The bioinorganic chemistry and associated immunology of chronic beryllium disease†

Brian L. Scott,* T. Mark McCleskey,a Anu Chaudhary,b Elizabeth Hong-Gellerb and S. Gnanakaranč

Received (in Cambridge, UK) 4th December 2007, Accepted 28th February 2008
First published as an Advance Article on the web 9th May 2008
DOI: 10.1039/b718746g

Chronic beryllium disease (CBD) is a debilitating, incurable, and often fatal disease that is caused by the inhalation of beryllium particulates. The growing use of beryllium in the modern world, in products ranging from computers to dental prosthetics (390 tons of beryllium in the US in the year 2000) necessitates a molecular based understanding of the disease in order to prevent and cure CBD. We have investigated the molecular basis of CBD at Los Alamos National Laboratory during the past six years, employing a multidisciplinary approach of bioinorganic chemistry and immunology. The results of this work, including speciation, inhalation and dissolution, and immunology will be discussed.

Introduction

The element beryllium was discovered by the French chemist Vauquelin in 1798 as a component of the mineral beryl, which has the stoichiometry [Be₃Al₂(SiO₃)₆].¹ Metallic beryllium was isolated in 1828 independently by Bussy and Wohler, but was not used extensively until it was discovered in 1920 that a 2% addition of beryllium to copper produced an alloy six times stronger than the original material.² Beryllium is extremely lightweight, is six times stiffer than steel, has a high melting point (1285 °C), a high heat-adsorption capacity, and is nonmagnetic and corrosion resistant.³ Because of these unique physical properties, beryllium has found wide use in modern industry, including computers and telecommunications equipment (springs, switches, relays, and connectors), appliances (optical laser scanners in copy machines and airport luggage handlers), automotive applications (air bag sensors, ignition switches, power steering systems), sports equipment (golf clubs and bike frames), and even dental prosthetics.²,³ Moreover, beryllium has the lowest thermal neutron absorption cross-section of any metal, which has led to its use by the United States and other nuclear weapon states in the fabrication of nuclear weapons.³ Beryllium is mined from naturally occurring silicates including beryl [5% by weight beryllium] and bertrandite (Be₄(OH)₂Si₂O₇, 15% by weight beryllium). In the year 2000 the US used 390 tons of beryllium with an estimated value of $140 million.⁶

The utility of beryllium is greatly complicated by its negative health effects. Most notably, exposure to beryllium, in certain susceptible individuals, causes a granulomatous disorder of the lungs, called chronic beryllium disease (CBD), which is a debilitating, incurable, and often fatal disease.⁷–⁹ In the current biomolecular understanding of how beryllium triggers CBD, beryllium inhaled into the lungs is detected by antigen
Cases of CBD have also been documented in as many as 134,000 in private industry and government workers who have been potentially exposed to beryllium, and currently 26,500 DOE and Department of Defense (DOD) workers have CBD or are sensitized against beryllium. However, inhalation is the only mechanism that has been proven to cause CBD. Other health effects of beryllium have been documented as well, and include cancer (beryllium is a Class A Environmental Protection Agency (EPA) carcinogen), and dermatitis. Beryllium also has the ability to impact enzyme function, DNA synthesis, protein phosphorylation, and cell division.

The combination of beryllium’s utility, toxicity, and widespread use make it imperative to better understand the chemistry of beryllium under biological conditions, and how this chemistry leads to disease, and potential cures and therapeutics. Our six-year study of beryllium at Los Alamos National Laboratory stems from the fact that over 300 Department of Energy (DOE) Workers have CBD or are sensitized against beryllium, and are at risk of developing the disease at a later date. According to a recent National Institute for Occupational Safety and Health (NIOSH) publication, there are currently 26,500 DOE and Department of Defense (DOD) workers who have been potentially exposed to beryllium, and as many as 134,000 in private industry and government combined. Cases of CBD have also been documented in European countries as well (Britain, Canada, France, Germany, Sweden, Israel, Japan, and Russia). The genetic susceptibility of CBD and BeS holds important clues to the molecular role that beryllium plays in the disease. The disease has been linked to particular alleles of the MHC class II molecule, HLA-DP, which is composed of a heterodimer of peptide chains denoted α and β and normally displays peptides derived from pathogen proteins to induce a host immune response [Fig. 2]. In particular, susceptibility has been most strongly associated with HLA-DPβ1 (β chain) alleles that encode a negatively charged glutamic acid at position 69 (Glu69) of the β chain. Two polymorphic regions with significant charge differences at positions 55–56 and 69 on the DP β chain were apparent in the susceptible alleles [Table 1]. Since Glu69, and the negatively charged aspartic acid and glutamic acid at positions 55 and 56 frequently coexist in disease relevant HLA-DP alleles, it is suggested that polymorphisms contribute to disease development after beryllium exposure. What is particularly striking about these polymorphisms is that the disease alleles have significantly larger numbers of carboxylate residues over the non-disease alleles. This observation, coupled with the propensity of beryllium to form clusters in the presence of carboxylate based ligands, led us, and others, to believe that the clustering of carboxylate residues in disease alleles were likely binding sites for beryllium and possibly the beryllium antigen.

Several published small molecule crystal structures suggest possible binding modes of beryllium in the peptide binding groove of HLA-DPβ1. The negatively charged residues at positions 55 and 56 in disease alleles are important for beryllium binding, and thus markers of disease [Table 1]. Since Glu69, and the negatively charged aspartic acid and glutamic acid at positions 55 and 56 frequently coexist in disease relevant HLA-DP alleles, it is suggested that polymorphisms contribute to disease development after beryllium exposure. What is particularly striking about these polymorphisms is that the disease alleles have significantly larger numbers of carboxylate residues over the non-disease alleles. This observation, coupled with the propensity of beryllium to form clusters in the presence of carboxylate based ligands, led us, and others, to believe that the clustering of carboxylate residues in disease alleles were likely binding sites for beryllium and possibly the beryllium antigen.

