

Antigen selection in IgE antibodies from individuals with chronic rhinosinusitis with nasal polyps

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ABSTRACT

Background: It is generally assumed that all immunoglobulin isotopes develop under antigen selection pressure, leading to dramatic increases in antigen-binding affinity. As activated B cells proliferate, somatic mutations accumulate in the regions of the immunoglobulin gene associated with antigen binding. Emerging evidence from studies investigating mutations in variable region sequences of IgE antibodies suggest that IgE may develop under less selection pressure than other isotopes. Recent studies have focused on IgE mutation patterns in sequences from the blood of allergic individuals. There is, however, little evidence of these patterns in IgE sequences isolated from tissue.

Methods: Seminested reverse-transcription polymerase chain reaction was used to amplify the V region of IgE sequences from nasal tissue of individuals with chronic rhinosinusitis with nasal polyps (CRSwNPs). IgE sequences were analyzed for evidence of antigen selection and compared with previously reported IgE sequences from other inflammatory and allergic disorders and nonallergic individuals.

Results: IgE sequences were successfully amplified from four individuals with CRSwNPs. Of 217 sequences amplified, 38 were unique, 31 of which were from tissue. Identification of the IGHV, IGHD, and IGHJ genes making up each sequence showed overrepresentation of an unusual gene in one individual, but otherwise normal gene usage. Mutation analysis revealed that only 5 of the 31 unique sequences from tissue show clear evidence of antigen selection.

Conclusion: With little influence from antigen selection, IgE antibodies are unlikely to be highly specific for antigens. Consequently, these findings have significant implications for the relevance of specific IgE, e.g., *Staphylococcus aureus* enterotoxin or fungal-specific IgE, in CRSwNP pathogenesis. Whether specific IgE expression is tightly related to pathogenesis or is merely a byproduct of B-cell interactions in local mucosa with colonizing organisms remains unresolved.

(Am J Rhinol Allergy 24, 416–421, 2010; doi: 10.2500/ajra.2010.24.3538)

Chronic rhinosinusitis (CRS) is a heterogeneous group of disorders characterized by inflammation of the paranasal mucosa; its etiology is poorly understood.¹ There is no clear link between CRS with polyps (CRSwNPs) and seasonal allergies,² and polyp patients are not always atopic;³ however, high levels of local mucosal IgE and tissue eosinophils are found in polyp tissue.⁴ Elevations in IgE specific to *Staphylococcus aureus* enterotoxins (SAEs), a “superantigen” with powerful immunostimulatory properties, have been shown in nasal polyp tissue,⁵ and chronic colonization and stimulation by superantigens has been hypothesized as a causative or disease-modulating element in CRSwNPs.⁶ Similarly, fungal-specific IgE is detected in some CRSwNP patients, especially those with eosinophilic mucin,⁷ and fungal allergy has been suggested as a driving pathogenic force in these patients.⁸ Detailed investigation of the humoral response to fungal antigens in CRSwNP patients shows little evidence of a relationship between fungal sensitivity and pathogenesis,⁹ and the clinical significance of fungal sensitivity remains unclear.⁸

B-cell development is tightly regulated with distinct stages and checkpoints to eliminate self-reactive cells, while ensuring the formation of functional antibodies (Fig. 1).¹⁰ First, the germline immunoglobulin gene is rearranged *via* somatic recombination to form a functional gene for expression. The entire immunoglobulin heavy

chain (IGH) gene loci encode many variable (IGHV), diversity (IGHD), and junctional (IGHJ) segments and one of each is combined to form a functional gene.¹¹ During an immune challenge, naive B cells are activated and proliferate within germinal centers in the lymph nodes. Within these cells, point mutations accumulate in the V, D, and J antibody genes.¹² Selective forces ensure that cells with favorable mutations, which increase antigen-binding affinity, survive and rapidly divide, while those with unfavorable mutations are eliminated. The result is a subset of B cells that are highly specific for a given antigen as a result of “antigen selection.”

