British Journal of Industrial Medicine 1989;46:364–369

Histocompatibility antigens in a population based silicosis series

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ABSTRACT Individual susceptibility to silicosis is suggested by the lack of a uniform dose response relation and by the presence of immunological epiphenomena, such as increased antibody levels and associated diseases that reflect altered immune regulation. Human leucocyte antigens (HLA) are linked with immune response capability and might indicate a possible genetic susceptibility to silicosis. Forty nine silicotic subjects were identified from chest radiographs in a population based study in Leadville, Colorado. They were interviewed for symptoms and occupational history and gave a blood specimen for HLA-A, -B, -DR, and -DQ typing and for antinuclear antibody, immune complexes, immunoglobulins, and rheumatoid factor. Silicotic subjects had twice the prevalence of B44 (45%) of the reference population and had triple the prevalence of A29 (20%), both of which were statistically significant when corrected for the number of comparisons made. No perturbations in D-region antigen frequencies were detected. B44-positive subjects were older at diagnosis and had less dyspnoea than other subjects. A29-positive subjects were more likely to have abnormal levels of IgA and had higher levels of immune complexes. This study is the first to find significant HLA antigen excesses among a series of silicotic cases and extends earlier reported hypotheses that were based on groups of antigens of which B44 and A29 are components.

Silicosis is associated with immunological epiphenomena, such as an increased prevalence of antinuclear antibody, rheumatoid factor, immune complexes, and raised immunoglobulins.[1-4] Decreased numbers of suppressor T-lymphocytes and a corresponding increase in the helper to suppressor ratio in peripheral blood have also been shown.[5] Silicamacrophage interactions are thought to trigger the cascade of inflammatory and fibrotic events involved in cell mediated immune responses.[6] Silica may also affect immune regulation, resulting in increased risks of myobacterial infection[7] and connective tissue disease.[3 8-10] These immunological correlates of exposure to silica dust, coupled with the absence of a uniform dose response relation for the development of silicosis, have spurred investigators to look for factors associated with individual susceptibility to silicosis.

Human leucocyte antigens (HLA) are linked with the immune response capability and are a logical target for a study of possible genetic susceptibility to silicosis. Although most disease associations with HLA antigens are in the D-region, prior studies of silicotic subjects have been limited to A and B locus antigens,[11-13] and none has reported statistically significant findings when the probability values were corrected for the number of antigens tested. Weak associations at A and B loci often occur because of linkage disequilibrium with D-region antigens, which reflect immune response genes. We report here the first study examining D-region antigens in addition to A and B locus antigens in a population based series of silicotic cases. We also present immunological test data to assess the possible relation between HLA antigens and autoimmune epiphenomena in silicosis.

Methods

We identified 34 non-Hispanic, white silicotic subjects, aged 40 or over, in a community based random sample prevalence study of respiratory disease in Leadville, Colorado, of whom 33 agreed to be tested. In addition,

Accepted 6 June 1988

we invited the 21 current employees of a local hardrock mining company who carried a diagnosis of silicosis and lived in Leadville, of whom 16 agreed to be tested. Cases of silicosis were defined radiologically as individuals with an occupational history of exposure to silica dust and a profusion of small opacities of 1/0 or greater using the 1980 International Labour Organisation classification.[14] Chest radiographs of the 49 subjects were read blindly by a certified B reader of pneumoconiosis films as part of a larger study of 161 subjects with and without silica exposure. Where discrepancies existed between the radiological diagnosis and prior diagnoses, old chest radiographs were reviewed to clarify classifications as silicotic or non-silicotic, without reference to tissue typing results.

After informed consent, a trained interviewer completed a standardised questionnaire[15] for each participant, supplemented by questions regarding silicosis and occupational history in mining and other dusty trades. Dyspnoea was defined as an affirmative answer to the question "Do you have to walk slower than people of your own age on the level because of breathlessness?" Participants gave a blood specimen for immunological tests and HLA typing.

