

Biased use of V_H5 IgE-positive B cells in the nasal mucosa in allergic rhinitis

Heather A. Coker, PhD,^{a*} Helen E. Harries, MBiochem,^a Graham K. Banfield, FRCS,^b Victoria A. Carr, RGN,^b Stephen R. Durham, MD,^b Elfy Chevetton, FRCS,^c Paul Hobby, MSc,^a Brian J. Sutton, PhD,^a and Hannah J. Gould, PhD^a
London, United Kingdom

Background: IgE antibody-producing B cells are enriched in the nasal mucosa in patients with allergic rhinitis because of local class switching to IgE. The expressed IgE V_H genes also undergo somatic hypermutation *in situ* to generate clonal families. The antigenic driving force behind these events is unknown.

Objective: To examine the possible involvement of a superantigen in allergic rhinitis, we compared the variable (V_H) gene use and patterns of somatic mutation in the expressed IgE heavy-chain genes in nasal biopsy specimens and blood from allergic patients and the IgA V_H use in the same biopsy specimens and also those from nonallergic controls.

Methods: We extracted mRNA from the nasal biopsy specimens of 13 patients and 4 nonallergic control subjects and PBMCs from 7 allergic patients. IgE and IgA V_H regions were RT-PCR amplified, and the DNA sequences were compared with those of control subjects. We constructed a molecular model of V_H5 to locate amino acids of interest.

Results: We observed a significantly increased frequency of IgE and IgA V_H5 transcripts in the nasal mucosa of the allergic patients compared with the normal PBMC repertoire. Within IgE and IgA V_H5 sequences in the nasal mucosa, the distribution of replacement amino acids was skewed toward the immunoglobulin framework regions. Three of 4 nonintrinsic hotspots of mutation identified in the V_H5 sequences were in framework region 1. The hotspots and a conserved V_H5-specific framework residue form a tight cluster on the surface of V_H5. **Conclusion:** Our results provide evidence for the activity of a superantigen in the nasal mucosa in patients with allergic rhinitis. (*J Allergy Clin Immunol* 2005;116:445-52.)

Key words: Human, allergic rhinitis, V_H5, superantigen, B lymphocyte, mucosa

Abbreviations used

CDR: Complementarity-determining region

FWR: Framework region

R/S: Replacement/silent mutation ratio

IgE and its receptors are central to allergic disease, manifested in different target organs, including the nose (allergic rhinitis), the lung (allergic asthma), the skin (atopic dermatitis), and the gut (allergic gastroenteritis). IgE binds to effector and antigen-presenting cells bearing IgE receptors (FcεRI, CD23, or both) in mucosal tissues associated with the target organs mediating the allergic response. In addition, IgE-mediated allergen presentation to T helper cells might lead to renewed IgE antibody synthesis, epitope spreading, and exacerbation of allergic sensitivity. A large number of genetic and environmental risk factors for allergy have been identified, providing broad insight into the pathogenesis of allergic disease. The factors that determine the susceptible target organ in different individuals exposed to the same allergens are, however, unknown. We suggest that these factors might be localized in the target organ and might include superantigens.

Snow and coworkers¹⁻⁵ have observed a bias in the repertoire of IgE heavy-chain variable (IgE V_H) regions in asthma, which exhibited the hallmarks of a superantigen. There are 51 V_H genes grouped into 7 gene families (V_H1-V_H7), varying in size from 22 members in V_H3 to 1 or 2 members in V_H5, V_H6, and V_H7.⁶ Each V_H region has 3 framework regions (FWR1-FWR3) alternating with 3 complementarity-determining regions (CDR1-CDR3). The FWRs determine the structural framework for the antigen-binding sites of the CDRs. The CDR sequences are inherently more prone to somatic hypermutation during affinity maturation of antibodies in the immune response, whereas the FWR sequences are relatively conserved.⁷ In peripheral blood B cells of healthy individuals, the proportion of expressed V_H regions from different V_H families generally reflects the size of the family.⁸ In asthma, however, Snow and coworkers found an overabundant use of the minor V_H5 family in IgE in the blood, lung mucosa, and spleen of asthmatic patients¹⁻³

From ^aThe Randall Division of Cell and Molecular Biophysics, King's College London; ^bUpper Respiratory Medicine, National Heart and Lung Institute, Imperial College, London; and ^cthe Department of Respiratory Medicine and Allergy, Guy's Hospital, London.

*Dr Coker is currently affiliated with Clare Hall Laboratories, Cancer Research UK, South Mimms, Hertfordshire, United Kingdom.

Supported by the Clinical Research Committee Royal Brompton and Harefield Hospitals NHS Trust. HAC and HEH were supported by BBSRC PhD studentships, and HG and SRD were supported by project grants from Asthma UK (grant no. 03/055) and the MRC (grant no. G0200485).

Received for publication August 25, 2004; revised March 23, 2005; accepted for publication April 22, 2005.

Available online June 17, 2005.

Reprints of this article are not available from the authors.

