Recent Advances in Understanding the Biomolecular Basis of Chronic Beryllium Disease: A Review

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Summary: In this review we summarize the work conducted over the past decade that has advanced our knowledge of pulmonary diseases associated with exposure to beryllium that has provided a molecular-based understanding of the chemistry, immunopathology, and immunogenetics of beryllium toxicity. Beryllium is a strong and lightweight metal that generates and reflects neutrons, resists corrosion, is transparent to X-rays, and conducts electricity. Beryllium is one of the most toxic elements on the periodic table, eliciting in susceptible humans (a) an allergic immune response known as beryllium sensitization (BeS); (b) acute beryllium disease, an acutely toxic, pneumonitis-like lung condition resulting from exposure to high beryllium concentrations that are rarely seen in modern industry; and (c) chronic beryllium disease (CBD) following either high or very low levels of exposure. Because of its exceptional strength, stability, and heat-absorbing capability, beryllium is used in many important technologies in the modern world. In the early 1940s, beryllium was recognized as posing an occupational hazard in manufacturing and production settings. Although acute beryllium disease is now rare, beryllium is an insidious poison with a latent toxicity and the risk of developing CBD persists. Chronic beryllium disease—a systemic granulomatous lung disorder caused by a specific delayed immune response to beryllium within a few months to several decades after exposure—has been called the "unrecognized epidemic". Although not a disease in itself, BeS, the innate immune response to beryllium identified by an abnormal beryllium lymphocyte proliferation test result, is a population-based predictor of CBD. Genetic susceptibility to CBD is associated with alleles of the major histocompatibility gene, human leukocyte antigen DP (HLA-DP) containing glutamic acid at the 69th position of the β chain (HLA-DPβ-E69). Other genes are likely to be involved in the disease process, and research on this issue is in progress. The current Occupational Safety & Health Administration permissible exposure limit of 2 μg/m3 has failed to protect workers from BeS/CBD. As a safe exposure limit that will not lead to BeS or CBD has not yet been determined, the realization that the risk of CBD persists has led to a renaissance in research on the effects of the metal on human health. Current data support further reductions in exposure levels to help minimize the incidence of CBD. Steps that would directly impact both the power of epidemiologic studies and the cost of surveillance would be to develop and validate improved screening and diagnostic tests, and to identify more genetic factors that affect either sensitization or disease process. The major focus of this review is the recent research on the cellular and molecular basis of beryllium sensitization and disease, using a multidisciplinary approach of bioinorganic chemistry and immunology. First we present a historical background of beryllium exposure and disease, followed by occurrence of beryllium in the environment, toxicokinetics, biological effects, beryllium lung disease, and other human health effects.

Keywords: CBD, pulmonary, granuloma, lung disease, cell-mediated immunity, lymphocyte, immunology, metal toxicity, epidemiology; genetic screening; genotype, hapolotype, HLA-DP antigens, HLA-DPβ1; antigen binding site, occupational exposure

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INTRODUCTION

Beryllium is a silver-gray metallic element found naturally in the earth's crust. The element is found in mineral forms in nature, and very small amounts of beryllium are ubiquitous in soil. Metallic beryllium has good resistance to alteration or chemical attack and when released naturally into the environment, is not easily altered to its toxic form. In manufacturing and production settings, however, beryllium dust, fumes, soluble salts have long been recognized as posing an occupational health hazard, primarily in the forms of acute beryllium disease following exposure to high beryllium concentrations that are rarely seen in modern industry or chronic beryllium disease (CBD) following high or very low exposures. Particle size, chemical form, concentration, and genetic factors play a role in determining whether a person develops beryllium disease. In susceptible persons, inhalation exposure to aerosols generated during primary beryllium production and machining of the metal, its alloys, or ceramics first leads to beryllium sensitization (BeS), an allergic immune response triggered by beryllium exposure that can progress to subclinical and clinical CBD or possibly to lung cancer. Beryllium sensitization can also be acquired via skin exposure. Although this route is not known to cause CBD directly, evidence has been found for increased risk of CBD upon inhalation of beryllium particulates in those individuals having dermal BeS (*vide infra*).

Although discovered in 1798, beryllium was not widely used in industry until the 1940s and 1950s. In industrial applications, beryllium can be used as a pure metal, mixed with other metals to form alloys, processed to salts that dissolve in water, or processed to form oxides and ceramic materials. Beryllium is a strong and lightweight metal that generates and reflects neutrons, resists corrosion, is transparent to X-rays, and conducts electricity. These unique characteristics make beryllium a superior material for use in many

applications. Although lighter than aluminum, beryllium is six times the specific stiffness of steel. Yet, beryllium's brittleness—the downside of its advantageous stiffness—increases the hazards that are associated with its toxicity. Unless ventilation and other controls are used, small particles and chips cf insoluble beryllium-containing materials break off during machining and other processes and spread through the air in the work area. The realization that beryllium disease persists has led to a renaissance in research on the effect of environmental beryllium on human health.

BERYLLIUM IN THE ENVIRONMENT

Natural Occurrence

The concentration of beryllium in the earth's crust generally ranges from 1 to 15 milligrams per kilogram (mg kg^{-1}) or parts per million (ppm). Beryllium never occurs as a free element in the environment, only as a compound in rocks, coal, oil, soil, and volcanic dust. Beryllium is emitted into the air and water from these sources by natural processes like erosion or by coal or oil combustion.

Theoretically, non-occupational exposure to beryllium could occur in soils, air, food, and water. Although no study has attempted to identify cases of beryllium sensitization from 'natural' sources, studies conducted by Donovan et al. /1/ revealed that approximately 1% of new employees with no known potential occupational or possible take-home exposures to beryllium were confirmed BeS-positive. In addition, no evidence has been found that beryllium biomagnifies within the food chain. In the atmosphere, beryllium particulates are removed by settling out or by precipitation.

Anthropogenic Sources

Metal extraction. Before the metal is extracted, beryllium ores are first converted to beryllium oxide (BeO) or beryllium hydroxide [$Be(OH)_2$], followed by conversion to beryllium chloride ($BeCl_2$) or beryllium fluoride (BeF_2). Finally, the pure metal is isolated by (1) an electric current or (2) a reaction with magnesium metal at high temperature. Fifteen percent of the beryllium used in the United States (US) is in the form of BeO, a white powder that can be made into many different shapes. Beryllium used in industry begins as a silicate ($BeSiO_3$), most commonly in beryl [$Be_3(Al_2(SiO_3))_6$].

Solubility. Certain beryllium compounds are soluble in water but many are not. Because of beryllium's small atomic size and +2 charge, the most stable beryllium compounds are formed with small anions like fluoride and oxide, where beryllium normally assumes a four coordinate, tetrahedral coordination geometry. The propensity for beryllium to form clusters with oxygen-based anions like carboxylates and hydroxides makes it particularly relevant in biological systems /2/. Beryllium is also capable of forming strong covalent bonds and can form organometallics, such as dimethyl beryllium [$Be(CH3)_2$] in the absence of water. Among work areas, beryllium has been identified in three crystalline forms: beryl, poorly crystalline BeO, and $Be(OH)_2$.

Environmental Distribution

Air. Beryllium is emitted to the atmosphere naturally as BeO by windblown dusts and volcanic particles, eventually falling on land or water in rain and snow or as dry particles /3/. Naturally occurring concentrations of beryllium in air are very low. The major source of its emission into the environment from anthropogenic sources is the combustion of fossil fuels (primarily coal), which releases Be-containing particulates and fly ash into the atmosphere. High concentrations are expected around coal-fired power plants and other facilities emitting beryllium. Other sources include the incineration of municipal solid waste and the production, use, and recycling of beryllium alloys and chemicals.

Airborne beryllium mass concentrations in aerosols generated during the industrial milling of bertrandite and beryl ores can range from 0.001 $\mu g/m^3$ (beryl ore grinding) to 2.1 $\mu g/m^3$ (beryl ore crushing) /4/. In low-energy input operation areas (ore crushing, hydroxide product drumming), respirable mass fractions of airborne beryllium-containing particles were reported to be < 20%, but in high-energy input areas (beryl melting, beryl grinding) > 80%.

Beryllium air concentrations in the US have typically been lower than the detection limit of 0.03 $ng.m^3$ (for detailed exposure information see /5/). The beryllium concentration in urban air is usually higher than in rural areas. Stack concentrations of toxic trace elements from coal-fired power plants were found to be 2 to 3 orders of magnitude greater than the range of ambient air concentrations for beryllium /6/, with those from US coal ranging from 1.8-2.2 mg kg^{-1} /4/. The US Environmental Protection Agency (US EPA) /7/) estimates that up to 180 metric tons of beryllium may be emitted each year from US coal combustion, with another 7.1 metric tons of beryllium being released each year from fuel oil. Doering and Akber /8/ reported in 2008 that the beryllium concentration in near-surface air in Brisbane, Australia measured over 4 years showed seasonal variations, with values above the annual mean occurring mainly in the spring and summer months of each year, similar to the seasonal rainfall pattern, and showing a statistically significant ($p < .001$) linear relation between monthly 7Be deposition and rainfall amount. According to the Argonne Laboratory fact sheet /9/, one cigarette contains about 0.5 to 0.7 µg beryllium, with about 5% to 10% escaping into sidestream smoke.

Water. Once deposited on land, BeO remains bound to the soil within the environmental pH range of 4-8. The insolubility of the compound prevents its release into groundwater. Beryllium naturally enters waterways through the weathering of rocks and soils containing the metal. The major sources of the anthropogenic release of beryllium to surface waters include discharges of waste water effluents from beryllium or related industries, runoff from Be-containing waste sites, and the deposition of atmospheric beryllium aerosols from industrial activities settling over water /10-11/. The estimated average concentration of beryllium in any fresh surface water is 1 µg/L or 1 ppb (part per billion) /8/. Most beryllium released into waterways will be retained in sediment and bound to soil particles, rendering it generally immobile. Insoluble beryllium compounds can remain in ocean water for a few hundred years before settling to the bottom of the ocean. Thus, the element is rarely a drinking water contaminant. The average concentration of beryllium in drinking water samples that were found to contain the metal was 190 ng L^{-1} or 0.01 to 0.7 ppb /9/. Beryllium is not easily altered to soluble forms because of its resistance to alteration or chemical attack.

Soil. Although the element occurs naturally in soil, the disposal of coal ash, incinerator ash, and industrial wastes can increase the amount of beryllium in soil. Water-insoluble beryllium can remain bound to the soil for thousands of years without moving deeper into the ground to enter groundwater. The average concentration of naturally occurring beryllium in US soils is 0.6 ppm, and levels typically range from 0.1 to 40 ppm (http://www.ead.anl.gov/pub/doc/Be.pdf). To put this number into perspective, a shovel full of typical soil weighing about one kg would contain about 1000 to 2000 µg of beryllium.

Concentrations in sandy soil are estimated to be up to 250 times higher than in interstitial water (water in the pore space between soil particles), with much higher concentration ratios found in loam and clay soils. Once in the environment, chemical reactions can change water-soluble beryllium compounds into insoluble forms and, conversely, water-insoluble beryllium compounds can change into soluble forms. The chemical form of beryllium is an important factor in its bioavailability and toxicology. Exposure to water-

soluble beryllium compounds in the environment generally poses a greater threat to human health for acute toxicity, whereas the inhalation of water-insoluble forms like BeO at low dose levels poses a threat to human health for CBD.

Food. The element is naturally present in various foods, with a median concentration of 22.5 μg kg^{-1} reported across 38 different food types, ranging from less than 0.1 μg kg^{-1} to 2,200 μg kg^{-1} /9/

Environmental Transport

For beryllium attached to particulate matter, the particle size determines the residence time in air, with most being found on particles with diameters of < 2.5 μm /12/. The transport of beryllium from the atmosphere to terrestrial and aquatic surfaces occurs through wet and dry deposition. The dry deposition rate of aerosol particles is a function of particle size, wind speed, and surface roughness. The process of wet deposition of airborne beryllium involves wash-out involving the scrubbing of particles from the air by rain and rain-out involving their attachment to aerosols in clouds /5/. Although small dust particles of beryllium in the air can fall out of the air onto surface water, plant surfaces, and soil either spontaneously or with rain or snow, extremely small beryllium particles can remain in the air for about 10 days. Beryllium is carried to rivers, lakes, and oceans by the process of land erosion. Only a small amount of beryllium is transported to surface waters from the land by wind-blown soil /13/. In addition to the beryllium found naturally in minerals, beryllium metal and the compounds remaining after human mining and processing can be released back into the environment as landfill waste.

