Genetic Factors Modify the Risk of Developing Beryllium Disease

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ABSTRACT

Chronic beryllium disease (CBD) is a debilitating, granulomatous lung disease that occurs in 1 to 5% of exposed workers. Beryllium stimulates a major histocompatibility Class II-restricted, TH1, CD4+T cell-mediated immune response. The immunological component of the illness, coupled with the small subset of beryllium workers who develop disease, led researchers to hypothesize that genetic factors modify risk of disease. Analysis of human leukocyte antigen (HLA) genes, the T cell receptor, and tumor necrosis factor (TNF)-α focused on three critical steps in the development of beryllium specific immunity. Molecular epidemiological analysis of the association of HLA-DP, -DR, and -DQ has implicated *HLA-DPB1*^{E69} allelic variants in disease; however, its role in sensitization is unclear. A single report suggested association between *HLA-DQB1*^{G86} and progression from sensitization to disease. A beryllium-specific binding motif was identified in CBD-derived T cell clones. Beryllium-stimulated proliferation using HLA-DPB1*0201 and TCRAV22S1/TCRBVb3 T cell receptors (TCRs) confirmed beryllium specificity of these molecules. The G/A transition at -308 in the TNF-α promoter was associated with high concentrations of TNF-α in bronchoalveolar lavage and to disease severity. Although these studies are continuing, the data confirm the role of genetic factors in the cellular response to beryllium.

KEYWORDS: Beryllium disease, risk factors, HLA, T cell receptor, TNF-α

Objectives: Upon completion of this article, the reader should understand gene susceptibility (to beryllium in particular) in occupational disease.

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Beryllium is an alkaline earth metal that occupies the fourth place in the periodic table of the elements. As the forty-fourth most abundant element, it occurs naturally in the environment, has an atomic weight of 9.012 and is dull gray in color. Beryllium has unique physicochemical properties. It is lightweight but stiffer than steel, nonsparking, noncorrosive, and has a melting point of 1560°K.[1]

Beryllium was discovered by the Frenchman Nicholas Louis Valquelin in 1798; however, commercial

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manufacture of beryllium products did not begin until after beryllium–copper–aluminum alloy was patented in 1923.[2] With its high conductivity, beryllium, as a metal, an oxide, or an alloy, has become indispensable in the manufacture of electrical switches, triggers for nuclear weapons, mast-mounted sights for ships, and mirrors for tanks. In the aerospace industry, beryllium is found in precision tools, satellites, gyroscopes, rockets, and the Hubble telescope. It is also x-ray opaque and used as a window in mammography equipment. Beryllium has everyday utility in cell phones, personal computers, camera shutters, springs, microwave devices, pen clips, and dental prostheses.[2]

The first evidence of the toxicity of beryllium was observed in the fluorescent lamp industry in the mid-1940s with the diagnosis of acute beryllium disease (ABD).[3,4] In 1949, the Atomic Energy Commission implemented an 8-hour exposure limit of 2 μg/m^3, and ABD was brought under control.[5] Subsequently, a second condition, consisting of a debilitating, persistent, granulomatous lung disease, was observed and designated chronic beryllium disease (CBD).[6,7] Although involvement of liver, spleen, skin, lymph node, kidney, and muscle has been noted in CBD, the lung is the primary site of disease.[8]

EXPOSURE

Environmental exposure to beryllium in food, water, air, and soil is commonplace, but the average concentration, in the absence of a polluting event, is generally well below the Environmental Protection Agency (EPA) standard. For example, the permissible concentration of beryllium in drinking water is 4 μg/L, and the average measurable concentration is 0.19 μg/L with a range of 0.1 to 1.22 μg/L.[2]

Exposure to beryllium concentrations high enough to initiate sensitization is most frequently associated with beryllium workers. On average, 1 to 5% of exposed workers become sensitized to beryllium.[6,9,10] After a variable latency period, on average 3 to 10 years, most sensitized individuals develop pulmonary, noncaseating granulomas. Studies suggest that 10% of the sensitized population progress to granulomatous disease each year.[6]

