Immunologic Effects of Beryllium Exposure

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Abstract

Metal-induced hypersensitivity is driven by T-cell sensitization to metal ions. Although numerous metals are associated with the development of diffuse parenchymal lung disease, beryllium-induced hypersensitivity is the best-studied to date. This review focuses on the interaction between innate and adaptive immunity that leads to the development of chronic beryllium disease. After beryllium exposure, activation of the innate immune system occurs through the engagement of pattern-recognition receptors. This activation leads to cell death, release of alarmins, and activation and migration of dendritic cells to lung-draining lymph nodes. These events culminate in the development of an adaptive immune response that is

characterized by beryllium-specific, T-helper type 1-polarized, CD4⁺ T-cells and granuloma formation in the lung. The unique ability of beryllium to bind to human leukocyte antigen-DP molecules that express a glutamic acid at position 69 of the β -chain alters the charge and conformation of the human leukocyte antigen-DP-peptide complex. These changes induce post-translational modifications that are recognized as non-self. In essence, the ability of beryllium to create neoantigens underlies the genesis of chronic beryllium disease, and demonstrates the similarity between beryllium-induced hypersensitivity and autoimmunity.

Keywords: granuloma; lung; T cell; major histocompatibility complex; inflammation

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Although certain metals, such as iron, magnesium, and zinc, are required for biological processes (e.g., functioning as enzymatic cofactors, impacting nucleic acid structure, and facilitating oxygen transport) (1, 2), exposure to other metals can result in the development of an injurious immune response, characterized by activation of both innate and adaptive immunity. Metal-induced hypersensitivity is defined as a type IV delayed-type hypersensitivity response that is mediated by CD4⁺ T-cells specific for major histocompatibility complex (MHC) class II (MHCII)/peptide-metal complexes. Clonal expansion of metal-reactive CD4⁺ T-cells requires activation of pattern-recognition receptors and presentation of these metal-containing

complexes by activated dendritic cells (DCs) to naive CD4⁺ T-cells. The resultant T-cell activation and interleukin-2 secretion culminates in the expansion of metal-reactive memory CD4⁺ T-cells. Upon re-exposure to the metal, these memory T-cells are reactivated, resulting in further expansion, cytokine and chemokine release, cellular damage, and inflammation. In the case of chronic beryllium (Be) disease (CBD), sensitization promotes alveolitis that can progress to granuloma formation in the lung and fibrosis, leading to cor pulmonale and, ultimately, death.

Certain metals can bind to the MHCII-peptide complex generating neoantigens that are recognized by CD4⁺ T-cells as foreign or nonself (3, 4). These

post-translational modifications indicate similarities between hypersensitivity reactions and autoimmunity (2, 4). Genetic susceptibility to some metal-induced hypersensitivities is associated with the expression of particular MHCII alleles. For example, Be-induced hypersensitivity is linked to human leukocyte antigen (*HLA*)-*DPB1* alleles expressing a glutamic acid at the 69th position of the β -chain (β Glu69) (5–10). Metals may also serve as adjuvants (e.g., aluminum hydroxide) by engaging innate pattern-recognition receptors and promoting DC activation and maturation, thus enhancing the magnitude of the adaptive immune response. In this review, we highlight recent advances in our

understanding of the mechanisms of Be-induced activation of innate and adaptive immunity and the generation of an inappropriate immune response in genetically susceptible individuals.

Beryllium-induced Activation of Innate Immunity

Be can function as an antigen as well as an adjuvant in immune responses (11), thus potentially enhancing its own pathologic response. Lee and colleagues (12) showed that the adjuvant properties of Be stem from its ability to induce interferon- γ secretion. After vaccination, adjuvants typically operate via engagement of pattern-recognition receptors that induce activation and maturation of antigen-presenting cells. Be induces the release of inflammatory chemokines, cytokines, and reactive oxidative species from macrophages and DCs (13–17). In addition, Be exposure induced phosphorylation of mitogen-activated protein kinase p38 and nuclear factor κ B activation in monocyte-derived DCs, enhancing Be-specific CD4⁺ T-cell secretion of interferon- γ and tumor necrosis factor- α (18). McKee and colleagues (19) showed that intratracheal Be exposure in mice resulted in necrotic/necroptotic cell death and rapid release of alarmins, such as DNA and interleukin-1 α , into the alveolar space (Figure 1A). Interleukin- α release was independent of the inflammasome, and was required for neutrophil accumulation in the lungs after Be exposure (19). *In vitro* Be exposure has been shown to induce apoptosis (20, 21); however, apoptosis is a noninflammatory event, and is not associated with the release of damage-associated molecular patterns.

