

# THORAX

## Editorials

### HLA genetics and allergic disease

The underlying cause of asthma, hay fever, and eczema in children and young adults is atopy, a disorder characterised by persistent immunoglobulin E (IgE) responses to extrinsic protein allergens. The phenotype is comprised of many factors, including total and allergen-specific IgE titres, skin prick test positivity, medical history, and provocation tests of bronchial reactivity. A broad definition of atopy may include 40% of the population,<sup>1</sup> while up to 10% of the population of western European countries suffers from asthma. Thus, it is hardly surprising that genetic analysis of atopic asthma has been difficult, despite strong evidence for heritability.

Currently, worldwide searches are underway for genetic loci regulating the IgE response and particular interest has centred on the  $\beta$  subunit of the high affinity IgE receptor (FC $\epsilon$ RI $\beta$ ) located on chromosome 11q and expressed on mast cells and basophils.<sup>2-9</sup> While claims have been made that FC $\epsilon$ RI $\beta$  is a major gene for atopy, with a strong bias towards maternal inheritance,<sup>7</sup> these findings have not been confirmed by all other studies.<sup>6,9</sup> More recently claims have also been made that interleukin 4 (IL-4) or a closely linked gene on chromosome 5q regulates IgE production in a non-antigen specific (non-cognate) fashion.<sup>10</sup> These exciting findings are currently under investigation in many laboratories. In addition to genes that control the overall level of expression of the atopic phenotype, however, important genetic influences are exerted over the recognition of allergenic antigens and the subsequent propagation of the immune response involving T cells, B cells, and IgE synthesis. In particular, genetic epidemiological studies in atopic subjects have shown several significant associations between particular human leucocyte antigen (HLA) class II genotypes and specific (cognate) allergic IgE responses

to antigens. A major international workshop is now underway to define further the contribution of HLA class II genes to house dust mite allergic asthma in family based studies. It is therefore an appropriate time to review findings from past and current studies of HLA genetics and allergic disease, mostly performed in studies of unrelated individuals, leading up to this important international collaborative clinical and laboratory study.

The classical human major histocompatibility complex (MHC) or HLA molecules are encoded by two highly polymorphic gene families located in a 3600 kb region of chromosome 6p (fig 1). The resulting HLA molecules – the most polymorphic found in humans – are membrane-bound glycoproteins that bind processed antigenic peptides and present them to T cells. The HLA class I A, B, and C molecules are each composed of an MHC encoded heavy chain (MW 45 kD), non-covalently associated with a non-polymorphic polypeptide  $\beta_2$ -microglobulin (MW 12 kD) encoded on chromosome 15 (fig 1). These HLA class I antigens, expressed on virtually all nucleated cells and platelets, function to present peptides of largely endogenous origin to CD8+ T cells which, in the main, are of cytotoxic function.

In contrast, HLA class II molecules, comprising three main subclasses (DR, DQ and DP) are found on a more restricted range of cell types including B cells, activated T cells, the monocyte/macrophage lineage, and are also interferon gamma (IFN $\gamma$ ) inducible. An expressed class II molecule consists of an  $\alpha$  chain (MW 31–34 kD) encoded by an A gene, non-covalently associated with a  $\beta$  chain (MW 26–29 kD) encoded by a B gene (fig 1). Both A and B genes may be polymorphic, but most polymorphism resides in the B genes (fig 1). Expressed class II molecules serve to present peptides of largely exogenous origin to CD4+ T cells, mostly of “helper” phenotype.

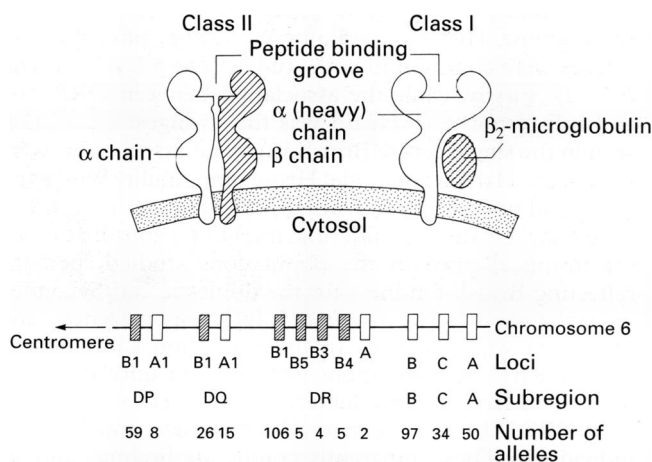


Figure 1 Classical HLA class I and II molecules and the genes encoding them. A genes = open boxes; B genes = hatched boxes. Number of allelic variants currently described is derived from Bodmer et al.<sup>14</sup> (Modified from Thorsby and Ronningen<sup>13</sup>).

