Animal Models of Beryllium-induced Lung Disease

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The Inhalation Toxicology Research Institute (ITRI) is conducting research to improve the understanding of chronic beryllium disease (CBD) and beryllium-induced lung cancer. Initial animal studies examined beagle dogs that inhaled BeO calcined at either 500 or 1000°C. At similar lung burdens, the 500°C BeO induced more severe and extensive granulomatous pneumonia, lymphocytic infiltration into the lung, and positive Be-specific lymphocyte proliferative responses \textit{in vitro} than the 1000°C BeO. However, the progressive nature of human CBD was not duplicated. More recently, Strains AU and C3H/HeJ mice were exposed to Be metal by inhalation. This produced a marked granulomatous pneumonia, diffuse infiltrates, and multifocal aggregates of interstitial lymphocytes with a pronounced T helper component and pulmonary \textit{in situ} lymphocyte proliferation. With respect to lung cancer, at a mean lung burden as low as 17 µg Be/g lung, inhaled Be metal induced benign and/or malignant lung tumors in over 50% of male and female F344 rats surviving ≥1 year on study. Substantial tumor multiplicity was found, but K-ras and p53 gene mutations were virtually absent. In mice, however, a lung burden of approximately 60 µg (∼300 µg Be/g lung) caused only a slight increase in crude lung tumor incidence and multiplicity over controls in strain AU mice and no elevated incidence in strain C3H mice. Taken together, this research program constitutes a coordinated effort to understand beryllium-induced lung disease in experimental animal models. — Environ Health Perspect 104(Suppl 5):973–979 (1996)

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Introduction

The toxicity of beryllium (Be) and its compounds has been a topic of concern for some 60 years, even though earlier reports dealt with this issue (1). Concerns were largely driven by reports of Be-induced toxicity in humans in Europe in the 1930s and in the United States in the 1940s (2). After approximately 1950, the acute form of Be-induced lung disease was largely eliminated due to the establishment of workplace exposure limits, but the chronic form of the disease is still of concern. Chronic beryllium disease (CBD) is characterized by progressive, noncaseating granulomatous inflammation of the lung that may be fatal. CBD occurs in only approximately 3% of exposed individuals and it has been suggested that a genetic predisposition is involved (3). Numerous animal models of Be-induced toxicity were investigated in the 1940s, despite a 1943 report by the U.S. Public Health Service (4) that erroneously exonerated Be as the causative toxic agent. The early work was brought together in 1947 with the Sixth Saranac Symposium (5), a galvanizing meeting for investigators dealing with industrial hygienic and toxicologic concerns for Be.

Over the subsequent decades, numerous reports were published from animal experiments involving Be exposures. These include the production of pneumonitis in animals inhaling Be compounds comparable to that seen in humans, the induction of osteosarcomas in rabbits injected with beryllium oxide (BeO) and zinc Be silicate (6), the production of lung tumors in rats inhaling beryllium sulfate (BeSO₄) (7), and the characterization of differing immune responses in two strains of guinea pigs (8). A full review of this work is beyond the scope of this article; other recent reviews and summaries of the literature describing the health effects (9–11) and biokinetics (12) are available.

This article provides an overview of studies of the inhalation toxicity of Be conducted at the Inhalation Toxicology Research Institute (ITRI). These studies, which began in 1982, are described below.

Studies at the Inhalation Toxicology Research Institute

The ITRI is conducting research to improve the understanding of CBD and to examine Be-induced lung cancer. Central to these efforts have been field studies of Be-containing aerosols likely to be found in the workplace, development of laboratory model aerosols mimicking workplace aerosols, detailed physicochemical characterization of these materials, and use of these aerosols in laboratory animal models. The following sections describe aerosol and physicochemical studies, efforts to develop an animal model having the key features of human CBD, and studies of Be-induced carcinogenesis.

