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*IMMUNOLOGICAL REVIEW*

# Immunogenetic basis of environmental lung disease: lessons from the berylliosis model

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*Immunogenetic basis of environmental lung disease: lessons from the berylliosis model. C. Saltini, M. Amicosante, A. Franchi, G. Lombardi, L. Richeldi. ©ERS Journals Ltd 1998.*

**ABSTRACT: The role of genetic factors has been hypothesized in the pathogenesis of a number of chronic inflammatory lung diseases. The genes of the major histocompatibility complex (MHC) locus on human chromosome 6 have been identified as important determinants in diseases caused both by inorganic and organic compounds such as beryllium, gold, acid anhydrides, isocyanates and grass pollens. Since many environmental factors are the determinants of the immunopathogenesis of asthma, pulmonary granulomatous disorders, hypersensitivity pneumonitis and fibrotic lung disorders, an understanding of the interaction between environmental factors is crucial to epidemiology, prevention and treatment of these disorders.**

**Berylliosis is an environmental chronic inflammatory disorder of the lung caused by inhalation of beryllium dusts. A human leukocyte antigen class II marker (HLA-DP Glu69) has been found to be strongly associated with the disease. In *in vitro* studies, the gene has been shown to play a direct role in the immunopathogenesis of the disease. In human studies, the gene has been shown to confer increased susceptibility to beryllium in exposed workers, thus suggesting that HLA gene markers may be used as epidemiological probes to identify population groups at higher risk of environmental lung diseases, to identify environmental levels of lung immunotoxicants that would be safe for the entire population and to prevent disease risk associated with occupation, manufactured products and the environment.**

**Studies on the associations between human leukocyte antigens and chronic inflammatory lung disorders are reviewed in the context of the berylliosis model.**
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As the site of gas exchange, the 50–100 $m^2$ of the respiratory tract epithelial surface is burdened by antigenic and nonantigenic particles present in over 7,000 L of air inhaled daily. The lung uses two general mechanisms to dispose of these particles: the bronchial clearance systems to remove them and inflammatory and immunocompetent cells to inactivate and destroy them. Experimental animal studies indicate that the lung contains a compartmentalized mucosal immune system capable of initiating immune responses. In humans, the density of immune cells recovered from the lower respiratory tract is several-fold greater than that of the blood. T-lymphocytes dominate the cellular milieu of the alveolar surface, which is almost entirely comprised of memory T-cells that are quite likely to have the ability to mount strong immune responses [1]. Thus the lung, like the gut and the skin, is a site where foreign antigens may initiate normal defensive responses, but also abnormal, damaging immune reactions. In this context, the lung is an ideal organ model for the study of the immunogenetic basis of hypersensitivity and inflammatory reactions to environmental agents.

## Environmental exposure and chronic inflammatory disorders of the lung

A growing list of environmental agents has been implicated in the pathogenesis of chronic inflammatory lung disorders. Exposures to inorganic compounds lend themselves as excellent study models for the interaction between environmental and genetic factors, owing to the defined chemical nature, quantifiable exposure levels and *in vitro* reproducibility of exposure conditions. Fumes or gases of Cd, Be, Co, Hg, Mn, Ti, Sb or Zn may cause acute chemical pneumonitis and bronchitis [2]. Zn, Cd, Cu, Mn and Al oxide fumes are the cause of a nonfibrosing acute alveolitis, also known as metal fume fever. This reaction resembles the organic dust toxic syndrome, a self-limiting immediate or semi-delayed flu-like syndrome in which the intense polymorphonuclear leukocytes (PMN)-dominated alveolitis is associated with the production of pro-inflammatory cytokines after exposure to organic dusts [3, 4].

Crystalline $SiO_2$, fibrous (asbestos) and nonfibrous (mica, kaolin) silicates cause interstitial lung disorders, the "classic pneumoconioses", while nonfibrogenic "benign pneumoconioses" are caused by exposure to Fe, Ba and Sn. Inhalation of mica, talc and lipids may cause foreign body-like granulomas, as an aspecific phagocytic response towards nonbiodegradable agents [5]. Be [6], Zr [7–9], Ti [10] and Al [11] are the cause of lung sarcoid-like granulomatous disorders. Exposure to tungsten carbide (WC) and Co complexes is associated with a giant cell (GIP) or a desquamative (DIP) interstitial pneumonitis and lung fibrosis, known

as hard metal lung disease [2]. Organic dusts from animals, vegetables or micro-organisms can also cause hypersensitivity pneumonitis (HP) [12]. The best known examples of HP are farmer's lung, bird breeder's disease and the summer-type HP in Japan [13]. HP can also be caused by small organic compounds, among them isocyanates [14].

Finally, a number of metals, chemicals and defined organic allergens has been implicated in the incidence of occupational asthma, the most frequent work-related respiratory disease in which both immunoglobulin (Ig)E-mediated allergy and the nonallergic bronchial hyperresponsiveness (BHR) can be observed [15]. Allergic occupational asthma is characterized by serum production of metal-specific IgE antibodies and positive prick and/or patch skin testing, all suggesting type I and IV immune reactions [2, 16]. BHR, typified by Al-induced potroom asthma, is more likely to be mediated by inflammatory mechanisms [2, 17]. Among known agents of occupational asthma are metals (Pt, Co, Cr, Ni and Zn) and organic chemicals (isocyanates and anhydrides). Asthma is also caused by a number of nonoccupational exposures. The allergenic agents that are best characterized are short ragweed, rye grass pollen and the house dust mite (HDM).

## Interaction between genetic and environmental factors in the berylliosis model

### *Structure and function of the immune response genes*

Major histocompatibility complex (MHC) molecules, or human leukocyte antigens (HLA) in humans were identified by Benacerraf and McDevitt [18] in 1972 as the immune response genes, *i.e.* the genes dictating the individual ability to recognize and mount an immune response against antigens (fig. 1). The HLA molecules are receptors whose function is to collect peptides inside the cell and present them on the cell surface for recognition by T-cells, as part of the mechanism for identifying foreign antigens and producing an immune response [19, 20]. Two types of molecule are included in this complex: HLA class I molecules, present on most cell types, which usually present peptides derived from any protein synthesized in a cell [19], while class II molecules, which are expressed on a limited number of cell types (*i.e.* antigen-presenting cells (APC) such as macrophages, B-cells and dendritic cells), present peptides derived from protein occurring in the endosomal/lysosomal compartment, including endogenous as well as phagocytosed antigens [20].

