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THE SPECIFICITY AND REGULATION OF T-CELL RESPONSIVENESS TO ALLERGENS

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KEY WORDS: allergy, T cells, HLA, cytokines, immunomodulation

Abstract

CD4⁺ T lymphocytes initiate and regulate both the specific and nonspecific effector mechanisms of allergic immune responses. The definition of immunogenic components within allergens has allowed the specificity of the T-cell repertoire to be examined, and the refinement of their molecular structure is now facilitating the mapping of functional epitopes. Nevertheless, the immunological mechanisms that distinguish between atopic and nonatopic states remain unclear; as knowledge of the complex network of cytokines in the regulation of immune responses unfolds, this problem may be resolved. Population genetics indicates that susceptibility to allergic disease is partly determined by products of the major histocompatibility gene complex. The use of molecular genetic probes and monoclonal T cells demonstrates the functional importance of both the HLA-DRAB1 and -DRAB3 gene products in T-cell recognition of allergen. Collectively, this information has made it possible to develop in vitro models examining the modulation of responsiveness to allergens.

INTRODUCTION

The induction of allergen-specific immunoglobulin E (IgE) and the activation of polymorphonuclear granulocytes characterize the allergic immune response in atopic individuals, who comprise 25–30% of the total population. The reason some individuals mount an IgE response to allergens, whereas others fail to do so, is not understood. When sensitized airways or the skin of atopic subjects is provoked by exposure to the specific allergen, a biphasic response often occurs. Cross-linking of specific IgE bound to Fc_ϵ receptors ($Fc_\epsilon R$) on the primary effector cells, mast cells, or basophils results in an immediate or early reaction, due to the release of primary mediators. In contrast, the subsequent secondary or late phase response (LPR) is largely inflammatory. The early response occurs within minutes of allergen exposure and is ascribed mainly to the release of granule-associated mediators. The late response starts after 4–6 hr and normally terminates within 24–48 hr. During this response cells are infiltrated by eosinophils, neutrophils, macrophages, lymphocytes, and basophils, which subsequently release secondary mediators including prostaglandins, leukotrienes B_4 and C_4 , and platelet activating factor (1, 2).

Considerable research effort has been focused on the specific and non-specific effector arcs of allergic immune responses in humans, while the regulatory role of $CD4^+$ T lymphocytes has been overlooked, despite clear demonstration that the induction of IgE synthesis is T-cell dependent (3). However, the realization that T and B cells may recognize distinct epitopes within a protein antigen (4) has encouraged investigation of the cellular and molecular mechanisms underlying T-cell recognition of allergen (5). Similarly, while the specificity of IgE directed against a number of allergenic components has been defined at the epitope level (6), comparable analysis of the T-cell repertoire reactive with allergen has been neglected. Additionally, activated T cells are now known to be involved in allergic inflammation via the release of selected lymphokines that regulate the differentiation, activation, and expansion of granulocytes (7). Increased knowledge of the mechanisms by which the T cells control IgE synthesis in atopic individuals and regulate the differentiation of allergic effector cells allows new approaches to immunotherapy to be identified.

This review summarizes recent developments in the evaluation of allergen recognition by $CD4^+$ T lymphocytes and their regulatory role in the human immune response to allergen.

IDENTIFICATION OF T-CELL EPITOPES

Both functional and biochemical studies have demonstrated that the generation of cellular immune responses depends upon antigen receptors on

T cells (TCR) recognizing peptidic fragments of foreign proteins associated with products of the major histocompatibility complex (MHC), that are expressed on the membrane of accessory cells (8, 9). Definition of the molecular interactions in the formation of these ternary complexes has advanced particularly from examination of the genetic organization of individual chains of TCR heterodimers (10, 11) and resolution of the crystal structure of HLA class-I molecules (12, 13). In a comparison of primary sequences, the structure of the antigen-combining site of class-II MHC molecules is predicted to be similar to that of MHC class I (14), although this will need to be confirmed by crystallographic studies. Based on this, several models have been proposed to predict those sequences within a protein that have the characteristics to bind to MHC molecules (15, 16). However, as peptides must also be recognized by TCR, the prediction of T cell epitopes based only on their ability to bind to MHC molecules is insufficient, and at present more direct approaches must still be used for the localization of T cell epitopes in antigens including allergens.

Allergen-Specific T-Cell Responsiveness of Atopic and Nonatopic Individuals

The ability has been demonstrated of peripheral T cells from allergic individuals to proliferate to allergens, including ragweed (17–20), grass pollens (21, 22), *Dermatophagoides* spp. (house-dust mite; 23–25), honey bee venom (26), murine urinary protein (27), nickel, cobalt, and chromium compounds (28), and cat antigens, *Felis domesticus* I (*Fel d* I; Ref. 29). The relationship between the degree of atopic disease that an individual possesses and the magnitude or ability of their T cells to respond to the relevant allergen has been debated. Several studies suggest that allergen-reactive T cells are detectable in primary in vitro stimulations in both allergic and normal subjects (29–31). The ability to clone house dust mite-specific T cells from nonatopic individuals (31), for example, indicates that T cells reactive with allergen are not limited to the allergic population, as was previously suggested (20, 23). Furthermore, responses may be observed in the majority of cat or ragweed induced primary T-cell cultures, from allergic as well as nonallergic subjects (29). In contrast, generation of long-term T-cell lines specific for cat or ragweed allergens is more prevalent in association with the particular allergy.

Experimental Approaches in the Analysis of T-Cell Antigen Specificity

Whether or not T cells sensitized to a particular allergen are able to respond to closely related species variants of that allergen provides information on

the specificity and cross-reactivity of antigen recognition. This fact is well illustrated by examination of responsiveness to *Dermatophagoides* spp. Although at the polyclonal level no quantitative differences appear in the response to *Dermatophagoides pteronyssinus* (*D. pteronyssinus*) or *Dermatophagoides farinae* (*D. farinae*), the major component of a *D. farinae*-induced, long-term T-cell line was species-specific, demonstrating minimal cross-reactivity to *D. pteronyssinus* (25). This observation was confirmed with T-cell clones (32); it contrasts with the humoral response to house-dust mite which is extensively cross-reactive between the two major species (33). The value of this approach in analyzing the specificity of the T-cell repertoire is limited, however, in that it provides no information on the individual proteins recognized.