0091-6749/\$30.00

© 2005 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2005.04.032

and in the blood of the majority of 6 asthmatic patients.⁵ Overabundant use of V_H5 was also reported in the blood of 3 patients with atopic dermatitis⁹ but not in another study of 2 patients with atopic dermatitis.¹⁰ By contrast, in the blood of 2 subjects with peanut allergy, Snow and coworkers observed an overabundant use of V_H1, suggesting the activity of a peanut-associated superantigen.¹¹

B-cell superantigens, similar to T-cell superantigens, act by binding to immunoglobulin FWRs, leading to clonal amplification of all members of the family. Because the number of CDRs in the B-cell repertoire (millions) is vastly greater than the number of V_H families (7), this results in biased use of the selected family.¹² Two previously identified B-cell superantigens, *Staphylococcus aureus* protein A and HIV gp120, selectively expand B cells expressing V_H3.¹²⁻¹⁴ The interaction of protein A with the FWR of V_H3 can be seen in the crystal structure of a complex with the V_H3 Fab fragment of an IgM antibody.¹⁵ Superantigen-selected B cells might also exhibit a skewed distribution of amino acid substitutions away from CDRs toward the FWR.^{7,16} The frequent occurrence of point mutation hotspots in DNA sequences that are intrinsically more susceptible to hypermutation is a further criterion for CDR- versus FWR-oriented selection; the sequences WRC and WA, which occur more frequently in the CDRs, are recognized as intrinsic hotspots of mutation.¹⁷ These 2 features of superantigen selection of B cells were also linked to the V_H5 overabundance in asthma and atopic dermatitis,^{2,9} which is consistent with the influence of a superantigen and in contrast to the lack of such influence observed in V_H5 B cells from normal spleen.¹⁸

In a previous study of the expressed V_H regions in allergic rhinitis, we presented evidence of local clonal expansion, somatic hypermutation, and class switching in the nasal mucosa in patients with allergic rhinitis.¹⁹ In this study we present a detailed analysis of IgE and IgA V_H family use in the nasal mucosa in patients with allergic rhinitis.

METHODS

Samples from patients with allergic rhinitis and nonallergic control subjects

Male and female donors with allergic rhinitis aged between 18 and 55 years were recruited for this study. The allergic status of the donors was assessed on the basis of medical history, skin prick tests, and, where possible, serum allergen-specific IgE (RAST). Of the 11 tissue samples from the nasal mucosa, 10 originated from patients with multiple allergies (CD6, JB7, CM10, HD14, SO16, AP19, SJ24, TL25, CA30, and SLT1) who were allergic to grass and also allergens such as animal dander or house dust mite, to which they could be perennially exposed. One tissue sample (HD17) originated from a patient with only grass pollen allergy. The samples were taken throughout the year, with the patient with only grass pollen allergy undergoing biopsy within the grass pollen season. Of the 11 nasal mucosa samples analyzed, 10 were biopsy specimens taken from the inferior turbinate, and one (SLT1) consisted of a piece of an inferior turbinate removed by surgery to alleviate nasal obstruction. In

agreement with previous authors,^{9,19} we were unable to use healthy subjects as a control group for IgE because PCR amplification of IgE was only successful in the allergic subjects. Instead, we recruited 4 nonallergic subjects (AA2, MTS3, KB5, and SK6) and used biopsy specimens from 2 of the allergic patients (TL25 and CA30) plus 2 additional patients with multiple allergies (JC1 and IB4) to examine V_H use in IgA.

Volunteers were recruited from the Royal Brompton Hospital Allergy Clinic or by advertisement in the local press to donate a nasal biopsy specimen. None had received immunotherapy, and any medication was discontinued at least 2 weeks before nasal biopsy. Biopsies were performed at the Royal Brompton Hospital, London, United Kingdom, and processed as described previously.²⁰ All such work had the approval of the local ethics committee and the patients' written informed consent. Blood samples were taken from 7 of the 16 patients who also donated nasal biopsy specimens (CD6, JB7, CM10, HD14, SO16, HD17, and AP19). PBMCs, including B cells, were isolated from these samples, as described previously.¹⁹ The tissue sample from the inferior turbinate resulted from operations performed at Guy's Hospital, London, United Kingdom, with the approval of the Guy's Research Ethics Committee and also with the patient's written informed consent.

Amplification and analysis of V_H region sequences

Total RNA was extracted from both the nasal mucosa and PBMC samples, cDNA was produced, and V_H-C ϵ sequences were PCR amplified by using the proofreading Pfu DNA polymerase (Promega, Madison, Wis) from IgE-positive B cells. The V_H-C ϵ PCR products were then cloned and sequenced. This entire procedure has been described in detail previously.¹⁹ V_H-C α sequences were amplified from cDNA in the same way as V_H-C ϵ sequences, replacing the C ϵ -specific primers with nested primers specific for C α : C α 1, 5'-TTTCGCTCCAGGTCACAC-3'; C α 2, 5'-GGGAAGAAGCCCTGGACCAGGC-3'. The annealing temperature for the second round of PCR was adjusted from 65°C to 69°C. Assignment of the V_H genes and their somatic mutations was carried out according to their homology with the germline sequences detailed on the VBase database (www.mrc-cpe.cam.ac.uk).

Only unique sequences were included in the analysis, meaning that repeat copies of identical sequences and also sequences that originated from clonally related B cells (determined by an identical CDR3/FWR4 signature region) were included only once in the analysis. However, when sequences did originate from related B cells, each unique mutation isolated from the family members was included in the mutational analysis.

Statistical significance was determined by the use of χ^2 analysis with the Yates correction for continuity (generating a more conservative calculation of significance when smaller data values are used).

Molecular modeling of V_H5 antibody structure

Because no V_H5 antibody crystal structure is available, a model was generated for the V_H5-51 germline sequence on the basis of the known structure of the most closely matched antibody (PDB code: 1CGS). The heavy-chain CDR1 and CDR2 (H1 and H2) loop lengths were identical in both antibody sequences, and the heavy-chain CDR3 (H3) loop and the light chain (V_L) domain structures were taken directly from 1CGS to produce a complete Fv model. No steric clashes were detected after substitution of the V_H5-51 sequence. The model was generated by using HOMOLGY and displayed with INSIGHT II (Accelrys, Cambridge, United Kingdom).

