Linking Genetic Susceptibility and T Cell Activation in Beryllium-induced Disease

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Chronic beryllium disease (CBD) is a granulomatous lung disorder caused by beryllium (Be) exposure in the workplace. It is characterized by the accumulation of Be-specific CD4$^+$ T cells in the lung as well as persistent lung inflammation, culminating in the development of lung fibrosis. CBD occurs in 2 to 16% of Be-exposed workers depending on the individuals' genetic susceptibility and the characteristics of the exposure. Genetic susceptibility to Be-induced disease has been linked to major histocompatibility complex class II molecules. In particular, HLA-DP alleles possessing a glutamic acid at the 69th position of the β-chain (βGlu69) are most strongly linked to disease susceptibility. The HLA-DP alleles that present Be to T cells match those implicated in the genetic susceptibility, suggesting that the HLA contribution to disease is based on the ability of those molecules to bind and present Be to T cells. However, the structural features of βGlu69-containing HLA-DP molecules that explain the disease association remain unknown. We have recently crystallized HLA-DP2, which is the most prevalent of the βGlu69-containing HLA-DP molecules. Its unique structure, which includes surface exposure of βGlu69, provides an explanation of the genetic linkage between βGlu69-containing HLA-DP alleles and Be-induced disease.

Keywords: beryllium; granuloma; human; lung; T cells

Beryllium (Be) is a lightweight metal with unique chemical and physical properties that make it ideally suited for use in high-technology industries, such as aerospace, ceramics, electronics, and defense (1). Beryllium exposure primarily occurs through inhalation in workers involved in machining Be-containing products. It is estimated that approximately 200,000 current and at least 1 million total workers have been exposed to Be in the United States alone (2, 3). Depending on the nature of the exposure and the genetic susceptibility of the individual, chronic beryllium disease (CBD) develops in 2 to 16% of these subjects (4–8). Thus, CBD remains an important public health concern.

Workplace screening of Be-exposed workers has identified individuals sensitized to Be who have no evidence of lung disease. These Be-sensitized subjects demonstrate a Be-specific immune response in peripheral blood but have no clinical, radiographic, or histopathologic features of CBD (1, 9). A subset of Be-sensitized subjects progress to CBD at a rate of 6 to 8% per year (9). CBD is characterized by the presence of noncaseating granulomatous inflammation that primarily affects the lung, although other organs may be involved (1, 10, 11). The diagnosis depends on the detection of a Be-specific immune response in blood and/or lung and the presence of noncaseating granulomas and/or mononuclear cell inflammation on a biopsy specimen (12). The histopathologic features of CBD are identical to those seen in sarcoidosis (13, 14). Due to the persistence of Be in the lung years after the cessation of exposure (15, 16), the natural history of CBD is characterized by a gradual decline in lung function, with one-third of untreated patients historically progressing to end-stage respiratory insufficiency (17).

Recently, major advances in our understanding of the immunopathogenesis of Be-induced disease have occurred. This review will focus on the link between major histocompatibility complex class II (MHCII) molecules, which have been strongly linked to disease susceptibility, and activation of Be-responsive CD4$^+$ T cells.

CBD IS A CD4$^+$ T CELL–MEDIATED DISORDER

The healthy human lung contains few lymphocytes. In comparison, the lungs of patients with CBD are characterized by a CD4$^+$ T cell alveolitis (18–22). Evidence suggests that these CD4$^+$ T cells play a critical role in the immunopathogenesis of CBD, while CD8$^+$ T cells play a minor, if any, role in the disease process (18, 19, 21, 22). Using an *in vivo* skin model of granulomatous inflammation, the development of granulomas was preceded by the influx into skin of CD4$^+$ T cells expressing identical T cell receptors (TCRs) to those clones found in the bronchoalveolar lavage (BAL) of the same patients, confirming the importance of these antigen-specific CD4$^+$ T cells in the initiation of the granulomatous response (23). Furthermore, in the lungs of patients with CBD, a remarkably large number of Be-responsive CD4$^+$ T cells accumulate (24). Be-specific T cells are specific for patients with CBD (i.e., cells expressing these TCRs are not found in the lungs of patients with other granulomatous or autoimmune diseases) and persist at high frequency in patients with active disease (21). These cells also express markers of previous activation and exhibit an effector memory T cell phenotype (characterized by expression of CD45RO and the loss of the lymph node homing receptors, CD62L and CCR7) (20, 24, 25). After Be recognition, CD4$^+$ T cells undergo clonal proliferation and secrete T helper 1 (Th1)-type cytokines such as interleukin-2 (IL-2), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) (Figure 1) (24, 26). Th2-type cytokines are not detected in the lungs of patients with CBD (24, 26). Importantly, IFN-γ and TNF-α promote macrophage accumulation, activation, and aggregation, resulting in the initiation of the granulomatous response.