Individual proteins isolated by affinity chromatography from ragweed, grass pollens, and house dust mite have been examined for their capacity to stimulate T-cell responses. To prevent a bias in the analysis of the T-cell repertoire by using only proteins selected by antibodies, allergen fractionated and immobilized on nitrocellulose has been used to identify immunogenic components. With this technique the specificity of T-cell clones and lines induced with *D. farinae* has been demonstrated for both group I and group II allergens, with the latter comprising the bulk of the T-cell repertoire (25). Recognition of affinity-purified group I and II allergens from both *D. pteronyssinus* and *D. farinae* confirmed the T-cell specificities. Analysis of the polyclonal response revealed that additional proteins of house-dust mite were able to stimulate T cells (Figure 1). One of these had an apparent mol wt of 58 kd, potentially corresponding to α

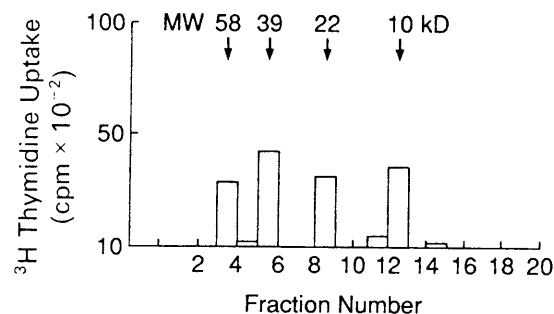


Figure 1 Pattern of reactivity of peripheral human T lymphocytes to fractionated house dust mite. *D. farinae* mite body extract was fractionated by separation on SDS-PAGE under denaturing conditions, transferred to nitrocellulose and then added to proliferation assays containing unfractionated peripheral T cells (27). Stimulation of the T cells was correlated with the incorporation of tritiated thymidine.

amylase (60 kd); the protein was recently defined serologically as the group IV allergen of *Dermatophagoides* spp. (34).

Mapping of T-Cell Epitopes

The ability to localize T-cell epitopes in allergens has been hindered by the lack of sequence data. Recently, however, proteins from honey bee venom, ragweed, rye grass, and house-dust mites have been cloned and sequenced. Therefore, it is now possible to define T-cell epitopes within allergens using the traditional approach of synthesizing overlapping peptides over the entire sequence of the protein antigen of interest. This allows the assessment of potential differences in the specificity at the epitope level of the T-cell repertoire, not only within the atopic population but between atopic and nonatopic individuals. Sublibraries of recombinant antigens provide an alternative approach for localization of epitopes (35). The best-studied system has been that of the group I allergen of *D. pteronyssinus*. The specificity of T-cell clones initially induced with recombinant *Der p* I (36) has allowed the subsequent identification of three epitopes [Ser(45)-Gly(73), Val(89)-Cys(117) and Phe(111)-Arg(139)]. Minimal T cell-stimulatory epitopes of eleven [Asp(56)-Cys(65)] and twelve [Asn(107)-Cys(117)] amino acids, respectively, were found (H. Yssel, P. Schneider, T. Nakamoto, J. de Vries, personal communication). Similarly, cloned *Der p* I reactive cells induced with house dust mite extract have been isolated that respond to residues His(85)-Asp(109) (R. O'Hehir, M-C. Kuo, W. Thomas, J. Lamb, unpublished results). Use of truncated recombinant *Der p* I in the analysis of polyclonal T-cell responses of individuals allergic to house-dust mites revealed the presence of additional epitopes located at the amino terminus (residues 1-56; W. Thomas, personal communication). Nonatopic individuals failed to respond to recombinant *Der p* I, confirming an earlier report based on use of the affinity purified protein (23). Use of synthetic peptides to screen the T-cell repertoire reactive with *Der p* I revealed that residues 81-102 were stimulatory in a number of subjects (R. O'Hehir, J. Lamb, unpublished results).

Certain individuals allergic to honey bee venom possess IgE specific for apamin, which is one of the minor allergens detected in venom. To examine the T-cell epitopes present in this 18 amino-acid peptide, murine T cell hybridomas were generated (37), and they revealed that one form of T-cell epitope present in apamin necessitated the unfolding of the two disulfide bonds present in native apamin. The protein also had two additional T-cell epitopes, located at the amino and carboxyl termini, respectively. However, the fine specificity of these T-cell epitopes was not mapped. A panel of murine T-cell hybridomas specific for the major allergen of honey bee venom, phospholipase A₂ (PLA₂; Ref. 38), demonstrated, in four

mouse strains with different H-2 haplotypes, the presence of seven T-cell epitopes defined by peptides of approximately 20 amino acids. Additionally, three T-cell hybridomas were examined that required the presence of an intact disulfide structure, implying that either the T-cell epitope must involve amino acids across the disulfide bridge or that the conformation of the T-cell epitope is dependent on the disulfide bonds. This result may be particularly relevant to allergy as allergens may contain a high representation of disulfide bonds.

T cells from individuals allergic to mouse urinary protein cross-react on rat α -2 euglobulin and react to the 80–108 peptide from the rat protein (39). A single T-cell epitope in ragweed, *Ambrosia artemisiifolia* (*Amb a* I) (residues 54–65), has been defined for three T-cell lines from three DR1 individuals whose T cells were responsive to *Amb a* I (40). These limited examples demonstrate the presence of T-cell epitopes in some allergens but fail to define the fine structure of a complete set of T-cell epitopes within any allergen studied to date.

The management of the disease states of allergy and asthma may involve the T cell-directed control mechanisms of isotype switching during an antibody response. The investigation of T-cell epitopes that enhance the switch to IgE will be critical to our understanding of allergy. Despite minimal serological cross-reactivity between the perennial rye grass, *Lolium perenne*, pollen allergens *Lol p* I and either *Lol p* II or *Lol p* III, sequence analysis demonstrated a region of homology between all three proteins, supporting the suggestion that a shared T-cell epitope might account for specific IgE synthesis (41). Current experimental systems for determining binding of peptides to MHC class-II proteins, together with the ability to isolate allergen-specific T-cell clones, will allow specific immune recognition of the rye grass allergens to be explored in detail.

The ability to define T-cell epitopes in other allergens is suggested by results showing that naturally occurring, protease-derived fragments of various allergens will stimulate a T cell-proliferative response from T cells primed to the whole allergen. With murine T-cell activation as a measure of the presence of epitopes, it has been demonstrated that there are functional sites in *Amb a* I (42), protease fragments from bovine serum albumin (BSA, Ref. 43), protease treated rye grass pollen extract (44); peptic fragments of short ragweed extract, and PLA₂ (A. Pesce, J. Michael, personal communication). In addition, T cells from humans allergic to rye grass pollen will proliferate to rye grass-pollen extract fragmented by cyanogen bromide (45). The ability to define "immunodominant" and "disease-related" T-cell epitopes within allergens is of potential practical value in the design of immunotherapeutics.