### Table 1: HLA-DP β chain amino acid residues for disease and non-disease alleles.

<table>
<thead>
<tr>
<th>DPB1 alleles</th>
<th>55</th>
<th>56</th>
<th>57</th>
<th>58</th>
<th>61</th>
<th>64</th>
<th>67</th>
<th>68</th>
<th>69</th>
<th>74</th>
<th>82</th>
<th>84</th>
<th>85</th>
</tr>
</thead>
<tbody>
<tr>
<td>*0401 non Glu69</td>
<td>A</td>
<td>A</td>
<td>E</td>
<td>Y</td>
<td>S</td>
<td>D</td>
<td>E</td>
<td>E</td>
<td>K</td>
<td>K</td>
<td>D</td>
<td>E</td>
<td>G</td>
</tr>
<tr>
<td>*0101 non Glu69</td>
<td>A</td>
<td>A</td>
<td>E</td>
<td>Y</td>
<td>S</td>
<td>D</td>
<td>E</td>
<td>E</td>
<td>K</td>
<td>D</td>
<td>D</td>
<td>E</td>
<td>E</td>
</tr>
<tr>
<td>*0301 non Glu69</td>
<td>D</td>
<td>E</td>
<td>D</td>
<td>Y</td>
<td>S</td>
<td>D</td>
<td>E</td>
<td>K</td>
<td>E</td>
<td>K</td>
<td>D</td>
<td>E</td>
<td>E</td>
</tr>
<tr>
<td>*0201 common Glu69</td>
<td>D</td>
<td>E</td>
<td>E</td>
<td>Y</td>
<td>S</td>
<td>D</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>G</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>*0601 rare Glu69</td>
<td>D</td>
<td>E</td>
<td>D</td>
<td>Y</td>
<td>S</td>
<td>D</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
</tr>
<tr>
<td>*0801 rare Glu69</td>
<td>D</td>
<td>E</td>
<td>E</td>
<td>Y</td>
<td>S</td>
<td>D</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
</tr>
<tr>
<td>*0901 rare Glu69</td>
<td>D</td>
<td>E</td>
<td>E</td>
<td>Y</td>
<td>S</td>
<td>D</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
</tr>
<tr>
<td>*1001 rare Glu69</td>
<td>D</td>
<td>E</td>
<td>E</td>
<td>Y</td>
<td>S</td>
<td>D</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
</tr>
<tr>
<td>*1301 rare Glu69</td>
<td>A</td>
<td>A</td>
<td>E</td>
<td>Y</td>
<td>S</td>
<td>D</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
</tr>
<tr>
<td>*1601 rare Glu69</td>
<td>D</td>
<td>E</td>
<td>E</td>
<td>Y</td>
<td>S</td>
<td>D</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
</tr>
<tr>
<td>*1701 rare Glu69</td>
<td>D</td>
<td>E</td>
<td>D</td>
<td>Y</td>
<td>S</td>
<td>D</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
</tr>
<tr>
<td>*1901 rare Glu69</td>
<td>E</td>
<td>A</td>
<td>E</td>
<td>Y</td>
<td>S</td>
<td>D</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
</tr>
</tbody>
</table>

Fig. 1 Immunopathogenesis of CBD. Upon inhalation of beryllium, antigen presenting cells such as macrophages present beryllium to CD4+ T cells resulting in T cell activation. Cytokine release (arrows) by both APC and T cells marks the immune response to beryllium.

Fig. 2 Homology model of an HLA-DP antibody indicated in CBD (*/201; Table 1). The antigen binding groove resides between the two yellow α-helices. The residues listed in Table 1, glutamic acid (E), aspartic acid (D), tyrosine (Y), and serine (S), are shown as ball and stick representations (carbon as gray; oxygen as red). The far left residue is 55, and the far right residue is 82.
groove of HLA-DP. For example, the structures depicted in Fig. 3 show how beryllium forms clusters in the presence of carboxylate, carbonate, hydroxo, oxo, alkoxide, and phenoxide ligands.16,18,19 These chemical functionalities can be thought of as mimics for the amino acid residues aspartic and glutamic acid, tyrosine, serine, and threonine that are all present in the binding groove of HLA-DP. There are three possible scenarios for binding beryllium in the HLA-DP molecule: (1) beryllium serves alone as the antigen, and is a predominantly inorganic species, (2) beryllium is bound to a peptide fragment resulting from a previous beryllium–protein interaction, and (3) beryllium is not part of the antigen, but binds adjacent to the binding groove, changing its shape, and allowing the antigenic peptide to bind. An additional possibility is that beryllium is not directly involved in the immune response; it does not bind to HLA-DP or the T cell but only binds adjacent to the binding groove, changing its shape, and allowing the antigenic peptide to bind. An additional possibility is that beryllium is not directly involved in the immune response; it does not bind to HLA-DP or the T cell but only creates an antigenic peptide through its interaction with a specific protein or peptide in the body.

At the time we began our study of beryllium chemistry, and its associated immune response, there was very little known regarding beryllium speciation, particularly in biological environments. Newman et al. pointed out in 2002 that “there is a paucity of data concerning the mechanisms by which Be forms complexes with other compounds or reacts with biologically important molecules, macromolecules and macromolecular systems”.20 The delay of the onset of disease that can vary from months to decades highlights the importance of Be speciation that must change in some fashion over time to trigger the immune response. Our work has addressed the disease from inhalation and dissolution, to speciation, to immune response, and has employed a multidisciplinary team from the chemical and biological sciences. We will discuss how these results have shed light on the following critical questions regarding the role of beryllium in CBD: (1) what is the speciation of beryllium under biological conditions and how does beryllium interact with proteins? (2) How is BeO dissolved under physiological conditions? And (3) what is the cellular response to beryllium complexes?

**Speciation**

CBD can be triggered by very low Be exposures (<2.0 µg m⁻³) and the delay of symptoms can vary from 1 to over 20 years. This combination suggests that a Be lung burden can remain in the lung as an inert species and that an environmental change alters the speciation resulting in the development of disease. A fundamental understanding of beryllium speciation under physiological conditions is needed to understand the kinetics of particle dissolution, migration of the Be through the cellular compartments, and the ultimate role of Be in formation of the antigen that elicits the immune response of CBD.

Aqueous chemistry of beryllium near the biological pH range of 5–7 is very limited due to the precipitation of Be(OH)₂(s) (Kₛᵣ = -20).16 At more acidic conditions the speciation of beryllium in the absence of ligands has shown that Be(H₂O)₂²⁺ dominates below pH 3.5. As the pH increases, hydrolysis leads to the formation of Be(OH)(H₂O)₃⁻ and then clusters begin to form and Be₃(OH)(H₂O)₆³⁻ (Fig. 3b), which exists as a cyclic six-membered ring structure, is the predominant species in solution from approximately pH 4.5 to 5.5 (at 2 mM Be).16 Between pH 4 and 5.5 there have been a variety of hydroxide structures, in addition to the trimer, that have been isolated and characterized from their crystal structures. Above pH 5.5, Be precipitates as a polymeric hydroxide.

