

Chapter 9

Silicates and Autoimmunity

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Abstract Inhalation of particulate matter is associated with a number of acute and chronic disorders including autoimmune rheumatic diseases. The strongest evidence for a link with autoimmune disease comes from epidemiological studies describing the association of occupational exposure to crystalline silica dust with the systemic autoimmune diseases SLE and RA. Very little is known regarding the mechanism by which silica exposure leads to systemic autoimmune disease. However, in the case of silicosis, there is an extensive research literature that can help identify disease processes that may precede development of autoimmunity. The pathophysiology of silicosis consists of deposition of particles into the alveoli of the lung where they cannot be cleared. Ingestion of deposited particles by alveolar macrophages initiates an inflammatory response which then stimulates fibroblasts to proliferate and produce collagen. Silica particles are enveloped by collagen leading to fibrosis and nodular lesions. These findings are consistent with an autoimmune pathogenesis that begins with activation of the innate immune system leading to proinflammatory cytokine production, inflammation of the lung leading to activation of adaptive immunity, breaking of tolerance, autoantibodies, and tissue damage. The variable frequency of these features following silica exposure suggests significant genetic involvement and gene/environment interaction in silica-induced autoimmunity.

Keywords Silica • Asbestos • Silicosis • Autoimmunity • Animal model

9.1 Introduction

Particulate matter (PM) is a complex mixture of extremely small particles and liquid droplets. It is made up of a number of components, including acids, organic chemicals, metals, and soil or dust particles. Particulate matter is categorized according to size, which defines its facility to be retained in the lungs. PM10 (particles up to 10 μm in diameter) deposit in the nasal passages or larger airways,

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while PM_{2.5} (particles smaller than 2.5 μm in diameter) can reach the alveoli [1]. Inhalation of particulate matter is associated with a number of acute and chronic disorders [1, 2] including autoimmune rheumatic diseases [3, 4]. The strongest evidence for a link with autoimmune disease comes from studies describing the association of exposure to crystalline silica with the systemic autoimmune diseases systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), systemic sclerosis (SSc), and anti-neutrophil cytoplasmic antibodies (ANCA)-related vasculitis [5]. Another silicate, asbestos, has been linked with RA and the presence of autoimmune features in the absence of diagnosed disease [5, 6]. In this chapter, the relationship between exposure to silicates and autoimmunity is examined with particular reference to silica and asbestos; the controversial relationship between silicone-containing breast implants and autoimmunity [7] is not reviewed here because, although silicone contains silicon, they have very different physical and chemical properties. The role of animal models in replicating observations of silicate-induced human autoimmunity is discussed. The limited information on possible mechanisms of silicate-induced autoimmunity, deduced from both human and animal studies, are compared and contrasted, and themes for future research suggested.

9.2 Silica and Autoimmunity in Humans

Silica (SiO_2) is an oxide of silicon and can exist in mineral form as well as being produced synthetically. Crystalline silica exists in seven types or polymorphs. Quartz is the most common form in nature and exists in two forms, α - and β -quartz, with α -quartz being the only stable form under normal conditions [8]. Occupational exposure to respirable crystalline silica ($<10 \mu\text{m}$) occurs in many situations where materials containing crystalline silica, such as rocks, are reduced to dust or when fine particles containing silica are disturbed. Occupations include drilling, mining, sand blasting, grinding, and cutting and are often referred to as the dusty trades [9, 10]. While oral ingestion of silica is essentially nontoxic, inhalation of crystalline silica dust can lead to silicosis, airway disease, cancer, and autoimmune diseases [9]. Silicosis is characterized by chronic inflammation and scarring in the upper lobes of the lung and can be classified based on the amount inhaled, time course, and duration of exposure as chronic simple silicosis, accelerated silicosis, and acute silicosis (silicoproteinosis) [8, 9, 11]. Chronic simple silicosis is the most common form and occurs after 10–15 years of exposure to low to moderate levels of respirable crystalline silica. A hallmark of the disease is the presence of silicotic nodules. A complicated form occurs when smaller lesions amalgamate to form nodules of greater than 2 cm. Accelerated silicosis occurs in a shorter time span, 5–10 years after first exposure to higher exposure levels. Acute silicosis can develop within weeks to several years following exposure to extremely high levels of respirable crystalline silica. It has a rapid onset of symptoms and is the most severe form of silicosis. The pathophysiology of silicosis, especially the

chronic form, consists of deposition of particles into the alveoli of the lung where they cannot be cleared. Ingestion of deposited particles by alveolar macrophages initiates an inflammatory response which then stimulates fibroblasts to proliferate and produce collagen. Silica particles are enveloped by collagen leading to fibrosis and nodular lesions [9].

