## ORIGINAL ARTICLE

# Immunoglobulins, complements and autoantibodies in 58 workers exposed to silica*

**HERMANO ALBUQUERQUE DE CASTRO*, CAROLINA GIMENES DA SILVA*, ALFRED LEMLE****

**Background:** The primary work-related lung disease in Brazil is silicosis. Its pathogenic agent is the dust of crystalline free silica ($SiO_2$; silicon dioxide). The inflammatory process of silicosis is not yet well understood.

**Objective:** To analyze, through immunologic laboratory evaluation, including nonspecific and specific immunity, the profile of IgG, IgM, IgA, C3, C4 and autoantibodies in the serum of workers, with or without silicosis, exposed to silica.

**Methods:** Fifty-eight male workers were studied. All had been exposed to silica. Immunologic, radiologic and functional evaluations were made. The immunoglobulins IgG, IgA, and IgM, the complement system components C3 and C4, and the autoantibodies were assessed.

**Results:** Chest X-rays were normal in 20 of the 58 workers and compatible with silicosis in 38. Among the 38 who were positive, IgG values were, on average, higher than in the group with normal X-rays ($p < 0.05$). There were no significant differences in average values of IgA, IgM, C3 or C4 ($p > 0.05$). The percentage of autoantibody positivity was higher in the silicosis group than in the group with normal X-rays.

**Conclusion:** The increased levels of IgG in patients with silicosis constitutes an important discovery. It may represent continuity of the granulomatous reaction, even when the individual is no longer being exposed to silica. However, further studies are necessary in order to increase understanding of the mechanism involved in the silicosis immunologic process.

**Key words:** Lung diseases. Silicosis. Occupational diseases. Immunoglobulins

Study carried out at * CESTEH/ENSP/FIOCRUZ, ** Faculdade de Medicina da Universidade Federal do Rio de Janeiro.
Correspondence to: Rua Leopoldo Bulhões, 1480. Manguinhos. RJ – CESTEH/ENSP/FIOCRUZ – CEP: 21041-210. E-mail: castro@ensp.fiocruz.br
**Submitted: 25 June 2003. Accepted, after revision: 17 March 2004.**

## INTRODUCTION

Silicosis is the primary work-related lung disease in Brazil and in the world. Its pathogenic agent is the dust of crystalline silicon dioxide (free silica). Free silica in the lungs provokes an inflammatory cellular response that originates in pulmonary alveolar macrophages[1,2].

Silicosis is a granulomatous disease that causes an increase in inflammatory cells and stimulates antibody production. Histological examination reveals the participation of precipitated antigen-antibody complexes[3], especially antinuclear autoantibodies. In the acute, accelerated form of the disease, autoimmune diseases are frequently present[1,4]. Individuals engaged in occupations such as sandblasting are often diagnosed with silicosis and typically present with multiple opacities and cavitations, as well as high levels of serum antinuclear antibodies[5,6].

Some authors have reported an increase in the number of polymorphonuclear neutrophils in workers exposed to free silica. Lugano et al.[7] reported that macrophages exposed to silica *in vitro* released a neutrophil chemotactic factor, possibly leukotriene B4.

Another important factor in the development of the disease is individual susceptibility, through which we have attempted to explain the incidence of the disease among workers exposed to the same conditions. Individual differences are characterized a lack of uniformity in immunological changes and in the dose-response relationship.

The intensity of local and systemic immune responses demonstrates the importance of silica as an immunogenic stimulator, as evidenced by hypergammaglobulinemia and the presence of circulating autoantibodies[8,9]. In addition, several authors have reported that the number of lymphocytes in bronchoalveolar lavage is related to increased serum levels of all types of immunoglobulins (up to 3 times higher than normal values), complement components, rheumatoid factor (RF) and circulating immune complexes[8,10,11].

The objective of this study was to analyze, in workers exposed to silica, the levels of various substances. Workers with or without silicosis were evaluated through immunologic laboratory testing. We measured A, G and M immunoglobulins (IgA, IgG, and IgM, respectively) and the complement components 3 and 4 (C3 and C4). In addition, we assessed anti-DNA, antimitochondrial, antithyroglobulin, thyroid antimicrosomal, anti-smooth muscle and antinuclear antibodies.