HLA class I molecules are single-chain integral glycoproteins of about 45 kDa noncovalently associated with the $\beta_2$-microglobulin, while class II molecules consist of a heterodimeric integral glycoprotein composed of tightly but noncovalently linked $\alpha$- and $\beta$-glycoprotein chains of about 30 kDa each. Both class I and class II molecules are highly polymorphic and three isotypes are coexpressed for each type (HLA-A, -B and -C for class I; and HLA-DR, -DP and -DQ for class II). Three-dimensional structural information is available for both class I and class II molecules, although this information is restricted to a few isotypes [21]. As members of the Ig superfamily, the HLA molecules have a typical organization in functional domains that reflect the genomic organization and both molecules share a similar three-dimensional structure. Class I molecules present three extramembrane domains ($\alpha$1, $\alpha$2 and $\alpha$3), a transmembrane portion and a cytoplasmatic tail; each class II chain contains two extramembrane domains, a transmembrane portion and a cytoplasmatic tail. The HLA-class I $\alpha$1 and $\alpha$2 domains and the first domain of each HLA-class II chain ($\alpha$1 and $\beta$1) are responsible for the binding of the peptide and for the interaction with the T-cell receptor (TCR). The class I $\alpha$3 domain, together with the $\beta_2$-microglobulin, and the class II second domain chain ($\alpha$2 and $\beta$2), together with the first one, are responsible for the assembly of the molecules and the interaction with the CD8 and CD4 molecules, respectively. The transmembrane domain anchors the molecules at the membrane surface. The cytoplasmatic tail has a role in signal transduction [22, 23].

Radiographic analysis revealed that HLA class I and class II molecules share a similar structure. For both molecules, the peptide-binding domain, also called the peptide-binding groove, is composed of eight strands of an antiparallel $\beta$-sheet (four for each class I domain or class II chain) as a floor and two antiparallel helical regions as the sides (one for each class I domain or class II chain) (fig. 2). Critical differences in the helical regions between HLA class I and class II molecules determine a closed peptide-binding site for class I and an open one for class II

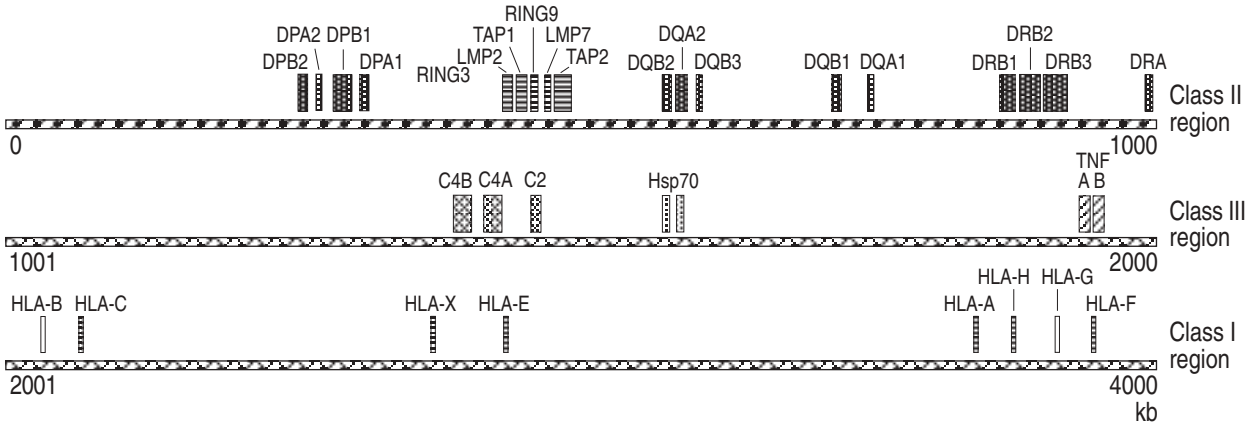


Fig. 1. – Map of the human major histocompatibility complex (MHC) locus. ▦ : Class II alpha and beta genes; ▤ : ABC transporter and proteasome-like genes; ▩ : complement genes; ▨ : tumour necrosis factor genes; ⁞⁞ : Hsp70 genes; ▭ : class I genes; HLA: human leukocyte antigen.

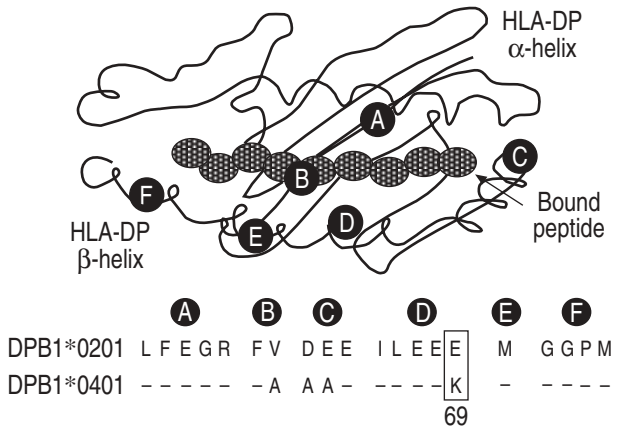


Fig. 2. – Structure of the human leukocyte antigen (HLA)-DP molecule. Shown below the diagram are the amino acid sequence changes in the five variable domains of the HLA-DP β-chain gene (in region D the polymorphism at position 69 is shown).

molecules [21, 24]. This difference permits the binding of peptides 8–9 residues long to class I and of peptides 15–24 amino acid residues long to class II molecules. In both molecules the polymorphic residues are confined in the peptide-binding groove and provide the mechanisms by which different HLA alleles or isotypes selectively bind peptides of different sequences. The properties of the side-chain of the amino acids present in the peptide-binding groove determine which peptides will be able to bind to that particular HLA molecule [25]. HLA class I molecules, which present peptides derived from any protein synthesized in a cell to CD8+ cytotoxic T-lymphocytes, dictate immune responsiveness against viral, tumour or foreign cellular antigens. In contrast, HLA class II molecules, which present peptides derived from the endosomal compartment (including endogenous as well as phagocytosed antigens) to CD4+ helper T-lymphocytes, dictate immune responsiveness against microbial and environmental antigens and against allergens [26].

With this background, the genes encoding HLA molecules are the prime candidate as susceptibility genes to immune and allergic lung disorders caused by specific environmental antigens and allergens.

### *Berylliosis*

Berylliosis is probably the best understood of the environmental chronic inflammatory disorders. It is characterized by the accumulation of CD4+ T-cells and macrophages in the lower respiratory tract in response to Be inhalation, noncaseating granuloma formation and, eventually, fibrosis. Pathologically, it is indistinguishable from Al- and Ti-induced granulomas and from sarcoidosis.

Exposure to Be is the cause of acute and chronic disorders of the skin and the lung. High levels of Be salts cause acute dermatitis, conjunctivitis, rhinitis and a pneumonitis similar to the metal fume fever syndrome induced by Al and other metals. Exposure to lower levels of insoluble Be dust, if protracted, may be the cause of berylliosis, which affects 2–5% of exposed workers [6]. Since the immunotoxicity of Be was recognized in the 1950s and hypersensitivity was suspected as the important mechanism in disease incidence, the levels of Be in the air in the industrial environment have been reduced over 100-fold and strict industrial hygiene measures implemented. While acute disease has been eliminated, chronic disease has not, suggesting that prevention may need to be targeted at the more sensitive segment of the exposed population.