A number of epidemiological studies support the association of silica exposure with autoimmune diseases in humans [5, 12–16]. It is unclear if silicosis is required for the expression of silica-induced autoimmune diseases, although high exposure levels were associated with SLE [10]. Other studies identified an association between the intensity of exposure and the production of autoantibodies but found no relationship between autoantibodies and silicosis [17, 18]. Importantly, autoantibodies specific to connective tissue diseases (SLE, SSc) were found in exposed individuals including anti-DNA, anti-SS-A/Ro, anti-SS-B/La, anti-centromere, and anti-topoisomerase I [17, 18]. A study of silicotics found no association between histocompatibility antigens and ANA, RF, or serum IgG and IgM; however, there was increased prevalence of B44 and A29 which supported previous observations of a link between HLA and silicosis [19] although the nature of the requirement requires further study. In some individuals the presence of disease-specific autoantibodies preceded the appearance of autoimmune disease [18]. Several studies found increased occurrence of different diseases in individual study populations [10]. Because clinical features and autoantibodies can overlap between diseases, this suggests that silica exposure may trigger a common mechanism among systemic autoimmune diseases.

Although the relative risk of a specific connective tissue disease (CTD) following silica exposure can increase manyfold [16], with the exception of rheumatoid arthritis (RA), most occur at 1 % or less in silica-exposed populations [5, 10, 16, 17]. However, the prevalence of non-disease-specific immunological features associated with autoimmunity occurs in far greater numbers. Antinuclear autoantibodies (ANAs) can be found in up to 30 % of silica-exposed individuals without CTD symptoms, with higher frequencies and titers associated with the development of CTDs [17, 20]. In patients with silicosis, hypergammaglobulinemia can occur in over 65 % of patients [21], and ANA prevalence can be 34 % or higher [22, 23]. ANA often occurs in association with increased cytokines [24]. Even in ANA-negative individuals [25, 26], silica exposure can be associated with changes in cell surface markers and cytokines [26, 27]. Proinflammatory cytokines and inflammation in the lung are thought to be precursors to silicosis [28] which can occur in 47–77 % of individuals with adequate follow-up after silica exposure [29]. End-stage renal disease due to silica exposure occurs in about 5 % of exposed individuals [29]. The mechanism of silica nephropathy is unclear but appears to have two components: a direct nephrotoxicity and induction of autoimmune disease [30]. These findings are consistent with a disease progression that begins with activation of the innate immune system leading to proinflammatory cytokine production, inflammation of the lung leading to activation of adaptive immunity, breaking of tolerance, autoantibodies, and renal damage [9, 28, 31, 32].

9.3 Asbestos and Autoimmunity in Humans

Asbestos consists of six naturally occurring silicate minerals with a characteristic external shape (crystal habit) consisting of long, thin fibrous crystals, with each visible fiber consisting of microscopic fibrils that can be released by abrasion [8]. There are two classes: serpentine and amphibole. Chrysotile is one of the three different polymorphs of the serpentine class; the other two are antigorite and lizardite. Amphibole fibers are needle-like and include the following members: amosite, crocidolite, tremolite, anthophyllite, and actinolite. Chrysotile, unlike amphibole, is sensitive to acidic environments and disassociates from its crystalline state [33]. This may help explain differences in chronic inflammation and pathology, including cancer, induced by exposure to these two forms of asbestos [6, 8, 33]. Prolonged exposure to asbestos results in asbestosis, a chronic lung disease caused by scarring of the lung tissue, which has a pathophysiology similar to that described above for silicosis [34].

