerzel et al. (2).

Bearing these limitations in mind, in this article we have shown: 1) that the pattern of V_H gene family usage can differ fundamentally between atopic entities, and 2) that the IgE response in preschool children with atopic dermatitis is significantly less focused than in childhood asthma.

A biased V gene usage in IgE transcripts from adult patients with atopic dermatitis has been reported by several authors (reviewed in Refs. 1 and 25). It has been hypothesized that superantigen-like activation might play a role in B cell maturation during the allergic immune response. In contrast with classical Ags, superantigens are bound to the FWRs of the Ab, irrespective of the CDR specificity. An excessive usage of V_H families that are normally infrequently used in secondary immune responses might hence be indicative of a superantigen-like activation of B cells (1). However, not all studies on immunogenetic characteristic of the IgE response in atopic eczema found a biased V_H usage (7, 8).

In this study, the distinct asymmetry of V_H gene usage in a collection of 200 functional IgE sequences from children with eczema is notable. Unlike IgE transcripts in allergic asthma, which reflected germline complexity, pattern of V_H gene family usage in atopic dermatitis showed a significant overrepresentation of the V_{H2} and V_{H4} gene family. The observation that the otherwise scarcely used V_{H4} gene family was the most frequently used by IgE transcripts from atopic dermatitis patients in our study is of particular interest. The *S. aureus*-derived enterotoxin D acts as a potent B cell superantigen by inducing survival of V_{H4}-expressing B cells (26). Other staphylococcal superantigens, however, favor usage of genes from the V_{H3} family (27, 28). Kozłowski et al. reported that IgM transcripts produced in response to *S. aureus* protein A are enriched for V_{H3} H chains (29).

S. aureus can be isolated from the skin of most patients with atopic dermatitis (3), and in a pediatric survey, 81% of children with atopic eczema were colonized (4). The putative pathogenetic contribution of *S. aureus* products during distinct stages of the inflammatory immune response in atopic dermatitis is the subject of ongoing discussion. It has been tried to reconcile divergent classifications of different phenotypes of atopic dermatitis (reviewed in Ref. 3). In summary, immunoactive proinflammatory mediators from *S. aureus* might play a role in perpetuating and enhancing the allergic inflammation in atopic dermatitis.

Plasma cells are rare in peripheral blood, especially IgE-producing plasma cells. Because blood samples from the infants in our study had to be relatively small, we would have expected a comparably low clonal diversity. However, against our expectations, the clonal diversity was greater than anticipated and even greater in the blood from atopic dermatitis patients than from asthma patients. In fact, the 156 unique functional IgE sequences from children with atopic eczema pertained to 111 different clonotypes, resulting in a markedly high clonal diversity (for secondary repertoires) of 0.69. These experimental findings do not support the hypothesis that the samples might have been less populated by plasma cells. These observations may hence indicate a polyclonal IgE response that targets a larger set of Ags. This notion is supported by the low somatic mutation rate in IgE transcripts from children with atopic dermatitis and the absence of positive CDR selection strength. In summary, the IgE response in children with atopic dermatitis is weakly focused and displays reduced clustering of replacement mutations in the CDR. However, considering the principal methodological limitations of mathematical models to infer Ag selection (30), we would be very cautious to draw any further conclusions on this matter.

How can our results be reconciled with Lim et al. (8), who reported that the IgE repertoire from atopic patients showed no bias in V_H gene usage? A first main difference to this study is the

differing age structure of the two collectives (young preschool children versus adults). As outlined earlier, the pathogenesis of atopic dermatitis is sequential by nature with a dominance of different immune mechanisms in distinct phases of the natural disease course (3). Hence drivers of the inflammation may change over time and vary in different age groups. The second major difference is the inclusion of mixed atopic entities (eczema + rhinitis + asthma). In this study, children with concomitant allergic asthma were excluded from the atopic dermatitis group and vice versa. In contrast with this separation of atopic entities in this study, several previous publications (2, 8) included patients irrespective of their atopic comorbidities. However, because the nature of the IgE response may vary between distinct atopic entities, the heterogeneous phenotypes might explain part of the observed differences.

In summary, IgE repertoires vary significantly between groups of children with different atopic diseases. Compared with children with asthma, IgE transcripts from preschool children with atopic dermatitis are significantly less mutated, clonally less focused, and display reduced clustering of replacement mutations in the CDR. Although in principle a classical B2 type immune response, the IgE response in preschool children with atopic dermatitis displays a substantial overusage of the V_{H2} and V_{H4} gene families, which are otherwise scarcely used in secondary Ab repertoires. In conclusion, we consider our data reconcilable with the hypothesis that superantigen-like activation contributes to maturation and selection of the IgE response in preschool children with atopic dermatitis.

