PREDOMINANCE OF HISTOCOMPATIBILITY ANTIGENS W18 AND HL-A1 IN MINERS RESISTANT TO COMPLICATED COALWORKERS' PNEUMOCONIOSIS*

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Abstract—The possibility that an association exists between susceptibility or resistance to coal workers' pneumoconiosis (CWP) and the distribution of blood group and histocompatibility (HL–A) antigens was tested in a sample of Pennsylvania and West Virginia coal miners with a high prevalence of CWP. Unrelated miners, who were matched by geographic region for age and years in mining, were then divided into three groups composed of men with (1) no radiographic evidence of CWP, (2) simple CWP, and (3) complicated CWP (progressive massive fibrosis or PMF). The distribution of twenty-three HL–A antigens was determined by a two-stage lymphocyte microcytotoxicity technique. The frequency for each antigen was calculated for the total sample (n = 277) and for each geographic region and disease category.

Blood-group antigen frequencies were compared by disease category and geographic region on participants in the study in an attempt to detect bias due to ethnic stratification. There were no differences noted within disease categories. One region was found where some stratification might be present.

The serum concentrations of complement and three immunoglobulin classes were measured in an effort to assess disturbances of humoral immunity. No apparent clinical significance could be attributed to the differences in mean values which existed between experimental groups.

When examined at the $P = 0.05$ level there was an excess of the W18 histocompatibility antigen in miners who had no evidence of disease but whose histories of coal-dust exposure were comparable to the two disease groups. The frequency of W18 in miners with simple CWP was approximately that expected for a heterogeneous population of Caucasians. The relative risk of complicated CWP was approximately 300% less in miners who possessed antigen W18 than in those lacking this antigen.

These results suggest an association between W18 and resistance to the development of progressive massive fibrosis and that HL–A1 is associated with resistance to the development of both simple and complicated CWP.

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INTRODUCTION

Coal workers' peumoconiosis (CWP) exists in two distinct forms—simple and complicated. The former occurs when the quantity of retained coal dust exceeds the clearance capacity of the lung and is recognized by the presence of small opacities in the chest radiograph. Three categories of simple CWP are defined according to the extent and profusion of these opacities. Simple CWP is associated with minimal respiratory impairment (MORGAN *et al.*, 1972). The second form of CWP, progressive massive fibrosis (PMF), usually occurs on a background of categories 2 or 3 simple CWP and is characterized by the development of large fibrotic masses. PMF is an irreversible process that is often associated with respiratory impairment, permanent disability and premature death (LAPP and SEATON, 1971). The proportion of miners with PMF varies from 0.5% in Utah and Colorado to 3% in central Pennsylvania and approximately 14% in the anthracite miners of Eastern Pennsylvania (MORGAN *et al.*, 1973). It has been suggested that undefined host factors are important in the development of complicated CWP. One of the suspected factors may involve immune responsiveness, since an increased frequency of antinuclear antibodies has been noted in patients with CWP, especially in association with PMF (LIPPMANN *et al.*, 1973; SOUTAR *et al.*, 1974). Whether immunological factors play a role in pathogenesis of this disease remains uncertain (MAJOR *et al.*, 1975).

It is now clear that in animals the genetic region which codes for the major histocompatibility antigens also carries genes important for disease susceptibility (GREEN, 1974). These observations have provided the basis for numerous studies in man of the possible association between genes of the HL–A system and disease. Convincing and consistent differences in the frequency of particular HL–A or related genes have been demonstrated in patients with several diseases of obscure aetiology.

The most widely accepted explanation for the established associations involves immune response (Ir) genes, some of which are linked closely to loci which specify serologically defined and lymphocyte activating determinants of the HL–A system. The fact that the majority of coal miners with simple CWP do not develop PMF raises the possibility that genetic factors may be important in the disease. We therefore compared the frequency of HL–A antigens within a population of active and retired miners in the coal fields of Pennsylvania and northern West Virginia. Our objective was to determine whether particular HL–A antigens were associated with susceptibility or resistance to CWP.

SUBJECTS AND METHODS*

The population under study consisted of 277 unrelated coal miners (273 Caucasian, 2 American Negro, and 2 American Indian) selected on a volunteer basis from a population of miners examined in the first and second series of the United States National Coal Study (MORGAN *et al.*, 1973). The subjects were divided into three experimental groups that were closely matched according to geographic region for age in years (mean 56; range 39–68) and years in mining (mean 34; range 1–54).