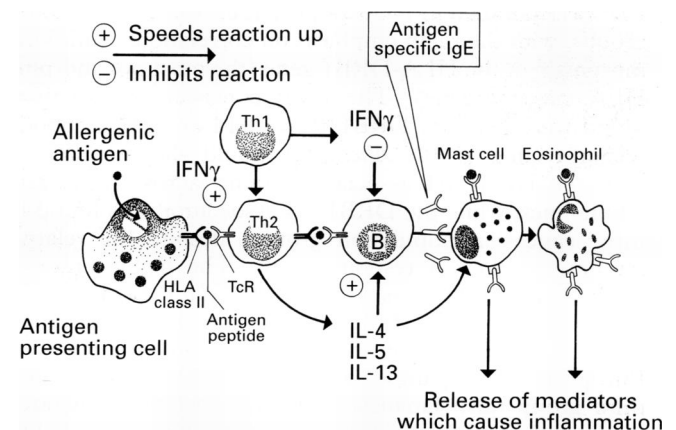


Figure 2 From antigen to allergic response showing interaction of HLA class II plus antigenic peptide and T cell receptor (TcR) on Th2 helper T cells. IFN $\gamma$  = interferon  $\gamma$ ; IL = interleukin.

In both class I and class II molecules the extensive molecular polymorphism is almost entirely confined to the peptide binding groove of each molecule<sup>11,12</sup> and this polymorphism determines which antigen-derived peptides may be bound and presented to T cells via their T cell receptors which, in the presence of appropriate co-stimulatory signals, leads to activation of those T cells recognising the HLA-peptide complex. Because of the pivotal role of HLA class II molecules in particular in regulating the immune response, combined with their extensive polymorphism, it is not surprising that particular HLA class II alleles have been implicated in susceptibility to a wide range of diseases known to have an immunological basis. Atopic allergy is no exception and the most probable role of the interaction between the HLA-peptide complex and T cell receptors in setting up the allergic reaction is shown in fig 2.

Initial immunogenetic studies of the possible role of HLA class II polymorphism in atopy, dating from the early 1980s onwards, concentrated on pollen allergens, and it has been clearly shown that increased IgE production to ragweed (*Artemisia artemisiifolia*) pollen allergens *Amb a V*, *Amb t V*, *Amb p V*, and *Amb a VI* occurs in individuals expressing DRB1\*1501 (DR2)-associated haplotypes<sup>15-18</sup> (the World Health Organisation HLA nomenclature committee now specifies a four number code to define each allele of each HLA locus at the DNA level, with two number codes defining the previous, lower resolution serological equivalents). Further studies have established population associations between DRB1\*03 and DRB1\*11 alleles and immune responses to rye grass (*Lolium perenne*) antigens.<sup>19,20</sup> In addition, it has now been established that products of the HLA-DRB1, DRB3, and DRB5 genes are able to restrict the recognition of house dust mite (*Dermatophagoides* spp) determinants<sup>21,22</sup> in line with population associations demonstrated in the 11th International Histocompatibility Workshop.<sup>23</sup> While several other single centre studies have found a number of additional HLA class II-allergen specific IgE associations in population based studies – for example, positive associations between DRB1\*07 and DQB1\*0201 and IgE response to the main olive pollen allergen *Ole e I*,<sup>24</sup> DRB3\*0101 and IgE responsiveness to the birch pollen allergen *Bet v I*,<sup>25</sup> and DRB1\*0101, DQA1\*0101, DQB1\*0501 and IgE responsiveness to the chironomid non-biting midge allergen *Chi t I*<sup>26</sup> – the most comprehensive investigation of HLA and allergy performed to date is still that of the 11th International Histocompatibility Workshop.