In contrast to silica, there is insufficient evidence that asbestos exposure is linked to autoimmune diseases [5, 6]. A case-control study of current and former residents of Libby, Montana, who were exposed to vermiculite contaminated with asbestos found an association with development of RA [35], but no medical records were evaluated to confirm the self-reported diagnoses. Another small case-control study in Sweden found an association with asbestos exposure in men with newly diagnosed RA [36]. A number of studies have suggested an association of asbestos exposure with immune activation or features of autoimmunity (e.g., elevated immunoglobulins, RF, ANA, or ANCA) [5, 6]. For the most part, these studies produced variable results making a definitive assessment of the potential of asbestos exposure to elicit autoimmunity difficult. This may stem in part from the difficulty in defining the contribution of asbestos and silica in concurrent exposures, identifying the important features of asbestos fibers in inflammation and disease, and identifying appropriate study cohorts and diagnostic criteria [5, 6, 34, 37].

9.4 Animal Models of Silica-Induced Autoimmunity

Although there is great confidence that silica exposure is a significant risk factor for human autoimmunity [38], there are few studies of silica-induced autoimmunity in nonhuman species [9, 34, 35]. The paucity of animal studies led a recent National Institutes of Environmental Health Sciences (NIEHS) workshop to note that the lack of a suitable animal model of silica-induced autoimmunity is a critical barrier to progress in understanding how silica exposure leads to autoimmunity [39]. Induction of autoimmunity following silica exposure has been examined in the SLE-prone NZM2410 mouse [40] and in the brown Norway rat [41, 42]. Intranasal instillation of 1 mg of crystalline silica (Min-U-Sil 5, with an average crystal length of 1.5–2.0 μM) twice over a two-week period resulted in exacerbation of

autoimmunity in NZM2410 mice compared to controls over the 22-week observation period. Silica-exposed mice had reduced survival, increased proteinuria, circulating immune complexes, renal deposits of IgG and C3, autoantibodies, and pulmonary inflammation and fibrotic lesions. Unlike their human counterparts, the mice had reduced levels of serum IgG. A follow-up study confirmed the reduced IgG and IgG1 as well as showing increased proinflammatory cytokines in bronchoalveolar lavage fluid (BALF), increased B1a B cells and CD4⁺ T cells in lymph nodes, and an alteration in the ratio of CD4⁺ T-to-CD4⁺CD25⁺ T cells [43]. Brown Norway rats exposed to 3 mg of sodium silicate (NaSiO₄) by oral or subcutaneous administration once a week for 5 weeks developed differing autoantibody responses. After 7 and 14 weeks, it was found that subcutaneous administration resulted in a greater number of ANA-positive animals, but specific autoantibodies (i.e., anti-double-stranded DNA, anti-Sm, anti-SS-A, anti-SS-B) were infrequently detected [42]. A subsequent study revealed that most of the ANA-positive samples also had anti-RNP reactivity [41]. The only other studies to examine the effect of silica on autoimmunity employed its toxic effects to deplete macrophages in a chicken model of autoimmune thyroiditis [44] and in rat [45, 46] and mouse [47] models of diabetes.

9.5 Animal Models of Asbestos-Induced Autoimmunity

Ironically, even though less is known of the relationship between asbestos and human autoimmunity compared with silica, there have been more studies using animal models and asbestos or asbestos-like material. Exposure of female C57BL/6 mice to two doses of 60 μg of Korean tremolite one week apart resulted in an increased frequency of ANA, anti-DNA, anti-SS-A/Ro, and IgG renal deposits [48]. Exposed mice also had decreased percentage of CD4⁺CD25⁺ T cells as well as decreased serum IgG. To explore possible differences in response to amphibole and chrysotile, female C57BL/6 mice were exposed to Libby 6-Mix or intermediate chrysotile by intratracheal instillation twice over 3–4 weeks (60 μg in total). While both materials induced inflammatory responses, only amphibole asbestos increased the frequency of ANA and levels of IL-17 [49]. In a companion study female C57BL/6 mice were exposed to erionite, an asbestos-like fibrous material, and compared to responses to amphibole (Libby 6-Mix, Korean tremolite) and chrysotile asbestos [50]. Erionite-treated mice had increases in serum ANA, IL-17, and TNF-α as well as renal deposits of IgG. Libby 6-Mix amphibole also elicited increased ANA but chrysotile did not. These studies highlight the importance of the properties of asbestos fibers in determining inflammation and autoimmunity.

