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Immunotoxicology of Beryllium Lung Disease

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Abstract

Beryllium induces non-caseating granulomatous inflammation in humans exposed to the metal dust or fumes in both occupational and non-occupational settings. The resulting condition, chronic beryllium disease (CBD), affects principally the lungs, lymphatics, and skin and continues to plague modern industry. Beryllium exerts several important immunotoxic effects, including induction of a beryllium-antigen specific adaptive immune response and the triggering of inflammatory and innate immune responses. Genetic susceptibility plays a role in CBD adaptive immune responses, mainly mediated through single nucleotide polymorphisms in HLA-DP and, to a lesser extent, HLA-DR. The adaptive response is characterized by influx and proliferation of CD4+ central and effector memory T cells expressing Th1 cytokines. Insights into the immunopathogenesis of CBD have implications for the understanding of other immune-mediated granulomatous disorders and for metal antigen behavior.

Key words: beryllium, berylliosis, Chronic Beryllium Disease, granuloma, metal immunotoxicity

Introduction

Beryllium, the second lightest metallic element on the period table, causes immune-mediated, non-caseating granulomatous inflammation in the lungs, skin, and other organs of people who have been exposed to beryllium dust or fume at work. The major effects include a T-cell mediated adaptive immune response seen in the blood, called 'beryllium sensitization' and the lung-predominant disease called Chronic Beryllium Disease (CBD). This paper addresses the mechanisms by which beryllium exerts its immunotoxicologic effects, resulting in human disease. CBD has provided a unique set of insights into the mechanisms underlying the granulomatous disorders, which include those caused by infectious agents and their antigens, organic antigens, autoantigens, other metal antigens, and idiopathic disorders such as sarcoidosis. The central hypothesis regarding not only CBD but many other granulomatous disorders is that granulomatous inflammation results from an immunologic 'over-reaction' to triggering antigens in genetically susceptible individuals, involving both adaptive and innate immunity, with pivotal roles for CD4+ T cells, the cascade of proinflammatory cytokines, and possibly a failure of normal down-regulatory mechanisms.

Exposure

CBD continues to occur in modern, beryllium-using industries in much of the world, including North and South America, Europe, the Middle East, Russia, and Asia (1). Industries that use beryllium metal, beryllium-containing alloys, and beryllium oxide include aerospace and aircraft, telecommunications, computers, defense, energy, electronics, dental, metal recycling, alloy machining, and alloy manufacturing, among others. In many of these industries in which efforts have been made to conduct medical surveillance, contemporary cases of CBD have been observed. The risk is wide-ranging, with beryllium sensitization and CBD occurring in certain high risk groups, such as beryllium alloy machinists, beryllium ceramics workers, construction workers and other dust disturbers, as well as in lower risk groups including security guards, front office workers, bystanders, and family members. The risk exists for individuals even at levels of exposure well below permissible exposure limits (1, 2).

Health Consequences

The majority of people exposed to beryllium develop no health problems. However there continues to be a high incidence of beryllium sensitization, chronic beryllium disease, and, more rarely, cases of acute berylliosis from higher level

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exposures. IARC recognizes beryllium as a human and animal carcinogen (2), based on sufficient epidemiologic evidence in humans and multiple animal studies using many forms and doses of beryllium as the inciting agent. Lung cancer is considered the main concern.

Immunopathogenesis

Investigators first hypothesized that CBD results from an immune response because of the clinical and epidemiologic evidence that conventional rules of dose/response did not predict who developed disease (3). In the 1940s, cases occurred in the neighborhood surrounding a beryllium manufacturing plant in the United States, at rates similar to the rate seen among plant workers, despite much lower community beryllium exposure levels. These observations were further supported in the 1950s by the demonstration of delayed hypersensitivity to beryllium skin patch testing (4). Since that time, we have learned that CBD results from an exuberant adaptive immune response to beryllium.