THE ROLE OF T-CELL CYTOKINES IN ATOPIC DISEASE

The roles of allergen-specific IgE and mast cells are well established in the etiology of immediate type hypersensitivity. Similarly, the involvement of eosinophils, neutrophils, and basophils has been appreciated in late phase reactions associated with atopic disease. Emphasis has been placed on mediators of allergic and inflammatory reactions such as histamine, leukotrienes, and prostaglandins. Cytokines, which influence IgE production, early mediator release, and late phase inflammatory responses, have only recently been studied in detail.

The Role of T Cells in IgE Production

Although it has been apparent for many years that T-cell help is required for the production of IgE by B cells (3), only recently has it been possible to begin a careful dissection of the precise role played by T cells—an analysis made possible by the advent of T-cell cloning and the availability of recombinant cytokines and blocking antibodies specific for these cytokines.

Studies in both mouse and human systems have shown that IL-4 is required for IgE synthesis by B cells previously primed with antigen (46). The mechanism involves signaling the B cell to switch from the production of IgM to the production of IgG₁ and IgE in the mouse, or to IgG₄ and IgE in the human (47). The molecular mechanisms of this switch may involve a specific induction of recombinase and an increased accessibility of the relevant regions of the Ig heavy-chain DNA. Early experiments showed that addition of recombinant IL-4 to unfractionated peripheral blood leukocytes from both atopic and nonatopic donors causes an increase in the secretion of IgE as well as enhancement of the proliferation of activated B cells. In mouse models, the addition of antibodies directed against IL-4 can greatly reduce an on-going IgE response induced by infection of the mice with the parasitic helminth *Nippostrongylus brasiliensis*. More recently, IgE production was shown to be more effectively inhibited by anti-IL-4 receptor antibodies than by anti-IL-4 antibodies. Furthermore, anti-IL-4 antibodies appear capable of blocking ongoing IgE responses in the mouse (46). These results have not been reproducibly confirmed in vitro in human systems. However, several reports suggest that anti-IL-4 may decrease the amount of IgE produced in IL-4-dependent IgE production (48). Thus, the precise role of IL-4 in ongoing IgE production remains to be determined, although its function as a switch factor has been well established.

The roles of other T cell-derived lymphokines in the production of IgE

have been studied only recently. IL-6 may provide a signal to enhance ongoing IgE synthesis, since it upregulates Ig production in an isotype-nonspecific fashion (49). In experiments using anti-IL-6 antibodies, total Ig secretion from activated B cells may decrease. However, the addition of exogenous IL-6 to monocyte-depleted cultures upregulates the production of IgE. It is interesting to note that a variety of other cell types such as monocytes, B cells, and endothelial cells as well as T cells are able to produce IL-6 (50). Thus, IL-6 may provide an important isotype-nonspecific signal to enhance IgE synthesis. In similar experiments, another T-cell product, IL-5, was shown to increase IL-4-dependent IgE production (51). Other studies have shown that IL-2 may also upregulate IgE production (52), although this finding is not conclusive. Many single human T-cell clones are capable of producing all of these cytokines; therefore clonal expansion of allergen-specific T cells producing these molecules may enhance IgE production.

In an attempt to approximate the allergen-specific *in vivo* situation, house dust mite-specific T-cell clones derived from atopic and nonatopic donors were examined for their ability to provide help for *in vitro* total IgE production by autologous B cells (31). Use of this approach showed that T-cell clones from an atopic individual could produce IL-4 and provide help for IgE synthesis. In contrast, mite-specific T-cell clones from a nonatopic individual, with undetectable IL-4 mRNA failed to support IgE production even in the presence of exogenous IL-4. A recent report has confirmed and extended these observations (52a). Although the *in vivo* situation is certainly more complex, these studies emphasize the importance of the quality of T-cell help provided to the B cell in the genesis of allergic responses. Closer examination of the properties of the T-cell clones generated from atopic and nonatopic individuals may provide important clues concerning the origins of the allergic phenotype.

In further studies using a panel of alloantigen-specific T-cell clones, certain of these clones, although able to produce IL-4, were not able to support IgE synthesis. These results suggest that, although IL-4 is required for IgE synthesis, it is not sufficient alone; the presence of a T cell-derived low molecular weight B-cell growth factor (LMW-BCGF) is necessary (53). LMW-BCGF, recently cloned (54), supports the growth of certain populations of B cells and is also involved in Ig production. In the same study, a signal involving T cell-B cell contact was required for optimal production of IgE, although the precise nature of the signal is unclear. The role of cell surface structures such as the low affinity IgE receptor (Fc_εIIR, CD23) in IgE synthesis has been explored (55). CD23 expression on B cells, T cells, macrophages, and eosinophils is induced by the addition of IL-4. Both surface CD23 and soluble CD23 (IgE-BF) appear to be

involved in the control of IgE production, although the mechanisms remain obscure (55, 56). Recombinant sCD23 added to cultures of leukocytes increases IgE production, and anti-CD23 antibodies are able to inhibit ongoing IgE production.

The effects of T cell-derived lymphokines are not limited to the upregulation of IgE production; these lymphokines appear capable of negatively regulating IgE synthesis. Initial studies revealed that IFN- γ or α were capable of decreasing IgE production. However, some T-cell clones producing both IL-4 and IFN- γ are still able to stimulate IgE production by B cells (48). IL-4 has been shown to downregulate the production of IFN- γ by T cells (57). Thus, IL-4 and IFN- γ appear to be antagonists in the cascade of events leading to IgE production. The level of IgE produced may, at least in part, be dictated by the balance between the production of these two cytokines. A recently described T cell-derived cytokine, called cytokine stimulation inhibitory factor (CSIF, now termed IL-10), has recently been described as having similarly negative effect on IFN- γ production by mouse T cells (58, 58a). However, CSIF may inhibit the production of a spectrum of lymphokines that exert a negative effect on IgE production. Thus, CSIF may exacerbate allergy by indirectly upregulating the production of IgE.

The results of the experiments reviewed above, indicate that the immediate phase of the allergic response is influenced by the quality and quantity of the T-cell help (in the form of cell-cell contact and lymphokines) provided to the allergen-specific B cells. However, it is still not clear whether the switch to IgE, and subsequent ongoing IgE synthesis, may be accomplished by the addition of cell-free T cell-derived lymphokines to primed B cells.