Environmental Transformation

Little is known about the environmental fate of beryllium. Presumably beryllium metal converts to the more stable BeO exposure to air for prolonged periods. It has been postulated, based on theoretical calculations, that in aqueous solutions BeO can then hydrolyze to form $[Be_3(OH)_3(H_2O)_6]^{3+}$ and associated clusters, which are soluble at environmentally relevant pH values /14-17/. Until recently, the mechanisms by which the element forms complexes with other compounds or reacts with biologically important molecules, macromolecules, and macro-molecular systems have not been well studied. Recent studies have shown that the dissolution of even high-fired BeO can be greatly enhanced by the presence of phosphate or through complexation with compounds, such as citrate /18/. The speciation of beryllium in biological systems will be discussed in subsequent sections.

Occupational Exposure

Occupational exposure to beryllium occurs where the chemical is mined, processed, or converted into metal, alloys, and other chemicals, as well as when machining metals containing beryllium, recycling beryllium from scrap alloys, or using beryllium products. Processes that create employee exposure in such industries typically involve machine shop, metalworking, and finishing processes, such as machining, sanding, stamping, grinding, crushing, lapping, and sintering. Today, beryllium has a wide range of applications in modern industrial processes, including aerospace, automotive, biomedical, defense, energy, fire prevention, manufacturing, scrap recovery, and telecommunication /19-20/. Beryllium exposure also occurs in numerous industries that use earth-based materials like feed stock into their products, such as ceiling tiles, charcoal, detergent, cosmetics, mineral supplements, and others.

Beryllium is also present in industries that do not intentionally produce or process the metal, such as in abrasive blasting operations where coal or copper slag is used as a substitute for sand; in spot or seam welding of specialized Be-copper electrodes; in welding processes where beryllium is in the electrode, the flux or rod, or the substrate

alloy being fabricated; and when recycling metals and other materials from computers and electrical products. People who work in beryllium manufacturing, fabricating, and reclaiming industries have a greater probability than non-occupational groups of inhalation exposure. Even though high concentrations of surface and airborne beryllium, mostly in the form of beryllium oxide and beryllium metal, have been documented in many workplaces, large-scale systematic surveys designed to identify occult beryllium exposures in private industry have not been conducted /21/.

Historical background. From the 1940s through the 1960s, the US Atomic Energy Commission (AEC, now the US Department of Energy [DOE]) and the Department of Defense (DOD) were the primary users of beryllium in the US. Beryllium-containing materials were first used in the following major markets: Aerospace 1940s, Appliances 1950s, Automotive 1950s, Computer 1940s, Dental 1960s, Maritime 1930s, Oil Exploration 1960s, Plastics (molds) 1950s, Recreational 1950s, Telecommunications 1940s, and Recycle 1950s. Over the years, however, technological advances have changed the specific products using beryllium.

During World War II, three cases of acute 'chemical pneumonia' (later termed acute beryllium disease) were first reported /22-23/. After 1945, exposure to beryllium dust became a public health problem following the first reports of a 'delayed chemical pneumonitis' (later termed chronic beryllium disease) in individuals exposed to beryllium compounds /24-26/. Cases of both acute and chronic lung diseases were reported from AEC laboratories, as well as from fluorescent-lamp factories. Between 1947 and 1948, data gathered by the International Agency for Research on Cancer (IARC) revealed extremely high concentrations of beryllium in the workplace /27/. In US beryllium extraction facilities, concentrations of > 1000 $\mu g/m^3$ were not unusual. Exposures measured late in 1946 ranged from 110 to 4710 $\mu g/m^3$ in the furnace area of an extraction plant. Concentrations of 590-43,300 $\mu g/m^3$ were found by AEC investigators in the Lorain, Ohio, plant in 1947-48 /28/.

In 1949, the AEC recommended the first occupational exposure limit (OEL) for beryllium of 2.0 $\mu g/m^3$. That limit was adopted by American Conference of Governmental Industrial Hygienists (ACGIH) /29/, the National Institute for Occupational Safety and Health (NIOSH), the Occupational Safety and Health Administration (OSHA) /30/, the American Industrial Hygiene Association /31/, and the American National Standards Institute /32/, and remains intact today. Today this standard is widely acknowledged to be insufficiently protective, and workers exposed to levels below the standard have developed beryllium-related disease /33/.

The identification of beryllium as an occupational and environmental hazard led to control measures in 1950. For occupational air exposures, a permissible level of 2.0 $\mu g/m^3$ was established based on an 8-h time-weighted average (TWA); the 30-min maximum peak level was set at 25 $\mu g/m^3$, and in air surrounding factories beryllium concentrations must not exceed 0.01 $\mu g/m^3$. As reports have shown that the 2.0 $\mu g/m^3$ standard is not protective, ongoing research is anticipated to support the development of one or more scientifically sound standards for the different chemical forms of beryllium /34/. For additional international, national, and state regulations and guidelines regarding beryllium in air, water, and other media, see summary in Table 8-1 of the ATSDR Toxicological Profile on Beryllium /5/.

Current beryllium exposures. At the end of the 20th century, Cullen et al. /35/, Kreiss et al. /36/, and Stange et al. /37/ reported CBD (and/or BeS) in workers exposed to average beryllium concentrations of 0.52, 0.55, or 1.04 $\mu g/m^3$, respectively. In 1999, DOE considered the OEL of 2 $\mu g/m^3$ to be inadequate to protect worker health and established a tenfold lower action level of 0.2 $\mu g/m^3$ at DOE facilities /38/. Additionally, a beryllium surface guideline was set at 3 $\mu g/100$ cm^2 for operational

areas in which workers could be exposed to beryllium; a second guideline for the surfaces of equipment and other items to be released to the general public or for use in DOE non-beryllium work areas was set at 0.2 μg/100 cm^2. Henneberger et al. /39/ calculated that as of 2004, at least 26,500 DOE and DOD workers had been potentially exposed to beryllium.

Recently, beryllium has become prevalent in the manufacture of beryllium-containing products, as well as in the salvage of materials containing beryllium. An assessment survey of occupational exposure to beryllium was conducted in France between late 2004 and the end of 2006 /40/. The results of this study indicated airborne beryllium concentrations in excess of the occupational exposure limit value of 2 μg/m^3 recommended in France. Metallurgy and electronic component manufacturing represented the activities and occupations in which workers had the highest arithmetic mean exposures to beryllium. Surface contamination levels were also high and frequently exceeded the thresholds recommended by different bodies.

Current beryllium exposures have been noted in diverse sectors (reviewed in http://ww.chronicberyll-iumdisease.com/exposure/ex_industries.htm#list). In addition to the US government facilities that use beryllium, potential exposure risks may exist at certain private plants. Henneberger et al. /39/ reported that overall, 54,400-134,000 workers in government and private industry had potential exposure to beryllium in the US, of which 26,400 to 106,000 were workers in the private sector. This count does not include former workers, contract workers, and construction workers exposed in beryllium using facilities.

By far the greatest use of beryllium metal is in alloys. Beryllium-copper alloys are present in electric connectors and relays, switches in auto-mobiles, telecommunication equipment, computers, home appliances, cellular phones, and connectors for fiber optics /5,41/. Beryllium oxide is used in high-technology ceramics, electric insulators, military vehicle armor, rocket nozzles, crucibles, laser structural components, automotive ignition systems, and radar electronic countermeasure systems /5,19,42/. Beryllium-nickel alloys are used in automobile air bags. A beryllium-aluminum alloy is used in fighter planes, helicopters, and missile systems. Transparency to microwaves has enabled its use in microwave devices.

As beryllium has the lowest thermal neutron absorption cross-section of any metal, th e metal has been used since the 1950s in nuclear technology, including weapons and reactors /43/. Bombardment by alpha particles, as from radium or polonium, produces neutrons and in this capacity beryllium has been used as a neutron trigger or initiator for nuclear weapons /44/. The US DOE uses beryllium metal to fabricate weapons components and to facilitate a number of weapons-related experiments.

In 2008, Sanderson et al. /45/ evaluated the presence of beryllium surface contamination in a US conventional munitions plant as an indicator of possible past beryllium airborne and skin exposure and used these measurements to classify job categories by potential level of exposure. Surface samples collected from production and non-production areas of the plant and at regional industrial reference sites with no known history of beryllium use were analyzed for beryllium mass content using inductively coupled plasma-atomic emission spectroscopy and the results were expressed as μg Be/100 cm^2. Beryllium was detected in 87% of samples collected at the munitions plant. Surprisingly, 72% of the samples collected at regional reference sites, at which beryllium was not known to have been used, also had quantifiable concentrations of beryllium. Two munitions plant samples from areas near sanders and grinders were above the DOE surface contamination limit of 3.0 μg/100 cm^2.

A recent pilot study exploring the occurrence of surface deposited beryllium in a random sample of 29 worksites in the Midwest US reported beryllium wipe concentrations above the analytical limit of

quantitation (0.035 μg beryllium per sample) at 79% of the sites tested /46/. In fact, the beryllium surface concentration for 11% of the 145 wipe samples taken at the worksites exceeded 0.2 μg/100 cm^2 and 4% of the samples exceeded beryllium concentrations of 3 μg/100 cm^2. The beryllium surface contamination criteria used by the US Department of Energy (DOE) (10 CFR 850.30) state that surfaces contaminated with beryllium dusts and waste must not exceed a removable contamination level of 3 μg/100 cm^2 during non-operational periods and that employers must provide protective clothing and equipment where surface levels exceed 3.0 μg/100 cm^2. Although a measurable surface beryllium concentration indicates only the potential for beryllium exposure, the workers and management at the 29 non-DOE-related worksites were unaware of the surface contamination. The diagnosis of CBD includes a sound occupational history and an abnormal beryllium lymphocyte proliferation test (BeLPT) (*vide infra*). Without a history of exposure to beryllium and a positive BeLPT, CBD patients could be misdiagnosed as having sarcoidosis, a different inflammatory disease of the lungs that has a similar clinical presentation but unknown etiology /47/.

Community Exposure

Today, toxicologically relevant exposure to beryllium appears to be almost exclusively confined to occupational settings. The general population could be exposed to trace amounts of beryllium, however, through the inhalation of air, the consumption of food and water, or by skin contact with air, water, or soil containing beryllium.

Historical background (1948-1951). During 1947-1948, cases of community-acquired CBD began to be reported from Loraine, Ohio /48-49/ and from Reading, Pennsylvania /50-51/. Between 1948 and 1969, cases of community-acquired CBD continued to be reported in household contacts of beryllium workers and in individuals living in neighborhoods surrounding beryllium facilities /48,52-53/. As the exposures in these reported community cases occurred before controls were instituted to reduce beryllium emissions, some community exposure levels at that time were likely to be similar to current occupational exposures.

Although the amount of beryllium measured in communities surrounding beryllium manufacturing facilities was significantly lower than in the workplace (100-1,000 μg/m^3), a similar prevalence of disease was reported /48,54/. This apparent paradox was attributed to the smaller particle size of beryllium emitted to the outside air as compared with beryllium particles inside the plant, and to community exposures occurring over a 24 hour period 7 days a week /48,51,55/. Moreover, 'drag-out' of beryllium from an industrial source could have been a likely cause of some cases /56/. Community exposures within one quarter of a mile of the plant, where most cases were identified, averaged 2.1 μg/m^3. In residents living at least three quarters of a mile away from a beryllium facility, where the airborne beryllium concentration was estimated to range from 0.01 to 0.1 μg/m^3, no cases of CDB were detected. The overall conclusion then was that individuals could develop CBD from beryllium exposures that would generally be considered incidental, namely, from exposures not directly related to inhaling workplace air /57/.