**Abbreviations used in this paper:**

| | |
|---|---|
| ANF | – Antinuclear factor |
| C3 | – Complement component 3 |
| C4 | – Complement component 4 |
| IgA | – Immunoglobulin-A |
| IgG | – Immunoglobulin-G |
| IgM | – Immunoglobulin-M |
| RF | – Rheumatoid facto |

## METHOD

Fifty-eight male outpatients were studied. All had been exposed to silica for at least 8 months in industrial workplaces. Patients with clinical and radiological evidence predictive of acute infections, including respiratory viral infections, were excluded. Radiographs were examined by 3 different specialists, in accordance with guidelines established by the International Labor Office in 1980[12].

On the basis of radiological findings, patients were classified as normal (0/-, 0/0, and 0/1 readings), level 1 (1/0, 1/1, and 1/2 readings), level 2 (2/1, 2/2, and 2/3 readings), or level 3 (3/2, 3/3, and 3/+ readings). A chest X-ray reading higher than 1/0 was considered confirmation of a diagnosis of silicosis.

Radial immunodiffusion assays, employing Behring Nor-Partigen® (IgG-HC, IgA, IgM, C3 and C4) plates, were used to assess IgG, IgA, IgM, C3 and C4. Antinuclear factor (ANF) was detected according to the method described by Lima et al.[13], using a substrate of mouse liver imprint stored at -20°C. The detection of thyroid autoantibodies (antimicrosomal and antithyroglobulin antibodies) was performed by semiquantitative determination of thyroid antimicrosomal and antithyroglobulin antibodies by microagglutination technique. The test is based on the agglutination of gelatin particles attached to microsomal and thyroglobulin antigens, extracted from human thyroid tissue and purified. The serum of the individuals studied was tested in dilutions of at least 1:100. The normal (expected) result from this test is negative. Anti-DNA, antimitochondrial and anti-smooth muscle antibodies were assessed by indirect immunofluorescence on slides with *Crithidia Luciliae*, mouse liver, and mouse stomach, respectively. A latex agglutination test was used to detect RF. When latex agglutination was positive, the Waaler-Rose test was performed. Both tests were performed in accordance with techniques described by Lima et al.[13] . Serum samples were stored at -20°C, and diluted to 1:30 prior to testing.

Results were expressed in terms of either means (with standard deviations) or positivity. Means were analyzed using Student's *t*-test when there was normal (homogeneous) distribution of data. Pearson's chi-square test was used as a nonparametric test for association tables (2 x 2). Significance level was set at $p < 0.05$ for all statistical tests.

All workers included in the study signed an informed consent form after having read it aloud.

## RESULTS

Of the 58 silica-exposed workers studied, 20 had normal chest X-rays, and 38 presented radiological findings compatible with silicosis. Of the 38 who were positive, 24 were classified as level 1, 9 as level 2, and 5 as level 3. None presented signs of collagen disease during the study. Mean age was 43.3 ± 9.9 years.

Overall mean time of exposure to silica was 191.60 ± 102.30 months. The highest mean was in level 1 patients (212.41 ± 143.68 months) and the lowest was in patients with normal chest X-rays (142.80 ± 80.63 months). In the population studied, 81% were smokers.

The means of IgG, IgA, and IgM values are described in table 1. This table shows that the only statistically significant difference between the group with normal chest X-rays and the group with silicosis was in mean IgG ($p = 0.03$).

The means and standard deviations for C3 and C4 values are detailed in Table 2. The differences in these means were not statistically significant ($p > 0.05$).

The percentages of positivity in the group studied were as follows: ANF, 20.6%; RF or latex agglutination test, 3.4%; Waaler-Rose test, 3.4%; anti-DNA antibody, 20.6%; antimitochondrial antibody, 1.7%; antithyroglobulin antibody, 1.7%; thyroid antimicrosomal antibody, 3.4%; and anti-smooth muscle antibody, 1.7%. The proportions are described in Table 3. In the results of the non-parametric test, no statistically significant differences were found between the group with normal chest X-rays and the silicosis group.

TABLE 1
Serum immunoglobulin levels in individuals with normal chest X-rays and in those diagnosed with silicosis (categorized by radiological level)

| | *n* | IgG (mg/dL) Mean ± SD | IgA (mg/dL) Mean ± SD | IgM (mg/dL) Mean ± SD |
|---|---|---|---|---|
| NORMAL X-RAY | 20 | 1713.3 ± 401.0* | 371.0 ± 149.9 | 206.2 ± 68.9 |
| SILICOSIS | 38 | 2015.8 ± 670.6* | 387.5 ± 126.0 | 195.2 ± 65.3 |
| LEVEL 1 | 24 | 2057.0 ± 693.6 | 384.8 ± 133.2 | 202.7 ± 63.0 |
| LEVEL 2 | 09 | 2035.5 ± 642.6 | 365.3 ± 123.8 | 180.6 ± 74.8 |
| LEVEL 3 | 05 | 1782.4 ± 702.3 | 440.2 ± 97.3 | 185.6 ± 67.4 |

