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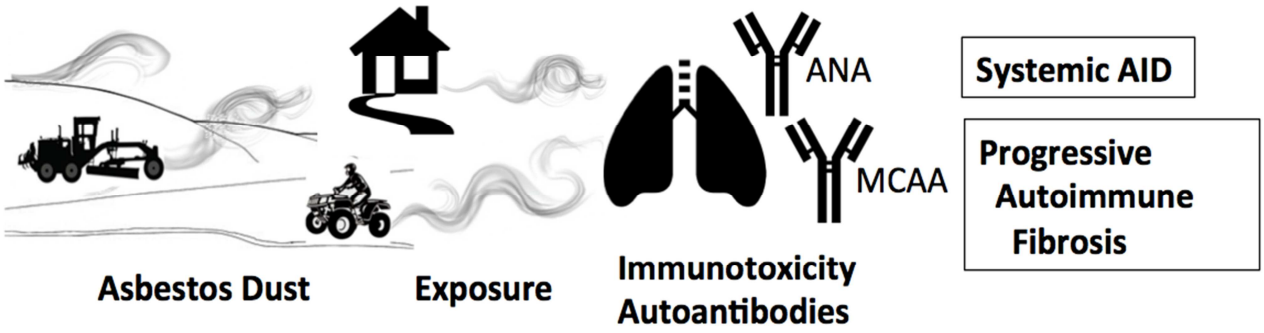
Graphical Abstract (Same as Figure 3)



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ABSTRACT

The study of asbestos immunotoxicity is generally applied toward understanding the mechanisms that lead to its infamous outcomes, mesothelioma and asbestosis, rather than as an outcome itself. However, emerging evidence suggests that asbestos exposure has critical inflammatory and autoimmune effects. Although crystalline silica is broadly accepted as an exposure trigger for systemic autoimmune diseases (SAID), the literature supporting asbestos as another SAID trigger is limited. Challenges for establishing causality between asbestos exposure and autoimmunity include small, often occupationally-exposed cohorts, a tendency to focus on carcinogenicity or lung pathology, and poor characterization of fiber type in a given exposure scenario. However, a growing set of studies strongly supports inclusion of amphibole asbestos (AA) as an environmental trigger for autoimmunity. Both human and animal studies have revealed that AA, but not the common commercial asbestos (chrysotile), drives autoantibody production, alters cytokine profiles, and is associated with autoimmune disease. The potential public health impact of these findings are highlighted in the growing awareness of "naturally occurring asbestos" in geographic locations where it was not previously predicted to occur, leading to environmental exposures in wide areas of the world as a component of dust. As climate change brings warmer and dryer conditions to the more arid parts of the world, wind-blown mineral dusts containing asbestos may become more common. It is essential that epidemiologists, clinicians and regulatory agencies become aware of this emerging risk to health by an environmental immunotoxicant.

Key words: Environmental, Autoimmunity, Naturally Occurring Asbestos (NOA), Pleural Fibrosis, Libby Amphibole

Abbreviations: ANA, antinuclear autoantibodies; AA, amphibole asbestos; ARD, asbestos-related diseases; ATSDR: Agency for Toxic Substances and Disease Registry; CARD, Center for Asbestos Related Diseases (Libby, MT); CDC, Center for Disease Control (and Prevention); EPA, Environmental Protection Agency; LA, Libby Amphibole; NOA, naturally occurring asbestos; SAID, systemic autoimmune diseases; SLE, systemic lupus erythematosus, aka lupus.

1.1 INTRODUCTION

Asbestos is a fibrous mineral that is highly resistant to destruction by heat or other forces. It has been used commercially for decades, providing valuable materials for insulation, brake linings, and construction. It continues to be mined in several countries and used in manufactured products around the world, including the United States. The term "asbestos" historically refers only to commercial fibers in two families, serpentine (chrysotile) and amphibole asbestos (AA). The commercial amphiboles include only five types of fibers, classified for purposes of understanding their properties related to extraction and materials fabrication. In this review, however, the term is used more broadly, to include not only the regulated commercial forms but also the unregulated fibers that have similar properties.