In 1988, the US EPA /7/ established the exposure guidelines for beryllium designed for the general population. For inhalation exposures, the reference concentration (RfC) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude or greater) of a continuous inhalation exposure of the human population (including susceptible subpopulations) that is likely to be without an appreciable risk of deleterious health effects during a lifetime. A lowest observed-adverse-effect level (LOAEL) of 0.20 μg/m^3 emerged from observations in a 1996 occupational-exposure study /36/ and from a

community-exposure study conducted in 1949 /58/. That value was adjusted by applying two uncertainty factors of 3 to account for the poor quality of the exposure assessments in such studies and to account for use of an LOAEL instead of a no-observed-adverse-effect level (NOAEL), resulting in an RfC of 0.02 $\mu g/m^3$.

Recently diagnosed cases. As further surveillance was not carried out in community exposures and additional cases were not reported after 1969, the risk of community-acquired CBD remained unrecognized until recently. In 2008, Maier et al. /59/ reviewed the medical records from individuals residing in a community surrounding the beryllium manufacturing facility in Reading, Pennsylvania. The estimated average exposure to beryllium was 0.015-0.028 $\mu g/m^3$, possibly with peak exposures > 0.35 $\mu g/m^3$ based on historic beryllium air sampling (1958). The results revealed cases of CBD meeting current immunologic diagnostic criteria exposure among the residents. As occupational or potential para-occupational exposure was excluded, these cases were deemed attributable to industry-generated beryllium pollution. Cases of CBD classified as 'definite' required (a) an abnormal BeLPT in blood or bronchoalveolar lavage cells (BAL) (*vide infra*), and (b) biopsy evidence of granulomatous inflammation for diagnosis. 'Probable' cases of CBD either displayed an abnormal blood test to beryllium and radiographic evidence consistent with disease, or met the epidemiologic criteria for CBD based on Beryllium Case Registry criteria (*vide infra*). Of the 16 cases of potential community acquired-CBD evaluated, 8 definite cases were identified (5 definite and 3 probable). Among the cases, the initial year of residence began between 1943 and 1953, continuing until 1956-2001, with most patients diagnosed years after exposure cessation. The authors anticipate that not only have cases been misdiagnosed in this community but also that more cases of CBD will be diagnosed in the future. Therefore, healthcare providers should continue to consider CBD in the differential diagnosis of patients with respiratory disease who reside near beryllium facilities.

TOXICOKINETICS

The toxicokinetic profile of beryllium compounds varies, with the more soluble forms undergoing greater systemic absorption, distribution, and urinary elimination. No animal model of human CBD developed to date accurately mimics all aspects of the human disease. Hence, ATSDR /5/ offers words of caution regarding the extrapolation of animal studies to humans. Although many experimental animal studies reported granulomatous inflammation in the lungs, the histopathology of such lesions does not resemble CBD in humans, and the effects are either transient or not consistently associated with beryllium-specific immune responses.

Beryllium compounds are absorbed primarily through the lungs, but information on humans is insufficient to determine the rate and extent of the deposition and absorption. The absorption of inhaled beryllium depends on the size and solubility of the particles and on the activity of alveolar macrophages, among others. The US EPA /60/ defines air particles according to a bimodal distribution as fine (< 1 µm aerodynamic equivalent diameter [d_{ae}]) and coarse (> 2.5 µm d_{ae}). Studies often report such results as PM_{10} (particle diameter below 10 µm d_{ae}). In the respiratory tract, 50% of particles of size PM_{10} will penetrate beyond the larynx. Generally, beryllium particles emitted into the atmosphere from anthropogenic processes, such as oil or coal combustion for electric power generation, are emitted as BeO /61/. Beryllium and its compounds are biotransformed once in the body (*vide infra*), and soluble beryllium salts are partially converted to less soluble forms in the lung /62/.

Following deposition in animals, beryllium slowly clears from the lung in a biphasic manner. Soluble compounds are cleared rapidly by dissolu-

tion in respiratory tract fluid, whereas insoluble particles are deposited in the upper respiratory tract and tracheobronchial tree and slowly cleared by mucociliary transport. Insoluble particles deposited in the pulmonary regions are cleared primarily by alveolar macrophages and translocation and solubilization mechanisms, with clearance half-times ranging from 2.5 days to a little over 2 years /63-64/. In humans, the presence of insoluble beryllium in the lung has been detected many years after occupational exposure has ceased /5/.

As most beryllium compounds do not dissolve easily, beryllium is poorly absorbed through the gastrointestinal tract /65-66/ or through intact skin /67/. Skin ulceration beryllium-exposed workers occurred only after the skin was accidentally cut or abraded /68/. The average concentrations measured in human organs were reported to be 0.21 ppm in lungs; 0.08 ppm in brain; 0.07 ppm in both kidney and spleen; 0.04 ppm in each of liver, muscle, and vertebrae; 0.03 ppm in heart; and 0.02 in bone, but the route of exposure (occupationally or environmentally) was not provided nor whether the organ samples were obtained at biopsy or autopsy /69/.

The primary routes of elimination of absorbed beryllium are urine and feces. In 1980, eight men were accidentally exposed for 10 days, 4 to 6 hours per day, to $BeCl_2$ (0.8 ng Be/m^3). The urine and blood beryllium levels increased fourfold above the levels of 0.1 ng Be g^{-1} of either blood or urine found in unexposed individuals /70/. After an accidental leakage of beryllium dust in a laboratory, 25 workers who were exposed to an undetermined concentration for 10-20 hours were monitored /71/, yielding a calculated biological half-time of beryllium of 2-8 weeks. Reeves /72/ concluded that the urinary excretion of beryllium is irregular and not useful for diagnostic purposes.

BIOLOGICAL EFECTS OF BERYLLIUM

Three distinct biological effects after beryllium exposure have been described in the literature—immune system effects, antiproliferative effects, and possible carcinogenic effects /73-74/. The potential for putative carcinogenicity will be discussed later in a separate section.

Immune System Effects

The effects of the beryllium ion on the immune system are complex. Metals are frequent inducers of a variety of T-cell mediated human diseases. Among these, CBD /75/, cobalt hard metal lung disease /76/, and contact hypersensitivities to a variety of metals, metal dusts, and other soluble metallic substances are prominent examples /77/. Beryllium particles can trigger both innate (inflammatory) and acquired immune responses. The innate immune functions of airway epithelial cells are significant in the pathogenesis of all major diseases of the lung /78-79. The airway epithelium responds to such exposures by increasing the local production of cytokines, chemokines, and antimicrobial peptides that function as regulators of inflammation and immunity.

T-lymphocyte response. Functionally distinct CD4+ T-helper (Th) cell subsets (Th1 and Th2) are characterized by the patterns of cytokines they produce. In culture, memory CD4+ T cells respond vigorously to beryllium salts by producing pro-inflammatory, Th1-type inflammatory cytokines—chemicals inciting the recruitment of alveolar macrophages, followed by alveolitis and subsequent granuloma development. The T-cell response, as assessed by the BeLPT test described below, is dependent on the frequency of *central memory* T cells /80/, whereas the Th1 cytokine secretion leading to granulomatous inflammation and disease is dependent upon the activity of *effector memory* T cells /81/. Effector memory T cells provide rapid effector function within the target organ, whereas central memory T cells proliferate in the lymph node to generate increased numbers of effector cells /82-83/.

Beryllium lymphocyte proliferation test. The chronic form of beryllium disease is characterized by the development of large numbers of lymphocytes within the lungs that can be measured by instilling and withdrawing fluid from a portion of lung (BAL) and counting the numbers and types of cells recovered /84-86/. The BeLPT is an in vitro test that measures lymphocyte hyperproliferation in BAL cells and peripheral blood mononuclear cells in response to beryllium compounds, such as beryllium sulfate ($BeSO_4$) /87/. Laboratories call a test *abnormal* if lymphocytes react strongly, *normal* if lymphocytes do not react, or *borderline* if the response is weak or indeterminate. An abnormal result must be confirmed to assure appropriate referral for CBD medical evaluation.

As with any other screening test used by the medical community, this assay has certain limitations, namely, the results of a BeLPT may appear abnormal when a person is not sensitized or normal when a person actually is sensitized. In one study, the test was reported to show low sensitivity with a high rate of false normal results (31.7%) in known sensitized individuals /88/. Another study found limited inter- and intra-laboratory reproducibility of abnormal BeLPT results /89/. Although concerns have been raised over the limitations of the blood BeLPT /90/, DOE considers the test to be a reliable tool for screening individuals for BeS /91/. A published evaluation /88/ of the commonly used BeLPT method used for 12,194 current and former workers at 18 DOE sites found the test to positively identify BeS at a rate comparable to other widely accepted medical tests. Thus, BeS could be called a biomarker of CBD, with a sufficient predictive value to make it useful both for screening for identifying individuals with CBD and for the surveillance of populations to understand the prevalence and risk factors for CBD.

Cytokines/chemokines production. The antigen-specific inflammatory response is a cell-mediated process coordinated by cytokines that stimulate both humoral (antibody) and cellular immune responses and activate phagocytic cells, which ingest and destroy foreign matter (phagocytosis). Chemoattractant cytokines (chemokines) promote the migration of cells of the immune system to a site of infection. Cellular and molecular studies have demonstrated that beryllium exposure induces specific cytokines and chemokines responses that modulate host immunity. In the presence of APCs, the beryllium antigen, which may be a Be-peptide complex, induces strong proliferative responses of BAL CD4(+) T cells, induces the production of super-optimal concentrations of the proinflammatory cytokines interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and interleukin-2 (IL-2); while upregulating several T-cell surface markers that promote T-T antigen presentation /92-95/. In beryllium disease, alveolar macrophages express elevated levels of mRNA for TNF-α and IL-6, but not for IL-1β. Additionally, increased levels of IL-2 and IL-2 receptors are involved in T-lymphocyte proliferation /96-97/. Using BAL cells from CBD patients in short-term tissue culture, Tinkle et al. /98/ evaluated cytokine protein levels using ELISA and T-lymphocyte proliferation. Beryllium stimulated the release of TNF-α, IL-6, IL-2, and IFN-γ, but not IL-4. The Be-stimulated IFN-γ release remained elevated after IL-2 levels returned to baseline.

Immunogenetics/Role of the MHC

Recent technological advances have shed light on genetic associations in complex disease. The genes of the vertebrate major histocompatibility complex (MHC) locus, which is located on human chromosome 6, have been identified as important determinants in diseases caused by both inorganic and organic compounds. The MHC complex comprises two types of molecules:

1. Class I molecules expressed on most cell types and usually present peptides derived from any protein synthesized in a cell /99/; and

2. Class II molecules expressed only on a limited number of specialized cell types, denoted antigen-presenting cells (APC), comprising B cells, macrophages, and dendritic cells).

The loci of the MHC, called HLA (human leukocyte antigen) in humans, encode cell-surface glycoproteins that collect peptides inside the cell and present them on the cell surface for recognition by T-cells as part of the mechanism for identifying foreign antigens and producing an immune response /100/. The T-cell receptor (TCR), a cell-surface antibody-like molecule, is responsible for recognizing antigens bound to MHC molecules.

In contrast to the cytosolic proteins presented by Class I, the peptides presented by Class II molecules derive from extracellular proteins that have undergone endocytosis, lysosomal digestion, and binding to the Class II molecule before migrating to the plasma membrane. Class II molecules interact exclusively with CD4+ (helper) T-lymphocytes, which determine the immune responsiveness against microbial or environmental antigens and allergens /101/. The MHC Class II (HLA D) locus contains at least three defined sub-regions, HLA-DP, HLA-DQ, and HLA-DR, each coding for heterodimers comprising two homologous, non-covalently linked glycopeptides, denoted as α and β chains, both anchored in the membrane.

Haptenization. A hapten is a small molecule that can elicit an immune response only when attached to a large carrier, such as proteins or peptides. Metals can (a) haptenize peptides to generate neoantigens that can be bound by HLA molecules and induce allergy or (b) bind directly to the HLA molecule, thus inducing hypersensitivity to an altered self-HLA /100/. Research to date suggests the following chain of events: following inhalation, the beryllium ion forms a complex with an as yet uncharacterized peptide or protein in the body, thereby creating a Be-antigen that, in turn, is selected as a specific antigen/hapten for presentation to T cells.

