HLA-A1 and Coalworkers' Pneumoconiosis[1-3]

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SUMMARY

It has been suggested that undefined host factors are important in the development of complicated coalworkers' pneumoconiosis and that at least one of these factors may involve immune responsiveness. Studies of various coal miner populations have produced evidence supporting this concept, including reports of marked variation in the prevalence of antinuclear antibody and rheumatoid factor, an implied role for lung reactive antibody in the pathogenesis of disease, and an apparent increased susceptibility in certain persons to the development of progressive massive fibrosis.

Tandem review of these data followed by information linking several human diseases of obscure cause with the genetic constitution of the major histocompatibility complex had prompted us to explore the possibility of an association between histocompatibility (HLA) antigen frequencies and coalworkers' pneumoconiosis.

To test the possibility of such an association, a sample of coal miners from Pennsylvania and West Virginia were studied during a 3-year period. In each of 4 geographic regions, 358 subjects were matched for age and years in mining, and were then divided into 3 groups consisting of (*1*) miners with progressive massive fibrosis, (*2*) miners with simple coalworkers' pneumoconiosis, and (*3*) miners with no radiographic evidence of coalworkers' pneumoconiosis (control group). The frequency of HLA-A1 in a combined grouping of miners with simple and complicated coalworkers' pneumoconiosis (21.6 per cent) was significantly lower than that in the control group (31.3 per cent; $P \simeq 0.045$) or a reference group (32 per cent) of white Americans ($P \simeq 0.006$). This apparent significant disparity between groups should be viewed with caution. When probabilities were corrected for the number of antigens tested, the difference was no longer significant for the deficiency for the HLA-A1 specificity. Nevertheless, overly conservative interpretation of preliminary data can preclude the recognition of frequency differences that, although weak, are potentially real and would therefore require individual scrutiny to establish or reject in an independent study.

Introduction

Two distinct forms of coalworkers' pneumoconiosis are recognized. Simple coalworkers' pneumoconiosis, the most common form is a reaction to coal dust alone and develops when the capacity of the lung to eliminate inhaled particles is overwhelmed. Simple coalworkers' pneumoconiosis is associated with minimal respiratory impairment (1). By way of contrast, complicated coalworkers' pneumoconiosis, or progressive massive fibrosis, usually develops on a background of category 2 to 3 simple coalworkers' pneumoco-

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niosis and may progress in the absence of further exposure to coal dust. Progressive massive fibrosis is defined radiographically by the presence of one or more opacities larger than 1 cm in diameter and is often associated with severe respiratory impairment (2), pulmonary disability, and decreased longevity (3). Necessary for the development of progressive massive fibrosis is an appreciable dust burden plus another factor as yet unknown. The question has been raised whether certain susceptibility factors favor the development of disease in some miners but not in others (4). Several studies have examined the role of immunologic factors in the pathogenesis of coalworkers' pneumoconiosis (5–7); although there is more positive evidence for an immunologic abnormality in the complicated form of the disease, the question remains unresolved. Likewise, there is no conclusive evidence that genetic factors play a role in susceptibility to simple coalworkers' pneumoconiosis or to progressive massive fibrosis. In a preliminary report, we presented HLA phenotype data for 277 coal miners (8, 9). We now present the combined results for a total of 358 West Virginia and Pennsylvania coal miners.

Materials and Methods

Subjects. The sample under study consisted of 358 unrelated male coal miners (354 whites, 2 American blacks, and 2 American Indians) selected on a volunteer basis from a population of miners examined in the United States National Coal Study (10). Letters were mailed to more than 9,000 subjects; approximately 1,000 responded that they would be willing to participate in the proposed study. Those with a diagnosis of massive fibrosis were set aside from the respondent group, and the remaining miners who had simple coalworkers' pneumoconiosis or no evidence of disease were matched to these cases of progressive massive fibrosis on the basis of relevant variables. These variables included geographic region of employment, age in years (mean, 55.1 years; range, 32 to 68 years), and years in mining (mean, 31.8 years; range, 1 to 54 years). Three groups were therefore established within each region. The first group consisted of 118 miners (33 per cent) with progressive massive fibrosis. The second group was composed of 109 miners (30 per cent) with a diagnosis of simple coalworkers' pneumoconiosis. The third (control) group consisted of 131 miners (37 per cent of the total) with no radiographic evidence of disease. We therefore minimized the possibility of multiple bias within the total population by imposing uniformity of variables within all geographic regions studied. The areas concerned were eastern Pennsylvania (anthracite mines), central Pennsylvania (high rank bituminous coal from the Johnstown area), western Pennsylvania, and northern West Virginia (lower rank bituminous coal from the Pittsburgh seam) and southern West Virginia (higher rank coal).

