

AUTOIMMUNITY PHENOMENA AND ALTERATIONS OF HUMORAL IMMUNOLOGICAL RESPONSES IN SILICOTIC PATIENTS

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INTRODUCTION

In the course of silicosis a variety of humoral alterations may occur.^{1,2,3,6,10-14} So far it is not known, if these humoral alterations are the cause or the consequence of the disease. The most important humoral alterations occurring in silicosis can be divided into three groups: 1) Parameters indicating a stimulation of macrophages: lysozyme, angiotensin-converting enzyme (ACE), fibronectin; 2) Effects on humoral immunity: immunoglobulins, complement, response to antigens; 3) Autoimmunity phenomena: antinuclear antibodies (ANA), circulating immune complexes (CIC), rheumatoid factors.

In our investigations on silicotic patients the following aspects were of particular interest:

1. Are serological changes, from which an influence of quartz-containing dust on the immune system and specific macrophage functions can be concluded, detectable in miners and other silicotic patients?
2. Are such changes detectable only when radiologically manifest silicosis exists or are they already detectable in exposed persons without silicosis?
3. Is there an influence of the quality of quartz containing dust on immunological disturbances?

MATERIALS AND METHODS

In this context, we studied four groups: 34 miners who had radiologically manifest fibrosis following exposure for 15 years and 40 miners who showed no radiological signs of silicosis even after exposure for more than 30 years. In addition 48 stone masons and quarriers were under investigation. For comparison a group of 25 non-exposed men was included.^{7,8}

The following parameters were studied in serum or plasma: lysozyme, angiotensin-converting enzyme (ACE), fibronectin, the complement component C_{3c}, immunoglobulins of classes IgG, IgA, IgE and IgM, circulating immune complexes (C₃, Clq, IgG, IgA, IgM bound in the complexes were detected here), and antinuclear antibodies (ANA). The methods used have been described previously in detail.^{5,8}

RESULTS AND DISCUSSION

Lysozyme, ACE and fibronectin which indicate macrophage stimulation (for review s.8) revealed significantly higher values in coal mine workers with and without pneumoconiosis in comparison to the control group. (Table I)

It has to be emphasized that the significantly increased values

Table I
Parameters Indicating Macrophage Stimulation in Silicotic Patients

	<u>COAL MINE WORKERS</u>		<u>CONTROL</u>
	<u>with silicosis</u>	<u>without silicosis</u>	
Lysozyme	8.25(±3.19)^a	6.75(±1.56)^a	5.00(±1.73)
(ng/l)	(n=34)	(n=39)	(n=25)
ACE	391.71(±99.98)^a	367.69(±84.77)^b	297.84(±77.17)
(U/l)	(n=34)	(n=39)	(n=25)
Fibronectin	65.47(±14.06)^a	58.84(±6.57)^a	38.42(±6.54)
(ng/l)	(n=14)	(n=5)	(n=25)

In parenthesis standard deviation, n=number of cases

Significantly increased over control a (p < 0.001; students t-test)
b (p < 0.01)

of these parameters were already detected in coal mine workers without radiological signs of fibrosis.

The determination of immunoglobulins of classes IgE and IgM showed no differences between the four groups. Also the concentration of the immunoglobulins of classes IgG and IgA was unchanged in miners with or without pneumoconiosis in comparison to control. In contrast, concentrations of IgG and IgA in sera of silicotic stone masons and quarriers were significantly increased over the values of the control group. Such variable findings concerning the IgG and IgA levels in sera of silicotic patients have also been reported by other authors. Beside data which are not striking¹¹ increases in the immunoglobulin classes IgG and IgA were reported.^{1,4} An important component of non-specific humoral immune response is the complement, which gets activated in cascade fashion by the classical or alternative pathway. In this process the complement component C₃ plays a central role, since it is involved in both pathways. Furthermore, of all complement components in serum, C₃ shows the highest concentration. Results of evaluation of the complement component C₃ in sera of the four groups were the following: coal miners with silicosis and silicotic stone masons and quarriers revealed the highest values. The differences to the control group were highly significant ($p \leq 0.001$).

It is especially remarkable that also coal miners without clinical signs of pneumoconiosis possessed high values of C₃, significantly different from controls ($p \leq 0.001$).

Of importance is the occurrence of circulating immune complexes (CIC) in sera of the four groups. For detection of immunoglobulins bound in these immune complexes we used the method of polyethylene glycol-precipitation and the radial immunodiffusion for subsequent determination of IgG, IgA and IgM.

Silicotic patients revealed significantly higher values of CIC than the non-exposed control group. It is remarkable that a significant increase of circulating immune complexes was already detected in coal miners without clinical signs of silicosis. Formation of immune complexes activates complement by the classical pathway. On the contrary, activation by the alternative pathway is not dependent on the presence of an antigen-antibody-reaction. Specifically, the latter one is activated by certain substances, such as bacterial endotoxins, macrophage enzyme elastase, and also by quartz dust.