The majority of research performed on asbestos has studied only the commercial forms of asbestos and has focused on cancer and asbestosis as outcomes of occupational exposures. Due to new discoveries, this limited research scope is no longer appropriate [1, 2]. Fibrous minerals not previously classified as asbestos must now be recognized as a part of the human "exposome", a component of lifetime exposures that may impact health. There are several non-occupational exposure pathways occurring world-wide from airborne release [3]. Such exposures carry risk even at low concentrations of fibers released from rocks and soils by construction, road building, recreation, or dust storms [3-6]. This is becoming a highly significant public health issue, especially in arid regions such as the southwestern United States due to a combination of increased population growth, development, and increasing aridity caused by climate change [7].

1.2 Paradigm Shifts: Mineral Fiber Terminology and Non-Cancer Outcomes

Research on environmental asbestos exposures has revealed the need to distinguish between the two families of asbestos. Chrysotile asbestos is well known as the most common commercial form of asbestos, due to its ability to be woven into many kinds of materials. Therefore, regulatory standards for asbestos are based largely on occupational exposures to chrysotile, which is known to increase risk for mesothelioma, pulmonary carcinoma, and interstitial fibrosis (asbestosis). However, it seems to be less pathogenic than amphibole asbestos (AA) [8], which makes up many of the known environmental exposures. The immunotoxicity of chrysotile appears to be quite distinct from that of AA [9], leading to different health outcomes, but this needs further study.

Despite decades of research supporting immune dysfunction from asbestos exposure, asbestos has not been designated as a trigger for autoimmunity (reviewed in [10, 11]), and autoimmune outcomes have never been incorporated into asbestos risk assessments. We propose the possibility that this is because the literature historically has not clearly differentiated between types of asbestos in autoimmune studies, and that AA, but not chrysotile, may be an environmental trigger for SAID [11].

1.3 Theory: Amphibole asbestos as a trigger for autoimmune outcomes.

What is needed is a paradigm shift in the way we evaluate health effects of exposure to fibrous dusts. This will mean careful evaluation of fiber-specific risk, going beyond the commercial fibers. The data reviewed below calls for continued studies into immune dysfunction from asbestos exposure, specifically comparing amphibole with chrysotile, and strongly supports the ability of asbestos to impact ultimate disease outcomes through its immunotoxicity. Specifically, AA has been linked to serum autoantibodies [11-13], and an

increased risk of systemic autoimmune diseases (SAID) [14], but chrysotile has not [9]. Interestingly, chrysotile's ability to cause cancer may result from a combination of its carcinogencity plus its inhibition of the anti-cancer immune response [9, 15], including T_H1 and T_H17 cytokines which are implicated in autoimmunity and are triggered by AA [9, 16].

2.1 Libby's Lessons

In 1999, Pulitzer-winning journalist, Andy Schneider, revealed to the world that the mining and use of asbestos-contaminated vermiculite in Libby, Montana was causing a high rate of morbidity and mortality [17]. The suffering manifested as typical asbestos-related diseases: mesothelioma, asbestosis and pleural fibrosis. However, a federally-funded screening program also revealed that an elevated proportion of the population was reporting systemic autoimmune diseases (SAID), such as Systemic Lupus Erythematosus (SLE). In 2001, a team from the University of Montana, Missoula, was asked to assist the CDC/ATSDR in screening the residents of Libby for scientific evidence of an autoimmune outcome. First, testing for antinuclear autoantibodies (ANA) was performed on serum donated from screening participants. ANA are commonly used to assist with diagnosis of SAID, and despite background levels in healthy people, they are considered a valuable tool for screening and assessment of people with autoimmune symptoms. That study demonstrated that the frequency and titers of ANA in Libby residents were significantly higher than in an age- and sex-matched group from nearby Missoula, MT [13]. Second, the team analyzed self-reported ATSDR survey data of more than 7300 Libby screening subjects for diagnoses of SAID. In 2006, this second study associated asbestos exposure with increased risk of SLE, scleroderma and rheumatoid arthritis [14]. In 2009, a report of the ANA profiles of the Libby cohort revealed that the most common ANA patterns were consistent with SLE, with elevated frequencies of antibodies to dsDNA, RNP, and Ro52 [18]. There was also a high frequency of antibodies to topoisomerase, also called Scl-70, indicative of scleroderma. These autoantibody patterns, especially at the elevated titers seen in Libby, suggest a pathogenic process according to current thinking [19]. Subsequently, the Libby Epidemiology Research Program (LERP) found that among the 4779 patients who have undergone health screening at the Center for Asbestos Related Diseases (CARD, Libby MT), the rate of diagnosed SLE is over 1%, well above the CDC's reported U.S. prevalence of 0.05%.

