Editorials

T cell receptor genetics, autoimmunity and asthma

Histological examination of asthmatic airways reveals epithelial damage, microvascular leakage, mucus hypersecretion, deposition of collagen beneath the basement membrane, and a mixed cellular infiltrate dominated by T cells and eosinophils. Interestingly, the histology of occupational and late onset “intrinsic” asthma is similar to that of atopic asthma, implying a common response to diverse insults. Irrespective of aetiology, airway inflammation leads to hyperresponsiveness of the bronchi which is manifest as bronchial irritability, fluctuating peak expiratory flow measurements, and a tendency to bronchoconstrict on exertion or when exposed to non-specific respiratory irritants. A considerable body of evidence has now accumulated suggesting that asthma is an immunological disorder in which T lymphocytes serve to orchestrate the inflammatory response. Increased numbers of T cells are found in the bronchial mucosa of asthmatics, both above and below the basement membrane, most of which are CD4+ (T helper/inducer subset) and CD45RO+ (memory subset). T cells that express markers of activation are increased in number both when recovered by lavage and in mucosal tissue biopsies, with a positive correlation between the degree of T cell activation and non-specific bronchial hyperresponsiveness. Increased numbers of activated T cells are found in the peripheral blood during acute episodes of asthma and their numbers decrease as the acute exacerbation resolves. Treatment with an inhaled corticosteroid for six weeks leads to a dramatic decrease in the mucosal content of mast cells and eosinophils in parallel with reduced expression of markers of T cell activation. Moreover, a subset of patients with chronic asthma with clinical resistance to glucocorticoids has associated abnormalities of T cell response to these drugs which cannot be attributed to differences in glucocorticoid receptors or pharmacokinetics.

Our understanding of the precise role (or roles) of T cells in asthma has been hampered by a lack of knowledge about the specificity of airway T cells in health and disease. Recent cloning studies suggest that relatively few airway T cells recognise relevant airborne allergens, which raises serious questions about the significance of expression of activation markers by a high proportion of T cells. Are these T cells recognising as yet unidentified foreign antigens, are they reacting against internal or self antigens, or are they perhaps only partially activated cells which are functionally anergic? Some of these issues can be explored by analysis of the T cell antigen receptor repertoire in the asthmatic airway, applying techniques which have been used successfully to address similar questions in relation to autoimmune diseases.

There are two alternative T cell antigen receptors (TcR), both of which are heterodimer structures. Most T cells use the αβ-TcR while 2–4% of peripheral blood T cells use the phylogenetically more primitive γδ-TcR. The αβ-TcR recognises antigens or, more accurately, fragments of antigens that are bound to MHC (major histocompatibility complex) molecules. CD4+ T cells bind class II MHC molecules on so-called antigen presenting cells and are principally concerned with handling exogenous antigens. CD8+ T cells recognise antigens in the context of MHC class I molecules and are primarily concerned with killing infected or neoplastic host cells. The αβ-TcR is encoded by multiple gene segments which are uniquely rearranged in each T cell and its clonal descendants. For practical purposes this recombination process is irreversible and, once a “readable” recombination has been made, each T cell and its clonal descendants continue to express that unique TcR and hence retain the same specificity for antigen. The antigen-combining region of the TcR is assembled by recombination of variable (V), diversity (D), and joining (J) segments. The TcRα gene locus contains over 100 V segments and over 100 J segments, while the TcRβ gene locus contains over 75 V segments, 13 J elements, and two D segments. The Va and VB gene segments can be clustered into families which share common genetic features and whose protein products can be identified by family-specific monoclonal antibodies. To date, 30 Va and 24 VB families have been identified in man. During T cell development the TcRα genes are rearranged sequentially to bring a Jα next to a Va, then the Vβα is brought next to the constant region Cα gene to form a TcRa gene that can be transcribed. Similarly, the TcRβ gene is assembled by rearrangement of Dβ to Jβ, then a VB is brought next to the DJβ and, finally, the VDJβ is transcribed together with the adjacent Cβ gene (fig 1). Additional diversity in the rearranged TcRα and TcRβ genes is generated by random nucleotide additions at each of the joining sites. Both the α and β chains have three points of contact with the MHC molecule and the antigenic fragment contained within the MHC molecule. These are termed “complementarity determining regions” (CDRs): the CDR1 and CDR2 regions bind to polymorphic framework regions of the MHC molecule, while the CDR3 region constitutes the antigen-combining site (fig 2). Broadly speaking, the CDR1 and CDR2 regions are formed from the framework regions of the V segment while the CDR3 is formed from the V(D)J junction region (fig 2). Hence, differences in the framework structure contributed by the various V gene families permit the TcR to bind to MHC molecules in slightly different configurations, while the hypervariability of the
Germline

\[ V_{\beta_1} \quad V_{\beta_2} \quad V_{\beta_3} \quad D_{\beta_1} \quad J_{\beta_1} \quad C_{\beta_1} \quad D_{\beta_2} \quad J_{\beta_2} \quad C_{\beta_2} \]