Aerosol and Physicochemical Studies

Initial ITRI studies focused on proposed uses of Be as a plasma limiter in fusion devices and soon expanded to include the potential uses of Be in structural, navigational, and nuclear reactor systems for space. Beryllium aerosols formed under industrial and applied research conditions were collected and examined (13); materials included machining-generated Be metal and BeO aerosols, stock Be metal and BeO...
powders, and aerosols derived from electron or laser beam impaction on Be blocks, and Be particles from a research fusion device. Particles of respirable size were found in all cases; particle morphology ranged from branched-chain aggregates in the case of laser vaporization to irregular shapes produced by the other operations. Additional efforts were made to characterize aerosols produced by the machining of Be metal, BeCu, or BeNi alloys; for a given machining operation, a greater percentage of the Be metal aerosol was found in the respirable size fraction than in either alloy (14). During this period of extensive aerosol development, an overview for practicing engineers was also prepared on the history of Be dispersion, regulations and industrial hygiene practices related to Be, and perspectives on the health risks of using Be (15).

Efforts were begun to mimic these workplace and research aerosols with appropriate surrogate aerosols produced under well-controlled laboratory conditions. Model aerosols for a radioisotope-labeled $^{7}$BeO generated from the nebulization of a $^{7}$Be(OH)$_2$ suspension and calcined at either 500 or 1000°C were developed (16). A laboratory laser vaporization technique capable of generating branched-chain aggregate aerosols of either Be metal (when operated under an argon atmosphere) or BeO (when operated under air) was also developed (17). Finally, a method employing dry-powder aerosolization with size fractionation using an aerosol cyclone was developed for an industrial preparation of Be metal powder (18).

An extensive quality control program was begun to certify and compare the chemical and physical properties of the laboratory aerosols. This program involved determination of particle morphology and geometric size, aerodynamic size, specific surface area, density, dissolution characteristics, chemical form, crystallinity, and composition (19–21). The work was complemented with in vitro toxicity studies in cell cultures in which for a given Be compound, short-term toxicity appeared to be governed by the amount of specific surface area of the preparation, and thus presumably the surface available for dissolution of Be ions (22).

Important features of these laboratory model aerosols include the production of particle sizes ranging from several tenths to 2 μm in mass median aerodynamic size, thus making the aerosols of optimal size for deposition in the alveolar compartment of the lung; thorough physical and chemical characterization, as described above; and ability to generate exposure atmospheres over a wide range of concentrations, thus permitting a wide range of lung burdens to be delivered in relatively short times. This latter point is particularly true for Be metal; the aerosolization system for this material can provide mass concentrations ranging from several tenths of μg/m$^3$ up to over 1 g/m$^3$ in a nose-only inhalation chamber (18).

Studies of Be-induced Granulomatous Lung Disease

**Studies in Dogs.** Studies of Be-induced granulomatous lung disease began with an examination of the toxicokinetics of 500 and 1000°C BeO in the beagle dog. An associated goal was the possible development of a CBD model. Justification for this approach included the clear indication from the literature of the importance of BeO preparation temperature on Be disposition and toxicity following inhalation (23), and the need for biokinetic data describing the disposition of these two BeO preparations. The beagle dog was selected because it represents a good biokinetic model for the disposition of other important elements (24), is amenable for the collection of toxicokinetic data and monitoring of pulmonary responses (using periodic radiographs and intrapulmonary lavage), and has immunological responses similar to those of humans (25).

