Genetic analysis of IgE

IgE antibodies have a central role in the mechanism by which environmental antigens cause allergic disease. Once IgE antibody has been induced by exposure to allergens, it can bind to the isotype-specific FceRI receptors on the surface of leucocytes, platelets, and mast cells. Binding of antigen can then crosslink the IgE and induce activation and degranulation of the Fce bearing cell with release of a range of chemical mediators. Two distinct forms of FceR are found on different cell types. The classical high affinity IgE receptor, FcεRI, is present on mast cells and basophils and is implicated in anaphylactic responses as well as atopic allergy. Crosslinking of two FcεRI molecules triggers degranulation with release of histamine and other preformed chemical mediators, together with generation of several newly formed mediators including leukotrienes and prostaglandins. More recently, a lower affinity receptor for IgE, FcεRII (CD23), has been described. This receptor is present on B lymphocytes, platelets, eosinophils, macrophages, and activated T cells, and is upregulated in atopy, both on blood mononuclear cells and on cells present in lesional skin of patients with atopic dermatitis. Although of low affinity, CD23 is known to participate in regulation of IgE synthesis, and antibodies against CD23 inhibit IgE production both in vitro and in vivo. The FcεRII receptor also has the potential to contribute to chronic allergic reactions by facilitating antigen presentation of low levels of allergen bound to circulating IgE. Expression of CD23 appears to be induced on cells of the bronchial epithelium in patients with asthma, and a direct role for CD23 in activation of epithelial cells has been suggested by the finding that production of endothelin can be induced by exposure to IgE/anti-IgE complexes.

Clearly, IgE, either alone or in complex form, has the capacity to induce and maintain allergic disease. However, in spite of this central role, there has been little information available until recently on the structure of IgE antibodies. One reason for this is that the amount of IgE in normal serum is very low, usually <1 μg/ml, partly because most IgE molecules are attached to the surface of blood basophils and tissue mast cells. Although serum levels can be substantially higher in atopic patients, they still remain below 1 mg/ml, making conventional immunochromatographic analysis difficult. In fact, most of our knowledge of the primary structure of human IgE has been obtained by studies on two of the rare IgE myeloma proteins designated ND and PS. A further complicating factor is the high susceptibility of IgE to proteolytic degradation. Recent advances in the techniques of molecular biology have made it possible to circumvent many of these analytical difficulties and have opened the way to probing not only the general structural features of IgE, but also the detailed amino acid sequences of individual antibodies. A review of the overall domain structure of IgE, with particular reference to the Fce region, has recently been published. This article will focus on the opposite end of the IgE molecule, the antigen binding domain, with particular reference to the variable region of the heavy chain, V_{H}, illustrated in fig 1.

**Genetic analysis of the variable region of antibodies**

Antibodies bind to antigen via the variable regions of the heavy and light chains. The "contact points" for antigen lie in the hypervariable region or complementarity determining regions (CDRs), of which there are three in each heavy and light chain. The disposition of the CDRs and the intervening framework regions is shown in fig 1. As might be expected, the CDRs have immense variability in amino acid sequence among antibodies of different specificity. However, the framework sequences of the variable regions, although less variable than the CDRs, are also considered to have some influence on the binding of antibody to antigen.

During the process of genetic recombination which generates functional genes able to encode the heavy chains of antibody molecules, genes of the variable (V_{H}) regions are juxtaposed to those of the constant regions. The constant region of the μ chain is initially used so that IgM can be synthesised but, under the influence of antigen and T cells, immunoglobulin class switching to IgG, IgA, or IgE can occur. When the class of immunoglobulin formed is changed to IgE (isotype switching), the presence of either IL-4 or IL-13 is required, together with engagement of the CD40 molecule on B cells by CD40L on T cells, basophils, or mast cells. Specificity for antigen lies initially in the sequences of the V_{H} region chosen for recombination and, for the CDR3 region, in the amino acids generated by nucleotide additions or losses during the recombination process. However, further changes in amino acids occur by somatic hypermutation. This process, which occurs in the germinal centre of the lymph node, introduces nucleotide substitutions across the variable region gene sequence, generating amino acid changes in the encoded protein sequence. The introduction of mutations represents a device used by the B cell to create heterogeneous antibody sequences which then provide greater opportunity of binding to external antigen. Under the influence of antigen selection, the mutations can result in particular amino acid sequences in the CDRs which interact with antigen. This means that, for IgE, the efficiency of recognition of allergen may be affected by (1) the nature of the V_{H} gene chosen, (2) the events of recombination, and (3) subsequent somatic mutations.

