

Molecular genetics of human immune responsiveness to pollen allergens

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Introduction

Atopic allergy is a complex, immunologically-based disease that is dependent on specific IgE antibody (Ab) responses to environmental antigens (allergens) [1]. In order to understand this disease, we need to appreciate the *multifactorial* (multiple genetic and environmental) determinants which lead to the allergen specificity and the different clinical manifestations – allergic rhinitis, asthma, atopic dermatitis, etc. For genetic analyses, we believe that the simplistic notions of single atopy-determining genes (reviewed in [2–5]) simply have to go; but, equally importantly, we have to frame our hypotheses appropriately, to choose our study populations and families carefully and ask the right questions; otherwise, we will become eternally bogged down in the complexity of the problem. Our primary focus here will be on the immunobiology of allergy, particularly of allergen-specific immune responsiveness. Another area of relevance genetically is the (presumably) multiple non-immunologic factors which influence the characteristic pathophysiologies found in different classes of allergies (cf, [6]).

In regard to immunogenetic factors in atopic allergy, let us consider the following postulates.

- 1) Clinically, the principal causative determinant of a specific allergy is specific IgE Ab response(s) to one or more components of an environmental allergen complex(es), such as a pollen or an animal dander.
- 2) The sum of all the specific IgE Ab responses to individual allergens within such allergen complexes is reflected in the total serum IgE level; only allergen-specific IgE is present in the serum (except perhaps in parasitic infestations and certain pathologic states such as myeloma).
- 3) Since virtually everyone has measurable total serum IgE levels, virtually everyone is “immunologically atopic” to *some* allergen(s); whether this is actually recognized *clinically* by the patient and/or the clinician depends primarily on the prevalence of the allergen(s). Often the offending allergen(s) in subjects who are believed to be clinically non-atopic, but who have measurable serum IgE, go undetected by the limited panel of allergen extracts used for skin-test and/or RAST screening.
- 4) We believe that, *from an immunological point of view*, to talk of the inheritance of an “atopy” gene is largely irrelevant, since virtually everyone is atopic in this sense. It is more appropriate to talk about the genetic determinants of: a) overall IgE responsiveness, and b) specific responsiveness (MHC, TcR, Ig genes), and how they *influence* the expression of the various clinical phenotypes characteristic of atopic disease.

IgE genetics

While the genetics of IgE responsiveness (of any specificity) is not straightforward, IgE-regulating genes are probably the most important non-environmental determinants of allergy [7, 8]. The available evidence points to the involvement of a major gene in IgE regulation [8] that is not linked to HLA [9]. Different modes of inheritance for this postulated IgE-regulating gene have been suggested based on the distribution of total serum IgE levels in different families [7]. However, three studies [7, 10, 11] suggest that “high” serum IgE levels are controlled by a recessive IgE-regulating gene. Meyers et al [11] have provided evidence that 37% of the distribution of IgE levels in 42 large families is determined genetic factors – half of which can be attributed to a recessive gene. The principal candidate genes for IgE regulation are those which control the levels and/or function of certain cytokines, particularly IL-4, which is known to be critical for IgE responsiveness; also, the expression of FcεRII on B and/or T cells may also play a role (see de Vries and Delespesse elsewhere in this volume).

Thus, there is a clear relationship between IgE regulation and the clinical expression of atopy, but the apparent mode of inheritance of “atopy” depends on how one defines the disease clinically [12].

Whether these data relate to a recently postulated dominant "atopy gene" linked to a marker on chromosome 11q [13] is presently unclear.

Genetics of specific immune responsiveness

The present confusion in the area of the genetics of IgE regulation can be contrasted with the relative clarity of our knowledge of the molecular genetics of factors responsible for specific immunity, including IgE Ab responses. This difference stems from the fact that the identity of the major genes determining the induction phase of the response appears to be established – specifically, Class II major histocompatibility complex (MHC) and T-cell receptor (TcR) α and β genes [14]. The products of these genes, Class II (or Ia) molecules and the TcR, together with an antigen (Ag) fragment derived from previous "Ag processing" within the APC, are able to form a trimolecular complex bridging an Ag-presenting cell (APC) and a CD4-positive helper T cell (Fig. 1).