Laboratory procedures. Blood samples were obtained during a period of 30 months and were transported to the laboratory in lymphocyte transport bags (Life Med. Corp. and Edwards Laboratories). More than 140 antisera were used to define 11 HLA-A and 15 HLA-B locus antigens using a 2-stage microcytotoxicity technique (11). Antisera used to define HLA-A1 were MF 8/69, Peo 1/69, Rag 4/71, D66-17058, Hesprick 3-06-05, Margo 12/72, and Hinnant 8/72.

For the purpose of analysis, subtypic specificities (e.g., Aw23, Aw24, Aw26, Aw30, Aw31, Aw33, Aw36, Bw38, and Bw39) were included in the broader specificities with which they are associated. The ABO, Rh_0 (D), and MN blood groups were determined by standard techniques (AABB Technical Methods and Procedures, 1974).

Results

To determine the extent of genetic stratification in miners of the 4 regions of West Virginia and Pennsylvania and to evaluate the effectiveness of matching within each region, we determined the ABO, Rh, and MN blood groups of 352 of the 358 miners (98 per cent). The distribution of blood group frequencies among miners in the 4 geographic regions is shown in table 1. In miners from central Pennsylvania, the frequency of blood group A was significantly lower and the frequency of blood group AB was significantly greater than those of miners from the other 3 regions. The distribution reflects the large proportion of miners of German and Czechoslovakian ancestry in this region. The frequency of blood group M was significantly lower and the frequency of group MN was significantly higher among the eastern and western Pennsylvania coal miners compared to those of coal miners from central Pennsylvania and West Virginia, reflecting the predominance of Scottish, Irish, and English descendants in the latter 2 regions. However, as shown in table 2, there were no significant differences in blood group antigen frequencies among the progressive massive fibrosis, simple coalworkers' pneumoconiosis, and control groups. This suggests that ethnic balance among the 3 groups of miners was achieved by the matching procedure.

The HLA frequency distribution is shown in table 3. There were no significant differences in the frequencies of HLA antigens between the

TABLE 1

BLOOD GROUP FREQUENCIES AMONG COAL MINERS, BY REGION

Blood Group	Distribution by Region: Southern (W. Va.) (n = 81) (no.)	(%)	Western (Pa.) (n = 109) (no.)	(%)	Central (Pa.) (n = 97) (no.)	(%)	Eastern (Pa.) (n = 65) (no.)	(%)	χ^2
0	38	46.9	44	40.4	44	45.4	23	35.4	2.52
A	35	43.2	48	44.0	26	26.8	30	46.2	9.15*
B	5	6.2	13	11.9	15	15.5	9	13.8	3.93
AB	3	3.7	4	3.7	12	12.4	3	4.6	8.63*
Rh (D)+	65	80.2	91	82.6	76	78.4	47	72.3	2.69
Rh (D)−	16	19.8	19	17.4	21	21.6	18	27.7	2.69
M	34	42.0	24	22.0	42	43.3	13	20.0	18.65*
N	20	24.7	23	21.0	16	16.5	10	15.4	2.81
MN	27	33.3	62	56.9	39	40.2	42	64.6	19.95*

*For $\chi^2 = 7.81$ with 3 degrees of freedom, P = 0.05.

progressive massive fibrosis group and the simple coalworkers' pneumoconiosis group. Therefore, both of these groups were combined for comparison with the control (no disease) group. As shown in table 3, the combined pneumoconiosis groups and the control group differed with respect to the frequency of HLA-A1. The frequency of HLA-A1 among the combined pneumoconiosis groups (21.6 per cent) was significantly less than that among the control group (31.3 per cent) ($\chi^2 = 4.16$; P ≃ 0.045, uncorrected). Moreover, the frequency of HLA-A1 among the control subjects was almost identical to that reported among white U.S. subjects (32 per cent) (12). When the frequency of HLA-A1 in that reference group of 503 persons is compared to the frequency among miners with coalworkers' pneumoconiosis, the significance of the difference is increased ($\chi^2 = 8.29$; P = 0.006, uncorrected); however, the difference does not reach significance when corrected for the number of antigens tested (P = 0.089). The prevalence of coalworkers' pneumoconiosis among miners with HLA-A1 relative to that among miners lacking HLA-A1 (i.e., the relative risk) was 0.60.