Recombination

\[ V_{\beta_1} \quad D_{\beta_1} \quad J_{\beta_1} \quad C_{\beta_1} \quad D_{\beta_2} \quad J_{\beta_2} \quad C_{\beta_2} \]

Translation

\[ NH_2 \quad COOH \]

Splicing

\[ V_{\beta_1} \quad V_{\beta_2} \quad D_{\beta_1} \quad J_{\beta_1} \quad C_{\beta_1} \quad D_{\beta_2} \quad J_{\beta_2} \quad C_{\beta_2} \]

Figure 1: Organisation of the human TcR\( \beta \) locus and the process of sequential recombination to form a readable TcR\( \beta \) product, followed by transcription, splicing, and translation. V\( \beta \) families are shown as clustered gene segments for clarity. A total of 75 V\( \beta \) germline sequences have been identified, falling into 24 families. D, J, and C segments are duplicated and either set can be used, with intervening DNA being deleted during recombination.

Complementarity determining regions (CDRs)

\[ \alpha \quad \beta \quad \gamma \quad TcR \]

Genetic derivation of TcR

\[ C_{\alpha} \quad C_{\beta} \quad J_{\alpha} \quad J_{\beta} \quad D \]

MHC

\[ V_{\alpha} \quad \alpha \quad \beta \quad \gamma \]

Figure 2: Contribution of V, D, and J gene segments to the complementarity determining regions (CDRs) of the T cell antigen receptor.

V(D)J joining process leads to an almost infinite range of possible amino acid residues in the antigen recognition site, and hence the ability to recognise an almost infinite variety of antigens.

In normal subjects the peripheral blood TcR repertoire is highly diverse, with an estimated 10^10 functional TcRs resulting from the use of different V, D, and J segments in forming TcR\( \alpha \) and \( \beta \) chains. During fetal life T cells are processed in the thymus and autoreactive T cells are eliminated by deleting clones which recognise unmodified self-MHC antigens. In later life autoreactive clones may be either deleted or inactivated, but if such clones inadvertently become active they may trigger autoimmunity disease. Increasingly, however, it is becoming clear that many diseases which have been thought to be autoimmune in origin are, in fact, responses to hitherto unidentified foreign antigens. The pattern of TcR gene usage in the adult organism is thus heavily influenced by MHC haplotype. During life the expressed TcR repertoire may be modified by proliferation of T cells responding to antigenic stimulation. In addition, selective expansion of cells expressing one particular TcR V gene family may occur through exposure to so-called superantigens. Superantigens are proteins which trigger T cell proliferation by binding, not to the antigen-combining sites, but to family-specific determinants on the TcR. This leads to polyclonal proliferation of T cells which share common V genes but have random antigen specificity. The best known superantigens are the staphylococcal enterotoxins, but a number of other superantigens have been described. Peripheral blood TcR usage appears to be quite stable within healthy individuals over time; the best evidence for this derives from studies of healthy monozygotic twin pairs. However, if one of the twins develops autoimmune disease the expressed peripheral blood TcR repertoire can diverge. It is noteworthy that the repertoire is more stable in the CD4+ subset than in the CD8 + T cell subset. Interestingly, a marked selective divergence of the peripheral blood CD8 + TcR repertoire has been reported in a single twin pair discordant for asthma.

For various reasons the peripheral blood T cell population may not reflect the repertoire in diseased organs. For example, subsets of T cells possess specialised homing receptors which allow them to localise to the skin or mucosal regions and hence the site, and hence the site, of the disease. T cell proliferation may result in expansion of oligoclonal T cell populations sharing DJ segments, while T cell interaction with superantigens would select a predominant TcR V\( \beta \) family with differing DJ segments. In contrast, non-specific T cell recruitment would result in a heterogeneous TcR repertoire at the inflammatory site, reflecting populations in blood.

Examples of TcR repertoire selection at sites of autoimmunity disease have been reported in man and in animal models. In multiple sclerosis, analysis of T cells infiltrating demyelinated plaques has shown oligoclonal expansion of T cells expressing V\( \beta_3 \), V\( \beta_8 \), V\( \beta_12 \), V\( \beta_24 \), and V\( \beta_27 \). However, not all these V\( \beta \) families were found in all patients or in all plaques. In experimental autoimmune encephalomyelitis, the mouse model of multiple sclerosis, some disease prone strains show highly restricted usage of V\( \beta \) families, especially V\( \beta_3 \) and V\( \beta_13 \), as well as V\( \alpha_2 \) and V\( \alpha_4 \). Moreover, monoclonal antibodies to these TcR families can be used to prevent and cure experimental autoimmune encephalomyelitis. Similar restricted usage of TcR V\( \beta \) genes is also seen in murine autoimmune uveitis. In other human autoimmune diseases such as thyroiditis and rheumatoid arthritis similar processes of selection are apparent, especially at disease sites with increased expression of a restricted range of V\( \alpha \) or V\( \beta \) genes in diseased tissue.