Genetic Susceptibility to CBD

A number of genes are known to be involved in the risk of sensitization and disease. The prevailing view is that most individuals must first be sensitized before beryllium in the lungs can cause the lung damage of CBD, based on studies showing that almost all individuals with CBD are also sensitized /96,102/. Susceptibility to BeS and CBD is predominantly associated with the alleles of Class II HLA-DP that contain glutamic acid (E) at the 69th position of the β chain (HLA-DPβ-E69) (*vide infra*). As other susceptibility genes may not yet have been discovered, research on this issue is still in progress.

The Be-antigen bound to HLA-DPβ-E69 is presented by APC to a Be-specific CD4+ T-lymphocyte clone, resulting in a series of biochemical events leading to T-cell activation /103-105/. Progression to CBD appears to depend on the genotype and phenotype of the exposed individual. Although workers having HLA-DPβ-E69 appear to be at the greatest risk of developing CBD, this allele is also present in up to 48% of Be-exposed people who do not have CBD, meaning that many people with this allele do not develop the disease. Such resistance could be due in part to other genetic considerations that will be discussed in the section on biomolecular research /106/.

Allelic polymorphism in MHC genes has long been known to determine the immune response variation to individual antigens, including auto-antigens, thereby making a major contribution to susceptibility and resistance in many diseases /107-109/. Specific genetic polymorphisms in MHC class II and proinflammatory genes have been investigated in BeS and CBD, as well as the role of TCR expression and other potential modifier genes. Although such polymorphisms for genetic susceptibility could be used to identify disease risk, ethical and social concerns have limited the value of wide-scale genetic screening in occupational settings. Thus, large scale genetic screening in the workplace is not currently recommended /110-

111/, and the future usefulness of genetic testing remains to be determined. In the industrial environment, both genetic and occupational factors appear to have at least an additive effect for risk of beryllium disease /112/.

Antiproliferative Effects

Normal cells do not divide indefinitely. After completing a sufficient number of cell divisions, cells exit the cell cycle and enter replicative senescence, a state of irreversibly arrested proliferation and altered differentiated function /113-114/, presumably to protect mammals from developing cancer. The most potent general effect of the beryllium ion is the production of cell-cycle arrest with premature expression of the principal markers of senescence /115/. Beryllium salts inhibit cell growth in cultured chick embryo fibroblasts and skeletal muscle cells /116/; and in rat liver cells $BeSO_4$ arrests the cell cycle at the G1 phase /117/. Similar cell-cycle effects have been reported in primary cultures of human skin fibroblasts and in human lung fibroblasts /118/. These experiments will be discussed in the section on biomolecular studies.

ROUTES OF HUMAN EXPOSURE

Exposure to environmental levels of beryllium can occur by breathing air, eating food, or drinking water containing the element.

Respiratory Exposure

Most beryllium exposures that cause disease are related to some aspect of beryllium processing. When any form of beryllium is milled, lathed, deburred, sanded, polished, or otherwise machined, the process liberates micron- and submicron-sized particles that deposit deeply and invisibly in the lungs. As beryllium particles are inhaled into the lungs and upper respiratory tract, the major pathway for human exposure is through airborne particles of beryllium metal, alloys, oxides, and ceramics. Cold working of alloys containing beryllium (milling, lathing, machining) have a much lower potential for generation of fine particulates /119/. Hot working (includes heat treating) tends to produce micron and submicron particles more readily. As naturally occurring beryllium can be inhaled from cigarette smoke, smokers breathe higher levels of beryllium than nonsmokers.

The metal is mined commercially from the naturally occurring silicates bertrandite and beryl. An occupational study, with limited sample size, performed by Deubner et al. in 2001 /56-57/ suggested that the actual mining of beryllium presents less risk of CBD or beryllium sensitization than do the other production activities that follow the mining. The authors hypothesized that exposure to the natural ore, beryl or bertrandite, may pose less of a risk for developing CBD as compared with exposure to beryllium oxide particulates. Beryllium has been identified in at least 535 of the 1,613 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (see http://www.epa.gov/superfund/sites/npl).

Ingestion Exposure

Beryllium has been detected in only 5% of 1,577 drinking water samples obtained throughout the US Beryllium is naturally present in various foods, with a median concentration of 22.5 $\mu g\ kg^{-1}$ reported across 38 different food types, ranging from less than 0.1 $\mu g\ kg^{-1}$ to 2,200 $\mu g\ kg^{-1}$. When compared with other harmful elements like lead and chromium, however, beryllium exposure through food is not significant. Children can be exposed to a limited extent by ingesting soil.

Dermal Exposure

Skin exposure to beryllium has long been acknowledged as a possible contributor to sensiti-

zation /120/. A group in Canada aimed to calculate dermal and inhalation exposures and apply the results to the case study of a recycling SPL industry /121/. The daily dose for the respiratory route was calculated to be 0.022 $\mu g\ kg^{-1}\ d^{-1}$, whereas the daily doses for the dermal route varied between $0.027 \times 10^{-7}\ \mu g\ kg^{-1}\ d^{-1}$ and $0.025 \times 10^{-3}\ \mu g\ kg^{-1}\ d^{-1}$, indicating that the dermal daily dose exposure was negligible. The authors caution that the case study did not involve the handling of contaminated items by the workers, which does lead to significant dermal exposure if care is not taken. Recently the contribution of dermal exposure has resurfaced.

HUMAN HEALTH EFFECTS

The major adverse health of effects of beryllium exposure are respiratory effects following inhalation exposure, gastrointestinal effects following oral exposure, and skin effects following dermal exposure. In humans, inhalation appears to be the primary route of exposure to beryllium. Exposure to airborne beryllium-containing particles can cause three distinct pulmonary conditions:

1. *Acute beryllium disease*, an acute chemical pneumonitis characterized by dyspnea, cough, and chest pain (now considered rare due to education and increased hazard mitigation);
2. *Beryllium sensitization* (BeS), Exposure to *beryllium* can lead to *sensitization*, a cell-mediated allergic-type response that is measured by the BeLPT or other measures of the specific immune response to beryllium, but who do not yet have any evidence of disease;
3. *Chronic beryllium disease* (CBD), a condition characterized by a granulomatous or mononuclear cell infiltration of the lung and an abnormal Be-LPT response or positive skin patch test in blood, lung, or skin /122/.

Occupational exposure to high concentrations of soluble beryllium compounds can result in acute beryllium disease, whereas exposure to both high and relatively low concentrations ($< 0.5\ \mu g/m^3$) of soluble or insoluble beryllium compounds can result in CBD.

Acute Beryllium Disease

In the past, acute beryllium disease was acquired by inhaling high concentrations of beryllium ($> 1\ mg/m^3$). In 1943, workers in the US who were exposed while extracting BeO from beryllium ore contracted the disease /22/. The onset of severe respiratory symptoms usually occurred over several weeks, with the autopsy results of fatal cases showing pulmonary edema, mononuclear cell exudate, fibrosis, nodules; one had well-defined granulomas /48/. With treatment and removal from exposure, most patients recovered over several months, yet upon renewed exposure, the condition recurred /22/. By 1945, 170 cases of acute beryllium poisoning had been reported, of which 5 died. Among the fatalities reported, pathologic changes showed edema, infiltration with mononuclear inflammatory cells, alveolar cell proliferation or desquamation, and the absence of granulomas /123/. Improvements in industrial hygiene have essentially eliminated acute beryllium disease in the US

Chronic Beryllium Disease and Sensitivity

The spectrum of CBD has three stages: Be sensitivity, subclinical CBD (detectable since 1999), and clinical CBD /21/. Although BeS (characterized by a consistently abnormal BeLPT) can progress to CBD, not all sensitized individuals develop CBD. In subclinical CBD, individuals are sensitized to beryllium and have histologic evidence of lung granulomas, but show no clinical signs. Although slight alterations in lung function during exercise have been observed in ~60% of individuals with subclinical CBD, no other

consistent alterations in lung function were found. Individuals with clinical CBD, on the other hand, are Be-sensitized, have histologic evidence of lung granulomas and respiratory symptoms, changes on chest radiographs, and/or altered lung function /5/.

Beryllium sensitization and/or CBD have been detected at exposure levels of 0.5 $\mu g/m^3$. High concentrations of soluble salts can also result in CBD. Cases of subclinical CBD and BeS have been detected during the past decade /37,58,124-125/. Nevertheless, respiratory disease is not likely to occur from exposure to beryllium levels in the general environment because the ambient air levels of beryllium are 0.03-0.2 ng/m^3. Yet in humans, CBD development has been reported following long-term exposure to relatively low concentrations (< 2 $\mu g\ m^{-3}$) of beryllium (expressed as BeO) /35,129-130/.

Inhalation exposure. Chronic beryllium disease is characterized by a debilitating and incurable damage of lung tissue /52,131-134/. Inhalation is the only mechanism that has been proven to cause CBD. In the respiratory tract, the epithelial cells lining the airways are the primary defensive contact against inhaled foreign substances like particulate beryllium. Hence, some but not all genetically susceptible individuals develop an inflammatory reaction that principally targets the respiratory system and skin. Beryllium sensitivity and CBD remain an important occupational and environmental health concern. According to Newman and co-workers /84,96,135/, sensitization precedes clinical CBD.

Role of skin exposure. As expected reductions in BeS/CBD cases did not ensue following regulatory reductions in inhalation exposure to beryllium, the role of skin exposure in BeS and CBD has been gaining attention /124,136-137/. Moreover, the results of several workplace epidemiologic studies failed to support the hypothesis that dose by the respiratory route determines the risk of disease. Thus, whether CBD is related only to direct pulmonary exposures to beryllium, or whether sensitization or disease outcome or both are also related to dermal exposure or to systemic burdens of beryllium is not clear. The analysis by Deubner et al. /56-57/ indicated that incidental routes of exposure could contribute to the total absorbed doses of beryllium that exceed simple airborne inhalation exposures. The authors found unpublished reports of BeS, as measured by BeLPT, in persons who had been used as 'normal' controls having no known occupational exposure to beryllium. Other potential mechanisms leading to BeS are currently being studied.

Whether less soluble particulate forms of beryllium (metal, oxides, and alloys), the primary media of work exposure, can penetrate human skin remains an open question. Workers who have skin lesions that presumably increase beryllium uptake /138/ have been reported to have an increased risk of CBD /89,139/. Cummings et al. /140/ described the effects of a comprehensive prevention program in one BeO ceramics plant targeted at both respiratory and dermal protection. Although the implementation of the extensive use of personal protective equipment (PPE) and administrative changes geared to reducing beryllium in the air, on all work surfaces, and on skin has substantially decreased the rate of BeS, the effectiveness of PPE is unclear. For example, Dufresne et al. /141-142/ recently conducted an exposure assessment at a copper-beryllium alloy facility and removed beryllium from the surfaces by serial wipe sampling at concentrations exceeding the US DOE standard limit of 0.2 $\mu g/100\ cm^2$. Air beryllium concentrations correlated strongly with the degree of contamination of work surfaces, and concentrations on work surfaces, gloves, and skin correlated as well. The authors concluded that from a safety standpoint in occupational settings, workers should be offered skin protection and respiratory protection if they must handle devices made of Be-Cu alloy.

If followed by respiratory exposure, skin exposure to other occupational and environmental sensitizers has been shown to lead to systemic

sensitization that can progress to lung disease /142/. The role of skin in the development of other systemic, as well as dermatologic, immune diseases supports the potential of skin exposure. Skin as a potential route of exposure and sensitization has several important implications for pathogenesis, risk factors, diagnosis, and the prevention of immune-mediated diseases like CBD and asthma. For example, the risk associated with beryllium could depend on its route of entry. Soluble metals and liquids could make beryllium more bioavailable to the skin, whereas respirable particles and vapors would be more bioavailable to the lung; If skin exposure can lead to sensitization, then regulatory standards based on air concentrations, even if very low, could fail to prevent sensitization or to abolish the risk of CBD.

Pathology. In Be-exposed persons, the main pathologic processes that can lead to CBD can be described as follows (see http://www.nap.edu/catalog/ 12464.html):

A. Beryllium sensitization: Between 1% and 15% of exposed workers become sensitized to beryllium following exposure. The incubation period for sensitization is as short as a few months of exposure (1,129,143/. Beryllium sensitivity induced through dermal contact could make the individual more susceptible to CBD should they subsequently inhale beryllium /120/.

