

## Clinical Application of Beryllium Lymphocyte Proliferation Testing

Barbara P. Barna,<sup>1</sup> Daniel A. Culver,<sup>1</sup> Belinda Yen-Lieberman,<sup>2</sup> Raed A. Dweik,<sup>1</sup> and  
Mary Jane Thomassen<sup>1,3\*</sup>

*Departments of Pulmonary and Critical Care Medicine,<sup>1</sup> Clinical Pathology,<sup>2</sup> and Cell Biology,<sup>3</sup> The Cleveland Clinic  
Foundation, Cleveland, Ohio 44195-5038*

Because of its unique properties, beryllium has become widely used in a variety of industrial applications, including inertial guidance systems, turbine rotor blades, laser tubes, rocket engine liners, springs, aircraft brakes and landing gear, ball bearings, injection and blow mold tooling, electrical contacts, automotive electronics, X-ray tube windows, spark plugs, electrical components, ceramic applications, gears, aircraft engines, oil and gas industries, welding electrodes, computer electronics, and golf clubs. It is extremely light, with a high modulus of elasticity (stiffness), a low coefficient of thermal expansion, high thermal and electrical conductivities, and a high melting point. Pure beryllium metal is useful in the nuclear industry as a moderator to slow neutrons, increasing the effectiveness of fission. More frequently, it is formulated as an alloy or an oxide.

### BERYLLIUM LUNG DISEASE

It has been estimated that up to 800,000 U.S. workers have been exposed to beryllium, with the potential to develop beryllium sensitization or chronic beryllium disease (CBD) (32). Occupations with the highest risk involve processes that generate particulates, especially metal production and machining. In general, the risk of disease is proportional to the intensity and duration of exposure to beryllium (24–26). However, well-documented cases have occurred in susceptible individuals with seemingly brief and trivial exposures, such as residents living near beryllium processing plants or secretaries working in offices attached to factories (13, 25, 34). Beryllium particulates may also be liberated into the air in unexpected settings, such as after the collapse of the World Trade Center (29). However, disease has not been noted in users of beryllium-containing products.

A further concern is the adequacy of current beryllium exposure limits, which were developed in 1948 (12). A number of studies have demonstrated that sensitization and disease can occur even in work environments with levels substantially lower than the current 8-h daily time-weighted-average exposure limit of 2  $\mu\text{g}/\text{m}^3$  (6, 24, 26). Most likely, factors other than the mean air beryllium concentration correspond more closely with risk. Such factors might include the concentration of alveolar-deposited beryllium particles (0.01 to 5  $\mu\text{m}$  in diameter), total number of particles, particle morphology, chemical form of beryllium, duration of exposure, dermal exposures, or amplitude of peak exposure values (21, 22, 24, 37).

As a result of industrial control measures, acute beryllium disease is rare today, occurring mainly in the context of industrial accidents (12). In contrast, CBD has increasingly been recognized as a result of the increased utilization of the beryllium lymphocyte proliferation test (BE-LPT). The following laboratories currently provide the BE-LPT in the United States: (i) Center for Epidemiologic Research (DOE patients only), Oak Ridge Institute for Science and Education, Former Beryllium Worker Medical Surveillance Program, ORISE/CER, P.O. Box 117, Mail Stop 45, Oak Ridge, TN 27831-0117 [phone, (865) 241-6152; fax, (865) 576-3194]; (ii) Cleveland Clinic Foundation, Department of Clinical Pathology, L40, 9500 Euclid Ave., Cleveland, OH 44195-0001 [phone, (216) 444-2200 or (800) 223-2273, ext. 48844 or 55763; fax, (216) 445-8160]; (iii) Hospital of the University of Pennsylvania, Pulmonary Immunology Laboratory, 833 BRB II/III, 421 Curie Blvd., Philadelphia, PA 19104-4283 [phone, (215) 573-9905; fax, (215) 573-4469]; (iv) National Jewish Center for Immunology and Respiratory Medicine, Cellular Immunology Tests, Pulmonary Division and Occupational/Environmental Division, 1400 Jackson St., Denver, CO 80206 [phone, (303) 388-4461]; and (v) Specialty Laboratories, Inc., OncQuest, 2211 Michigan Ave., Santa Monica, CA 90404-3900 [phone, (310) 828-6543 or (800) 421-4449; fax, (310) 586-7275].

Since the onset of disease is insidious and prolongation of exposure may hasten progression, there has been widespread recognition of the utility of beryllium screening tests for at-risk populations. Furthermore, the first symptom may occur many years after cessation of beryllium exposure. Prolonged latency (up to 40 years) from first exposure to disease is not uncommon (13, 35). The incidence and pace of progression from subclinical to symptomatic CBD are poorly characterized (7).