It has to be emphasized that we found remarkable high concentrations of C₃ in sera of silicotic patients despite the fact that high concentrations of circulating immune complexes were present, which are known to bind and consume C₃. Results suggest that quartz dust induces activation of complement by the alternative pathway leading to increased values of C₃ in sera.^{8,14}

An important sign of autoimmunity phenomena is the occurrence of antinuclear antibodies (ANA) beside the presence of circulating immune complexes (CIC). For detection of ANA we used the method of indirect immunofluorescence. The kidney cell line CV-1 from *Cercopithecus aethiops* has been employed as the substrate. In this study 114 sera of non-exposed persons served as a control. In sera of non-exposed

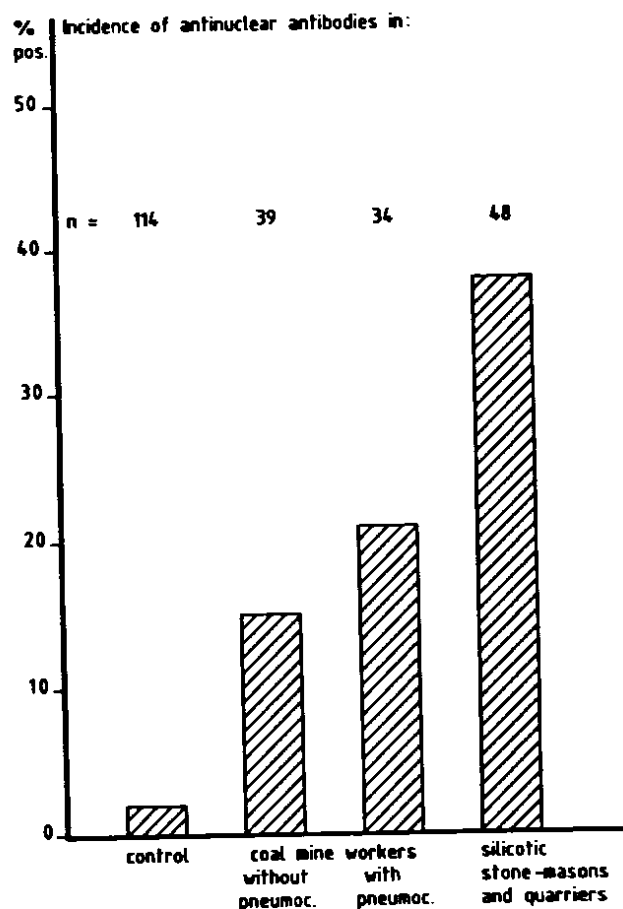


Figure 1. Incidence of antinuclear antibodies.

persons ANA were found in 2% of cases. In contrast, persons exposed to quartz-containing dusts are showing a remarkably higher incidence of ANA. So we found in sera of coal mine workers without pneumoconiosis ANA in 15% of cases, while in sera of coal mine workers with pneumoconiosis ANA were found in 21%. A nearly doubled incidence of ANA of 38% was found in sera of stone masons and quarriers (Figure 1). It is known that pattern of immunofluorescence of cell nuclei can be membranous, homogeneous, speckled and nucleolar depending on the reaction with the corresponding antigen. The pattern is schematically shown on Figure 2. Although all patterns of nuclear immunofluorescence were observed in silicotic patients, preferentially the type of homogeneous and speckled immunofluorescence was seen. As an example, in Figure 3 pattern of homogeneous immunofluorescence observed with ANA-containing serum from a silicotic patient is shown. As demonstrated by detection of ANA, the quality of quartz-containing dust has a great influence on the occurrence of immunological disturbances. The determination of all above described parameters is demonstrating almost the same effect: the alterations do not only occur when radiologically manifest silicosis exists, but are sometimes already found in miners without radiological fibrosis. These findings illustrate that quartz-containing dusts stimulate not only those functions of the macrophages which induce fibrosis,¹⁶ but

Pattern of Immunofluorescence of Cell Nuclei and corresponding Antigen in Presence of Anti-Nuclear Antibodies

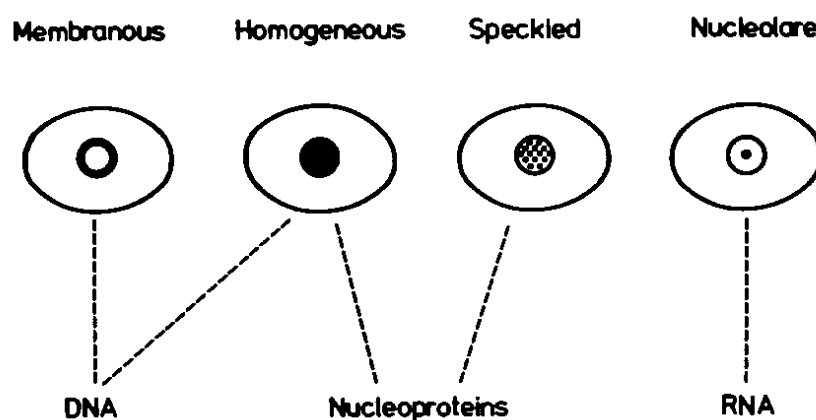


Figure 2. Pattern of immunofluorescence of cell nuclei and corresponding antigen in presence of anti-nuclear antibodies.