B. Chronic disease pathology. In sensitized individuals, beryllium exposure can result in the formation of granulomas (inflammatory cells surrounding beryllium particles) in the lung that reduce oxygen exchange. The symptoms include fatigue, non-productive cough, gradually progressive shortness of breath, chest pain, anorexia, weight loss, fevers, night sweats, and joint and muscle pain. The clinical picture includes cyanosis, hypertrophic osteoarthropathy (digital clubbing), and dry bilateral rales on auscultation. Less frequently lymphadenopathy, hepatomegaly, splenomegaly, skin lesions, and thyromegaly can occur. Pulmonary infiltrates are usually seen on chest X-ray, with arterial blood gas analyses typically showing hypoxemia /144/.

Once CBD is clinically apparent, the disease process can continue to progress if left untreated. Although some patients with clinical CBD have been reported to go into remission if treated, or at least to stabilize clinically upon removal from exposure, most studies indicate that disease progression is the general rule, even after the patient leaves the workplace /145-147/. No cure has yet been found for CBD. Although the lung is the principal organ involved, the granulomatous inflammatory response can affect the skin, liver, and lymphatic system as well /123,148/.

Hardy and Tabershaw /149/ reported the first cases of CBD in 1946, a delayed chemical pneumonitis among former employees of fluorescent-lamp plants and industrial plants that refined and manufactured beryllium metal and beryllium alloys. At that time, the clinical and morphologic characteristics of chronic berylliosis (now known as CBD) were established, namely, symptoms of tachypnea with inspiratory rales and tachycardia. By 1948, the risk of disease among beryllium workers was variable and increasing with the intensity of airborne exposure /150/; CBD progression could vary among individuals as well. Despite an extensive drop in respiratory exposures to beryllium after regulatory measures were imposed, the disease continues to occur in exposed workers in the US because of the growing number of new applications of beryllium-containing materials in industry. Cases are also being reported outside the US /39,40,151-154/. Hence, the chronic form of the disease is global and far from being eradicated. Cases of CBD and Be-S among beryllium industry workers can be expected to increase.

The disease can be caused by virtually any form of beryllium dust or fumes, ranging from beryllium alloys (for example, aluminum-, copper-, nickel-, and magnesium-Be) and beryllia ceramics to pure metal /155/. Once inhaled, beryllium can initiate

the disease in the body at any time. A single exposure is sufficient for a lifelong risk of developing the disease. The suggested latency period is quite variable and can range from a few months to 30 or 40 years or more after first exposure. A true latency period may be difficult to ascertain because even when CBD is suspected, fulfilling current diagnostic criteria can be challenging. Moreover, as beryllium can be one of many metals present in an alloy, a history of beryllium exposure can be difficult to obtain because workers might not be aware of exposure.

Because CBD is caused by an allergic reaction to beryllium, very little exposure is required to cause the chronic form of the disease. Before the widespread BeLPT surveillance program, strict adherence to the standards of 2 $\mu g.m^3$ as an 8-h time-weighted average and a 30-min peak standard of 25 $\mu g.m^3$ /58/ appeared to be sufficient to protect workers from CBD. Certain individuals, however, have developed CBD after short exposures below the current occupational exposure limits of 2 $\mu g.m^3$. In 2000, Wambach and Tuggle /156/ recommended a protective 8-hour TWA level of 0.1 $\mu g/m^3$ for occupational exposure to beryllium.

The diagnosis of CBD, based upon criteria established by the Beryllium Case Registry, includes the following: evidence of significant beryllium exposure, abnormalities in chest x-ray and lung function tests, presence of beryllium in lung or other tissue, presence of granulomatous and/or mononuclear cell inflammation in a biopsy specimen, and a proliferative response of blood or lung T cells to beryllium salts in vitro /157/. As the diagnosis is complicated by sarcoidosis, a similar lung disease of unknown etiology that shares virtually all of the symptoms of CBD /158-159/, the only sure diagnosis of CBD would include elemental analysis for beryllium from lung biopsy. Because CBD is insidious, developing slowly over time, workers may have the disease for years without knowing it, and with progression, the disease can sometimes be fatal.

Immunopathogenesis of CBD

Research during the past decade has advanced our understanding of the epidemiology of CBD and improved our knowledge of the immune basis and immunogenetics of this disease. This section will focus on the latest developments in chemistry and immunology /84,160/ that have led to major advances in the understanding of Be-related diseases.

The CBD process begins when the immune system recognizes the beryllium particles. Sterner and Eisenbud /52/ first proposed an immunologic mechanism of CBD in 1951. The immunopathogenesis of CBD and beryllium sensitivity depends on the development of a typical antigen-specific, T lymphocyte-mediated immune response (delayed hypersensitivity type) to beryllium that releases inflammatory mediators. An accumulation of inflammatory cells in the lung leads to the formation of a noncaseating granuloma, mononuclear cell infiltrates (predominantly epithelioid cells and lymphocytes), and fibrosis. After beryllium skin-patch testing of CBD subjects, an analysis of TCRs in the development of granulomas suggested that clonal T cells, similar to those found in the lung, are mobilized from the blood and infiltrate the affected tissue /157/. Around the beryllium particles, the stimulated T cells form balls called 'granulomas', nodules consisting mainly of epithelioid macrophages and other inflammatory and immune cells, as well as an extracellular matrix, that interfere with the normal lung function. The lungs become stiff and lose their ability to transfer oxygen from the air into the bloodstream. Disease prevalence has often been underestimated because of the long latency period of the disease and because of its elusive clinical and pathological presentation /75,103/.

Disease Progression

Although CBD is a progressive disease, the rate of progression can vary among individuals. Many

years after cessation of exposure /161/, beryllium can persist within the lungs of individuals, thereby resulting in continuous antigenic stimulation /162/ that could facilitate ongoing Be-specific immune responses. The number of longitudinal studies of progression of BeS to CBD is limited. An early study by Rom et al. /163/ of 82 milling and mining workers relied on only one abnormal result of an early version of the BeLPT to determine sensitization. Therefore, the finding that none of 13 beryllium-sensitized workers developed CBD during the following 3 years, with a few showing a possible reversal of sensitization, is questionable.

A small study by Kreiss et al. /36/ found that three of six nuclear weapons BeS workers progressed to CBD over a 2-year time period. One additional individual was found to have progressed to CBD on his fourth follow-up evaluation, 9.5 years after the initial BeS diagnosis, an overall rate of 68% progression. Machinists showed a sensitization rate of 14.3% compared with a rate of 1.2% among other employees. Machining process daily weighted average (DWA) estimates of exposure accounted for most DWAs exceeding the 2.0 $\mu g/m^3$ OSHA standard, with 8.1% of machining DWAs being above the standard.

Newman et al. /164/ recently reported that 31% of 55 BeS individuals who had shown no evidence of CBD upon initial lung biopsy developed CBD on subsequent clinical evaluation over a mean follow-up period of 3.8 years (range, 1.0 to 9.5 years). The authors estimated a conversion rate from BeS to CBD of 6% to 8% per year. Chronic disease was defined as developing granulomas and/or mononuclear cell infiltrates on repeat transbronchial lung biopsy. One individual was diagnosed as having CBD based on the development of a significant BAL lymphocytosis (56% lymphocytes), an abnormal BAL BeLPT, shortness of breath and fatigue, and decrements in other measures of physiology. No statistically significant difference in age, gender, race, ethnicity, or smoking status at time of initial evaluation between those who progressed and those who remained sensitized was noted. Thirty-eight of the fifty-five (69%) remained beryllium sensitized without disease after an average follow-up time of 4.8 years (range, 1.7-11.6 years). No differences in baseline chest x-ray, pulmonary function, and/or exercise tolerance test measures were found for those who remained sensitized compared with those who progressed. Those who progressed were no more likely to have current beryllium exposure at the time of their CBD diagnosis then those who remained sensitized. Further followup of 11 of the 17 who developed CBD revealed that some showed declines in lung-function. Additional longitudinal follow-up of the remaining sensitized individuals will be important to determine how many of the original cohort will eventually progress to disease. The presence of BAL lymphocytosis at the time of baseline evaluation and being a beryllium machinist were identified as the only significant risk factors for progression from BeS to CBD. The time from first exposure to the development of CBD, ranging from 3.5 to 44.5 years, implies that those with BeS remain at lifelong risk of progressing to CBD. One limitation of this study is that individuals who may have had normal pathology on first evaluation and who then develop abnormal pathology on subsequent evaluation could have been missed on the initial evaluation because of sampling error. Moreover, the lack of personal exposure monitoring data for most individuals means that measuring quantitative beryllium exposure is impossible. Interestingly, two progressors had only incidental or bystander exposure.

CARCINOGENIC POTENTIAL OF BERYLLIUM

Debate has raged over the carcinogenic potential of beryllium. The risk to workers depends considerably on their work tasks. The National Toxicology Program /165-166/ lists beryllium and certain of its compounds—beryllium-aluminum alloy, $BeCl_2$, BeF_2, $Be(OH)_2$, BeO, $BeSO_4$, beryllium

phosphate, beryllium zinc silicate, and beryl ore—as substances reasonably anticipated to be carcinogens. The IARC /27,167/ classifies beryllium and beryllium compounds in Group 1, carcinogenic to humans. The US EPA /7/ classifies inhaled beryllium in Group B1, a probable human carcinogen /6/. NIOSH /30/ recommends that the element be treated as a potential human carcinogen and advises a 10-hour TWA not to exceed 0.5 $\mu g/m^3$. The US EPA inhalation unit risk for carcinogens is the upper-bound excess lifetime cancer risk estimated to result from continuous exposure to an agent at a concentration of 1 $\mu g/m^3$ (reviewed in /168/). For beryllium, the unit risk calculated in 1987 was estimated to be 2.4×10^{-3} per $\mu g/m^3$, based on a 1970s occupational-exposure study by Wagoner et al. /169/. To date, the US EPA has issued no new reassessment of beryllium cancer risks. Based on the original unit risk value stated by the US EPA, air concentrations of 0.04, 0.004, and 0.0004 $\mu g/m^3$ are estimated to result in respective cancer risks of 1×10^{-4}, 1×10^{-5}, and 1×10^{-6}.

The Committee on Beryllium Exposures of the National Academy of Sciences agrees with the other agencies that the balance of the evidence supports a conclusion that beryllium is likely to be a human carcinogen. The committee concludes that a meaningful cancer dose-response assessment cannot be conducted until more information is available on existing or new worker cohorts: complete work history, possible exposure to other carcinogens, and better exposure histories (see http://books.nap.edu/catalog.php?record_id=12464).

Epidemiologic Studies on Beryllium and Cancer

Several epidemiologic studies have reported excess lung cancer deaths among Be-exposed employees /28,170/ (reviewed in 171/), but unequivocal conclusions could not be made due to little or lack of consideration of smoking history or exposure to other potential lung carcinogens, or due to underestimating expected cancer deaths in control populations /172/. Early studies showed an increased risk of lung cancer among beryllium-exposed workers and among workers with acute beryllium disease or CBD, being more pronounced among those with acute beryllium disease (SMR = 2.32) than among those with CBD (SMR = 1.57) /173/. An IARC report /171/ stated that several aspects of studies by Steenland and Ward /173/ support the conclusion that beryllium is a human carcinogen. Briefly, this conclusion was based on the consistency of lung cancer excess in most locations studied, (b) greater excess cancer risk in workers hired before 1950 when beryllium levels were much higher than in subsequent decades, and (c) the highest risk of lung cancer in individuals with acute beryllium disease and at the facility with the greatest proportion of acute beryllium disease. The IARC also pointed out the following limitations: absence of individual exposure measurements, relatively low excess lung-cancer risks, and absence of any mention of exposures to other lung carcinogens in the workplace. Noteworthily, studies implicating beryllium as an occupational carcinogen examined lung cancer in cohorts exposed in the 1930s and 1940s, before industrial hygiene controls were in place and when concentrations were orders of magnitude higher than permitted today. Since then, statistically significant increases in lung cancer have been difficult to demonstrate in workers exposed to lower levels /172/.