In individuals affected by berylliosis, Be-sensitized lung T-cells are dominated by CD4+ CD45RO+ memory T-cells [27], which are polyclonal [28], although they may express a limited set of T-cell antigen receptor variable region genes [29] and recognize Be as a specific MHC restricted antigen/hapten [28]. Consistent with the disease immunopathology, T-cells in the lower respiratory tract of patients with berylliosis are T-helper (Th)1 T-cells, *i.e.* they produce interleukin (IL)-2 and interferon (IFN)-γ, the macrophage-activating cytokine driving the granulomatous reaction [30]. The finding that Be is recognized as a specific HLA class II-restricted hapten/antigen prompted the search for immune response genes associated with Be hypersensitivity. RICHELDI *et al*. [31] found in a retrospective study of 32 cases that the HLA class II HLA-DP allele HLA-DPB1*0201 was associated with disease "risk" and the allele HLA-DPB1*0401 was associated with disease "protection". Sequence analysis showed that susceptibility to berylliosis was associated with the polymorphic sequence coding for a lysine to glutamic acid change in the fourth variable domain D (amino acids 67–69) of the β-chain of the HLA-DP gene (HLA-DP Glu69) (fig. 2). This association was confirmed in a cohort study of 137 Be ceramic workers, where an 80% frequency of HLA-DP Glu69 was found among individuals with berylliosis *versus* 35% in exposed controls [32]. Similar results were recently obtained in a larger cohort of 650 Be-exposed workers (L. Richeldi, K. Kreiss and C. Saltini, unpublished data). Interestingly, STUBBS *et al*. [33] confirmed the association of berylliosis with HLA-DP Glu69; in addition, they found that HLA-DR allelic variants were also associated with disease.

What is the function of HLA-DP in the pathogenesis of berylliosis? In the context of the current knowledge of the interaction of metals with the human immune system, it may be hypothesized that the HLA-DP gene functions as either 1) the specific immune response gene conferring upon the carrier's HLA molecules a higher affinity for Be or a Be/protein complex, hence the ability to select Be as a specific antigen/hapten for T-cell presentation; 2) a deoxyribonucleic acid (DNA) disease marker linked to some nearby gene(s) influencing the individual's immune responsiveness; or 3) a nonspecific immune response gene directing the immune response towards the preferential expression of a type-1 T-cell response and, hence, towards chronic inflammation and autoimmunity.

Firstly, the possibility that HLA-DP may function as the primary immune response gene in berylliosis is supported by the observation of LOMBARDI *et al*. [34] who, consistent with earlier T-cell studies [27, 28], found that Be-specific T-cell clones from individuals with berylliosis were activated only when Be was presented in the context of HLA-DP molecules expressing glutamic acid in position 69 of the β-chain. Recent data indicating that T-cell production of IFN-γ in response to Be is also restricted by HLA-DP lend further support to this hypothesis (A. Franchi, H. Wiedemann and C. Saltini, manuscript in preparation) (fig. 3).

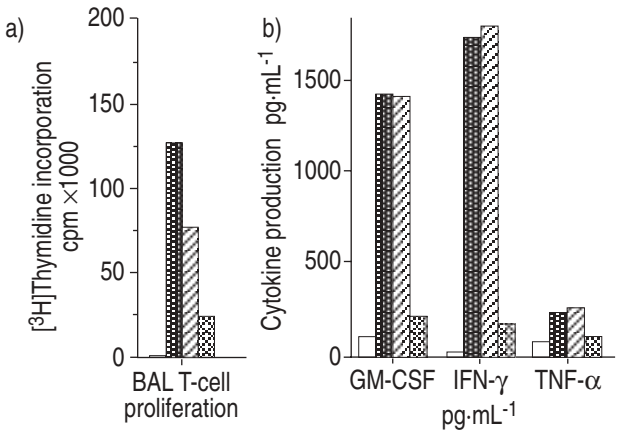


Fig. 3. – Role of human leukocyte antigen (HLA)-DP in beryllium (Be) presentation and T-cell activation. The diagram summarizes the results of the effect of the monoclonal antibodies L243 (anti-DR) and B7/21 (anti-DP) on a) bronchoalveolar lavage (BAL) T-cell proliferation and b) production of granulocyte macrophage-colony-stimulating factor (GM-CSF), interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α) on stimulation with Be of lung T-cells from patients with berylliosis. □ : Cell; ▦ : Be 100; ▨ : +anti-DR; ▩ : +anti-DP.

Current concepts of metal recognition by T-cells are that metals can haptenize peptides, thereby generating neoantigens which can be bound by HLA molecules and induce allergy or can bind directly to the HLA molecule, thus generating an altered self-HLA and thereby inducing hypersensitivity. In nickel-allergic individuals, nickel binds to histidine residues on peptides, thus making antigenic a multiplicity of peptides. In gold hypersensitivity, gold binds directly to the HLA molecule [35]. Although the nature of the Be antigen has not yet been clarified, Be may bind directly to the HLA-DP molecule, thereby generating an altered self-HLA and an immune reaction (fig. 4). This possibility is consistent with the finding of a direct role of specific allelic variants of the HLA genes in gold- and Be-induced lung hypersensitivity. Thus, HLA genes may function as primary susceptibility genes or specific immune response genes in these hypersensitivity diseases.

Alternatively, HLA-DP Glu69 might simply be a DNA marker linked to other nearby immune response genes with direct influence on disease susceptibility. In this regard,

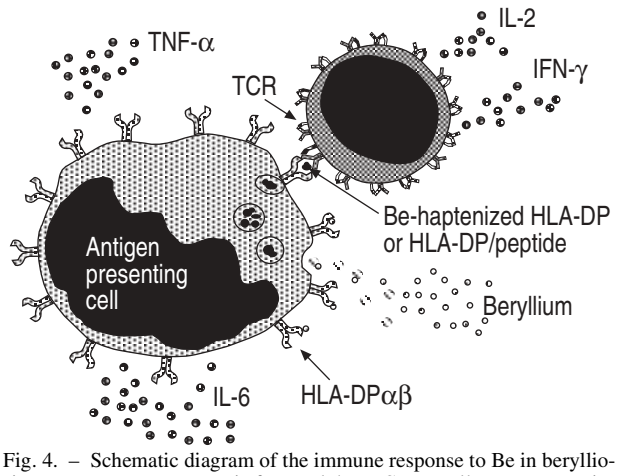


Fig. 4. – Schematic diagram of the immune response to Be in berylliosis. TNF-α: tumour necrosis factor-alpha; TCR: T-cell receptor; IL: interleukin; IFN-γ: interferon-gamma; HLA: human leukocyte antigen.

the studies on the association of the tumour necrosis factor (TNF)-α gene with chronic inflammatory and autoimmune disorders such as rheumatoid arthritis (RA) and diabetes may suggest that in berylliosis the association of HLA-DP with disease might be the consequence of linkage disequilibrium with the TNF gene (fig. 5) or other cytokine genes. Two lines of evidence oppose this possibility. Firstly, the HLA-DP gene is only weakly linked to the other HLA locus genes, including the TNF-α gene [36, 37]. Secondly, although berylliosis is associated with the TNF-α gene, this association is weaker than that with HLA-DP (see [31] for the analysis of the *Nco*1 TNFβ polymorphism (fig. 6) and L. Richeldi, K. Kreiss, B. Zhen, H. Wiedemann and C. Saltini, unpublished data, for the analysis of the TNFα-308 polymorphism). It cannot be excluded, however, that other chromosome 6 genes, linked to the HLA-DP gene and strongly associated with susceptibility to berylliosis, will be identified. The finding that hard-metal lung disease, a disorder driven by increased oxidant production, is strongly associated with HLA-DP Glu69 [38], supports the possibility that HLA-DP Glu69 may be linked with inflammation-driving genes.