Overt CBD generally indicates advanced disease, which may not be reversible. Common manifestations include the insidious onset of exertional dyspnea, nonproductive cough, fatigue, arthralgias, and chest pain (45, 46). Nonpulmonary organs, including the skin, liver, spleen, myocardium, kidneys, salivary glands, and bone, may also be affected (20, 46). Lung function tests may demonstrate restrictive, obstructive, or mixed physiology, usually with a decreased pulmonary diffusing capacity. Chest radiographs are often normal in early disease; with progression, diffuse infiltrates culminating in end-stage fibrosis typically occur. Clinical or radiologic features are often indistinguishable from those of sarcoidosis. Unless a detailed lifetime exposure history engenders clinical suspicion or a BE-LPT is positive, CBD may be misdiagnosed as sarcoidosis (34).

The diagnosis of CBD is usually made by bronchoscopy with bronchoalveolar lavage (BAL) and transbronchial (forceps) biopsy. Lavage fluid typically reveals a lymphocytosis in indi-

\* Corresponding author. Mailing address: Department of Pulmonary and Critical Care Medicine, The Cleveland Clinic Foundation, Desk A90, 9500 Euclid Ave., Cleveland, OH 44195. Phone: (216) 444-4429. Fax: (216) 444-5172. E-mail: thomasm@ccf.org.

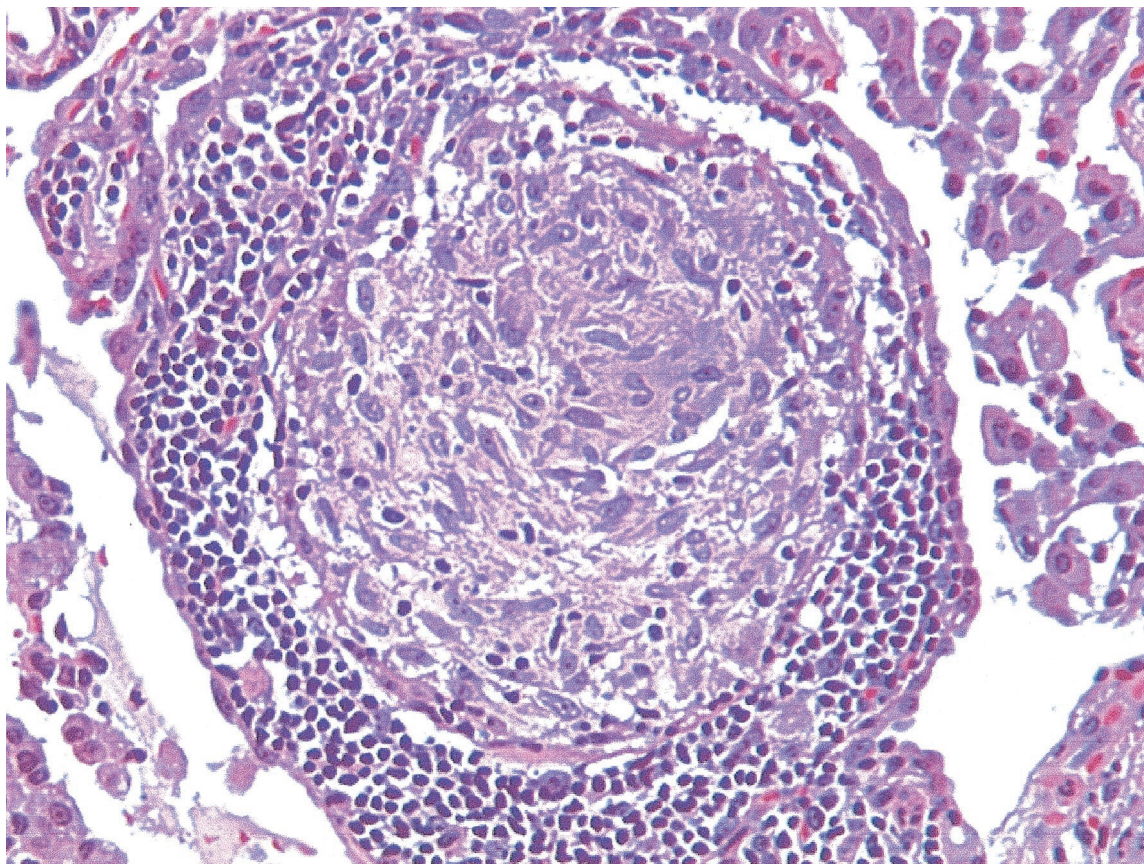


FIG. 1. Nonnecrotizing granuloma typical of CBD. A rim of lymphocytes surrounds a core of epithelioid cells. This example also shows a prominent central multinucleated giant cell. Hematoxylin and eosin staining was used. Magnification,  $\times 125$ .

viduals with both beryllium sensitization and CBD, with mean values of 12% and 41 to 53%, respectively (35, 36). The percentage of BAL lymphocytes may correlate with the severity of physiologic and radiographic disease (33). In CBD, biopsy reveals evidence of nonnecrotizing granulomas and lymphocytic interstitial infiltration (Fig. 1). The granulomas are histologically indistinguishable from those due to other granulomatous disorders, such as sarcoidosis (16). Prolonged treatment with corticosteroids is usually necessary for patients with significant symptoms or physiologic derangements. The response to therapy is usually excellent, but occasionally oxygen therapy is required, and a few patients progress to end-stage lung disease.