As such it is impossible to match the sub-groups according to dust exposure in that

* Mention of brand names does not constitute endorsement by the U.S. Public Health Service.

for the most part it can be assumed that miners with simple CWP and PMF are likely to have been exposed to greater amounts of respirable coal dust than have miners with category 0 (ROGAN *et al.*, 1973). None the less, this need not always be so since the development of CWP is also related to the efficiency of lung clearance. For the purposes of this study, it can also be assumed that miners who have worked for many years but who have clear lungs nevertheless have had fairly heavy dust exposure but for reasons as yet not understood have not developed CWP.

Group I consisted of 97 miners (35% of the total) with no radiologic evidence of disease. Group II was composed of 87 (31%) miners with a diagnosis of simple pneumoconiosis. Group III consisted of 93 (34%) miners with PMF. The number of subjects in each experimental group was similar in each of three defined geographic regions in Pennsylvania and West Virginia (Table 1, Fig. 1).

TABLE 1. HISTOCOMPATIBILITY (HL-A) ANTIGENS FREQUENCIES TABULATED ACCORDING TO CWP DISEASE CATEGORY AND GEOGRAPHIC REGIONS

HL-A antigens		Distribution within disease category				Distribution within regions		
		Group I (no disease)	Group II (simple disease)	Group III (complicated disease)	Groups II + III (simple and complicated)	Western region	Central region	Eastern region
	n= (23)	n= (97)	n= (87)	n= (93)	n= (180)	n= (109)	n= (97)	n= (71)
		% no.	% no.	% no.	% no.	% no.	% no.	% no.
LA Locus	1	35.1 (34)	20.7 (18)	21.5 (20)	21.1 (38)	24.8 (27)	28.9 (28)	23.9 (17)
	2	51.6 (50)	54.0 (47)	49.5 (46)	51.7 (93)	55.0 (60)	45.4 (44)	54.9 (39)
	3	19.6 (19)	29.9 (26)	22.6 (21)	21.6 (47)	22.9 (25)	26.8 (26)	21.1 (15)
	9	22.7 (22)	19.5 (17)	27.9 (26)	23.9 (43)	22.9 (25)	19.6 (19)	29.6 (21)
	10	16.5 (16)	13.8 (12)	7.5 (7)	10.6 (19)	13.8 (15)	13.4 (13)	9.9 (7)
	11	12.4 (12)	8.0 (7)	12.9 (12)	10.6 (19)	11.9 (13)	10.3 (10)	11.3 (8)
	W19	5.2 (5)	3.5 (3)	5.4 (5)	4.4 (8)	4.6 (5)	3.1 (3)	7.0 (5)
	W28	4.1 (4)	8.0 (7)	7.5 (7)	7.8 (14)	3.7 (4)	2.1 (2)	16.9 (12)
	W29	7.2 (7)	6.9 (6)	11.8 (11)	9.4 (17)	10.1 (11)	8.2 (8)	7.0 (5)
FOUR Locus	5	12.4 (12)	14.9 (13)	11.8 (11)	13.3 (24)	9.2 (10)	11.3 (11)	21.1 (15)
	7	21.7 (21)	21.8 (19)	24.7 (23)	23.3 (42)	27.5 (30)	15.5 (15)	25.4 (18)
	8	21.7 (21)	18.4 (16)	12.9 (12)	15.5 (28)	16.5 (18)	17.5 (17)	19.7 (14)
	12	19.6 (19)	28.7 (25)	26.9 (25)	27.8 (50)	23.9 (26)	28.9 (28)	21.1 (15)
	13	6.2 (6)	10.3 (9)	4.3 (4)	7.2 (13)	7.3 (8)	6.2 (6)	7.0 (5)
	W5	18.6 (18)	12.6 (11)	20.4 (19)	16.7 (30)	16.3 (21)	17.5 (17)	14.1 (10)
	W10	11.3 (11)	5.7 (5)	7.5 (7)	6.7 (12)	11.0 (12)	6.2 (6)	7.0 (5)
	W14	3.1 (3)	2.3 (2)	5.4 (5)	3.9 (7)	6.4 (7)	2.1 (2)	1.4 (1)
	W15	16.5 (16)	10.3 (9)	16.1 (15)	13.3 (24)	11.9 (13)	14.4 (14)	18.3 (13)
	W16	2.1 (2)	1.1 (1)	3.2 (3)	2.2 (4)	1.8 (2)	2.1 (2)	2.8 (2)
	W17	7.2 (7)	4.6 (4)	7.5 (7)	6.1 (11)	8.3 (9)	6.2 (6)	4.2 (3)
	W18	15.5 (15)	10.3 (9)	4.3 (4)	7.2 (13)	12.8 (14)	5.2 (5)	12.7 (9)
	W22	5.2 (5)	6.9 (6)	7.5 (7)	7.2 (13)	6.4 (7)	4.1 (4)	9.9 (7)
	W27	9.3 (9)	16.1 (14)	12.9 (12)	14.4 (26)	5.5 (6)	20.6 (20)	12.7 (9)