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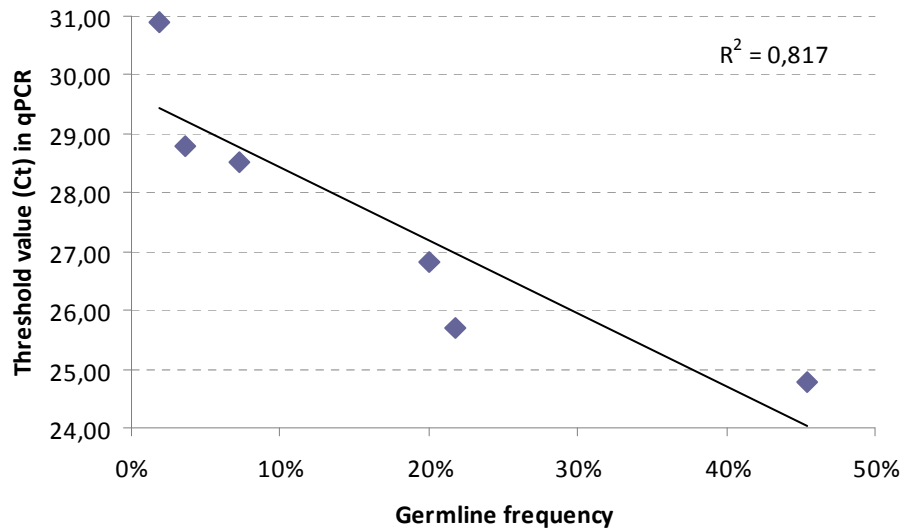
Disclosures

The authors have no financial conflicts of interest.

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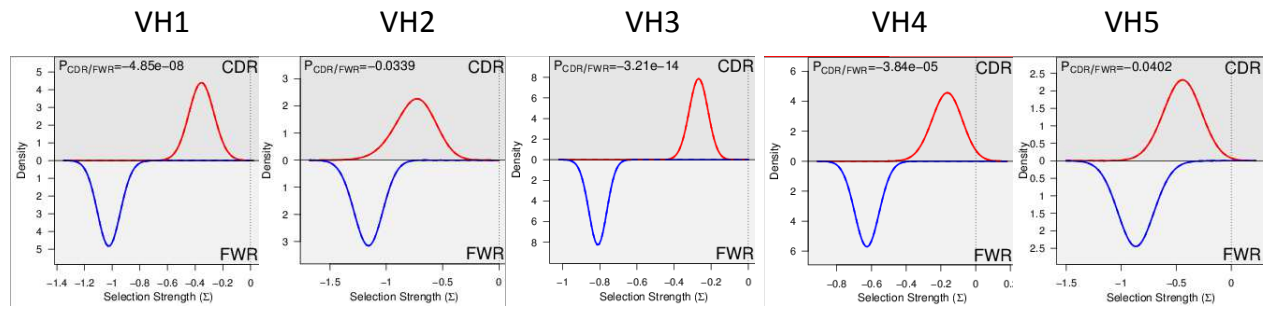
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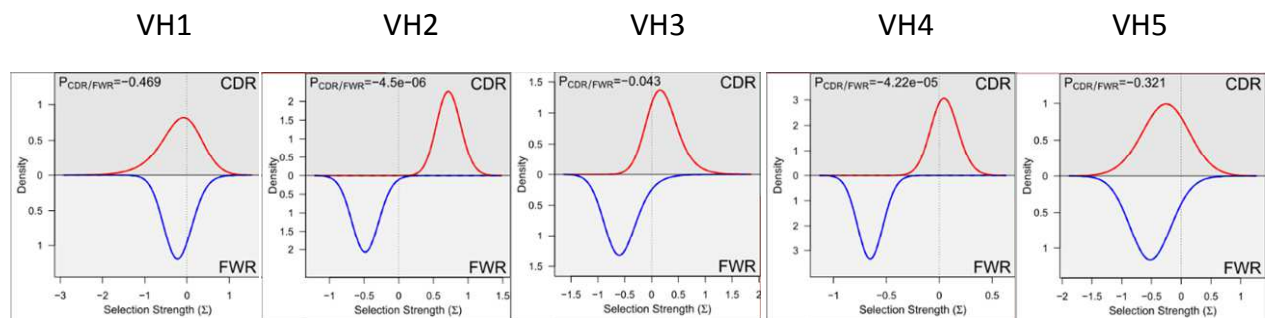
Supplemental Figure 1: Quantitative PCR of IgM transcripts shows strong correlation between the cycle threshold of V_H forward primers and the germline frequency of the corresponding V_H family

The x-axis shows the naturally occurring frequency due to germline configuration. On the y-axis, the cycle threshold value (Ct) in the quantitative IgM PCR is shown. Overall, we observed a high correlation between germline frequency and order of appearance in the qPCR.

Allergic asthma



Atopic dermatitis



Supplemental Figure 2: Antigen-selection strength of IgE-transcripts (assessed by BASELINE) differs between allergic asthma and atopic dermatitis

IgE transcripts from children with allergic asthma showed significant negative selection strength for framework regions (FWR) and for complementarity determining regions (CDR). In the atopic dermatitis group, no positive CDR selection strength was detected for any V_H family but IGHV2.