#### BERYLLIUM SENSITIVITY TESTING

**Development of laboratory methods.** Hypersensitivity to beryllium compounds in beryllium-exposed individuals was first described in 1951 by Curtis, who used cutaneous beryllium patch testing (8). In the late 1960s, the advent of *in vitro* techniques for studying cellular immune responses led to development of laboratory methods for detecting beryllium sensitivity (9). In 1970, Hanifin and associates demonstrated beryllium-induced release of migration inhibition factor in peripheral blood cells of patients with CBD (19). Soon after, Deodhar and coworkers reported a correlation between the clinical status of CBD and *in vitro* responses to beryllium in an LPT (10). This test utilized peripheral blood mononuclear cells

and was initially called a lymphocyte transformation test because abnormal (elevated) responses to beryllium sulfate challenge *in vitro* were determined by morphological quantitation of lymphoblasts (10). The test was modified to utilize tritiated thymidine uptake as an indicator of response (50). In all of these early studies, highly elevated responses to beryllium were observed in patients with clinically verified CBD, but moderately elevated responses were also noted in a few healthy beryllium-exposed individuals, suggesting sensitization to the metal (6, 49).

In 1982 Epstein and colleagues described a positive BE-LPT using pulmonary immune cells obtained from a CBD patient by BAL (14). The patient had histologic evidence of granulomas and a positive BE-LPT in peripheral blood cells as well (14). A subsequent study comparing beryllium-exposed workers with healthy controls and sarcoidosis patients suggested that elevated BAL responses in the BE-LPT were higher than those of blood cells in biopsy-proven CBD, and overall, the BE-LPT with BAL cells was more sensitive and specific than that with blood (40). To date, however, there has not been a well-designed study comparing BE-LPT data obtained with blood and BAL. In practice, the BAL BE-LPT is most commonly used as part of the evaluation for CBD in patients with a positive blood BE-LPT or clinically suspected CBD. Beryllium-specific immune responses in CBD have been shown to be mediated by CD4-positive T lymphocytes recently characterized as effector memory cells (15, 43).

Kreiss and coworkers reevaluated the BE-LPT with blood cells as a workplace screening tool and reported good reproducibility, justifying continued evaluation of the test (27). They also noted elevated blood LPT responses in a small group of subjects who were asymptomatic at the time of testing. Clinical evaluation of these individuals revealed histopathologic evidence of granulomas in some patients and elevated BE-LPT responses in BAL cells. The investigators concluded that the test might be helpful in preventing clinical CBD by allowing early diagnosis of subclinical disease (27). Although intuitively attractive, this conclusion is currently unconfirmed due a paucity of natural history data. In a subsequent study, Kreiss and associates further evaluated the BE-LPT in workers exposed to beryllium in ceramics manufacture (28). The results indicated that the BE-LPT exhibited a high positive predictive value (100%) for beryllium disease, further justifying its use as part of medical surveillance (28). Later studies, however, have not shown this to be a consistent finding.

**Current status of the BE-LPT.** The BE-LPT is highly useful for distinguishing CBD from sarcoidosis in the differential diagnosis of granulomatous lung disease. The assay may also detect asymptomatic beryllium hypersensitivity and subclinical CBD, as noted above. One of the major problems encountered with the assay, however, is a high level of variability. The practice of having two laboratories test split blood samples from a given individual is reported to identify more cases of beryllium sensitization than would have been the case for either laboratory alone (26, 31). Strikingly, each laboratory in the study generated approximately half of the total cases, suggesting that variability might relate to the cell populations within the sample itself rather than the laboratory (26). Results from a larger industrial study reviewing the use of BE-LPT as a surveillance tool confirmed this variability (11). A single abnormal blood BE-LPT was found to have a positive predictive value of only 39%, with considerable intra- and interlaboratory variability (11).

