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

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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 coworkers[1-5] 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 patients[1-3]

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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 dermatitis[9] 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-Cε sequences were PCR amplified by using the proofreading Pfu DNA polymerase (Promega, Madison, Wis) from IgE-positive B cells. The V_H-Cε PCR products were then cloned and sequenced. This entire procedure has been described in detail previously.[19] V_H-Cα sequences were amplified from cDNA in the same way as V_H-Cε sequences, replacing the Cε-specific primers with nested primers specific for Cα: Cα1, 5′-TTTCGCTCCAGGTCACAC-3′; Cα2, 5′-GGGAAGAAGCCCT-GGACCAGGC-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 HOMOLOGY and displayed with INSIGHT II (Accelrys, Cambridge, United Kingdom).