Preliminary Communications

Leucocyte Antigens and Disease: 1. Association of HL-A2 and Chronic Glomerulonephritis

British Medical Journal, 1969, 2, 424–426

Summary: The HL-A2 leucocyte antigen was found to be present in a significantly higher percentage of 485 patients with chronic glomerulonephritis than in 428 normal control subjects. No correlation could be shown between any of the ABO groups and chronic glomerulonephritis. There was no statistically significant difference in the frequency of HL-A2 when the controls were compared with 280 patients with a variety of other diseases. The trend toward an excess of HL-A2 positives among patients with glomerulonephritis was also seen in a study of 108 sibships, though the difference fell short of statistical significance. It was concluded that an HL-A2 positive person was about 1·5 times as likely to develop chronic glomerulonephritis as an HL-A2 negative person.

Introduction

A statistical association between the red cell ABO antigens and certain disorders of the gastrointestinal tract (Aird *et al.*, 1954) and other body systems (Roberts, 1957) is now generally accepted. It is of some relevance, therefore, to inquire whether a similar association exists between certain diseases and the antigens of the white cell series. This question is particularly pertinent in view of the advances made in the serological typing of leucocytes in the past few years (see Curtoni *et al.*, 1967). Methods of typing have become more or less uniform, and several discrete antigenic specificities are now recognized by a system of international nomenclature (HL-A system) (World Health Organization Nomenclature Committee, 1968). The genetic determinants of these HL-A antigens appear to constitute a highly complex chromosomal region (Ceppellini *et al.*, 1967) with considerable haplotype differences among different ethnic groups. During the past four years leucocytes of over 6,000 subjects have been typed in this laboratory principally in connexion with kidney transplantation. The incidence of HL-A2 leucocyte antigen was noted to be higher among patients with chronic glomerulonephritis than among normal controls. The higher incidence has persisted over the four years of testing, and appears to be of statistical significance in this preliminary study.

Materials and Methods

The 485 patients diagnosed as having chronic glomerulonephritis by their referring physicians and 708 control subjects included in this study were of Caucasian origin and came from five geographically scattered transplant centres in North America. The control subjects comprised two groups. The first group of 428 subjects were either healthy kidney donors not related to the affected patients or cadaver donors with head injury. None were considered to have any evidence of underlying kidney disease. The second group of controls consisted of 280 patients with diseases other than that of the kidney. They included: congenital biliary atresia and other liver diseases in 76, rheumatic and ischaemic heart disease in 164, primary intracranial tumours in 31, and miscellaneous diseases in 9. The sib data consisted of 236 children from 108 families—100 with two, four with three, and two with four children each, and two with seven and nine children respectively. In each family only one child had glomerulonephritis, the others acting as controls. All subjects were typed in the four-year period between 1964 and 1968 by using the lymphocytotoxicity technique previously described (Terasaki *et al.*, 1967). Though they were typed for several leucocyte antigens, the subjects were classified in this study only according to whether they had the HL-A2 antigen or not. During the four-year interval HL-A2 was the most easily defined of the leucocyte antigens, 10 to 20 antisera of HL-A2 specificity being routinely used. Erroneous typing would therefore have been the least likely in typing for this antigen.

Results

Red cell ABO types were available for 445 patients with chronic glomerulonephritis and 409 normal controls. The percentage distribution of the ABO groups was as follows (the first figure applies to patients with glomerulonephritis): Type O 40·9%, 41·6%; Type A 45·6%, 44·9%; Type B 10·6%, 10·5%; and Type AB 3·2%, 2·9%. The difference in frequencies between the patients and controls for each of the ABO groups was not statistically significant ($P=0·9$).

The result of correlating the presence or absence of the HL-A2 antigen with glomerulonephritis in the general population is shown in Table I. Among the nephritic subjects, 252 of the 485 (52%) were positive for the HL-A2, whereas only 181 of the 428 (42·3%) controls were positive for the antigen. This 10% difference was statistically highly significant ($P=0·005$). The 6% difference in the frequency of HL-A2 between patients with glomerulonephritis and 280 patients with other diseases when considered as controls was not statistically significant. Patients with diseases other than chronic glomerulonephritis had an HL-A2 frequency of 46·4%, which was not significantly different from the normal controls ($P=0·25$). Likewise, the frequency of HL-A2 among patients with biliary atresia and other liver diseases (46·1%) and rheumatic and ischaemic heart diseases (49·4%) did not differ significantly from the normal controls, though the number of patients in each category of diseases was small.