Because the BE-LPT remains the primary test used for assessing CBD risk in large populations, laboratories performing the test (see Beryllium Lung Disease section above) formed a working group, the Committee to Accredite Beryllium Sensitivity Testing, to address technical issues (23). The consensus BE-LPT method utilizes challenge of peripheral blood mononuclear cells with three incremental doses of beryllium sulfate solution at two different culture periods, thus providing six different responses for analysis (reviewed in reference 17). Tritiated thymidine is added to cultures 1 day before harvest, and uptake of radiolabeled thymidine is measured as counts per minute with a beta counter. Final results are expressed as a stimulation index (SI), where the mean counts per minute of control (beryllium-free) cultures are divided into the mean counts per minute of each beryllium-exposed culture. The test is interpreted as abnormal if an SI of 3.0 or higher occurs in two of six beryllium-exposed cultures. An SI of 3.0 or higher in one of six cultures is considered borderline, and an SI of 3.0 or higher in zero of six cultures is considered negative. The least-absolute-values method of statistical analysis has been proposed as a means to improve identification of borderline and abnormal BE-LPT results (17).

## GENETIC TESTING

Genetic differences in individuals susceptible to a beryllium-induced granulomatous lung disease were first recognized in strain 2 and 13 guinea pigs (3). One of the strengths of the BE-LPT has been to provide a standard by which to identify CBD and beryllium hypersensitivity for studies of genetic markers in humans. The current status of genetic testing in CBD has recently been reviewed (32). The influence of human leukocyte antigen (HLA) markers in the HLA-DP subregion, specifically HLA-DPB1<sup>Glu69</sup>, on susceptibility to CBD was first reported by Richeldi et al. (39). Since then other investigative groups have also confirmed an association between HLA-DPB1<sup>Glu69</sup> and beryllium disease risk in exposed workers (32). This haplotype has also been found to be related to beryllium sensitization in some studies (41, 47). Others have noted an association of HLA-DR<sup>Arg74</sup> with beryllium sensitization but not with CBD (42). Surveillance studies of beryllium industry employees suggest that an HLA-DPB1<sup>Glu69</sup> haplotype combined with environmental exposure in a specific job task such as machining confers an additive risk of CBD (38). Testing for carrier status in a preemployment population, however, is problematic not only because of ethical issues but because HLA-DPB1<sup>Glu69</sup> is a haplotype frequently encountered within the general population (48). With a CBD disease frequency of 5% in exposed workers, the positive predictive value of this test has been calculated to be only 8.3 to 14.3% in different racial groups (48).

## APPLICATION OF THE BE-LPT: LESSONS FROM A CLEVELAND CLINIC STUDY

As reviewed above, an elevated blood BE-LPT response may indicate asymptomatic beryllium hypersensitivity or CBD (overt or subclinical). The recommended follow-up procedure to diagnose these conditions is a pulmonary evaluation with BAL for BE-LPT and transbronchial biopsy for histologic examination. Fifty-seven beryllium-exposed individuals with abnormal blood BE-LPTs were identified over a 2-year period, during which time approximately 1,800 blood BE-LPTs were performed in the Cleveland Clinic Foundation laboratory. Biopsy results initially revealed lung granulomas (considered biopsy-proven CBD) in 24 of 57 individuals (2, 4). Age, sex, work years, and smoking history of granuloma-positive and -negative individuals were not significantly different. In BAL analyses of the granuloma-positive group, however, the percentage of BAL lymphocytes (37%) and the (SIs) of BAL BE-LPTs (SI = 80) were significantly higher ( $P = 0.0001$ ) than those in the granuloma-negative group (9% and 0.8, respectively) or in healthy controls (4% and  $\leq 3.0$  SI, respectively) (Fig. 2). Abnormal BAL BE-LPTs occurred in 20 or 24 (83%) of the granuloma-positive group but also in 5 of 32 (16%) of the granuloma-negative group (Fig. 2). Follow-up of these granuloma-negative individuals with abnormal BAL BE-LPTs indicated that within 5 years, two of these individuals became granuloma positive. Thus, of the initial 57 subjects with abnormal blood BE-LPTs given pulmonary evaluation, 46% (26 of 57) had biopsy-documented CBD, or a total of 1.4% of the initial 1,800 individuals tested. These findings are consistent with prior reports indicating that approximately 40 to 50% of beryllium-exposed subjects presenting with abnormal blood