In this Workshop specific IgE (and IgG where measured) responsiveness to eight highly purified allergens (*Lol p V*, *Amb a V*, *Par o I*, *Bet v I*, *Cry j I*, *Fel d I*, *Alt a I*, and *Der p I*) was examined in 1006 atopic patients in 13 population groups, with the main emphasis on correlations with polymorphism of the HLA-DRB1 gene (the most polymorphic HLA class II gene). The most consistent associations found were between DRB1\*04 and 14 and IgE antibody responsiveness to the *Alternaria* mould allergen *Alt a I*, with the DRB1\*04 association found across racial and ethnic lines, while the DRB1\*14 association was found in three caucasian population groups, despite the relative rareness of *Alt a I* responsiveness. The highest rate of antibody responsiveness was towards *Fel d I* (the major cat allergen), with DRB1\*15 showing a positive association with *Fel d I* IgE antibodies in two study groups (one European, one Japanese) and a negative association in a third group (also Japanese). Other associations included confirmation of the well known association between IgE response to the *Amb a V* allergen and DRB1\*15 (in US caucasians), positive associations between DRB1\*04 (in Swedish caucasians) and DRB1\*03 (in Italian caucasians)

and IgE responsiveness to the house dust mite allergen *Der p I*, plus a negative association between DRB1\*11 and IgE antibodies to *Bet v I* in Swedish and Bulgarian populations.

Thus, the 11th International Histocompatibility Workshop and similar more limited studies have shown that, while there is still considerable uncertainty over the exact nature of particular HLA class II associations with specific IgE responsiveness to defined allergens (the DRB1\*15 association with antibody response to *Amb a V* is still the only consistent association seen in several studies), such associations do indeed occur. The goal of future studies is to establish definitively the exact nature and relative significance of these HLA genetic contributions to cognate IgE response to allergen, and then to determine the relative genetic contribution to both cognate and non-cognate IgE response in allergy. To achieve the first of these aims two new approaches will be required. Firstly, it will be necessary to move on from population based studies of unrelated atopic individuals to family based analyses which permit additional valuable sib-pair tests for genetic linkage to be performed. Secondly, the advent of comprehensive DNA-based techniques for HLA class II DR, DQ, and DP typing should now permit full molecular characterisation of segregating HLA class II haplotypes in the families concerned.<sup>27</sup> One recent family based study shows the value of this approach.<sup>28</sup> In this study of 431 British caucasian subjects in 77 nuclear and seven extended families recruited through allergy or asthma clinics, HLA-DRB1 and DPB1 genotype frequencies were determined by polymerase chain reaction (PCR) based DNA typing and specific IgE responses to *Der p I* and *Der p II* (from the house dust mite), *Alt a I*, *Can f I* (from the dog), *Fel d I* and *Phl p V* (from the timothy grass, *Phleum pratense*). The results showed a number of weak associations between HLA-DRB1 alleles and IgE responses to the test allergens, of which the strongest association was between DRB1\*01 and *Fel d I* responsiveness. This association was also supported by excess sharing of the HLA haplotype concerned in affected sibling pairs, as was the association between IgE responsiveness to *Alt a I* and DRB1\*04, demonstrating the advantage of studying multicase families in HLA and disease association studies. Logistical regression analyses to control for reactions to more than one allergen also showed that an apparent association between IgE response to *Phl p V* and DRB1\*04 was due to the presence of many individuals showing an IgE response to both *Alt a I* and *Phl p V*.

It is informative to set the results from this large, single centre family based study alongside those of the 11th International Histocompatibility Workshop, since three allergens were common to both studies (*Der p I*, *Alt a I*, and *Fel d I*). Firstly, while the association between DRB1\*01 and IgE response to *Fel d I* was the strongest association seen in the single centre British study, no associations were seen in the 11th International Histocompatibility Workshop which did not include a British patient cohort. This difference may be due to differing levels of exposure to the sensitising allergen in the populations studied, perhaps reflecting British fondness for the domestic cat! Secondly, an association between *Alt a I* IgE responsiveness and DRB1\*04 was observed. In the Workshop study similar HLA-DRB1\*04 associations were seen in unrelated Bulgarian and Israeli atopic subjects. Conversely, associations with DRB1\*14 were seen in US caucasian and Swedish individuals. These apparently conflicting findings may be elucidated by further studies of complete HLA class II haplotypes in these populations. For example, the same DQB1 alleles can be found on some DRB1\*04 and DRB1\*14 haplotypes and thus the most precise as-