TABLE I.—*Distribution of the HL-A2 Leucocyte Antigen Among Patients with Chronic Glomerulonephritis and Controls in the General Population*

Category		Total No.	% HL-A2+	χ^2*	P
Chronic glomerulonephritis		485	52	8·14	0·005
Normal controls		428	42·3	—	—
Other diseases	All patients	280	46·4	1·02	0·25
	Liver disease	76	46·1	0·24	0·65
	Heart disease	164	49·4	2·14	0·15

*χ^2 calculated in comparing each with controls.

A more critical analysis of the association of HL-A2 with glomerulonephritis was done by an examination of 108 sibships. In the 100 paired sibs in which only two sibs from a family had been tested, the affected and healthy sibs both had HL-A2 in 36 pairs and both lacked the antigen in 35 pairs (Table II). Of the remaining pairs 29 were informative in that one member of the pair was HL-A2 positive and the other HL-A2 negative. In 19 pairs of sibs only the affected sib had the antigen, whereas in 10 only the normal sib was HL-A2 positive. Sibships from which more than two sibs were tested are listed in Table III.

The significance of the different distribution of HL-A2 antigen among patients and control sibs was assessed by association analysis similar to that used by Clarke *et al.* (1956) in their study of ABO groups and duodenal ulcer (see Appendix for method). Of the 108 sibships only 35 were informative for analysis of association from within family comparisons. The

TABLE II.—*Distribution of the HL-A2 Antigen Among 100 Pairs of Sibs*

Patient	+	+	−	−	Total
Sib	+	−	+	−	
No.	36	19	10	35	100

TABLE III.—*Distribution of the HL-A2 Antigen Among Sibs in Eight Families with Three or More Children*

No. of Sibs*	Patient	Sibs	
		+	−
3	−	2	1
3	−	0	3
3	−	1	2
3	+	2	1
4	+	4	0
4	+	3	1
7	−	6	1
9	+	4	5

*Including patient.

analysis did not indicate a statistically significant association ($S=8{\cdot}96$, $\chi^2=2{\cdot}29$, $P=0{\cdot}15$). In view of the small number of sibships it is not surprising that an association failed to be asserted. If consideration were restricted to families with two sibs that were unalike for HL-A2, about 100 families would be required for an even chance of establishing a significant association. This conclusion is based on an analysis of the relative likelihood of acquiring glomerulonephritis depending on the presence of HL-A2 antigen. The indication from Table I is that an HL-A2 positive person is about 1·5 times as likely to acquire glomerulonephritis as an HL-A2 negative person. The value of the ratio developed from all the data in 100 sibships in Table II, 1·67, is quite consistent with that from Table I, as is the ratio $19/10=1{\cdot}9$ based on sibs not alike for HL-A2. If the value of 1·5 is assumed for the ratio, then the HL-A2 positive sib will have glomerulonephritis in approximately 60% of HL-A2 unalike sib pairs, while the HL-A2 negative sib will have the disease in 40% of the pairs. About 100 such sib pairs would be needed for an even chance of distinguishing this from the 50-50 split obtained if there were no association.

Discussion

This study has indicated the existence of a possible association between the HL-A2 leucocyte antigen and chronic glomerulonephritis. The association is fairly strong at a population level, and the general tendency for an excess of HL-A2 positive persons among nephritics is also reflected in the limited sibship data. If these results are borne out by further work, it may have important implications in the aetiology of chronic glomerulonephritis and in the genetic polymorphism at the HL-A locus.

As noted by Fraser Roberts (1957), certain pitfalls have plagued the studies of association of ABO groups and disease. The present study cannot be considered to be free from these defects:

Numbers.—In order to show a statistically significant correlation between the ABO groups and disease large numbers of patients and controls are essential. Figures in the range of several thousands rather than hundreds have been suggested. The number of patients (408) and controls (728) utilized in the present study is considerably short of that regarded as optimal for demonstration of a statistical association, though as noted by Fraser Roberts (1957) the association of peptic ulcer with group O was already noticeable with only 172 patients in Buchanan and Higley's (1921) early work.