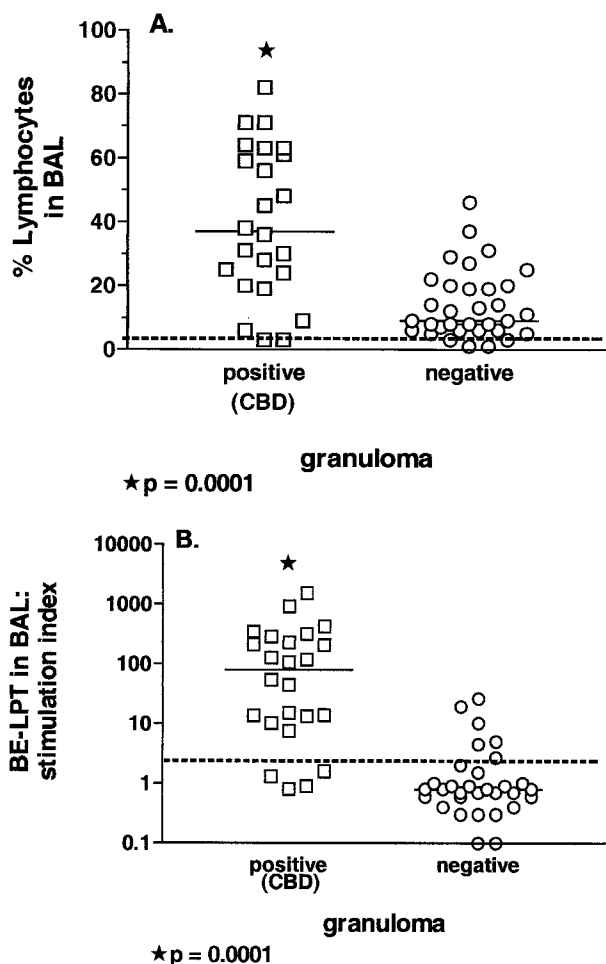


FIG. 2. Analyses of BAL cells in beryllium-exposed individuals with abnormal blood BE-LPTs. Pulmonary evaluation and transbronchial biopsy were carried out on 57 beryllium-exposed individuals with abnormal blood BE-LPTs. The diagnosis of CBD was confirmed by granulomatous histopathology in 24 individuals. Significant differences ( $P = 0001$ ) were noted between granuloma-positive (CBD) and granuloma-negative (BE-sensitized) individuals in the percentage of BAL lymphocytes (A) and BAL BE-LPT responses (B). Dotted lines indicate upper limits for healthy controls.

BE-LPTs are ultimately found to have biopsy-proven CBD (11, 26, 31) (Fig. 3).

**FUTURE DIRECTIONS**

New approaches for detection of beryllium sensitivity and CBD will likely include investigation of additional genes. Polymorphisms in the tumor necrosis factor alpha promoter appear to confer risk for beryllium sensitization and affect the rate of CBD development as well as the clinical severity of CBD (1, 30, 42). The effects of tumor necrosis factor alpha appear to be independent of HLA-DP (1). Single-nucleotide polymorphism analysis has been applied to the HLA-DPB1<sup>Glu69</sup> variant and may be helpful in other diagnostic applications (5). Differential display has been used to analyze mRNA responses of beryllium-challenged cells in CBD patients (18). The results suggested that a small percentage of mRNA responses were specific to beryllium and not generated by challenge with other

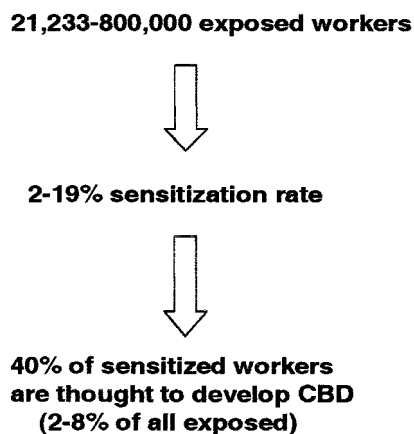


FIG. 3. Rates of beryllium sensitization and disease. Estimates of the number of exposed workers vary widely. Rate of sensitization refers to a positive blood BE-LPT, whereas diagnosis of CBD requires sensitization and histopathologic demonstration of nonnecrotizing granulomas. Approximately 40% of sensitized individuals are found to have CBD, although many CBD cases are clinically silent at the time of diagnosis.

stimuli (18). Additional studies are needed to define the spectrum of genetic alleles and molecular changes associated with CBD as opposed to beryllium sensitivity. Novel functional assays are also needed to improve detection of in vitro responses to beryllium challenge. Recently, an ATP-monitoring test to measure lymphocyte activation was approved by the Food and Drug Administration for use in assessing immune function status in transplant patients (44). This relatively simple assay may herald a new generation of rapid in vitro methods for monitoring lymphocyte function and may potentially revolutionize beryllium sensitivity testing.