A dose–response pilot study in dogs using BeO treated during generation at 500°C indicated that granulomatous lung lesions were present 1 month after exposure (26). Additional dogs were subsequently exposed by inhalation to $^{7}$BeO that had been treated at 500°C during generation and subsequently calcined at either 500 or 1000°C. Dogs received mean lung burdens of either 17 or 50 μg/kg body weight; control dogs received a sham exposure (experimental design given in Table 1). Groups of dogs (2 dogs per time point per calcination temperature per lung burden level) were sacrificed at various times through 1 year after exposure (a total of 28 dogs) to measure $^{7}$Be content in various tissues (27), and to evaluate lung and lung-associated lymph node lesions (28). Another group of dogs was held for periodic assessment as described below. As expected, the BeO prepared at 500°C was cleared from the lung more rapidly than the 1000°C material (clearance half-times of 72 and 210 days, respectively). Beryllium cleared from the lung was either excreted (principally in feces at early times after exposure, later in urine) or translocated primarily to bone and liver. Through 1 year after exposure, lung lesions observed included macrophage hyperplasia, granulomas, fibrosis, alveolar epithelial cell hyperplasia, and lymphocytic infiltrates. These lesions were generally more extensive or severe in dogs exposed to the 500°C BeO, and peaked in relative severity at 2 months after exposure.

Twenty dogs (4 controls and 4 each per calcination temperature per lung burden) were not sacrificed, but were followed by periodic radiography and collection of blood and intrapulmonary lavage fluids for assessment of cell types and performance of standard in vitro lymphocyte proliferation assays (LPA) to detect Be-specific immune responses (28). In blood, positive LPA results were observed only sporadically in all exposure groups. Lymphocytes constituted

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**Table 1.** Experimental design for study of beagle dogs exposed to BeO calcined at 500 or 1000°C.

| Sacrifice time (days after exposure) | Controls | Mean lung burden achieved and BeO calcination temperature
|-------------------------------------|----------|--------------------------------------------------|
|                                     |          | 17 μg BeO/kg$^2$                                 |
|                                     |          | 500°C | 1000°C | 500°C | 1000°C |
| 8                                   | –        | 2$^a$ | 2$^a$ | –     | –     |
| 32                                  | –        | 2     | 2$^b$ | –     | –     |
| 64                                  | –        | 2     | 2$^b$ | 2     | 2$^b$ |
| 180                                 | –        | 2$^c$ | 2     | 2     | 2$^d$ |
| 360                                 | –        | 2$^c$ | 2$^e$ | 2     | 2$^d$ |
| –1100                               | 4        | 4$^f$ | 4$^f$ | 4$^f$ | 4$^f$ |

$^a$Dogs sacrificed from 8 to 380 days after exposure to examine biokinetics and histopathologic effects of BeO. Dogs sacrificed at approximately 1100 days after exposure were reexposed to 500°C BeO (mean initial lung burden of 74 μg BeO/kg) at approximately 900 days after first exposure and were used to examine the immunopathologic effects of BeO. Additional details have been published (25,26,28). $^b$Single, acute, nose-only inhalation exposure. $^c$Control dogs were sham-exposed to filtered air only. $^d$For each BeO preparation temperature, mean lung burden (after completion of rapid clearance phase of BeO deposited on conducting airways) normalized by body weight at time of exposure for each dog. $^e$Number of dogs per group; equal numbers of males and females. A dash (–) indicates no dogs exposed at the indicated conditions.

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over 50% of lung lavage cells 3 months after exposure in a group of four dogs inhaling 500°C BeO, then percentages declined to less than 30% by 7 months after exposure. The positive LPA results observed were most prominent in dogs that inhaled the 500°C BeO to achieve the 50 μg/kg lung burden. These results generally peaked 6 to 8 months after exposure, then declined. There was substantial variability among individual dogs, and an apparent shift toward more T-helper lymphocytes when lymphocyte numbers were elevated relative to controls. Additional work using cloned lung lymphocyte T-cell lines suggested a Be-specific, major histocompatibility complex Class-II-mediated nature of the response (29).

Continued monitoring of the dogs through 2 years after exposure, however, coupled with the Be lung clearance data and the decrease in the relative severity of lung toxicity from 2 months to 1 year in the sacrificed dogs, suggested that toxic reactions to Be had largely resolved. Thus, although these results were promising, a model of the progressive nature of CBD was not developed. To determine if potential immune memory of Be might result in responses greater in either magnitude or duration than seen after the first exposure, the dogs were reexposed by inhalation to 500°C BeO approximately 2.5 years after their first exposure to result in a mean lung burden of 74 μg/kg (30). The influx of lymphocytes, reactivity of the lymphocytes as revealed by the LPA, and lung lesions upon sacrifice 6 months after exposure were similar to those seen after the first exposure, indicating that these responses did not appear to be cumulative using this exposure regime.