Heparinized peripheral blood specimens for HL–A typing were transported to the laboratory in Terasaki lymphocyte transport bags (Lifemed Corporation, Campton, California) at ambient temperature. Lymphocytes were separated from other blood cells by a standard ficoll-renograffin technique. The HL–A phenotypes were determined by means of a two-stage microcytotoxicity technique (AMOS *et al.*, 1969). About 100 antisera, obtained from local sources, from Dr Amos, and from the National Institutes of Health were used to detect 9 antigens of the LA (A) series and 14 antigens of the FOUR (B) series. At least three antisera were used to define each specificity. Sera of coal miners were screened for lymphocytotoxic antibodies.

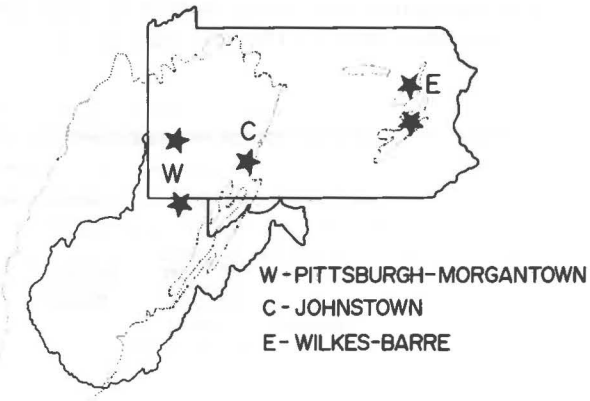

FIG. 1. Geographic regions of Pennsylvania and West Virginia from which coal workers were selected on a volunteer basis for inclusion in the study. The eastern (E) region mines anthracite coal, the central (C) region mines high-ranked bituminous coal and the western (W) region mines lower-ranked bituminous coal.

The ABO, $Rh_o(D)$ and MN blood groups were determined by standard immunohaematological techniques (MILLER *et al.*, 1974). ABO typing results were verified by back-typing with the serum. All $Rh_o(D)$ negative typings were re-examined for the presence of the Du component and all serum specimens were screened for the presence of atypical antibodies with a commercial pooled erythrocyte suspension prepared for that purpose.

Total haemolytic complement levels were determined titrimetrically according to a modification of standard protocol (MAYER, 1961) on serum stored at −70°C prior to assay. CH_{50} units were calculated by probit analysis of the data.

Serum immunoglobulin (IgG, IgA, IgM) concentrations were determined with a microfluoronephelometric autoanalyser (Technicon Corporation, Terrytown, N.Y.) according to the recommended procedure (RITCHIE *et al.*, 1973).

The frequency of a given HL–A antigen or blood group in simple CWP or complicated CWP was compared to its frequency in the CWP free control group using the chi-square test. The frequency of each antigen in the three geographic regions was likewise compared with the chi-square method to test for disturbances of HL–A or blood-group frequencies caused by ethnic stratification.

The relative risk (x) was calculated by the formula:

$$x = \frac{ad}{bc}$$

where a and b are the numbers of affected subjects carrying or lacking a given antigen, respectively, and c and d are the numbers of controls carrying or lacking the antigen, respectively.

RESULTS

The distribution of blood group frequencies by disease category and by geographic region is shown in Table 2. There are no significant differences in the frequency of these antigens when disease categories are compared, indicating that susceptibility or resistance to CWP is not associated with any of these antigens. However, there were differences in the frequencies of blood groups A. M and MN in the central region when compared to the eastern and western regions. The data reflect the different origins of the populations under study.

TABLE 2. BLOOD GROUP FREQUENCIES ENCOUNTERED IN U.S. REFERENCE POPULATION, COMBINED SAMPLE POPULATION, CWP DISEASE GROUPS AND GEOGRAPHIC REGIONS

