Immune mechanisms in beryllium lung disease

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■ The role of the immune system in the pathogenesis of beryllium lung disease has been suspected for years. The observation of cutaneous hypersensitivity to beryllium led to the development of the lymphocyte blast transformation test; the test clearly distinguishes between healthy subjects, who show little or no blast transformation response, and patients with beryllium lung disease, who demonstrate significant responses. The degree of blast transformation also correlates with the severity of the clinical disease. Animal studies have demonstrated the importance of histocompatibility antigens in development of the disease, and support the participation of cellular immune mechanisms.

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OUR UNDERSTANDING of the clinical and pathologic aspects of beryllium lung disease can be credited largely to Dr. H.S. VanOrdstrand's insight and dedication during a 40-year period that began in the early 1940s. VanOrdstrand and his associates were the first to describe a "chemical pneumonia" in workers at a beryllium plant involved in extraction of beryllium oxide.[1]

The first three cases studied by VanOrdstrand appeared to represent the acute form of beryllium-induced lung disease, in which the irritating or toxic effects of inhaled beryllium compounds were thought to be directly responsible for the chemical pneumonia. This initial report was followed by others in the United States and Europe.

Because of the widespread use of beryllium in various industries, its potential pathogenic effect on the lung became a serious occupational health concern. In the late 1940s and early 1950s, chronic forms of this disease were beginning to be recognized, but the pathogenic mechanisms involved in the chronic disease were not clearly understood until much later.

To date, more than 1,000 cases of beryllium-induced lung disease have been described in the literature. The epidemiologic aspects of this disease have been the subject of several recent reviews.[2–4]

Dr. VanOrdstrand suspected early on that host factors, such as immune sensitization, play a major role in the development of the chronic form of this disease. The insidious nature of the disease, the long latent period between exposure and onset, and the histopathology of the lung lesion (a beryllium granuloma with mononuclear and lymphocytic infiltrate) were all strong, although indirect, evidence of participation of the immune system.

LYMPHOCYTE TRANSFORMATION TEST

In 1951, Curtis[5] described cutaneous hypersensitivity to beryllium and, based on that observation, developed the beryllium patch test for the diagnosis of beryllium disease.[6] A positive patch test was thought to

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reflect delayed hypersensitivity to beryllium compounds in patients with beryllium lung disease. Lack of sensitivity and quantitation prevented widespread use of these skin tests in studying patients with beryllium lung disease or workers in beryllium industries.

By the late 1960s, in vitro techniques were available to study delayed hypersensitivity or cellular immunity to a given agent. These techniques, such as measurement of migration inhibition factor (MIF) and T lymphocyte blast transformation response, had the advantages of high sensitivity, specificity, and reproducibility, as well as quantitation. With persistent urging by Dr. VanOrdstrand, cellular immunologic studies were begun that involved patients with beryllium lung disease and beryllium industry workers, as well as animal models.

Hanifin and associates[7] had observed the release of MIF during the interaction of a patient's lymphocytes and beryllium sulfate in vitro. The MIF studies had some technical disadvantages that made it difficult to apply them on a large scale.

In our laboratory, we explored the lymphocyte blast transformation (proliferation) technique as a means of studying cellular immunity to beryllium compounds. The procedure involved isolating the subject's lymphocytes and observing the blast transformation phenomenon in the presence of beryllium sulfate. The number of blast cells formed was determined either by morphologic counting or by quantitative study of the uptake of tritiated thymidine.

Our initial studies, published in 1973,[8] included 35 patients with documented beryllium lung disease; 30 beryllium industry workers without disease; 22 normal, healthy subjects; and 12 patients with other granulomatous lung diseases such as sarcoidosis. The degree of blast transformation was quantitated and the test results clearly distinguished between normal, healthy subjects who showed little or no blast transformation response and patients with beryllium lung disease who demonstrated significant responses. Moreover, there was good correlation between the severity of the clinical disease and the degree of blast transformation.

We subsequently expanded the study population to include large numbers of beryllium workers from around the country, as well as patients with other lung diseases, particularly sarcoidosis.[9] Our laboratory has applied the lymphocyte blast transformation test to a wide variety of drug and other hypersensitivities.[10] The studies carried out over the past two decades in patients with chronic beryllium lung disease, beryllium industry workers, and patients with other chronic lung diseases have confirmed the value of this test in studying patients with beryllium lung disease.

CLINICAL APPLICATIONS

Williams and Williams have extensively studied the applications of the lymphocyte transformation test. They introduced significant modifications in the test to improve sensitivity and specificity.[11] They compared the lymphocyte blast transformation test with the migration inhibition test in the detection of beryllium hypersensitivity[12] and concluded that the beryllium lymphocyte transformation test was the most sensitive and reproducible. They therefore recommended its use in the diagnosis of disease and in monitoring exposed workers.