Epidemiological analyses of workplace exposures showed that disease prevalence varies by exposure type and intensity.[11] The exposure limit for most work environments remains 2 μg/m^3 (time weighted average)[12]; however, the failure of this exposure limit to decrease the rate of disease motivated the Department of Energy to set an exposure limit of 0.2 μg/m^3 for their facilities. A recent report by McCawley and colleagues has linked beryllium particle number and particle size, not beryllium mass measurements, with increased disease prevalence.[13] These data are consistent with observations that job tasks that generate ultrafine beryllium dust, such as machining and lapping, convey the highest risk of disease.[11] Historically, pulmonary exposure to beryllium was considered the primary route for sensitization. Recent evidence suggests that sensitization may be related to skin exposure[14,15] or to the systemic burdens of beryllium.[16]

IMMUNOPATHOLOGY OF CHRONIC BERYLLIUM DISEASE

Beryllium Sensitization

The beryllium-stimulated immune cell response is a major histocompatibility (MHC) Class II-restricted, T helper 1, CD4+ T cell response.[17] Consistent with the model of a cell-mediated immune response (CMIR), beryllium, probably in association with a peptide and in the context of human leucocyte antigen (HLA), is presented by an antigen-presenting cell to the T cell (Fig. 1). Activation of the T cell causes clonal expansion and the generation of beryllium-specific memory T cells. This cellular process is driven by proinflammatory cytokines, including TNF-α and interleukin (IL)-6.[18,19] A TH1 (T helper 1) cytokine response has been demonstrated in vitro with beryllium-stimulated bronchoalveolar lavage (BAL) cell production of interferon gamma (IFN-γ) and IL-2.[17,20]

Several laboratories confirmed the requirement for HLA–beryllium–TCR interaction by blocking beryllium-stimulated CBD BAL lymphocyte proliferation with monoclonal antibodies directed against the Class II complex.[8,17] The HLA-DP antibody provided the strongest inhibition of the beryllium lymphocyte proliferation test (BeLPT), 50 to 90%, and the antibody directed against HLA-DR blocked cell proliferation by 30 to 50%. Multiple studies have investigated the identity of the beryllium-presenting HLA, and Fontenot and colleagues performed similar studies to determine the identity of the TCR.[21] The molecular specificity of the HLA–beryllium–TCR interaction, coupled with the 1 to 5% sensitization rate in the exposed worker population, suggests genetic susceptibility to beryllium.[22–25]

Chronic Beryllium Disease

CBD is characterized by chronic pulmonary inflammation, T cell alveolitis and phagocyte accumulation, granuloma formation, calcific inclusion, and deposition of fibrotic tissue.[8,26] The pathogenesis of CBD is similar to other pulmonary granulomatous diseases, and the pulmonary lesions in beryllium-, aluminum-, and titanium-induced granulomas are virtually indistinguishable from each other and from sarcoidosis.[27,28]

Noncaseating granuloma formation is the hallmark pathobiological response to persistent beryllium lung burden. In the response to antigen, the CD4+ T cells release proinflammatory molecules that attract