The extent to which a B cell has developed under selection pressure can be determined by analyzing replacement mutations in the IGHV (variable region) sequence.¹³ Selection results in a characteristic clustering of replacement mutations in the complementarity determining regions (CDRs) of the variable region,¹⁴ while the surrounding framework regions of the gene are relatively unmutated.¹³ A random model of mutation, previously described by Dahlke and colleagues, shows what the expected distribution of mutation of mutations should be if they are accumulating randomly, without any influence from selection pressure.¹⁵ In this model, the probability of a random mutation occurring in the CDRs is 0.26, calculated by determining the relative mutability of nucleotides in the germline immunoglobulin sequences. Mutational hotspots and the frequency of mutations in the surrounding framework regions of the CDRs were used to model the random accumulation of mutations, without bias from selection pressure. Studies of sequences from blood of allergic and nonallergic individuals have shown, using this model, that a mutation bias is not readily apparent in IgE, suggesting that IgE-expressing B cells are subject to less selection pressure than those expressing IgG.¹⁵

In CRS, elevations of secreted IgE are found in lymphoid tissue and fluids around nasal polyps,⁶ and cells expressing IgE are enriched in mucosal tissues.^{16–17} Growing evidence suggests that proliferation of these cells can occur locally in the mucosa, in the absence of the specialized cellular interactions that regulate selection and maturation inside germinal centers.^{18–21} In this study, we report a set of IgE sequences obtained from the turbinate tissue and sinus mucosa of individuals with CRSwNPs that show little evidence of antigen selection. Our results support the notion that the IgE response develops

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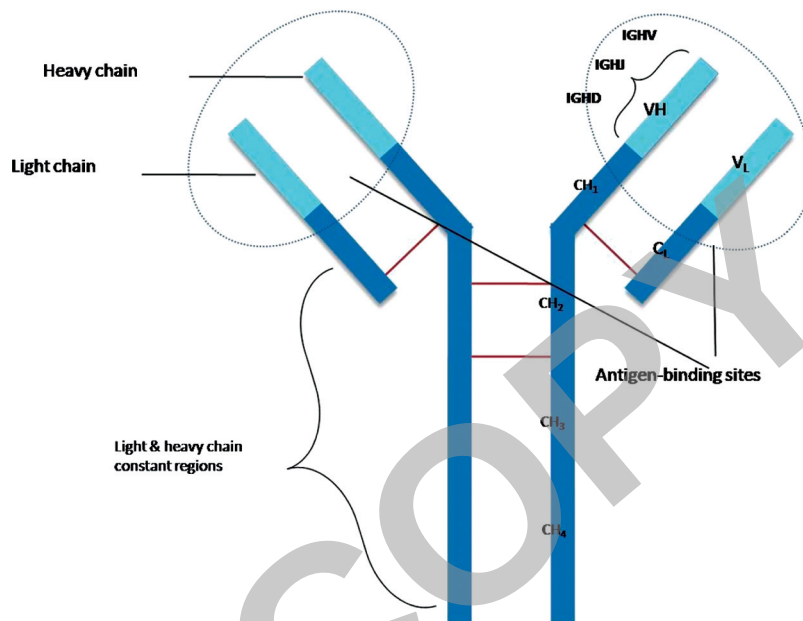
Supported by a grant from the National Health and Medical Research Council R. J. Harvey receives grant support, and is a speaker/consultant for NeilMed, Medtronic, and/or Schering Plough. The remaining authors have nothing to declare pertaining to this article

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Figure 1. Generalized antibody structure. Immunoglobulin isotypes share a common structure, with two identical heavy chains, and two identical light chains. The inner, heavy chains are encoded by the heavy chain genes (IGH). The outer light chains are encoded by the light chain genes. On both the heavy and the light chains are the constant regions, which are encoded by the CH and CL genes for the heavy and light chains, respectively. The constant region determines both the isotype and the physiological functions of the immunoglobulin. The antigen-binding regions of the antibody are encoded on the heavy chain by the IGHV, IGHD, and IGHJ heavy chain genes. Mutations in the genes encoding this region result in conformational changes in the antigen-binding sites of an antibody. Disulphide bonds link the light chain to the heavy, and disulphide bonds join the two heavy chains (bond locations are simplified).



in a fundamentally different fashion to the IgG response and raise questions about the relevance of SAE- and fungal-specific IgE in CRSwNP pathogenesis.