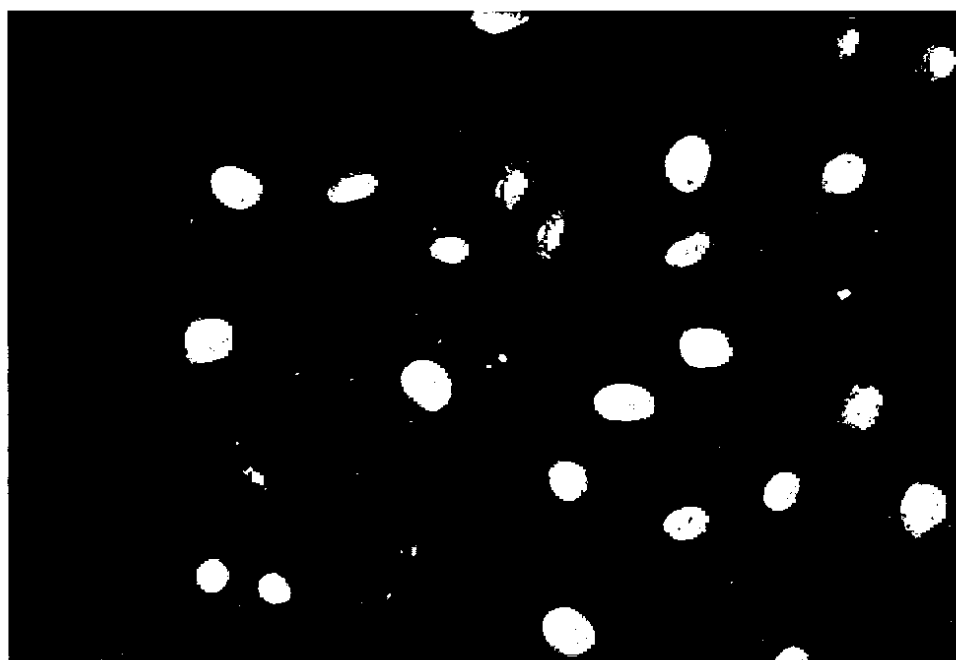


Figure 3. Pattern of homogeneous immunofluorescence of ANA.

at the same time the immunoregulatory functions and that disturbances in the latter functions are detectable before fibrosis or independently thereof.

Important recent findings demonstrate a cross-reactivity of anti-DNA antibodies with proteoglycans, cardiolipin and other phospholipids as well as with the intermediate filament vimentin.^{3,9,15} These reports allow a new view on autoimmunity in silicotic patients.

SUMMARY AND CONCLUSIONS

1. In silicotic patients the following humoral disturbances and autoimmunity phenomena are found:

- a. Indicators for macrophage stimulation, such as lysozyme, angiotensin-converting-enzyme (ACE), and fibronectin are increased.
- b. Effects on humoral immunity are seen by an increase of complement component C₃ and in part by an increase of immunoglobulins IgG and IgA.

Autoimmunity phenomena are shown by occurrence of antinuclear antibodies and of circulating immune complexes, containing C₃, IgG, IgA and IgM.

2. The above mentioned alterations do not only occur when manifest silicosis exists, but are also found in miners without radiologically fibrosis. These findings illustrate that quartz-containing dusts stimulate not only those functions of the macrophages which are involved in fibrosis, but also lead to immunoregulatory dysfunctions which are detectable before incidence or independently of a clinical manifest fibrosis.

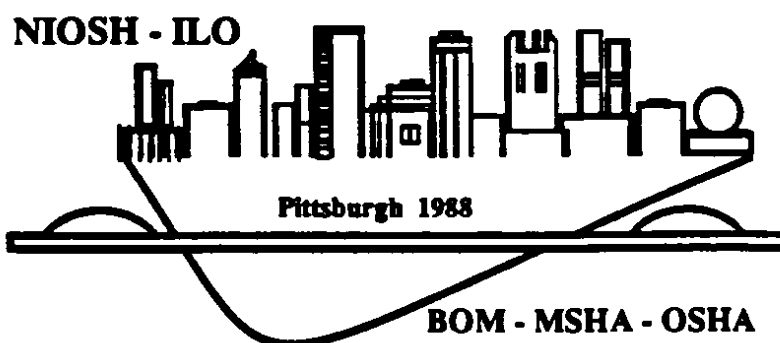
3. As demonstrated by incidence of ANA, the quality of quartz-containing dust has a great influence on the manifestation of autoimmunity phenomena.

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