**SUMMARY**

Beryllium is a lightweight metal with unique properties that render it ideal for use in nuclear, aerospace, electronics, ceramics, and metallurgy industries. Up to 5% of beryllium-exposed individuals develop CBD, a granulomatous disorder characterized by T-lymphocyte-mediated hypersensitivity to beryllium. Although CBD is clinically indistinguishable from sarcoidosis, the BE-LPT provides a means for differential diagnosis. A positive blood BE-LPT is specific for beryllium hypersensitivity, which may be associated with CBD (subclinical or overt) at the time of testing. As a follow-up step, individuals may undergo pulmonary evaluation, including bronchoscopy, transbronchial biopsy, BAL, and BAL BE-LPT. There is some evidence that the sensitivity of the BE-LPT for CBD is higher in BAL than in blood, but both tests show some variability, and histopathologic verification of granulomas is required for CBD diagnosis. Genetic analyses have shown an association of HLA-DPB1<sup>Glu69</sup> (a relatively common phenotype within the general population) with CBD. Further studies are needed to continue to elucidate beryllium hypersensitivity and CBD on the molecular level.

**REFERENCES**

1. Amicosante, M., F. Berretta, A. Franchi, P. Rogliani, C. Dotti, M. Losi, R. Dweik, and C. Saltini. 2002. HLA-DP-unrestricted TNF-alpha release in beryllium-stimulated peripheral blood mononuclear cells. *Eur. Respir. J.* 20:1174-1178.