The TcR repertoire has a direct influence on the type and strength of immunological responsiveness to a variety of antigens and it is conceivable that the combination of T cell repertoire and the HLA haplotype may predispose individuals to atopy. In strains of mice which show strong IgE responses the T cells which regulate IgE production appear to belong to a restricted range of TcR families. In humans an association has recently been found between responsiveness to particular major allergens and genetic polymorphisms in the TcR V\( \alpha \) region of chromosome 14. This study suggests that a gene (or genes) within the TcR V\( \alpha \) region modifies specific IgE responses, although it is not direct evidence of repertoire restriction. Within individuals cloned allergen-specific human T cells show HLA-DR restricted and HLA-DP restricted responses to house dust mite allergens, reflecting known HLA population associations and consistent with the concept that responses to immunodominant regions of processed antigens are strongly correlated with the MHC type rather than with the antigen itself. However, at the population level, initial indications are that particular epitopes on house dust mite antigens may be presented to T cells by several different MHC class II alleles and other studies have found
Figure 3  PCR analysis of T cell antigen receptor V gene usage in blood and bronchoalveolar lavage fluid (BAL) in two patients with mild atopic asthma. Note variable intensity of TcR bands in blood, reflecting heterogeneity in Vβ family usage. BAL lanes show general reduction in intensity due probably to smaller total amount of TcR cDNA in BAL sample. In (A) note a single markedly increased intensity band for Vβ12/13 in BAL fluid relative to blood. In (B) note the general proportionality of blood and BAL lanes except for Vβ7 which is over-represented in BAL fluid and Vβ14 which appears in blood but is not seen in BAL fluid.

no strong associations between HLA type and T cell reactive regions on the major house dust mite allergen Der p I. Nevertheless, the T cell response of an individual to antigen will depend on the available T cell repertoire which, in turn, may be influenced by the HLA haplotype of the individual. Furthermore, skewing of the expressed TcR repertoire within the CD4+ and CD8+ T cell subset populations may result from interaction of these subsets with HLA class I and class II molecules, respectively, during development, since the natural murine immune response to inhaled soluble protein antigens has been shown to involve both MHC class I restricted CD8+ and MHC class II restricted CD4+ T cells.

There is relatively little information about the influence of the expressed T cell repertoire on T cell responses in the human lung. In lungs obtained at necropsy from patients without lung disease monoclonal antibody staining has revealed an over-representation of T cells bearing Vβ9 and Vβ15 antigens. The most extensive studies in disease states have been performed in sarcoidosis. These studies have variously reported increased numbers of Vβ9 T cells without predominant junctional sequences, and increased numbers of T cells bearing Vβ3 in patients with the HLA-DR3 haplotype. Two recent studies suggest that the T cell accumulation in sarcoid alveolitis arises through two distinct processes—a local oligoclonal expansion of T cells and an apparently non-specific accumulation of T cells with an extremely diverse Vβ repertoire. In asthma it is possible that chronic airways inflammation may be a form of autoimmune disease, with allergen playing a part in the early phases of development but becoming less important as the disease moves towards a more chronic form of inflammation. This would be consistent with the more exuberant cellular infiltration seen in intrinsic asthma, and the presence of autoantibodies against epithelial antigens in most cases of intrinsic asthma. The increased numbers of CD8+ cells in biopsy samples from patients with intrinsic and occupational asthma provide further support for the suggestion that asthma may be a form of autoimmune disease in which viruses or chemical haptons modify intrinsic antigens which, in turn, are targeted by CD8+ cytotoxic cells. Determination of the frequency of usage of TcR V gene families and junctional analysis among T cells isolated from asthmatic airways may allow us to identify T cell populations responding to various stimuli and provide insight into the forces which shape the T cell population of the airways in asthma. Preliminary data are fairly limited: Vα gene usage has been examined in bronchial biopsy samples from six subjects using a non-quantitative RT-PCR technique. Biopsy samples from atopic asthmatic subjects showed mRNA for a mean of 4-8 TcR families compared with 11-1 TcR families in a group of normal subjects. In our own preliminary studies we have found variable patterns of TcR mRNA expression in bronchoalveolar lavage fluid samples from patients with mild atopic asthma (fig 3), suggesting that some patients may have expansion of single TcR families in a superantigen type response, while others show a more diverse airways TcR repertoire. With the increasing availability of high affinity family-specific monoclonal antibodies the time is now right for a more detailed examination of this issue.

Conclusions

The accumulation of lymphocytes at the site of inflammation in the lung arises as a consequence of three mechanisms: (1) non-specific recruitment of T cells to the lung, (2) selective trafficking of subpopulations of cells bearing lung-specific homing molecules, and (3) local proliferation of T cells responding specifically to antigenic or superantigenic stimulation within the lung. Evaluating the repertoire of T cells at the site of inflammation in the lung should help to differentiate the relative contributions of each of these processes. Flow cytometry with family-specific monoclonal antibodies will give initial indications of selection, but analysis of CDR3 sequences is required to distinguish between responses to immunodominant peptides and responses driven by superantigens. In addition, we need to address the extent of variability of the airways T cell repertoire in comparison with blood from the same subjects, with special attention to the role of MHC in shaping the intrinsic TcR repertoire.

University Medicine, Southampton General Hospital, Southampton SO16 6YD, UK

A J FREW

J DASMAHAPATRA


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A J Frew and J Dasmahapatra

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