R.B.C. blood group	Distribution within		Distribution within disease category			Distribution within regions		
	U.S. reference population	Combined sample population	Group I (no disease)	Group II (simple disease)	Group III (complicated disease)	Western region	Central region	Eastern region
		n= (271)	n= (95)	n= (85)	n= (91)	n= (109)	n= (97)	n= (65)
	%	% no.	% no.	% no.	% no.	% no.	% no.	% no.
O	45	40.9 (111)	44.2 (42)	41.2 (35)	37.4 (34)	40.4 (44)	45.4 (44)	35.4 (23)
A	40	38.4 (104)	36.8 (35)	34.1 (29)	44.0 (40)	44.0 (48)	26.8 (26)	46.2 (30)
B	11	13.7 (37)	12.6 (12)	17.7 (15)	11.0 (10)	11.9 (13)	15.5 (15)	13.9 (9)
AB	4	7.0 (19)	6.3 (6)	7.1 (6)	7.7 (7)	3.7 (4)	12.4 (12)	4.6 (3)
Rh_o+	85	80.4 (218)	85.3 (81)	78.8 (67)	76.9 (70)	83.5 (91)	79.4 (77)	77.0 (50)
Rh_o-	15	19.6 (53)	14.7 (14)	21.2 (18)	23.1 (21)	16.5 (18)	20.6 (20)	23.0 (15)
M	28	29.2 (79)	31.6 (30)	30.6 (26)	25.3 (23)	22.0 (24)	43.3 (42)	20.0 (13)
N	22	18.1 (49)	16.8 (16)	16.5 (14)	20.9 (19)	21.1 (23)	16.5 (16)	15.4 (10)
MN	50	52.8 (143)	51.6 (49)	52.9 (45)	53.9 (49)	56.9 (62)	40.2 (39)	64.6 (42)

One source of bias in studies of association between HL–A antigens and disease results from ethnic differences between the disease and control samples, since the frequencies of certain HL–A antigens vary between different population groups. In the current study this source of bias was minimized by having equivalent numbers in the control and disease groups from each geographic area. It was of interest, nevertheless, to compare HL–A antigen frequencies in the total miner population with another study

involving normal Caucasians. In Fig. 2, the HL–A frequencies observed in the current study are compared with those reported by ALBERT *et al.* (1972) for a population of mixed Caucasians. The HL–A frequencies between the two studies were comparable except for lower frequencies of W19, W14, W15 and W16 in the miners observed. These differences are attributed, at least in part, to a lack of sera to consistently detect the W30, W31 and W19.6 specificities of the W19 complex and relatively weak antisera for W14, W15 and W16.

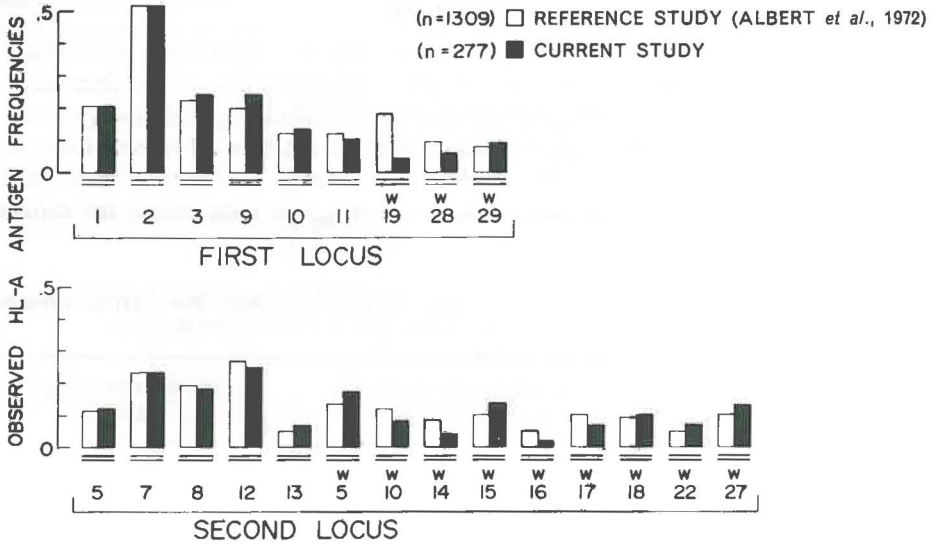

FIG. 2. Comparison of HL–A frequencies observed in the current study of coal workers' pneumoconiosis with those frequencies reported in a reference population of mixed Caucasians.

The distribution of HL–A antigen frequencies is shown in Table 1. HL–A1 was significantly more frequent in the group without evidence of CWP than in the group with simple CWP (Fisher's exact probability = 0.022) or the group with complicated CWP (Fisher's exact probability = 0.027). The frequency of HL–A1 in both CWP groups is equal to the observed frequency of this antigen in a heterogeneous Caucasian population (ALBERT *et al.*, 1972). Antigen W18 occurred significantly (Fisher's exact probability = 0.008) more frequently among miners with no radiographic evidence of disease than in those with PMF. The frequency of W18 in the group with simple CWP is essentially equal to the expected frequency for Caucasians (ALBERT *et al.*, 1972).

When the distribution of HL–A antigens was examined for regional balance, it was found that significant disparities for the W28 specificity existed within the eastern region when it was compared to both the central and western areas. An additional imbalance was evident between the central and western sectors with regard to the W27 antigen. Whether these differences are due to serological, statistical, ethnic bias, or a combination of factors has not been determined.