While it is experimentally known that the beryllium dication forms multinuclear species in water, a theoretical investigation of the thermodynamics of the cluster formation had never been undertaken prior to our work. We have used quasi-chemical theory, which partitions the problem into inner shell (quantum mechanical) and outer shell (dielectric continuum), and *ab initio* molecular dynamics to calculate the fundamental speciation at low pH. These calculations have reproduced the tetrahedral, four coordination of beryllium observed in X-ray scattering experiments on aqueous solutions of BeCl₂.21 The calculations also predicted a p𝐾ᵣ of 3.8 for Be(H₂O)₂²⁺, which agrees very well with the measured value of 3.5. The A𝐻 of hydrolysis of Be(H₂O)₄³⁺ to Be(OH)(H₂O)⁺ was calculated to be -14.4 kcal mol⁻¹, in good agreement with the experimental value of -22.1 kcal mol⁻¹.22

In order to better understand the speciation of beryllium at biologically relevant pHs, where insoluble hydroxides are normally present, we set out to find a set of ligands that could stabilize beryllium in the 5–7 range. The ligands also had to be biologically relevant. Most aqueous research has focused on monomeric BeL or BeL₂ complexes at low pH, where a wide variety of carboxylate, phenolate and phosphate complexes have been reported.16,19,23 As the pH increases (> ~ 5.8), most ligands are unable to compete with the formation of Be(OH)₂(s).16 Only a few monodentate ligands (F⁻) and bidentate ligands (malonate) have been shown to solubilize Be above pH 5.8 and these eventually form Be(OH)₂ as the pH is increased further ≈ 7.5.24 Citric acid on the other hand is an excellent ligand for Be.23,25 Citric acid (CA, I) solubilizes Be at molar concentrations across the entire pH range, and also has biologically relevant chemical functionality. Potentiometric studies form the basis for most Be binding studies in aqueous...
systems but can be difficult to interpret as bridging hydroxides deprotonate. Early work on citric acid had led to two different interpretations of the speciation. We used a combination of $^{13}$C and $^{9}$Be NMR spectroscopy to demonstrate that Be is bound in a 2 : 1 Be : CA ratio. As the ratio of Be : CA was varied (pH = 9) from 0.05 : 1 to 2 : 1 the $^{9}$Be NMR spectrum remained unchanged, with peaks at ~3.5 and ~1.9 ppm. At the 2 : 1 ratio the Be species is soluble above pH 5.9 in molar concentrations. Mass spectral data at 10 μM CA and 20 μM Be further supports the 2 : 1 ratio, even at low concentrations, with a parent ion peak at 519 g mol$^{-1}$ corresponding to a Be$_2$(CA)$_2$ dimer as proposed by Vanni and co-workers. Our results, which could clearly identify the existence of free ligand by $^{13}$C NMR spectroscopy, suggested that the binding constants for the Be$_2$(CA) or Be$_4$(CA)$_2$ species are significantly higher than previously reported and that these species dominate in solution at both high and low Be concentrations.

Comparison of strong binding of CA to the very weak binding with tricarboxylic acid$^{25}$ indicated that the aliphatic alcohol of CA played a critical role in Be binding. Further studies comparing a variety of ligands that contained aliphatic alcohols and carboxylic acids that could support Be binding in a 2 : 1 Be : ligand ratio demonstrated the preferred geometry for binding involves a bridging aliphatic alcohol and carboxylates to form a unit consisting of one five-membered and one six-membered ring. Binding and deprotonation of the aliphatic alcohol group in CA is rare, but has been observed in the case of Al$^{3+}$. These results have a significant impact in terms of the potential sites for binding in the body. The ability to deprotonate aliphatic alcohols opens up the possibility of Be binding to Ser, Tyr, and Thr residues adjacent to Glu or Asp carboxylates as well as the potential for serving as a bridge between carboxylate residues and alcohol rich carbohydrates. Such sequences are observed in HLA-DP alleles that have been indicated in CBD (Table 1).

The results with CA binding were used to select ligands for Be binding with rigid backbones that could support the unit with a bridging phenoxide (instead of the alkoxide in CA). In this manner we could introduce a tyrosine-like functionality into our study. The ligands 2-hydroxyisophthalic acid (HIPA) and 2,3-dihydroxybenzoic acid (DHBA) were the first ligands purposely chosen to bind polynuclear Be units (Fig. 4). HIPA has two six-membered ring binding pockets with carboxylate groups on either side of the phenoxide functionality, and DHBA has one six-membered and one five-membered ring binding pocket made from the two phenol groups and the carboxylic acid. Absorbance and fluorescence changes in the aromatic ligands were used to determine the binding constants directly. Both HIPA and DHBA bind Be strongly at physiological pH values in a Be : L ratio of 2 : 1 with stability constants of 17.5 and 18.4 ([LBe]/[L][Be]) and 27.0 and 28.5 ([LBe$_2$]/[LBe][Be]), respectively. The [LBe]/[L][Be] binding constants are higher than any previously reported values for Be binding and the [LBe$_2$]/[LBe][Be] binding constants are so high that the Be$_2$L complex dominates at concentrations as low as 1 μM. As in the CA work the mass spectrometry data show a parent ion peak that indicates dimerization to form Be$_4$(HIPA)$_2$(H$_2$O)(OH)$^+$. The DHBA ligand fluoresces strongly at 402 nm as Be binds. This fluorescence has been used to detect Be at very low levels in HEPES buffer at pH 7. A 10 μM solution of DHBA can be used to determine 50 nM of Be (4.5 pg mL$^{-1}$). Other fluorescent indicators for Be with similar detection limits based on simple BeL binding have severe interferences from other metals such as aluminium and iron.$^2$ Aluminium has a similar size to Be and the O–O distance between the two nearest chelating oxygens in a tetrahedrally bound Be and an octahedrally bound Al are identical to within 0.02 Å, resulting in significant interference. The DHBA ligand is designed to bind with the Be–O–Be motif, which results in a tremendous selectivity for Be. In a pH 7 solution with 10 μM of DHBA and a cocktail of metal ions including Al, Fe, Cr, Cu, Zn, Cd, Pb, all at 10 μM each, the background fluorescence remains minimal and Be can be detected down to 200 nM Be (1.8 ng mL$^{-1}$). These fluorescent indicators may make it possible to monitor Be in cells directly. The bincuclear chelating ligands have been the most successful at dissolving Be across the entire physiological pH range.