**Studies in Cynomolgus Monkeys.** To refine and extend these observations in dogs, the immunopathologic responses to Be were examined in cynomolgus monkeys (*Macaca fascicularis*). One important reason to extend these studies to nonhuman primates was the lack at that time of appropriate antibodies against the various T-lymphocyte subpopulations in the dog. The monkeys were exposed by bronchoscopic, intrabronchiolar instillation to either BeO calcined at 500°C or to Be metal (31). A separate lung lobe received an instillation of the saline vehicle alone. The masses of Be used ranged from 0.4 to 38 μg for the BeO, and 1.0 to 148 μg for the Be metal; the differing amounts were used because they were estimated to provide roughly equimolar amounts of the Be⁺⁺ ion dissolved from the particles over the 6-month study.

The monkeys underwent bronchoalveolar lavage at various times after instillation. The animals were sacrificed at either 80 or 180 days after exposure for evaluation of lung histopathology. Compared to control lung lobes, the numbers of lymphocytes obtained from exposed lobes were elevated at 14, 30, and 90 days postexposure (dpe) in the Be metal-exposed animals, and at 60 dpe in the BeO-exposed monkeys. Be-specific *in vitro* lymphocyte proliferation occurred at 14, 60, and 90 dpe in lymphocytes from Be-exposed lung lobes only; no Be-specific lymphocyte proliferation was observed in BeO-exposed animals. Lung lesions in Be-metal-exposed monkeys included focally intense, interstitial fibrosis, marked hyperplasia of the alveolar epithelium, and variable lymphocytic infiltrates. Some Be metal-exposed animals had discrete immune granulomas characterized by tightly organized lymphocytic cuffs surrounding epithelioid macrophage aggregates. When present, lesions in BeO-exposed monkeys were rare and much less severe.

Thus, lung lesions having certain features of CBD were successfully produced in the cynomolgus monkey and were accompanied by Be-specific immune responses. Furthermore, the results suggested that Be metal produced more severe lesions than the BeO prepared at 500°C. However, the experimental design precluded an examination of whether the pulmonary responses increased over time or resolved, as was observed in the dogs. Largely because of the expense of working with the nonhuman primates and the related inability to study more than a few individuals, this line of investigation was not pursued.

**Studies in Rodents.** To determine if mice that inhaled Be develop responses that mimic human CBD, female strains A/J and C3H/HeJ mice were exposed to a Be-metal aerosol to achieve mean initial lung burdens of 47 μg or 64 μg, respectively (32). The mice were sacrificed 28 weeks after exposure. Cells were harvested from peripheral blood, spleen, and bronchial lymph nodes of both exposed and control mice. Be-specific *in vitro* lymphocyte proliferation was assayed, but responses were seen only in the positive control samples in which the lymphocytes were exposed to phytohemagglutinin.

The right cardiac lung lobes from selected mice were inflated with a cryopreservative agent, frozen, then sections were cut and reacted with antibodies to detect mouse B, helper T, and suppressor T lymphocytes. Remaining lung lobes were fixed and sectioned for standard histopathology; this examination revealed a marked, multifocal, granulomatous pneumonia with mild interstitial fibrosis, perivascular and interstitial mononuclear (lymphocytes, plasma cells, monocytes, and macrophages) cell infiltrates, and multifocal interstitial mononuclear cell aggregates. Multinucleated giant cells were common; most were of the foreign-body type, but Langhans giant cells were also found. Immunohistochemical examination showed that these interstitial mononuclear cell aggregates were of two types: some consisted primarily of helper T cells and Be-containing macrophages (microgranulomas), while others consisted of a central zone of B cells and a peripheral zone of helper T cells. Helper T cells, which were the majority of lymphocytes in the lungs of Be-exposed mice, were located in the aggregates described above, in the interstitium within foci of granulomatous inflammation, and in perivascular cuffs. Suppressor (CD8⁺) T cells were infrequent and scattered within the lesions.