The immunological tests included antinuclear antibody, rheumatoid factor, immune complexes, and quantitative IgG, IgA, and IgM levels. Antinuclear antibody was determined by an indirect immunofluorescence procedure with HEp-2 cells (Immuno-Concepts, Sacramento, CA). Rheumatoid factor was detected by latex agglutination (Behring Diagnostics, La Jolla, CA). Circulating immune complexes were detected using an ^{125}I-C1q binding assay.[16] IgG, IgA, and IgM were quantitated by nephelometry (Beckman ICS, Beckman Instruments, Inc, Fullerton, CA).

We performed typing for HLA-A and HLA-B antigens by the lymphocyte microcytoxicity assay[17] under standard NIH incubation conditions.[18] For HLA-DR and HLA-DQ, we used extended incubation times[17] and B cells purified by incubation on nylon wool columns.[19] We purchased well characterised HLA reagents from One Lambda, Los Angeles, CA. In two individuals DRw6 was assigned by likelihood in the presence of DQw1 and DR3 reactivity.

We compared antigen prevalences at A and B loci in the silicotic cases with data for more than 1000 North American whites published in 1980[20] and for a similar number of international whites published in 1984,[21] using Yates's chi-squared test or Fisher's exact probability test for small expected numbers. For D-region antigens, comparison was made only with 1984 frequencies, since the more recent published data are more robust in terms of numbers and quality of typing.[21] We compared continuous variables for subgroups of silicotic cases, grouped by presence or absence of an antigen, by Student's t test; for categorical variables we used Yates's chi-squared or Fisher's exact tests.[22]

Results

The 49 silicotic subjects who took part represented 89% of the non-Hispanic white population identified as having silicosis. Table 1 gives their age, radiological findings, dyspnoea, and exposure findings. Seventy eight per cent had smoked cigarettes. All but five men had worked at a local molybdenum mine, although many had also held jobs at other mines. Of the remaining five, two had several decades of exposure to assay sample grinding for the hardrock mining industry, one had worked at hardrock mills for 30 years, and two had worked at other hardrock mines.

The prevalences of HLA-A and HLA-B locus antigens for the cases of silicosis and two white referent groups are presented in table 2. Statistically significant differences exist at the 0·05 probability level between both referent groups and the silicotic cases only for three antigens: B44, A29, and B27. When correction for multiple comparisons is made by multiplying the computed probability by the number of comparisons made (49), the prevalences of B44 and A29 remain statistically significantly different between the silicotic cases and the larger 1984 white reference group ($p = 0·044$ and $p = 0·029$, respectively). The prevalence of B44 is 45% in the silicotic group, about double that in the referent group. The prevalence of A29 is 20%, about three times that in the referent group.

Table 3 gives the prevalences of HLA-DR and HLA-DQ locus antigens for the silicotic cases and 1984 white referent group. Although DQw2 and DQw3 are overrepresented among the cases of silicosis, this had probably occurred by chance alone, when the probability values are multiplied by the number of comparisons made. The frequency of DQw1 was also somewhat increased, suggesting increased sensitivity for detecting DQw specificities in this study.

Table 1 *Characteristics of 49 Leadville cases of silicosis*

Characteristic	*Mean (y)*	*Range (y)*	*No*
Age	60·4	38–83	49
x Ray profusion:			
1/0, 1/1, 1/2	—	—	30
2/1, 2/2, 2/3	—	—	14
3/2, 3/3, 3/+	—	—	5
x Ray large opacities	—	—	5
Complaints of dyspnoea	—	—	15
Years in mining	27·7	5–58	49
Age at silicosis diagnosis	44·4	30–59	33
Latency to diagnosis	21·0	9–42	33
Time since 1st silica exposure	37·1	12–66	49

Table 2 *Per cent prevalences of HLA-A and HLA-B antigens in cases of silicosis and two referent groups*