CBD offers an excellent model for the study of the mechanisms underlying granulomatous inflammation because we can use beryllium salts as antigen to stimulate the antigen-specific T-cell response. In a landmark 1970 paper, Hanifin and colleagues examined the *in vitro* effects of beryllium salts on monocytes and lymphocytes, first describing the concentration-dependent lymphocyte proliferation response (5). This initial observation has since been converted by our group and others into a reproducible assay known as the beryllium lymphocyte proliferation test (BeLPT) (6, 7). This test can be performed on mononuclear cells collected from either blood or lung washings, called bronchoalveolar lavage. Typically, beryllium sulfate is used in commercial assays, although beryllium oxide, beryllium chloride, beryllium fluoride and beryllium-ferritin adducts have all be shown to induce a similar T-cell response. Use of the BeLPT over the last 20 years has not only revolutionized the clinical approach to CBD but has enabled researchers to make progress in unraveling the immunopathogenesis and immunogenetics of the disease (8). Although the majority of research on beryllium immunology has centered on antigen presenting cells (APC) and antigen-specific T cells, it is important to take note of some of the early events that promote the adaptive immune response to beryllium by creating a microenvironment that encourages the inflammatory response. One of the earliest events following inhalational exposure to beryllium is disruption of the alveolar-capillary membrane, resulting in exudation and alveolar epithelial cell injury, which, presumably, create an opportunity for interaction between antigen and APC (9). Additional early events that may contribute to sensitization include the ability of lymphocytes to migrate toward beryllium, seeing it as a chemoattractant (10), and the local, beryllium-induced production of proinflammatory cytokines, especially Tumor Necrosis Factor-alpha (TNF) (11).

The major lymphocyte populations involved in the beryllium response are helper T cells (CD4+), as suggested by both *in vitro* studies and the high proportion of lymphocytes recovered from the lungs of patients with this disorder (12). Like the clinically-similar disease sarcoidosis, the lymphocytes from affected organs in CBD are functionally Th1 type CD4 cells that express markers of previous activation (CD45RO+) (13). Bronchoalveolar lavage cells from patients with CBD express elevated levels of mRNA for both TNF-α and IL-6 but not IL-1β. In addition, when lymphocytes from sensitized individuals are stimulated with beryllium salts, they produce IL-2 as an early event followed by sustained production of IFN-γ. The Th2 cytokine IL-4 is not produced and its addition to beryllium-stimulated cells from patients with CBD does little to modify the commitment of the T cells toward proliferation and Th1 cytokine production. The proliferation and cytokine production is partially blocked by antibody to IL-2 (14–16).

Researchers have further characterized the lymphocytes in CBD, recognizing them both as the key target for antigen presentation and as a major source of mediators that promote the ongoing granulomatous response. Multiple studies of T cell phenotype and function in CBD indicate that specific T cell clones emerge early in the disease course. Studies show there is an oligoclonal expansion and recruitment of T cells bearing certain T cell receptor variable regions specific for CBD into areas with beryllium leading to a granulomatous response (17, 18). The specific T cells found in target organs are primarily effector memory cells, as they express CD11a but not CD62L or CCR7 (16, 19). In addition, many of these cells do not require CD28 stimulation to produce and secrete cytokine (20).

In one interesting study, we performed serial skin biopsies following the application of beryllium sulfate skin patch tests in patients with CBD (21, 22). The early skin lesions contained diverse, non-clonal T cell populations, followed over the next several days to weeks by the appearance of specific T cell clones. Based on their T cell antigen receptors and on the homology of the complementarity determining regions (CRD3), we showed that the clones that emerged in the skin granulomas in response to beryllium were the same as those which had accumulated in the lungs of these patients. There is no single "pathologic T cell" in CBD, rather there appear to be a limited, small number of T cells that recognize the antigen (8, 18, 23). These clones persist in the lungs over time, based on our studies of T cell receptor oligoclonality and conservation of the complementarity determining regions of their T cell antigen receptors, even when analyzed on serial bronchoalveolar lavage cell specimens.