A subgroup of the mice received injections of a 5-bromo-2-deoxyuridine (BrdU) solution 2 days to 1 hr before sacrifice to label the nuclei of replicating cells. This treatment revealed lymphocyte proliferation within microgranulomas, perivascular cuffs, and the lymphoid aggregates. Unfortunately, because different groups of mice received lung cryosection immunohistochemistry and the BrdU labeling, the BrdU technique could not permit the identification of either the proliferating lymphocyte subtype or Be specificity of the response.

No substantive differences in response between the two murine strains were observed; other strains have not been examined. The observed chronic lung lesions parallel those seen in human CBD cases in several important respects: morphologically, with the helper T cells constituting the primary lymphocytic component, and proliferatively, with the pronounced *in situ* lymphocyte replication (Table 2). However, the Be specificity of these responses must be demonstrated before the disease in mice can be considered an animal model of human CBD. Efforts in this area continue.

**Studies of Be-induced Cancer**

**Studies in Rats.** Studies of Be metal-induced cancer began at ITRI as part of a
Table 2. Comparison of responses between human chronic beryllium disease cases and strains A/J and C3H/HeJ mice inhaling beryllium metal.a

| Response                                      | Humans | Mice |
|-----------------------------------------------|--------|------|
| Microgranulomas/mononuclear infiltrates       | +      | +    |
| Significant lymphocytic component             | +      | +    |
| Accumulation of helper T cells                | +      | +    |
| Lymphocytic infiltration in vivo              | */?    | +    |
| Be-specific in vitro                          | +      | */?  |
| Delayed hypersensitivity                      | +      | */?  |

*aTwo strains of mice received a single, acute, nose-only inhalation exposure to result in mean initial lung burdens of 47 μg (for strain A/J) or 64 μg (for strain C3H) Be metal; experiment described in text. bKey to responses: + = response observed; */? = response probably occurs but has not been definitively proven; */? = response not observed but a systematic examination of the response was not performed; */? = existence of response not known.

A larger program to study the cancer risks from exposures to combinations of radiation and other agents. Pertinent to this article, a study is being conducted in rats exposed to Be metal and/or plutonium dioxide (239PuO₂) (33,34). The following discussion relates primarily to rats exposed only to Be metal within the larger study; the Be portion of the design of this study is given in Table 3.

Groups of F344/N rats (raised in the ITRI barrier facility) were designated for single, nose-only exposure to Be metal to result in lung burdens of approximately 50, 150, or 450 μg. This involved exposures of 10 to 41 min to Be metal mass concentrations of 470 to 960 mg/m³. Control rats received filtered air alone. Following exposure, groups of rats were designated for serial sacrifice at times ranging from 8 to 450 dpe for determination of the quantity of Be within the lungs and for assessment of presence or progression of lung lesions.

Exposure to the highest level of Be metal (target lung burden of 450 μg) proved acutely lethal to a substantial fraction of the rats (35). Thirty-seven percent of male and 49% of female rats died approximately 2 weeks after exposure. The lungs of these rats were characterized by a severe hemorrhagic pneumonia (36). This acute mortality was not observed in rats exposed to lower lung burdens of Be metal. Inhaled Be metal also decreased long-term survival in a dose-dependent manner (37). For both genders, median survival times of Be metal-exposed rats were similar to those of controls in groups receiving the lowest target lung burdens, and were approximately 80% those of controls at the highest lung burdens.