Over the last few years, because of the application of molecular biological techniques, there has been an ex-
plosion in our knowledge of the immunoglobulin V genes.\textsuperscript{15} We now know that there are about 51 potentially functional V\textsubscript{H} genes, divisible into seven families (V\textsubscript{H}1-V\textsubscript{H}7), with sequence similarity between family members being >80\%. It is evident that certain V\textsubscript{H} genes are used preferentially for antibodies against particular antigens. The largest studies have been concerned with auto-antibodies, where a dramatic example of selective V\textsubscript{H} gene usage is seen in the cold reactive pathological anti-red cell antibodies. For these antibodies the V\textsubscript{H}4-21 gene, a single member of the V\textsubscript{H}4 family, appears mandatory, leading to the conclusion that interaction with the red cell antigen is via a conserved framework sequence present in all V\textsubscript{H}4-21 genes rather than the conventional CDRs.\textsuperscript{17} Similar but less dramatic selective V\textsubscript{H} gene usage has been shown for several human antibodies against exogenous antigens such as those against staphylococcal protein A, where the V\textsubscript{H}3 family is preferred,\textsuperscript{18} or those against Haemophilus influenzae type b capsular polysaccharide, where an asymmetric usage of the V\textsubscript{H}3b subfamily has been observed.\textsuperscript{19} The fact that these antibodies appear to interact with antigen via uncon-"sidered" somatic hypermutation has led to the concept of B cell “superantigens”. Superantigens may be defined as antigens which bind to more conserved regions of the antigen receptor which are outside the conventional binding site, thereby being less specific in their binding requirement than conventional antigens. They have been described in detail for T cells – for example, the enterotoxin of Staphylococcus aureus which causes toxic shock syndrome.\textsuperscript{20}

The pathogenic action of the enterotoxin is caused by the fact that the less specific binding results in stimulation of a large number of T cells. Although this example represents a pathological consequence, the ability of superantigens to stimulate a range of T or B cells may be advantageous in an early immune response.

Analysis of V\textsubscript{H} regions of IgE

The ability of the polymerase chain reaction (PCR) to amplify small amounts of DNA has meant that the V\textsubscript{H} genes involved in encoding IgE are now accessible. By using primers specific for the full range of V\textsubscript{H} families in combination with a primer for the constant regions of IgE (Ce), the V\textsubscript{H}-Ce nucleotide sequences of IgE synthesised by B cells from blood of patients with atopic dermatitis have been obtained.\textsuperscript{21} The sequences have shown some surprising features, the first being the presence of a high number of transcripts from the V\textsubscript{H}5 family. In addition, extensive somatic hypermutation of the genes has been found, with evidence for “hot spots”. These “hot spots” represent identical mutational changes seen in several different V\textsubscript{H}5 sequences.\textsuperscript{21} However, there is no evidence for accumulation of mutations leading to replacement amino acids in the CDRs which would be a characteristic expected of an antigen-selected sequence, leaving open the question of the functional significance of the observed mutational pattern.

We were able to apply a similar genetic analysis to IgE synthesised by B cells from a patient with hypersensitivity to house dust mite who died as a result of an asthmatic attack.\textsuperscript{21} In this case the patient’s spleen was examined, affording a rare opportunity to probe the splenic B cell IgE response. The results obtained, using nested PCR to increase sensitivity, confirmed the suspected increased usage of the V\textsubscript{H}5 family by IgE.\textsuperscript{22} The sequences were again highly somatically mutated and revealed no evidence for antigen selection. We have now investigated B cells from the blood of a further seven asthmatic patients with hypersensitivity to house dust mite with essentially similar results (manuscript in preparation). In this more extended investigation we have found that both the available genes in the V\textsubscript{H}5 family (V5-51 and V5p32) are used. Representative deduced amino acid sequences from the two V\textsubscript{H}5 genes used by IgE in a single patient are shown in fig 2. The location of the CDRs, intervening framework regions, and the J region in the sequence can be compared with the expanded V\textsubscript{H}4 illustrated in fig 1. Comparison of the IgE derived sequences with the sequence of the germ line V\textsubscript{H}5 genes (fig 2) indicates that there has been extensive somatic hypermutation leading to replacement amino acids. However, these tend to be scattered across the V\textsubscript{H} region rather than being concentrated in the CDRs as would be expected from antigen selection. These mutational patterns are similar to those reported for the IgE from splenic B cells\textsuperscript{22} and from patients with atopic dermatitis.\textsuperscript{21} We have also confirmed the presence of “hot spots” in the V\textsubscript{H}5 sequences. Overall, V\textsubscript{H}5 gene usage by IgE in patients with asthma appears to be similar to that reported for patients with atopic dermatitis.\textsuperscript{21}