The importance of interaction of the T cell antigen receptor and specific HLA molecules in the so-called "trimolecular complex," has been well-demonstrated in CBD. Studies show that macrophages have the capacity to phagocytose beryllium (5, 24). These cells can then lead to lymphocyte proliferation, even in the absence of added beryllium salts. The immune response to beryllium is restricted to HLA class II molecules, first comfirmed by studies that showed that the blood and lung T cell proliferative response can be blocked by antibodies to class MHC II but not MHC class I molecules (25–27). In a landmark paper, Richeldi et al. showed that the majority of beryllium susceptibility comes from HLA-DPB1 isomers bearing a glutamic acid at position 69 in what is thought to be one of the hypervariable regions of the HLA molecule (25, 26). This observation has since been confirmed by other investigators studying many different beryllium-exposed populations (28–

37). Overall, greater than 80% of patients with beryllium sensitivity or with CBD carry a variant of the gene that has Glu69 on the HLA-DPB1. This single nucleotide polymorphism is present in approximately 45% of the general population, but is statistically much more common among those who develop beryllium sensitization or disease. Glu69 homozygosity has been associated with a higher relative risk of CBD versus sensitization without disease, suggesting indirectly that the density of HLA-DPB1 Glu69 molecules on the surface of an antigen presenting cell may influence disease pathogenesis (29, 37).

The HLA-DPB1 Glu 69 polymorphism is functional and directly involved in antigen presentation (27, 28). The actual antigen is still not known; however, beryllium does bind to the MHC molecule directly and its ability to bind may be related to the charge at the antigen binding cleft. Thus, it is possible that beryllium is binding to the HLA-DP molecule directly, displacing CLIP, or might still be processed intracellularly, and transferred to the cell surface with the HLA-DP/peptide complex. There are approximately 106 different HLA-DPB1 alleles of which 36 carry a glutamic acid at position 69. Modeling studies suggest that position 69 is integral to the actual antigen binding cleft (38). In addition, beryllium susceptibility seems to increase the greater the negative charge in the cleft. Future research may yet determine the precise nature of beryllium antigen. This determination may be aided by the recent discovery that HLA-DR variants, such as HLA-DRB1 Glu71, confer increased risk for beryllium sensitization in individuals who are HLA-DPB1 Glu69 neg (37–40).

Other important, but not completely understood, factors important in the immunopathogenesis of CBD include exposure factors, the role of innate immunity, and the mechanisms behind the progression from granulomatous inflammation to fibrosis. It is well known that exposures affect disease risk as certain job categories carry a much higher risk of disease (2). In addition, the genetic background of the individual may interact with exposure to help define any given individual's risk of sensitization and disease, although there remains little evidence in the published literature to support this assertion (41).

Innate immunity also appears to play an important role. Notably, beryllium's adjuvant properties have been known for more than 30 years, in hindsight, probably reflecting the high production of beryllium-induced TNF. While the epidemiologic evidence supporting a functional TNF gene promotor polymorphism is contradictory, the observation remains that TNF is produced at extraordinarily high levels in response to beryllium both in vitro and in vivo. In addition, beryllium induces a high rate of apoptosis in human alveolar macrophages as well as in macrophage cell lines (42–44) with concomitant generation of oxygen free radicals. These events may not only facilitate the accumulation of inflammatory cells in the lung and contribute to the inflammatory milieu that promotes T cell activation and proliferation, driving the granulomatous inflammation but could potentially contribute to the ability of beryllium to persist in the lung. For example, if macrophages undergo apoptosis when they ingest beryllium, they are likely to release their contents, including beryllium, making it available for reuptake by other inflammatory cells and possibly by dendritic cells involved reputed to be involved in antigen presentation. This activation of innate immunity may prove to be critical for the subsequent development and maintenance of beryllium-specific immunotoxicity. Future directions in beryllium research will elucidate the structure of beryllium antigen, determine how the immune response to beryllium is regulated, including further research on beryllium gene regulation (45), examine the impact of beryllium-induced oxidative stress on both adaptive and innate immune responses (44), and lead to more sensitive immunoassays for beryllium sensitization and disease and to better treatment options, beyond the use of glucocorticoids.

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