2. **Barna, B. P., S. Burgess, B. Jacobs, T. N. Markham, M. J. Thomassen, and H. P. Wiedemann.** 1995. Beryllium lung disease: association of biopsy findings with bronchoalveolar lavage lymphocyte content and immune response to beryllium. *Am. J. Respir. Crit. Care Med.* **151**:A712.
3. **Barna, B. P., S. D. Deodhar, T. Chiang, S. Gautam, and M. Edinger.** 1984. Experimental beryllium-induced lung disease. I. Differences in immunologic responses to beryllium compounds in strains 2 and 13 guinea pigs. *Int. Arch. Allergy Appl. Immunol.* **73**:42–48.
4. **Barna, B. P., R. A. Dweik, C. F. Farver, D. Culver, B. Yen-Lieberman, and M. J. Thomassen.** 2002. Nitric oxide attenuates beryllium-induced IFN $\gamma$  responses in chronic beryllium disease: evidence for mechanisms independent of IL-18. *Clin. Immunol.* **103**:169–175.
5. **Cai, H., P. S. White, D. Torney, A. Deshpande, Z. Wang, B. Marrone, and J. P. Nolan.** 2000. Flow cytometry-based minisequencing: a new platform for high-throughput single-nucleotide polymorphism scoring. *Genomics* **66**:135–143.
6. **Cullen, M. R., J. R. Kominsky, M. D. Rossman, M. G. Cherniack, J. A. Rankin, J. R. Balmes, J. A. Kern, R. P. Daniele, L. Palmer, G. P. Naegel, K. McManus, and R. Cruz.** 1987. Chronic beryllium disease in a precious metal refinery. Clinical epidemiologic and immunologic evidence for continuing risk from exposure to low level beryllium fume. *Am. Rev. Respir. Dis.* **135**:201–208.
7. **Culver, D. A., and R. A. Dweik.** 2003. Chronic beryllium disease. *Clin. Pulm. Med.* **10**:72–79.
8. **Curtis, G. H.** 1951. Cutaneous hypersensitivity due to beryllium. *Arch. Dermatol.* **64**:470–482.
9. **Deodhar, S. D., and B. P. Barna.** 1991. Immune mechanisms in beryllium lung disease. *Clev. Clin. J. Med.* **58**:157–160.
10. **Deodhar, S. D., B. P. Barna, and H. S. Van Ordstrand.** 1973. A study of the immunologic aspects of chronic berylliosis. *Chest* **63**:309–313.
11. **Deubner, D. C., M. Goodman, and J. Iannuzzi.** 2001. Variability, predictive value, and uses of the beryllium blood lymphocyte proliferation test (BLPT): preliminary analysis of the ongoing workforce survey. *Appl. Occup. Environ. Hyg.* **16**:521–526.
12. **Eisenbud, M.** 1982. Origins of the standards for control of beryllium disease (1947–1949). *Environ. Res.* **27**:79–88.
13. **Eisenbud, M., and J. Lissou.** 1983. Epidemiological aspects of beryllium-induced nonmalignant lung disease: a 30-year update. *J. Occup. Med.* **25**:196–202.
14. **Epstein, P. E., J. H. Dauber, M. D. Rossman, and R. P. Daniele.** 1982. Bronchoalveolar lavage in patient with chronic berylliosis: evidence for hypersensitivity pneumonitis. *Ann. Intern. Med.* **97**:213–216.
15. **Fontenot, A. P., S. J. Canavera, L. Gharavi, L. S. Newman, and B. L. Kotzin.** 2002. Target organ localization of memory CD4<sup>+</sup> T cells in patients with chronic beryllium disease. *J. Clin. Investig.* **110**:1473–1482.
16. **Freiman, D. G., and H. L. Hardy.** 1970. The relation of pulmonary pathology to clinical course and prognosis based on a study of 130 cases from the U. S. beryllium case registry. *Hum. Pathol.* **1**:25–44.
17. **Frome, E. L., L. S. Newman, D. L. Cragle, S. P. Colyer, and P. F. Wambach.** 2003. Identification of an abnormal beryllium lymphocyte proliferation test. *Toxicology* **183**:39–56.
18. **Gaede, K. R., U. Mamat, M. Schlaak, and J. Muller-Quernheim.** 2000. Analysis of differentially regulated mRNAs in peripheral blood monocytes of berylliosis patients after in vitro stimulation. *J. Mol. Med.* **78**:293–299.
19. **Hanifin, J. M., W. L. Epstein, and M. J. Cline.** 1970. In vitro studies of granulomatous hypersensitivity to beryllium. *J. Investig. Dermatol.* **55**:284–288.
20. **Hardy, H. L., and I. R. Tabershaw.** 1946. Delayed chemical pneumonitis occurring in workers exposed to beryllium compounds. *J. Ind. Hyg. Toxicol.* **28**:197–211.
21. **Henneberger, P. K., D. Cumro, D. D. Deubner, M. S. Kent, M. McCawley, and K. Kreiss.** 2001. Beryllium sensitization and disease among long-term and short-term workers in a beryllium ceramics plant. *Int. Arch. Occup. Environ. Health* **74**:167–176.
22. **Kent, M. S., T. G. Robins, and A. K. Madl.** 2001. Is total mass or mass of alveolar-deposited airborne particles of beryllium a better predictor of the prevalence of disease? A preliminary study of a beryllium processing facility. *Appl. Occup. Environ. Hyg.* **16**:539–558.
23. **Kreiss, K., F. Miller, L. S. Newman, A. Ojo-Amaize, M. Rossman, and C. Saltini.** 1994. Chronic beryllium disease—from the workplace to cellular immunology, molecular immunogenetics, and back. *Clin. Immunol. Immunopathol.* **71**:123–129.
24. **Kreiss, K., M. M. Mroz, L. S. Newman, J. Martyny, and B. Zhen.** 1996. Machining risk of beryllium disease and sensitization with median exposures below 2 micrograms/m<sup>3</sup>. *Am. J. Ind. Med.* **30**:16–25.
25. **Kreiss, K., M. M. Mroz, B. Zhen, J. W. Martyny, and L. S. Newman.** 1993. Epidemiology of beryllium sensitization and disease in nuclear workers. *Am. Rev. Respir. Dis.* **148**:985–991.
26. **Kreiss, K., M. M. Mroz, B. Zhen, H. Wiedemann, and B. P. Barna.** 1997. Risks of beryllium disease related to work processes at a metal, alloy, and oxide production plant. *Occup. Environ. Med.* **54**:605–612.