METHODS

Whole blood and sinus and turbinate tissue specimens were obtained from four individuals with chronic inflammatory sinus disease attending tertiary rhinology clinics for endoscopic sinus surgery (Table S1). The University of New South Wales and St. Vincent's and the Mater Health Human Research Ethics Committees approved this research, and written informed consent was obtained from each donor. Processing of specimens for sequencing and analysis of sequences were performed at UNSW. Sequencing was performed by the Ramaciotti Center for Gene Function Analysis, UNSW.

Sequences obtained in this study were compared with an IgE sequence database previously compiled by our laboratory. This comparative database, with 263 IgE sequence from 24 individuals, contains a combination of published sequences from the GenBank and ImMunoGeneTics (IMGT) immunoglobulin sequence databases (21 individuals)²² and sequences amplified by our laboratory.¹⁵ Amplified sequences were from two nonallergic donors (60 sequences) and an individual with atopic dermatitis (76 sequences),¹⁵ and all additional sequences were obtained from published studies investigating individuals with allergic rhinitis, dermatitis, peanut allergy, and asthma (127 sequences). An IgG database with 152 sequences, used as a point of reference for mutation analysis, was compiled by identifying previously reported IgG sequences in IMGT.¹⁵

RNA Extraction, Seminested Polymerase Chain Reaction (PCR), and Gene Sequencing

Tissue samples were transported in RNAlater RNase inactivation solution (Ambion, Austin, TX) to preserve mRNA integrity. Total RNA was extracted using the method described by Chomczynski and Sacchi.²³ cDNA was synthesized using SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA.). Seminested PCR was used to amplify rearranged IgE heavy chain genes from the start of the VH region, to the beginning of the ϵ -constant region (CH1 of the IgE constant region). Mixed forward primers, specific for the most commonly expressed IGHV gene families, VH1, VH3, and VH4, were used. Preferential VH gene use is proportional to gene family size,

and these three largest IGHV gene families are used in ~85% of human immunoglobulin sequences.²⁴ Reverse primers were specific for the constant region of the IgE heavy chain (CH1), with the binding site for the secondary PCR located internally in the product of the primary PCR (Table S2). Amplified IGHE sequences were purified and then cloned using the pGEM-T Easy Vector (Promega, Madison, WI.). Plasmids were harvested from random colonies, and inserts were sequenced.

V Gene Sequence Alignment and Mutation Analysis

VDJ gene rearrangements were identified by aligning sequences with the expressed IGHV repertoire using the iHMMune-align program.²⁵ Clonally related sequences, defined as those with shared V, D, and J genes and N regions, and a high degree of shared mutations, were identified using IMGT-VQuest²⁶ sequence alignment. Replacement mutations in the CDRs were identified from the iHMMune-align sequence partitioning output. Total mutation number and the number of replacement mutations in CDRs 1 and 2 were used to determine the influence of antigen selection on the sequences amplified. A sequence was said to have clear evidence of antigen selection if the proportion of replacement mutations in the CDRs was greater than that predicted by a model of the accumulation of random mutations. The upper 95% confidence interval of this random model, previously described by Dahlke and colleagues, is graphically represented in Fig. 2. Any sequence falling outside the upper confidence limit is likely to be the product of antigen selection because the proportion of mutations in the CDRs compared with total V region mutations (M_v) is greater than what is expected from the random model.¹⁵

Statistical Analysis

The total number of V region mutations (M_v) from IgE sequences in this study, as well as the frequency distribution of these, was compared with the M_v of previously reported IgE sequences.¹⁵ Comparison was also made to database-derived IgG for reference. The significance of differences in M_v between sequence sets was analysed using Student's *t*-test. Differences in the frequency of sequences in each data set with varying numbers of total mutation were tested with the χ^2 -test.