The human Class II (HLA-D) region contains DR, DQ, and DP subregions, each of which includes one or more A and B genes which are expressed as the α and β polypeptide chains of Class II MHC molecules [14]. With the exception of DRA, the expressed genes are highly polymorphic (*ie*, there are many different alleles at each A and B locus). The second exons contain the most polymorphic residues: notable "hot spots," or allelic polymorphic regions (APRs) encode amino acid (aa) residues which point into the Ag-binding grooves of the Class II molecules (from the β -pleated sheets or the α -helices), and are probably involved directly in binding to the Ag fragment (Fig. 2, [15, 16]).

The use of molecular biological and cellular immunological techniques, particularly recombinant DNA technology and T-cell cloning, greatly facilitates study of the Ia–Ag–TcR complex. All three components of the complex merit study; but the specific regions which interact to form the trimolecular complex are of particular interest. The portion of the Ag fragment interacting with the Ia molecule is referred to as the "agretope" and the part which interacts with the TcR as the "T-cell epitope."

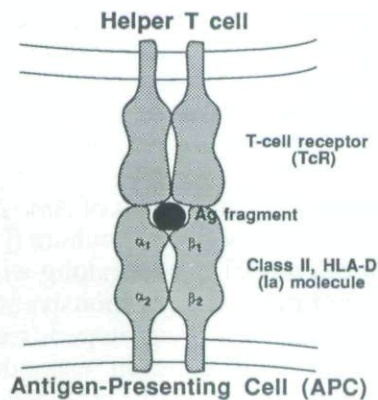


Fig. 1. The Ia–Ag–TcR complex [14]

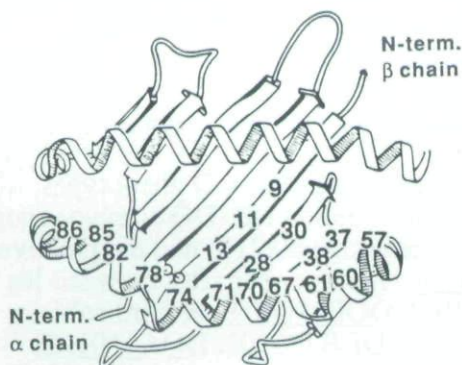


Fig. 2. The Ag-binding groove of a modeled HLA-DR molecule as viewed from the TcR. Numbered residues of DR β chain are predicted to point into the groove (from [15, 16]).

Defining the molecular genetic basis of specific immune responsiveness to allergens

In order to explore the molecular basis of specific immune responsiveness to allergens, we have employed the following approach. First, we have analyzed for possible statistical associations between specific HLA-D types (determined by serological and/or MLR typing) and specific immune responsiveness of allergic subjects to highly purified allergens (defined by measurement of serum IgE and IgG Ab levels by double Ab radioimmunoassay, before or after ragweed immunotherapy (Rx)). Second, we have selected those favorable instances where there are marked HLA-D associations with the presence of specific serum Abs. We have then investigated the allelic polymorphic regions of the HLA-DRB, DQB and DQA genes which encode aa residues implicated in binding to the Ag fragment (*cf*, Fig. 2). This has been done by amplifying the second exons of DRB, DQB and DQA genes by means of the polymerase chain reaction (PCR), followed by sequencing and/or SSO-dot-blot analyses of the amplified DNA fragments. Third, we have isolated Ag-specific T-cell clones and used them to define more precisely the HLA-D molecule involved in Ag presentation as well as the agretope/T-cell epitope of the Ag. On-going studies will analyze of the TcR α and β genes. Finally, we are studying the cDNA sequences of allergen cDNAs in order to investigate the nature of Ia/T-cell determinants further by means of site-directed mutagenesis. These studies are presented and reviewed in detail elsewhere [14, 17–21].

Table 1. Significant associations of HLA with specific antibody responsiveness toward highly purified pollen allergens in atopic Caucasoid subjects.

Systematic Name	M _r	Major HLA Association	Westinghouse subjects		Clinic patients		Overall p values*
			+ve	-ve	+ve	-ve	
<i>Ambrosia artemisiifolia</i> and <i>A. trifida</i> (short and giant ragweeds):							
<i>Amb a V</i>	5,000	DR2/Dw2	9/9 (100%)	20/83 (24%)	27/29 (93%)	10/56 (18%)	<10 ⁻⁹
<i>Amb a VI</i>	11,500	DR5	11/13 (85%)	14/103 (14%)	6/15 (40%)	4/66 (6%)	<10 ⁻⁶
<i>Amb t V</i>	4,400	DR2/Dw2	3/4 (75%)	0/13 (0%)	3/3 (100%)	1/7 (14%)	<10 ⁻³
<i>Lolium perenne</i> (perennial rye):							
<i>Lol p I</i>	27,000	DR3	23/70 (33%)	9/65 (14%)	14/39 (36%)	2/28 (7%)	<10 ⁻³
<i>Lol p II</i>	11,000	DR3	11/28 (39%)	21/107 (20%)	9/19 (47%)	7/48 (15%)	10 ⁻³
<i>Lol p III</i>	11,000	DR3	13/30 (43%)	19/105 (18%)	13/23 (57%)	3/44 (7%)	<10 ⁻⁵