In order to better compare the association between HL–A antigens and susceptibility/resistance to disease, it is frequently useful to determine the relative risk factor (WOOLF, 1955). The relative risk indicates how many times more frequently the disease

occurs in individuals carrying the antigen than in those lacking it. Accordingly, an increased frequency of an antigen in the disease group gives a risk factor above unity and indicates a positive association. Conversely, decreased frequencies in the disease group yield a relative risk factor of less than 1 (negative association). The relative risk factors calculated for the present study are shown in Fig. 3. The data indicate a

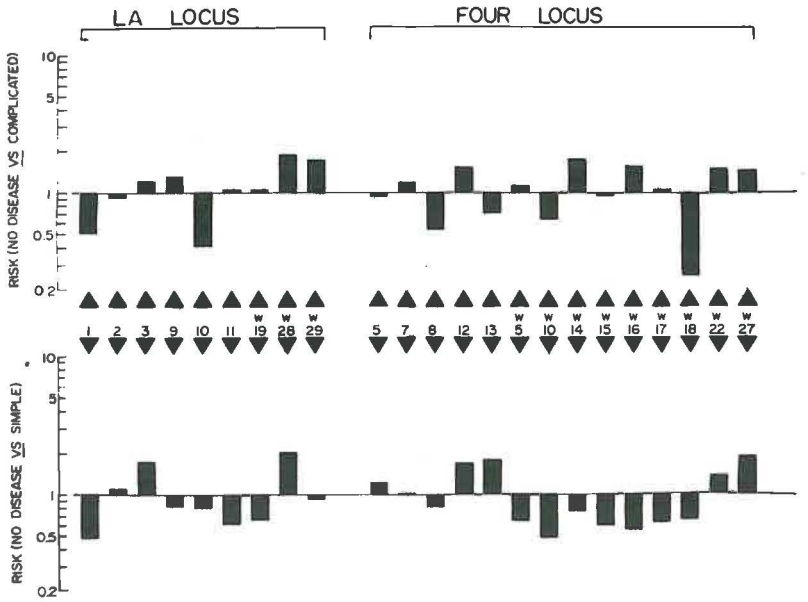

FIG. 3. Relative disease risk factors for twenty-three tested histocompatibility antigens in a population of unrelated coal miners. Relative risk for both simple and complicated CWP is expressed in reference to miners showing no radiographic evidence of CWP.

moderately strong negative association between antigen W18 and PMF (relative risk 0.25). Thus, miners carrying this antigen were about 300% less likely to develop complicated CWP than those who lacked the antigen. A weaker negative association was obtained for HL-A1 and both forms of CWP (relative risk = 0.51 and 0.48 for simple CWP and PMF respectively).

Serum haemolytic complement concentrations were determined in 250 of the miners participating in the study. The results expressed in CH_{50} units/ml are shown in Fig. 4. When the CH_{50} values were compared between disease categories, the mean complement concentrations were equivalent: 101, 98 and 103 CH_{50} units for the group without CWP, simple CWP and complicated CWP, respectively. The mean values between geographic regions were 95, 112 and 93 CH_{50} units in miners from western, central and eastern areas, respectively.

Likewise, there was no apparent clinical significance to the differences in the serum concentrations of IgG, IgA or IgM when compared by disease group but the mean values in the eastern region (177 mg/100 ml) and western region (186 mg/100 ml) were somewhat greater than in the central region (96 mg/100 ml) (Table 3). Cytotoxic antibodies were observed in only 1 of 217 sera.