One of the interesting aspects in the speciation of beryllium has been the observation that the natural form of beryllium, beryl (Be$_3$Al$_2$(SiO$_3$)$_6$), has not led to any known cases of CBD$^{33}$ in the beryllium mining industry. CBD is associated only with the processed forms of beryllium such as BeO, Be(s) and BeF$_2$. The beryl structure has the striking feature that there are no Be–O–Be linkages despite the high concentration of Be. This stands out in contrast to the observations of the strong binding behavior of ligands designed to have Be–O–Be linkages. All of the beryllium bound oxygens in beryl are Si–O units that bridge between aluminium and beryllium. We recently investigated the interaction of CA with Be and Al and reported that the complex (NH$_3$)$_4$[Be$_2$Al$_2$(CA)$_4$] was

![Fig. 4](image-url) Biologically relevant ligands chosen to support the formation of five- and six-membered rings containing beryllium.
formed in quantitative yield by neutralizing an acidic solution containing Al(NO$_3$)$_3$, Be(NO$_3$)$_2$ and citric acid with aqueous NH$_3$.$^{32}$ The mixed metal complex is highly soluble in water ($\sim$ 3 M) from pH 3.5 to 10.5. The $^9$Be NMR spectrum exhibits one peak at 1.84 ppm indicating that the Be resides in the six-membered chelated ring of citrate and the $^{27}$Al NMR spectrum shows a broad singlet at 14.0 ppm. The singlet in the $^{27}$Al NMR spectrum (in contrast to the four peaks observed for the 1 : 1 aluminium citrate complex) combined with the observation that the $^{13}$C NMR spectrum has only one resonance for each of the carbon atoms from citric acid suggests that the mixed metal complex is highly favored thermodynamically and the dominant species in solution at the Be : Al : citric acid ratio of 1 : 1 : 2. An X-ray diffraction study of the Be : Al : CA$_2$ complex revealed an interesting four-metal complex with a core of two Al and two Be ions in an eight-membered ring with bridging O atoms from the $\alpha$-hydroxyl groups of the four bound CA ligands (Fig. 5). This core structure is similar to the larger core of beryl that contains six Al and six Be atoms in an alternating pattern bridged by oxygens from Si–O units in the mineral. All of the remaining sites (2 for each tetrahedral Be and 4 for each octahedral Al) are occupied by carboxylates of the CA resulting in a highly soluble hexa-polyanion.

A complication for studying the speciation of beryllium in the body is the low solubility of beryllium in phosphate media above pH 3. Phosphate is commonly encountered in cellular systems and is on the order of 1 mM in blood serum as inorganic phosphates.$^{33,34}$ Even in the presence of excellent chelating ligands such as DHBA, beryllium precipitates in the presence of a phosphate buffer. The first stable beryllium complex isolated within a phosphate medium was (NH$_3$)$_4$Al$_6$Be$_6$(CA)$_4$(PO$_4$)$_8$ based on the Be$_2$Al$_2$CA$_4$ complex. The phosphate complex is highly soluble at pH 7 (>1 M), stable in the presence of phosphate media (0.5 M), and has been characterized by X-ray diffraction.$^{32}$ The Be-O-Al units are bridged by $\alpha$-hydroxyl groups from the CA ligands as in the Be$_2$Al$_2$(CA)$_4$ complex, but the phosphate structure has a large cluster with six Be-O-Al units stitched together by phosphate groups. The structure is templated by two additional phosphate groups in the center of the molecule with each phosphate group binding three aluminiums with one unbound P–O$_{free}$ bond aligned on the C$_3$ axis of the molecule.

**Beryllium as tetrahedral proton**

Polynuclear ligands have opened up the study of aqueous Be chemistry under physiological pH conditions, but there are also reports of monomeric ligands that function under the same pH conditions. The fluorescent indicators chromotropic acid and 10-hydroxybenzo[a]quinoline-7-sulfonate (CRA and HBQS; Fig. 4) bind beryllium well at pH 7. HBQS is an extraordinary ligand even at pH 12 and is part of a commercial beryllium detection system that can selectively detect beryllium at concentrations <1 nM.$^{35,36}$ In order to understand if there was any commonality between the binding of monomer and polynuclear binding ligands we re-examined the binding of Be to a wide variety of monomeric ligands. After considering Be binding across a wide range of ligands and careful re-examination of the proton NMR spectra of the polynuclear ligands in aprotic media, we have proposed a new binding concept that focuses on the comparison of beryllium to H$^+$. The common aspect in Be binding appears to be that Be displaces H$^+$ in many “strong hydrogen bonds” where Be as a “tetrahedral proton” (O–Be–O angle is tetrahedral opposed to the nearly linear O–H⋯O angle) is thermodynamically preferred (Scheme 1).$^{37}$

![Scheme 1](image)

Strong hydrogen bonds occur when the distance between the two heteroatoms, typically O–H⋯O or O–H⋯N, is shorter than the sum of the van der Waals radii and the energy barrier to hydrogen transfer between two atoms is very low (on the order of the O–H vibrational zero-point-energy). This unique bond allows for access to the basic site without breaking the strong covalent O–H bond by simply moving the proton to the more acidic site. These bonds are readily identified through significant NMR shifts in the range of 10–22 ppm. All of the ligands that have strong hydrogen bonds as identified by their NMR shift bind Be well. This is true even for the polynuclear species, as a 1 : 1 solution of Al and CA shows a strong hydrogen bond at 17 ppm upon Al binding. Within the series of mononuclear ligands, binding constants increase as the pK$_a$ of the proton and basicity of the site increases (Fig. 6). The strongest mononuclear ligands [chromotropic acid, sulfosalicylic acid, and HBQS; Fig. 4] have pK$_a$ values >13 and are capable of solubilizing Be at physiological pH. Ligands with “strong hydrogen bonds” and high pK$_a$ values make for ideal binding sites for Be as a tetrahedral proton and provide a unique kinetic pathway for Be binding.