Analysis of the requirements for IL-4 production by T cells has only recently begun but has the potential to yield insights into the control of IgE production, which also involves cytokines additional to IL-4. Certain types of antigen-presenting cells such as those present in mucosal tissues may enhance IL-4 production. Indeed, murine models suggest that T cells from lymph nodes draining mucosal tissues may produce more IL-4 than do T cells from the spleens of the same animals (59). The same study reported that the androgen steroid dihydroepiandrosterone (DHEA) induces more IL-2 by activated T cells and that macrophages contain a high level of DHEA-sulphatase which converts DHEA-sulphate to active DHEA. Thus, certain presenting cells may regulate the production of cytokines selectively. The role of subsets of antigen-presenting cells in the signaling of T cells to produce a spectrum of lymphokines that would be favorable for IgE production has become a center of focus. Epidermal Langerhans cells appear to favor the production of IL-4 from long-term

cultures of T cells stimulated with antigen, to the exclusion of IL-2 (60). However, T cells activated early produced IL-2 as well as IL-4, even when Langerhans cells were used as presenting cells. Therefore, so-called "memory" T cells might produce more IL-4 than "naive" T cells. Interestingly, Langerhans cells coated with IgE are much more efficient at presenting antigen to T cells, which efficiency may lead to more IL-4 production (61).

The affinity of the accessory cell-T cell receptor interaction may also influence differential lymphokine secretion. However, if allergens were generally presented by subsets of antigen-presenting cells that preferentially produced peptides, inducing heightened IL-4, IL-5, IL-6, and LMW-BCGF secretion, then levels of allergen-specific IgE may be enhanced.

T Cell-Derived Cytokines and Mast Cells

T cell-derived cytokines influence the immediate allergic response by their direct action on nonspecific effector cells (including mast cells loaded with allergen-specific IgE) through their high affinity receptors for IgE (Fc ϵ IR). Cytokines influencing the "releasability" of histamine and other mediators, and the number and activation state of mast cells in the tissues, may exacerbate the allergic response.

The growth and differentiation of mast cells are influenced by granulocyte/macrophage colony stimulating factor (GM-CSF), IL-3 and IL-4 (62). Many of the studies to date have been performed on cultures of rodent mast cells, which have proved more amenable to study than human mast cells. GM-CSF has the ability to recruit mast cell precursors. The multipotential colony stimulating factor IL-3 is a potent mast cell growth factor, and it may have the additional ability to help recruit hematopoietic stem cells into the mast cell lineage. IL-4 may also have some role in the growth and differentiation of mast cells (63). However, many of these findings remain to be duplicated in human mast cell cultures, which have only recently been developed. IL-9, a T cell-derived T-cell growth factor previously known as P40 or TCGF-III, has recently been implicated in the growth and differentiation of mouse mast cells (64, 64a). Interestingly, IFN- γ , a T-cell product known to downregulate IgE production, has also been shown to inhibit mouse mast cell growth (65).

Clearly, as growth and differentiation factors for mast cells, IL-3, IL-4, and IL-9 may influence the number and activation state of mast cells in the tissues. Additionally, T-cell factors such as IFN- γ may reduce the number of mast cells in the tissues. Another category of cytokines known as histamine releasing factors (HRF) were first isolated from monocytes (66) but appear to be a broad category of mediators, some of which are

produced by activated T cells. HRFs appear to "prime" mast cells for the release of histamine and to increase the amount of IgE-dependent histamine release, which may enhance the allergic response. The most well defined HRFs have been studied in human basophil systems as discussed below, although there are reports of HRFs that increase the release of histamine from mouse mast cells.

Currently receiving much attention is the finding that mast cells, when degranulated by an encounter with allergen to which they have surface bound IgE, are capable of producing cytokines generally considered T-cell products. Mouse mast cell tumor lines (67) and non-T non-B mouse spleen cells (68), when loaded with IgE and cross-linked with specific antigen or anti-IgE, secrete IL-3, IL-4, IL-5, IL-6, and GM-CSF, among other cytokines. The release of these cytokines is not immediate, as in the case of histamine, but requires RNA and protein synthesis. Although these results are thus far restricted to rodent systems human mast cells will probably also prove capable of producing these cytokines. Therefore, mast cells, degranulated by cross-linking of allergen-specific IgE, may provide positive feedback for the production of more IgE and the recruitment of additional mast cells and other inflammatory cell types to the site of the allergic reaction.

T Cell-Derived Cytokines and the Late Phase Reaction

The late phase of the allergic response (LPR) seems to be initiated as a consequence of an IgE-mediated immediate-type hypersensitivity reaction. The phase involves a localized inflammatory reaction characterized by an influx of basophils, activated eosinophils, neutrophils, and lymphocytes. The role of T cell-derived cytokines in the LPR has not been established; however, their effects on the cell types responsible for the LPR have been investigated.

Large numbers of eosinophils are known to accumulate at the site of the LPR (69). These cells appear to contribute to the inflammatory response by degranulation, which releases a variety of mediators such as eosinophil major basic protein, eosinophil cationic protein, and eosinophil-derived neurotoxin, which cause tissue damage. It has been known for many years that eosinophilia due to infestation with parasites is T-cell dependent (70). IL-3 and GM-CSF, both T-cell products, influence the differentiation of eosinophils (71), and recently, T cell-derived IL-5 has come to appear necessary for their growth and differentiation. IL-5 was originally defined by its ability to induce eosinophil colony formation in mouse bone marrow cultures (72) and to cause eosinophilia in animals administered with recombinant IL-5.

The effect of other cytokines on eosinophil growth and differentiation

continues to be actively investigated. No potent eosinophilic chemotactic factors have yet been described, although IL-5 and TNF- α appear to have weak chemotactic activity (73). Mouse models have shown a striking eosinophilia in animals injected with tumor cells transfected with the mouse IL-4 gene (74). Whether or not this occurs through the stimulation of IL-5 production remains to be discovered. In addition to its effect on other granulocytes, IL-4 may also promote eosinophil growth in vivo. IgA may be important in the allergen-specific degranulation of eosinophils, since IgA coupled to beads enhances eosinophil degranulation (75); this fact implies that IgA may facilitate degranulation in vivo. IL-4 and IL-5 influence the production of IgA (76); therefore, these cytokines may be important in the development of the LPR, as may transforming growth factor— β (TGF- β)—which acts as a switch factor for IgA (46).