In 2001, Sanderson et al. /50/ conducted a large study using a nested case-control design in an occupational cohort and controls by attained age. Exposure was characterized as employment duration, cumulative exposure, average exposure, and maximum exposure, which was truncated at the age of death of the lung cancer case. Cases were compared with controls without adjustment for covariates. Unlagged exposure was not importantly increased in cases. With 10- and 20-year lagging of exposures, cases had much higher exposures than controls. Subsequently, three additional analyses on the methodology in that study have been reported. In a reexamination of the

results, Levy et al. /174/ recognized that case-control differences in age at hire would introduce confounding of lagged exposure; controlling for that factor resulted in a decrease in all case-control differences in 10-year lagged exposure. Schubauer-Berrigan et al /175/ then added date-of-birth and age-at-hire confounding to the analysis, which further reduced the odds ratios for cumulative exposure at a lag of 10 and 20 years and for average exposure and maximum exposure at a lag of 20 years, with only a minimal decrease in the odds ratios for average exposure and maximum exposure at a lag of 10 years.

In 2004, Brown et al. /176/ published a case-control study of lung cancer and internal doses of plutonium among workers at the Rocky Flats plant in Colorado. This study also obtained information on smoking histories and on cumulative exposures to four lung carcinogens. No associations were found between lung cancer mortality and cumulative exposures to asbestos, beryllium, hexavalent chromium, or nickel.

Genotoxicity Studies and Beryllium Exposure

No study has been identified by ATSDR regarding genotoxicity in humans after inhalation, oral, or dermal exposure to beryllium or its compounds. Overall, the genotoxicity assays of soluble beryllium compounds are inconsistent, possibly due to each of the different chemical forms of beryllium having a unique effect on mutagenicity. Soluble beryllium compounds appear to be weakly genotoxic. Although mammalian test systems exposed to beryllium compounds have provided evidence of mutations, chromosomal aberrations, and cell transformations, bacterial assays were predominantly negative. $BeCl_2$, $Be(NO_3)_2$, $BeSO_4$, and BeO were not mutagenic in the *Salmonella typhimurium* reverse mutation assay /177-180/, nor in mutation assays with *Escherichia coli pol A*, *E. coli* WP-2 *uvr A*, or *Saccharomyces cerevisiae* /5,7,181-182/. Although $BeSO_4$ yielded positive genotoxicity results in bacterial rec assays /183-185/, the incidence of micronucleated polychromatic erythrocytes did not increase in bone marrow /186-187/. Morita et al. /186/ found that the micronucleus assay is generally less sensitive to metals and their compounds. Lung tumors in F344/N rats treated with $BeSO_4$ showed no mutations of the *p53* or *c-raf*-1 gene, yet weak mutations were found in the K-*ras* gene /188/.

In the *B. subtilis* rec assay /189/, $Be(NO_3)_2$ was genotoxic and induced clastogenic alterations in cultured mammalian cells. Beryllium chloride was mutagenic in the *E. coli* forward-mutation assay /190/, the *Photobacterium fischeri* assay /191/, and the *B. subtilis* rec assay with spores /189/, and was mutagenic for cultured mammalian cells as well /192-194/. Both $BeSO_4$ and $BeCl_2$ induced gene mutations in whole mammalian cell cultures /193-194/. Only one study has shown that $BeSO_4$ can induce chromosomal aberrations in mammalian cells /195/, with other studies reporting that this compound does not /1 78,187/. Beryllium sulfate did not affect DNA-repair in mammalian cells nor induce un-scheduled DNA synthesis in primary rat hepatocytes /196-197/.

OTHER HEALTH EFFECTS

Other systemic effects are not common and are usually secondary to CBD or related to extra-pulmonary granulomatous lesions. The US EPA /7,181/ noted that systemic effects of inhaled beryllium other than those seen with CBD would be expected to occur only after exposure much greater than the lowest doses that induce CBD or cancer. No human studies were identified that could be used to derive an oral reference dose (RfD), the maximum acceptable oral dose of a toxic substance. Only a few studies have been conducted to evaluate mortality rates associated with CBD /28,173,198-199/. Among Be-exposed workers, excess mortality has been observed for heart disease, diseases of the respiratory system (for example, emphysema, pneumoconiosis), and

diseases of the genitourinary system /28,173,198/ (cited in /106/).

Dermatitis

Exposure to beryllium dust or to soluble beryllium compounds can cause a skin disease characterized by poor wound healing and wart-like bumps /200/. A skin rash can also be one of the early symptoms of CBD /120,201 /. Metal-induced allergic diseases are diagnosed on the basis of tests with metal antigens, including skin tests, the radioallergosorbent test for specific antibody, LT test, macrophage migration inhibition test, and provocation test. For example, a delayed skin hypersensitivity reaction occurred in 30% to 60% of pre-sensitized guinea pigs challenged with copper-beryllium alloys and aluminum-beryllium alloy /202/. If beryllium particles become embedded in the skin through a scrape or cut, then skin ulcers may occur. For the wounds to have any chance of healing, the beryllium particles must be removed.

Reproductive Effects

A hazard assessment of environmentally relevant doses conducted by the US EPA /181/ concluded that the potential of beryllium to induce developmental and/or reproductive effects has not been adequately assessed. The agency identified only one study of reproductive and developmental outcome in workers, a case-control study /203/ examining the effect of parents' occupational exposure, including beryllium, on the risk of stillbirth, preterm delivery, and small-for-gestational-age infants. No reproductive or developmental effects after paternal occupational exposure to beryllium were found. Neither ATSDR /5/ nor ACGIH /29/ have described any human studies.

Cardiovascular Effects

In humans, ATSDR /5/ concluded that the right atrial and ventricular hypertrophy described by Hardy and Tabershaw in 1946 /149/ are not likely to be due to direct toxicity to the heart, but rather occur as a response to impaired lung function. From animal observations, ATSDR concluded that the effects of beryllium compounds on the cardiovascular system probably represent compensatory increases in cardiac musculature due to pulmonary fibrosis caused by inhalation exposure, and that decreased arterial oxygen tension reflects the reduced ability of the lung to oxygenate blood.

Hematologic Effects

Information from human case studies /23,204/ has not revealed any significant effect on hematology parameters or blood chemistry. Animal studies have shown that intermediate-duration, high-dose exposures can cause anemia in some species but not in others /5/.

Hepatic Effects

Information regarding hepatic effects in humans after inhalation exposure to beryllium and its compounds is limited. A 10-month follow-up of laboratory workers exposed to an undetermined concentration of beryllium chloride over a period of 10 to 20 hours showed no increase in liver enzymes, serum glutamic oxaloacetic transaminase, or serum glutamic pyruvic transaminase /71/. Autopsy revealed hepatic necrosis in only 1 of 17 individuals exposed to beryllium in a plant that manufactured fluorescent lamps /149/. In animals, hepatic effects have been observed only at lethal exposure levels after inhalation exposure to beryllium and its compounds.

Renal Effects

Kidney stones and an excess of calcium in the blood and urine, which are seen quite frequently in patients with CBD, are only suggestive and cannot be absolutely attributed to beryllium disease /205/. In one cohort mortality study of workers employed

at beryllium manufacturing facilities, Ward et al. /28/ reported an increased risk of death from chronic and unspecified nephritis, renal failure, and other renal sclerosis.

Overall Evaluation of Human Health Risk

Even when human data are available, risk assessment is inherently uncertain and often controversial. The risks associated with exposure to a hazard can be expressed by individual lifetime risk, annual population risk, the percentage or proportion of increase in risk, and loss of life expectancy /206/. Bailar and Bailer /207/ introduced several concepts about risk assessment, including hazard identification, dose-response modeling, exposure assessment, and risk characterization. Not every person exposed to a potential hazard will exhibit an adverse response. The frequency or magnitude of an adverse response generally depends on the degree and extent of exposure to a hazard, possibly with a threshold below which no risk is apparent. Moreover, the risk for any individual depends on intrinsic factors like age, gender, prior or concurrent exposures to other hazards, and on the level of detoxifying enzymes. Subgroups (infants, the elderly, and those with impaired immune systems) could be at unusually high risk. As data for the direct measurement of human risk are often absent or seriously inadequate, biological processes can sometimes cause low-level exposures to be almost as hazardous as high-level exposures /208/.

Although no linear relation has been found between the level of exposure to beryllium and the risks of BeS and CBD, the highest exposures are associated with an increased risk. Insoluble particles of small diameter are probably associated with increased risk. An environmental change is likely to alter the speciation resulting in the development of disease /160/ (*vide infra*). The results of one small exposure study at a beryllium mining and extraction facility suggest that a specific form of beryllium might affect the likelihood of developing CBD. Specifically, exposure to beryl and bertrandite ore dusts or to beryllium salts, in the absence of exposure to BeO particulates, appears to pose a lower risk for developing CBD /56/.

BIOMOLECULAR RESEARCH

The genetic susceptibility of BeS and CBD pointed the way toward defining the molecular role of beryllium in the disease, providing a unique opportunity to apply inorganic chemistry and immunology to explore the disease process at the molecular level.

Be-Ferritin

Beryllium binds to the ubiquitous iron storage and transport protein ferritin, a high molecular weight protein (MW > 400,000 Da) comprising 24 subunits /209/, each composed of four α-helices that form parallel cylinders with an external shell and a phosphorylated internal core that binds ~4,500 ferric atoms in a crystalline inorganic complex. Beryllium binds tightly to the ferritin subunit core region but not to the protein shell, with no displacement of iron from the core /210/. These findings are important because Sawyer et al. /200/ found that a Be-ferritin adduct acts as a "Trojan horse", triggering the proliferation of Be-ferritin-specific CBD BAL T cells and inducing the apoptosis of BAL macrophages. The induction of apoptosis in the same lung macrophage population that is Be-metal-specific and independent of lung granulomatous inflammation, while triggering T-cell proliferation in CBD, supports the hypothesis that this dual effect might explain the persistence of Be-antigen and a failure to delete Be-reactive T cell clones in the lungs of patients with CBD.

Beryllium Cation

All known DNA polymerases require either Mg^{2+} or Mn^{2+} as an added divalent cation for

activity /202/, which in cells is presumably Mg^{2+}. Beryllium cation apparently cannot substitute for Mg^{2+} as a metal activator /213-214/. Beryllium cation has been shown to interfere with the in vitro enzyme function /215/ and fidelity of DNA synthesis /216-217/, with protein phosphorylation /218/, and with cell division /118/.

The beryllium cation is the only divalent cation that is smaller than the physiological cations Mg^{2+} and Ca^{2+}, and its small ionic radius gives Be^{2+} exceptional charge density. Because of its unique properties, Be^{2+} has the potential to bind tightly to clefts in proteins that are inaccessible to other cations. These considerations make it possible for beryllium to exert biological effects that are unlike those of any other metal salt.

At the molecular level, the most potent general effect of Be^{2+} is the production of cell-cycle arrest, causing a specific block at the G1 phase /117,219/. The major cell-cycle regulatory proteins that are associated with replicative senescence are the tumor suppressor protein p53, p21 (cyclin-dependent kinase inhibitor 1A), and p16, a senescence regulator that prevents cell cycle progression mediated by the tumor suppressor pRb (retinoblastoma protein) /220/. The expression of p16 is tightly controlled by p53. Lehnert et al. /118/ showed that both p53 and p21 protein levels increase after treatment with relatively high doses of $BeSO_4$ (0.1-100 μM). Further, Shannon et al. /221/ found that a 24 h treatment of young presenescent human fibroblasts with 3 μM beryllium sulfate increases p21 mRNA by > 200%. Longer periods of exposure increased mRNA and protein levels for both p21 and p16(Ink4a). Beryllium sulfate treatment also induced senescence-associated β-galactosidase activity (SA-β-gal) in a dose-dependent manner. Additionally, beryllium sulfate caused the p53 protein to associate with its DNA binding site in the promoter region of the *p21* gene, indicating that p53 transcriptional activity is responsible for the large increase in p21 mRNA elicited by beryllium.