Finally, the HLA-DP gene marker associated with berylliosis may be a Th1 marker. Berylliosis screening programmes using a blood Be-specific lymphocyte proliferation test (BeLPT) as a disease marker, allowed the identification of Be-sensitized individuals who have an immune reaction to beryllium, but do not have the disease, as determined by a negative transbronchial biopsy [6]. As individuals with Be-specific sensitization may not progress from hypersensitive to granulomatous lung disease [39], the observation that the prevalence of HLA-DP Glu69 was higher among clinically identified subjects [31] than in subjects identified by screening [32], together with the observation that it was also significantly higher among individuals with disease than in those with lone hypersensitivity (L. Richeldi, K. Kreiss, B. Zhen, H. Wiedemann and C. Saltini, unpublished data), supports the hypothesis that the carriage of the HLA-DP gene may confer greater susceptibility to granulomatous inflammation in exposed individuals. Although the HLA-DP gene has been associated with the Th2 type response to egg albumin in egg allergy [40], this hypothesis is consistent with the observations that HLA-DP Glu69 is associated with the Th1 reactions of sarcoidosis [41] and the generation of alloreactive type-1 T-cells in transplant rejection [42].

What role may a genetic susceptibility marker play in the epidemiology, diagnosis, prevention and treatment of berylliosis and other chronic inflammatory disorders?

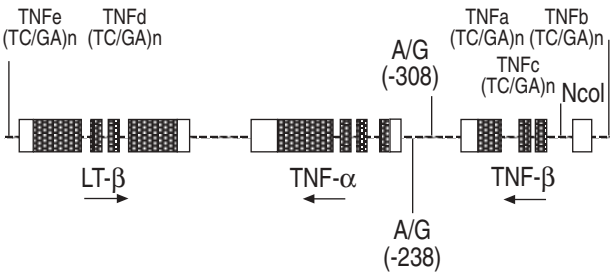


Fig. 5. – Schematic representation of the tumour necrosis factor (TNF) α and β genes with genetic polymorphisms and microsatellite markers. LT: lymphotoxin; A/G: adenine to guanine substitution.

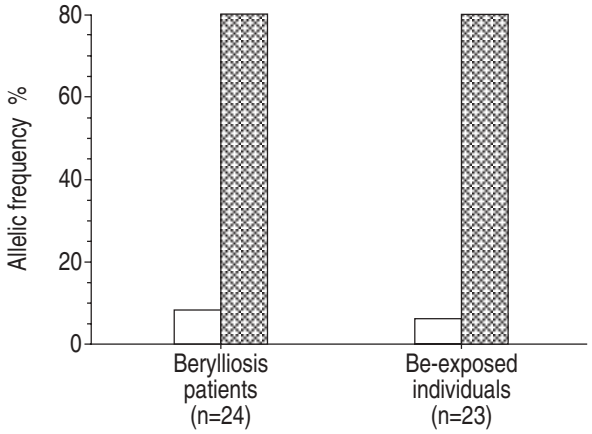


Fig. 6. – Frequency of the *Nco*1 polymorphism of the tumour necrosis factor (TNF)-β gene in pulmonary berylliosis and in Be-exposed individuals. No differences between groups were found. □ : TNFB*1; ▨ : TNFB*2. (From [31].)

In berylliosis, the HLA-DP Glu69 marker shows a strong association with the lung granulomatous reaction to Be, *i.e.* with disease, and a less strong association with sensitization to Be in the absence of disease, *i.e.* a positive blood test without lung granulomas. The studies of L. Richeldi and coworkers have shown that the carriage of the HLA-DP Glu69 marker was associated with an eight-fold increase in the rate of disease in workers with a history of higher exposure to Be, thereby suggesting that genetic and exposure factors may have an additive or multiplicative effect (table 1). Contrary to the observations with environment-related cancer models where genetic factors were irrelevant to risk determination at higher exposure levels [43], in the berylliosis model susceptibility increases disease risk at high levels of exposure. This model shows that estimating exposure-related disease risk for the whole exposed population greatly underestimates the risk of the more susceptible workers. In the cohort studies mentioned above, machining processes corresponded to a risk of 3.2% for two-thirds of machinists, *i.e.* those not expressing the HLA-DP Glu69 marker, and a risk of 25% for those expressing the marker. The exposure at which the genetically susceptible population is protected would certainly protect the less susceptible two-thirds of the population as well.

## Association of HLA with chronic inflammatory disorders of the lung

### *Asthma*

Asthma is a disease of the airways characterized by chronic inflammation with infiltration of lymphocytes, eosinophils and mast cells, along with epithelial desquamation and thickening of the bronchial mucosa, leading to airway narrowing and wheezing in response to a variety of stimuli. Asthma is caused by, or associated with atopy and/or BHR. Family and population studies have strongly indicated asthma as a complex genetic disorder. Four phenotypic markers have been used in the search for the asthma gene(s): 1) total serum IgE levels; 2) immediate reactivity in skin tests to aeroallergens and specific IgE levels; 3) BHR to physical and pharmacological stimuli; and 4) a history of wheezing in the clinical diagnosis of asthma. The genetic loci tentatively associated with asthma are a locus on chromosome 11q13 in close proximity to the IgE receptor and a locus on chromosome 5q31 located near a cytokine gene cluster comprising the IL-4, IL-5 and IL-13 genes and near the $\beta_2$-adrenergic receptor gene. These associations, however, have not been confirmed in all studies, possibly owing to varying diagnostic criteria and differences in the ethnic groups tested [44].

The association of HLA genes with asthma has been explored by many investigators, with conflicting results. Consistent with the antigen-presenting function of HLA molecules, HLA genes have not been associated with hyper-IgE, bronchial hyperresponsiveness, wheezing or with the diagnosis of asthma *per se*. Contrary to earlier reports [45], few studies have associated HLA with asthma *per se* or with atopy. Among others, a British and a Greek study found a weak association of atopic asthma with the haplotype HLA-A1, B8, DR3 [46, 47] and a French family study with the extended haplotype DR53 (HLA-DR4, DR7, DR9) [48]. However, no associations were found in American, British or Chinese studies [49–51].