Neutrophils accumulate at the site of the late phase of the allergic response and cause inflammation through release of a variety of mediators, including a number of proteases. A T cell-derived cytokine, IL-8, also termed *neutrophil activating peptide* (NAP1) (77) serves to activate neutrophils and leads to their degranulation. In addition, IL-8 apparently plays some role as a histamine releasing factor (78). Injections of IL-8 lead to infiltration of neutrophils at the site of injection (79). TNF- α and IL-1 cause a similar infiltration, probably by increasing the production of IL-8. TNF- α increases the adherence of neutrophils to endothelial cells, an effect which may be explained by the observation that TNF-induced IL-8 increases cell surface expression of adhesion molecules such as the CD11/CD18 complex on neutrophils (80). Thus, a particular spectrum of T cell-derived cytokines may control the extent of the late phase of allergic response due to neutrophils by promoting their growth, differentiation, degranulation, and ability to invade sites of inflammation.

Mounting evidence points to the basophil as a major effector cell in the pulmonary LPR following inhaled allergen challenge. As in the case of mast cells, a number of the T cell-derived cytokines are capable of influencing the growth and differentiation of basophils. Several of the colony stimulating factors, including IL-3, are known to influence the differentiation of basophils (7). IL-8 in the presence of IL-3 has been shown to increase the IgE-dependent release of histamine from basophils (81). The proposed role of IL-3 is to provide a "priming" effect for histamine release from basophils. Recent studies demonstrate that histamine releasing factors may upregulate the release of mediators from basophils (82). HRFs generally increase release from basophils loaded with IgE in the presence of specific antigen, but a low level of release has been shown to be IgE-independent (7). In these studies, HRFs derived from a number of cell

types, including T cells, were active in assays involving basophils coated with IgE from atopic patients. There is some suggestion that plasma containing IgE from nonatopic individuals is unable to provide an efficient signal for degranulation of human basophils (82). IgE from atopic patients is termed IgE+, since it efficiently leads to basophil degranulation. The exact nature of IgE+ remains to be determined, although post-translational modifications have been suggested. If such a difference does exist, it is possible that T cell cytokines may play some role in controlling IgE+ production.

GENETIC INFLUENCES ON IMMUNE RESPONSIVENESS TO ALLERGENS

Although elevated levels of specific IgE are common to all individuals affected by atopic diseases, the identification of the genetic factors controlling atopy has failed. This appears to be due primarily to incomplete knowledge of the mechanisms of IgE regulation (83) and to the possibly polygenic nature of inherited susceptibility to allergies (84, 85).

Non-MHC Linked Genetic Regulation

Asthma and rhinitis are multifactorial conditions, the expression of which depends upon both genetic and environmental influences. While it is generally accepted that predisposition to allergic diseases together with a specific liability to develop asthma or rhinitis is inherited, no clear mode of inheritance is recognized (86, 87). Detailed segregation analysis of atopy, based on the evaluation of total serum levels of IgE, was performed in a large number of families. This investigation examining linkage between 21 genetic markers provided evidence against the codominant, recessive, or dominant models of inheritance of atopy. The study did not substantiate purely environmental models of inheritance (88). The vertical transmission of the atopy phenotype was examined in extended and nuclear families in which individuals demonstrated allergic immune reactions to a variety of common allergens, clinically characterized by symptoms compatible with atopic disease, the presence of positive skin tests, and elevated serum IgE. The results of this study suggested autosomal dominance as the mode of inheritance. Subsequent molecular genetic linkage studies on the family members supported the indication that the transmission of atopy was a dominant trait and allowed a putative "atopy gene" to be mapped to chromosome 11q (89). From an immunological point of view it is interesting to note that a number of T cell activation antigens, including CD3, CD5, and CD26, are also located on this chromosome.

HLA-Associated Responsiveness to Allergens

GENETIC EPIDEMIOLOGICAL STUDIES Analysis of responsiveness to defined proteins from pollen allergens has provided much of the information on the linkage between HLA and allergic disease. In extensive population studies examining specific IgE and IgG antibodies to Ra5 (*Amb a V*), after natural exposure to ragweed pollen, responsiveness correlated with expression of the HLA-DR2Dw2 phenotype (90, 91). Despite a lack of serological cross-reactivity, *Amb t V* the homologue of *Amb a V* from *Ambrosia trifida* (giant ragweed), is also DR2Dw2-associated, suggesting that recognition was directed toward a shared determinant (92). Homology present in the region 17–26 may determine DR2Dw2-associated responsiveness. The investigation of individuals with specific IgE to both proteins supported this conclusion, in that all were DR2Dw2+ (93). Competition studies using *Amb a V* reactive T-cell clones demonstrated that both *Amb t V* and *Amb p V*, from *Ambrosia psilostachya* (western ragweed), inhibit the presentation of *Amb a V* by DR2Dw2 accessory cells, suggesting all three members of the *Amb V* allergen family are DR2Dw2-restricted (19). Collectively, these studies and subsequent population analyses (94) support a positive association between immune recognition of the *Amb V* allergens and the DR2Dw2 phenotype.

In genetic epidemiological studies an association of the HLA-DR5 (DRw11) haplotype and the presence of *Amb a VI* specific IgE was observed (95, 96). However, not all studies on specific responsiveness to pollen antigens have revealed HLA associations. No significant correlation has yet been observed for *Amb a I* or *Amb a III*. The recognition of these allergens may be directed toward multiple determinants obscuring any HLA associations.

MOLECULAR ANALYSIS OF HLA CLASS-II REGULATION Since the initial studies in which the DR2Dw2 specificity was determined by serology and mixed lymphocyte reaction, polymorphism of DR2 molecules has been demonstrated from DNA sequences. To further examine the polymorphism of DR2-associated responsiveness to *Amb a V*, restriction fragment length polymorphism (RFLP) was performed, retrospectively, on a proportion of the subjects used in the initial studies with DR β , DQ α , and DQ β probes (97). This study revealed that most of the *Amb a V* reactive individuals had RFLPs consistent with DR2Dw2, whereas the phenotype of the *Amb a V* nonresponder group was DR2Dw2 negative. Patterns of hybridization using allele-specific oligonucleotide probes on genomic DNA from DR2+ subjects, amplified by the polymerase chain reaction (PCR), also supported the *Amb V* responsiveness/DR2Dw2 association (98). *Lolium perenne* (rye grass) has been studied to establish HLA-allergen associations, both in