*HLA antigen**	*Silicotics (n = 49)*	*North American whites (n = 1029)*[20]	*Whites (n = 1061–1082)*[21]
A1	28·6	25·7	26·4
A2	55·1	46·6	49·5
A3	18·4	26·0	24·6
A11	8·2	12·5	12·2
A23	10·2	5·0	2·8 (p = 0·015)
A24	18·4	12·8	19·6
A26	6·1	7·2	6·3
A28	10·2	9·9	9·2
A29	20·4	8·1 (p = 0·007)	5·7 (p = 0·0006)
A30	2·0	5·1	6·9
A31	2·0	6·2	5·7
A32	8·2	7·1	7·6
Aw33	2·0	3·4	2·8
B7	24·5	18·7	21·7
B8	14·3	17·1	18·3
B13	6·1	5·3	5·7
B14	4·1	9·5	7·3
B18	8·2	9·7	10·7
Bw22	4·1	5·4	5·5
B27	16·3	7·5 (p = 0·049)	6·7 (p = 0·020)
B35	14·3	15·6	19·9
B38	4·1	6·2	4·9
B39	4·1	3·6	3·9
B44	44·9	26·1 (p = 0·006)	23·1 (p = 0·0009)
B45	2·0	1·4	0·8
B49	4·1	4·7	3·6
B51	4·1	9·3	12·0
Bw52	2·0	2·8	3·9
Bw57	14·3	7·2	5·7 (p = 0·025)
Bw60	10·2	11·0	7·5
Bw61	2·0	2·0	4·2
Bw62	6·1	9·5	11·8

*The following specificities were not detected but were included in the number of antigens tested (49) for correcting p values: A25, B37, B41, Bw50, and Bw63. p Values in the table are not corrected for the number of comparisons.

To elucidate possible clinical ramifications of the HLA antigens that occurred more frequently among silicotic subjects we compared exposure estimates, radiological categories, symptoms, and immunological abnormalities of silicotic subjects with and without B44, A29, and B27 antigens (table 4). No differences were found in any of the three antigen comparisons for mean age, smoking history, *x* ray profusion distribution, presence of large radiographic opacities, years of mining, or latency from first exposure to diagnosis of silicosis for those 33 men whose diagnosis preceded the present study. Nevertheless, the age at diagnosis of silicosis was somewhat higher for those men who had B44 antigen than those without it (47·1 ± 5·8 SD *v* 41·9 ± 7·1, p = 0·03). The symptom of dyspnoea was less common among B44-positive silicotic subjects (13·6% *v* 44·4%, p = 0·04, relative risk = 0·197 with confidence interval of 0·047–0·829).

The only immunological differences among silicotics grouped by presence or absence of an antigen were found for those cases having HLA-A29. A29-positive cases were more likely to have abnormally high levels of IgA than were A29-negative cases (70% *v* 30·8%, p = 0·03). Mean IgA levels in A29-positive and A29-negative cases, however, did not significantly differ (388 *v* 306). A29-positive cases also had higher average levels of immune complexes than A29-negative cases (18·1 *v* 12·8, p = 0·01). No difference existed for any of the comparisons by antigen for antinuclear antibody, IgG, IgM, or rheumatoid factor.

Table 3 *Per cent prevalences of HLA-DR and HLA-DQ antigens in cases of silicosis and referent whites*

HLA antigen	*Silicotics (n = 49)*	*Whites (n = 963–1008)*[21]
DR1	22·4	18·1
DR2	24·5	29·1
DR3	24·5	22·5
DR4	24·5	23·8
DR5	18·4	26·5
DRw6	22·4	21·1
DR7	26·5	22·5
DRw8	6·1	5·9
DRw9	4·1	1·6
DQw1	63·3	54·2
DQw2	49·0	32·9 (p = 0·03)*
DQw3	57·1	41·2 (p = 0·039)*

*p Values are not corrected for the number of comparisons made.