Technical Errors.—Of particular relevance to the present study is the question of whether or not technical errors in typing leucocytes could have contributed to the results. Erroneous typing was particularly likely during the first and second year of the four-year period of study when the overall error rate may have been high. All during this period, however, HL-A2 was the one antigen which stood out as particularly easy to type, and several operationally monospecific anti-HL-A2 antisera were available. The consistency of typing for HL-A2 during the four-year period of study was assessed by dividing the patients and controls into three equal groups according to the time they were typed. In all three groups a persistent and consistent excess of HL-A2 positives of 8–10% was observed among patients with chronic glomerulonephritis when compared with normal persons tested in the same time period. The frequency of HL-A2 antigen in the earliest typed patients and their controls was 52·3 and 42·1% respectively, compared with 49·7 and 40·2% in those typed most recently. It should also be noted that precise agreement in typing for the HL-A2 antigen was noted when 100 persons were typed independently by our laboratory and six other leucocyte typing laboratories (see Curtoni *et al.*, 1967).

Racial Stratification.—An important objection that was raised when studying the ABO groups and disease was the possibility that patients and controls might not be drawn from an entirely corresponding population. Though such a possibility cannot be excluded, racial statification might be less likely in the present study in view of the mixed nature of the North American Caucasian population. As noted by Clarke *et al.* (1956), environmental and genetic variables are reduced by a study of sibships in which the unaffected sib acts as a control. The fact that in the present study the excess of HL-A2 positives among sibs with glomerulonephritis fell short of statistical significance is not surprising in view of the small numbers. None the less, the evidence is sufficiently encouraging to be deemed worthy of further study.

Disease Classification.—Ideally, only well-defined and specific disease entities should be selected when associations are studied. Though the present study purports to include only cases of chronic glomerulonephritis, the accuracy of this diagnosis could be questioned because of the difficulties inherent in the differential diagnosis of end-stage renal disease. It is conceivable for patients diagnosed as having chronic glomerulonephritis to include some cases of other diseases, notably chronic pyelonephritis. On the other hand, readily diagnosable conditions such as polycystic disease could be excluded with a reasonable degree of certainty.

In spite of these pitfalls, which cannot be entirely discounted, the findings of the present study are not without certain interest. The leucocyte group HL-A6 was shown to be possibly associated with Hodgkin's disease in a study involving 41 patients (Amiel, 1967). No statistically significant association could be found for chronic lymphocytic leukaemia and HL-A antigens (Kourlisky *et al.*, 1968). Though the association between HL-A2 and chronic glomerulonephritis cannot be regarded as definitely established at this early stage, the evidence presented is strong enough to open up future avenues of research in which other HL-A antigens could profitably be studied for association with a variety of other diseases.

Appendix: Method of Analysis of Sibship Data

In our analyses all family groups are treated on an equal basis by scoring each family so as to have variance = 1 independently of the number of sibs. The score used was:

$$S=\frac{NX-AB}{\sqrt{\frac{A(N-A)\,B(N-B)}{N-1}}}$$

where N is the total number of sibs, A is the number with glomerulonephritis, B is the number with the HL-A2 antigen, and X is the number with both glomerulonephritis and HL-A2 antigen. Families for which all sibs are alike either for the HL-A2 antigen or for the presence of glomerulonephritis were not scored. The scores are added and the $(\text{Total})^2/M$ (M= number of families scored) is approximately chi-square with one degree of freedom if there is no association.

This investigation was supported in part by Research Grants AM 02375, AM 07513, and AI 04444 from the National Institute of Health, United States Public Health Service, and Contract PH 43 65 994 from the National Institute of Allergy and Infectious Diseases. Computing assistance was obtained from the Health Sciences Computing Facility, UCLA, sponsored by NIH Grant FR-3.

R. PATEL,* M.R.C.P., M.R.A.C.P.,
MAX R. MICKEY, PH.D.,
PAUL I. TERASAKI, PH.D.,
Department of Surgery and the Department of Biomathematics, School of Medicine, University of California, Los Angeles.

* Present address: Division of Artificial Organs, Cleveland Clinic Foundation, Cleveland, Ohio.

REFERENCES

Aird, I., Bentall, H. H., Mehigan, J. A., and Roberts, J. A. F. (1954). *British Medical Journal*, **2**, 315.

Amiel, J. L. (1967). In *Histocompatibility Testing*, p. 79, edited by E. S. Curtoni, P. L. Mattiuz, and R. M. Tosi. Copenhagen, Munksgaard.

Buchanan, J. A., and Higley, E. T. (1921). *British Journal of Experimental Pathology*, **2**, 247.