Work from several laboratories has instead indicated that HLA genes may be associated with responsiveness to specific protein or peptide allergens (table 2). In allergy to ragweed, sensitization to the specific allergen Amb aV is associated with HLA-DR2 and that to Amb aVI with HLA-DR5 [63, 64] (table 3). Among subjects with allergy to the rye grass pollen, sensitization to the allergens Lol pI and Lol pII is associated with HLA-DR3 and that to Lol pIII with both HLA-DR3 and DR5 (table 3). Interestingly, antigen-specific T-cells are restricted by the respective allelic variants of the HLA-DR molecule [65]. Allergy to HDM seems to be associated with the HLA-DQ gene which is different from grass pollen allergy. Prick test reactivity to *Dermatophagoides farinae* has been found to be associated with HLA-DQA1*0301 but not with the DR53 haplotype identified in the French study quoted above [51] (table 3). O'Brien *et al*. [66] found an association between *in vitro* T-cell reactivity to the *D. pteronissimus* Der p2 antigen-derived peptides and the HLA-DQ7 antigen. In the context of the observation that Der p2 is recognized by T-

Table 1. – Prevalence of berylliosis by marker and job history

| Genetic marker | Nonmachinist | Machinist | Total |
|---|---|---|---|
| HLA-DP Glu69-negative | 0/55 (0) | 1/31 (3.2) | 1/86 (1.2) |
| HLA-DP Glu69-positive | 1/25 (4.0) | 4/16 (25.0)‡ | 5/41 (12.2)** |
| Total | 1/80 (1.3) | 5/47 (10.6)+ | 6/127 (4.7) |

Data are presented as number with percentages in parentheses. HLA: human leukocyte antigen. **: p=0.01 compared with HLA-DPB1Glu69 negative workers; +: p=0.02 compared with nonmachinist; ‡: p=0.05 compared with HLA-DPB1Glu69-negative machinists. (Reproduced from [32].)

Table 2. – Human leukocyte antigen (HLA) association with hypersensitivity

| Disease | [Ref.] | HLA allele associated with susceptibility |
|---|---|---|
| Berylliosis | [31]* | DPB1Glu69 (HLA-DP2-like) |
| Gold-induced HP | [52, 53] | DR1 |
| Bird breeder's disease | [54–59] | B8, B40, DR3, DW6 DR3, DR7 |
| Farmer's lung | [60–62] | B7, DR2 |

HP: hypersensitivity pneumonitis.

Table 3. – Human leukocyte antigen (HLA) association with obstructive lung disorders

| Disease | [Refs.] | HLA allele associated with susceptibility | Specific antigens |
|---|---|---|---|
| Ragweed asthma | [63, 64] | DR2, DR5 | Amb aV, Amb aVI |
| Rye grass asthma | [65] | DR3, DR3, DR5 | Lol pI, Lol pII, Lol pIII |
| House dust mite asthma | [66, 67] | DQ7, DQA0301 | Der p2, *Dermatophagoides farinae* |
| Anhydride asthma | [68] | DR3 | Trimellitic anhydride |
| Isocyanate asthma | [69, 70] | HLA-DQB0503, 0201/0301 | |

cells by the HLA-DR molecule, but not by the HLA-DQ or HLA-DP [67], it has been hypothesized that HLA-DQ functions as a T-cell suppressor antigen in HDM-induced asthma [51, 66]. HLA-DQ has also been implicated in aspirin-induced asthma in a small American study [71], but the data were not confirmed in a study of British and German aspirin-sensitive asthmatics [72].

Occupational asthma has been defined by the Industrial Injuries Advisory Council of Great Britain as "variable air flow limitation caused by sensitization to a specific agent encountered at work and excluding other occupational causes of variable air flow limitations not due to sensitization". The definition of occupational asthma must include: 1) reversible air flow impairment; 2) work relatedness; 3) documented sensitization if allergic; or 4) nonspecific hyperresponsiveness [73]. Occupational asthma affects hundreds of thousands of workers throughout the industrialized world. More than 200 agents causing asthma in the work environment have been identified, among them a number of reactive small chemicals such as metals, anhydrides and isocyanates which may react as haptens with endogenous proteins generating neoantigens.

There is the credence, in occupational asthma as in contact dermatitis, that a subpopulation of exposed individuals may be more "sensitive" to environmental agents since they have "leaky" airways due to atopy or to injured bronchial epithelium [73]; however, since the identification of causative agents is critical to the diagnosis, occupational asthma provides an excellent model with which to test the hypothesis on individual susceptibility to allergy: HLA genes appear to be a major component of susceptibility to chemicals inducing occupational asthma, suggesting that they may function as specific immune response genes in these reactions (table 4). Although isocyanate-induced asthma has been considered by many authorities to be an irritant-induced type of asthma, it has been shown that T-cells from subjects with diisocyanate-induced asthma react *in vitro* against the offending agent. Bernstein *et al*. [91] showed that T-cells from diisocyanate-induced asthmatics expressed a biased TCR repertoire, consistent with the expansion of Vβ 1 and Vβ 5 genes in isocyanate-stimulated T-cells, thus indicating a specific immune reaction. Diisocyanate-induced asthma was associated with the HLA-DQB1*0503, 0201/0301 alleles of the HLA-DQ gene in a study by Bignon *et al*. [69] and, hence, Balboni *et al*. [70] suggested that disease susceptibility may be associated with the expression of a specific amino acid residue (aspartic acid 57) on the HLA-DQ molecule β-chain. No association with HLA was found in the study by Bernstein *et al*. [91].

Table 4. – Human leukocyte antigen (HLA) association with fibrotic lung disorders

| Disease | [Ref.] | HLA allele associated with susceptibility |
|---|---|---|
| HMLD (cobalt) | [38] | HLA-DPB1Glu69 |
| Asbestosis | [74–82] | B27, DR5, DR53, DQw2, B12(1) |
| CWP, silicosis | [83–90, 98, 99] | A19, B21, B29<br>A1, B8, B21, B45<br>B18, DR8<br>B44<br>B54, DR4, DR53 |

HMLD: hard-metal lung disease; CWP: coal worker's pneumoconiosis.

In anhydride-induced asthma, Young *et al*. [68] showed a significant excess of HLA-DR3 in 30 asthma cases with a positive radioallergosorbent test, compared with 30 expos-ed controls without specific IgE. The excess of HLA-DR3 was associated with the presence of IgE against trimellitic anhydride, indicating HLA class II proteins as an important determinant in the generation of a specific IgE response to inhaled haptens.

### *Hypersensitivity pneumonitis*

*Gold-induced hypersensitivity pneumonitis*. The administration of colloidal gold to patients with RA may cause skin and lung hypersensitivity. The studies of F. Sinigaglia have shown that the response to gold by individuals with the allergy is maintained by T-cells recognizing gold in an antigen-specific fashion. In contrast to nickel, which can haptenize any peptide carrying a histidine residue and can therefore react with a large repertoire of antigenic moieties, gold is thought to bind directly to the HLA molecule. The studies of F. Sinigaglia and coworkers indicate that this very property may make gold an immunosuppressive and a immunogenic agent at the same time. By binding to the HLA molecule, it may block the responses to the autoantigen(s) of RA. However, for the same reason it may transform certain HLA molecules into neoantigens [35].