Discussion

The present report concludes the first reported study of HLA antigens in coalworkers' pneumo-

TABLE 2

BLOOD GROUP FREQUENCIES AMONG COAL MINERS WITH AND WITHOUT PNEUMOCONIOSIS

Blood Group	Distribution by Disease Group: PMF (n = 116) (no.)	(%)	Simple CWP (n = 107) (no.)	(%)	Control (No Disease) (n = 129) (no.)	(%)	χ^2
O	50	43.1	43	40.2	56	43.4	0.29
A	48	41.4	39	36.4	52	40.3	0.62
B	11	9.5	17	15.9	14	10.9	2.40
AB	7	6.0	8	7.5	7	5.4	0.43
Rh (D)+	87	75.0	83	77.6	108	83.7	2.98
Rh (D)−	29	25.0	24	22.4	21	16.3	2.98
M	35	30.2	37	34.6	41	31.8	0.51
N	23	19.8	21	19.6	25	19.4	0.01
MN	58	50.0	49	45.8	63	48.8	0.42

Definitions of abbreviations: PMF = progressive massive fibrosis; CWP = coalworkers' pneumoconiosis.

P = 0.05 for $\chi^2 > 5.99$ with 2 degrees of freedom.

TABLE 3

DISTRIBUTION OF HLA ANTIGENS AMONG APPALACHIAN COAL MINERS

HLA Antigen	Group						χ^2*	
	PMF (I) (n = 118)		Simple CWP (II) (n = 109)		No Disease (III) (n = 131)			
	(*no.*)	(%)	(*no.*)	(%)	(*no.*)	(%)	I vs II	I+II vs III
A1	27	22.9	22	20.2	41	31.3	0.24	4.16*
A2	57	48.3	58	53.2	69	52.7	0.55	0.13
A3	30	25.4	33	30.3	29	22.1	0.67	1.37
A9	31	26.3	25	22.9	29	22.1	0.34	0.29
A10	10	8.5	12	11.0	19	14.5	0.42	1.90
A11	18	15.3	12	11.0	16	12.2	0.89	0.07
A28	10	8.5	5	4.6	4	3.1	1.39	2.09
A29	12	10.2	6	5.5	12	9.2	1.69	0.16
Aw19†	9	7.6	9	8.3	10	7.6	0.03	0.01
B5	13	11.0	16	14.7	14	10.7	0.68	0.34
B7	29	24.6	29	26.6	26	19.8	0.12	1.50
B8	15	12.7	20	18.3	27	20.6	1.38	1.56
B12	33	28.0	28	25.7	31	23.7	0.15	0.45
B13	4	3.4	10	9.2	7	5.3	3.28	0.10
B14	7	5.9	3	2.8	6	4.6	1.36	0.01
B18	8	6.8	12	11.0	19	14.5	1.26	2.77
B27	13	11.0	17	15.6	14	10.7	1.04	0.49
Bw15	18	15.3	11	10.1	24	18.3	1.36	2.03
Bw16	3	2.5	1	0.9	5	3.8	0.86	1.43
Bw17	8	6.8	5	4.6	9	6.9	0.50	0.19
Bw22	9	7.6	8	7.3	6	4.6	0.01	1.17
Bw35	26	22.0	15	13.8	21	16.0	2.62	0.24
Bw40	9	7.6	8	7.3	14	10.7	0.01	1.07

Definitions of abbreviations: PMF = progressive massive fibrosis; CWP = coalworkers' pneumoconiosis.

*P = 0.05 for $\chi^2 > 3.84$ = with 1 degree of freedom.