Sulfosalicylic acid (SSA, Fig. 4) is an excellent model system since it has a rigid backbone structure, a strong hydrogen bond (NMR shift of 16.3 ppm) and a high pK$_a$ (>13). The crystallographic O–H⋯O angle has been reported as 155.32°. This represents a pre-organized binding site, where beryllium...
Beryllium in proteins

Recent results of Be binding to transferrin have supported this binding concept. Transferrin typically transports iron throughout the body via an iron binding pocket that has 1 Asp, 2 Tyr, 1 His and a carbonate ligand from solution. Binding of Be to transferrin was strong enough to allow retention of the Be after repeated size selective filtration and was confirmed directly by $^9$Be NMR spectroscopy with a broad peak at 1.1 ppm. Surprisingly, binding was observed for both the apo (aTF) and the indium-loaded protein. The stoichiometry of binding to the apo and holo forms of transferrin was found to be 12 and 7, respectively. A search of the crystal structures of the apo and holo forms of transferrin found 12 and 6 strong hydrogen bond sites, respectively. This agrees quite well with experiment. Be binding to transferrin is of particular interest since it may offer a pathway for Be migration into the cellular systems as it does for iron.

A more specific binding model for beryllium in transferrin was developed through the comparison of $^9$Be NMR line shifts in transferrin:Be$_{12}$ with small, strong hydrogen bonded molecule mimics. This approach was taken since a quantum calculation on the full protein was not computationally possible. As a first step, the $^9$Be NMR line shifts were calculated for complexes of HIPA$^2^-$, DHBA$^2^-$, and SSA$^2^-$ (Table 2), and we found good agreement at the HF/3-21G(d) level of theory. Next, we calculated the $^9$Be NMR line shifts for two small molecule mimics of the proposed transferrin binding sites; these complexes were Be(H$_2$O)$_2$(phenoxide)(acetate) and Be(H$_2$O)$_2$(methoxide)(acetate). The first complex, with the phenoxide ligand, is a mimic for the strong hydrogen bonded tyrosine–glutamate/aspartate sites in transferrin. The second complex, with the methoxide ligand, is a mimic for the strong hydrogen bonded sites serine–glutamate/aspartate and threonine–glutamate/aspartate in transferrin. The calculated values, 1.66 ppm for the phenoxide mimic, and 1.72 ppm for the methoxide mimic, agree quite well with the 1.1 ppm (broad) for transferrin:Be$_{12}$. These predictions, coupled with the database binding site analysis, indicate that the beryllium dication is binding to the strong hydrogen bonds between residues Asp/Glu and Thr/Tyr/Ser. The $^9$Be NMR spectrum is invaluable, because it offers the only direct observation of Be binding. The concentrations required can make it challenging for many protein experiments. However, the combination of highly soluble transferrin and multiple bound beryllium dications enabled relatively easy observation of the NMR shift.

Other target proteins such as the MHC–II receptors, HLA-DP, that have been genetically linked to CBD are more difficult to synthesize and solubilize at high concentrations. To overcome this difficulty with MHC molecules researchers have made shorter polypeptides (RTLs) that encompass the peptide binding site of the MHC receptor, and are known to fold into the effective antigen binding portion of the HLA-DP antibody. Most important, the RTLs designed to mimic the disease associated HLA-DP molecules stimulate T cell proliferation in the presence of beryllium. Moreover, the RTLs are soluble enough to allow use of $^9$Be NMR spectroscopy to determine binding. For the first time, the binding of beryllium to an HLA-DP disease associated allele, in the form of an RTL, has been observed. The $^9$Be NMR studies, combined with UV/Vis spectroscopy to determine protein concentration, and ICP-AES to determine beryllium concentration, showed that the binding groove binds 6.5 beryllium atoms per RTL. The RTL used in this study, RTL600, was based on the HLA-DP disease allele *0201 (Table 1). In addition, RTL700, based on the non-disease allele *0401 (Table 1), did not bind any beryllium. These studies are consistent with an earlier modeling study that predicted clusters of beryllium in the peptide binding groove of HLA-DP.

Table 2 Calculated and measured $^9$Be NMR shifts for transferrin:Be$_{12}$ and relevant small molecule mimics

<table>
<thead>
<tr>
<th>Complex</th>
<th>$^9$Be shift (calc.)</th>
<th>$^9$Be shift (expt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(HIPA$^2^-$)Be$_2$(H$_2$O)$_2$</td>
<td>3.40</td>
<td>2.97</td>
</tr>
<tr>
<td>(DHBA$^2^-$)Be(H$_2$O)$_2$</td>
<td>4.52, 2.65</td>
<td>4.60, 2.54</td>
</tr>
<tr>
<td>(SSA$^2^-$)Be(H$_2$O)$_2$</td>
<td>3.25</td>
<td>2.80</td>
</tr>
<tr>
<td>Be(H$_2$O)$_2$(phenoxide)(acetate)</td>
<td>1.66</td>
<td>—</td>
</tr>
<tr>
<td>Be(H$_2$O)$_2$(methoxide)(acetate)</td>
<td>1.72</td>
<td>—</td>
</tr>
<tr>
<td>Transferrin:Be$_{12}$</td>
<td>—</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Fig. 6 Plot of pK$_a$ versus beryllium binding, log $K_a$, for 11 different ligands based on literature values.$^{16,18}$
Theory of beryllium binding

While the quasi-chemical results showed good agreement (vide supra), the technique is computationally expensive, and thus cannot be used on larger protein systems. In this vein, we have recently developed a new potential energy function for beryllium that has a very simple form and can easily be used in large-scale simulations of the biomolecules.\(^{39}\) Due to its small size and divalent character, the beryllium ion presents a difficult challenge for representing its interactions with a simple empirical energy function in biomolecular simulations. Specifically, the charge transfer interaction with water may exhibit substantial covalent character. In the past, three-body potentials have been shown to be necessary for the aqueous Be\(^{2+}\) ion to reproduce the correct coordination numbers in classical simulations. With this new simple two-body potential, \textit{ab initio} derived Lennard-Jones parameters and a +2 charge at Be were able to effectively reproduce the structural properties of the aqueous Be\(^{2+}\) ionic complex in explicit aqueous solution. This accomplishment enabled us to probe Be\(^{2+}\) interactions in HLA-DP molecules associated with chronic beryllium disease using all-atom molecular dynamics simulations.