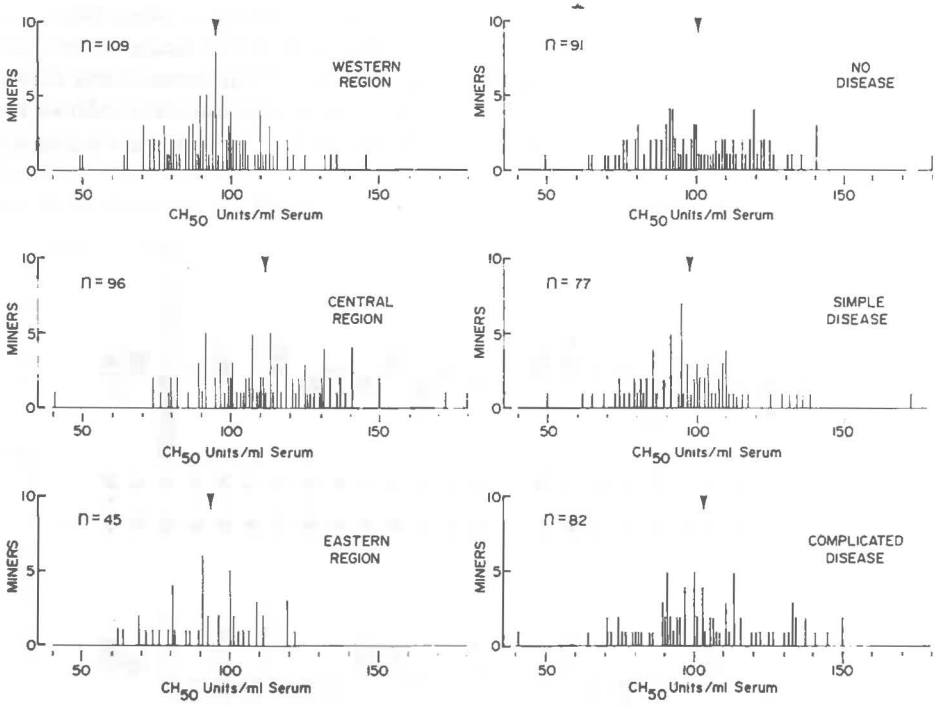

FIG. 4. Distribution of serum haemolytic complement concentrations within the various geographic mining regions and within each experimental disease group. Mean (m) values are indicated by the solid arrows.

TABLE 3. SERUM IMMUNOGLOBULIN LEVELS ENCOUNTERED WITHIN COMBINED SAMPLE POPULATION, CWP DISEASE GROUPS AND GEOGRAPHIC REGIONS

Serum immunoglobulin fraction		Distribution within disease category				Distribution within regions		
		Combined sample population	Group I (no disease)	Group II (simple disease)	Group III (complicated disease)	Western region	Central region	Eastern region
IgG	n	275	97	86	92	109	97	69
	m±s*	1172±267	1120±235	1195±294	1206±267	1242±283	1112±234	1146±263
IgA	n	271	95	85	91	108	95	68
	m±s	259±145	222±127	277±148	281±155	277±155	272±144	211±120
IgM	n	269	95	84	90	106	96	67
	m±s	152±106	150±97	156±112	149±110	186±114	96±59	177±115

* m±s = mean ± s.d. (mg/100 ml).

DISCUSSION

The significant observation in this study was the increased frequency of the HL–A1 and W18 antigens in miners without radiographic evidence of pneumoconiosis. The differences were significant at the 5% probability level, but not when multiplied by the number of antigens tested as recommended by SVEJGAARD *et al.* (1974). Since this latter method is conservative, it is entirely possible that the increased frequencies of HL–A1 and W18 are real and not due to chance. With twenty-three tests (one for each antigen surveyed) this conservative approach amounts to performing each test at the $\alpha = 0.002$ level instead of $\alpha = 0.05$, and thus true differences might be overlooked. However, HL–A1 and W18 were found significant and had values of $\alpha = 0.04$. If the tests were independent and there were no differences for any of the twenty-three antigens, then using the binomial probability function, one finds that the probability of finding at least one test with $\alpha \leqslant 0.04$ out of twenty-three would be about 0.61, and the probability of finding at least two tests with $\alpha \leqslant 0.04$ would be about 0.23. Thus we acknowledge the possibility that our findings could be due to chance, but because of the probing nature of this study, one might reserve judgements on these two antigens until a future study involving only HL–A1 and W18 is undertaken as suggested by SVEJGAARD *et al.* (1974).

It was also found that neither of these antigens were abnormally distributed within the three geographic regions. Thus, if these differences are real, no reason was found to attribute the differences to geographic location.

Approximately a dozen diseases show consistent and significant associations with HL–A antigens (SVEJGAARD *et al.*, 1975). In essentially all previously established associations with disease, there is an increased frequency of one or more antigens as compared to controls. An increased frequency in a disease group indicates that the alleles confer susceptibility to disease. We interpret the increased frequency of the HL–A1 and W18 alleles in the miner group without evidence of CWP as indicating resistance to development of pneumoconiosis. It should be noted that with the possible exception of W18 in the complicated CWP group, the antigen frequencies are essentially those expected for normal Caucasians. Thus, it is the group without disease which appears to be different. For this reason, larger numbers of subjects in the CWP groups would be needed relative to the control group. The data from simple and complicated CWP can be pooled since they do not differ significantly. When the relative risks are calculated on this basis the relative risk factor for HL–A1 is 0.50 and for W18 is 0.43. Expressed differently this indicates that the risk of CWP is 100% and 133% less in miners who carry HL–A1 or W18. This magnitude of relative "resistance" to CWP is approximately the same as the increased susceptibility for insulin-dependent diabetes associated with HL–A8 (SVEJGAARD *et al.*, 1975). By comparison, the classical association between blood group 0 and duodenal ulcer confers a relative risk of 1.33 (an ncreased risk of 33%) for blood group 0 individuals compared to non-0 individuals iVOGEL and HELMBOLD, 1972).