The Libby 6-Mix amphibole has also been used in animal studies to determine if asbestos exposure can exacerbate RA [51] based on the observation that amphibole exposure in Libby, Montana, was associated with increased risk of RA in humans [35]. Prior to induction of arthritis by either collagen or peptidoglycan-polysaccharide (PG-PS) injection, female Lewis rats received either Libby

amphibole or amosite by intratracheal instillation over a 13-week period. Asbestos exposure consisted of a range of doses from 0 to 5 mg in total. Neither exposure affected development of collagen-induced arthritis or production of RF or anti-cyclic citrullinated peptide antibodies. Prior exposure to Libby amphibole reduced features of disease in the PG-PS model. However, both exposures elicited ANA in PG-PS and non-arthritis controls but not rats receiving collagen injections. A follow-up publication attempted to identify the ANA specificity following Libby amphibole exposure and to determine if other features of systemic autoimmunity were present [52]. Although elevated ANA was detected as early as 8 weeks after exposure to the highest dose of Libby amphibole, this could not be explained by reactivity to an extract of soluble nuclear antigens (ENA) or selected nuclear antigens (e.g., Sm, RNP, SS-A/Ro, SS-B/La, Scl-70, DNA). Asbestos exposure was not associated with changes in kidney histology or renal deposition of immunoglobulin or complement, although evidence of proteinuria was found. Intriguingly, almost all of the sera reactive with the soluble nuclear extract were found to react with the Jo-1 antigen, a cytoplasmic protein. Antibodies to Jo-1 are specific for histidyl-tRNA synthetase which is one of a group of autoantibodies against aminoacyl-tRNA synthetases (ARS) which are the key features of the anti-synthetase syndrome (aSS), characterized in part by interstitial lung disease [53]. This raises the interesting possibility that an anti-Jo-1 response may indicate interstitial lung disease following asbestos exposure. However, it remains to be determined if this autoantibody response is common among different forms of asbestos, if it occurs following exposure to other silicates and whether it plays a role in disease pathogenesis.

9.6 Mechanisms of Silicate-Induced Autoimmunity

9.6.1 Human Studies

Very little is known regarding the mechanism by which silica exposure leads to systemic autoimmune disease particularly in relevant patient populations [5]. However, a number of observations, made in a cohort of Japanese brickyard workers diagnosed with silicosis, have been argued as providing insight into immune abnormalities that arise following silica exposure and that might precede development of autoimmune disease [54]. These studies come with two caveats. First, none of the patients studied had evidence of symptoms of autoimmune diseases [54], specifically sclerotic skin, Raynaud's phenomenon, facial erythema, or arthralgia [31]. Thus, the absence of disease makes it difficult to determine if any of the observations gathered are relevant to the development of systemic autoimmune disease. Moreover, as the patient population appears to number less than 100 [55–57], the chance of any individual patient developing a specific systemic autoimmune disease is relatively remote given the low prevalence of systemic

autoimmune diseases like RA [58] and SLE [59] in the Japanese population. Second, there is conflicting data concerning the relationship between development of silicosis and autoimmune disease following silica exposure [10]. Therefore, it is unclear if a population of silicotics is the most relevant group in which to study immune abnormalities that may herald impending development of systemic autoimmune disease following silica exposure.

This group of patients was described as having higher titers of ANA than a control group [54], including antibodies to DNA topoisomerase I (also known as anti-Scl-70) [60], an autoantibody response useful in predicting scleroderma patients at higher risk for interstitial fibrosis/restrictive lung disease [61]. Other studies identified the presence of autoantibodies against CD95/Fas [62], caspase-8 [63], and desmoglein [64]. These findings support other observations of a range of autoantibody specificities in silica-exposed individuals [17, 18, 20] together with evidence of a disease-related specificity. The observation that apoptosis-related molecules (CD95/Fas, caspase-8) were targets of autoantibodies led to an examination of Fas expression which identified increased levels of soluble Fas, presence of alternatively spliced versions of Fas lacking the transmembrane domain, and increased expression of decoy receptor 3 (DcR3) which functions to inhibit Fas [54]. These findings suggested inefficient Fas-mediated cell death in silicosis which may allow prolonged survival of self-reactive lymphocytes. Analysis of cell surface markers, coupled with *in vitro* responses to silica particles, of peripheral blood cells from silicosis patients suggested the presence of two T cell populations. The first being chronically activated T cells resistant to Fas-mediated apoptosis, and the second chronically activated T regulatory cells sensitive to Fas-mediated apoptosis [31, 54]. These findings have received some support from animal model studies where alterations in the ratio of CD4⁺-to-CD4⁺CD25⁺ T cells have been found following silica exposure of NZM2410 mice [40], and reduced CD4⁺CD25⁺ T cell numbers have been found in C57BL/6 mice exposed to asbestos [48]. However, it is clear that much more probing of mechanism needs to be done especially with larger study populations if there is to be insight into how silicates induce and/or exacerbate autoimmunity and autoimmune disease in humans.