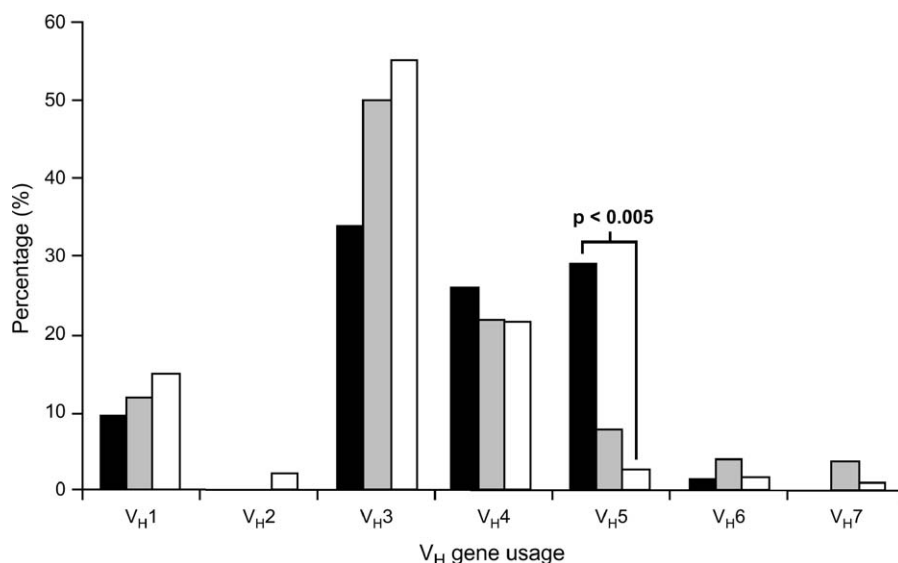


FIG 1. V_H gene use in allergic nasal mucosa IgE-positive B cells. Sixty-two sequences from 11 allergic nasal mucosa samples (filled bars) and 50 sequences from 7 allergic PBMC samples (gray bars) were pooled to compare V_H gene use. V_H gene use in normal PBMCs (open bars) was also included for comparison.²¹

RESULTS

IgE V_H gene use

We examined a total of 62 in-frame V_H region sequences derived from the nasal mucosa of the 11 patients with allergic rhinitis* and 50 V_H region sequences from the PBMCs of 7 of these patients.† There was a clear difference in the V_H gene use observed in IgE B cells from the nasal mucosa of the allergic patients compared with that expected on the basis of the normal genomic PBMC repertoire²¹ and also compared with that observed in IgE-positive PBMCs from the allergic patients (Fig 1).²¹

There was a highly significant decrease in the use of V_H3 by IgE-positive B cells in the allergic nasal mucosa (34%) compared with that expected on the basis of the normal repertoire in PBMCs (55%) observed by previous workers ($P < .005$, χ^2 analysis).²¹ This was not, however, significant when compared with that observed in the allergic PBMCs (50%; $P > .1$, χ^2 analysis).

There was also a highly significant increase in the use of V_H5 in the nasal mucosa (29%) compared with that observed by previous researchers in normal PBMCs (2.9%; $P < .005$, χ^2 analysis). The increased use of V_H5 in the allergic nasal mucosa was significant when compared with the 8% V_H5 use observed in the allergic IgE-positive PBMCs ($P < .025$, χ^2 analysis). There was no significant difference in the use of V_H5 in the allergic PBMCs compared with that expected on the basis of the normal PBMC repertoire (8% vs 2.9%; $P > .1$, χ^2 analysis).

These data were taken from sequences pooled from multiple patients. The occurrence of V_H5 sequences across the PBMC samples appeared to be evenly distributed (with the 4 V_H5 sequences occurring in 4 of the 7 different PBMC samples). This was in contrast to the distribution of V_H5 sequences in the samples from the nasal mucosa, in which the 18 V_H5 sequences were isolated from only 5 of the 11 patients. There was a particularly high level of V_H5 use in the nasal mucosa of patient CA30, in whom 11 of 18 sequences were from unrelated B cells expressing V_H5 IgE-positive sequences. However, even when this patient was excluded from the analysis, the increased V_H5 IgE use (14%) in the allergic nasal mucosa compared with that expected on the basis of the normal PBMC repertoire was still highly significant ($P < .005$, χ^2 analysis), although the significance between the allergic nasal mucosa and allergic PBMCs was lost ($P > .1$, χ^2 analysis).

Hotspots of mutation in IgE V_H5 sequences from the allergic nasal mucosa

All of the sequences isolated from nasal biopsy specimens exhibited evidence of somatic hypermutation. Somatic mutations evident within 17 distinct V_H5 sequences were compared with those from 19 randomly chosen non-V_H5 sequences to determine whether there was evidence to suggest that the overuse of V_H5 in the allergic nasal mucosa might have been a consequence of B-cell selection (eg, by the presence of nonintrinsic hotspots of mutation).

The percentage variability of each codon was used to identify apparent hotspots of somatic hypermutation (Fig 2). The percentage mutation (including both silent and replacement mutations) and the percentage mutation at each nucleotide were also analyzed (data not shown),

*Nucleotide sequences submitted to Genbank, accession numbers AY640472–AY640533.

†Nucleotide sequences submitted to Genbank, accession numbers AY640534–AY640583.

