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Erionite induces production of autoantibodies and IL-17 in C57BL/6 mice

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ABSTRACT

Background: Erionite has similar chemical and physical properties to amphibole asbestos, which induces autoantibodies in mice. Current exposures are occurring in North Dakota due to the use of erionite-contaminated gravel. While erionite is known to cause mesothelioma and other diseases associated with asbestos, there is little known about its effects on the immune system.
Objectives: We performed this study to determine whether erionite evokes autoimmune reactions in mice.
Methods: Bone marrow derived macrophages (BMDM) were used to measure toxicity induced by erionite. Cytokine production by BMDM and splenocytes of C57BL/6 mice was examined by bead arrays and ELISA following exposure to erionite, amphiboles and chrysotile. Wild type C57BL/6 mice were exposed to saline, erionite, amphibole asbestos (Libby 6-Mix) or chrysotile through intratracheal instillations at equal mass (60 μg/mouse). Seven months after exposure, sera were examined for anti-nuclear antibodies (ANA) and IL-17. Immunohistochemistry was used to detect immune complex deposition in the kidneys.
Results: Erionite and tremolite caused increased cytokine production belonging to the T_H17 profile including IL-17, IL-6, TGF-β, and TNF-α. The frequency of ANA was increased in mice treated with erionite or amphibole compared to saline-treated mice. IL-17 and TNF-α were elevated in the sera of mice treated with erionite. The frequency of immune complex deposition in the kidneys increased from 33% in saline-treated mice to 90% with erionite.
Conclusions: These data demonstrate that both erionite and amphibole asbestos induce autoimmune responses in mice, suggesting a potential for adverse effects in exposed communities.

Introduction

Erionite is a fibrous material classified in a group of minerals called zeolites. Erionite occurs naturally when volcanic ash reacts with ground water and forms fibrous masses inside the hollow spaces of rock. Erionite fibers share some morphologic and chemical similarities with amphibole asbestos. They are both mixtures of long and straight mineral fibers composed of long chain silicon oxides (averaging $Si_{14}O_{36}$ and Si_8O_{22}, respectively) with various cations depending on the specific type of fiber (Carbone et al., 2012). Both asbestos and erionite exposures can cause pleural fibrosis and malignant mesothelioma, and there have been extraordinarily high rates of mesothelioma in villages located in central Turkey where the residents have been exposed to erionite (Carbone et al., 2011).

Many geologic formations contaminated with erionite have been found throughout the western United States (Carbone et al., 2011; Van Gosen et al., 2013). Several gravel pits that are contaminated with erionite have been excavated in western North Dakota (Dunn County Erionite Workgroup, 2010). Since the 1980s, this gravel has been used to construct more than 300 miles of local roads, parking lots, baseball fields, playgrounds and other areas (Carbone et al., 2011). Activity based sampling demonstrated that exposures in Dunn County (0.175 fibers/cm^3) were higher or similar to those found in Turkish villages (0.0431–0.221 fibers/cm^3) experiencing mesothelioma rates as high as 1 in 1000. This is compared to rates of 1–15 per 10^6 in most areas of the United States even where asbestos exposure is high (Carbone et al., 2011). Fortunately, a rise in mesothelioma cases among exposed populations in North Dakota has not yet been observed. Of note, in Karain, a Turkish village with over 50% of all deaths (1990–2006) due to mesothelioma, it was reported that the average latency (time between exposure and the development of disease) for mesothelioma was 53.8 years (Metintas et al., 2010). Therefore, given the long latency between the development of mesothelioma and the relatively recent use of erionite-contaminated gravels in North Dakota (ND), it may still be too early to observe this health outcome. A recent study of Dunn County, ND, road workers, without a history of asbestos exposure, did report evidence of fibrotic pleural disease consistent with erionite exposures (Ryan et al., 2011). Thus, given the demonstrated toxicity and health effects associated with erionite exposures, it is

Abbreviations: ANA, antinuclear autoantibodies; BMDM, bone marrow derived macrophages; SLE, systemic lupus erythematosus; TGF, transforming growth factor; TNF, tumor necrosis factor; CBA, cytometric bead array.

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important to implement preventive public health actions to reduce exposures and to follow exposed workers and communities for adverse effects and any opportunities for early medical interventions.

A population in Libby, Montana has been exposed amphibole asbestos due to the mining of contaminated vermiculite. Not only has this population been suffering from increased incidence of mesothelioma and other pulmonary diseases, but screening done by the Agency for Toxic Substances and Disease Registry (ATSDR) in 2000–2001 found that 6.7% of the people screened were diagnosed with a systemic autoimmune disease including systemic lupus erythematosus (SLE), scleroderma, and rheumatoid arthritis, where the expected prevalence for these three diseases is less than 1% (Pfau et al., 2005). The Libby population was also found to have higher levels of autoantibodies when compared to a population with no known exposure to amphibole asbestos (Pfau et al., 2005). Further study of the Libby population showed that the risk for being diagnosed with a systemic autoimmune disease (SAID) increases with increased exposure to amphibole asbestos, supporting the hypothesis that asbestos exposure is linked to autoimmunity (Noonan et al., 2006).