The significance of the increased usage of V\textsubscript{H}5 genes of the V\textsubscript{H}5 family to encode IgE is not yet clear, but the selective use of certain V\textsubscript{H} genes is reminiscent of the antibody responses against staphylococcal protein A or Haemophilus influenzae capsular polysaccharide\textsuperscript{18} and is consistent with stimulation by a B cell superantigen. What is envisaged there is that the B cell superantigen binds to conserved framework regions of the selected V\textsubscript{H} genes. The result of this prolific binding would be to stimulate all B cells that use the selected V\textsubscript{H} gene. Consequently, large amounts of antibody of relatively low affinity would be secreted, which could be useful in an early immune response. In the case of IgE, components of common allergens such as house dust mite may similarly act as superantigens, binding to the framework regions of V\textsubscript{H}5 encoded immunoglobulin expressed by B cells.\textsuperscript{22} Following stimulation, these B cells would mature, undergo somatic hypermutation and, in atopic individuals, switch to production of IgE. If allergens are binding outside the CDR sequences there would be no selection pressure by allergens for introduction of particular amino acids at the conventional “contact points” of the CDRs of V\textsubscript{H}. This could account for the fact that there is indeed no detectable clustering of replacement amino acids in the CDRs. An alternative explanation for the lack of clustering is that

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**Figure 2** Two examples (E1 and E2) of deduced amino acid sequences of the VH region of IgE, encoded by the V\textsubscript{H}5 family, from blood B cells of a patient with asthma. Comparisons are made with the closest germ line genes, the two members of the V\textsubscript{H}5 family, V\textsubscript{H}5-1 and V\textsubscript{H}5-2. The CDR sequences are indicated. Upper case: replacement mutations; lower case: silent mutations.
chronic stimulation by allergen could generate neutral mutations that may shift the pattern towards apparent randomness.21

So far the genetic approach has been applied to available V_{H}-Ce sequences obtained from circulating B cells in the patient, with no evidence that those IgE molecules are specific for allergen. The relative difficulty of obtaining sequences from non-allergic individuals suggests that the identified sequences are relevant to the disease process,21 but it is clearly desirable to obtain functional data. To achieve this we are now expressing the identified sequences as recombinant Fab molecules, and these will be assessed for reactivity to candidate allergens.21 Although the light chain in the original IgE remains unidentifiable at present, it is likely that the major contribution to specificity lies in V_{H},24 so we are currently using a common light chain to construct the Fab molecules.

Further application of VH gene analysis of IgE

The VH sequences from individual IgE antibodies provide useful markers for B cell clones.24 For each B cell the CDR3 sequence provides a "clonal signature" which is characteristic of only that B cell. It is therefore feasible to design specific oligonucleotide primers based on CDR3 sequence, in order to track clonally related B cells in patients by PCR. The technology is similar to that used to detect minimal residual disease in patients with acute leukaemia. For normal B cells engaged in an immune response it is possible to use PCR to follow a single B cell from IgM production through to IgE, or to other immunoglobulin classes. By this procedure, it has been shown already in patients that a single B cell clone can synthesise IgM, IgE, and IgG.25 The ability to follow individual B cells through immunoglobulin class switching events provides an opportunity to investigate the factors which influence this mechanism in vivo. The isotype switch to IgE underlies the development of allergy, and the balance between immunoglobulin classes and IgG subclasses could be crucial to disease manifestation. Molecular tracking of B cell behaviour during disease progression or during desensitisation protocols could provide more definitive data than are provided by current serological investigations.

A second opportunity provided by genetic probes is to investigate the distribution of IgE positive B cells. These cells have been tentatively identified in bronchial tissue of allergic patients26 and PCR can be used to confirm this. It may also be of interest to see if the VH gene pattern in local B cells reflects that of B cells in blood.

Concluding remarks

The genetic approach is at last allowing access to the structure of the antigen binding site of IgE antibodies. Even the limited investigations which have been carried out so far have revealed several unusual features of the site. The first surprise is that there is selective usage of the minor V_{H}5 family to encode IgE. One interpretation of this which has wide biological implications is that common allergens may be acting as "superantigens" for B cells. The second finding is that the VH genes are highly somatically mutated, indicating that the B cells have been exposed to the mutation mechanism in the germinal centre of lymph nodes. IgE positive B cells can be found in blood and spleen, and it remains to be seen if they are present, and possibly selected, in local sites such as the bronchi.

Application of the technology for tracking B cell clones at different sites, for probing variant members of a clone which have switched to other immunoglobulin classes, and for expressing IgE derived Fab molecules for functional analysis is certain to enlarge our understanding of the role of IgE in allergic disease.

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