2.2 Epidemiologic Evidence and the Imprecision of the Term Asbestos

Despite the emerging evidence from Libby, plus a history of studies describing similar results in other asbestos-exposed cohorts, asbestos has not yet been designated as an environmental trigger for SAID [10, 20]. However, another silicate dust, crystalline silica, is strongly associated with SAID (reviewed in [21, 22]). This difference triggered mechanistic studies to examine possible similar pathways to autoimmunity by both silica and asbestos, but no consensus arose, with some evidence that the immune dysfunction pathways were different [23] and some showing that they were similar [24-26]. The problem may be that the term "asbestos" is too imprecise because of the many forms of mineral fibers to which humans are exposed. When the literature was reviewed based on fiber types, a pattern emerged in which AA was linked with autoantibodies and SAID, but chrysotile was not (reviewed in [11, 27]). To our knowledge, there is only one study comparing ANA frequency in cohorts exposed exclusively to amphibole or chrysotile. In that case, amphibole (LAA) increased the frequency of ANA above expected levels, but chrysotile (in a cohort of New York pipe insulators) did not [27]. There were, however, weaknesses in this study including the small size of the chrysotile

cohort and in comparing a purely occupational exposure (pipe insulators) with a mixed occupational/environmental exposure (Libby). However, comparable exposure populations are rare, making such studies in humans very difficult.

3.1 Mouse Studies of Asbestos Immunotoxicity

The immunotoxicity of asbestos starts with inflammation, driven by oxidative stress and cytokine production (Figure 1). The effects of asbestos in mice and cell culture studies include "frustrated phagocytosis", oxygen radical formation, inflammasome activation, DNA damage, and apoptosis [28-30]. These early outcomes of interaction with mineral fibers appear to drive the ultimate disease outcomes, and may be related to specific size, mineralogy, and chemistry of the fibers. Current studies are even finding similarities between the inflammatory effects of asbestos and elongated nanomaterials, such as nanotubes [31]. The mechanisms of AA immunotoxicity appear to be similar to those of crystalline silica, but distinct from chrysotile. For example, one group in Japan has contrasted the immune effects of silica and chrysotile asbestos, demonstrating that although silica clearly generates an immune dysfunction supportive of autoimmunity, chrysotile is immunosuppressive particularly in terms of an anti-cancer T_H1 responsiveness [15, 23, 32]. Similar results were seen when comparing the effects of amphibole to chrysotile in mice, where chrysotile inhibited T_H1 and T_H17 cytokines, but AA induced them [9, 16] (Figure 2).

Studies in rodents have demonstrated the ability of AA to trigger ANA production [33, 34]. In mice, immune complex deposition and kidney pathology have been noted, suggesting a lupus-like disease manifestation [16, 33]. Amphibole asbestos (including both tremolite and LAA) also triggers T_H17 cytokines both *in vitro* (spleen cells) and *in vivo* (mouse serum), which are implicated in autoimmunity [9, 16]. Chrysotile, however, did not trigger ANA, kidney pathology, or T_H17 cytokines [9]. These studies strongly support the hypothesis that only AA is similar to silica in producing an immune dysfunction that predisposes to autoimmunity. This may clear the way for studies of AA specifically as an environmental trigger for SAID.