9.6.2 *Animal Studies*

The scarcity of studies on silica-induced autoimmunity in animal models significantly restricts discussion of mechanisms that might explain development of disease [65]. However, clues to the sequence of events that may eventually lead to autoimmunity may be found in the inflammation and pathology that follows crystalline silica exposure. Strain-specific responses have been found for exposure to silica and induction of silicosis [66–68]. Six different strains exposed to 5 mg of α -quartz by intratracheal instillation showed three different levels of response after 4 weeks [67]. The most responsive strains included the DBA/2, C57BL/10, and BALB/c, while the C57BL/6 and C3H/He had intermediate responses and the

CBA/J the least response. However, all strains had extensive disease compared to saline controls. Comparison of the fibrotic response to intratracheally administered silica also showed strain-dependent responses in eight strains with the C57BL/6 showing the greatest hydroxyproline content and CBA/J the least [68]. Breeding studies and genome-wide linkage analysis identified quantitative trait loci (QTL) on chromosome 4 and suggestive QTL on chromosomes 3 and 18 [68]. Inflammation and fibrosis can be uncoupled in NMRI mice by administration of anti-inflammatory molecules which reduced inflammation and proinflammatory cytokines (IL-1 β and TNF- α) but had no significant effect on fibrosis or expression of the fibrogenic cytokines TGF- β and IL-10 [69]. Intense silicosis does not develop with inhalation of amorphous quartz silica, but the more biologically active polymorph crystalline silica produces more extensive disease in an orderly dose-time relationship with little disease activity observed before 3–4 months after exposure [66]. C3H/HeN mice demonstrated histopathological silicotic lesions and enlarged bronchus-associated lymphoid tissue (BALT) and increased lung wet weight; bronchoalveolar lavage (BAL) recovery of macrophages, lymphocytes, and neutrophils; and total lung collagen (hydroxyproline). BALB/c mice developed slight pulmonary lesions, while autoimmune-prone MRL/MpJ mice demonstrated prominent pulmonary infiltrates with lymphocytes, and NZB mice developed extensive alveolar proteinaceous deposits, inflammation, and fibrosis [66]. BALT was particularly numerous in the MRL/MpJ. These regions are of particular interest as recent studies argue that inducible BALT provides a niche for T cell priming and B cell education [70]. The differences in response among strains were attributed to genetic differences, involving histocompatibility loci and other unrelated genetic differences among the strains [66]. These findings show that strain differences exist in response to silica exposure and that a genetic predisposition to autoimmunity is associated with more aggressive pulmonary inflammation arguing that gene-environment interaction may be important in development of frank autoimmune disease.

9.6.2.1 Silica-Induced Autoimmune Phenotypes in Mice

Another critical barrier to progress in understanding the link between particulate exposure and autoimmunity is the lack of criteria for identifying human autoimmune disease phenotypes associated with environmental-induced autoimmunity [71]. However, in the case of silica, there is a rich literature of human and animal research because of the well-documented association with occupational disease [9, 10, 16, 28, 29, 31]. The silica-induced immunological responses in mice and rats include lung inflammation [66, 67, 69], proinflammatory cytokines [72–75], activated adaptive immunity [76–79], autoantibodies [40, 42, 48, 51, 52], and renal disease [40, 48].

The immunological responses to silica which lead to autoimmunity are believed to begin with an inflammatory response in the lung associated with production of proinflammatory cytokines which leads to recruitment of the adaptive immune

system characterized by helper CD4⁺ T cell activation and reduced T regulatory cell function [28, 31, 80]. This milieu allows breaking of tolerance and autoantibody production and tissue pathology [10, 20, 25]. The first pathological indices following silica exposure are pulmonary inflammation followed by fibrosis which, although nonspecific in terms of autoimmune disease, are common in inbred mice, and both high and low responders show similar patterns of inflammation and fibrosis which are specific to silica exposure [67]; autoimmune-prone mice appear to have more pronounced responses [66]. Whether silicosis is a precursor of autoimmunity [10] is less clear as inflammation requires only cells of the innate immune system [81], and pulmonary inflammation can be dispensable for the development of fibrosis [69].