This new two-body classical potential captures several structural, reactive and spectroscopic properties of the Be\(^{2+}\) ion complex in water.\(^{39}\) It reproduces the correct radial distribution function and coordination numbers for this cation in classical simulations of explicit aqueous solution when compared to published X-ray and NMR measurements. This potential also produces a well-established hydrogen bonding between the first and second solvation shells. These calculations have also yielded a tetrahedral four coordinate [Be(H\(_2\)O)\(_4\)]\(^{2+}\) species. Moreover, an \textit{ab initio} calculation on a snapshot of the MD calculation gives the best vibration mode of the aqueous beryllium to date. The ability to accurately calculate beryllium coordination environments using molecular dynamics is a crucial step in being able to predict binding of beryllium in proteins of interest. Current comparative all-atom molecular dynamics studies that are considering the differences between disease and non-disease alleles could shed much needed microscopic insight on the roles of conformational flexibility and water on binding.

Inhalation and dissolution

Aerosol particles of beryllium that are inhaled into the lung are typically found in phagolysosomes within macrophage cells in the pulmonary tissue. While it is known that beryllium particles in these vacuoles (pH = 4.8) undergo dissolution,\(^{40}\) there has not been any significant work performed to address the interface between particulate and solution, where dissolution actually takes place. The interactions of ligands with the particulate form are especially important in the speciation and bioavailability of Be. BeO is one of the more commonly encountered forms of Be in commercial industry,\(^{41}\) but the extremely low solubility has made dissolution studies difficult. Members of our team have performed theoretical studies on the different surfaces of BeO that show the exposed surface face can influence the thermodynamics significantly.\(^{42}\) These studies show that the (0 0 1) face of BeO has the greatest reactivity in water, and becomes hydroxylated during the reaction. This hydroxylated surface is probably a reactive intermediate in the dissolution process, leading to soluble beryllium clusters in solution, such as [Be\(_3\)(OH)\(_3\)(H\(_2\)O)\(_6\)]\(^{3+}\) (Fig. 3b).

New techniques for reliably dispensing uniform aliquots of BeO have enabled dissolution studies not previously possible. Recent results on the dissolution of BeO particles in HEPES and the phosphate buffers at pH 7 have shown that ligands and proteins can significantly enhance dissolution of particulate BeO.\(^{43}\) In the absence of phosphate BeO is insoluble even over a period of months. Addition of apo-transferrin, DHBA or CA substantially increases the dissolution, achieving concentrations \(>1 \mu M\) in all cases over a 30 week period. This is a biologically relevant level as LPTs show that the blood and lung will generate an immune response to concentrations of 1 \(\mu M\) Be and higher. (As mentioned earlier, the lymphocyte proliferation test (LPT) measures the immune response as the hyperproliferation of CD4\(^+\) T cells.) Phosphate buffer significantly enhances the dissolution rate and there is a synergistic effect with phosphate and CA that leads to 75 \(\mu M\) Be solutions in only 20 weeks. These preliminary studies suggest that both small biological ligands and protein-based binding agents can significantly enhance the rates of dissolution for high fired BeO at pH 7. It is possible that environmental changes around BeO particles in the lung during phagocytosis could significantly alter the dissolution process depending on phosphate concentration and the availability of ligands in the form of proteins or CA. An environmental based change in the dissolution rate of BeO could explain the highly variable onset time observed for CBD.

The recent increased understanding of aqueous Be speciation has resulted in several highly soluble Be complexes at physiological pH as described earlier. These soluble complexes have been used in conjunction with the blood LPT to examine the bioavailability of a variety of soluble Be complexes. Ideally low bioavailability would give insights for potential chelation agents and suggest pathways for developing a cure. Using blood from patients known to be Be sensitized based on an LPT with Be\(_2\)SO\(_4\), lymphocyte proliferation was then studied in response to the most stable complexes known including the Be–Fe–CA\(_2\), Be–Al–CA\(_2\), and Be\(_2\)DHBA.\(^{44}\) All of the soluble Be species illicit a strong immune response in the LPT (Fig. 7). The response is very similar to that observed with Be\(_2\)SO\(_4\). The observation of such a strong immune response implies that either there exists an equally strong site in the physiological system that can competitively extract the Be or the backbone of the CA species is processed at some point during the test to liberate free Be species in solution. The fact that such strong immune responses are seen both from the aliphatic CA complexes and the phenyl-based DHBA indicates ligands are not simply degraded to liberate free Be. The change in backbone structure would be expected to alter the kinetics of ligand metabolism and change the immune response if the ligand were being metabolized to release the Be. Instead, a similar immune response is observed suggesting that the beryllium is accessible based on competitive binding with a biological site for beryllium. The site would have to be competitive with
DHBA which has a sub picomolar binding constant. These results are consistent with the idea that “strong hydrogen bonds” form good Be binding sites. Although not all proteins have “strong hydrogen bond sites”, several new high resolution crystal structures have shown that there are other proteins like transferrin that have O–O distances indicating “strong hydrogen bonds” on the protein surface. Newman et al. recently reported that Be bound to ferritin elicited an LPT response similar to that of BeSO₄ in sensitized individuals. Ferritin is an iron storage protein that exists as a protein shell with a complex iron oxide–phosphate core. There are only two openings: one is a 5.6 Å pore lined by six carboxylate groups, and the second is a hydrophobic opening of 2–3 Å. The holo protein is capable of binding over 800 equivalents of beryllium. This is an even more pronounced example of how the backbone of the ligand does not appear to alter the ability of the body to respond to beryllium even when sequestered in a protein shell. These sites may provide kinetically accessible and strong thermodynamic binding sites that allow Be to easily move through cellular systems.

**Cellular response**

As mentioned previously, individuals with CBD exhibit specific peripheral blood and alveolar CD4⁺ helper T cell proliferation upon exposure to Be. The molecular mechanisms by which Be stimulates the host immune system and promotes disease progression from Be sensitization to CBD remain poorly understood. Additionally, it is not clear how the critical balance between inflammatory and suppressive mediators secreted by immune cells, such as cytokines shown in Fig. 1, that are maintained in healthy individuals, may be lost in CBD. The cytokine family consists of small signaling proteins that function in cell communication during the host immune response to inflammatory and infectious disease. Cytokines play such roles as triggering of antibody production against specific pathogens and maturation of T cells to attack pathogens. In addition, a special class of cytokines, termed chemokines, or chemoattractive cytokines, signal immune cells to migrate to the site of infection to more efficiently combat infection.