Another effect observed in this combined exposure study was a striking reduction in the lung’s ability to clear Be and other materials (33,37). Clearance of 239Pu from the lung in rats also inhaling Be metal was best modeled by a single-component, negative exponential function having a half-time of some 500 days. This effect was independent of the level of Be metal examined. In contrast, 239Pu clearance in rats not also exposed to Be was best modeled by a two-component, negative exponential, and the clearance half-time for the first component (which accounted for approximately 80% of the 239Pu lung burden) was about 35 days. For a given level of 239PuO₂ exposure, the coexposure to Be metal with the associated reduction in lung 239Pu clearance served to increase the total potential life-span radiation dose to the lung by a factor of approximately three, compared to controls. This phenomenon has subsequently been examined in more detail (below).

The most notable result from this study was the carcinogenicity of Be metal to the lungs of the F344/N rats; these data have been reported in abstract form (38,39). The most prevalent neoplasm observed was the bronchiolar/alveolar adenocarcinoma having alveolar, papillary, or tubular patterns. Other tumors observed included adenosquamous carcinomas and squamous cell carcinomas. In addition, substantial multiplicity of lung tumors within the same animal was observed.

In four groups of 30 male and 30 female rats each receiving mean Be metal initial lung burdens of 40, 110, 360, and 430 μg Be, tumors became apparent by 14 months after exposure, and a crude incidence of 64% of the rats developed lung tumors over their lifetimes (40). An analysis in the Be-induced rat lung adenocarcinomas of genes frequently mutated in human lung cancers (the oncogenes K-ras and c-raf-1, and the tumor suppressor gene p53) revealed few alterations. Direct sequencing of exons 1 and 2 in 24 tumors did not reveal any mutations in K-ras codons 12, 13, or 61. A more sensitive technique revealed codon 12 base pair transversions in 2 of 12 tumors examined, suggesting K-ras oncogene activation was a rare, late event in the carcinogenic process. No p53 gene mutations were observed through either immunohistochemical techniques or direct sequencing of exons 5 through 8, nor were c-raf1 mutations evident by Southern blot analysis. Thus, the mechanisms underlying the production of pulmonary adenocarcinomas from inhalled beryllium in the rat do not involve gene dysfunctions common with human non-small-cell lung cancer.

As a result of the level of carcinogenicity observed in this study, additional rats (CDF(F344)/CrlBR, Charles River Laboratories, Raleigh, NC) have been exposed to lower lung burdens of Be metal (Table 3) and are being observed. Target initial lung burdens for this portion of the study range from 0.3 to 50 μg. The goal of this work is to define dose–response relationships between lower lung burdens of

Table 3. Experimental design for study of Be metal carcinogenicity in F344 rats.a

| Planned initial lung burden of Be metal (μg) | Study Phase I | Study Phase II | Total rats (no.) |
|---------------------------------------------|---------------|----------------|-----------------|
| 0                                           | 208           | 270            | 478             |
| 0.3                                         | –             | 288            | 288             |
| 1.0                                         | –             | 288            | 288             |
| 3.0                                         | –             | 288            | 288             |
| 10                                          | –             | 288            | 288             |
| 50                                          | 240           | 156            | 396             |
| 150                                         | 240           | –              | 240             |
| 450                                         | 240           | –              | 240             |
| Total                                       | 928           | 1578           | 2506            |

aAs described in the text, this is a part of a larger study of the carcinogenicity of combined exposures of rats to Be metal and 239PuO₂; this table describes the portion of the study in which rats receive no radiation treatment.
bPlanned level of initial lung burden resulting from a single, acute, nose-only inhalation exposure to Be metal.
cNumber of animals per group; equal numbers of male and female rats. A dash (—) indicates no rats exposed at the indicated conditions.
Be metal and lung cancer and to reproduce in F344/Crl rats the findings described above in F344/N rats.