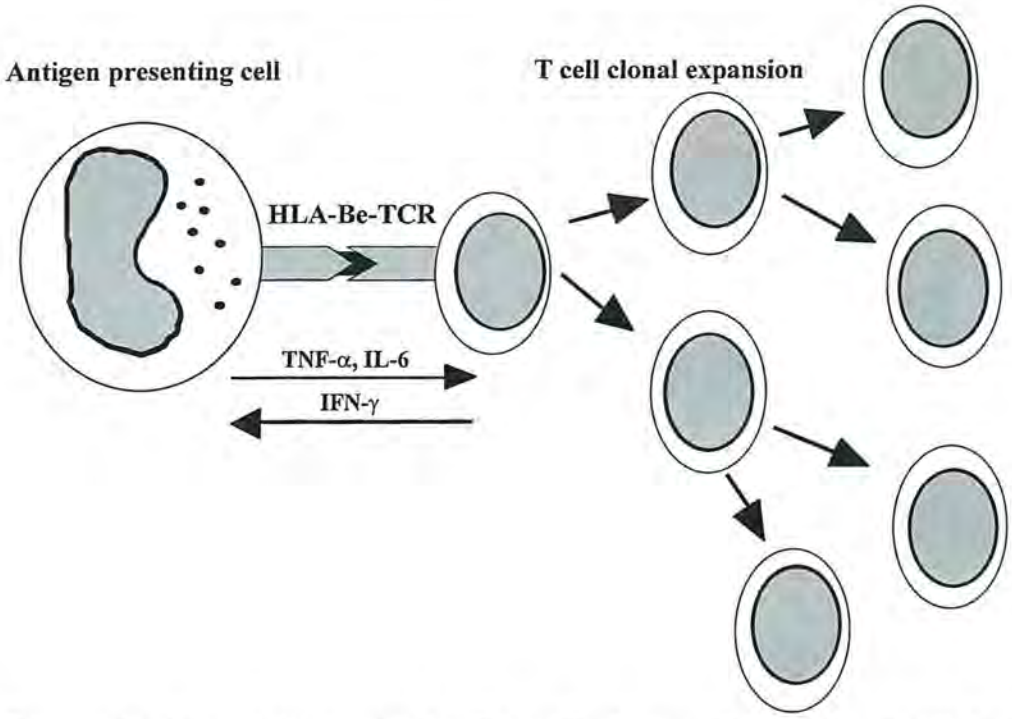

Figure 1 The interaction of human leukocyte antigen, beryllium, and T cell receptor (HLA–Be–TCR) is pivotal in the development of beryllium sensitization and disease. TNF-α, tumor necrosis factor alpha; IL-6, interleukin 6; IFN-γ, interferon gamma.

macrophages and other inflammatory cells to the site of antigen deposition.[17,29] Antigen persistence maintains the CMIR, and, over time, inflammatory cells, and the CD4+ T cells in particular, accumulate in the lung. This proinflammatory microenvironment promotes macrophage differentiation into multinucleated giant cells (MGCs). The granuloma forms as a discrete nodule containing a cluster of MGCs surrounded by a ring of densely packed lymphocytes.[7,8] Laminated calcific densities can be observed in the MGCs.

Little is known about the mechanism that causes pulmonary fibrosis in CBD. The pathogenesis of dust-induced fibrosis relates chronic activation of macrophages to activation and proliferation of fibroblasts.[24] Increasing numbers of fibroblasts in the pulmonary interstitium in CBD patients and the close association of the profibrotic, basic fibroblast growth factor (bFGF)-positive mast cells with beryllium granulomas has been shown.[30,31]

With this understanding of beryllium sensitization and disease, we next examine the genetic identity of HLA and TCR and the contribution they make to beryllium disease susceptibility. Investigations into a role for HLA used a molecular epidemiological approach. The TCR studies focused on sequence analysis of beryllium-stimulated T cell clones derived from a limited number of individuals. Finally, we will review the data correlating a polymorphism in the TNF-α promoter region with risk of disease and disease severity.

HUMAN LEUKOCYTE ANTIGEN

The HLA Class II molecules, designated HLA-DP, -DQ, and -DR, are highly polymorphic transmembrane heterodimers located on antigen-presenting cells. They are composed of alpha (A) and beta (B) chains. The antigen-binding groove is composed of a platform of eight antiparallel beta sheets and two antiparallel alpha helices. The structural and electrical configuration of this site is determined by the sequence of amino acids lining the groove, which, in turn, is dictated by genetic recombination during transcription. The protein topology then dictates the antigen-binding requirements.

Molecular Epidemiology

The observation that only a subset of beryllium-exposed workers develop CBD is suggestive of a host factor or factors that modify disease risk. Biological confirmation of the MHC-restricted, beryllium-stimulated, proliferative response in sensitized cells suggested a survey of the HLA genes.[17] Thus genetic variants of the HLA-DP, -DQ, and -DR loci were evaluated for their association with risk of CBD. These genes are all located on chromosome 6p12.3 (Fig. 2).[32]

A series of molecular epidemiological case-control and cross-sectional studies among people employed in or formerly employed by the beryllium industry implicated several supratypic markers in *HLA-DPB1* with CBD.[22] Most notably *HLA-DPB1* alleles encoding a