Since erionite and amphibole asbestos share some physical properties and can cause similar diseases, the purpose of this study was to determine whether erionite is able to stimulate autoimmune responses, also similar to amphibole asbestos. Immune cells, specifically cultured macrophages and mixed splenocytes, were exposed to erionite *in vitro* and examined for various cytokines that have been implicated in autoimmune disorders: in particular, interleukin-17 (IL-17), which is produced by T Helper 17 (T_H17) cells. T_H17 cells form in the presence of TNF-α, IL-6 and TGF-β, which are produced by innate immune cells including macrophages (Furuzawa-Carballeda et al., 2007). Some studies have shown that IL-17 plays a part in the pathogenesis of rheumatoid arthritis by demonstrating elevated levels of IL-17 in synovial fluids of diseased joints and activation of osteoclasts (Kotake et al., 1999). Elevated serum IL-17 has been demonstrated in individuals with SLE, but the role of IL-17 in SLE is still unclear (Afzali et al., 2007).

Given the potential role of cytokines of the T_H17 lineage in autoimmune diseases, it was hypothesized in this study that immune cells *in vitro* and *in vivo* would express T_H17 cytokines after exposure to amphibole asbestos, which has been associated with autoimmunity in the Libby, MT population. Also, since erionite and amphibole asbestos share similar physical characteristics, it is also hypothesized that erionite will evoke a similar response by immune cells to produce T_H17 cytokines.

Autoantibodies against ubiquitous antigens are hallmarks of systemic autoimmune diseases (Darrah and Andrade, 2013). The presence of these antibodies was examined using C57BL/6 mice exposed to erionite through intratracheal instillations. Mice were also exposed to saline only, amphibole asbestos, and chrysotile asbestos, which has not been associated with autoimmunity. A study done by Pfau et al. in 2008 demonstrated increased autoantibodies in C57BL/6 mice exposed to an amphibole asbestos, tremolite (Pfau et al., 2008). However, to our knowledge, this type of study has not been done using erionite. Therefore, this study went on to assess how erionite affects certain immune parameters that are associated with autoimmunity *in vivo*.

Methods

Fibers. Erionite collected at Rome, Oregon, was provided by the U.S. Environmental Protection Agency (EPA) as a characterized sample of purified mineral fibers. Transmission Electron Microscopy (TEM) characterization of the EPA erionite was provided by Dr. Jed Januch, EPA (Table 1). Pure Korean tremolite asbestos (amphibole) was provided by Dr. Ann Wylie, University of Maryland. Libby 6-Mix asbestos containing tremolite, other amphibole asbestos forms, and closely related asbestiform fibers of winchite and richterite, was provided by the U.S. Geological Survey (Meeker et al., 2003). Chrysotile was provided by the National Toxicology Program by Dr. Dori Germolec (National Toxicology Program, 1990). Wollastonite was kindly provided by Dr. Andrij Holian (University of Montana). All fibers were suspended in sterile phosphate buffered saline (PBS, pH 7.4), and sonicated (Branson Ultrasonics, Danbury, CT) for 5 min prior to use to minimize aggregation of the fibers. Concentrations used in cell cultures are given in $\mu g/cm^2$ with the observation that the fibers precipitated to the bottom of the well, with densities being 2.5 g/cm^2 for erionite (Dogan et al., 2008), 2.6 g/cm^2 for intermediate chrysotile (National Toxicology Program, 1990), and 3.0 g/cm^2 for tremolite (Webber et al., 2008).

Mice. All experiments on mice were approved by the Idaho State University Institutional Animal Care and Use Committee (IACUC, protocol #692-0213). The mice used were female wild type C57BL/6 (Idaho State University Animal Care Facility, breeders from Jackson Labs, Bar Harbor, Maine). This strain is not genetically predisposed to systemic autoimmune responses, and was chosen in order to a) test the autoimmune responses in a genetic background that did not already produce disease, and b) to be able to compare to other published studies of ANA in mice (Ferro et al., in press; Pfau et al., 2008). These mice were housed under specific pathogen free (SPF) conditions in ventilated cages (Tecniplast, West Chester, PA) with 12 hour light–dark cycle, constant temperature (22 °C) and humidity (45%), and *ad libitum* food and water.

Bone marrow derived macrophages. To examine innate immune system cells, we used bone marrow derived macrophages (BMDM) as a model for alveolar, pleural or peritoneal macrophages. The bone marrow used was from C57BL/6 mice and collected and differentiated as previously described (Overocker and Pfau, 2012). The media used for these cells was RPMI 1640 1× with L-glutamine and 25 mM HEPES (Mediatech, Manassas, VA), supplemented with 10% fetal bovine serum (FBS, Atlanta Biologicals, Lawrenceville, GA) and penicillin–streptomycin solution (Sigma, St. Louis, MO). All cultures were maintained in a humidified 5% CO_2 incubator at 37 °C.

Cell viability. The CyQUANT Proliferation Assay (Invitrogen, Eugene, OR) quantifies cell proliferation or death in culture based on the amount of DNA, using a green fluorescent dye, CyQUANT GR, that binds to nucleic acids. A cell suspension of BMDM macrophages was obtained in media at a concentration of 10^6 cells ml^{-1}. One hundred microliters

Table 1
Summary characteristics for fibers used in this study.

Fiber	Length (um)	Diameter (um)	Aspect ratio (L/D)	Surface area (m^2/g)	Reference	Source
Libby 6-Mix	7.21[a]	0.61[a]	11.8	5	Hillegass et al. (2010)	USGS
Korean tremolite	5.49[a]	0.32[a]	17.16		Bernstein et al. (2003)	Dr. AG Wylie, Univ. of Maryland
Intermediate chrysotile	0.82[b]	0.089[b]	8.435	20.2 ± 0.1	National Toxicology Program (1990)	NIEHS
Oregon erionite	6.21[a]	0.83[a]	13.37[a]		Januch, J.	EPA
	4.15[b]	0.53[b]	9.23[b]		TEM data	

[a] Mean.
[b] Median.