Hakala *et al*. [52] showed that gold-induced hypersensitivity pneumonitis is associated with HLA-DR1 in Finnish RA patients and the same association was found by Partanen *et al*. [53]. Strikingly, in RA patients with gold hypersensitivity described by Romagnoli *et al*. [92], the T-cell response to gold was restricted by HLA-DR1, leading to the hypothesis that gold may be able to haptenize, or "neoantigenize", the HLA-DR1 molecule.

*Organic dust-induced hypersensitivity pneumonitis*. Exposure to a variety of organic dusts causes chronic hypersensitivity lung reactions. Extrinsic allergic alveolitis, or HP, is caused by repeated exposure to organic dusts, most of which are products from animals, vegetables or microorganisms. A few small organic compounds can also cause HP. The best known examples of HP are farmer's lung, bird breeder's disease and, in Japan, the summer-type HP. Farmer's lung is caused by the inhalation of *Actinomyces thermophilus rectivirgula*, grown in mouldy

hay. Bird breeder's disease results from exposure to bird droppings and summer-type HP is caused by exposure to the micro-organism *Trichosporon cutaneum*. The typical HP patient presents with flu-like symptoms developing 4–6 h after exposure. Respiratory symptoms may worsen insidiously with repeated exposure leading to severe respiratory dysfunction, anorexia and weight loss. HP patients have positive skin tests and serum-precipitating antibodies against the offending agent. HP is an immunologically mediated disorder: exposure to the dusts trigger the activation of macrophages and the release of granulocyte chemotactic factors, leading to the accumulation of granulocytes in the lower respiratory tract during the first hours after exposure. A T-cell alveolitis follows, which is dominated by CD4+ T-cells in the acute stage and by CD8+ cytotoxic T-cells in the chronic phase. Tissue lesions are characterized by: 1) interstitial infiltrates of mononuclear cells without eosinophilia; 2) scattered, poorly formed granulomas without necrosis; and 3) bronchiolitis. The theory that HP lesions are the consequence of an exaggerated reaction to specific antigen(s) or superantigen(s) has been suggested by the observation of expansion of both CD8+ and CD4+ T-cells bearing selected TCR-α- and β-chain variable-region genes [60].

The disease affects <10% of exposed individuals and it has been suggested that individual susceptibility factors may play a role in its pathogenesis. A number of studies have addressed the association of hypersensitivity pneumonitis with HLA genes. In 1977, Allen *et al*. [54] described the inhibition of antigen-specific T-cell responses by anti-HLA antiserum, postulating an immune response gene for HP. They evaluated the association between HP and HLA genes and found an increased frequency of HLA-Bw40 in bird breeder's disease. In 1978, Sennekamp *et al*. [55] found that the HLA-B8 gene was strongly associated with bird breeder's disease. Nonsignificant associations with hypersensitivity to avian antigens were reported by Berril and van Rood [56] for HLA-DW6 and by Muers *et al*. [57] for HLA-DR3. This observation was later confirmed by Rittner *et al*. [58], who found a strong association between bird breeder's disease and HLA-DR3 in a study of 116 German pigeon breeders. Selman *et al*. [59] described the association with HLA-DR7 and haplotype HLA-B35, DR4, the postulated Hispanic equivalent of the Caucasian HLA-B8, DR3, reported in Mexican bird breeders.

In farmer's lung, Khomenko *et al*. [61], in 1985, ob-served an increased frequency of HLA-B7 and disease and Wahlstrom *et al*. [60] of HLA-DR2, whereas no association was found in a study of 37 unselected, end-stage HP patients by Nowack and Goebel [62]. In summer-type HP, a Japanese study by Ando *et al*. [93] showed an association with HLA-DQw3.

### *Fibrotic lung disorders*

A number of dusts are capable of inducing fibrotic lung disorders. The most notable examples are asbestos, coal, silica and hard metals. Current concepts of the pathogenesis of dust-induced fibrotic lung disorders are that dusts may directly stimulate the activation of macrophages, inducing the production of inflammatory cytokines, resulting in chronic inflammation, activation and proliferation of fibroblasts and progressive fibrosis and lung dysfunction. Although immunological mechanisms have been hypothesized in these diseases, they have not yet been convincingly substantiated.

*Hard metal lung disease*. Exposure to hard metals, *i.e.* a mixture of WC and Co, or to Co alone, may be the cause of allergic dermatitis, asthma, pulmonary fibrosis and, possibly, of HP. Co allergic dermatitis is thought to affect <5% of exposed individuals, suggesting that individual susceptibility might play a role in the prevalence of allergy. There are, instead, uncertainties as to the epidemiology of pulmonary fibrosis. Although the disease has been considered a rare event, two reports have claimed prevalence figures between 20 and 30% [94]. Fibrosis induced by Co is characterized by the accumulation of large numbers of macrophages and giant cells in the alveolar spaces with diffuse interstitial fibrosis. Lymphoid infiltrates are not prominent and the role of T-cells is uncertain. In this regard, the observation that exposed individuals develop either fibrosis or asthma might negate the role of an immune reaction to Co in pulmonary fibrosis. A study evaluating HLA-A, -B, -C and -DR genes in 853 hard-metal workers, 39 of whom had skin sensitization to Co, found no association with any of the genes tested [95]. Another study evaluated DQA, DQB, DRB, DPA and DPB polymorphisms in a group of patients including 38 Co-sensitive, 26 Cr-sensitive and 70 Ni-sensitive individuals [96], with no significant results. With regard to Co-induced lung fibrosis in hard-metal workers, Potolicchio *et al*. [38] showed that the disease is very strongly associated with the HLA-DP gene, as all patients in the study expressed allelic variants of HLA-DP coding for the lysine to glutamic acid substitution at position 69 of the β-chain (table 3), a finding even more impressive given the disparate ethnic origin of the study subjects. Such a finding calls into question the current concept of disease pathogenesis, since such a strong association would imply a direct role of HLA-DP in the interaction with the offending agent. Alternatively, as a recent phenotypic study based on TNF localization in the affected tissue has claimed that hard-metal lung disease may be associated with exaggerated TNF-α production [97], one might hypothesize that HLA-DP is in tight linkage disequilibrium with a nearby gene(s) regulating the fibrogenic response.

*Asbestosis*. Asbestos is a fibrous material widely used in industry because of its insulating properties against heat and cold, incombustibility, great tensile strength and flexibility properties. Exposure to asbestos is the cause of pulmonary fibrosis in 4–15% of individuals with consistent exposure, with pleural fibrosis occurring in 3–50% of exposed workers. Although there is no demonstrable lymphocyte reaction against asbestos, asbestos-exposed workers have been reported to have altered immune reactions characterized by an increased prevalence of rheumatoid factor, antinuclear antibodies, elevated levels of circulating Igs and IgA immune complexes [98], suggesting that asbestos-induced immune alterations may play a role in disease pathogenesis.