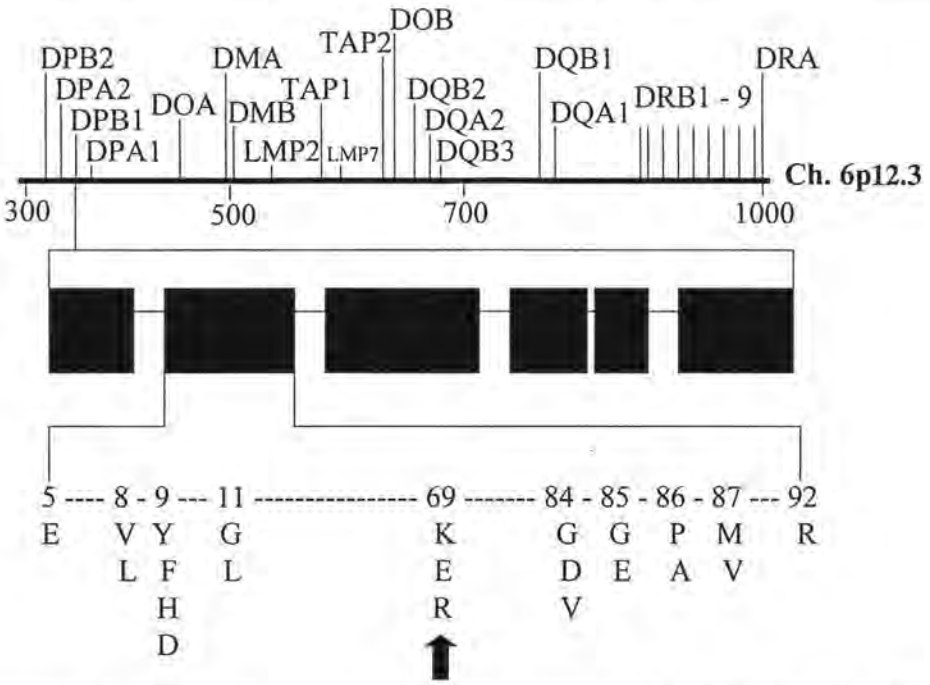

Figure 2 Chromosome 6p, indicating the relative positions of major histocompatibility (MHC) Class II loci. The *HLA-DP* locus is found at 6p12.3.

glutamic acid at the amino acid residue in the sixty-ninth position (E69) of the mature protein was associated with very high odds of CBD (OR = 76, 95% CI = 18–322). At this time, a total of only about 20 different alleles were known, and the predominant alleles coding for E69 and associated with CBD were those in the *HLA-DPB1*02* family. The most common allele, encoding a lysine at the amino acid residue in the sixty-ninth position (K69) of the mature protein, *HLA-DPB1*0401* was associated with reduced disease susceptibility (OR = 0.2, 95% CI = 0.1–0.6). These data were also biologically consistent. Modeling the structure of HLA-DP analogous to the known structure of HLA-DR positioned the sixty-ninth amino acid of the mature protein in the antigen-binding groove.[33]

Four years later a cross-sectional study by the same group investigated this question in current workers and former workers at a beryllium plant in Tucson, Arizona.[23] Six cases of disease were reported; however, this was approximately representative of the true disease prevalence (1–5%) compared with the control group (n = 121). The results were similar, yielding a high odds of disease associated with inheritance of *HLA-DPB1*^{E69} (OR = 10.1, 95% CI = 1.4–89.6), or alleles encoding glutamic acid at position 69. They also demonstrated that machinists, a high exposure group, were also at higher risk for disease (OR = 9.4, 95% CI = 1.1–83.1). The gene–environment interaction question as to whether these factors are additive or multiplicative is not yet resolved.

A study of beryllium-exposed workers or former workers, 20 CBD cases and 75 case controls, was performed using high-resolution, allele-specific DNA sequencing.[34] These data were consistent with the studies of Richeldi and colleagues,[22,23] in that they identified a strong association of CBD with inheritance of *HLA-DPB1*^{E69} (OR = 22.9, 95% CI = 4.8–108.2). However, the high-resolution, allele-specific DNA sequencing strategy allowed detailed scrutiny of other regions of the gene. These studies showed that elevated risk of CBD was associated with alleles coding for amino acids at positions 8 (valine–V), 9 (histidine–H or tyrosine–Y), and 11 (leucine–L) and at positions 84 to 87 (aspartic acid–D, E, alanine–A, and V, respectively). Inheritance of alleles coding for V8, H/Y9, and L11 at positions 8, 9, and 11 conferred a greater risk of CBD (OR = 9.0, 95% CI = 2.6–31.6) than did inheritance of alleles coding for L, phenylalanine–F, and glycine–G. Similarly, alleles coding for D84, E85, A86, and V87 at positions 84 to 87 also conferred a greater risk of CBD (OR = 9.8, 95% CI = 2.6–36.6) compared with inheritance of alleles coding for G, G, P, methionine–M. The amino acid residues in positions 8 to 11 and 84 to 87 primarily distinguish the relatively common *HLA-DPB1*0201* and *HLA-DPB1*0202* that code for E69 from the rarer *HLA-DPB1*^{E69} alleles (e.g., *HLA-DPB1*1701*).