The question as to whether resistance to CWP is dominant or recessive could be approached if the HL–A antigens themselves were responsible for the association in which case it could be determined whether the relative resistance is the same for heterozygotes as for homozygotes. However, if the HL–A antigens are merely passive

markers for associated disease-resistance genes then the situation is more complicated. If the reasonable assumption is made that the unknown alleles at the LA and FOUR loci are infrequent, then there is little error in regarding individuals carrying only one antigen of the LA and FOUR series as homozygotes. In this regard, there was not an increased number of two or three antigen phenotypes in the CWP free group of miners as compared to the CWP groups. This may suggest that resistance is a dominant character.

Most of the established HL–A associations with disease involve antigens of the FOUR (B), C or D loci. The accepted explanation is that the associations between FOUR antigens and disease involve immune response (Ir) genes rather than HL–A antigens themselves. This explanation assumes that certain FOUR-locus genes and Ir genes are in linkage disequilibrium. Since the antigens implicated in the present study (HL–A1 and W18) belong to the LA and FOUR series, respectively, the evidence neither supports nor detracts from the Ir gene explanation. However, the strongest association is with W18, a second locus antigen. The hypothetical Ir gene involved in resistance to CWP could be involved with the way in which coal dust is handled by the body.

The question as to whether the apparent association between resistance to CWP is due to a particular haplotype (HL–A1, W18) cannot be answered since family studies have not been carried out.

Recently, MERCHANT *et al.* (1975) reported a significant increase in the frequency of antigen W27 in a group of asbestos workers with definite or suspected asbestosis. An association between W27 and severe asbestosis was also suggested. Despite some similarity between CWP and asbestosis these initial HL–A studies are not in agreement in regard to which, if any, HL–A antigens are definitely associated with pneumoconiosis.

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DISCUSSION

R. THOMAS: Have you looked at rosette formation in T cells in these exposed cases?

Dr MAJOR: No, we have not. The collection and processing of blood specimens taken from within the eastern parts of Pennsylvania was encumbered logistically and from a technical standpoint we had the minimum number of cells required for HL-A typing. Western Pennsylvania was less of a problem making re-collection of blood for rosette testing a possibility, yet this would exclude the high-risk anthracite areas so I am not sure this would be worthwhile. We have tentative plans to continue this study in the western United States in areas of low incidence of CWP. Perhaps T cell rosette formations could be addressed as a part of this proposed work which could provide information on the status of the delayed response in exposed cases.

D. LIDDELL: (i) One interpretation of the "relative risks" for HL–A1 and W18 is that there was a shortfall of these types of antigen in complicated CWP. However, the frequencies of these types were high in "normal" cases, i.e. where no disease was found. Is there an explanation?

(ii) If the frequencies of ALBERT *et al.* (1972) are to be expected in your selection (*not* population) of miners, should they not arise without disease? Surely not averaged over your complete selection? Are the observed differences in the non-diseased real or artefactual?

Dr MAJOR: (i) I attempted to explain that we felt that the "higher" frequency level for the W18 antigen which was observed in the "no disease" group could possibly be related to an innate constitutional ability by this group to resist the development of disease when exposed to a compromising dust burden. We found that the frequency of this antigen in the "simple" disease category reflected almost exactly those frequencies observed in the general population and in our overall combined population, which makes interpretation difficult. The frequency for the W18 antigen which occurred in the "complicated" category (approximately one-half the normal value) could indicate that the diminished presence of this antigen must be linked to a susceptible posture for the development of progressive massive fibrosis.

(ii) We had no idea before our data were analyzed that our "no disease" or control group would not be characterized by the expected HL-A frequencies reported by ALBERT, *et al.* (1972) unless ethnic statifications were demonstrated within the population studied. The "no disease" group was standardized with the other disease groups by age and longevity of employment and were therefore assumed to have been exposed to comparable dust burdens. I really cannot comment beyond this particular point.

G. BERRY: Presumably there was no *a priori* interest in the W18 antigen as compared with the other twenty-two antigens, it was simply that W18 gave the most significant difference between the three groups? If the fact that W18 was the *most* significant of twenty-three possibilities were taken into account when carrying out the significance test, would differences between the groups still be significant for this antigen?

Dr MAJOR: We have examined 23 antigens and perhaps we should have divided our 0.05 probability level by that number before proclaiming our rejection of the null hypothesis (at least that has been suggested). Such a procedure would technically preclude in multiple testing the recognition of frequency differences which occur by chance alone. However, if we adopted this more conservative approach, we might well be missing differences that are actually there and so we decided to adopt a less conservative procedure in a preliminary study and lay ourselves open to the possibility of error in order to avoid excluding any possible differences at this time.