Be-HLA-DPβ1-E69

An analysis of MHC Class II genes in patients with CBD revealed a positive association with a glutamic acid residue at the 69th position of the HLA-DPβ1 gene (HLA-DPβ1-E69), with an almost 10-fold increase of disease risk reported in exposed individuals /112/. At least 43 HLA-DPB1 gene variants with Glu69 also have other DNA sequence variations (polymorphisms). Molecular epidemiologic studies have shown that certain variants appear to convey a low to moderate risk of CBD (for example, HLA-DPβ1*0201, ~3-fold increased risk); some convey an intermediate risk (for example, HLA-DPβ1*1901, ~5-fold); whereas others convey high risk (for example, HLA-DPβ1*1701, >10-fold) /222/. Grouping alleles by the relative negative charge on the molecules for which they code suggested that those alleles associated with the most negatively charged proteins carry the greatest risk of BeS and disease.

Although the inheritance of HLA-DR antigens differs from that of the HLA-DP antigens, DR also plays a role in BeS. Indeed, CBD patients without a Glu69-containing HLA-DP allele have shown an increased frequency of HLA-DR13 alleles possessing a glutamic acid at position 71 of the β-chain (corresponding to position 69 of HLA-DP). Using fibroblasts expressing mutated HLA-DP2 and –DR13 molecules, Bill et al. /223/ showed that Be-recognition is dependent on a glutamic acid residue at the identical position in both HLA-DP and HLA-DR, suggesting a critical role for this amino acid in beryllium presentation to Ag-specific CD4+ T cells. The results of that study demonstrated that a single amino acid residue of the MHC class II β-chain dictates beryllium presentation and potentially, disease susceptibility. In the absence of Glu69, however, the HLA-DRβ1 alleles can still function in beryllium presentation, increasing the risk of CBD /81/. Rossman et al. /224/ reported that HLA amino acid epitopes on HLA-DRβ1 and HLA-DQβ1, in concert with or

independently of HLA-DPβ1-E69, may be associated with progression to CBD.

Moreover, the frequent coexistence of Glu69 and the negatively charged aspartic acid and glutamic acid at positions 55 and 56 in disease-relevant HLA-DP alleles suggested that such polymorphisms contribute to disease development after beryllium exposure /160/. HLA-DPβ1-E69 is not only a progression marker for CBD but also a marker for a type of susceptibility to Be-hyper-sensitivity that is not specific for CBD /225/. Homozygosity in Glu69 also acts as a functional marker associated with markers of CBD severity. Workers with CBD and/or sensitization were reported to be more likely to be homozygous HLA-DPβ1-E69 than workers without disease or sensitization ($p < .001$) /226/. In the determination of berylliosis risk, the TNF-α (TNFA-308*2) allele was independently associated with HLA-DP-E69 /227/.

Whether all 43 of the known HLA-DPβ1 alleles coding for Glu69 convey equal risk of CBD is unknown. Snyder et al. /228/ used three-dimensional models of HLA-DPβ1 proteins encoded by these alleles and calculated the electrostatic potential at the molecular surface of each HLA-DP. These comparisons identified the specific characteristics near the *antigen-binding pocket* that distinguish the different HLA-DP allotypes. The differences in electrostatics originate from the shape, specific disposition, and variation in the negatively charged groups around the pocket. The more negative the pocket potential, the greater the odds of developing CBD as estimated from reported epidemiologic studies. The results indicated that adverse impact is caused by charged substitutions in positions 55, 56, 69, 84, and 85, namely, the exact same loci identified as genetic markers of CBD susceptibility. Subsequently Snyder and coworkers /229/ found that Glu69 alleloforms of MHC class II antigen-presenting proteins having the greatest negative surface charge convey the highest risk of CBD on the one hand, and on the other hand, irrespective of allele, convey equal risk of BeS. In addition, the data suggest that the same alleles that cause the greatest risk of CBD are also important for the progression from BeS to CBD. Alleles conveying the highest risk code for Glu26 in a constant region and for Glu69, aspartic acid 55 (Asp55), Glu56, Asp84, and Glu85 in the hypervariable regions of the HLA-DPβ1 chain. Together with the calculated high binding affinities for beryllium, their results suggest that an adverse immune response, leading to CBD, is triggered by chemically specific metal-protein interactions.

Be-Antigen-Binding Studies

Beryllium antigen binding to an HLA-DP molecule and its presentation by APCs to T lymphocytes initiates the primary immune response to beryllium, characterized by the hyperproliferation of CD4+ T cells and release of proinflammatory cytokines /100/. During the past decade, a group at the Los Alamos National Laboratory (LANL) in New Mexico, USA has been at the forefront of biomolecular research on CBD, using a multi-disciplinary approach of bioinorganic chemistry and immunology to study the chemical relation between beryllium, antigen, and HLA-DP. The revelation that over 300 DOE workers in the DOE complex either have CBD or are sensitized against beryllium inspired their 6-year study of beryllium.

Beryllium speciation. The delay of disease onset that can vary from months to decades implies that beryllium speciation must change in some fashion over time to trigger the immune response. The LANL researchers have addressed the disease from inhalation and dissolution, to speciation, to the immune response, employing a multidisciplinary team from the chemical and biological sciences. The LANL group /160/ postulated that as CBD is triggered by very low beryllium exposures (< 2.0 mg m^{-3}), with a delay of symptoms varying from 1 to > 20 years, a beryllium lung burden is likely to remain in the lung as an inert species until an environmental change alters the speciation, resulting

in the development of disease. Therefore, a fundamental understanding of beryllium speciation under physiological conditions was prerequisite for understanding the kinetics of particle dissolution, migration of the beryllium through the cellular compartments, and the ultimate role of beryllium in formation of the antigen that elicits the immune response of CBD.

A more complete picture emerged of the speciation of beryllium under biological conditions, including interactions with proteins and subsequent immunologic consequences. As the aqueous chemistry of beryllium near the biological pH range of 5-7 is very limited by the precipitation of $Be(OH)_2(s)$ /230/, the researchers synthesized several new biologically relevant small molecule complexes containing beryllium that were soluble at biologically relevant pHs and demonstrated that beryllium prefers to bind in specific sites through the displacement of an H^+ in a strong hydrogen bond /231/. Extending the idea to the protein transferrin revealed that beryllium does indeed saturate all available strong hydrogen bond sites in that protein, where beryllium as a 'tetrahedral proton' (the O–Be–O angle is tetrahedral as opposed to the nearly linear O–H···O angle) is thermodynamically preferred.

Antigen binding site. As part of the study of beryllium, a homology model of the HLA-DP protein was developed /2/. Analyses of the sequences of the HLA-DPβ1 and HLA-DPα1 alleles, which are the most common among CBD patients, revealed several carboxylate-rich regions in the peptide-binding cleft, containing many hard Lewis base sites that could provide bonding opportunities for beryllium, which is a hard Lewis acid. Quantum chemistry calculations and structural database results supported the presence in the HLA-DP binding cleft of beryllium clusters, bridged by carboxylate, hydroxo, and/or oxo ligands. Scott et al. /160/ postulated that these ligands could be mimics for the amino acid residues aspartic and glutamic acid, tyrosine, serine, and threonine that are present in the binding groove of HLA-DP.

The observation that disease alleles have significantly larger numbers of carboxylate residues over the non-disease alleles, coupled with the propensity of beryllium to form clusters in the presence of carboxylate based ligands /230/, led the LANL team and others /222/ to believe that the clustering of carboxylate residues in disease alleles were the likely binding sites for beryllium and possibly for the beryllium antigen (see Figure 1). Moreover, ligands with 'strong hydrogen bonds' and high pKa values are ideal binding sites for beryllium as a tetrahedral proton and provide a unique kinetic pathway for beryllium binding.

The first experimental proof of beryllium binding in HLA-DP was demonstrated using RTLs that stimulate T cell proliferation when exposed to beryllium. The RTLs are short polypeptides that encompass the peptide binding site of the MHC receptor and fold into the effective antigen binding portion of the HLA-DP antibody. Consistent with earlier theoretical studies /2/, the experimental evidence showed approximately six beryllium atoms in the HLA-DP binding groove, indicating that the beryllium antigen probably contains or is made up solely of a beryllium-containing cluster. This new paradigm of beryllium binding can be used as a model for beryllium binding in proteins.

Cellular Immune Response to Beryllium

The primary cellular immune response to beryllium begins with the inhalation and subsequent speciation of beryllium and the presentation of beryllium by APCs to CD4+ T cells. During the secondary response, memory CD4+ T cells in the lung respond vigorously to beryllium by producing proinflammatory Th1-type cytokines. The presence of Be-stimulated inflammatory cytokines leads to the recruitment of alveolar macrophages, alveolitis, and subsequent granuloma development. The molecular mechanisms by which beryllium initiates this process and stimulates progression from beryllium sensitization to CBD are currently being

Fig. 1: Ribbon representation of an HLA-DP protein implicated in chronic beryllium disease. The antigen binding groove is bounded on the top left and right by alpha helices and on the bottom by beta sheet structural elements. The carboxylate and alcohol containing amino acid side chains, which are potential locations for beryllium binding, are shown in the ball-and-stick representation (oxygen is black, carbon is gray, and hydrogen is white).

unraveled. The finding of am abnormal Be-LPT against very tightly binding complexes like 2,3-dihydroxybenzoic acid implied that the propensity for the beryllium antigen to form and to stimulate T-cell production is very high /160/. Cellular and molecular studies using the PBMC/DC cell model (peripheral blood mononuclear and dendritic cells) from healthy individuals revealed that beryllium exposure induces specific cytokine and chemokine responses that modulate host immunity.

Intercellular adhesion molecule-1 (I-CAM1). The adhesion molecule I-CAM1, an inducible member of the immunoglobulin gene superfamily, is expressed on the surface of immune cells (reviewed in /232/). The molecule interacts with the cell-surface receptor proteins integrin leukocyte function associated antigen-1 (LFA-1, CD11a/CD18) and integrin Mac-1 (CD11b/CD18), as well as with CD43 and others. I-CAM1 on endothelium plays an important role in the migration of (activated) leukocytes to inflammation sites. I-CAM1 expression on alveolar macrophages is a good marker of activity and disease outcome in sarcoidosis, an inflammatory lung disease of unknown origin having symptoms similar to CBD /233-234/. Moreover, the soluble form of intercellular adhesion molecule-1 (sI-CAM1), derived from alveolar macrophages, is a serum parameter of inflammatory activity reflecting the stage of sarcoid inflammation /235/.

Although long known as the primary contact for inhaled foreign substances, small airway epithelial cells (SAEC) were used only recently by the LANL group to study Be-induced inflammatory responses by examining the regulation of I-CAM1 in SAEC upon exposure to $BeSO_4$ (reviewed in /160/. Rodriguez et al. /236/ found that beryllium exposure specifically induces I-CAM1 expression on the SAEC cell surface and the release of soluble I-CAM1 into the extracellular medium. The finding that anti-I-CAM1 antibodies inhibited the Be-stimulated adhesion of SAEC to the macrophage cell-line THP1 indicated that Be-induced adhesive properties of SAEC are due at least in part to I-CAM1 expression. Real-time PCR analysis revealed that I-CAM1 gene transcription is specifically elevated in Be-stimulated SAEC, suggesting that the increased cell surface expression of I-CAM is due to de novo transcription. These studies support a model in which I-CAM1 in lung epithelial cells may play a role in directing immune cells to the lung and in activating a Be-specific immune response in beryllium hypersensitivity disease.

Recent studies have begun to link I-CAM1 to the development of CBD. Using Be-specific CD4+ T cell lines derived from the BAL fluid of CBD patients, Fontenot et al. /237/ showed that stimulation by Be/APC upregulates ICAM-1 within 24 h, and that this increase is inhibited by anti-LFA-1 antibody. The results suggest that LFA-1 and I-CAM1 interactions play an important role in the cell-cell adhesion processes involved in CBD disease progression and granuloma formation.

Tumor necrosis factor-α. The ability to produce high levels of TNF-α protein in response to beryllium exposure is a unique property of Be-specific $CD4^+$ T cells. Fontenot et al. /237/ demonstrated that purified CD4+ T cells produce significant amounts of IFN-γ and TNF-α upon exposure to beryllium even in the absence of APC, but require exogenous IL-2 for survival. A restricting anti-MHC class II monoclonal antibody (mAb) completely eliminated Be-induced T-cell proliferation during self-presentation and significantly reduced IFN-γ and TNF-α production. Subsequently, Sawyer et al. /238/ reported that Be-exposed CBD BAL cells upregulate TNF-α protein production in a transcription-dependent manner, with a Be-specific increase in nuclear levels of AP-1 and NF-κB transcription factors.