Discussion

This is the first study of any pneumoconiosis to report significant associations with histocompatibility antigens, when probability values are corrected for the number of antigens tested. Specifically, two antigens were present in excess in silicotic cases, A29 and B44. No clinically useful parameters correlated with the presence of either antigen. It is also of particular interest that despite the immunological epiphenomena noted by others in silicosis, our cases showed no strong D-region associations. DR and DQ typing, first reported here, yielded no statistically significant excesses, although DQw2 and DQw3 may be of interest in future studies as prior hypotheses.

Our findings of excess A29 and B44 are particularly important, in the light of publications by others on HLA antigens among cases of silicosis, asbestosis, and coalworker's pneumoconiosis. We did not confirm the suggestion by Koskinen *et al* that the Aw19, B18 phenogroup is increased in silicotic subjects.[13] We have, however, refined their finding of an increased prevalence of Aw19, of which A29 is a component (along with A30, A31, A32, and Aw33). Several studies of cases of asbestosis have shown excesses of B12, of which B44 is a component.[23–25] Whereas other reports fail to confirm an excess of B12 in asbestosis[26–28] or coalworker's pneumoconiosis,[29–31] these reports are limited by small numbers, lack of statistical power, or selection bias. There is no reason to assume that

Table 4 *Characteristics of Leadville cases of silicosis by HLA antigen presence*

Characteristic	*B44-positive (n = 22)*	*B44-negative (n = 27)*	*A29-positive (n = 10)*	*A29-negative (n = 39)*	*B27-positive (n = 8)*	*B27-negative (n = 41)*
Age:						
Mean (SD)	61·7 (10·5)	59·4 (11·7)	60·9 (10·6)	60·3 (11·4)	65·4 (12·4)	59·4 (10·8)
Range (y)	46–83	38–80	38–74	43–83	51–80	38–83
x Ray profusion:						
1/0, 1/1, 1/2	50·0%	70·4%	50·0%	64·1%	62·5%	61·0%
2/1, 2/2, 2/3	40·9%	18·5%	20·0%	30·8%	25·0%	29·3%
3/2, 3/3, 3/+	9·1%	11·1%	30·0%	5·1%	12·5%	9·8%
Large opacities on *x* ray	13·6%	7·4%	20·0%	7·7%	0%	12·2%
Dyspnoea	13·6%*	44·4%	30·0%	30·8%	62·5%*	24·4%
History of cigarette smoking	72·7%	81·5%	80·0%	76·9%	100·0%	73·2%
Years in mining:						
Mean (SD)	27·3 (8·3)	28·0 (9·6)	27·1 (7·3)	27·8 (9·4)	32·4 (13·3)	26·8 (7·8)
Range	5–40	11–58	13–35	5–58	11–58	5–40
Age at silicosis diagnosis:						
Mean (SD)	47·1* (5·8)	41·9 (7·1)	47·6 (7·6)	43·6 (6·6)	42·8 (6·9)	44·7 (7·0)
Range (No of observations)	37–58 (n = 16)	30–59 (n = 17)	37–59 (n = 7)	30–58 (n = 26)	35–50 (n = 4)	30–59 (n = 29)
Latency (from first exposure to diagnosis):						
Mean (SD)	22·6 (6·8)	19·5 (8·2)	20·7 (9·4)	21·1 (7·3)	25·8 (12·9)	20·3 (6·7)
Range (No of observations)	11–34 (n = 16)	9–42 (n = 17)	11–34 (n = 7)	9–42 (n = 26)	14–42 (n = 4)	9–34 (n = 29)
Time since first silica exposure						
Mean (SD)	38·4 (11·9)	36·1 (13·6)	36·1 (13·4)	37·4 (12·7)	46·1* (13·8)	35·4 (11·9)
Range	22–66	12–58	12–54	17–66	29–58	12–66

*p < 0·05 by Student's *t* test, Fisher's exact, or Yates's chi squared tests.

increased genetic susceptibility to silicosis would parallel susceptibility to asbestosis or coalworker's pneumoconiosis, as reflected in histocompatibility antigens.