For statistical analysis of mutation patterns, the proportion of se-

Frequency distribution of V region mutations in immunoglobulin datasets

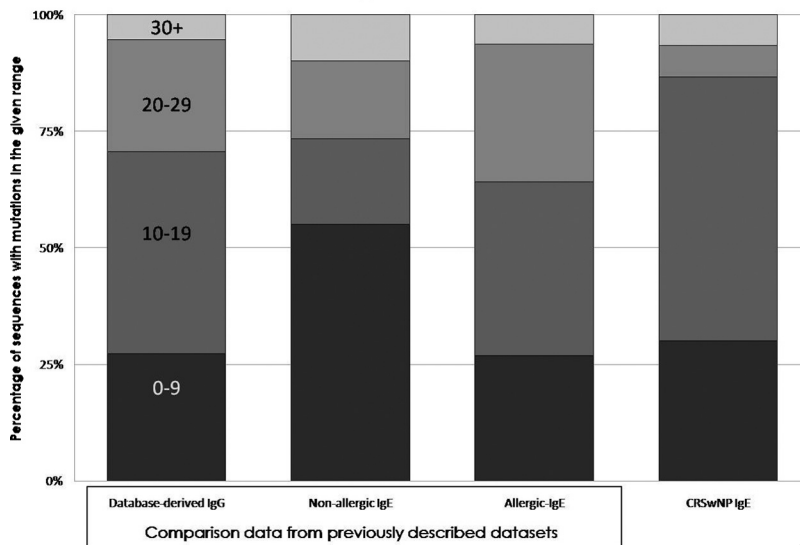


Figure 2. Frequency of human nonallergic IgE, allergic IgE, reference IgG, and chronic rhinosinusitis with nasal polyp (CRSwNP) IgE with varying numbers of total mutation. In contrast with IgG, a high percentage of CRSwNP IgE sequences have fewer than 20 mutations throughout the V region (87 and 71%, respectively). In nonallergic IgE, 55% of the sequences have fewer than nine mutations. As a result, there is a significant difference between nonallergic IgE and CRSwNPs ($p < 0.05$); however, CRSwNPs and allergic IgE sequences are not significantly different ($p > 0.05$). Low total mutation in IgE suggests that it undergoes a short proliferative stage only, limiting the number of mutations that accumulate in the sequence.

quences in each data set with evidence of antigen selection was counted, and the differences in proportion between data sets were compared. The significance of these differences was tested using Fisher's exact test, assuming the normal distribution.

RESULTS

Gene Usage in IgE Sequences

In total, 217 IgE sequences were amplified from four different individuals. Sequences were obtained from sinus mucosa in all four individuals. In one individual, IgE sequences were also obtained from turbinate tissue and the peripheral blood mononuclear cell (PBMC). Of the 217 sequences, 86% were duplicates—identical sequences amplified from either the same template cDNA or from multiple strands of mRNA expressed by the same or identical B cells or plasma cells. Thirty-eight sequences (14%) were unique; seven sequences from PBMCs, shown in Table 1, are not included in the mutation analysis here, but were, however, amplified for reference. Nineteen different V genes were identified in the unique sequences (Table 1). The alleles VH3-23*01 and VH3-66*01 were used most often. VH3-66*01, which is an unusual allele,²⁴ was found in 5 of 23 sequences from one donor (donor 04, Table 1).

Identification of Clonally Related Sequences

Clonally related sequences were defined as those with identical V, D, and J genes and nucleotide additions and with some shared mutations. Two sets of clonally related sequences were identified from one donor. The first pair, both turbinate tissue sequences, had nine shared mutations, and each sequence had a further unique mutation (see Table 1, donor 04, sequences T1 and T4). The second pair, of which one sequence was derived from PBMC and the other from turbinate tissue, shared 4 mutations, and the PBMC sequence had five further unique mutations (Table 1, donor 04, sequences PBMC7 and T2).

Mutation Analysis of IgE Sequences: Total Mutation Rate and Frequency Distribution

All sequences amplified from tissue had at least one mutation, with a mean total mutation number (M_v) of 13 (Table 1). Of 31 sequences from tissue, 30% had 9 or fewer mutations, and the majority (56%)

had between 10 and 19 mutations (Fig. 2). No significant difference ($p > 0.05$) was found between the M_v of sequences from this study¹³ and the M_v of either database-derived IgG (M_v 15), allergic IgE (M_v 12.4), or nonallergic IgE (15.9), suggesting similar baseline rates of random mutation in IgE and IgG isotypes.

Analysis of the frequency distribution of mutations showed a significant difference in the number of IgG sequences with >20 mutations ($p < 0.05$; Fig. 2). No significant difference in distribution was found between the CRSwNP IgE and reference allergic IgE ($p > 0.05$). A high percentage of nonallergic sequences has fewer than nine mutations, resulting in a significant difference between the frequency distribution in this set and the CRSwNP IgE ($p < 0.05$; Fig. 2).