Ceppellini, R., Curtoni, E. S., Mattiuz, P. L., Miggiano, V., Scudeller, G., and Serra, A. (1967). In *Histocompatibility Testing*, p. 149, edited by E. S. Curtoni, P. L. Mattiuz, and R. M. Tosi. Copenhagen, Munksgaard.

Clarke, C. A., Edwards, J. W., Haddock, D. R. W., Howel-Evans, A. W., McConnell, R. B., and Sheppard, P. M. (1956). *British Medical Journal*, **2**, 725.

Curtoni, E. S., Mattiuz, P. L., and Tosi, R. M. (editors) (1967). *Histocompatibility Testing*. Copenhagen, Munksgaard.

Kourlisky, F. M., Dausset, J., Feingold, N., Dupuy, J. M., and Bernard, J. (1968). In *Advance in Transplantation*, p. 515, edited by J. Dausset, H. Hamburger, and G. Mathe. Copenhagen, Munksgaard.

Roberts, J. A. F. (1957). *British Journal of Preventive and Social Medicine*, **11**, 107.

Terasaki, P. I., Vredevoe, D. L., and Mickey, M. R. (1967). *Transplantation*, **5**, Suppl. 1057.

World Health Organization Nomenclature Committee (1968). *Bulletin of the World Health Organization*, **39**, 483.

Medical Memoranda

Rare Complication of Tracheostomy: Erosion of Innominate Artery

British Medical Journal, 1969, 2, 426

During the past 20 years there has been a remarkable change in the whole concept of tracheostomy, and the advent of positive-pressure ventilators has revolutionized the treatment of all forms of ventilatory insufficiency. However, tracheostomy should not be regarded as a "harmless" procedure (Watts, 1963). Erosion of the innominate artery is a rare complication. Three fatal cases due to erosion of the innominate artery have been reported by Lunding (1964) and one death on the 77th day by Stiles (1965). Davis and Southwick (1956) described two cases of erosion of an anomalous innominate artery.

A case of tracheostomy in which death was due to erosion of the innominate artery on the 15th postoperative day is described.

CASE REPORT

A man aged 20 was admitted to hospital as a medical emergency on 3 November 1968. He complained of a cough, difficulty in swallowing, and paraesthesia in both hands. Thirty-six hours previously he had experienced difficulty in focusing his right eye and had noticed numbness of the face on both sides. A diagnosis of "viral encephalitis" was made. On 8 November the E.N.T. unit was contacted on account of respiratory failure. Tracheostomy was performed under general anaesthesia with endotracheal intubation. A 13-mm. cuffed Portex tracheostomy tube was inserted and connected to a Bennett respirator for intermittent positive-pressure respiration. He was making a slow recovery, but on 23 November, 15 days postoperatively, there was massive haemorrhage through the tracheostomy and 4 to 5 pints (2·3 to 2·8 litres) of blood was sucked out from the tracheobronchial tree. In spite of all efforts he collapsed and died.

Post-mortem Examination.—A plastic cuffed tracheostomy tube is present in the trachea. The cuff of the tube has eroded through the posterolateral wall of the trachea on the right side 3 cm. above the carina. The trachea at the site of the cuff shows marked inflammatory changes and is abnormally dilated. Both lungs show widespread bronchopneumonic changes with blood in the bronchial tubes. An erosion, size about 1 by 1 cm., is present in the innominate artery. Microscopical examination of the brain stem and spinal cord show considerable anterior horn cell damage. Appearance suggests poliomyelitis.

In this case the cause of tracheal perforation is not clear. Care was taken to deflate the cuff of the tube regularly every four hours and not to overinflate it.

I wish to thank Dr. G. Watkinson, Mr. Charles Smith, and Dr. J. Kinnell for permission and guidance in writing this case report. I am indebted to Dr. D. MacKinnon and Dr. G. A. C. Summers, of the Department of Pathology, County Hospital, for their helpful comments.

P. N. PATHAK, F.R.C.S.ED., D.L.O.,
E.N.T. Registrar, Fulford Hospital, Fulford, York.

REFERENCES

Davis, J. B., and Southwick, H. W. (1956). *Annals of Surgery*, **144**, 893.
Lunding, M. (1964). *Acta Anaesthesiologica Scandinavica*, **8**, 181.
Stiles, P. J. (1965). *Thorax*, **20**, 517.
Watts, J. McK. (1963). *British Journal of Surgery*, **50**, 954.