*$p < 0.05$
IgG: immunoglobulin-G; IgA: immunoglobulin-A; IgM: immunoglobulin-M; SD: standard deviation

TABLE 2
Complement component levels in individuals with normal chest X-rays and in those diagnosed with silicosis (categorized by radiological level)

| | C3 (mg/dL) Mean ± SD | C4 (mg/dL) Mean ± SD |
|---|---|---|
| NORMAL X-RAY | 125.3 | 40.8 ± ?9.2 |
| SILICOSIS | 133.0 ± 36.0 | 46.1 ± 14.0 |
| LEVEL 1 | 128.7 ± 30.3 | 46.4 ± 14.1 |
| LEVEL 2 | 118.8 ± 25.1 | 39.5 ± 11.5 |
| LEVEL 3 | 181.0 ± 44.0 | 56.4 ± 13.2 |

C3: complement component 3; C4: complement component 4; SD: standard deviation.

TABLE 3
Incidence of autoantibodies in individuals with normal X-rays and in those with silicosis

| | *n* | ANF N/P | RF/LAT N/P | WAAL N/P | DNA N/P | AMA N/P | ATA N/P | MIC N/P | ASMA N/P |
|---|---|---|---|---|---|---|---|---|---|
| NORMAL | 20 | 14/6 | 20/0 | 20/0 | 14/6 | 20/0 | 20/0 | 20/0 | 20/0 |
| SILICOSIS | 38 | 32/6 | 36/2 | 36/2 | 32/6 | 37/1 | 37/1 | 36/2 | 37/1 |

N: negative; P: positive; ANF: antinuclear factor; RF: rheumatoid factor; LAT: latex test; WAAL: Waaler-Rose test; DNA: anti-DNA antibody; AMA: antimitochondrial antibody; ATA: antithyroglobulin antibody; MIC: thyroid antimicrosomal antibody; ASMA: anti-smooth muscle antibody

## DISCUSSION

Immune disturbances seem to be directly related to disease progression in workers exposed to free silica in the workplace.

Several authors have reported the significance of the specific humoral response in silicosis disease progression[2]. Greater numbers of polyclonal immunoglobulins, both in blood and in bronchoalveolar lavage fluid, have also been reported in silicosis[5,10,14].

Karnick et al.[15], in a study of workers exposed to silica and presenting or not presenting silicosis, reported a mean IgG value of 1835.44 mg/dL. When the authors compared those workers to those in the control group (mean of 1373 mg/dL), they found a statistically significant difference. There was a slight increasing trend related to the duration of the exposure to silica. However, when they analyzed the differences of the means of immunoglobulins in the group of workers exposed to silica, the authors reported a significant difference in IgM values between the group with simple silicosis (144.64 mg/dL) and the group without silicosis (187.53 mg/dL). That means there was an increase in IgM values in the group exposed to silica but not diagnosed with the disease. The same was not observed for IgG and IgA. A difference between IgG values was also detected: in the group of silicosis patients whose chest X-rays revealed conglomerations and multiple opacities (types A, B and C), the mean IgG was 2193.68 mg/dL, whereas in the workers without silicosis, the mean was 1860.85 mg/dL.

Nigam et al.[4] studied 19 workers exposed to silica. Of the 19, 7 were diagnosed with silicosis and 12 were not. The authors also included 19 controls (individuals not exposed to silica) and reported a statistically significant difference between the two groups only in mean serum IgG (exposed: 2318.00 mg/dL; non-exposed: 1663.00 mg/dL). Values for IgM, IgA, C3 and C4 were slighter higher in silica-exposed subjects, although not attaining the level of statistical significance. The authors also reported significantly higher levels of IgA in the group diagnosed with silicosis than in the group exposed to silica but not diagnosed with silicosis.

Nagaoka et al. and Miossec et al.[16,17] reported a significant difference in IgG levels between groups of workers exposed to silica (with or without silicosis) and control groups of individuals not exposed to silica. The authors of both studies describe the participation of some immunoglobulins in silicosis progression.

The limiting factor of the present study was the lack of a control group not exposed to silica, hindering the comparison with other studies on immunoglobulins and autoantibodies. However, the intragroup analysis, i.e. the evaluation of the group of workers exposed to free silica and diagnosed or not diagnosed with silicosis yields information indicative of immunological changes. We highlight the fact that there are a greater number of cases presenting radiological changes compatible with early-stage silicosis, i.e. milder cases.