**Studies in Mice.** The carcinogenicity of inhaled Be metal is being examined in two strains of mice: A/J mice, which are susceptible to either spontaneous or chemically induced lung cancer, and C3H/HeJ mice, a strain that is relatively resistant to lung cancer induction (41). Groups of mice were exposed to Be metal to result in group mean initial lung burdens of 47 μg Be (A/J) or 64 μg Be (C3H). Serial sacrifices were conducted to yield lung tissue for histologic examination, molecular analysis of gene changes in the carcinogenic process, and analysis of Be for dosimetry and lung clearance data.

Histopathological analyses of the lungs have been completed (42). Compared to control mice, the crude incidence of lung tumors in Be metal-exposed A/J mice is slightly elevated (46% in exposed vs. 37% in controls) and in C3H/HeJ is slightly decreased (5% in exposed mice vs. 10% in controls). In addition, tumor multiplicity is slightly increased in the exposed A/J mice compared to that in controls. The potential statistical significance of these data and the multiplicity and time-to-tumor data are being analyzed. Be exposure reduced survival for both strains. In a logrank test (Breslow test; SAS P1L, SAS Institute, Cary, NC), this reduction in survival was statistically significant for strain C3H mice (p = 0.042) but only marginally so for strain A/J mice (p = 0.077). Both exposed and control strain A/J mice appeared to have slightly greater survival times than C3H mice; however, neither of these differences were statistically significant (p > 0.05).

An additional topic of ongoing analysis in this study is the potential for mutations in the K-ras oncogene (43). Preliminary data suggest that K-ras gene mutations are more common in the mouse lung tumors than in the rat lung tumors, but mutational hotspots are lacking within the gene, which suggests that Be is not acting as a genotoxic carcinogen.

**Studies of Acute and Chronic Inflammatory Lung Disease in Rats and Mice.** In concert with the cancer studies in rats and mice described above, the nature of acute and chronic responses to inhaled Be metal have been examined through 1 year after exposure in both species. Male F344/N rats were exposed to Be metal to result in lung burdens ranging from 0.32 to 100 μg (about 0.2–85 μg Be/g lung tissue), then sacrificed at 8, 16, 40, 90, 210, and 365 dpe (44). The Be metal aerosol was mixed with an aerosol of 85Sr-labeled fused aluminosilicate particles (85Sr-FAPs), a relatively insoluble particle used as a tracer particle to study clearance from the lungs. Control rats received the 85Sr-FAPs alone. Be exposure significantly retarded 85Sr-FAP lung clearance in all exposure groups, except for the lowest lung burden (0.32 μg) where clearance was slightly retarded but not statistically different from that in controls. In addition, lung burdens of 10 or 100 μg Be induced minimal to mild acute and chronic inflammation, hyperplasia of the alveolar epithelium, and early-occurring fibrosis, whereas a lung burden of 1.8 μg caused only late-occurring, minimal chronic inflammation and alveolar epithelial hyperplasia. The histological changes were generally accompanied by alterations in the enzyme, protein, and cellular components of bronchoalveolar lavage fluids.

A virtually identical study was also performed in female C3H/HeJ mice (45). Mice received both 85Sr-FAP tracer particles and Be metal lung burdens of 1.7 to 34 μg (about 14–280 μg Be/g lung). A lung burden of 1.7 μg Be had some measurable but minimal effect on lung clearance, the 2.6-μg Be lung burden was intermediate in effect, and lung burdens of 12 or 34 μg Be induced a substantial reduction in pulmonary clearance of the 85Sr-FAP. Histological evaluation of the lungs revealed granulomatous pneumonia at later times, an increased number and size of interstitial lymphocytic aggregates, and interstitial infiltration of mononuclear cells. Findings were most pronounced in the two highest lung burden groups, although a minimal granulomatous pneumonia was observed in many of the mice in the 2.6-μg lung burden group. As with the rats, indications of lung damage revealed by bronchoalveolar lavage generally mirrored the lung histology results.

These studies in rats and mice provide dose–response data describing the effects of inhaled Be metal on lung toxicity. The most striking difference in lung pathology between the two species is the marked component of interstitial lymphocytic aggregates in the mouse; lymphocytes are not a substantial component of the response in rats. A comparison between the two species is shown in Figure 1, in which Be metal lung burdens are divided by control animal lung weight in an attempt to normalize the data for comparison.