Mutation Analysis of IgE Sequences: Evidence of Antigen Selection in Amplified Sequences

Of the 31 unique sequences amplified from tissue, five showed evidence of antigen selection (Fig. 3). These five sequences were from three different individuals (Table 1, donor 02 sequence S3; donor 04, sequences S1, S3, and T8; and donor 07, sequence S4). M_v in these five sequences ranged from 6 to 36, suggesting that there is no relationship between total mutation number (M_v) and evidence of antigen selection. Comparison between sequences from CRSwNPs and reference IgG showed that a significantly lower proportion of CRSwNP IgE sequences have clear evidence of antigen selection ($p < 0.05$; Fig. 4). No difference in this proportion exists between IgE from this study and the previously reported reference IgE sequences¹⁴ ($p > 0.05$; Fig. 4).

DISCUSSION

IgE sequences from CRSwNP individuals are relatively unmutated and show little evidence of antigen selection

The focus of this study is not whether allergic versus nonallergic processes are responsible for IgE pathogenesis in CRS. Rather, it is an investigation of what influences selection pressures have in shaping the IgE antibody repertoire in CRS. Although the low number of individuals studied here is a limitation, the number of sequences obtained (38) is comparable with recent IgE work using similar methods. Studies of IgE are inherently difficult given its low concentration

Table 1 Unique sequences by donor

Donor	Sequence ID	V Gene	D Gene	J Gene	Total	R _{CDR1&2}	R _{CDR} /M _V
02	S1	IGHV1-2*02	N/A	IGHJ4*02	18	3	0.1667
02	S2	IGHV3-23*01	IGHD5-5/18*01	IGHJ4*02	17	6	0.3529
02	S3	IGHV3-21*01	N/A	IGHJ3*02	36	15.5	0.4306
02	S4	IGHV3-33*01	IGHD3-9*01	IGHJ4*02	8	2	0.2500
02	S5	IGHV3-23*01	N/A	IGHJ4*02	23	7	0.3043
02	S6	IGHV3-33*01	IGHD6-13*01	IGHJ6*02	11	4	0.3636
04	PBMC1	IGHV3-48*03	IGHD3-22*01	IGHJ4*02	20	9	0.4500
04	PBMC2	IGHV3-9*01	IGHD3-3*01	IGHJ6*02	10	3	0.3000
04	PBMC3	IGHV3-30*18	IGHD2-21*01	IGHJ4*02	11	3	0.2727
04	PBMC4	IGHV3-21*01	IGHD6-19*01	IGHJ6*02	13	6	0.4615
04	PBMC5	IGHV3-9*01	IGHD2-15*01	IGHJ4*02	6	2	0.3333
04	PBMC6	IGHV3-23*01	IGHD5-5/18*01	IGHJ4*02	7	4	0.5714
04	PBMC7	IGHV3-64*01	N/A	IGHJ4*02	9	4	0.4444
04	PBMC8	IGHV3-66*01	N/A	IGHJ4*02	15	4.5	0.3000
04	S1	IGHV3-66*01	IGHD3-9*01	IGHJ6*02	12	6	0.5000
04	S2	IGHV3-74*01	N/A	IGHJ6*03	8	3	0.3750
04	S3	IGHV3-23*01	IGHD6-19*01	IGHJ3*02	10	7	0.7000
04	S4	IGHV3-13*01	IGHD6-19*01	IGHJ4*02	16	4	0.2500
04	S5	IGHV3-33*01	IGHD7-27*01	IGHJ4*02	13	6	0.4444
04	S6	IGHV3-7*01	IGHD3-3*01	IGHJ4*02	10	3	0.2804
04	S7	IGHV3-66*01	IGHD2-2*y	IGHJ4*03	16	6	0.3750
04	T1	IGHV3-66*01	IGHD3-10*01	IGHJ5*02	10	3	0.3000
04	T2	IGHV3-64*01	N/A	IGHJ4*02	4	2	0.5000
04	T3	IGHV3-9*01	IGHD6-19*01	IGHJ5*01	16	5	0.3125
04	T4	IGHV3-66*01	IGHD3-10*01	IGHJ5*02	10	3	0.2857
04	T5	IGHV3-64*01	IGHD5-5/18*01	IGHJ4*02	9	4	0.4211
04	T6	IGHV1-18*01	IGHD2-2*x	IGHJ4*02	4	1	0.2500
04	T7	IGHV3-30*01	N/A	IGHJ6*03	17	4	0.2353
04	T8	IGHV3-23*01	IGHD3-22*01	IGHJ1*01	4	3	0.7500
06	S1	IGHV3-33*04	IGHD5-12*01	IGHJ4*02	41	11	0.2683
06	S2	IGHV3-11*03	IGHD6-13*01	IGHJ4*02	9	4	0.4444
07	S1	IGHV3-53*01	IGHD6-19*01	IGHJ4*03	5	1	0.2000
07	S2	IGHV3-33*01	IGHD1-26	IGHJ6*02	4	2	0.5000
07	S3	IGHV3-53*01	IGHD2-2*z	IGHJ3*02	10	4	0.4000
07	S4	IGHV5-51*01	N/A	IGHJ4*02	10	6	0.6000
07	S5	IGHV1-2*02	N/A	IGHJ3*01	13	5	0.3846
07	S6	IGHV1-2*02	IGHD1-26	IGHJ4*03	20	8	0.4000
07	S7	IGHV3-66*02	IGHD1-26	IGHJ5*02	4	2	0.4444