Although the vast majority of Be-specific T cells are compartmentalized to the lung, a greater frequency of antigen-specific cells in blood strongly correlates with the extent of lung inflammation, as measured by the severity of the CD4$^+$ T cell alveolitis (27). These findings raise the possibility that determination of the

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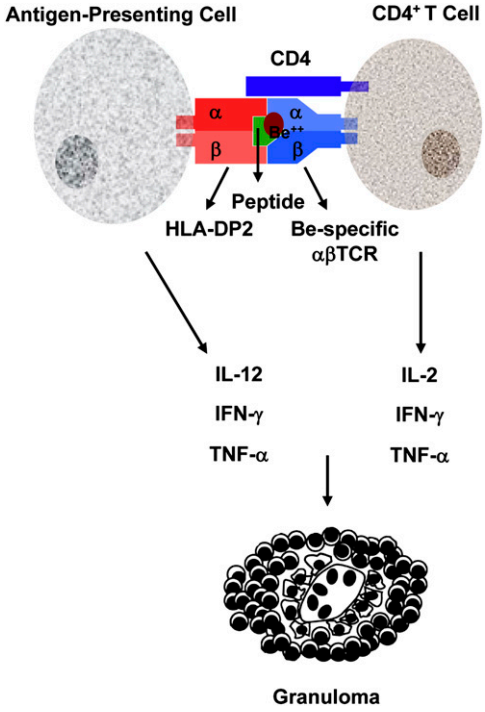

Figure 1. Immunopathogenesis of chronic beryllium disease. Following the inhalation of Be-containing particulates, antigen-presenting cells expressing a βGlu69-containing HLA-DP molecule present Be to $CD4^+$ T cells resulting in T cell activation, proliferation, and Th1-type cytokine production.

number of circulating Be-specific T cells may provide a glimpse of the inflammatory response occurring in the lung without the need for invasive procedures.

GENETIC SUSCEPTIBILITY TO BERYLLIUM-INDUCED DISEASE

In addition to Be exposure in the workplace, genetic susceptibility plays a major role in the immunopathogenesis of CBD. Susceptibility to CBD has been linked to HLA-DP alleles that contain a glutamic acid at position 69 of the β-chain (βGlu69) (28–36). Richeldi and coworkers (28) showed that *HLA-DPB1*0201* was associated with the development of CBD, whereas *DPB1*0401* was protective. Sequence analysis showed that *DPB1* alleles with βGlu69, which includes *DPB1*0201*, were strongly associated with disease susceptibility (28). Other studies have confirmed these findings, documenting the presence of βGlu69-containing *DPB1* alleles in approximately 80% of patients with CBD compared with 30 to 48% of exposed controls (29–36). Recent studies have shown that βGlu69-containing *DPB1* alleles are a risk factor for the development of Be sensitization and not simply a marker of progression from sensitization to disease (32–35). In addition, rare HLA-DP alleles carrying βGlu69 (i.e., non-*HLA-DPB1*0201* alleles), more than the HLA-DP βGlu69 itself, were associated with CBD in some studies (30, 32, 33).

Approximately 20% of patients with CBD do not possess a βGlu69-containing *HLA-DPB1* allele, suggesting the importance of other MHCII molecules in the genetic susceptibility to Be-induced disease. In this subset of patients with CBD, an increased frequency of HLA-DR13 alleles was observed (33, 34). These alleles possess a glutamic acid at position 71 of the DR β-chain (βGlu71), which corresponds to position 69 of HLA-DP. Another study showed that the presence of a phenylalanine at position 47 of the β-chain of HLA-DR was independently associated with Be sensitization and CBD in those subjects without a βGlu69-containing HLA-DP allele (36).