The present study reveals a significant difference in the levels of IgG between the group with normal chest X-rays and that with chest X-rays compatible with silicosis. The same was not observed with IgA and IgM levels. However, higher IgM values were found in the group with normal X-rays, indicating there is a decreasing trend in IgM values as the disease progresses, i.e. as the disease becomes more severe.

IgG is an immunoglobulin that appears only after prolonged exposure to an injurious agent. Increased IgG values in patients with silicosis may indicate an inflammatory response due to the exposure to silica dust and the persistence of the granulomatous reaction, even after the cessation of exposure. During the immune response, IgM is the first circulating immunoglobulin produced by the plasmacytes. There is an increase in IgM levels immediately after the first contact with the antigen. Since IgM, as well as IgG, activates the system pathways, it is also more efficacious in complement fixation[18]. Increased IgM values may represent one of the initial immune responses in workers exposed to silica.

Karnik et al.[15] proposed a hypothesis regarding the involvement of humoral immune dysfunction in silicosis. They highlighted the fact that there is an increase in the production of antibodies, and that the longer the exposure to silica, the greater the humoral response – especially in silicosis patients presenting large opacities.

Mean serum levels of C3 and C4 in the group with normal chest X-rays were lower than those in workers with silicosis, but this difference was not statistically significant. Level 3 patients presented higher C3 and C4 values. Increased serum levels of complement components, especially C3, are fundamental for the activation of the classic and alternative complement system pathways[18]. During activation of the complement system, small fragments, generated from C3, C4, and C5 and known as C3a, C4a and C5a, are released. These fragments have unusual characteristics and act on the immune system. According to some authors, C3a, C4a and C5a may play important roles in the genesis of silicosis[1,2,19].

In the present study, there was a greater proportion of individuals with positive results for ANF and anti-DNA antibody (20.6%). Other authors reported various results, such as 44% positivity in a group of 39 sandblasters with silicosis[5], 26% in 53 patients with silicosis[8], 13.4% in 134 patients with silicosis[16] and 11% in 58 patients with silicosis[20]. A high prevalence of ANF positivity was generally reported in patients in the severe stage of the disease[5,16]. The RF antibody, which basically belongs to the IgM family, displays anti-IgG activity. Although only two (3.4%) of the level 1 patients tested positive for RF, a higher incidence of RF has been reported in the literature. Doll et al.[8] reported RF titers higher than 1:40 in 28% of 53 patients with silicosis, whereas Nagaoka et al.[16] reported 10.4% positivity for RF in 134 patients. Subra et al.[20], however, found no significant increases in levels of RF, C3 or C4.

Nigam et al.[4] reported one ANF-positive case among workers exposed to silica but not diagnosed with silicosis. However, they reported a higher incidence of immunocomplexes in silica-exposed workers that presented radiological changes than in those with normal X-rays.

Autoantibodies are antibodies that identify host tissues as antigens and may or may not harm the organism[21]. Autoantibodies can be found in small quantities in healthy individuals and probably contribute to immune homeostasis[22,23]. Connective tissue diseases can cause an increase in immune system autoaggression, harming the organism.

Some authors have reported higher numbers of serum autoantibodies in patients with silicosis, although the patients presented no autoimmune diseases[8]. Other authors have reported an increase in the incidence of autoimmune diseases in patients with silicosis[21,24,25]. Ângelo Papi[26] reported a high prevalence of systemic sclerosis in mine workers diagnosed with silicosis or anthracosis, hypothesizing that pulmonary fibrosis played a fundamental role in the genesis of sclerotic process. SLuis-Cremer et al.[24], in studying the relationship between rheumatoid arthritis and silicosis, reported that silica-exposed miners diagnosed with rheumatoid arthritis were more likely to develop silicosis than those without rheumatoid arthritis.

Based on our results and on those from other studies, the possibility that, in individuals exposed to silica, changes in both specific and non-specific immune responses occur must be considered.

## ACKNOWLEDGMENTS

The authors would like to thank Dr. Precy Grosmann and her team at the Immunology Laboratory of the UFRJ College of Medicine.