Antigen presenting cells (APCs) are thought to process an as-yet uncharacterized Be–peptide complex through the MHC class II antigen processing pathway. This Be–peptide complex would act as a foreign antigen to activate T cell proliferation and induce migration of peripheral blood lymphocytes to the lung to establish a Be-specific inflammatory response. The experimental evidence of beryllium binding to the MHC-II molecules, taken with the genetic susceptibility of those individuals with alleles having a higher number of carboxylate residues, suggests that the MHC-II receptor plays a major role in Be hypersensitivity. The response to Be exposure in cells derived from CBD patients encompasses a number of downstream effects, including pulmonary epithelial cell injury and production of high levels of the Th1 pro-inflammatory cytokines, such as IFN-γ and TNF-α.

Be exposure in non-diseased human and animal cell models has also been shown to stimulate a variety of cellular responses, including cell migration and growth inhibition. These immunomodulatory activities suggest that Be may trigger an innate immune response in non-CBD individuals as well. Although this immune response may not necessarily lead to manifestation of CBD symptoms in healthy individuals, the induction of lung inflammation and injury through Be-stimulated mechanisms of toxicity may significantly contribute to the pathogenesis of CBD in genetically susceptible individuals. To address these questions, we have used two different primary cell models derived from non-CBD individuals, donor-matched peripheral blood mononuclear and dendritic cells (PBMC/DCs) and small airway epithelial cells (SAEC). Using the PBMC/DC model, we have investigated specific induction of cytokine mediators in response to Be exposure using cell biological assays and global microarray analysis. We have also investigated effects of prior Be exposure on the innate immune response. In the SAEC model, we have examined Be-induced regulation of the cell adhesion molecule, intercellular adhesion molecule 1 (I-CAM1), which has been implicated in multiple inflammatory lung disorders. Aluminum sulfate (Al₂(SO₄)₃) was used as the control metal in these studies to elucidate Be-specific responses.

**Cytokine release**

Using the PBMC/DC model system, we have tested immune responses to BeSO₄ in cells from three healthy individuals. In support of our hypothesis that healthy individuals are able to regulate the immune response to Be, we find a critical balance in place wherein the release of proinflammatory cytokine IL6 is suppressed, and the release of suppressive cytokine IL10 is enhanced. This is in contrast to the Be-specific release of predominately proinflammatory cytokines in CBD. Furthermore, cytokine release in non-CBD cells can be correlated with alterations in intracellular phosphorylation, detectable as early as 90 min upon Be treatment. Specific pharmacological inhibitors can suppress Be-triggered phosphorylation and
reverse the inhibition of IL6 release, suggesting interplay between these two events.\textsuperscript{57}

To further study the specific signaling pathways activated by Be exposure, we have utilized global microarray analysis to examine changes in gene expression in the PBMC/DC model system.\textsuperscript{58} From these studies, we identified several chemokine genes, including members of the MIP and GRO families, that are upregulated in response to BeSO\textsubscript{4} compared to Al\textsubscript{2}(SO\textsubscript{4})\textsubscript{3}. Chemokines are chemotactic cytokines that have been implicated in mediating cell migration by establishing a concentration gradient directing other lymphocytes and macrophages to sites of inflammation.\textsuperscript{59} MIP-1\textalpha, MIP-1\beta, GRO1, GRO3, and Rantes are specifically upregulated at least three-fold in response to BeSO\textsubscript{4} in at least 4 out of 5 independent microarray experiments. To validate the microarray studies, we performed semi-quantitative RT-PCR and ELISA studies to examine transcription and secretion profiles for MIP-1\textalpha, MIP-1\beta, and GRO1. Again using PBMC/DCs from three different donors, we demonstrate that BeSO\textsubscript{4} stimulation generally exhibited an increased rate of both chemokine mRNA transcription and release compared to Al\textsubscript{2}(SO\textsubscript{4})\textsubscript{3} exposure, although variations among the individual donors do exist. MIP-1\textalpha and MIP-1\beta neutralizing antibodies can partially inhibit the ability of BeSO\textsubscript{4} to stimulate cell migration of PBMC/DCs in vitro. Taken together, these results suggest a model in which Be stimulation of PBMC/DCs 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 Be disease in susceptible individuals.

**Beryllium and adhesion proteins**

In addition to the chemokines, other immunomodulatory genes were identified in our microarray studies as being upregulated in response to BeSO\textsubscript{4} treatment, including the integral membrane proteins, integrin \beta3 and CD44. During response to chemotactic stimuli in the lung, upregulation of integrin function strengthens the adhesion between PBMCs and the vascular endothelium, allowing the PBMCs to rapidly transmigrate along the epithelium, extravascular compartment, and into the alveolar epithelial barrier.\textsuperscript{60} CD44 is the cell surface receptor for the extracellular matrix component, hyaluronan, and mediates the rolling of lymphocytes, as directed by chemotactic gradients, to inflamed endothelium and other tissues.\textsuperscript{61} Furthermore, increased expression of CD44 has been reported upon topical application of Be in mice.\textsuperscript{62}

To date, the great majority of Be studies have been performed in cells of hematopoietic lineage such as T cells and macrophages. In the respiratory tract, the epithelial cells lining the Airways represent the first defensive immunological barrier against inhaled foreign substances, such as particulate beryllium.\textsuperscript{63} However, despite their primary contact role, lung epithelial cells have not yet been explored as a model system to study Be-induced inflammatory responses. To investigate host immune responses to Be in lung epithelial cells, we have examined the regulation of the cell adhesion molecule I-CAM1 in SAEC upon exposure to BeSO\textsubscript{4}. I-CAM1 is an inducible member of the immunoglobulin gene superfamily that interacts with the \beta2 integrins CD11a/CD18 (LFA-1) and CD11b/CD18 (MAC-1) expressed on the cell surface of immune cells. These interactions are critical for transendothelial migration of lymphocytes and other immune cells into inflamed tissue during the host response. I-CAM1 expression is associated with a number of lung disorders, including allergy, cancer, and sarcoidosis, an inflammatory lung disease with similar symptoms to CBD but unknown etiology.\textsuperscript{64,65} In addition, levels of a soluble form of I-CAM1 (sI-CAM1) in the body have been found to correlate with the severity of various inflammatory disorders.\textsuperscript{66}