sociations may, in fact, be with a DQA1 or DQB1 allele or allele combination. Finally, in the British study no associations were found between IgE response to *Der p* I and any DRB1 or DPB1 allele. However, in the Workshop associations with DRB1\*04 and DRB1\*03 were seen in Swedish and Italian subjects respectively, as outlined above, although no associations were seen in US caucasian subjects. HLA-DPB1 associations were not examined in the Workshop but the possible role of DPB1 alleles in IgE responsiveness to *Der p* I has been a matter of debate since a negative association between DPB1\*0401 and allergic asthma was reported in a Colombian mulatto study population.<sup>29</sup> This association has been substantiated by the finding that a component of the T cell repertoire reactive with *Dermatophagoides* spp determinants in atopic individuals is HLA-DP restricted,<sup>30</sup> while DPB1\*0401 is negatively associated with both aspirin tolerant and intolerant asthma in German and British subjects.<sup>31</sup>

The role of particular HLA class II alleles in IgE responsiveness to *Der p* I determinants and the identification of HLA associated *Der p* I epitopes is currently the object of much study in several laboratories worldwide. In addition, the primary aim of the 12th International Histocompatibility Workshop now underway is to undertake a systematic analysis of the HLA immunogenetics of atopic house dust mite allergy using family based studies in several population groups. This proposed Workshop is thus a worthy successor to the 11th Workshop, building on its strengths and the valuable network of clinical and laboratory teams established, but with the added benefits of being more focused, using family rather than population studies, and making full use of accurate, high resolution DNA techniques for HLA class II gene polymorphism detection. The aim of the study, coordinated by Professor Malcolm Blumenthal of the University of Minnesota, is to collect at least 25 nuclear families from each participating centre with the proband and at least one sibling having mite sensitivity. Full health questionnaires will be completed for all individuals participating and core investigations will include skin prick test reactivity using standard methodology (for at least *Der p*, *Der f*, *Fel d* and *Lol p*), total and *Der p* I, *Der f* I, *Fel d* I, and *Lol p* I specific IgE and IgG determinations and comprehensive DNA based HLA class II typing. Complete HLA class II haplotype characterisation in affected and unaffected individuals will permit the most precise association between HLA class II marker and specific IgE response to be identified, thus confounding the effects of linkage disequilibrium (preferential associations between alleles of different loci) which is such a frustrating feature of HLA disease studies. Additional studies will focus on studies of T cell markers, including T cell receptor (TcR) variable (V) gene repertoire, and polymorphism of other "candidate" genetic markers, including those genes already implicated on chromosomes 5q and 11q. The Workshop will be completed by June 1996 and this comprehensive approach should clearly define the precise nature and relative significance of the HLA class II genetic contribution to house dust mite allergy in a number of diverse ethnic groups. By combining studies of HLA class II genes and other "candidate" genetic markers it will also be possible to dissect the relative genetic contributions to both cognate and non-cognate IgE responses in this particular allergy.

Finally, given that a number of positive and negative associations between particular HLA class II alleles and specific (cognate) IgE responses to allergens have now been established and are being characterised more accurately in ongoing studies, by what mechanisms may these HLA class II genetic associations with atopic allergy be operating? Positive associations between specific IgE responsiveness

and HLA class II alleles are consistent with the concept of strong CD4+ helper T cell (Th2) responses to immunodominant regions of processed allergenic antigen, regulating IgE-mediated hypersensitive responses (fig 2). Negative HLA class II associations may reflect weak processed antigen presentation by the expressed class II molecule in question, lack of T cell recognition or responsiveness, or more controversially, recognition by "suppressor" T cells. However, a third possible mechanism should not be ruled out, namely that the HLA haplotype of an individual influences the expressed T cell repertoire of that individual during thymic development, thus selecting for or deleting potential HLA plus allergen peptide-reactive T cells. In fact, there is now an increasing body of evidence that the T cell repertoire (as measured by TcR  $\beta$  chain V gene family usage) of an individual is indeed influenced by HLA haplotype, while skewing of the expressed TcR repertoire within the CD4 and CD8 T cell subsets may result from interaction of these subsets with HLA class II and I molecules, respectively, during development.<sup>32</sup> However, the degree and significance of these HLA-mediated effects on TcR repertoire in T cell-mediated diseases remains controversial.