†Includes Aw30, 31, 32, 33, 36.

coniosis. Our preliminary finding (8, 9) that HLA-A1 occurred less frequently in miners with radiographic evidence of pneumoconiosis than in those without pneumoconiosis was sustained by the additional data obtained from 83 miners from southern West Virginia. A log-linear model that accounts for all 3 factors (region, disease category, and presence of HLA-A1) confirmed the χ^2 value shown in this paper and provided evidence to suggest that the results from the southern (West Virginia) region were not different from those from the other regions. However, the previously reported excess of HLA-B18 among nondiseased miners was not significant in the final combined data reported here.

The observation of a decreased frequency of HLA-A1 among persons with coalworkers' pneumoconiosis remains to be explained. There were no apparent technical difficulties in the definition of HLA-A1 during the course of this study; moreover, control subjects, miners with simple coalworkers' pneumoconiosis, and miners with progressive massive fibrosis were typed concurrently. To determine whether serum from miners contained antibodies to lymphocytes that might interfere with HLA typing, we screened 314 sera from miners against a selected cell panel of 7 persons using a sensitive cross-match technique and an IgM antiglobulin technique. Two of the 7 cells possessed HLA-A1. None of the sera reacted in the direct cytotoxicity test. Five of the 314 (2 per cent) reacted with the antiglobulin technique. We believe, therefore, that it is unlikely that humoral factors interfered with the detection of HLA-A1.

We conclude that there is a weak negative association between HLA-A1 and coalworkers' pneumoconiosis. However, this association was not significant when corrected for the number of antigens tested, as suggested by some to avoid type 1 errors. In multiple testing, such a procedure would technically preclude the recognition of frequency differences that occur by chance alone. However, if this more conservative approach were taken, differences that are actually present might be missed. Therefore, if a con-

servative interpretation is to be adopted, further studies are needed to confirm this association.

Although most HLA and disease associations involve HLA-B region or D region antigens, at least one, hemochromatosis, apparently is associated primarily with HLA-A locus antigens. It has been suggested that disease susceptibility genes are located in various positions along the major histocompatibility complex and may therefore be found in gametic association with alleles of the HLA-A, -B, -C, or -D loci. It also has been suggested that HLA-B8 and, possibly, the HLA-A1, and -B8 haplotypes are associated with an enhanced immunologic reactivity to certain autoantigens.

An unusual feature of the apparent association between HLA-A1 and coalworkers' pneumoconiosis was that the frequency of the HLA antigen was decreased rather than increased in the diseased group. If our observation of a difference in the distribution of HLA-A1 antigen between the combined pneumoconiosis group and the control (no disease) group is accepted as significant, a number of explanations may be put forward. First, the higher incidence of HLA-A1 in the control group as opposed to the combined pneumoconiosis group might represent an increased resistance of this group to the development of coalworkers' pneumoconiosis. This explanation appears unlikely, because the prevalence of HLA-A1 in our control population does not differ significantly from that in the general white population. Conversely, the lower-than-expected prevalence of the HLA-A1 antigen among the diseased miners could suggest predisposition to the development of coalworkers' pneumoconiosis. If susceptibility to coalworkers' pneumoconiosis were, in this instance, directly related to the presence or absence of the HLA-A1 antigen, it might follow that the absence of A1 implies the presence of some other A-locus antigen that would influence predisposition to coalworkers' pneumoconiosis. Because we have no evidence for a direct relationship, this is speculative; but it is interesting to note that there was no increase in any of the A-locus antigens for which we tested. The possibility remains that there might have been such an increase in an A-locus antigen for which we did not test. Finally, the decreased prevalence of the HLA-A1 antigen in our combined pneumoconiosis group could reflect an increased morbidity/mortality among HLA-A1-positive persons with coalworkers' pneumoconiosis; thus, our diseased groups might represent a "survivor population." The possibility that susceptibility to coalworkers' pneumoconiosis depends on HLA-A1 homozygosity seems unlikely, because the proportion of miners with only one HLA-A locus antigen detected among the 3 groups did not differ significantly ($\chi^2 = 1.10$; data not shown). Nor did the groups differ significantly in the number of cases in which the single A-locus antigen was HLA-A1 ($\chi^2 = 2.07$). Finally, this study did not provide evidence that simple coalworkers' pneumoconiosis and progressive massive fibrosis involve different genes. Confirmation of the association and the pathogenetic implications will require further study.

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