The finding of overrepresentation of both A29 and B44 in our group of silicotic cases may not be surprising, since positive linkage disequilibrium exists for these two antigens. We also found increases in A2 and A23, which would be expected on the basis of linkage disequilibria with B44. Anticipated deviations in DR4 or DR7, however, were not observed despite pronounced linkage disequilibrium with B44.

Although excess of B27 did not maintain statistical significance in our study when the probability was corrected by the number of comparisons made, we report our finding because a prior hypothesis from published reports makes such stringent statistical treatment questionable. B27 had been increased in four published studies of asbestosis cases[27 28 32 33] and was not increased in two others[24 26]; in no instance was the association statistically significant when corrected for the number of comparisons made. Our B27-positive cases complained of dyspnoea more frequently than the remainder, suggesting a possible reason why clinic based series might have an overrepresentation of this antigen. We conclude that the association between B27 and pneumoconiosis, if it exists, is weak and of no predictive value in identifying individuals at greater risk of disease.

Three major issues have clouded the interpretation of earlier studies of the association of histocompatibility antigens and pneumoconioses. The first is the necessity to correct for the statistical likelihood of obtaining significant results in the setting of multiple comparisons. The second concerns the selection of appropriate referent groups. The third is case selection bias. Previous studies of silicosis have all suffered from one or more of these methodological considerations.[11-13] For example, Gualde and coworkers reported a decreased frequency of B7 among 75 silicotic subjects by comparison with 160 unexposed controls and 46 workers exposed to silica[11]; 80% of the silicotic cases had progressive massive fibrosis on chest radiograph and many had had pulmonary tuberculosis. These characteristics suggest a clinic based series of silicotic cases which may not be representative of most cases of silicosis, which are asymptomatic. The small referent groups used in that study result in low statistical power to detect any differences in the setting of multiple comparisons.

We have considered these three methodological issues in our study. Our comparison data, based on large published North American and international white series, are the best available and are internally consistent at HLA-A and HLA-B loci. We chose not to test a local comparison group because of our population based design: a silica exposed referent group from this small single industry town would be misleading, since those without silicosis might well be depleted of antigens present in excess among those with silicosis. We avoided a falsely enhanced genetic difference between silicotic and non-silicotic miners

from the same population by using an external reference group. The only plausible alternative explanation for our finding of excess B44 and A29 phenotypes among silicotic subjects is genetic isolation of this group compared with North American or international whites. Two lines of evidence make this alternative interpretation unlikely. Firstly, our earlier population based survey and the work of others[34] suggest considerable immigration to Leadville of adult study participants. Secondly, our silicotic cases have antigen prevalences remarkably similar to those found in the two referent groups, except for the statistically significant excesses of B44 and A29. In particular, DR antigen prevalences are close to robust published data, despite both positive and negative linkage disequilibrium known to exist between B44 and most DR antigens.[35]

We applied strict inclusion criteria and a careful case definition in establishing our silicotic cases from an employee group with yearly chest radiographs and from a random sample of the Leadville population tested in a community study of respiratory disease. That 16 of the 33 community silicotic cases did not have a previous diagnosis of silicosis testifies to the population based nature of this series. We achieved a high participation rate, reducing this potential source of bias. A potential limitation of our study may be an unquantifiable bias arising from selective emigration of symptomatic silicotic cases from Leadville, which has an elevation of 3100 metres. Since 10% of our case series had progressive massive fibrosis, which is often accompanied by respiratory symptoms, we have no evidence of an underrepresentation of severe silicosis.

The search for individual susceptibility to occupational lung disease is motivated by attempts at prevention. Whether our findings have any prognostic or screening value in workers exposed to silica depends on longitudinal follow up and replication in other population based series of silicotic cases.

We thank the health and safety staff of the Climax Molybdenum Company, field interviewer Agnes Fabian, study coordinator Marilyn Kehe, data analyst Donann Bottolfsen, and laboratory technologist Mary Beer.

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This work was supported, in part, by NIOSH grant number K01-OH00018.

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