Whether these innate pathways drive acute berylliosis in exposed individuals or contribute to Be sensitization in genetically susceptible individuals remain important unanswered questions. Under steady-state conditions, DCs maintain tolerance to innocuous substances (22). Conversely, Be exposure increased migration of classical DCs to lung-draining lymph nodes (2, 19). These activated DCs in lung-draining lymph nodes have upregulated costimulatory molecules and function to enhance primary and memory CD4⁺ T-cell responses via myeloid differentiation primary response 88 (known as MyD88)-dependent signaling pathways (Figure 1B).

Figure 1. Exposure of the lung to beryllium (Be) ions or particles results in association of these metal ions with glutamic acid at the 69th position of the β -chain of human leukocyte antigen (HLA)-DP molecules on the surface of antigen-presenting cells. Beryllium-induced cell death leads to the release of alarmins that engage pattern-recognition receptors. Activation of pattern-recognition receptors on dendritic cells promotes activation, maturation, and migration of dendritic cells to the lung-draining lymph nodes. In the lymph nodes, activated dendritic cells promote the expansion of beryllium-specific CD4⁺ T-cells that circulate back to the lung and mediate granuloma formation. IL = interleukin.

These data suggest that Be exposure releases damage-associated molecular patterns from dying and necrotic cells, resulting in the engagement of myeloid differentiation primary response 88–dependent receptors to enhance DC function. However, the upstream pattern-recognition receptor(s) responsible for activation of myeloid differentiation primary response 88 remains unknown.

CD4⁺ T-Cell Recognition of Human Leukocyte Antigen–DP–Peptide–Beryllium Complexes

Metal-induced hypersensitivity requires the generation and maintenance of a memory population of metal-specific CD4⁺ T-cells. In this regard, naive T-cell activation occurs upon engagement of T-cell receptors (TCRs) expressed on CD4⁺ T-cells with their cognate MHCII–peptide complex expressed on DCs (Figure 1B). Metals and other small-molecule sensitizers interact with MHCII–peptide complexes in multiple ways to activate T cells. Some metal cations (e.g., nickel) and small molecules (e.g., fluorescein)

act as haptens, interacting with amino acid residues on MHCII-bound peptides (23–25). Alternatively, metal–MHC interactions may alter the repertoire of peptides that are capable of binding to the MHCII molecule in the presence of the metal (26).

In the case of Be-induced hypersensitivity, genetic susceptibility was strongly associated with β Glu69-containing *HLA-DPB1* alleles (5–10). The most prevalent β Glu69-containing allele is *HLA-DPB1*02:01*, being expressed in 15–25% of white individuals (5, 8). Several groups have shown that Be presentation occurs primarily through β Glu69-expressing HLA-DP molecules (27, 28). Importantly, site-directed mutagenesis of this glutamic acid to either an alanine or lysine abolished Be-induced T-cell proliferation and interferon- γ secretion (27, 28). Thus, the molecular mechanism for the genetic linkage of β Glu69-expressing *HLA-DPB1* genes with Be-induced hypersensitivity is related to the ability of those proteins to bind and present Be to antigen-specific CD4⁺ T-cells (27, 28).

Although Be is a key component of the MHCII–peptide–Be complex,

specific peptides are required to activate Be-specific $\alpha\beta$ TCRs (29, 30). We identified Be-dependent mimotopes and self-peptides that bind to HLA-DP2 and activate Be-specific CD4⁺ T-cells derived from the lungs of patients with CBD (30) and the lungs of HLA-DP2-transgenic mice (31). These Be-dependent peptides expressed negatively charged aspartic and glutamic acid residues at p4 (pD4) and p7 (pE7) of the peptide (30). Importantly, the location of these peptide-derived amino acids, along with three glutamic acid residues derived from the HLA-DP2 β -chain, including β Glu69, created a cluster of negative charges within the footprint of the TCR, suggesting a role for these amino acids in the coordination of the Be cation. Recently, a quad-molecular complex of a Be-specific TCR bound to an HLA-DP2 mimotope–Be complex was crystallized to a resolution of 2.8 Å (Protein Data Bank ID code 4P4R) (3). In this structure, Be and an accompanying cation were bound to the acidic pocket of HLA-DP2-mimotope, with a characteristic coordination of Be in a tetrahedral manner (3). The addition of Be to the HLA-DP2–peptide complex induced changes in both charge and conformation through interactions of Be with carboxylate residues of β Glu69 and other HLA-DP2 oxygen molecules, stabilizing the HLA-DP2–peptide complex (3). However, Be and an accompanying cation were not solvent accessible, meaning that the Be-specific TCR bound the altered HLA-DP2–peptide complex with no direct interaction with the Be cation (3). Thus, Be is not functioning as a hapten, but rather indirectly generates neoantigens through changes in surface charge and topology of the HLA-DP2–peptide complex. This novel post-translational modification bypasses negative selection in the thymus. Importantly, other small molecules or drugs, such as abacavir, drive T-cell activation via similar mechanisms (32, 33).