* p values are given only for the most significant associations for analyses of IgE Ab data, except that IgG Ab data from immunized patients were used for *Amb t V*. No significant HLA-D associations were found for responsiveness to: *Amb a I* (M_r = 37,800), *Amb a III* (M_r = 12,300), *Phl p V* (M_r = 27,000), *Der f I* (M_r = 24,000) and *Der f II* (M_r = 14,500). (Adapted from [14] and unpublished.)

As shown in Table 1, certain specific IgE (and IgG) Ab responses to highly purified allergens exhibit marked, significant associations with particular HLA-DR/Dw types; whereas other responses, usually to more complex and/or more abundant allergens, show no significant associations in the populations studied. In the latter cases, several different agretopes may be recognized by different Class II molecules and/or DP molecules (not yet studied) may be involved in specific immune recognition, as discussed elsewhere [14].

We will review only our molecular biological and cellular immunological studies of *Amb a V*; data concerning minor *Amb a V Ir* genes and *Ir* genes for further allergens are reported elsewhere ([14, 17–21] and Zwollo et al, submitted). The simple *Amb a V* (45-aa peptide) model system (along with its *Amb V* homologues from other ragweed species) is particularly appropriate, since responsiveness is strongly associated with HLA-DR2 and Dw2 ("DR2.2"), suggesting that a major agretope is recognized by a single Class II molecule. We studied the APRs of DRB, DQB and DQA genes in responders and non-responders to *Amb a V*. We sequenced PCR-amplified DRB second exons of 17 ragweed-allergic patients and of DQB in 15 of these subjects, and used sequence-specific oligonucleotides (SSOs) in dot-blotting experiments to study the polymorphism in the APRs of DRB and DQB alleles of 67 patients and of DQA in 40 of these patients ([17, 20]; Zwollo et al, submitted). No *Amb a V* responder-specific sequences were found; but response-associated sequences were noted, namely, DRB1*1501, DRB5*0101, DQA1*0102 and DQB1*0602, which are normally found together (are in "linkage disequilibrium") on the common Caucasoid DR2(Dw2),DQw1 haplotype (see [22] for nomenclature of DNA sequences of HLA).

The crucial "breakthrough" came with the discovery of three unusual HLA-D phenotypes, which included so-called "recombinant haplotypes" not conforming to the common DR–DQ combinations in Caucasoids. One *Amb a V* responder from natural pollen exposure (subject #218) had the putative haplotype DRB1*1501, DRB5*0101, DQA1*0102, DQB1*0502. A responder after ragweed Rx (subject CSz) possessed DRB1*1501, DRB5*0101, DQA1*0102, DQB1*0603. A third subject (JMc), a non-responder even after extensive Rx, had DRB1*0301, DRB3*0101, DQA1*0102, DQB1*0602. (In each case, the unusual DQ sequence(s) within the haplotype are underlined.) The patterns of *Amb a V* responsiveness in these recombinants suggest that particular DR, but not DQ, sequences are involved in responsiveness – ie, the DR2.2-associated sequences (either DRB1*1501 or DRB5*0101), but not the DQ1.2-associated sequences (DQA1*0102 or DQB1*0602) are involved.