## REFERENCES


1. Davis GS. Pathogenesis of silicosis: current concepts and hypotheses. Lung. 1986;164:139-54.
2. Ziskind M, Jones RN, Weill H. Silicosis. Am Rev Respir Dis. 1986;113:643-65.
3. Schuyler M, Ziskind M, Salvaggio L. Cell-mediated immunity in silicosis. Am. Rev Respir Dis. 1977;116:147-51.
4. Nigam SK, Saiyed HN, Malaviya R, Suthar AM, Desai UM, Venkaiah K, et al. Role of circulating immune complexes in the immunopathogenesis of silicosis. Toxicol-Lett. 1990;51:315-20.
5. Jones RN, Turner-Warwick M, Ziskind M, Weill H. High prevalence of antinuclear antibodies in sandblasters silicosis. Am Rev Resp Dis 1976;113:393-4.
6. Costallat LTL, De Capitani E, Zambon L. Pulmonary silicosis and systemic lupus erythematosus in men: a report of two cases. Joint Bone Spine. 2002;69:68-71.
7. Lugano EM, Dauber JH, Elias JA. The Regulation of lung fibroblast proliferation by alveolar macrophages in experimental silicosis. Am Rev Respir Dis. 1984;129:767-71.
8. Doll NJ, Stankus RP, Mughes J. Immune complexes and autoantibodies in silicosis. I Allergy Clin Immunol. 1981;68:281.
9. Moseley PL, Monick M, Hunninghake GW. Divergent effects of silica on lymphocyte proliferation and immunoglobulin production. J Appl Physiol. 1988;65:350-7.
10. Ferreira A, Moreira JS, Caetano R, Gabetto JM, Quirico-Santos T. Caracterização imunofenotípica das subpopulações de linfócitos do lavado broncoalveolar de pacientes com silicose. J Pneumol. 2000;26:107-12.
11. Calhoun WJ, Christman JW, Ershler WB, Graham WG, Davis GS. Raised immunoglobulin concentrations in bronchoalveolar lavage fluid of healthy granite workers. Thorax. 1986;41:266-73.
12. ILO - International Labour Office. Guidelines for the use of ILO International Classification of Radiographs of Pneumoconiosis. Geneva: ILO; 1980. (Occupational safety and health Series, n. 22).
13. Lima AO. Métodos de laboratório aplicados clínica. 5a ed. Rio de Janeiro: Guanabara Koogan; 1977.
14. Ueki A, Isozaki Y, Tomokuni A. Autoantibodies detectable in the sera of silicosis patients. The relationship between the anti-topoisomerase I antibody response and HLA-DQB1* 0402 allele in Japanese silicosis patients. Sci Total Environ. 2001;270:141-8.
15. Karnik AB, Saiyed HN, Nigan SK. Humoral immunologic dysfunction in silicosis. Indian J Med Res. 1990;92(B):440-2.
16. Nagaoka T, Tobata M, Kobayashi K, Okada A. Studies on production of anticollagen antibodies in silicosis. Environ Res. 1993;60:12-29.
17. Miossec P, Youinou P, Cledes J, Clavier J, Philippon P, Ferec C, et al. Lowered Fc IgG receptor-bearing T lymphocytes correlate with non-organ-specific autoantibodies in silicosis. Int Arch Allergy Appl Immunol. 1984;73:212-5
18. Stites DP, Terr AI. Basic and clinical immunology. 7a ed. New Jersey: Prenttice-Hall; 1991.
19. Warheit DB, Overby LA, George G, Brody AR. Pulmonary macrophages are attracted to inhaled particles through complement activation. Exp Lung Res. 1988;14:51-66.
20. Subra JF, Renier G, Reboul P, Tollis F. Lymphopenia in occupational pulmonary silicosis with or without autoimmune disease. Clin Exp Immunol. 2001;126:540-4.
21. Naparstek Y, Plotz PM. The role of autoantibodies in autoimmune disease. Ann Rev Immunol. 1993;11:79-104.
22. Nossal GJ. Current concepts: immunology - the basic components of the immune system. N Engl J Med. 1987;316:1320-5.
23. Berneman A, Guilbert B, Eschrich S. IgG auto- and polyreactivities of normal human sera. Mol Immunol. 1993;30:1499-510.
24. Sluis-Cremer GK, Hessel PA, Hnizdo E, Churchill AR. Relationship between silicosis and rheumatoid arthritis. Thorax. 1986;41:596-601.
25. Rustin MH, Bull HA, Ziegler V, Mehlhorn J, Haustein UF, Maddison PJ, et al. Silica-associated systemic sclerosis is clinically, serologically and immunologically indistinguishable from idiopathic systemic sclerosis. Br J Dermatol. 1990;123:725-34.
26. Papi IA. Esclerose sistêmica. In: Bonomo I. Conhecimentos básicos de reumatologia. S.l.: s.n.; 1974. 35-43.