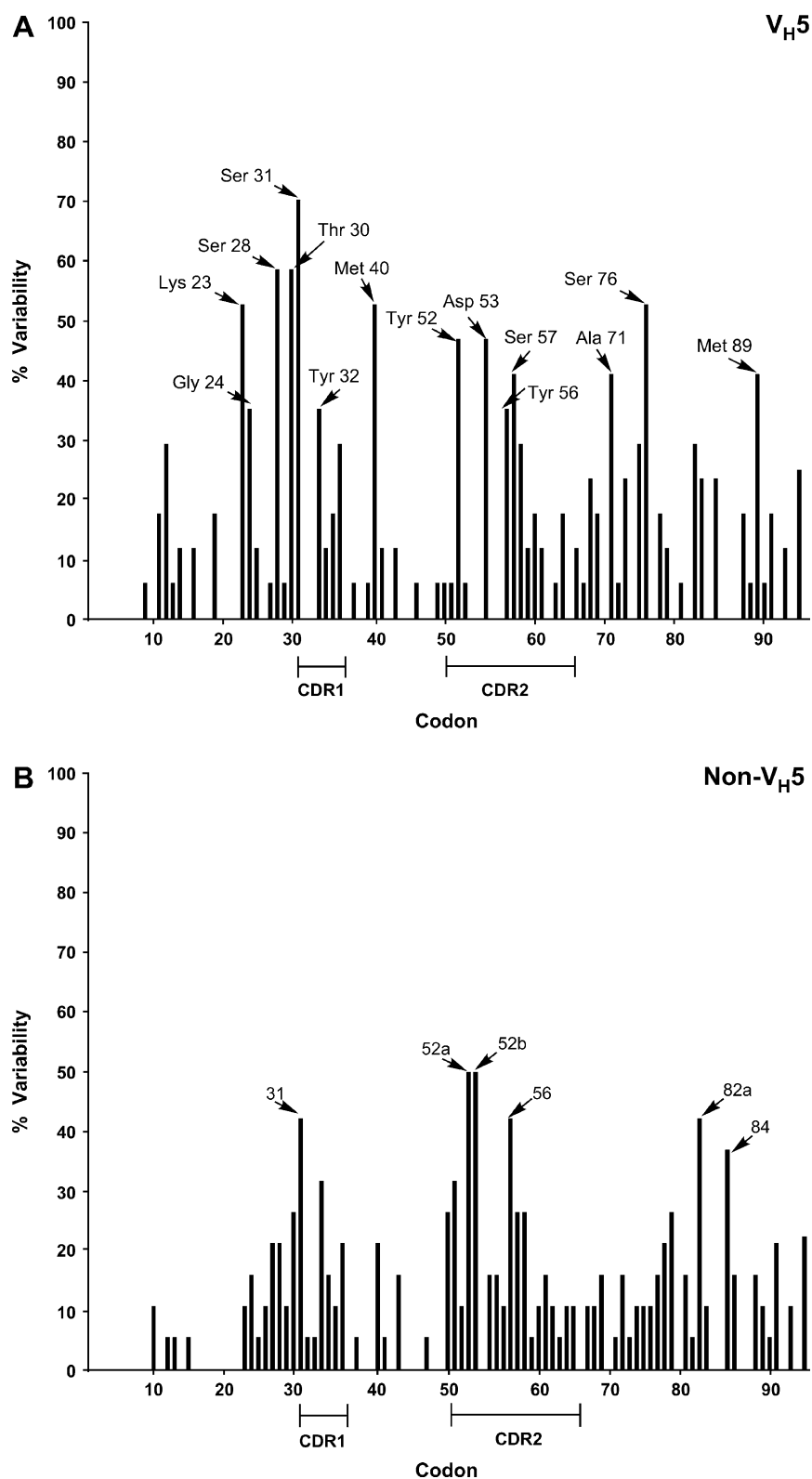


FIG 2. Distribution of somatic mutations across IgE V_H . **A**, Somatic mutations identified in 17 allergic nasal mucosa V_H5 sequences were pooled. Hotspots of mutation were identified on the basis of the percentage variability at each codon. **B**, Nineteen non- V_H5 sequences randomly chosen from the same cohort of allergic patients were subjected to the same analysis.

although neither identified any further hotspots of mutation to that evident from Fig 2.

Of the hotspots of mutation identified in Fig 2, those at Met 40 and Tyr 56 in the V_H5 sequences and at codons 52a, 56, and 84 in the non-V_H5 sequences, when examined in more detail, were disregarded because the mutations were spread evenly across the 3 nucleotide positions of the codon and had very different effects on the amino acid. The apparent hotspots at codon 52b and also codon 82a in the non-V_H5 sequences were also disregarded because codon 52b was mutated in both of only 2 sequences that used this polymorphic codon, and the mutations at codon 82a were divided between 3 Asn and 3 Ser residues, such that there were insufficient mutations of each for analysis.

The exact nature of the remaining apparent hotspots of mutation was examined in detail to determine whether the direction of mutation at each codon reflected the accepted trends of somatic hypermutation, as defined by previous authors.²² Those mutations that conformed to such a profile were defined as intrinsic, whereas those that clearly differed were defined as nonintrinsic and therefore likely to have been selected in response to antigen (Table I).²²

The only hotspot apparent in the non-V_H5 sequences was a commonly identified hotspot, Ser 31,²² which was classified as intrinsic. In contrast, although 9 of the 13 remaining hotspots identified in the V_H5 sequences also appeared to be intrinsic in nature, importantly, 4 apparent nonintrinsic hotspots were also identified. This is consistent with the non-V_H5 IgE molecules having been targeted toward a wide range of antigens, whereas the identification of nonintrinsic hotspots of mutation in the V_H5 sequences suggests that the V_H5 IgE molecules were targeted toward a limited number of antigens. The 4 nonintrinsic mutations evident in the V_H5 sequences were present at Lys 23(1), Gly 24(2), and Thr 30(2), each in FWR1, and also at Tyr 52(2), which was found in CDR2. According to Kabat's numbering, CDR1 incorporates codons 31 through 35, and CDR2 incorporates codons 50 through 65. FWR1 incorporates codons 1 through 30, FWR2 incorporates codons 36 through 49, and FWR3 incorporates codons 66 through 95.