epidemiological studies and at the molecular level. From population studies, responsiveness to *Lol p I*, *II*, and *III* is associated with HLA-DR3, based on specific antibody levels (99, 100), as is allergic sensitivity to the pollen of *Parthenium hysterophorus* (101). Comparison of their sequences indicates that *Lol p II* and *III* have 54% homology, providing an explanation for the serological cross-reactivity observed to the two proteins (41). Recognition of *Lol p I* is also DR3-associated, although antibodies to *Lol p I* are not cross-reactive on either *Lol p II* or *III* (102). The sequence of *Lol p I* is incomplete; there is, however, homology to *Lol p II* and *III* in the region 60–71, which may account for the association with DR3. Detailed analysis of specific responsiveness to *Lol p III* in two groups of skin-test-positive Caucasian individuals revealed a significant association with HLA-DR5, in addition to DR3 (103). This may be due to the presence of distinct epitopes in *Lol p III*, restricted by DR3 and DR5, respectively. Alternatively, a single sequence from *Lol p III* may bind equally to both alleles. Interestingly, analysis of one of the patient groups indicated a weak linkage of *Lol p III* reactivity with the DRw6 allele (103). The DRw52 supertypic specificities are encoded by the HLA-DRB3 gene products of the DR3, 5, 6 and 8 alleles. Therefore, if responsiveness to *Lol p III* were restricted by these class II molecules, it might account for the complex pattern of DR association. However, population analysis and hybridization studies using an oligonucleotide probe specific for the third variable region of DR3/DRw52 failed to establish a linkage between responsiveness to *Lol p III* and DRw52. Comparison of the amino acid sequences of the β chains of DR3 and DR5 revealed homology in the amino terminal half of the domains (residues 9–13, EYSTS), and conservation of this polymorphic region in DR3 and 5 was interpreted as determining the ability of *Lol p III* to bind preferentially to these two MHC class-II molecules.

HLA CLASS-II RESTRICTION SPECIFICITY OF T-CELL RECOGNITION The isolation of allergen-reactive T-cell clones encouraged investigation of the contribution of individual MHC class-II molecules in T-cell recognition at the molecular level. This approach has been informative where population analysis failed to demonstrate HLA associations. Direct evidence that HLA-DR, as opposed to -DP or -DQ gene products, function as major restriction elements in the recognition of allergen was based initially on inhibition of antigen-dependent responses of T-cell clones and lines with polyclonal (22) and monoclonal (32) antibodies. However, lacking appropriate antibodies to define subtle structural differences in HLA-D region gene products, allogeneic presenting cell panels were employed to define restriction specificity. The pattern of HLA class-II restriction of house dust mite-reactive T cell clones of different antigen specificities was observed

to be both heterogeneous and complex (2, 32). No obvious patterns of restriction consistent with the serologically defined specificities appeared, although subsequent analysis with murine fibroblasts transfected with DR5 (DRw12) confirmed that this DRB1 gene product was functional in the recognition of dust mite (104). Clonal analysis indicated that DR5 was also functional in the restriction of responsiveness to ragweed (R. Garman, R. O'Hehir, J. Lamb, unpublished results). The specificity of these T-cell clones appeared to be for the *Amb a* VI pollen antigen. It is important to emphasize that definition of fine antigen specificities and the association of these epitopes with the production of specific IgE would be required to draw conclusions on HLA associations from in vitro patterns of restriction specificity. Recognition of a dust mite epitope was mapped to a region of the β 1 third variable domain shared by three DR alleles (DR2, 5, and 8). Presentation by fibroblast transfectants and EBV-transformed B cells expressing HLA class-II molecules of the appropriate specificities confirmed a functional role for residues 70 and 71 in the recognition of this epitope (104). Furthermore, these T cells supported IgE synthesis in an allergen-dependent in vitro B-cell assay. The restriction specificity of T-cell clones reactive with *Amb a* V has also been mapped to the B1 gene of DR2Dw2 (DR2.2), supporting the population studies demonstrating an association between responsiveness to *Amb a* V and DR2Dw2 (19).

MHC class-II regulation of antigen recognition by those dust mite-reactive T-cell clones with broad patterns of restriction specificity, as assessed by presenting cell panels, was examined with HLA class-II transfected murine fibroblasts. Specific presentation by DRw52b + fibroblasts to T-cell clones that support IgE synthesis, and to the major component of an oligoclonal T-cell line, implied that the HLA-DRB3 gene products are functional in the recognition of *Dermatophagoides* (Figure 2). The role of the DRB3 gene products in the recognition of house-dust mite is unclear, particularly in view of their reduced expression as compared to DRB1 (105). Perhaps the expression and turnover of these class-II molecules on specialized antigen presenting cells in the lung are different (106). Assessment of epidemiological genetic studies is required to determine a potential HLA association between the HLA-DRB3 gene products and responsiveness to dust mite. Immune regulation by these MHC class-II gene products may in part account for the high prevalence of dust mite allergy. The observed complex patterns of antigen and restriction specificity of T-cell recognition of *Der p* I (H. Yssel, J. de Vries, personal communication; 104) suggest promiscuity in both peptide binding and T cell recognition (107).

The analysis of the restriction specificity of the interaction of allergen with T cells is limited, and expansion of this data base is required to establish HLA associations. However, population analysis of inheritance

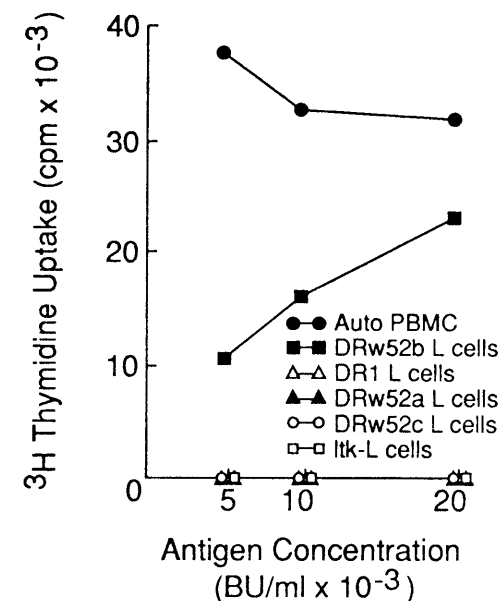


Figure 2 HLA-DRB3 (DRw52) restricted T-cell recognition of *Dermatophagoides* spp. Oligoclonal T cells reactive with house dust mite were maintained in long-term culture and examined for their capacity to recognize specific antigen presented by murine fibroblasts expressing HLA-D region molecules. Fibroblasts expressing DRw52b, histocompatible with the T cells but not the control transfectants, were able to induce T-cell proliferation. Proliferation was determined by the incorporation of tritiated thymidine. This figure (104) (copyright Oxford University Press) is reproduced by kind permission.

of allergic diseases argues against sole regulation by the HLA gene products. Potential interactions between non-MHC linked genes, for instance the "atopy gene" on chromosome 11q, are intriguing and suggest the possibility that such genes may determine overall predisposition to allergic disease, while the MHC haplotype influences the specificity of the allergic response.