Williams and Williams applied the modified beryllium lymphocyte transformation test to three groups: patients diagnosed with chronic beryllium disease, subjects suspected of having the disease, and healthy beryllium workers.[13] They found the test to be positive in all patients with definite disease and negative in those suspected of having the disease. In 2 of 117 healthy workers, the test was slightly positive, indicating both exposure and sensitization. Low-grade positivity was noted in our studies also.[9] Whether this low-grade positivity among healthy workers indicates high risk for development of the disease has not been answered definitively.

Cullen and co-workers at the Yale-New Haven Occupational Medicine Program have also demonstrated the importance of specific immunologic testing in patients suspected of beryllium exposure and lung disease.[14] They found that granulomatous lung disease had developed in five metal refinery workers originally diagnosed with sarcoidosis; immunologic testing subsequently proved these patients to have beryllium hypersensitivity.

Kreiss and colleagues evaluated the beryllium-specific lymphocyte transformation test as a workplace screening tool in Colorado and concluded that the test can identify minimally symptomatic cases of beryllium disease;[15] however, not all individuals with a positive test had beryllium disease at the time of their initial evaluation.

BRONCHOALVEOLAR LYMPHOCYTE STUDIES

Another approach to understanding immune mechanisms in chronic beryllium lung disease has involved study of the immunologic properties of

bronchoalveolar lymphocytes from patients with this disease. Epstein and associates[16] compared bronchoalveolar lymphocytes of patients who had beryllium lung disease with those of normal controls and observed a significant increase in the number and proportion of T cells in patients with the disease. The percentage of activated bronchoalveolar T cells was nearly five times that in the control group. Both peripheral blood and bronchoalveolar lymphocytes demonstrated blast transformation response to beryllium sulfate, but the response of bronchoalveolar lymphocytes was greater than that of peripheral blood lymphocytes. Based on these findings, they suggested that bronchoalveolar lavage cells may be more helpful in establishing the diagnosis of this disease.

Studies by Rossman and colleagues showed that lymphocyte transformation tests with beryllium salts, as performed on bronchoalveolar lavage cells, achieved a sensitivity of 100% in patients with chronic beryllium lung disease.[17] The specificity of the test was also 100% among normal volunteers and patients with sarcoidosis.

Saltini and co-workers[18] further explored immunologic properties of bronchoalveolar lymphocytes in patients with chronic beryllium disease and reported that the proliferation of T cells in response to beryllium in vitro was confined to the CD4 (helper) cells. The proliferation depended on the presence of both major histocompatibility complex class II antigens and functional interleukin-2 receptors. T cells from the patients' lungs had a significantly greater response to beryllium than did T cells from peripheral blood. Their studies clearly demonstrated that in patients with chronic beryllium disease, beryllium acts as a class II restricted antigen, causing proliferation of beryllium-specific helper T cells. A recent case report[19] of beryllium lung disease in identical twins further supports the role of histocompatibility antigens in initiating hypersensitivity to beryllium.

STUDIES IN ANIMAL MODELS

In 1981, we described[20] an experimental model for chronic granulomatous beryllium lung disease using inbred strain 2 guinea pigs that had received endotracheal injection of beryllium oxide. These animals had positive skin tests to intradermal injections of beryllium sulfate and their peripheral blood lymphocytes showed significant blast transformation to beryllium salts in vitro. Treatment with prednisone decreased severity of the lung disease as graded by histologic evaluation. Guinea pigs made immunologically tolerant to beryllium by intravenous or oral administration of beryllium sulfate developed minimal disease when challenged with endotracheal beryllium oxide.

In subsequent studies[21,22] we observed that strain 13 guinea pigs, another inbred strain, demonstrated striking resistance to development of granulomatous lung disease when administered the same amount of endotracheal beryllium oxide. These differences between strain 2 and strain 13 guinea pigs demonstrated the importance of histocompatibility antigens in development of the disease and the role of the immune system. In strain 2 guinea pigs, the disease as well as the delayed skin reactivity could be transferred by sensitized spleen cells. Furthermore, the disease could be produced in F1 hybrids of strain 2 and 13 animals, although it was considerably milder in strain 13 than in strain 2 guinea pigs. In general, these studies emphasized the involvement of genetically determined cellular immune mechanisms in beryllium lung disease.

The clinical and experimental animal data to date strongly support the participation of immune mechanisms in chronic beryllium lung disease. However, with precautionary measures introduced in various beryllium industries, the number of new cases described has decreased dramatically in the past few years.

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