A 1975 pilot study on asbestos-exposed workers with or without asbestosis found an association between asbestosis risk and the HLA antigen (B)w27 [74]. The finding was felt to be important since the same antigen is strongly

associated with ankylosing spondilitis, an autoimmune disorder frequently complicated by pulmonary fibrosis [75]. DARKE *et al*. [76] in a study of 78 diseased and 92 unaffected asbestos workers, also found an increase in the frequency of the B27 antigen, although this was nonsignificant. The association between the HLA-B27 gene and asbestosis was not confirmed in a study of 37 patients and 37 matched exposed controls by EVANS *et al*. [77]. In addition, EVANS *et al*. [77] found no association even when they combined their data with those of MATEJ *et al*. [78]. EVANS *et al*. [77] found, instead, that subjects with the HLA-B12 allele had more severe fibrosis. With the background that the B12 antigen had been found associated with cryptogenic fibrosing alveolitis, a disease similar to pulmonary asbestosis in the absence of asbestos exposure, they suggested that a gene in positive linkage disequilibrium with HLA-B12 could be responsible for the weak association observed. In contrast, HUUSKONEN *et al*. [79] found a "protective effect" of the HLA-A18 and HLA-B27 alleles in a small population of asbestos-exposed workers with and without asbestosis.

With regard to class II genes, BEGIN *et al*. [80] found no association with any class I or class II genes in a study of 72 French Canadian asbestos workers, 40 of whom had the disease. In a small study, SHIH *et al*. [81] evaluated HLA-DR and DQ genes in asbestos-induced pulmonary fibrosis and found an association with HLA-DRw53 and HLA-DQ2. In contrast, AL JARAD *et al*. [82], in a 1992 study of 99 asbestos workers, did not find any association between class I HLA-A and HLA-B or with class II HLA-DR and HLA-DQ genes, with the exception of a weak negative association of HLA-DR5 when their data were combined with the data of BEGIN *et al*. [80].

Based on a meta-analysis of the literature, AL JARAD *et al*. [82] attributed the weak associations found in the previous studies to the lack of stringent analytical criteria. Associations were thought to be type I errors due to the high number of comparisons rather than type II errors, or missed associations due to the small size of samples [82]. Thus, even considering that diagnostic standards for asbestosis, *i.e.* positive chest radiographic findings and bibasilar rales at auscultation, are insensitive and lead to an underestimation of the prevalence of disease, as shown by using the more sensitive high-resolution computed tomography (HRCT), a role of HLA genes in susceptibility to asbestos-induced pulmonary and pleural fibrosis is far from established.

*Coal worker's pneumoconiosis and silicosis*. Coal mining has been associated with a number of fibrotic lung disorders of varying severity, including: 1) chronic bronchitis and emphysema; 2) uncomplicated coal worker's pneumoconiosis; 3) massive pulmonary fibrosis and rheumatoid pneumoconiosis (or Caplan's syndrome); and 4) silicosis. Uncomplicated pneumoconiosis, affecting 20% of miners, is the most prevalent, while silicosis (12%) and progressive pulmonary fibrosis (5%) are the least common. It has been proposed that uncomplicated pneumoconiosis develops when the lung's capacity to eliminate coal dusts is overwhelmed. Progressive pulmonary fibrosis, which may develop after cessation of exposure, is often associated with immunological abnormalities and with severe lung damage and disability. Exposure to silica in a variety of occupations, including sandblasting and grinding, mining, quarrying and tunnelling and foundry work, is the cause of "classic" silicosis, a fibrotic lung condition similar to coal worker's pneumoconiosis, but characterized by a typical silica-induced lesion: the silicotic nodule. Silica toxicity is, at least in part, due to the ability of this material to trigger an oxygen radical reaction which is damaging to cellular structures.

Individual susceptibility to coal worker pneumoconiosis has been suggested by: 1) the lack of a uniform relationship between exposure dose and disease incidence; and 2) the association of pneumoconiosis with immunological abnormalities, such as increased prevalence of antinuclear antibodies and rheumatoid factor, as well as increased levels of Igs and immune complexes. HEISE *et al*. [83] found no strong association between progressive massive fibrosis, coal worker pneumoconiosis and HLA-A and HLA-B genes in a study of 358 north-eastern American miners. In contrast, WAGNER and DARKE [85], in a 1979 study of over 400 Welsh coal workers with or without pneumoconiosis, found an association between HLA-Bw21 and pneumoconiosis and between HLA-Bw45 and complicated pneumoconiosis. In a 1983 follow-up study, the same authors confirmed the presence of HLA-Bw45 in complicated pneumoconiosis with rheumatoid factor. They also found a weak association between HLA-A1 and B8 with complicated silicosis without rheumatoid factor [85]. KOSKINEN *et al*. [86] found an increased frequency of the HLA-Aw19 allele among Finnish silicosis patients, compared with silica-exposed unaffected subjects. Interestingly, they were able to demonstrate an increased frequency of the haplotype HLA-Aw19, B18 among those developing progressive fibrosis, suggesting a role for this haplotype in the pathogenesis of lung fibrosis. KREISS *et al*. [87] found a statistically significant increase in the frequency of the HLA-A29 and B44 alleles among 59 western American coal miners with silicosis compared with controls. They also found that B44-positive subjects were older at the time of diagnosis and were less symptomatic than other subjects, while A29-positive subjects were more likely to have pneumoconiosis-associated immune abnormalities, such as high levels of IgG and immune complexes.

While KREISS *et al*. [87] found no association with HLA-DR and HLA-DQ, RIHS *et al*. [88] found an in-creased frequency of HLA-DR8 in German miners dev-eloping silicosis with a rapid disease course, while the frequency of DR1 was increased and that of DRw52 de-creased in those not developing silicosis. Consistent with the notion that silicosis patients have more severe disease in the presence of RA or of rheumatoid factor [99], HONDA *et al*. [89] reported in 1988 similar increased frequencies of the HLA antigens Bw54, DR4 and DRw53 in silicosis and in RA patients. The same authors were later able to extend and confirm their observation in the same population of 46 Japanese silicosis patients, showing that the frequencies of HLA-Bw54, DR4 (Dw15), DRw53 and DQw4 were elevated. No association was found with the alleles of the HLA-A and HLA-DP genes [90]. Overall, the data suggest that HLA genes are variably associated in different geographical groups with disease presentation rather than with the disease *per se*.

Do HLA genes play a role in susceptibility to coal worker pneumoconiosis and silicosis? In a 1984 critique of the published literature, SLUIS-CREMER and MAIER [99] pointed to the inconsistency of the associations found in different

populations and the weakness of statistical results obtained and thus concluded, consistent with the notion that the basic mechanism of silica toxicity is independent on T-cell recognition and reaction, that it was unlikely that one or more HLA genes were involved in disease susceptibility. However, these weak associations may suggest linkage disequilibrium with other gene(s) in the HLA locus. As HONDA *et al*. [89] suggested in their 1988 work, this gene is likely to be in positive linkage disequilibrium with HLA-Bw54-DR4-DRw53 and in negative linkage disequilibrium with HLA-Bw52-DR2. Interestingly, ZHAI *et al*. [100] have recently presented preliminary evidence of 13 pneumoconiotic miners, suggesting that the TNF2 allele of the TNF-α gene is associated with susceptibility to pneumoconiosis, thus making the TNF-α gene the leading candidate gene for silicosis susceptibility.