Analysis of replacement/silent mutation ratio values

Because 3 of the 4 apparently nonintrinsic hotspots of mutation in the V_H5 sequences were unconventionally present in the framework region, further analysis of the ratio of replacement to silent mutations (R/S values) in the different sequences was carried out. The non-V_H5 group of sequences exhibited R/S values that were consistent with conventional antigen selection, with a significant difference between the CDR and the FWR (CDR, 3.46; FWR, 1.67; $P < .025$, χ^2 analysis). In stark contrast, there was no significant difference between the R/S values in the CDR and the FWR of the V_H5 group of sequences (CDR, 2.38; FWR, 2.06; $P > .1$, χ^2 analysis).

Because the V_H5 FWR R/S value was higher than expected and also because 3 of the 4 nonintrinsic muta-

TABLE I. The direction of somatic mutation at hotspots in V_H IgE sequences from the allergic nasal mucosa suggests the presence of 4 nonintrinsic hotspots in V_H5 sequences

Hotspot	From	To		O*	E†	
V _H 5						
Lys 23(1)	AAG	CAG	Gln	6	2	Nonintrinsic
		GAG	Glu	2	4	
		TAG	Stop	0	2	
Gly 24(2)	GGT	GAT	Asp	0	2	Nonintrinsic
		GCT	Ala	5	0	
		GTT	Val	1	4	
Ser 28(3)	AGC	AGA	Arg	3	1	
		AGG	Arg	2	2	
		AGT	Ser	4	6	
Thr 30(2)	ACC	AAC	Asn	1	1	Nonintrinsic
		AGC	Ser	4	1	
		ATC	Ile	2	5	
Ser 31(2)	AGC	AAC	Asn	7	7	
		ACC	Thr	5	4	
		ATC	Ile	0	1	
Ser 31(3)	AGC	AGA	Arg	1	1	
		AGG	Arg	0	1	
		AGT	Ser	6	5	
Tyr 32(3)	TAC	TAA	Stop	0	0	
		TAG	Stop	0	1	
		TAT	Tyr	4	3	
Tyr 52(2)	TAT	TCT	Ser	1	1	Nonintrinsic
		TGT	Cys	1	4	
		TTT	Phe	5	2	
Asp 53(3)	GAT	GAA	Glu	4	2	
		GAC	Asp	2	4	
		GAG	Glu	1	1	
Ser 57 (3)	AGC	AGA	Arg	0	1	
		AGG	Arg	0	1	
		AGT	Ser	7	5	
Ala 71(2)	GCC	GAC	Asp	0	1	
		GGC	Gly	0	1	
		GTC	Val	6	4	
Ser 76(2)	AGC	AAC	Asn	6	4	
		ACC	Thr	1	2	
		ATC	Ile	0	1	
Met 89(3)	ATG	ATA	Ile	4	3	
		ATC	Ile	0	2	
		ATT	Ile	1	0	
Non-V _H 5						
Ser 31(2)	AGC	AAC	Asn	5	3	
		ACC	Thr	0	2	
		ATC	Ile	0	0	

*O denotes the observed number of somatic mutations.

†E denotes the expected direction of mutation on the basis of previous research.²²

tions were present in FWR1, analysis of the R/S values of the individual FWRs in the V_H5 group of sequences was also carried out. FWR1 exhibited an R/S value of 2.16, FWR2 exhibited an R/S value of 1.06, and FWR3 exhibited an R/S value of 2.61, suggesting that although FWR2 was conventionally conserved in the V_H5 sequences, FWR1 and FWR3 contributed to the unusual overall FWR R/S value observed in the V_H5 sequences.

TABLE II. Regional diversification of IgE and IgA V_H sequences

Isotype	Biopsy specimen	Number of sequences							Total
		V _H 1	V _H 2	V _H 3	V _H 4	V _H 5	V _H 6	V _H 7	
IgE	CA30A	0	0	0	0	11	0	0	11
	CA30B	0	0	1	3	0	0	0	4
IgA	CA30A	1	0	8	1	2	0	0	12
	CA30B	2	0	6	1	0	1	0	10

IgA V_H gene use

If a superantigen was involved in the biased use of V_H5, this might be apparent in isotypes other than IgE. We first examined this possibility by using 2 adjacent biopsy specimens (CA30A and CA30B) from patient CA30.

As shown in Table II, all 11 IgE V_H sequences from biopsy specimen A but none of the 4 sequences from biopsy specimen B were V_H5. IgA exhibited a similar pattern, with 2 of 12 IgA V_H sequences in biopsy specimen A but none of 10 in biopsy specimen B being V_H5. Combining IgE and IgA results, 57% (13/37) of sequences in biopsy specimen A were V_H5, whereas 0% (0/14) of sequences in biopsy specimen B was V_H5. None of 13 V_H5 sequences in CA30 were related to any of the others, suggesting polyclonal activation of B cells expressing V_H5 sequences locally in the region of biopsy specimen A. The results for CA30 demonstrate the linkage between V_H5 bias in IgE and IgA and suggest that a B-cell superantigen might influence the selection of V_H in a local manner.