3.2 MCAA and Autoimmune Fibrosis in Mice

In addition to ANA, mice produce Mesothelial Cell AutoAntibodies (MCAA) in response to LAA exposure, and MCAA are associated with excess production of pleural collagen in AA-exposed mice [35]. Critically, MCAA instilled into the peritoneal cavity of naïve (non-asbestos exposed) mice can trigger collagen deposition, suggesting that MCAA can directly contribute to serous fibrosis [35]. The idea that antibodies that bind to collagen-producing cells can contribute to fibrosis is not new. Antibodies to fibroblasts have been implicated in autoimmune fibrotic diseases such as scleroderma [36, 37]. As a potential autoimmune phenomenon, therapeutic modalities for pleural fibrosis begin to emerge. Therefore, the possibility that pleural fibrosis includes an autoimmune process has precedence and public health significance.

4.1 Progressive Pleural Fibrosis as an Autoimmune Phenomenon

Another lesson learned from Libby is that pleural fibrosis is the most common manifestation of exposure and that LAA's pleural fibrosis is severe and progressive [38-40] unlike that seen with chrysotile. LAA's progressive and inflammatory pleural disease led to consideration of a potential contribution from immune dysfunction (Figure 3).

First, we demonstrated that LAA induces MCAA, which were associated with

radiographic changes in the pleura [12]. We then showed that MCAA induced collagen deposition by mesothelial cells *in vitro* [41], and that they were associated with pleural, but not interstitial, disease in a subset of CARD patients [27]. Currently, screening indicates that up to 35% of LAA-exposed subjects have MCAA, but that MCAA are rare in normal human serum (Libby Epidemiology Research Program and [27]).

4.2 Possible mechanisms of Asbestos-Induced Autoimmune Fibrosis

The above studies of anti-fibroblast and anti-mesothelial cell autoantibodies reveal that they can induce cytokine and collagen production that support fibrogenic processes. One pathway involves binding of antibodies to the Platelet-Derived Growth Factor Receptor, PDGFR-alpha [42, 43]. In mice, anti-fibroblast antibodies induced by LAA were shown to bind PDGFR-alpha, and to induce a myofibroblast phenotype with STAT-1 activation, all implicated in fibrogenic pathways [44]. A study of the gene expression initiated by exposure of mesothelial cells to asbestos-induced MCAA revealed that several pathways related to fibrogenesis were triggered, including both PDGFR-alpha and STAT-1 [45]. Further, two key transcription factors were activated, C/EBP-beta and HIF-alpha, both of which play roles in collagen matrix remodeling [45]. Pharmaceutical inhibition of either transcription factor reduced the collagen expression induced by MCAA, directly relating these LAA-induced autoantibodies with collagen matrix formation and thus fibrosis [45].

Clearly, antibodies to cellular receptors such as PDGFR-alpha suggest the possibility that blocking such an interaction could be therapeutic. Using mass spectrometry of surface antigens bound by LAA-induced MCAA, one study showed that surface plasminogen is a target for MCAA [46]. A commercial antibody to plasminogen, known to block plasminogen activity, increased collagen production from cultured mesothelial cells similarly to the MCAA, suggesting that a mechanism of MCAA-induced collagen production is by blocking plasminogen activity. The plasminogen/plasmin system is known to participate in collagen matrix remodeling in other forms of fibrosis [47], thus validating inhibition of this pathway as a mechanism for MCAA-induced fibrosis.

5.1 Conclusions and Implications for the Future

The goal of this research direction is to improve health through recognition and prediction of disease, improved screening, and early diagnosis. Recently, a panel of experts described three areas in which criteria need to be developed for establishing associations between environmental factors and autoimmunity [48]. Two of those would be establishment of criteria for defining environmental risk factors for diseases meeting diagnostic criteria, and new environmental autoimmune disorders not meeting current diagnostic criteria. Third, there is a need to identify environmental agents that trigger autoimmune disease in particular patients. As evidence is gathered from various research strategies, from epidemiology to animal models, "levels of confidence" can be applied to the strength of evidence. Unfortunately, we have rare opportunities to conduct prospective studies of asbestos-exposed populations. Environmental AA exposures are increasing, but we understand little about the immunologic consequences of these exposures. It seems reasonable and prudent to use emerging data from Libby and rodent studies to raise the level of concern for AA as a trigger for autoimmune disease, and to put effort and resources toward more research. Critically needed are more animal studies of fiber-specific comparisons of health outcomes, including immune dysfunction and autoimmunity in multiple strains. Also, any studies of asbestos effects in humans should include blood collection for evaluation of immune markers, including

autoantibodies.