Another important difference between the species appears to be the levels of Be lung burdens required to induce a toxic reaction in the lung; the rats are affected by the various changes described above at weight-normalized lung burdens substantially lower than those in mice.

**Discussion**

As noted in the introduction, a substantial body of toxicity studies of Be in animals exists (9–11). It can be difficult, however, to comprehend the effects of Be in animals from this work. Many of the studies, particularly the early ones, are plagued by problems such as confounding diseases within the animal colonies; use of inappropriate

| Disease                        | Animal | Response |
|--------------------------------|--------|----------|
| Lung cancer                    | Rats   | ? ± ? ± |
|                                | Mice   | ± ± ± ± |
| Chronic inflammation           | Rats   | − ± + ± |
|                                | Mice   | − − + + |
| Decreased lung clearance       | Rats   | − − + + |
|                                | Mice   | ± − + + |

Figure 1. Comparative responses of rats and mice following single, acute, nose-only inhalation exposure to beryllium metal to result in a range of initial lung burdens. Symbols: (+), response observed; (−), response minor or equivocal; (−), response not observed; (?), potential response currently being studied.
modes of exposure; failure to quantitate dose or disposition; or use of exposure materials that were poorly characterized, poorly described, or irrelevant to workplace exposures (9).

Our studies of granulomatous lung disease indicate that dogs and monkeys respond to Be with many of the responses seen in human CBD patients. These responses include granulomatous lung lesions having a significant lymphocytic component, and the presence of in vitro, Be-specific lymphocyte proliferative capability (46,47). However, the finding in dogs that both of these responses resolve CBD has not been achieved following the acute exposure modes used. In addition, further work with dogs and monkeys is not promising because of the substantial expense associated with working with these large-animal models and the related inability to examine the large numbers of subjects necessary with these outbred species. The potential for developing useful models of beryllium disease in rodents appears much more promising.

Work with F344 rats indicates that the lack of significant lymphocytic response to inhaled Be metal in this species renders it unsuitable for detailed immunopathogenic study (46). In mice, however, several parallels between murine and human responses were observed, most notably including the development of granulomas and/or mononuclear infiltrates having a pronounced helper T cell component (Table 2). Efforts continue to demonstrate Be-specific lymphoproliferative and delayed hypersensitivity responses in the mouse. This work is based on the premise that the development of a laboratory animal model having the significant features of human CBD will afford opportunities to study not only the cellular and molecular mechanisms of responses involved in the progression of CBD but also to examine both the influence of the physicochemical form of Be and the exposure mode (single, chronic, multiple) on disease outcome and the potential for therapeutic intervention.

Studies of the carcinogenicity of inhaled Be metal are being conducted in both rats and mice. A striking difference in response between these species is being observed. The F344 rat develops a relatively high crude incidence and multiplicity of lung tumors. These tumors, however, essentially lack mutations in genes commonly found to be mutated in various types of human cancers, including lung cancers. On the other hand, at doses that induce substantial carcinogenicity in rats, the carcinogenic response is weak in strain A mice and absent in strain C3H mice. Clearly, continued efforts are required to understand the similarities/differences in responses of rats versus those in mice, the molecular events surrounding Be-induced carcinogenesis, and the responses of these species to Be-containing compounds other than Be metal before these findings can be extrapolated to humans.

In conclusion, ITRI studies are oriented toward understanding events involved in the development of beryllium-induced, immune-mediated, chronic granulomatous lung diseases, and lung cancer. This research program constitutes an ongoing, coordinated effort to understand beryllium-induced lung disease in experimental animal models. Use of multiple species in this program increases the scientific basis for eventual extrapolation of the results from laboratory animal models to humans.

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BERYLLIUM-INDUCED LUNG DISEASE IN ANIMALS

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