Thirty-eight unique sequences amplified were analyzed for gene usage and mutation distribution. V and J genes are shown for each sequence and the D gene where it was identified. Multiple sequences were amplified from each donor from sinus mucosa (S), turbinate tissue (T). Sequences were also amplified from PBMCs and, although not included in mutation analysis, are included here for reference (PBMC). The iHMMune-align sequence partitioning program was used to determine both the total number of mutations in the V region and the number of replacement mutations in CDRs 1 and 2. The ratio of replacement mutations in the CDRs against total mutations (R_{CDR}/M_V) is used to determine the influence of antigen selection in a sequence.

CDR = complementarity determining region; PBMC = peripheral blood mononuclear cell.

relative to other immunoglobulin isotypes. Thus, insights have previously been reported in studies investigating IgE from few individuals.^{16,18,27-29} Although sequences reported here are from only four individuals, this study sought to establish what patterns in mutation are apparent in tissue and not firmly establish a definitive mutation analysis for CRS IgE sequences. In this study, comparative analysis was performed using reference IgE and IgG sequences from GenBank and IMGT public databases. Mutation analysis of IgE and IgG sequences from individual patient samples may be possible in the future using high-throughput 454 sequencing.

Failures of the immune response may be a significant contributor to CRSwNP pathogenesis.³⁰ Initial antigenic stimulation, from bacterial colonization and subsequent irritation of the mucosa, may trigger a chronic inflammatory response.³⁰ Chronic antigen exposure may result in accumulation of many mutations in immunoglobulin sequences; however, previously described IgE sequences are relatively unmutated.¹⁵ Likewise, the sequences reported here have few V

region mutations (Table 1, total mutations), suggestive of B cells that have undergone a short proliferative stage. Low relative mutation frequencies in these nasal tissue sequences suggest that IgE-mediated inflammation of the nasal mucosa in CRSwNPs can not be explained by chronic antigenic stimulation alone. Furthermore, antigenic stimulation does not appear to be an adequate explanation for the elevated levels of local IgE seen in CRSwNPs, especially because commensal bacteria are ubiquitous in the mucosal tracts and should presumably also interact with IgE.²⁸ A high proportion of sequences amplified in this study were duplicates, indicating little diversity in the CRSwNP IgE response. Proliferating cells may mutate infrequently, or a small number of unrelated B cells could be up-regulating their IgE expression to produce these duplicates. Although many IgE⁺ cells may be present in the mucosa on staining, the IgE repertoire in CRSwNPs and the range of antigens driving the response appear to be restricted.