Studies by Huang et al ([18] and submitted) using a panel of three cloned *Amb a* V-specific T cells from a DR2.2+ *Amb a* V-responder patient provided a definitive test of this hypothesis, and also allowed us to resolve which of the two DR2.2 Class II specificities, DR $\alpha\beta_1$ 2.2 or DR $\alpha\beta_V$ 2.2 (encoded by DRA/DRB1*1501 and DRA/DRB5*0101, respectively) is involved in responsiveness to *Amb a* V. The APCs from the three patients having recombinant haplotypes were used to test the importance of DR versus DQ molecules in *Amb a* V presentation to the cloned T cells. Huang et al found that the APCs of subjects #218 and CSz (together with *Amb a* V) were able to induce proliferation in each of the three T-cell clones, but the APCs of subject JMc could not. These data again point to the importance of DR: this was confirmed by showing that only anti-DR MAb, but not anti-DQ or anti-DP, was able to inhibit T-cell proliferation in cultures containing *Amb a* V, cloned T cells and autologous APCs. The question of whether DR $\alpha\beta_1$ 2.2 or DR $\alpha\beta_V$ 2.2 was involved in Ag presentation was resolved by use of the MAb Hu30, which reacts with DR $\alpha\beta_1$ 2.2, but not DR $\alpha\beta_V$ 2.2 [23]. Huang and associates showed that MAb Hu30 could specifically block the presentation of *Amb a* V to the T-cell clones by DR2.2+ APCs, clearly implicating DR $\alpha\beta_1$ 2.2 as the major *Amb a* V-Ir gene. It was further shown that APCs typing HLA-DR2.12+, but not those typing DR2.21+ or DR2.22+, could present *Amb a* V to the T-cell clones. The Ag presentation by DR2.12+ cells was also blocked by MAb Hu30.

The aforementioned data are entirely compatible with the known close aa sequence similarity of the Ag-binding domains of DR β_1 2.2 and DR β_1 2.12 on the one hand, and the differences between these sequences and those of DR β_1 2.21 and DR β_1 2.22 on the other (confirmed in our patients). DR β_1 2.2 and 2.12 differ only by val-gly at aa position 86, whereas DR β_1 2.2/2.12 and DR β_1 2.21/2.22 differ notably at aa positions 67, 70 and 71, including charge differences at positions 70 and 71; Table 2).

Table 2. The variable aa residues within DR β_1 2 subtypes. Charged residues (at neutral pH) are indicated.

Am. Acid Residue	DR β_1			
	2.2	2.12	2.21	2.22
67	Ile	Ile	Phe	Leu
70	Gln	Gln	Asp ⁻	Asp ⁻
71	Ala	Ala	Arg ⁺	Arg ⁺
86	Val	Gly	Gly	Gly

Ongoing studies

We have also begun to investigate the fragment of *Amb a* V involved in binding to the Ia and TcR molecules using the Ag-specific T-cell clones (Huang and Marsh, unpublished). This has been done using synthetic peptide fragments of *Amb a* V for direct presentation, or inhibition of *Amb a* V presentation, to the T-cell clones. Further studies will involve the use of mutant forms of *Amb a* V prepared by site-directed mutagenesis of *Amb a* V cDNA clones. In order to study the structure of the Ag-binding portions of the TcR α and β genes, PCR amplification can again be

used; single-stranded cDNA is prepared from mRNA from an Ag-specific T-cell clone using reverse transcriptase, amplified by the "anchored-PCR" [24] and sequenced.

Conclusions

The expression of atopic allergy is dependent on immunogenetic controls of both overall and specific IgE responsiveness. Probably several other genes, as well as a complex array of environmental factors influence the immunopathology of the disease. We have shown that a combination of molecular biological and cellular immunological technologies provides a powerful approach towards addressing the molecular genetic basis of specific immune responsiveness: here, the choice of a model system (*Amb a* V) has proved crucial in simplifying the problem. The genes responsible for IgE regulation and other factors influencing the expression of atopic allergy are presently unknown. The analysis of these genes will require a combination of modern approaches of genetic epidemiology, cellular immunology, gene mapping and sequencing technologies in families. The choice of families which exhibit clear-cut segregation of individual genes will likely prove to be crucial in these studies.

References

1. Ishizaka K, Ishizaka T. Human reaginic antibodies and immunoglobulin E. J Allergy 1968; 42: 330-63.
2. Schwartz M. Heredity in bronchial asthma. Acta Allergol 1952; 5 (Suppl 2): 1-288.
3. Bias WB. The genetic basis of asthma. In: Austen KF, Lichtenstein LM, eds. Asthma: Physiology, Immunopharmacology and Treatment. New York: Academic Press, 1973: 39-53.