9.6.2.2 Involvement of Innate Immunity in Silica-Induced Autoimmunity

The findings that the innate immune response is sufficient for the development of silicosis [81] beg the question as to which innate immune cells and pathways play predominant roles. Silica deposition in the lungs leads to participation of alveolar macrophages in an attempt to clear the particles. This involves scavenger receptors such as macrophage receptor with collagenous structure (MARCO) and can result in cell death [82]. It has been recently appreciated that such cell death allows release of endogenous stores of IL-1 α which precedes expression of IL-1 β and inflammation consisting predominantly of neutrophils [83]. IL-1 β expression requires activation of the inflammasome and caspase-1 in both silica and asbestos exposures [84, 85]. This appears to involve lysosomal destabilization particularly by particles less than 3 μm in length [86] and generation of reactive oxygen species (ROS) [87] leading to cathepsin B activation [84]. However, it is possible that lysosomal destabilization may not be essential for IL-1 β production if silica binding to the cell membrane results in potassium ion efflux [88]. In vitro studies suggest that chrysotile asbestos and amphibole (Libby 6-Mix) induce different inflammatory events with chrysotile better at inflammasome activation, while amphibole generated more ROS [89].

Proinflammatory cytokine expression has not been directly compared among different mouse strains, but several strains, including C57BL/6 and A/J, show increases in TNF- α , IL-1 β , IFN- γ , and IL-6, particularly in BAL fluid [75, 77, 90–92]. Neutralization of IL-1 β attenuates silica-induced lung inflammation and fibrosis in C57BL/6 mice [57] and inflammation, and macrophage apoptosis following silica exposure was found to be induced by IL-1 β and nitric oxide [76]. This is not unexpected given the importance of these cytokines to pulmonary inflammation and silicosis [28, 93]. Silica-activated macrophages expressed high levels of IL-10 in the lung of silica-sensitive but not silica-resistant strains of mice, indicating that besides chronic lung inflammation, a pronounced anti-inflammatory reaction may also contribute to the extension of silica-induced lung fibrosis and represent an alternative pathway leading to lung fibrosis [95]. Cytokine expression

can be transient lasting hours up to several days following exposure [75, 91]. However, cytokine expression is found in the blood of silica-exposed individuals [26, 27] suggesting that repeated exposure leads to systemic expression. Given that individual genes, such as TNF- α [94] and IFN- γ [93], are required for silicosis, it is possible that genetic heterogeneity in cytokine expression may significantly impact immune responses to silica.

9.6.2.3 Involvement of Adaptive Immunity in Silica-Induced Autoimmunity

Although innate immunity is sufficient for silicosis in mice [81], pulmonary inflammation is also characterized by the presence of T and B cells [93, 95–98], and anti-CD4 treatment reduces fibrosis [97]. Lupus-prone NZM mice have increased numbers of CD4⁺ but not CD4⁺CD25⁺ T cells following silica exposure [43]. This is reminiscent of the reduced expression of CD25 (IL-2R α) in human silicosis [24, 26, 99] and supports the suggestion that silica exposure results in the loss of T regulatory cell function [99, 100]. This may be associated with increased presence of soluble CD25 in silicosis [101] which has the ability to promote autoimmune disease and enhance Th17 responses through its ability to sequester IL-2 [102]. In experimental silicosis, production of IL-17A by $\gamma\delta$ T and Th17 cells induces acute alveolitis, but is not necessary for the development of the late inflammatory and fibrotic lung responses to silica [97].

Elevation in immunoglobulin levels in both serum and BAL has been observed in response to silica in rodents [79, 103, 104]; however, in autoimmune NZM2410 mice, serum IgG was reduced even though autoimmunity, including autoantibodies, was exacerbated [40, 43]. Asbestos exposure also reduced serum IgG while inducing autoantibodies in C57BL/6 mice [48]. Effects on immunoglobulin levels can also vary in human populations with studies showing increases [21, 105–107] or no change [26]. Other factors may also impact immunoglobulin levels including cytokines [79]. ANAs are quite common in both mice and rats following exposure to silica, asbestos, or sodium silicate with 50–100 % of animals being positive [40, 42, 48, 52] although this was influenced by the site of exposure [42] and the presence of ANA in control animals [48]. Although ANAs can exhibit disease specificity [108], they have been found to occur before clinical onset of disease with some specificities (e.g., anti-SS-B/La, anti-SS-A/Ro) more prevalent than disease-specific autoantibodies (e.g., anti-dsDNA, anti-Sm) [109]. Anti-dsDNA, anti-SS-B/La, and anti-SS-A/Ro have been found to occur in up to 50 % of rodents exposed to silica or asbestos [42, 48].