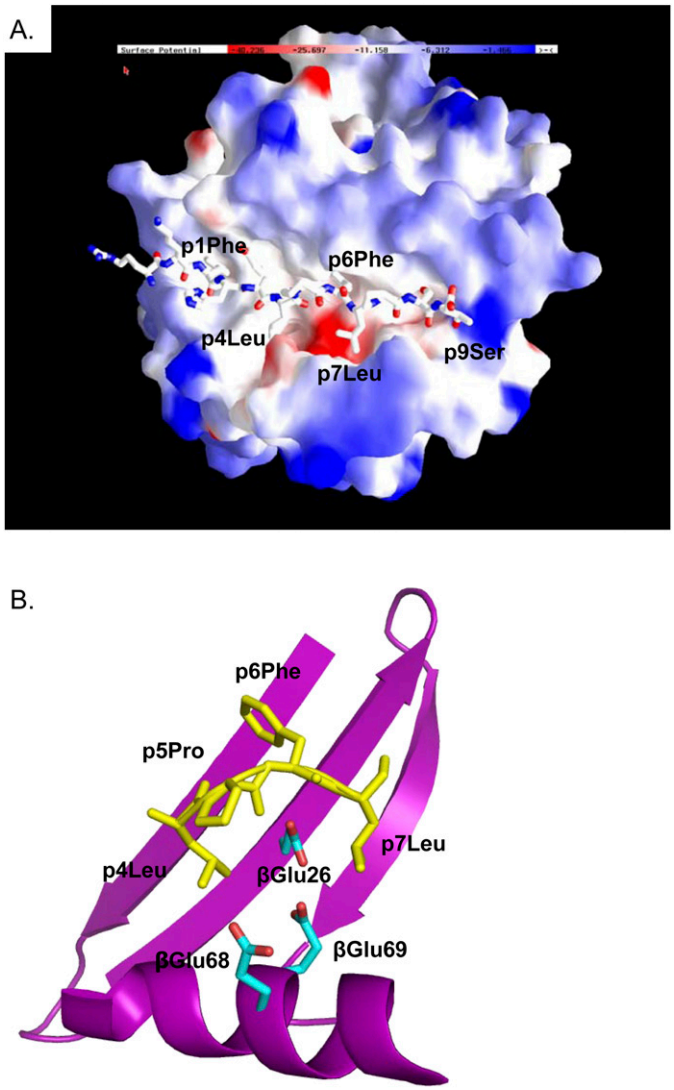

Figure 2. HLA-DP2 possesses a solvent-exposed acidic pocket which includes βGlu69. (*A*) The electrostatic surface charge of the HLA-DP2 molecule (with bound pDRA) is shown colored by the relative charge of the surface atoms (*red*, negative; *blue*, positive) (program GRASP). A wireframe representation of pDRA is shown with CPK coloring. (*B*) DP2 molecule is shown in the area between p4Leu and the DP2 β-chain α-helix with ribbon and wireframe representations of the DP2 β-chain (*magenta*) and pDRA (*yellow coloring*), respectively. Wireframe representations of the side chains of βGlu26, βGlu68, and βGlu69 are also shown.

BERYLLIUM PRESENTATION TO $CD4^+$ T CELLS

Human MHCII molecules have been implicated in susceptibility to various immune-mediated diseases. Due to the unknown initiating autoantigen(s) in the majority of these disorders, the mechanism for the association is poorly defined. Conversely, CBD allowed a determination of whether the association of HLA-DP molecules and disease susceptibility occurs at the level of antigen presentation. Using Be-specific $CD4^+$ T cells and either lymphoblastoid cells or human DNA-engineered mouse fibroblasts as antigen-presenting cells, investigators have

TABLE 1. AMINO ACID RESIDUES OF HLA-DPB1 ALLELES INVOLVED IN CHRONIC BERYLLIUM DISEASE SUSCEPTIBILITY

HLA-DPB1 Allele	Ability to present Be	Amino Acid Position*																	
		8	9	11	26	35	36	55	56	57	67	68	69	76	84	85	86	87
*DPB1*0101*	—	V	Y	G	**E**	Y	A	A	A	E	E	**E**	K	V	D	E	A	V
*DPB1*0401*	—	L	F	G	**E**	F	A	A	A	E	E	**E**	K	M	G	G	P	M
*DPB1*0402*	—	L	F	G	**E**	F	V	D	E	E	E	**E**	K	M	G	G	P	M
*DPB1*0501*	—	L	F	G	**E**	F	V	E	A	E	E	**E**	K	V	D	E	A	V
*DPB1*0201*	+	L	F	G	**E**	F	V	D	E	E	E	**E**	**E**	M	G	G	P	M
*DPB1*0601*	+	V	Y	L	**E**	F	V	D	E	D	E	**E**	**E**	M	D	E	A	V
*DPB1*1001*	+	V	H	L	**E**	F	V	D	E	E	E	**E**	**E**	V	D	E	A	V
*DPB1*1301*	+	V	Y	L	**E**	Y	A	A	A	E	E	**E**	**E**	M	D	E	A	V
*DPB1*1701*	+	V	H	L	**E**	F	V	D	E	D	E	**E**	**E**	M	D	E	A	V