Based on the observations concerning the relative frequencies of VH/YL-LFG (amino acids 8–11) and GGPM-DEAV (84–87), Wang and colleagues asserted

that, among the alleles coding for E69, the rarer variants were likely to carry a greater CBD susceptibility than the *HLA-DPB1*02* family of alleles.[34] These investigators observed that the *HLA-DPB1*02* family of alleles codes for LFG at positions 8, 9, and 11 and GGPM at positions 84 to 87, whereas the rarer *HLA-DPB1^{E69}* variants code for VH/YL and DEAV, respectively. Although the sample size was small, further analysis of their data tentatively suggests a hierarchical scheme for the odds associated with each *HLA-DPB1^{E69}*. We estimated the lowest odds for *HLA-DPB1*0201/2* (OR ~ 15, 95% CI ~ 3–85), the next highest was *HLA-DPB1*1901*, and so on to the next *HLA-DPB1*1301* < *HLA-DPB1*0901* = *HLA-DPB1*1001* < *HLA-DPB1*0601* < *HLA-DPB1*1701* (OR ~ 246, 95% CI ~ 38–1594). Two caveats should be mentioned in this analysis: in the case of individual alleles, a small sample size results in extremely large overlapping confidence intervals; and Wang and colleagues reported only 19 distinct alleles.[34] To date, 103 *HLA-DPB1* nucleotide sequence variants have been identified, of which 34 code for E69, 62 for K69, and 5 for arginine (R69).[35] Because the proportion of *HLA-DPB1* alleles coding for R69 is small, we will consider the E69 versus K69 variants for this review.

In addition to the simple association of CBD with *HLA-DPB1^{E69}*, Wang and colleagues also noted the impact of homozygosity for E69.[34] Their data suggested that the *HLA-DPB1^{E69}* homozygote was at increased risk compared with the *HLA-DPB1^{K69}/HLA-DPB1^{E69}* heterozygote. Subsequently, Wang and colleagues demonstrated similar associations between *HLA-DPB1^{E69}* alleles and sensitization.[35]

In both of these studies, Wang and colleagues investigated the distribution of the alpha chain, *HLA-DPA1* polymorphisms in both CBD[34] and sensitization.[35] The alpha gene of the HLA-DP heterodimer is located only 1 to 2 kb distal with respect to the beta locus, so it is expected that there is a high degree of linkage. There are four allelic subgroups of *HLA-DPA1* that encode the alpha chain (*HLA-DPA1*01, HLA-DPA1*02, HLA-DPA1*03, HLA-DPA1*04*), and in total there are 20 known alleles.[35] Wang and colleagues reported two alpha alleles, *HLA-DPA1*0103* and *HLA-DPA1*02011*.[34] For *HLA-DPB1^{E69}* alleles among controls, they found that *HLA-DPB1*0201* was exclusively associated with *HLA-DPA1*01*; similarly, among CBD cases *HLA-DPB1*0201* was associated with *HLA-DPA1*01* in seven of eight individuals. The sole exception was the association of an *HLA-DPB1*0201* with an *HLA-DPA1*02011* allele in one case of CBD. These data suggest that, in combination with rare *HLA-DPB1^{E69}* alleles, the strongly linked *HLA-DPA1*02* allele family (*HLA-DPA1*02011* and *HLA-DPA1*02012*) is associated with elevated risk of CBD (OR = 13.3, 95% CI = 2.1–17.5).[34] In the case of beryllium sensitivity, *HLA-DPA1*02* alleles were represented to about the same extent in both beryllium sensitized individuals and controls (OR = 1.4, 95% CI = 0.6–3.2).[35]

In a study of 22 CBD cases, 23 beryllium sensitized and 93 controls recruited at a beryllium manufacturing facility located in Elmore, Ohio, Saltini and colleagues[26] recently used a combination of high-resolution DNA sequencing and oligonucleotide hybridization to examine the frequency *HLA-DPB1^{E69}* alleles. They found *HLA-DPB1^{E69}* to be associated with CBD (OR = 3.7, 95% CI = 1.4–10.0) but not with sensitization (OR = 0.9, 95% CI = 0.3–2.2).