In conclusion, evaluation of asbestos health effects must a) include all mineral fibers, distinguishing chrysotile from AA, b) include non-cancer outcomes, particularly immunotoxicology and autoimmunity, and c) recognize the emerging public health risks of environmental sources of toxic mineral fibers, such as Naturally Occurring Asbestos (NOA).

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Figure Legends

Figure 1: Schematic of Early Cellular Events and Health Outcomes with Asbestos Exposure.

Figure 2: Hypothetical Model of Differential Effects and Outcomes of Amphibole Versus Chrysotile Exposure. Pie charts do not contain data, but represent simple models in which health outcomes for amphibole asbestos are predominated by fibrotic and autoimmune responses, and those for chrysotile tend to be cancer (mesothelioma and lung carcinomas).

Figure 3: Schematic Model for Hypothetical Induction of Autoimmune Responses through Environmental Exposures to Amphibole Asbestos. ANA = Antinuclear Autoantibodies; AID= Autoimmune Diseases; MCAA = Mesothelial Cell Autoantibodies

Figure 1

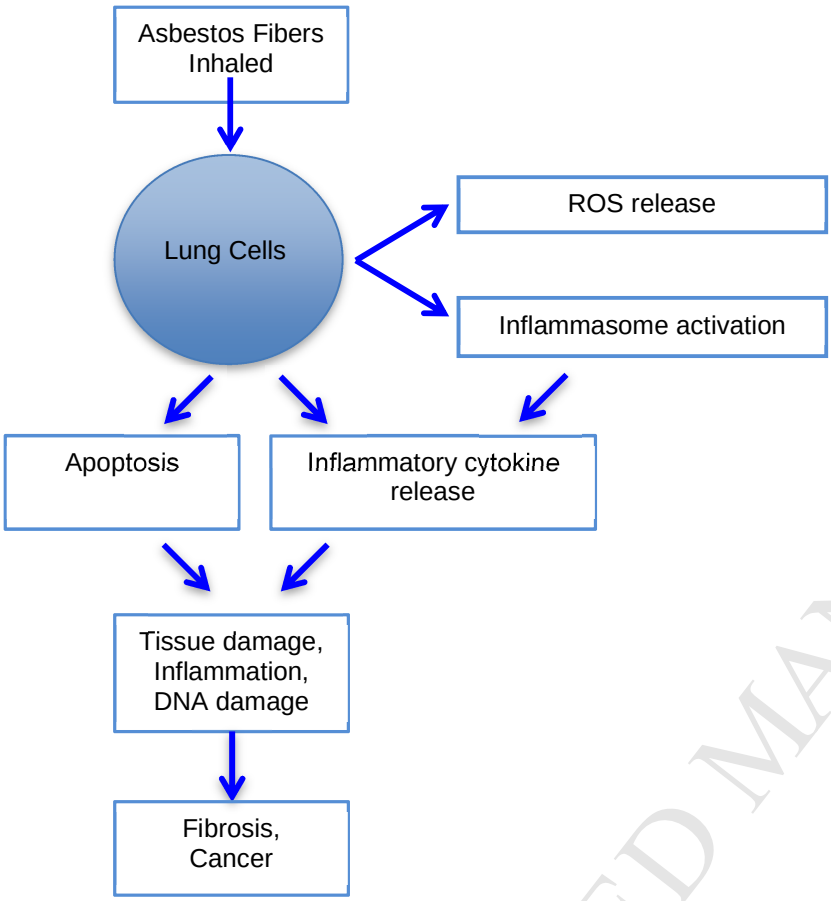

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Figure 2

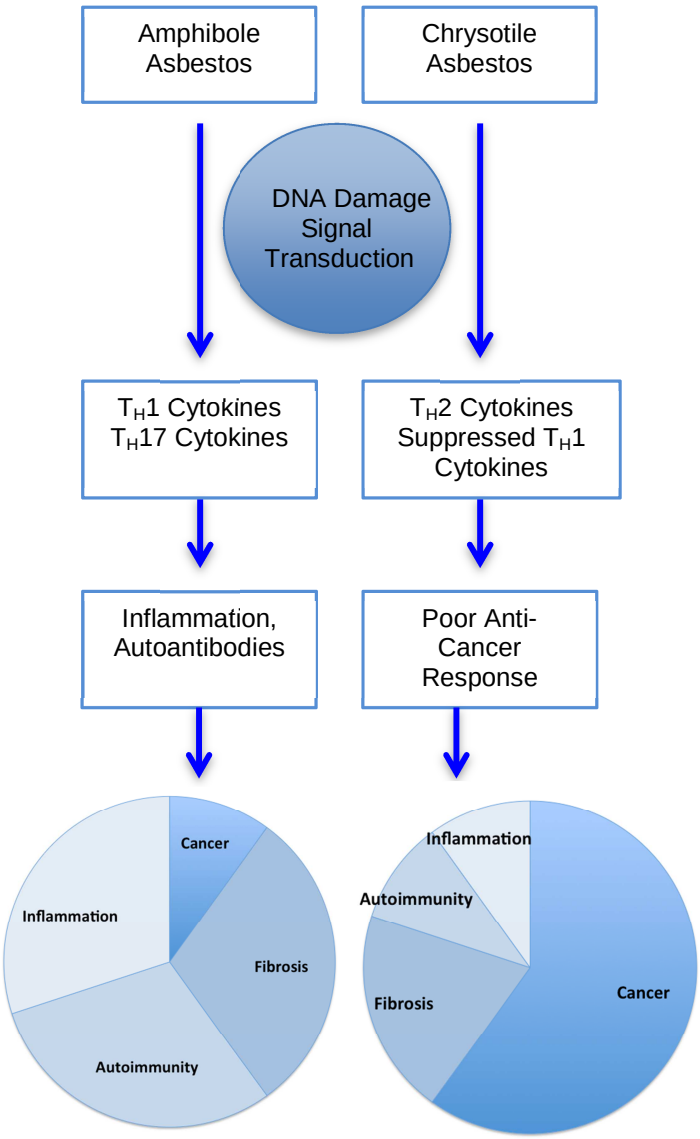

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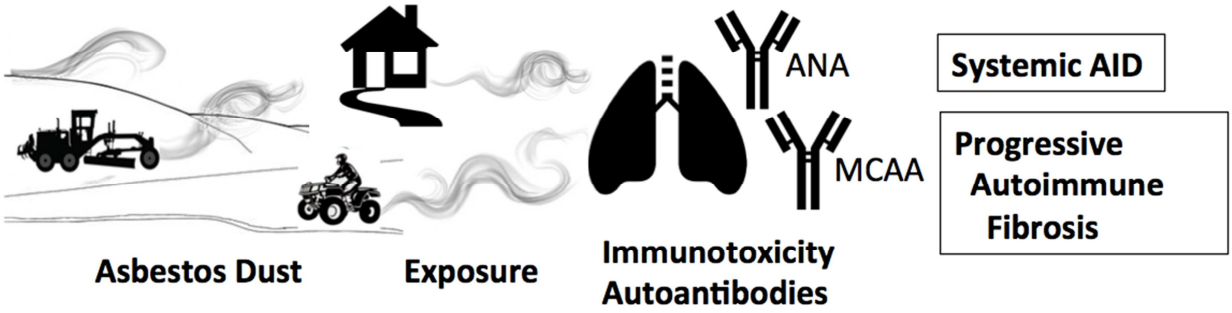
Figure 3



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Highlights:

- Amphibole Asbestos (AA) may be an environmental trigger for autoimmunity.
- AA can trigger autoantibodies associated with both systemic autoimmune diseases such as lupus, and autoimmune pleural fibrosis.
- Mouse studies corroborate findings regarding AA in exposed human populations.
- Naturally Occurring Asbestos (NOA) now presents an emerging public health risk.