The FWR and CDR R/S values for IgA V_H5 and non-V_H5 were determined and compared. R/S values for IgA non-V_H5 sequences in CA30 were comparable with those of IgE non-V_H5 sequences (CDR, 4; FWR, 1.4) and thus indicative of normal antigen selection. However, as observed for IgE V_H5, the FWR and CDR R/S values for IgA V_H5 sequences were similar (CDR, 4; FWR, 4.3).

On analysis of 73 IgA V_H sequences[‡] in 2 of the biopsy specimens from the original cohort (TL25 and CA30) plus 2 additional biopsy specimens (JC1 and IB4) from allergic patients, we observed V_H5 to be significantly higher than in normal PBMCs (14% vs 2.9%; $P < .005$, χ^2 analysis).²¹ The greater abundance of IgA-expressing compared with IgE-expressing B cells in the nasal mucosa allowed us to determine the IgA V_H use in the nasal mucosa of healthy nonallergic subjects. Of 55 sequences§ from 4 nonallergic donors, we found a 9% frequency of V_H5, which is also significantly different from that seen in normal PBMCs ($P < .05$, χ^2 analysis).²¹

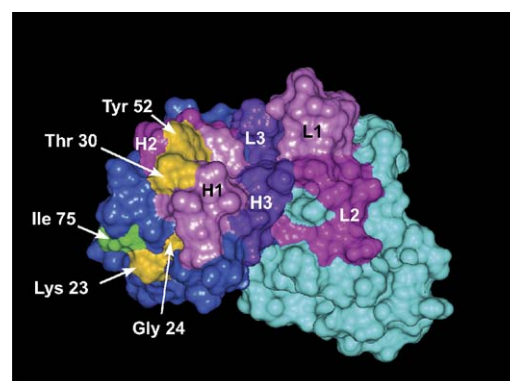


FIG 3. Location of nonintrinsic hotspots in the 3-dimensional structure. Hotspots Lys23, Gly24 and Thr30 (FWR1), and Tyr52 (CDR2) in V_H5 are shown in yellow on a homology model of V_H5-51. Exposed hydrophobic residue Ile75 is shown in green. CDRs are indicated, encompassing both Kabat and Chothia²⁴ definitions. The remaining V_H and V_L FWRs are shown in dark and light blue.

Location of nonintrinsic hotspots in the structure of IgE V_H5

The 4 nonintrinsic hotspots occurred at Lys 23(1), Gly 24(2), Thr 30(2), and Tyr 52(2). Of these, 3 were unusually situated within FWR1, with only Tyr 52(2) in CDR2. Therefore it would seem likely that the putative superantigen has contact with FWR1 and CDR2, although the R/S values imply that FWR3 might also be involved. The B-cell superantigen protein A has been shown to bind similarly to V_H3, interacting with FWR1, FWR3, and CDR2.^{12,13,15,23}

Because no crystal structure of a V_H5 is available, we constructed a model on the basis of the known structure of the most closely matched antibody. There are 2 V_H5 genes, V_H5-51 and V_H5-a, but only the former is expressed in the majority of the population. Fig 3²⁴ shows Lys 23, Gly 24, and Thr 30 in FWR1 and Tyr 52 in CDR2 in our model of V_H5-51.

When the locations of the 4 nonintrinsic hotspots are identified within this model, it is immediately apparent that they are clustered together at the edge of the conventional antigen-binding site, as defined by the 6 CDRs. Lys 23 and Gly 24 are adjacent to CDR1, and Thr 30 lies at the very boundary between FWR1 and CDR1; Tyr 52 lies at the boundary between FWR2 and CDR2. Also shown in Fig 3 is residue Ile 75, a hydrophobic surface residue in an unusually exposed location but conserved in the vast majority of V_H5 sequences. In fact, all V_H2, V_H3, V_H4, and V_H6 germline sequences have lysine at this position, whereas it is isoleucine only in V_H5. This cluster of residues might thus define at least a part of the site at which a putative superantigen might interact with IgE V_H5.

DISCUSSION

In our previous study of somatic hypermutation in V_H sequences in IgE-expressing B cells in the nasal mucosa of patients with allergic rhinitis, we observed that 2 of a total

[‡]Nucleotide sequences submitted to Genbank, accession numbers AY971069-AY971142

[§]Nucleotide sequences submitted to Genbank, accession numbers AY971012-AY971068

of 3 clonal families were derived from the V_H5 family, and we detected an IgA V_H5 clone related to one of the families.¹⁹ This hinted at a biased use of V_H5 in the tissue similar to that found in asthma and atopic dermatitis.^{1-5,9} We have therefore analyzed V_H sequences from a large cohort of patients with allergic rhinitis to test this hypothesis.

Our results have revealed a significant bias toward V_H5, even when one biopsy (CA30), which exhibited the most extreme bias, was excluded. A similar trend in the PBMCs from a subgroup of these patients did not reach statistical significance. The greater bias in tissue suggests that a local event caused the V_H5 bias. The similar trend in PBMCs might result from the displacement of V_H5-expressing B cells from the tissue and dilution in the pool of normal B cells in the circulation. IgA V_H sequences also exhibited a significant but less extreme bias toward V_H5 compared with that seen in IgE sequences. The demonstration of V_H5 bias in both IgE and IgA and colocalization in the nasal mucosa support the superantigen hypothesis. The putative superantigen might bind to the subset of B cells expressing V_H5, leading to selective proliferation or rescue from apoptosis. The smaller bias in IgA V_H5 might be due to the T_H2 environment of the nasal mucosa, which favors class switching to IgE in rapidly dividing B cells. We observed an even smaller IgA V_H5 bias in the normal nasal mucosa, which might hint that the bias precedes the development of allergy. Allergen-specific B cells might be further selected from this population.