Beryllium-Specific CD4⁺ T-Cells in Chronic Beryllium Disease

Although normal subjects have few lymphocytes in the bronchoalveolar lavage, the lungs of patients with CBD are characterized by a CD4⁺ T-cell

alveolitis (34–38). Importantly, this T-cell alveolitis in patients with CBD is composed of Be-responsive, T-helper cell type 1–polarized CD4⁺ T-cells, with up to 30% of bronchoalveolar lavage CD4⁺ T-cells being responsive to Be salts in culture (39, 40). These Be-specific CD4⁺ T-cells in bronchoalveolar lavage are effector memory T-cells (37, 40, 41) and recognize Be in a CD28 costimulation-independent manner (42). In addition, these cells upregulate markers of senescence (e.g., CD57) (43), as well as upregulating protein death receptor 1 (44) and cytotoxic T-lymphocyte protein 4 (45), coinhibitory receptors that negatively regulate T-cell function (46). Upregulation of protein death receptor 1 on Be-specific CD4⁺ T-cells suppresses Be-induced T-cell proliferation, thus potentially preserving lung function in the presence of persistent Be exposure. Be-specific CD4⁺ T-cells are also present in blood, and form the basis of the Be lymphocyte transformation test (47). In addition, the number of circulating Be-specific CD4⁺ T-cells positively correlated with the percentage of Be-specific CD4⁺ T-cells in the bronchoalveolar lavage, suggesting that the number of Be-responsive T cells in blood may serve as a biomarker for severity of T-cell alveolitis in CBD (48, 49).

The TCR repertoire of bronchoalveolar lavage CD4⁺ T-cells is skewed in CBD, with the presence of oligoclonal expansions that are consistent with conventional antigen recognition (34, 35). Importantly, the TCRs are specific for CBD, and are not seen in other granulomatous lung diseases, such as sarcoidosis (34, 35). Using Be-pulsed HLA-DP2–peptide tetramers, public V β 5.1⁺ TCRs have been identified in the lung CD4⁺ T-cell pool of all HLA-DP2–expressing patients with CBD (50). The frequency of these public CD4⁺ T-cells varies inversely with lung function and exercise capacity, suggesting that these public CD4⁺ T-cells play a pathogenic role in CBD (50).

Development of a Human Leukocyte Antigen–DP2–Transgenic Murine Model of Chronic Beryllium Disease

All studies to date suggest the critical importance of the cluster of glutamic

acid residues contributed by the β -chain of β Glu69-containing HLA-DP molecules in the initiation of Be-induced hypersensitivity. However, no inbred strain of mice expresses this triad of glutamic acids, likely explaining the inability to generate an adaptive immune response in mice. Mack and colleagues (31) exposed HLA-DP2-transgenic mice to beryllium oxide and identified peribronchovascular mononuclear infiltrates and an HLA-DP2–restricted, Be-specific CD4⁺ T-cells only in FVB/N mice expressing HLA-DP2. These findings established a murine model of Be-induced hypersensitivity that replicated many of the features of the human disease. In light of the rarity of CBD, this murine model will hopefully enhance the ability of investigators to study the interplay of innate and adaptive immunity in the establishment of a Be-specific immune response.

Conclusions

Recent work has indicated that both the innate and adaptive arms of the immune system are required for the development of beryllium-induced hypersensitivity. Enhanced understanding of the adjuvant properties of beryllium, together with the unique structural features of human leukocyte antigen–DP2, and beryllium-induced structural changes to the human leukocyte antigen–DP2–peptide complex, provides an explanation for the generation of chronic beryllium disease in genetically susceptible workers exposed to beryllium. The development of the human leukocyte antigen–DP2-transgenic murine model of chronic beryllium disease is a vital step in addressing many of the unanswered questions that have been impossible to address given the rarity of the disease. Along with decreasing workplace exposures to beryllium, we are hopeful that these future studies will contribute to the eradication of beryllium-induced disease during our lifetime. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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