9.6.2.4 Silica-Induced Nephropathy

Kidney disease is a complication of silicosis and may have an autoimmune component. Apart from evidence of immune deposits in autoimmune-prone NZM2410

mice given silica [40] and C57BL/6 mice exposed to asbestos [48], there has been little done to define the requirements for silica-induced nephropathy in experimental animals. The mechanism of silica nephropathy is unclear but appears to have two components: a direct nephrotoxicity and induction of autoimmune disease [30]. These findings are consistent with a disease progression that begins with activation of the innate immune system leading to proinflammatory cytokine production, inflammation of the lung leading to activation of adaptive immunity, breaking of tolerance, autoantibodies, and renal damage [9, 28, 31, 32]. The variable frequency of these disease features suggests significant genetic involvement and gene-environment interaction.

9.7 Genetic Requirements of Silica-Induced Autoimmunity

There is very little known about the genetic requirements of silica-induced autoimmunity; however, as noted above studies of silicosis in mice have identified significant genetic involvement. Silica-induced pulmonary inflammation is dependent on IFN- γ [93], but not Th2 cytokines such as IL-4 and IL-13 [79] or IL-12 [110]. Innate immunity mediates this process as SiO₂-induced inflammation and fibrosis can occur in the absence of T, B, NK T, or NK cells [81]. Notably, although acute lung inflammation requires IL-17 [78], chronic inflammation is dependent on type 1 IFN and IRF7 [72]. The NLRP3 inflammasome and caspase-1 and IL-1 β are also required for silicosis [85, 111–113], as is IL-1 α [83]. MyD88 which links TLR signaling to proinflammatory cytokine production is required for silica-induced inflammation but not fibrosis [114]. Deficiency of either scavenger receptors MARCO or CD204, expressed mainly on macrophages, impairs silica clearance and exacerbates silica-induced lung inflammation [115, 116]. Significantly, both MARCO and CD204 have been argued to promote tolerance to apoptotic cell material [117]. Thus, the immunological response to silica requires genes involved in both innate and adaptive immunity. There have been no studies identifying the genetic loci required for silica-induced autoimmunity. A quantitative trait locus (QTL) study of murine silicosis identified a major QTL on chromosome 4 and suggestive loci on chromosomes 3 and 18 [68] suggesting that responses to silica involve multiple genes.

9.8 Conclusions and Future Research

Epidemiological data has established a solid link between silica dust exposure and several systemic autoimmune diseases. However, limited research has been done to unravel the mechanism of this effect. This stems in part from a lack of suitable human cohorts to study but also limited studies toward development of a suitable animal model. The variable frequency of features of silica-induced inflammation

and subsequent autoimmune features in human subjects suggests significant genetic involvement as well as gene/environment interaction. This is reflected in the differences in response to silica by inbred mouse strains. Development of a model expressing this heterogeneity of response is unlikely to come from studies of inbred mice. This obstacle may be overcome by the use of outbred strains such as the Diversity Outbred [118] where genetic diversity may lead to a more appropriate representation of the heterogeneity of human responses. On the other hand, the extensive literature on silica-induced responses in the mouse including genetic requirements for both inflammation and fibrosis provides a foundation from which to explore the requirements for silicate-induced autoimmunity. A deeper understanding of the contribution of inflammation to fibrosis is required, as is an understanding of how both inflammation and fibrosis contribute to the induction and development of autoimmunity. The contribution of BALT also needs examination as this feature may be essential in the adaptive (auto)immune response following silica exposure, and the development of these discrete foci in the lung may be a site of either the genesis and/or expansion of autoreactive lymphocytes. These are just some of the unresolved issues that plague our understanding of particulate matter and particularly silica-induced autoimmunity. However, given the active research in this area, it is very likely that the next few years will see a significant increase in our understanding of the relevant mechanisms.

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