Antigen selection results in a characteristic clustering of replace-

Mutation analysis of tissue IgE sequences from CRSwNP individuals

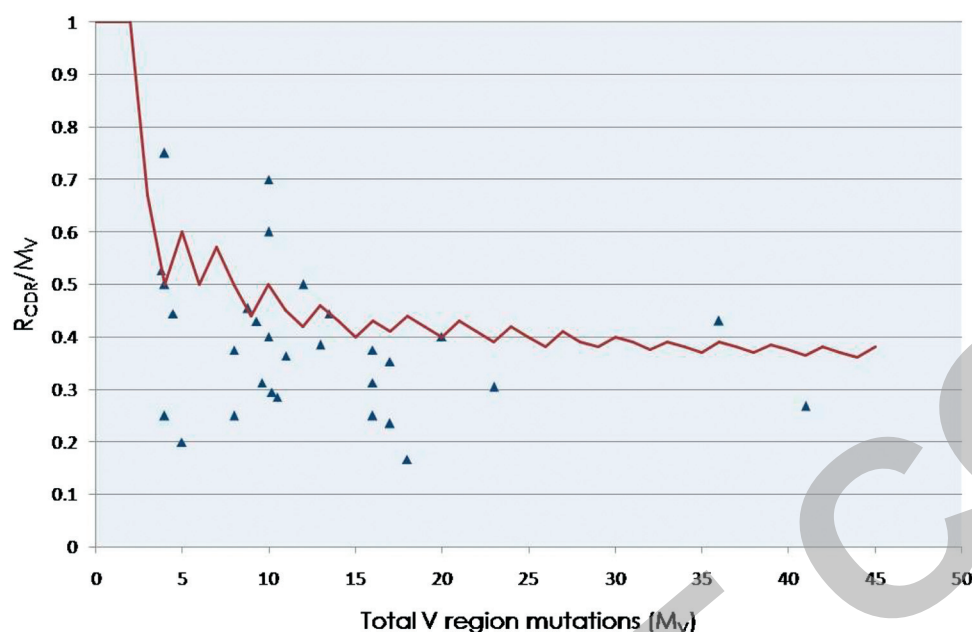


Figure 3. Under the influence of antigen selection, replacement mutations accumulate in the complementarity determining regions (CDRs) of a sequence. Sequences here have been compared with a model of random mutation, whereby the probability that a mutation is random ($p = 0.26$) is used to determine how mutations should accumulate when there is no selection pressure. The upper range of a 95% confidence limit is shown here as a line, and sequences that fall outside this range are likely to be the product of antigen selection.¹⁵ The total number of mutations in a sequence is plotted against the proportion of mutations in the sequence that are replacements in the CDRs to determine whether or not replacements have accumulated in such a way as to suggest selection pressure. Individual IgE sequences are represented here by diamonds; five sequences fall clearly outside the upper confidence limit. The lower confidence limit is not shown. The total number of mutations in a sequence is not related to the influence of antigen selection on that sequence; however, sequences are plotted against total mutation number because the clustering of mutations within CDRs changes as more mutations are accumulated.