L. MAGOS: Your conclusions are based on a static study which shows that, in groups of workers with pneumoconiosis, two antigens were less frequent than in miners without pneumoconiosis. However, a definite answer can be obtained only by a dynamic study which starts when the individual enters the mine for the first time. Supposing that the presence of the two antigens HL–A and W18 predisposed the miners to bronchitis, those who had these antigens might have left the mine before the development of CWP. In this case without the suggested relationship the frequency of HL–A and W18 must be lower in the group of workers with pneumoconiosis than in the control group.

Dr MAJOR: That is true in this situation if the HL–A antigens mentioned rendered miners more susceptible to bronchitis and as a result they elected to change their job to one where there was no dust at all (within or without the industry). As a result, one might indeed expect to observe smaller numbers of miners with these antigens in the group of progressive pneumoconiosis. However, this supposition does not explain the higher than expected frequencies of these antigens in the "no disease" category which is an observation in this report.

M. LIPPMANN: Do you have any data on exposure levels to support the assumption that the three groups had equivalent dust exposures? Years of mining are, at best, a very rough index of cumulative dust exposure. Furthermore, even with the same exposures, variations in pulmonary deposition and clearance could easily account for large differences in dust retention even when exposures are similar.

Thus, factors not considered in this study may well have much greater influences on the development of simple CWP or PMF.

Dr MAJOR: The clinical and technical information for this study was taken from our data base of miners examined in the first and second round of the U.S. National Coal Study. Participation of miners was on a volunteer basis which was the best we could achieve.

Dr MORGAN: Regarding coal dust exposure levels it is extraordinarily difficult, if not impossible, to select two groups of miners, one with PMF and the other with no pneumoconiosis, both of which have had comparable dust exposures.

M. LIPPMANN: I do not agree with that.

Dr MORGAN: Are you implying that miners with normal films have had the same dust exposure as men with categories 2 and 3 simple CWP. In general this would seem unlikely.

M. LIPPMANN: I would agree that there is a statistical association and that you are correct if that is all you are inferring, but in view of the variability in deposition and clearance for the same aerosol, there certainly could be an order of magnitude difference in dust retention in these miners and that is a different factor from the one I raised earlier as to whether the dust level was ten times higher for instance in one mine than another.

Dr MORGAN: We know from short term studies done by the U.S. bureau of Mines that dust levels were ten times higher in certain mines than in others; but I am beginning to see what you are suggesting—namely, individual susceptibility. This is a most difficult matter to deal with. There were no long term dust measurements made in the U.S. prior to 1969 and, therefore, it would be quite impossible to devise a study in which the groups were matched for dust exposure. Finally, I think it needs to be borne in mind that dust exposure, however much, will not affect one's tissue type.

D. C. F. MUIR: I cannot agree with Dr Morgan that years of underground exposure is an adequate measure of dust exposure.

I would like to ask Dr Major whether there was any evidence of consanguinity between members of the sub-groups in the population.

Dr MAJOR: We excluded all siblings and other relatives of miners participating in the study.

J. A. DICK: As the prevalence of PMF varies from coalfield to coalfield, can you explain why the levels of W18 should also vary? And is it possible that PMF causes the absence of W18?

Dr MAJOR: I would not concur that PMF causes the absence of W18 as I am under the impression that the W18 antigen should be with one from womb to tomb. Why does the prevalence of PMF vary from coalfield to coalfield? I do not know but I would suggest that this consideration should not influence our data as we circumvented this problem by selecting miners on a volunteer basis from all coalfields studied according to their diagnosed disease category, that is all regions of study and disease category, were balanced by equal numbers of individuals.

J. S. MCLINTOCK: There certainly are differences in the prevalence of PMF in different coalfields in Great Britain (and also in the U.S.A.), but when we looked at the attack rates of PMF and standardised for prevalence in each coalfield (see *Inhaled Particles III*, p. 933), we found the attack rate to be very close in almost all coalfields.

M. D. ATTFIELD: In the light of the statistical significance of your findings, will you be studying a further group of men to see if the two associations still remain significant?

Dr MAJOR: We have tentative plans to continue this study in southern West Virginia which will extend our work from Pennsylvania and northern West Virginia. We also wish to look at this same problem in miners from areas of low prevalence in the western United States such as in Utah and Colorado. As far as expanding our numbers of participants to any great extent we are limited by logistic and technical problems such as dealing with the wide dispersion of miner's residences in the hill and mountain areas and the transport of specimens from the field to the laboratory.

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IV

(IN TWO PARTS)

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