HLA-LINKED CONTROL OF ALLERGEN NONRESPONSIVENESS The lack of IgE responsiveness of nonatopic subjects to allergen, in some instances, has been attributed to the induction of specific suppression (108). The effects of depletion and reconstitution of CD8+ T cells on the response of peripheral blood mononuclear leukocytes to allergen implied that reactive CD4+ T-cell clones in nonatopic individuals were negatively regulated. Seasonal exposure to *Cryptomeria japonicum* causes 10–15% of Japanese subjects to suffer from cedar pollinosis. In this population, unlike that of

unaffected individuals, synthesis of specific IgE is observed after provocation with purified antigen. Genetic analysis suggests that the inheritance of low responsiveness in nonallergics is dominant, in negative linkage disequilibrium with HLA-DQw3 (109). The isolation of a DQw6-restricted cedar pollen-specific T-cell line from a healthy individual supports a functional role for DQ class-II molecules in the recognition of allergen (T. Sasazuki, personal communication). A recent investigation of the hereditary characteristics of allergic rhinitis, also in the Japanese population, has however indicated that inheritance is autosomal and recessive. Analysis by DNA typing revealed that the frequency of DQw3 increased in patients allergic to cedar pollen and *D. farinae*, compared with unaffected individuals (Y. Sadanaga, T. Ishikawa, personal communication). The disparity in these reports leaves inconclusive and controversial the functional role of DQ class-II molecules in regulating low responsiveness to allergen.

IMMUNOREGULATION OF IMMUNE REGULATION OF ALLERGENS

Despite the widespread clinical use of hyposensitization regimens, understanding is limited of the immunological mechanisms underlying allergen immunotherapy. A widely held view has been that the decrease in allergen-directed responsiveness was due to the generation of suppressor T cells (108, 110). This interpretation may now need to be reassessed in view of potential interactions of various T-cell cytokines produced from T cells modulated during hyposensitization. Decreases in T-cell proliferation (111, 112), IL-2 production (113), and the number of high affinity IL-2 receptors on the T cells from the allergen-stimulated cultures (114) following immunotherapy have all been reported. Despite the mechanisms mediating hyporesponsiveness, ideal immunotherapeutic agents are those that reduce the level of specific IgE without risk of inducing systemic anaphylactic reactions.

Interference with the formation of the ternary complex formed by TCR/MHC and antigen may allow the downregulation of responsiveness and may facilitate immunotherapy. From an immunological stand point, this downregulation entails the ability to establish peripheral anergy by directly regulating the T cells or by competition from non allergen-derived peptides at the level of the class-II MHC molecules.

Functional Inactivation of Allergen Reactive T Cells

Anergy induced by specific antigen in a nonimmunogenic form potentially allows only those "disease-related" T cells to be functionally inactivated or deleted by virtue of the unique specificity and clonal distribution of the

TCR. However, if multiple T-cell epitopes on different proteins are able to induce IgE in a specific allergic reaction, then practical aspects of this approach to therapy are complicated. Structural modification may be necessary to convert "disease-related" epitopes to forms that are no longer able to bind IgE. Studies on the induction of tolerance with peptides from hen egg lysozyme have indicated that those peptides that are "immuno-dominant" are also potent tolerogens (115). The ability of fragments to modulate immune responses to intact molecules has been analyzed for a number of proteins, but of these studies the regulation of the IgE response to BSA is of particular relevance to this discussion. Fragments of BSA ranging in size from approximately 40 to 300 residues, each containing a single serological determinant, when administered intravenously were able to downregulate the response to the intact protein. By adoptive transfer the suppression appeared to be mediated by T cells and required that the "helper" and "suppressor" epitopes be linked. Immunization with the fragments primed for antibody was directed against only the fragment used for immunization, and not other unrelated "helper" epitopes in the same protein (43). In other murine studies of the IgE antibody response to house-dust mite allergens, downregulation of the IgE antibody response was obtained by chemically modified antigen (116). The experimental approach of using fragments of allergen for hyposensitization has been adopted clinically and studied in detail in the manipulation of the response to bee venom (117). Patients were treated with P-1, a pepsin-derived fragment of bee venom PLA₂. No initial rise in IgE occurred following cutaneous injection of P-1, and the decrease in both PLA₂-specific IgE and IgG was rapid. Skin test sensitivity to PLA₂ was also reduced (117). Although following desensitization these individuals had immunological parameters similar to nonatopic individuals, the clinical benefit of this treatment was not assessed. Neither were the immunological mechanisms mediating the effects examined, although the authors speculated that the effect of pepsin hydrolysis of PLA₂ may be either to enhance the number of "suppressor" or to decrease the "helper" epitopes in the protein. Nevertheless, the induction of T cell nonresponsiveness would also explain their findings.

Animal studies demonstrated that when allergen in the form of an aerosol was repeatedly administered to mice and rats, the allergen induced transient IgE responses which were spontaneously downregulated despite continuing aerosol exposure (118). Subsequent rechallenge of these animals revealed that aerosol exposure had selectively suppressed IgE antibody production. The lack of specific responsiveness lasted for three months after the termination of aerosol exposure. From the results of adoptive transfer experiments, the mechanism of unresponsiveness

appeared to be mediated by CD8⁺ T cells. The cellular targets for the CD8⁺ T cells were CD4⁺ T cells; B cells were unaffected (119, 120). The downregulation of responsiveness to antigen appeared restricted primarily to high IgE responder strains (118–121). However, nanogram amounts of allergen, like those encountered in the normal environment, induced tolerance in low IgE responder animal strains; 1000-fold greater concentrations were required to achieve the same effect in high responder strains (120).