Although there was a significant IgE V_H5 bias in the 62 sequences analyzed, these sequences came from a minority (5/11) of the patient population. This must not be taken to indicate that a superantigen is likely to have been acting in only the 5 patients, however. We have demonstrated previously¹⁹ and again here, exemplified by the comparison of biopsy specimens A and B from patient CA30, that there are not necessarily any identical sequences or even clonal relationships between cells in adjacent 1- to 2-mm³ biopsy specimens. These biopsy specimens (10-20 mg) are less than 1% of the inferior turbinates (average weight, approximately 2 g). Thus sequences derived from a single biopsy specimen are not necessarily representative of the tissues in individual patients. Our V_H5 bias is based on sampling a population of patients. The absence of V_H5 sequences in 6 of 11 of the biopsy specimens does not imply that there are none elsewhere in the tissue. Superantigens acting in other regions of the tissue might still contribute to the “V_H5-negative patients” symptoms of allergy.

Our comparison of V_H5 and non-V_H5 R/S values and the positions of the nonintrinsic mutations within FWR, notably FWR1, provide additional evidence to support the superantigen hypothesis. Moreover, the 3 nonintrinsic hotspots in FWR1, along with one at the edge of CDR2, are clustered in our model of V_H5. These mutations might increase the affinity of the putative superantigen for V_H5. Similar interactions involving the edge of the conventional antigen-binding site and adjacent FWR residues have also been observed in other superantigen-antibody complexes:

antibodies that recognize the carbohydrate I/i red blood cell antigen are restricted to the V_H4-34 gene, and residues 23-25 in FWR1 are implicated in binding.²⁵ In addition, the crystal structure of the complex between a rheumatoid factor antibody and its autoantigen, IgG Fc, involved residues at the FWR1/CDR1 and FWR2/CDR2 boundaries, enabling the CDR to remain available for conventional antigen binding.^{26,27}

Remarkably, exactly the same features that we have observed in IgE V_H5 in allergic rhinitis were previously reported in asthma.¹⁻⁵ For example, V_H5 represented 29% and 33% of the IgE sequences in this allergic rhinitis study and a previous asthma study,² respectively. Exactly the same nonintrinsic hotspots, Lys 23(1), Gly 24(2), Thr 30(2), and Tyr 52(2), were observed in asthma¹ as in this study of allergic rhinitis but differing from those in atopic dermatitis (Gly 35 and Thr 95).²² The similarities between allergic rhinitis and asthma might not be surprising, given that the upper and lower airways are physically contiguous and exposed to some of the same aeroallergens, but are important, given the differing susceptibility of some individuals to asthma and others to rhinitis and that many individuals have both conditions. It might be fortuitous that the same minor V_H family appears to be overexpressed in both allergic rhinitis-asthma and (at least in one of the 2 studies) atopic dermatitis. The selection of different FWR mutations in the IgE V_H5 in atopic dermatitis, however, might point to the action of different superantigens.

What might be the putative B-cell superantigen associated with allergic rhinitis? About 37% of the population carries *Staphylococcus aureus* in the nasal mucosa.²⁸ Certain *S aureus* enterotoxins are characterized as T-cell superantigens that stimulate B-cell proliferation, IgE synthesis, or both.²⁹⁻³¹ Some of these or others might act as B-cell superantigens. If a B-cell superantigen binds as we suspect to FWR1 of V_H5, an allergen might still bind to the CDR on the immunoglobulin. This could have important consequences for the activation of B cells expressing V_H5 and also the mast cells and dendritic cells that capture IgE V_H5 in the tissue.

We thank Drs Rebecca Beavil, Pooja Takhar, Lyn Smurthwaite, and Graham Dunn (King's College London) for helpful discussions and practical advice.

REFERENCES

1. Snow RE, Chapman CJ, Frew AJ, Holgate ST, Stevenson FK. Pattern of usage and somatic hypermutation in the V_H5 gene segments of a patient with asthma: implications for IgE. *Eur J Immunol* 1997;27:162-70.
2. Snow RE, Djukanovic R, Stevenson FK. Analysis of immunoglobulin E V_H transcripts in a bronchial biopsy of an asthmatic patient confirms bias towards V_H5, and indicates local clonal expansion, somatic mutation and isotype switch events. *Immunology* 1999;98:646-51.
3. Snow RE, Chapman, Frew AJ, Holgate ST, Stevenson FK. Analysis of Ig V_H region genes encoding IgE antibodies in splenic B lymphocytes of a patient with asthma. *J Immunol* 1995;154:5576-81.
4. Snow RE, Chapman CJ, Holgate ST, Stevenson FK. Clonally related IgE and IgG4 transcripts in blood lymphocytes of patients with asthma reveal differing patterns of somatic mutation. *Eur J Immunol* 1998;26:3354-61.