In our studies, we find that Be exposure specifically induced I-CAM1 expression on the cell surface of SAEC and release of sI-CAM1 into the extracellular medium, compared to untreated and Al\textsubscript{2}(SO\textsubscript{4})\textsubscript{3}-treated controls.\textsuperscript{67} Furthermore, anti-I-CAM1 antibodies inhibited Be-stimulated adhesion of SAEC to the macrophage cell-line THP1, indicating that the Be-induced adhesive properties of SAEC are at least partly due 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 activating a Be-specific immune response in Be hypersensitivity disease. Recent studies have begun to link I-CAM1 to the development of CBD. An anti-LFA-1 antibody was shown to inhibit cell proliferation and cytokine release in a CD4\textsuperscript{+} T cell line derived from a CBD patient stimulated with antigen presenting cells and BeSO\textsubscript{4}.\textsuperscript{38} Stimulated CD4\textsuperscript{+} T cell lines also exhibited a marked increase in cell surface expression of I-CAM1 within 24 h following stimulation. These studies 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. Anti-LFA-1 antibodies that strongly reduce CD4\textsuperscript{+} T cell proliferation and cytokine release have been touted as potential lead candidates for intervention in CBD.

**Beryllium and innate immunity**

The effects of environmental factors, such as the presence of low levels of endotoxin, on host response to Be or the effects of prior exposures to Be on the host innate immune response have not been investigated previously. We hypothesized that Be exposure may alter the innate immune response to bacterial cell wall components such as lipopolysaccharide (LPS). We have examined the host response to LPS in the PBMC/DC model, using cells previously exposed to BeSO\textsubscript{4} in vitro.\textsuperscript{68} We find that Be-treated cells exhibit altered cytokine release and intracellular phosphorylation profiles in response to LPS. Results in this study suggest, for the first time, that individuals exposed to Be may have altered innate immune responses to bacterial infections. LPS-mediated secretion of IL10 is significantly inhibited, while IL1\beta release is enhanced, in cells from multiple healthy donors. Additionally, not all LPS-mediated responses are altered, as IL6 release is unaffected upon Be-treatment. BeSO\textsubscript{4}-treated cells show altered phosphotyrosine
levels upon LPS-stimulation, and Be inhibits the phosphorylation of signal transducer and activator of transcription 3 (STAT3), induced by LPS. This study suggests that prior exposures to beryllium could alter host immune responses to bacterial infections in healthy individuals, by altering intracellular signaling. Clinically, pathologically and radiologically, CBD is difficult to distinguish from pulmonary sarcoidosis, and susceptibility to disease for both has been associated with the MHC, HLA-DP allele. Cell wall-deficient bacteria are reported in tissue from patients with sarcoidosis and sarcoid BAL fluid reportedly contains notably higher levels of endotoxin. The occurrence of sarcoidosis appears to be influenced by both genetic susceptibility and environmental factors, and closely linked with bacterial infections. Whether these infections are the cause or effect of disease is still unclear. Further research will be required to determine whether bacterial infections play a role in pathogenesis of “sarcoidosis of known etiology”, that is, CBD.

Concluding discussion

The role of beryllium in CBD and BeS has provided us with a unique opportunity to apply inorganic chemistry and immunology to better understand disease at a molecular level. We were able to synthesize several new small molecule complexes containing beryllium that were soluble at biologically relevant pHs, and that also had biological relevance. These complexes demonstrated that beryllium prefers to bind in specific sites, namely through the displacement of an $H^-$ in a strong hydrogen bond. This idea was then extended to the protein transferrin, where it was shown that beryllium does indeed saturate all of the available strong hydrogen bond sites in that protein. This new paradigm of beryllium binding can be used in future studies as a model for beryllium binding in proteins.

The first experimental proof of beryllium binding in HLA-DP was also demonstrated using RTLs that stimulated T cell proliferation when exposed to beryllium. The resulting experimental evidence of approximately six beryllium atoms in the HLA-DP binding groove is consistent with earlier theoretical studies, which indicated that the beryllium antigen probably contains, or is solely made up of, a beryllium containing cluster. The answer to the final form of the beryllium antigen, whether or not it contains an associated peptide, will probably only be answered with a crystal structure of the MHC-II–beryllium antigen complex. Furthermore, our development of a potential energy function for beryllium will aid in elucidating the antigen through molecular dynamics simulations.

The propensity for the beryllium antigen to form, and proliferate T cell production is very high, as evidenced by a positive LPT against very tightly binding complexes such as DHBA. Moreover, the ability of this antigen to incite inflammatory responses that lead to disease was borne out in our immunological studies. From our cell and molecular studies using the PBMC/DC cell model from healthy individuals, we show that Be exposure induces specific cytokine and chemokine responses that modulate host immunity. In addition, we demonstrate the role of lung epithelial cells in Be-mediated upregulation of cell adhesion molecule expression as a means to enhance migration of immune cells to sites of inflammation. These studies provide mechanistic insight into host response to Be exposure and may contribute to a more comprehensive understanding of the onset of Be hypersensitivity disease.

The high binding affinity of the beryllium antigen, coupled with the dissolution of BeO, aided by readily available molecules such as citric acid and transferrin, will make the sequestration of beryllium in vitro extremely difficult. Antibodies and reagents directed against LFA-1 and ICAM1, however, do show promise in inhibiting progression of disease symptoms upon exposure. Additionally, the role of bacterial infections in pathogenesis of CBD requires further investigations.

Future studies will focus on structural and molecular dynamics (MD) studies of beryllium binding in proteins, and the imaging of beryllium in physiological systems. Specifically, small angle neutron and X-ray scattering on solutions of beryllium/HLA-DP complexes will potentially yield the distribution and number of beryllium atoms in the complexes. Attempts to grow crystals of beryllium–protein complexes to obtain three-dimensional crystal structures will also be attempted. Currently, comparative MD studies are being performed to elucidate the differences between disease and non-disease alleles that could shed insight on the roles of conformational flexibility and water on binding to Be$^{2+}$. Preliminary results reveal that the effect due to K69E mutation in the disease allele is non-local and cooperative, suggesting that the recognition of the beryllium antigen extends beyond position 69 in the binding groove. Finally, we are developing three- and four-coordinate ligands for kinetic isolation of Be and fluorescent imaging in cells.

Notes and references