In conclusion, while there remains considerable uncertainty as to the exact role of particular HLA class II genetic polymorphisms in triggering an allergen-specific IgE response, and the relative importance of specific versus total IgE responses in allergic disease, there can be little doubt that the human leucocyte antigen genes have a crucial role. The more systematic studies currently underway should provide further clarification of this, and results from the comprehensive 12th International Histocompatibility Workshop are awaited with great interest.

*Molecular Immunology Group,  
Tenovus Laboratory,  
Southampton General Hospital,  
Southampton SO16 6YD, UK*

W M HOWELL

*Immunopharmacology Group,  
University Medicine,  
Southampton General Hospital,  
Southampton SO16 6YD, UK*

S T HOLTGATE

- 1 Cookson WOCM, Hopkin JM. Dominant inheritance of atopic immunoglobulin E responsiveness. *Lancet* 1988;i:86-8.
- 2 Cookson WOCM, Sharp PA, Faux JA, Hopkin JM. Linkage between immunoglobulin E responses underlying asthma and chromosome 11q. *Lancet* 1989;ii:1292-5.
- 3 Morton NE. Major loci for atopy? *Clin Exp Allergy* 1992;22:1041-3.
- 4 Marsh DG, Meyers DA. A major gene for allergy - fact or fancy? *Nature Genetics* 1992;2:252-4.
- 5 Young RP, Sharp PA, Lynch JR, Faux JA, Lathrop GM, Cookson WOCM, et al. Confirmation of genetic linkage between atopic IgE responses and chromosome 11q13. *J Med Genet* 1992;29:236-8.
- 6 Moffat MR, Sharp PA, Faux JA, Young RP, Cookson WOCM, Hopkin JM. Factors confounding genetic linkage between atopy and chromosome 11q. *Clin Exp Allergy* 1992;22:1046-51.
- 7 Cookson WOCM, Young RP, Sandford AJ, Moffat MF, Shirakawa T, Sharp PA. Maternal inheritance of atopic IgE responsiveness on chromosome 11q. *Lancet* 1992;340:381-4.
- 8 Shirakawa T, Li A, Dzuboritz M, Dekker JW, Shaw AE, Faux JA, et al. Association between atopy and variants of the  $\beta$  subunit of the high-affinity immunoglobulin E receptor. *Nature Genetics* 1994;7:125-30.
- 9 Brereton HM, Ruffin RE, Thompson PJ, Turner DR. Familial atopy in Australian pedigrees: adventitious linkage to chromosome 8 is not confirmed, nor is there evidence of linkage to the high affinity IgE receptor. *Clin Exp Allergy* 1994;868-77.
- 10 Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich-Kautzky E, et al. Linkage analysis of IL-4 and other chromosome 5q 31.1 markers and total serum immunoglobulin E concentrations. *Science* 1994; 264:1152-6.
- 11 Parham P. Typing for class I HLA polymorphism: past, present and future. *Eur J Immunogenet* 1992;19:347-59.
- 12 Brown JH, Jardetsky TS, Gorga JC, Stern LJ, Urban RG, Strominger JL, et al. Three dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature* 1993;364:33-9.
- 13 Thorsby E, Ronningen KS. Role of HLA genes in predisposition to develop insulin-dependent diabetes mellitus. *Ann Med* 1992;24:523-31.
- 14 Bodmer JG, Marsh SGE, Albert ED, Bodmer WF, Dupont B, Erlich HA, et al. Nomenclature for factors of the HLA system, 1994. *Tissue Antigens* 1994;44:1-18.
- 15 Marsh DG, Hsu SH, Roebber M, Ehrlich-Kautzky E, Freidhoff LR, Meyers