Interleukin-6. Results from the Los Alamos group support the hypothesis that healthy individuals regulate the immune response to beryllium /239/. The authors demonstrated in cells

from three healthy individuals that after 5-10 hours of beryllium exposure, the proinflammatory cytokine IL-6 shows decreased release, whereas the suppressive cytokine IL-10 shows enhanced release. Furthermore, the Be-specific pattern of IL-6 and IL-10 release is dependent upon the induction of the threonine phosphorylation of a 45 kDa cytosolic protein (p45), as early as 90 minutes after beryllium treatment. This finding is in contrast to CBD patients, in whom the Be-specific release from BAL cells consists predominately of pro-inflammatory cytokines (IL-2, IFN-γ, TNF-α, IL-6) /93,98,240/.

Additionally, Chaudhary and coworkers /239/ examined the host response to the bacterial cell wall component lipopolysaccharide (LPS) in the PBMC/DC model, using cells previously exposed to $BeSO_4$ in vitro. In response to LPS, the Be-treated cells exhibited altered cytokine release and intracellular phosphorylation profiles. The results from this study suggested, for the first time, that individuals exposed to beryllium may have altered innate immune responses to bacterial infections. The LPS-mediated secretion of the suppressive IL-10 was significantly inhibited, whereas IL-1b release was enhanced in cells from multiple healthy donors. Not all LPS-mediated responses were altered, however, because IL-6 release was not affected by beryllium treatment.

Molecular Genetics

Taylor et al. /241/ evaluated the mutagenicity of $BeSO_4$ and the co-mutagenicity of beryllium with a known mutagen 1-methyl-3-nitro-1-nitrosoguanidine (MNNG), using a forward mutant detection system developed in *E. coli*. In this system, $BeSO_4$ was weakly mutagenic alone and significantly enhanced the mutagenicity of MNNG up to 3.5-fold over MNNG alone. Based on these results, a proteomic study was conducted to identify the proteins regulated by $BeSO_4$. Using the techniques of 2-DE and oMALDI-TOF MS, the group successfully identified 32 proteins that are differentially regulated by beryllium and/or MNNG in the *E. coli* test system. The results suggest several potential pathways for the focus of further research into the molecular mechanisms underlying Be-induced genotoxicity.

Gene-Environment Interactions

Chronic pulmonary berylliosis (CBD) has been proposed as a model of the interaction between the environment and genetic predisposition /242/. In a cross-sectional study of 127 workers, Richeldi et al. /112/ evaluated the interaction between HLA-DPβ1-E69 and beryllium exposure in a small population, in which the prevalence of berylliosis was associated with machining beryllium. The disease showed a higher prevalence (25%) in HLA-DPβ1-E69+ machinists than in HLA-DPβ1E69– machinists (3.2%, $p = .05$). Although the results of that study suggest a potent additive gene-environment interaction, the number of cases was very small (6), therefore, this finding must be confirmed in a larger cohort.

Polymorphisms in the HLA-DPβ1-E69 gene provide a classic example of how genetic susceptibility markers have a clear role in identifying disease risk in CBD. Polymorphisms in cytokine genes also affect the risk of CBD and a more severe disease.

At the Los Alamos Laboratory, microarray studies led to other insights into the mechanisms governing the beryllium immune response. Hong-Geller and coworkers /243/ used a mixture of PBMC/DC from a non-CBD source to identify genes that are specifically upregulated in response to stimulation by $BeSO_4$. Their finding of a threefold or higher upregulation of the chemotactic chemokines macrophage inflammatory proteins MIP-1α and MIP-1β, growth related oncogene factors GRO1 and GRO3, and RANTES in response to $BeSO_4$ suggested a mechanism involving chemokine concentration gradients to attract immune cells to the site of inflammation. Although variations occurred among individual donors, semi-quantitative

RT-PCR and ELISA methods examining the transcription and secretion profiles for MIP-1α, MIP-1β, and GRO1 following $BeSO_4$ stimulation generally showed an increased rate of both chemokine mRNA transcription and release when compared with aluminum sulfate exposure. Both MIP-1α- and MIP-1β-neutralizing antibodies partially inhibited the ability of $BeSO_4$ to stimulate the cell migration of PBMC/DC in vitro.

The ability of beryllium to regulate chemokine gene activation was demonstrated by the alteration of transcription factor RUNX[1] binding to the MIP-1α promoter consensus sequence following the incubation of PBMC/DC with $BeSO_4$. Taken together, the results suggest a model in which beryllium stimulation of PBMC/DC can modulate the expression and release of different chemokines, leading to the migration of lymphocytes to the lung and the formation of a localized environment for development of beryllium disease in susceptible individuals.

Transforming growth factor β1 gene. Transforming growth factor β1 (TGF-β1), a cytokine involved in mediating the fibrotic/Th1 response, displays several gene polymorphisms (–988 C/A; –800 G/A; –509 C/T; 10 T/C and 263 C/T) of known or suggested functional significance. Polymorphisms in the common TGFβ1 promoter, –509C/T, have been linked with risk, progression, and outcome of numerous diseases. Gaede et al. /153/ found an association between polymorphisms in the TGF-β1 gene and CBD in 59 CBD patients compared with 164 healthy Be-exposed controls. Although Jonth et al. /244/ found no significant differences between CBD cases and controls in TGF-β1 variants or haplotypes based on sequence-specific primer PCR analysis, the –509C and codon 10T implicated in lower levels of TGF-β1 protein production were significantly associated with disease severity indicators.

Programmed death-1 (PD-1) expression. The negative T-cell regulator, PD-1, is involved in signaling T cell death (apoptosis) and negatively regulates T-cell function /245-246/. The expression of PD-1 is upregulated in response to chronic exposure to various conventional antigens /247-249/. A high concentration of the non-conventional antigen, $BeSO_4$, induces apoptosis in BAL macrophages from subjects with CBD and/or BeS /250/. To determine the role of PD-1 in the response to beryllium, Palmer et al. /251/ measured PD-1 expression on blood and BAL CD4+ T cells from Be-sensitized and CBD subjects. The results showed that PD-1 expression was significantly higher on BAL CD4+ T cells than on peripheral blood T cells, with the highest expression on the Be-specific T cell subset. In addition, the expression of PD-1 on BAL CD4+ T cells directly correlated with the severity of the T-cell alveolitis. Increased expression of the PD-1 ligands, PD-L1, and PD-L2, on BAL CD14+ cells compared with blood was also seen. The addition of anti-PD-1 ligand mAbs augmented Be-induced CD4+ T cell proliferation, and an inverse correlation was seen between PD-1 expression on Be-specific CD4+ T cells and Be-induced proliferation. The results indicate that the PD-1 pathway is active in Be-induced disease and plays a key role in controlling Be-induced T cell proliferation.

CONCLUSIONS

A combination of immunology and bioinorganic chemistry research during the past decade has resulted in an unprecedented molecular-based understanding of chronic beryllium disease. The cell and molecular studies reviewed herein provide mechanistic insight into the host response to beryllium exposure and contribute to a more comprehensive understanding of the onset of beryllium hypersensitivity disease.

The study of beryllium under physiologically relevant pH conditions has been greatly extended

[1] protein controlling when genes are switched on or off

through the development several new, small biologically relevant molecular complexes that effectively bind and solubilize beryllium at pH 7. The resulting binding information demonstrates that beryllium prefers to bind in specific sites, namely through the displacement of an H+ in a strong hydrogen bond. The binding *via* strong hydrogen bonds has been shown to apply to Be protein interactions and presents an important framework to understand how beryllium is transported in the body and interacts with the immune system at a molecular level. This new model has already shown how beryllium binds to the iron transport protein transferrin, and suggests one possible pathway for its entry into lymphocytes.

The first experimental proof of beryllium binding in HLA-DP was achieved using short polypeptides (RTLs) that encompass the peptide binding site of the MHC receptor. The RTLs fold into the effective antigen binding portion of the HLA-DP antibody and stimulate T-cell proliferation when exposed to beryllium. The postulate that a beryllium cluster, bridged by carboxylate and alcohol containing amino acid residues, is an important structural feature of the beryllium antigen was placed on firmer footing by ^{9}Be NMR binding experiments. These binding experiments showed six beryllium atoms complexed to an RTL mimic indicated in CBD, while non-CBD alleles did not bind beryllium, reinforcing the importance of the Glu-69 mutation to the recognition of beryllium.

Immunologic studies showed a specific immune response to the beryllium antigen, marked by cytokine, chemokine, and the production of other immunomodulatory molecules. It was discovered that healthy individuals can regulate the balance between proinflammatory cytokine IL6 and the inflammatory suppressive cytokine IL10, but that this balance is upset in CBD patients. Additionally, the finding that beryllium exposure alters the innate immune response to bacterial cell wall components suggests that individuals exposed to beryllium could have altered innate immune responses to bacterial infections.

Currently, the diagnostic criteria for BeS and CBD rely heavily on the beryllium lymphocyte proliferation test. Hopefully, understanding the molecular mechanisms and genetic factors associated with sensitization and disease may lead to the development of new diagnostics and treatment for BeS and CBD. Results from immunologic studies combined with a more detailed understanding of the speciation of beryllium in proteins, could possibly open the door to therapies or cures for CBD. The identification that I-CAM1, associated with many lung disorders (cancer, sarcoidosis, allergies) is up regulated upon exposure to $Be(SO_4)$ in lung epithelial cells, opens the door to known antibody and reagent treatments. For example, reagents and antibodies that are known to react against I-CAM1 could be used to reduce the effects of the disease. The strong affinity of the HLA-DP molecule for beryllium will challenge chelation-type therapies once CBD has been initiated. A tremendous excess of the chelating agent would be required to compete for the Be, based on similar equilibrium values. If strong binding molecules, such as citric or salicylic acids, however, could be used to solubilize and remove beryllium from the body during onset (months to years), then the disease may be prevented or inhibited. There is hope that as a more detailed molecular picture of the beryllium antigen interaction develops, resulting from future research efforts, drugs to either prevent antigen formation or presentation may be developed.

Currently, the diagnostic criteria for BeS and CBD rely heavily on the beryllium lymphocyte proliferation test. Hopefully, understanding the molecular mechanisms and genetic factors that are associated with sensitization and disease may lead to the development of new diagnostics and treatment for BeS and CBD. For example, antibodies directed toward the cell-surface receptor protein LFA-1 strongly reduce $CD4^{+}$ T-cell proliferation and cytokine release and have been suggested as

potential lead candidates for intervention in CBD.

A national discussion is currently underway on the hazards of beryllium. The EPA has announced that it is revising its beryllium risk assessment document, and OSHA is moving to replace its current outdated workplace exposure standard /33/ (also see http://sefora.org/2008/07/02/beryllium-science-or-public-relations). Although genetic and occupational factors appear to have an additive effect for risk of beryllium disease, ethical and social concerns have limited the value of wide-scale genetic screening in occupational settings; hence large-scale genetic screening in the workplace is not currently recommended.

Some cases of CBD appear to have been misdiagnosed and more cases are likely to emerge in the future. Therefore, healthcare providers should continue to consider CBD in the differential diagnosis of patients with respiratory disease who reside near beryllium facilities. Steps that would directly impact both the power of epidemiologic studies and the cost of surveillance would be to develop and validate improved screening and diagnostic tests, and to identify genetic factors that affect either sensitization or disease process. Prevention programs should be developed that include the following:

- beryllium substitution when possible,
- eliminating as many job tasks involving exposure to beryllium particles as possible and minimizing the number of workers performing those tasks,
- stringent engineering and work-practice controls for any use of beryllium that could generate dusts, mists, fumes, or small particulates,
- process control and surface decontamination,
- guidance for minimizing potential worker exposure to beryllium during any handling or use, in conjunction with
- suitable medical surveillance to identify potentially beryllium-exposed workers, based on the best current understanding of beryllium disease and medical diagnostic tests available so that risks related to job tasks can be identified and reduced as soon as possible, and to detect beryllium sensitization and/or CBD at the earliest possible stage.

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