Recently, Rossman and colleagues, using oligonucleotide hybridization and allele-specific sequencing where necessary, also demonstrated an association between inheritance of *HLA-DPB1^{E69}* and susceptibility for beryllium sensitization (OR = 9.9, 95% CI = 2.8–35.3) and CBD (OR = 5.8, 95% CI = 1.8–18.4).[36] Although there was no statistical difference in *HLA-DPB1^{E69}* allelic frequencies between the sensitized and CBD groups (OR = 0.63, 95% CI = 0.30–1.35). In addition, the frequency (F) of *HLA-DPB1*02* family of alleles did not differ between cases (F = 0.18) and controls (F = 0.16). Contrary to Richeldi et al[23] and Saltini et al,[25] this result tends to support the contention by Wang and colleagues that the rare *HLA-DPB1^{E69}* alleles are more important for sensitization and disease than the *HLA-DPB1*02* family of alleles.[34,35] Consistent with Richeldi and colleagues[22] there was a significantly lower frequency of the *HLA-DPB1^{K69}* alleles *HLA-DPB1*0401* among sensitized and CBD (OR = 0.41, 95% CI = 0.20–0.85). However, a statistically significant higher allelic frequency of *HLA-DPB1*0402* was found among CBD cases with respect to controls (OR = 2.65, 95% CI = 1.00–7.01).

In addition to *HLA-DPB1^{E69}*, Saltini and colleagues also examined the frequency of *HLA-DRB*.[24] There are more than 240 *HLA-DRB* alleles that are classified by nine subgroupings (*B1–B9*). Essentially, there are two genuine loci: *HLA-DRB1* (the largest) with more than 180 alleles, and *HLA-DRB3/4/5* with more than 60 alleles. The additional loci, *B2, B6, B7, B8,* and *B9*, are pseudogenes.[37,38] An arginine residue can be found at position 74 of the mature proteins encoded by 13 alleles designated *HLA-DRB1* (e.g., *HLA-DRB1*03011*) and six alleles designated *HLA-DRB3* (e.g., *HLA-DRB3*0302*). We have adopted the same nomenclature as that used by Saltini et al[26]; therefore we describe this supratypic marker as *HLA-DRB^{R74}*. Saltini and colleagues found *HLA-DRB^{R74}* to be associated with sensitization (OR = 4.0, 95% CI = 1.5–10.1) but not with CBD (OR = 0.9, 95% CI = 0.3–2.6).

Rossman and colleagues[36] also considered the *HLA-DRB1* locus but not the *HLA-DRB3/4/5* locus.[24] Their data show an elevated frequency of *HLA-DRB1*0302*

among sensitized individuals but not those with CBD (OR = 5.96, 95% CI = 1.37–25.91). *HLA-DRB1*0302* is among those alleles coding for the HLA-DRB protein with arginine-R at position 74. These data also implicated *HLA-DRB1*1302* in sensitization but not CBD, and *HLA-DRB1*0901* in both. However, these post hoc findings were not robust enough to withstand the Bonferroni correction ($n = 31$).

Three *HLA-DQB1* alleles were implicated: *HLA-DQB1*0608* in sensitization, *HLA-DQB1*0604* in CBD, and *HLA-DQB1*0605* in both sensitization and CBD. A single amino acid epitope, *HLA-DQB1^{G86}*, that withstood correction for multiple comparisons was found at higher frequency in CBD cases than controls.[36] The frequency of this marker was also higher in the CBD group compared with the sensitized group ($p = 0.09$, borderline significant). Rossman et al concluded that these data indicate that *HLA-DQB1^{G86}* may be a susceptibility marker for progression from sensitization to disease.[36]

In summary of published molecular epidemiological studies the first three HLA-CBD studies reported very high ORs for the association of CBD susceptibility and inheritance of *HLA-DPB1^{E69}* (OR = 76, 95% CI = 18–322[22]; OR = 11.8, 95% CI = 1.3–108.8[23]; and OR = 22.9, 95% CI = 2.9–180.1[35]). However, the most recent studies have suggested this association to be more modest (OR = 3.7, 95% CI = 1.4–10.0 [25] and 5.8, 95% CI = 1.8–18.4[36]).