## Implications for the chronic inflammatory lung disorders of unknown origin

### *Pulmonary sarcoidosis*

Sarcoidosis is a chronic, multisystem granulomatous disorder. The immunopathology of pulmonary sarcoidosis is characterized by: 1) a macrophage-lymphocyte alveolitis; 2) formation of noncaseating granulomas in the peribronchial and perivascular interstitium of the lung; and 3) fibrosis. The alveolitis of sarcoidosis is dominated by activated macrophages releasing T-cell-activating lymphokines such as TNF-α [101] and by the accumulation of activated type-1 CD4+ T-cells expressing IL-2 receptors and HLA-DR surface molecules releasing IL-2 at exaggerated levels [102] and IFN-γ. These T-cells express biased repertoires of T-cell antigen receptor rearranged genes (see below), suggesting the exaggerated response to a single antigen.

Sarcoidosis presentation and clinical course vary. Stage I is characterized by prominent mediastinal lymph node reaction and rapid resolution, stage II by diffuse lung infiltration by granulomatous lesions and stage III by diffuse fibrosis and severe lung dysfunction. Interestingly, markers of exuberant local T-cell activation such as the increase in the number and proportions of the CD4 T-cells are associated with stage I disease and with rapid resolution.

Several lines of evidence suggest that sarcoidosis is due to environmental agents, including mycobacteria, and that susceptibility to sarcoidosis is inherited. In this regard, there is a definite prevalence of sarcoidosis in certain ethnic groups, such as the Irish, the Swedish and the North American Black population, and familial clusters are described in the literature.

Sarcoidosis has been associated with the DR3 allele of the HLA-DR gene [103–105]. The HLA-B8, DR3 haplotype has been associated with acute-onset, stage I resolving sarcoidosis [106–109] in Caucasians but not in West Indians [107]. HLA-DR5 has also been associated with sarcoidosis [103–105]. However, HLA-DR5 was also associated with nonresolving sarcoidosis in a Japanese study. In this regard, ISHIHARA *et al*. [110] hypothesized that sarcoidosis is associated with the HLA-DR antigen group including DR3, DR5, DR6 and DR8. Consistent with the notion that HLA-DR3 is very common among Caucasians but very rare among the Japanese, sarcoidosis is associated with the group of HLA antigens including DR3, DR5 and DR6 in Caucasians and HLA-DR5, DR6 and DR8 in the Japanese.

What is the role of HLA genes in sarcoidosis? As in berylliosis, where the HLA-DP gene seems to function as the gene restricting the presentation of Be, in sarcoidosis HLA genes may also function as immune response genes in restricting the presentation of specific antigen(s) or superantigen(s). GRUNEWALD and co-workers [111, 112] found that in Scandinavian sarcoid patients carrying HLA-DR3, CD4+ T-cells expressing the Vα 2.3 TCR gene accumulated in the lung and USUI *et al*. [113] found that in Japanese patients carrying HLA-DR12 T-cells accumulating in the lung expressed the Vβ 6 gene. These studies suggest the presence of specific antigen(s) or superantigen(s) with high affinity for the disease-associated HLA gene(s), and hence, a direct role for both the HLA and the TCR genes in disease susceptibility.

Alternatively, with the background that HLA-B8 and HLA-DR3, both associated with sarcoidosis in many studies, are in positive linkage disequilibrium with the TNF gene, it may be hypothesized that TNF is the primary sarcoidosis gene. However, ISHIHARA *et al*. [114] showed that the TNF-β gene *Nco*1 polymorphism is more weakly associated with disease than HLA-DR genes, leaving open the question of whether the disease course, rather than the disease itself, is associated with the TNF gene. In this regard, RICHELDI *et al*. [115] extended the study of MARTINETTI *et al*. [109] to show that stage I sarcoidosis was associated with the TNF2 allele of the TNF-α gene, which is asso-ciated with high TNF-α production. In the context that the TNF2 allele is in positive linkage disequilibrium with HLA-DR3 [116] and in negative disequilibrium with DR5, it may be hypothesized that in stage I sarcoidosis, as seems to be the case in silicosis, the expression of a "high TNF" genotype may play a role in the genesis of immunopathological manifestations of exuberant inflammation.

### *Pulmonary fibrosis*

Pulmonary fibrosis, also called cryptogenic fibrosing alveolitis (CFA) or idiopathic pulmonary fibrosis (IPF), is a chronic disorder of the lung characterized by the accumulation of inflammatory cells in the lower respiratory tract: macrophages accumulate in the alveolar spaces and the interstitium of the lung, accompanied by lymphocytes and polymorphonuclear leukocytes. Pulmonary fibrosis is a frequent complication of a number of autoimmune disorders including ankylosing spondilitis, a disorder tightly associated with HLA-B27 [117]. A familiar form of IPF has been described and pulmonary fibrosis is often observed in the course of the Hermanski Pudlack syndrome, a genetic disorder dominated by albinism and blood cell maturation abnormalities. The association between pulmonary fibrosis and HLA genes has been examined in several studies. Association with the HLA class I genes B7, B8 and B12 and the HLA class II gene DR2 was inconsistently found and not confirmed by published studies [118–121].

In conclusion, HLA associations have been clearly demonstrated for hypersensitivity and allergic lung disorders caused by specific antigens or haptens such as berylliosis,

gold hypersensitivity, certain forms of hypersensitivity pneumonitis, occupational asthma and respiratory allergy to specific antigens. A role for HLA genes has not been convincingly shown in fibrotic lung disorders; however, current evidence leads one to hypothesize on the role of non-HLA susceptibility gene(s) located in chromosome 6 and TNF-α seems to be a promising, although not the sole candidate in fibrotic lung disorders. In the context that the lung is continuously exposed to airborne agents and with the notion that distinct HLA alleles are associated with sensitization to specific respiratory antigens, it is conceivable that genetic HLA markers play a major role in determining susceptibility to a large number of environmental agents.

Could HLA markers be used as epidemiological probes to identify population groups at higher risk and prevent disease risk associated with occupation, manufactured products and the environment?

In the berylliosis model, the HLA-DP Glu69 marker is carried by 30–40% of the general population, as are other human leukocyte antigen markers of susceptibility to chronic inflammatory disorders, such as the HLA-DQ-Asp57 marker associated with insulin-dependent diabetes mellitus [122]. With the ethical and social problems arising from the risk of discrimination due to genetic testing [123], screening for susceptibility to occupational diseases using human leukocyte antigen markers does not seem applicable to disease prevention. In the cohort study of RICHELDI *et al*. [32], if the 40 workers carrying the hu-man leukocyte antigen marker were not employed bas- ed on preplacement genetic screening, five cases out of six would have been prevented, but 36 workers would have been denied employment. However, the same study indicates that genetic marker epidemiology is useful in determining 1) the size of the population that may be genetically susceptible to certain exposure-related risks; and 2) the levels of exposure that may be safe for these large genetically susceptible population segments. By means of genetic epidemiology research studies, exposure control may be designed to protect all workers and all people from respiratory hazards, thereby preventing a large number of disease cases.

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