Although the clinical protocols of hyposensitization mimic certain of the features of T cell-dependent low-zone tolerance and are able to decrease IgE levels in some cases, the immunological basis is ill-defined. In preliminary studies in vitro, functional inactivation of house dust mite-reactive cloned T cells that are able to support IgE synthesis could be demonstrated using a protocol of "low dose" hyposensitization (122). The helper T cells were initially activated by stimulation with autologous accessory cells and *D. farinae*, as would be encountered in vivo. Subsequent exposure to repeated escalating concentrations of a multideterminant polypeptide antigen mixture, derived from house dust mite, resulted in a 42% decrease in the response of the T cells to an immunogenic challenge. The decreased antigen responsiveness of the T cells was not attributable to cytotoxicity, for enhanced IL-2 proliferation was observed. This fact had been demonstrated in the in vitro model of T-cell nonresponsiveness using supraimmunogenic concentrations of free peptide (123). If "immunodominant" epitopes could be mapped by analyzing the specificity of T-cell responses using recombinant DNA sublibraries and synthetic peptides, structural modification of these epitopes might allow both their tolerogenic activity to be enhanced and their potential for IgE binding to be disrupted. Specific allergic immune responsiveness in atopic individuals, however, undoubtedly will not be limited to a single "immunodominant" epitope but will involve the recognition of multiple epitopes. Nevertheless, T cell receptor V β gene usage may be restricted, as appears to occur in certain autoimmune diseases (124, 125). An alternative approach may be to modulate the T cells specifically through shared characteristics of V β gene products. A number of antigens have been termed "superantigens" based on their ability to stimulate powerful polyclonal proliferative responses of T cells bearing particular V β gene products. This family of antigens includes the *Staphylococcus aureus* enterotoxins, and recent studies have demonstrated that certain of these toxins are able to tolerize human T cells in such a way that they fail to respond to an immunogenic challenge with their natural ligand, even though IL-2 responsiveness is retained (126). Similarly, in house dust mite-reactive T cells expressing V β 6 gene products, nonresponsiveness induced by *S. aureus* toxin E is associated with modu-

lation of the T cell antigen receptor from the cell surface (R. O'Hehir, J. Lamb, unpublished results). Clearly, the generation of reagents with a similar spectrum of tolerogenic activity, but lacking toxic activity, may provide an alternative modality for therapeutic intervention.

Inhibition of Responsiveness by Antigen Competition

The ability of peptides to compete for occupancy of the antigen combining site of MHC class-II gene products (127) theoretically provides the opportunity to regulate immune responses at the level of the ternary complex. By designing nonstimulatory, nonallergen derived peptides that bind with high affinity to class-II molecules of the appropriate specificity, the binding of the allergen fragment may be inhibited. Direct binding assays indicate that the interaction between peptides and MHC class-II molecules is permissive, in that a particular MHC class-II molecule is capable of binding a large number of distinct peptides and, similarly, a single peptide may bind to the gene products of many different MHC class-II alleles (107, 128). Despite this observed permissiveness in binding, variations in class-II MHC molecules regulate T-cell recognition by affecting both peptide binding and interaction with T cell receptors (8). Clearly, if competitive inhibition were to have a role in the regulation of allergic immune responses, then specificity of the blocking peptide for the disease-associated MHC class-II allele would be a prerequisite.

The role of nonallergen-derived peptides as competitors in the response to house-dust mite has been examined. Analysis of the MHC class-II restriction specificity of T-cell recognition of dust mite suggested that the DRB3 gene products functioned as the major restriction elements (104). In screening peptides for direct binding, an analogue of the carboxyl terminus of influenza virus haemagglutinin (HA; residues 307–319; with the naturally occurring tyrosine residue at position 309 substituted by serine) bound strongly to murine fibroblasts expressing DRw52b molecules. The HA analogue was able to inhibit in vitro both polyclonal responses to house dust mite and that of individual T-cell clones of different specificities. These facts imply that the competing peptide could prevent activation of the majority of dust mite-reactive T lymphocytes (129). The inability of the peptide to inhibit responsiveness of ragweed or *Fel d I* reactive T-cell clones to their natural immunogen demonstrated that overall T-cell responsiveness was not ablated, and that a certain degree of specificity of nonresponsiveness could be achieved.

In order to identify those components of house dust mite that were binding strongly to DRw52b, peptides derived from the group I and II allergens were examined for their ability to compete for the binding of the HA peptide. As observed in functional proliferation assays, the unfrac-

tionated extracts of dust mite and the group-I allergens, but none of the peptides, were able to compete for binding, indicating that those sequences with specificity for DRw52b had not been identified at the epitope level. Nevertheless, pulsing presenting cells that express only a single MHC class-II molecule with a complex mixture of antigens and testing their ability to compete for the binding of a peptide of known binding specificity is useful in the mapping of dominant restriction specificities. The choice of a nonstimulatory, nonallergen-derived peptide minimizes the potential risk of anaphylaxis. As precedent for such a method of regulating immune responses, Adorini et al (130) demonstrated that a self-peptide derived from murine lysozyme, and differing by only three amino acids from hen egg lysozyme (HEL), was able to inhibit presentation of HEL in vivo in a high responder strain. This is supported by the observation that non-stimulatory peptide analogues derived from myelin basic protein are able to prevent the induction of experimental allergic encephalomyelitis (125, 131). However, protection was mediated only if the competing antigen was administered at the same time as the immunogen. Such an approach may be particularly useful in the seasonal exposure to grass and tree pollen allergens. Clearly, the immune response of atopic individuals to allergen may be considered as aberrant, analogous to autoreactivity, in that benefit to the individual will come from the downregulation of the specific response.

CONCLUDING COMMENTS

Evidence continues to accumulate supporting a central role for CD4⁺ T cells in the induction and regulation of immune responsiveness following exposure to allergens. Nevertheless, knowledge of the antigen specificity, genetic regulation, and functional properties of those T cells capable of driving the allergic response is incomplete. Cloning of the major proteins of different allergens will allow the antigen specificity of the cellular immune response to be mapped in detail, and compared between allergic and nonallergic individuals. The restriction specificity of T-cell recognition may now be explored by the application of murine fibroblasts expressing HLA-D region gene products and allergen-reactive T-cell clones. Despite the molecular characterization and knowledge of the mode of action of many of the cytokines that influence both specific and nonspecific allergic effector mechanisms, those immunological signals that differentiate between the development of allergic responsiveness in atopic and non-atopic individuals remain to be defined. The practical benefit of understanding the immunological basis of allergic disease is that it will facilitate

the development of experimental models to examine potential protocols of hyposensitization.

ACKNOWLEDGMENTS

We are grateful to our colleagues who have contributed their results prior to publication. We also thank M. Chapman, M. Geftter, A. B. Kay, M-C. Kuo, H. Lowenstein, D. Marsh, T. Platts-Mills and W. Thomas for their enlightening discussions. The work was supported in part by the Medical Research Council (J. R. Lamb) and a Wellcome Senior Research Fellowship in Clinical Science (R. E. O'Hehir). The literature search for this review ended in August 1990.

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