T CELL RECEPTOR

The TCR is a heterodimeric transmembrane protein whose surface structure describes a groove composed of a beta sheet and two alpha helices. By genetic recombination, the TCRA locus encodes the A chain from V and J segments, and the TCRB locus encodes the B chain from V, D, and J loci. There are within the V region of the A and B chains three complementarity-determining regions (CDRs), which form the binding site for the HLA–antigen complex. CDR1 and 2 are encoded in the germline and positioned over the residues at either end of the antigenic peptide. CDR3 is positioned over the central peptidic residues and generated by genetic recombination. The variability of the CRD3 amino acid sequence and its central location make it a critical region in HLA–antigen–TCR recognition and binding.

TCR Sequence Homology

To determine the molecular identity of a beryllium-specific TCR, Fontenot and colleagues compared the TCR repertoire in the bronchoalveolar lavage (BAL) cells and blood cells, and observed an expansion of the TCR VB3 repertoire in BAL cells compared with blood, in 11 of 28 CBD subjects.[21] This repertoire persisted over time and could be expanded further by in vitro stimulation with beryllium salts. Sequencing the VB region of the TCR in T cell clones from five CBD patients, they reported a homologous TCR motif in the CDR3 region of VB3 (Fig. 3). These researchers determined that, for four of the individuals, the CDR3 region is seven to eight amino acids long and frequently has a conserved cysteine at amino acid position 90, a glycine or lysine at position 95, an invariant aspartic acid at position 96, and arginine or glutamine at position 97 (Fig. 3). This homology was observed among T cell clones derived from the same individual and among individuals.

Of the approximately 100 possible TCRA V combinations, eight clones from two CBD subjects expressing Vβ3 co-expressed the same A chain, TCRAV22S1. This V region was characterized by a leucine at amino acid position 92 in seven of eight clones, and in all clones, by serine or arginine at position 93 and an invariant asparagine at position 95 (Fig. 3).

To demonstrate the direct involvement of this TCR motif in beryllium-specific T cell clonal expansion, Fontenot used autologous, irradiated PBMCs and TCR-characterized T cell clones to demonstrate beryllium-specific T cell proliferation in vitro and the specificity of the TCR motif for antigen presented by HLA-DPB1 *0201. These findings are compelling and suggest that the combination of *HLA-DPB1^{E69}* and the TCR VB motif described above are important genetic components of the immunological response to beryllium in these patients. However, it is important to remember that the analysis of the TCR oligoclonality is based on T cells from 11 of 28 patients, and the final analysis on eight clones from two patients. These data lay the groundwork for the HLA–antigen–TCR interaction in CBD for a defined set of molecular interactions; however, the applicability of these findings to a larger patient population has yet to be determined.

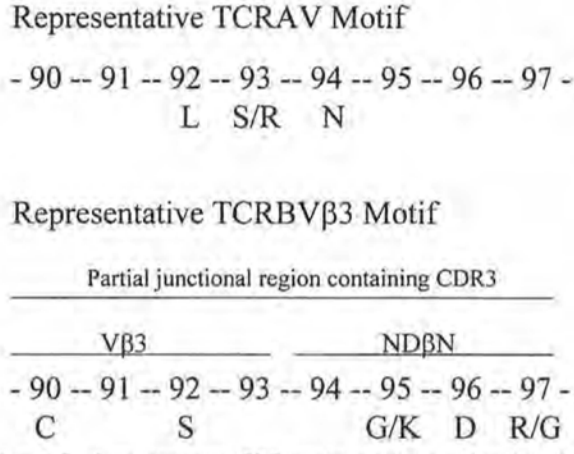

Figure 3 T cell receptor (TCR) alpha and beta chains identifying the conserved amino acid residues of a beryllium-specific TCR motif.