27. **Kreiss, K., L. S. Newman, M. M. Mroz, and P. A. Campbell.** 1989. Screening blood test identifies subclinical beryllium disease. *J. Occup. Med.* **31**:603–608.
28. **Kreiss, K., S. Wasserman, M. M. Mroz, and L. S. Newman.** 1993. Beryllium disease screening in the ceramics industry. *J. Occup. Med.* **35**:267–274.
29. **Lioy, P. J., C. P. Weisel, J. R. Millette, S. Eisenreich, D. Vallero, J. Offenberg, B. Buckley, B. Turpin, M. Zhong, M. D. Cohen, C. Prophete, I. Yang, R. Stiles, G. Chee, W. Johnson, R. Porcja, S. Alimokhtari, R. C. Hale, C. Weschler, and L. C. Chen.** 2002. Characterization of the dust/smoke aerosol that settled east of the World Trade Center (WTC) in lower Manhattan after the collapse of the WTC 11 September 2001. *Environ. Health Perspect.* **110**:703–714.
30. **Maier, L. A., R. T. Sawyer, R. A. Bauer, L. A. Kittle, P. A. Lympany, D. McGrath, R. Dubois, E. Daniloff, C. S. Rose, and L. S. Newman.** 2001. High beryllium-stimulated TNF- $\alpha$  is associated with the -308 TNF- $\alpha$  promoter polymorphism and with clinical severity in chronic beryllium disease. *Am. J. Respir. Crit. Care Med.* **164**:1192–1199.
31. **Markham, T. N.** 1996. Screening for chronic beryllium disease using beryllium specific lymphocyte proliferation testing. *Int. Arch. Occup. Environ. Health* **68**:405–407.
32. **McCanlies, E. C., K. Kreiss, M. Andrew, and A. Weston.** 2003. HLA-DPB1 and chronic beryllium disease: a HuGE review. *Am. J. Epidemiol.* **157**:388–398.
33. **Newman, L. S., C. Bobka, B. Schumacher, E. Daniloff, B. Zhen, M. M. Mroz, and T. E. King, Jr.** 1994. Compartmentalized immune response reflects clinical severity of beryllium disease. *Am. J. Respir. Crit. Care Med.* **150**:135–142.
34. **Newman, L. S., and K. Kreiss.** 1992. Nonoccupational beryllium disease masquerading as sarcoidosis: identification by blood lymphocyte proliferative response to beryllium. *Am. Rev. Respir. Dis.* **145**:1212–1214.
35. **Newman, L. S., K. Kreiss, T. E. King, Jr., S. Seay, and P. A. Campbell.** 1989. Pathologic and immunologic alterations in early stages of beryllium disease. Re-examination of disease definition and natural history. *Am. Rev. Respir. Dis.* **139**:1479–1486.
36. **Newman, L. S., M. M. Mroz, L. A. Maier, E. M. Daniloff, and R. Balkissoon.** 2001. Efficacy of serial medical surveillance for chronic beryllium disease in a beryllium machining plant. *J. Occup. Environ. Med.* **43**:231–237.
37. **Paustenbach, D. J., A. K. Madl, and J. F. Greene.** 2001. Identifying an appropriate occupational exposure limit (OEL) for beryllium: data gaps and current research initiatives. *Appl. Occup. Environ. Hyg.* **16**:527–538.
38. **Richeldi, L., K. Kreiss, M. M. Mroz, B. Zhen, P. Tartoni, and C. Saltini.** 1997. Interaction of genetic and exposure factors in the prevalence of berylliosis. *Am. J. Ind. Med.* **32**:337–340.
39. **Richeldi, L., R. Sorrentino, and C. Saltini.** 1993. HLA-DPB1 glutamate 69: a genetic marker of beryllium disease. *Science* **262**:242–243.
40. **Rossman, M. D., J. A. Kern, J. A. Elias, M. R. Cullen, P. E. Epstein, O. P. Preuss, T. N. Markham, and R. P. Daniele.** 1988. Proliferative response of bronchoalveolar lymphocytes to beryllium. *Ann. Intern. Med.* **108**:687–693.
41. **Rossman, M. D., J. Stubbs, C. W. Lee, E. Argyris, E. Magira, and D. Monos.** 2002. Human leukocyte antigen class II amino acid epitopes. Susceptibility and progression markers for beryllium hypersensitivity. *Am. J. Respir. Crit. Care Med.* **165**:788–794.
42. **Saltini, C., L. Richeldi, M. Losi, M. Amicosante, C. Voorter, E. van den Berg-Loonen, R. A. Dweik, H. P. Wiedemann, D. C. Deubner, and C. Tinelli.** 2001. Major histocompatibility locus genetic markers of beryllium sensitization and disease. *Eur. Respir. J.* **18**:1–8.
43. **Saltini, C., K. Winestock, M. Kirby, P. Pinkston, and R. G. Crystal.** 1989. Maintenance of alveolitis in patients with chronic beryllium disease by beryllium-specific helper T cells. *N. Engl. J. Med.* **320**:242–244.
44. **Softong, P. R., J. A. Rosebrock, J. A. Britz, and T. R. Kramer.** 2000. Measurement of T-lymphocyte responses in whole blood cultures using newly synthesized DNA and ATP. *Clin. Diagn. Lab. Immunol.* **7**:307–311.
45. **Sprince, N. L., D. J. Kanarek, A. L. Weber, R. I. Chamberlin, and H. Kazemi.** 1978. Reversible respiratory disease in beryllium workers. *Am. Rev. Respir. Dis.* **117**:1011–1017.
46. **Stoeckle, J. D., H. L. Hardy, and A. L. Weber.** 1969. Chronic beryllium disease. Long-term follow-up of sixty cases and selective review of the literature. *Am. J. Med.* **46**:545–561.
47. **Wang, Z., G. M. Farris, L. S. Newman, Y. Shou, L. A. Maier, H. N. Smith, and B. L. Marrone.** 2001. Beryllium sensitivity is linked to HLA-DP genotype. *Toxicology* **165**:27–38.
48. **Weston, A., J. Ensey, K. Kreiss, C. Keshava, and E. McCanlies.** 2002. Racial differences in prevalence of a supratypic HLA-genetic marker immaterial to pre-employment testing for chronic beryllium disease. *Am. J. Ind. Med.* **41**:457–465.
49. **Williams, W. J., and W. R. Williams.** 1983. Value of beryllium lymphocyte transformation tests in chronic beryllium disease and in potentially exposed workers. *Thorax* **38**:41–44.
50. **Williams, W. R., and W. J. Williams.** 1982. Development of beryllium lymphocyte transformation in chronic beryllium disease. *Int. Arch. Allergy Appl. Immunol.* **67**:175–180.