4. Black PL, Marsh DG. The genetic basis for atopic allergy in man. In: Segal MS, Weiss EB, eds. *Bronchial Asthma: Mechanisms and Therapeutics*. Boston: Little, Brown and Co, 1976: 53-64.
5. Marsh DG. Historical introduction. In: Marsh DG, Blumenthal MN, eds. *Genetic and Environmental Factors in Clinical Allergy*. Minneapolis: University of Minnesota Press, 1990: 3-9.
6. Marone G, Casolaro V, Celestino D, Bonini S. The role of genetic and environmental factors in the control of basophil and mast cell releasability. In: Marsh DG, Blumenthal MN, eds. *Genetic and Environmental Factors in Clinical Allergy*. Minneapolis: University of Minnesota Press, 1990: 153-60.
7. Marsh DG, Bias WB, Ishizaka K. Genetic control of basal serum immunoglobulin E level and its effect on specific reaginic sensitivity. *Proc Nat Acad Sci (USA)* 1974; 71: 3588-92.
8. Meyers DA, Marsh DG. Report on an NIAID-sponsored workshop on the genetics of total IgE levels in man. *J Allergy Clin Immunol* 1981; 67: 167-70.
9. Meyers DA, Hasstedt SJ, Marsh DG, Skolnick M, King MC, Bias WB, Amos DB. The inheritance of immunoglobulin E: linkage analysis. *Amer J Med Genet* 1983; 16: 575-81.
10. Gerrard JW, Rao DC, Morton NE. A genetic study of immunoglobulin E. *Am J Hum Genet* 1978; 30: 46-58.
11. Meyers DA, Beaty TH, Freidhoff LR, Marsh DG. Inheritance of total serum IgE (basal levels) in man. *Am J Hum Genet* 1987; 41: 51-62.
12. Borecki IB, Rao DC, Lalouel JM, McGue, Gerrard JW. Demonstration of a common major gene with pleiotropic effects on immunoglobulin E levels and allergy. *Genet Epidemiol* 1985; 2: 327-38.
13. Cookson WOCM, Sharp PA, Faux JA, Hopkin JM. Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. *Lancet* 1989; i: 1292-5.
14. Marsh DG. Immunogenetic and immunochemical factors determining immune responsiveness to allergens: studies in unrelated subjects. In: Marsh DG, Blumenthal MN, eds. *Genetic and Environmental Factors in Clinical Allergy*. Minneapolis: University of Minnesota Press, 1990: 97-123.
15. Bjorkman PJ, Saper MA, Samraoui B, Bennet WS, Strominger JL, Wiley DC. Structure of the human class I histocompatibility antigen, HLA-A2. *Nature* 1987; 329: 506-12.
16. Brown JH, Jardetsky T, Saper MA, Samraoui B, Bjorkman PJ, Wiley DC. A hypothetical model for the foreign antigen binding site of Class II histocompatibility molecules. *Nature* 1988; 332: 845-50.
17. Zwollo P, Kautzky EE, Ansari AA, Erlich HA, Marsh DG. Responsiveness to short ragweed allergen *Amb a V* correlates with specific HLA-DRB and DQB sequences. *Am J Hum Genet* 1989; 45: A232.
18. Huang SK, Marsh DG. Genetic restriction of human T-cell clones to short ragweed allergen, *Amb a V*. *Am J Hum Genet* 1989; 45: A196.
19. Marsh DG, Zwollo P, Ansari AA. Toward a total human immune response fingerprint: the allergy model. In: El Shami AS, Merrett TG, eds. *Allergy and Molecular Biology*. Oxford: Pergamon Press, 1989: 65-82.
20. Marsh DG, Zwollo P, Huang SK, Ghosh B, Ansari AA. Molecular studies of human response to allergens. In: Watson JD, Inglis J, eds. *Immunological Recognition*. Cold Spring Harbor Symposium 1990; 54: 459-70.
21. Ansari AA, Shenbagamurthi P, Kihara T, Zwollo P, Marsh DG. Molecular studies of human response to grass allergens. In: Schon AH, Kraft D, Kunkel G, eds. *Epitopes of Atopic Allergens*. Brussels: UCB Inst Allergy, 1990: 87-92.
22. Bodmer JG, Marsh SGE, Albert EA. Nomenclature for factors of the HLA system, 1989. *Immunol Today* 1990; 11: 3-10.
23. Kawai J, Ando A, Sato T, Nakasatsuji T, Tsuji K, Inoko H. Analysis of gene structure and antigen determinants of DR2 antigens using DR gene transfer into mouse L cells. *J Immunol* 1989; 142: 312-7.
24. Loh EY, Elliott JF, Cwirla S, Lanier LL, Davies MM. Polymerase chain reaction with single-sided specificity: analysis of T-cell receptor δ chain. *Science* 1989; 243: 217-20.

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