* Sequences for the different HLA-DP alleles are presented based on homology to *DPB1*0101*. The bolded amino acid residues at positions 26, 68, and 69 indicate the three glutamic acid residues that comprise the solvent-exposed acidic cluster of HLA-DP2 and are shared between the presenting HLA-DP molecules.

shown that most Be presentation occurs through HLA-DP (37, 38). However, only certain HLA-DP molecules are capable of presenting Be to pathogenic $CD4^+$ T cells (37). The *DPB1* alleles that mediate Be presentation match those implicated in disease susceptibility, providing a mechanism for the association of these HLA-DP alleles and disease susceptibility (37, 38). In addition, Be recognition required the presence of βGlu69, raising the possibility that a single polymorphic amino acid might dictate Be presentation and, more importantly, disease susceptibility. Studies using soluble HLA-DP molecules expressing βGlu69, but not HLA-DP molecules with a lysine at that position, corroborate these findings (39, 40).

In subjects without a βGlu69-containing HLA-DP allele, recent studies have shown that HLA-DR assumes the predominant role in Be presentation (36, 41). For example, HLA-DR13 molecules are capable of presenting Be to Be-responsive T cells (41), and mutational analysis revealed that the critical polymorphic amino acid in the DR13 molecules is βGlu71 (41). Thus, a hierarchy of Be presentation to $CD4^+$ T cells has been established: HLA-DP assuming the predominant role, with a minor role for HLA-DR and no known role for HLA-DQ.

STRUCTURAL BASIS FOR THE ASSOCIATION OF βGlu69-CONTAINING HLA-DP ALLELES AND DISEASE SUSCEPTIBILITY

The structures of at least 10 disease-associated HLA-DR and -DQ molecules have been reported. Conversely, the HLA-DP molecule has been recalcitrant to attempts at crystallization. As a result, the unique structural features of βGlu69-containing HLA-DP molecules that explain the disease association have remained unknown. Recently, we crystallized HLA-DP2 (*DPA1*0103*, *DPB1*0201*) in complex with a self-peptide derived from the HLA-DR α-chain (pDRA) (42) and solved its structure to a resolution of 3.25 Å (43). Although the overall structure of the DP2-pDRA complex was similar to that of other MHCII/peptide complexes, two unique structural features were identified that may explain the ability of HLA-DP2 to present Be to T cells. The first was a widening of the gap between the peptide and the β-chain α-helix, with DP2 having the largest gap of any of the 29 published MHCII structures (43). This region of the β-chain α-helix may be quite flexible (44), explaining the large variation in the width of this part of the binding groove. The second distinct feature of the DP2 peptide binding groove was an upward shift in the DR α-chain peptide such that the leucine at the p4 position of the peptide was solvent-exposed. As a result of these structural changes, an acidic pocket was solvent-exposed (Figure 2A) (43). This pocket was flanked by leucine residues at the p4 and p7 positions of the pDRA and the β-chain α-helix and was composed of three DP2 β-chain amino acids that contribute to the net negative surface charge of this pocket: βGlu68 and βGlu69 from the β-chain α-helix and βGlu26 from the floor of the peptide binding groove (Figure 2B) (43).

In addition to βGlu69, these findings raised the possibility that βGlu26 and βGlu68 of HLA-DP2 may be involved in Be coordination and presentation. Since these two amino acids are invariant among HLA-DP alleles (Table 1), their presence is not sufficient for Be presentation in the absence of βGlu69. Site-directed mutagenesis of βGlu26 and βGlu68 and expression of the mutated HLA-DP2 molecules on the surface of fibroblasts did not activate beryllium-specific T cells, confirming the importance of these additional glutamic acid residues in Be coordination and presentation (43). Of note, the HLA-DR13 alleles, which are linked to disease susceptibility and can present Be to Be-responsive T cells, also possess an acidic cluster composed of βAsp28, βAsp70, and βGlu71. Together, these findings add further support for the critical nature of this acidic pocket in Be coordination and subsequent T cell activation.

SUMMARY

The combination of genetic susceptibility studies, along with functional and structural research, strongly suggest that the acidic pocket of the HLA-DP2 binding groove is the Be binding site and provides an explanation for the genetic linkage of HLA-DP2 to the development of granulomatous inflammation in the Be-exposed worker. However, despite these recent advances in our understanding of the immunopathogenesis of Be-induced disease, important unanswered questions remain. For example, which MHCII-bound peptides are capable of completing the Be-responsive αβTCR ligand? Are Be-responsive T cells recognizing Be alone or conformational changes in the self-peptide induced by Be? Hopefully, answers to these and other questions will allow disease prevention in susceptible individuals as well as the development of new therapeutic strategies to prevent the development of fibrotic lung disease in patients with CBD.

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