Evidence of antigen selection in sequences from comparison datasets

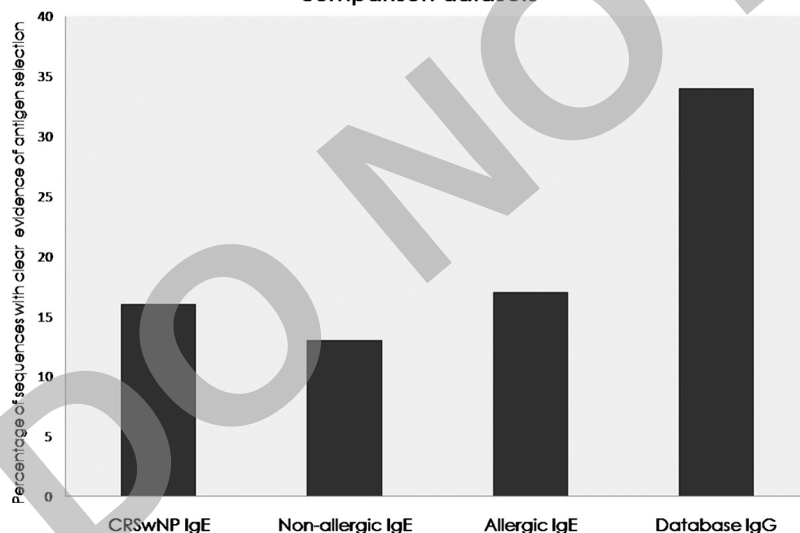


Figure 4. The proportion of sequences showing evidence in antigen selection differs between sequence groups. In the chronic rhinosinusitis with nasal polyp (CRSwNP) IgE data set, 5 of 31 (16%) sequences amplified had clear evidence of antigen selection. This is not significantly different to the proportion seen in either allergic or nonallergic IgE. Compared with a set of database-derived IgG, used as an indicator of what kind of proportions are to be expected when antigen selection is acting on sequences, a significantly higher proportion of IgG sequences (34%) shows evidence of antigen selection ($p < 0.05$).

ment mutations in the CDRs of the immunoglobulin V region gene.¹³ Although earlier IgE studies have investigated whether the distribution of mutations is “as expected,” no point of reference was used to determine what the expected distribution should be.¹⁵ Thus, sequences here are compared with the model described by Dahlke and colleagues, which uses statistical inference to show the expected distribution of mutations, if there were no selection pressure. The upper 95% confidence limit of this model can be graphically represented as a line that sequences are plotted against and those that fall below the line, *i.e.*, within the confidence limits of the model, are not likely to be the product of antigen selection (Fig. 3). Although the sequences amplified in this study do show evidence of mutation (see total mutations, Table 1), few of those mutations suggest selection

pressure. Only five of the sequences show clear evidence of antigen selection, *i.e.*, they fall outside the 95% confidence limit of the random model (Fig. 3). The proportion of sequences with evidence of antigen selection is similar to previously reported IgE sequences from PBMC of nonallergic¹⁵ and allergic individuals.¹⁵ In contrast, IgG-committed cells are understood to mature in germinal centers, where they are exposed to a high degree of selective pressure,¹² and this is apparent in the significantly higher proportion of IgG sequences showing evidence of antigen selection (Fig. 4). Maturation outside germinal centers, possibly along a recently proposed pathway that involves expansion of B cells that have undergone class switching to produce IgE and left the germinal center,³¹ could explain why IgE sequences accumulate random mutations as they proliferate in the absence of the

cellular interactions necessary for continued selection. Such cells, although they do have some mutations, are unlikely to have high antigen-binding affinity. Foci of lymphoid neogenesis and B-cell attractant chemokines have been detected in sinonasal tissues,³² and germinal centerlike structures have been found⁶; however, it is unclear the extent to which organized cellular interactions occur within these structures.

Random mutations likely accumulate with greater frequency when a high antigen burden is imposed on an individual. The IgE sequences reported here have few total mutations and significantly fewer sequences with evidence of antigen selection than is seen in an reference IgG data set.¹⁵ Taken with the growing body of evidence supporting local tissue synthesis of IgE and class switching in mucosal tissue, it is likely that relatively unmutated, low-affinity IgE is the product of local reaction in mucosa-associated lymphoid tissue (MALT). The detection of a pair of clonally related sequences in the same piece of turbinate tissue of one donor in this study, indicative of the presence of B cells with a common clonal ancestor, further supports a local source of IgE production. As such, IgE detected in the nose and paranasal sinuses may be completely unrelated to the allergic status of an individual. With reduced selection pressure during their development, B cells are unlikely to produce IgE antibodies that are highly antigen specific. Previously, the detection of SAE- and fungal-specific IgE in the tissue and serum of CRSwNP patients has led to much focus on the possible role of *S. aureus* or fungal superinfections in the pathogenesis of CRSwNPs. If local mucosal tissue is a source of relatively unspecific IgE, however, antigen-specific IgE found in tissue and blood may be completely unrelated to the disease status of CRSwNP individuals.

CONCLUSION

IgE in the paranasal mucosa may be nothing more than the product of local B-cell interactions with colonizing organisms within the sinuses. The sequence data presented here suggest that the majority of IgE found in CRSwNP tissue develops with little, if any, selection pressure. Consequently, it is unlikely to have high antigen specificity. Whether specific IgE expression is a significant factor in CRSwNP pathogenesis or is merely an epiphenomenon, secondary to local interactions, remains unresolved.

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