- DA, *et al.* HLA-Dw2: a genetic marker for human immune response to short ragweed pollen allergen Ra5. I. Response resulting primarily from antigenic exposure. *J Exp Med* 1982;155:1439-51.
- 16 Roebber M, Klapper DG, Goodfriend L, Bias WB, Hsu SH, Marsh DG. Immunochemical and genetic studies of *Amb t* V (Ra5G), an Ra homologue from giant ragweed pollen. *J Immunol* 1985;134:3062-9.
- 17 Marsh DG, Freidhoff LR, Ehrlich-Kautzky E, Bias WB, Roebber M. Immune responsiveness to *Ambrosia artemisiifolia* (short ragweed) pollen allergen *Amb a* VI (Ra6) is associated with HLA-DR5 in allergic humans. *Immunogenetics* 1987;26:230-6.
- 18 Marsh DG, Zwollo P, Huang SK, Ghosh B, Ansari AA. Molecular studies of human immune response to allergens. *Cold Spring Harbor Symp Quant Biol* 1989;54:459-70.
- 19 Freidhoff LL, Ehrlich-Kautzky EE, Meyers DA, Ansari AA, Bias WB, Marsh DG. Association of HLA-DR3 with human immune response to *Lol p* I and II allergens in allergic subjects. *Tissue Antigens* 1988;31:211-9.
- 20 Ansari AA, Freidhoff LR, Meyers DA, Bias WB, Marsh DG. Human immune responsiveness to *Lolium perenne* pollen allergen *Lol p* III (Rye III) is associated with HLA-DR3 and DR5. *Hum Immunol* 1989;25:59-71.
- 21 O'Hehir RE, Eckels DD, Frew AJ, Kay AB, Lamb JR. MHC-class II restriction specificity of cloned human T-lymphocytes reactive with *Dermatophagoides farinae* (house dust mite). *Immunology* 1988;64:627-31.
- 22 O'Hehir RE, Mach B, Berte C, Greenlaw R, Tiercy J-M, Bal V, *et al.* Direct evidence for a functional role of HLA-DRB3 gene products in the recognition of *Dermatophagoides* spp by helper T-cell clones. *Int Immunol* 1990;2:885-92.
- 23 Marsh DG, Blumenthal MN, Ishikawa T, Ruffilli A, Sparholt S, Freidhoff LR. HLA and specific immune responsiveness to allergens. In: Tsuji K, Aizawa M, Sasazuki T, eds. *HLA 1991. Proceedings of the Eleventh International Histocompatibility Workshop and Conference*. Vol I. Oxford: Oxford University Press, 1992: 765-7.
- 24 Cárđaba B, Vilches C, Martín E, Andrés B de, Pozo V del, Hernández D, *et al.* DR7 and DQ2 are positively associated with immunoglobulin-E response to the main antigen of olive pollen (*Ole e* I) in allergic patients. *Hum Immunol* 1993;38:293-9.
- 25 Sparholt SH, Georgsen J, Madsen HO, Svendsen UG, Schou C. Association between HLA-DRB3\*0101 and immunoglobulin E responsiveness to *Bet v* I. *Hum Immunol* 1994;39:76-8.
- 26 Tautz C, Rihs H-P, Thiele A, Zwollo P, Freidhoff LR, Marsh DG, *et al.* Association of class II sequences encoding DR1 and DQ5 specificities with hypersensitivity to chironomid allergen *Chi t* I. *J Allergy Clin Immunol* 1994;93:918-25.
- 27 Kimura A, Sasazuki T. Eleventh International Histocompatibility Workshop reference protocol for the HLA DNA-typing technique. In: Tsuji K, Aizawa M, Sasazuki T, eds. *HLA 1991. Proceedings of the Eleventh International Histocompatibility Workshop and Conference*. Vol I. Oxford: Oxford University Press, 1992: 397-419.
- 28 Young RP, Dekker JW, Wordworth BP, Schou C, Pile KD, Mathiesen F, *et al.* HLA-DR and HLA-DP genotypes and immunoglobulin E responses to common major allergens. *Clin Exp Allergy* 1994;24:431-9.
- 29 Caraballo L, Marrugo J, Jimenez S, Angelini G, Ferrar GB. Frequency of DPB1\*0401 is significantly decreased in patients with allergic asthma in a mulatto population. *Hum Immunol* 1991;32:157-61.
- 30 Higgins JA, Lamb JR, Marsh SGE, Tonks S, Hayball JD, Rosen-Bronson S, *et al.* Peptide-induced nonresponsiveness of HLA-DP restricted human T-cells reactive with *Dermatophagoides* spp (house dust mite). *J Allergy Clin Immunol* 1992;90:749-56.
- 31 Lympany PA, Welsh KI, Christie PE, Schmitz-Schumann M, Kemeny M, Lee TH. An analysis with sequence-specific oligonucleotide probes of the association between aspirin-induced asthma and antigens of the HLA system. *J Allergy Clin Immunol* 1993;92:114-23.
- 32 Reed EF, Tugulea SL, Suciú-Foca N. Influence of HLA class I and class II antigens on the peripheral T-cell receptor repertoire. *Hum Immunol* 1994;40:111-22.