TNF-α

Genetic variation in TNF-α is linked to susceptibility to autoimmune and infectious diseases and to disease progression.[39,40] The *TNF*-α gene is located in the class III region of the HLA complex, between the Class I and Class II loci. At least nine polymorphisms in the *TNF*-α promoter region have been described and linked to variability in TNF-α production.[41] A glycine/alanine (G/A) transition is located 308 base pairs upstream from the TNF transcriptional start site. The minor variant (A) is designated *TNF-α*02* and has been associated with greater mitogen-stimulated TNF-α production.[42–44] Additional studies have linked HLA-DR4 with high TNF-α production and HLA-DR2 with low TNF-α production.[45,46]

TNF-α is a potent proinflammatory cytokine in the beryllium-stimulated BAL CMIR.[18] Maier and colleagues hypothesized that polymorphisms in the TNF-α promoter contribute to the magnitude of the TNF-α response to beryllium and to the severity of disease.[47] Examining BAL cells from 20 CBD patients, 10 designated high TNF-α producers (TNF-α > 1500 pg/mL) and 10 low producers (TNF-α < 1500 pg/mL), they observed an association between the presence of the *TNF-α*02* allele and high production of TNF-α (OR = 13.5, 95% CI = 1.0–687.9; $p = 0.057$), an absence of the *HLA-DR4* allele ($p > 0.05$), the presence of *HLA-DPB1^{E69}* (OR = 5.5, 95% CI = 1.06–31.5; $p = 0.02$). Comparison of clinical measures of disease severity with TNF-α production showed that high producers had significantly more severe disease est radiographic score ($p = 0.02$), peak stimulation index in the BAL BeLPT ($p = 0.01$) and spirometric measurement of the ratio of forced expiratory volume at 1 second to forced vital capacity (FEV_1:FVC) ($p = 0.03$)]. However, comparison of TNF-α levels by *TNF*-α promoter genotype showed no significant difference ($p = 0.10$). These data link the TNF-α*02 allelotype to the magnitude of beryllium-stimulated BAL TNF-α production and to disease severity, and underscore the genetic complexity of CBD. The small patient sample size, the absence of a nonexposed control group, and correlative nature of most findings in this study preclude generalization of the findings to the CBD population at large.

Saltini et al examined the frequency of three genetic markers: *HLA-DPB1^{E69}*, *HLA-DRB^{R74}*, and *TNF-α*02* in his report, to determine if a combination of these genes would provide a marker of beryllium disease susceptibility.[26] Comparing 22 CBD patients with 23 beryllium-sensitized individuals and 93 control subjects, they found *TNF-α*02* to be associated with both sensitization and disease (OR = 7.8, 95% CI = 3.2–19.1). The frequency of this marker was higher in CBD patients than in controls and similar for both beryllium subgroups. When each comparison was tested separately, the difference between groups was not significant.[24] An attempt was also made to evaluate gene–gene interactions using a two by four chi square approach.[48] The strongest combined associations were between *TNF-α*02* and *HLA-DPB1^{E69}* in the CBD group (OR = 9.7, no CI given, $p < 0.05$ after correction for multiple analyses), and between *TNF-α*02* and *HLA-DRB^{R74}* in the beryllium sensitized group (OR = 9.9, no CI given, $p < 0.05$ after correction for multiple analyses). However, this approach lead to small numbers of CBD cases/sensitized in some cells and many expected associations, e.g., the independent association of *HLA-DPB1^{E69}* with disease, could not withstand the correction for multiple analyses.[24]

SUMMARY

The HLA–beryllium–TCR interaction is critical in the initiation of beryllium sensitization. Given the highly polymorphic nature of HLA and TCR and the small subset of exposed workers who become sensitized, the genetic (and hence, protein) structure of these molecules will significantly impact, if not determine, susceptibility to beryllium disease. Continued analysis of the immunologic mechanism for sensitization and determination of the relationship of these findings to larger patient populations will add a third component, response, to gene-exposure studies.

Disagreement among literature reports linking HLA to risk of disease highlights the genetic complexity of CBD and the need to formally address several critical questions. In particular, the differences in risk of disease that may be associated with *HLA-DPB1^{E69}* homozygosity and heterozygosity, the relationship of *HLA-DPB1^{E69}* and *HLA-DPB1^{R74}* with sensitization, and the association of *TNF-α*02* with sensitization and disease need to be delineated. Identification of a hierarchical order in the degree of susceptibility to CBD and/or beryllium sensitization conferred by specific *HLA-DPB1* allelic variants may be key in resolving these issues.

Answers to these questions constitute important factors in the development of our strategy to translate genetic information into public health benefit through appropriate control of exposure and therapeutic intervention.[49]

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