5. Snow RE, Chapman CJ, Stevenson FK. Allergen recognition sites in immunoglobulin E from patients with asthma. In: Holgate ST, Busse WW, editors. Lung biology in health and disease. New York: Marcel Dekker Inc; 1989. p. 323-41.
6. Cook GP, Tomlinson IM. The human immunoglobulin V_H repertoire. *Immunol Today* 1995;16:237-42.
7. Chang B, Casali P. The CDR1 sequences of a major proportion of human germline Ig V_H genes are inherently susceptible to amino acid replacement. *Immunol Today* 1994;15:367-73.
8. Brezinschek H-P, Brezinschek RI, Lipsky PE. Analysis of the heavy chain repertoire of human peripheral B cells using single-cell polymerase chain reaction. *J Immunol* 1995;155P:190-202.
9. Van der Stoep N, Van der Linden J, Logtenberg T. Molecular evolution of the human immunoglobulin E response: high incidence of shared mutations and clonal relatedness among ϵ V_H5 transcripts from three unrelated patients with atopic dermatitis. *J Exp Med* 1993;177:99-107.
10. Edwards MR, Brouwer W, Choi CHY, Ruhno J, Ward RL, Collins AM. Analysis of IgE antibodies from a patient with atopic dermatitis: biased V gene usage and evidence for polyreactive IgE heavy chain complementarity-determining region 3. *J Immunol* 2002;168:6305-13.
11. Janezic A, Chapman CJ, Snow RE, Hourihane JO, Warner JO, Stevenson FK. Immunogenetic analysis of heavy chain variable regions of IgE from patients allergic to peanuts. *J Allergy Clin Immunol* 1998;101:391-6.
12. Silverman GJ, Goodyear CS. A model B-cell superantigen and the immunobiology of B lymphocytes. *Clin Immunol* 2002;102:117-34.
13. Potter KN, Li Y-C, Capra JD. Staphylococcal protein A simultaneously interacts with framework region 1, complementarity determining region 2, and framework region 3 on human V_H3 encoded immunoglobulins. *J Immunol* 1996;157:2982-8.
14. Berberian L, Goodlick L, Kipps TJ, Braun J. Immunoglobulin V_H3 gene products: natural ligands for HIV gp120. *Science* 1993;261:1588-91.
15. Graille ME, Stura A, Corper AL, Sutton BJ, Taussig MJ, Charbonnier J-B, et al. Crystal structure of a Staphylococcus aureus protein A domain complexed with the Fab fragment of a human IgM antibody: structural basis for recognition of B-cell receptors and superantigen activity. *Proc Natl Acad Sci U S A* 2000;97:5399-404.
16. Shlomchik MJ, Marshak-Rothstein A, Wolfowicz CB, Rothstein TL, Weigert MG. The role of clonal selection and somatic mutation in autoimmunity. *Nature* 1987;328:805-11.
17. Rogozin IB, Pavlov YI. Theoretical analysis of mutation hotspots and their DNA sequence context specificity. *Mut Res* 2003;544:65-85.
18. Ellyard JJ, Avery DT, Phan TG, Hare NJ, Hodgkin PD, Tangye SG. Antigen-selected, immunoglobulin-secreting cells persist in human spleen and bone marrow. *Blood* 2004;103:3805-12.
19. Coker HA, Durham SR, Gould HJ. Local somatic hypermutation and class switch recombination in the nasal mucosa of allergic rhinitis patients. *J Immunol* 2003;171:5602-10.
20. Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, et al. Cytokine messenger RNA expression for IL-3, IL-4, IL-5 and granulocyte/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. *J Immunol* 1992;48:2390-4.
21. Brezinschek H-P, Dörner T, Monson NL, Brezinschek RI, Lipsky PE. The influence of CD40-CD154 interactions on the expressed human VH repertoire: analysis of V_H genes expressed by individual B cells of a patient with X-linked hyper-IgM syndrome. *Int Immunol* 2000;12:767-75.
22. Betz AG, Neuberger MS, Milstein C. Discriminating intrinsic and antigen-selected mutational hotspots in immunoglobulin V genes. *Immunol Today* 1993;14:405-11.
23. Randen I, Potter KN, Li Y, Thompson KM, Pascual V, Forre O, et al. Complementarity-determining region 2 is implicated in the binding of staphylococcal protein A to human immunoglobulin V_HIII variable regions. *Eur J Immunol* 1993;23:2682-6.
24. Chothia C, Lesk AM. Canonical structures for the hypervariable regions of immunoglobulins. *J Mol Biol* 1987;196:901-17.
25. Potter KN, Hobby P, Klijn S, Stevenson FK, Sutton BJ. Evidence for involvement of a hydrophobic patch in framework region 1 of human V4-34-encoded immunoglobulins in recognition of the red cell I antigen. *J Immunol* 2002;16:3777-82.
26. Corper AL, Sohi MK, Bonagura VR, Steinitz M, Jefferis R, Feinstein A, et al. Structure of human IgM rheumatoid factor Fab bound to its autoantigen IgG Fc reveals a novel topology of antibody-antigen interaction. *Nat Struct Biol* 1997;4:374-81.
27. Sutton BJ, Corper AL, Bonagura V, Taussig MJ. The structure and origin of rheumatoid factors. *Immunol Today* 2000;21:177-83.
28. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev* 1997;10:505-20.
29. Hofer MF, Harbeck RJ, Schlievert PM, Leung DYM. Staphylococcal toxins augment specific IgE responses by atopic patients exposed to allergen. *J Invest Dermatol* 1999;112:171-6.
30. Jabara HH, Geha RS. The superantigen syndrome toxin-1 induces CD40 ligand expression and modulates IgE isotype switching. *Int Immunol* 1996;8:1503-10.
31. Bachert C, Van Zele T, Gavaert P, De Schrijver L, Van Cauwenberge P. Superantigens in nasal polyps. *Curr Allergy Asthma Rep* 2003;3:523-31.