HLA ANTIGEN FREQUENCY OF NORTHERN IRELAND BLOOD DONORS

by

D. MIDDLETON
Transplantation and Clinical Immunology, Belfast City Hospital

J. MARTIN *
Transplantation and Clinical Immunology, Belfast City Hospital

HISTOCOMPATIBILITY (HLA) typing is primarily used to match kidney donors with awaiting recipients. However many studies have been carried out to determine the frequency of the HLA antigens in various diseases.

In this laboratory we have been requested to carry out such studies in several diseases, e.g. diabetes, ankylosing spondylitis, farmer’s lung, Plummer-Vinson stricture and flax byssinosis. In order to compare the antigen frequencies in these diseases with those of a normal healthy population we decided to type 200 blood donors. Obviously there are drawbacks to using blood donors as controls as their health is better than average and their age is not of either extreme. However, using blood donors provides a simple way to obtain the blood sample required for our testing and also gives a cross-section of social status within the population. One interesting and very important offshoot of this study is that we have 200 tissue-typed blood donors who could, if the need occurred, be used for platelet transfusion if their type was identical to that of a patient requiring platelets.

MATERIALS AND METHODS

Two hundred blood donors (125 male, 75 female) born in Northern Ireland were tissue typed for HLA–A and –B locus antigens. The number of blood donors from each county was proportional to the total population of that county. An extra 10 ml of blood from suitable donors was taken at the donor sessions into sodium citrate. The blood donors were typed within one day of bleeding.

A total of 72 Sera defining the following specificities were used:

- HLA – A1, A2, A3, A9, A10, A11, A28, A29
- HLA – B5, B7, B8, B12, B13, B14, B15, B17, B18, BW22, B27, BW35, B.40

The sera were either supplied by the National Tissue Typing Reference Laboratory, Bristol, or were sera from our own laboratory which have been tested for their activity both here and in Bristol. Typing was by a two stage

* Supported by a grant from the Northern Ireland Kidney Research Fund.
microlymphocytotoxicity test at 22°C (Nelson and Middleton 1975) and at least 3 sera were used to define each specificity.

The antigen frequency of the Northern Ireland blood donors was compared with the antigen frequency in 1,036 blood donors from Bristol, in 342 blood donors from Glasgow and in 453 blood donors from Eire using a Yates' corrected chi-squared analysis, with a correction for the number of antigens tested (Grumet et al, 1971).

The gene frequency of each of the antigens in the Northern Ireland blood donors was calculated using the formula: \[ p = 1 - (\sqrt{1 - f}) \] where \( p \) denotes the gene frequency and \( f \) the frequency of the corresponding antigen.

The tissue types of the Northern Ireland blood donors were analysed to find out if there were any differences in the antigen frequency due to sex, age or place of birth. In the latter analysis only blood donors born in Co. Down (n=43), Co. Antrim (n=48) and Belfast (n=42) were considered, as the number of blood donors born in Counties Derry, Tyrone, Armagh and Fermanagh were considered too small for statistical analysis. For the purpose of assessing the effect of age on HLA antigen frequency we divided the blood donors into two groups, 119 aged 16-35 and 81 aged 36-65.

RESULTS

Table 1 shows the frequency of HLA antigens in blood donors from Northern Ireland compared with the antigen frequency in blood donors from Bristol, Glasgow and Eire. Statistical differences at the 5 per cent level were found in the following antigens HLA - A1,-B7,-B8,-B14 and -B18 with Bristol, -A11 with Glasgow and -B12 with Dublin. Only one difference, HLA -B14 in the comparison with Bristol blood donors, is still significant after multiplying the initial \( P \) value by a factor off 21, the number of comparisons made between the two populations.
### Table 1

**Frequency of HLA antigens in blood donors from Northern Ireland, Bristol, Glasgow and Eire**

| HLA Antigen | N. Ireland Bl. Donors (n=200) | Bristol Bl. Donors (n=1036) | Glasgow Bl. Donors (n=342) | Eire Bl. Donors (n=453) |
|-------------|-------------------------------|-----------------------------|--------------------------|------------------------|
|             | No | %     | No | %     | No | %     | No | %     |
| 1           | 86 | 43    | 348 | 33.6a | 138 | 40.4  | 210 | 46.4  |
| 2           | 91 | 45.5  | 536 | 51.7  | 163 | 47.7  | 217 | 47.9  |
| 3           | 53 | 26.5  | 265 | 25.6  | 78  | 22.8  | 97  | 21.4  |
| 9           | 31 | 15.5  | 190 | 18.3  | 60  | 17.5  | 61  | 13.5  |
| 10          | 20 | 10    | 87  | 8.4    | 21  | 6.1   | 35  | 7.7   |
| 11          | 28 | 14    | 106 | 10.2   | 26  | 7.6f  | 55  | 12.1  |
| 28          | 10 | 5     | 38  | 3.7    | 31  | 9.1   | 34  | 7.5   |
| 29          | 16 | 8     | 78  | 7.5    | 16  | 4.7   | 41  | 9.1   |
| 5           | 13 | 6.5   | 87  | 8.4    | 21  | 6.1   | 26  | 5.7   |
| 7           | 67 | 33.5  | 265 | 25.6b  | 93  | 27.2  | 124 | 27.4  |
| 8           | 69 | 34.5  | 260 | 25.1c  | 105 | 30.7  | 156 | 34.4  |
| 12          | 54 | 27    | 254 | 23.7   | 114 | 33.3  | 163 | 36.9  |
| 13          | 5  | 2.5   | 45  | 4.3    | 15  | 4.4   | 9   | 2     |
| 14          | 30 | 15    | 72  | 7.2d   | 34  | 9.9   | 47  | 10.4  |
| 15          | 9  | 4.5   | 50  | 4.8    | 29  | 8.5   | 31  | 6.8   |
| 17          | 18 | 9     | 79  | 7.6    | 26  | 7.6   | 46  | 10.2  |
| 18          | 16 | 8     | 35  | 3.4e   | 19  | 5.6   | 34  | 7.5   |
| 22          | 8  | 4     | 55  | 5.3    | 13  | 3.8   | 16  | 3.5   |
| 27          | 11 | 5.5   | 77  | 7.4    | 27  | 7.9   | 26  | 5.7   |
| 35          | 16 | 8     | 114 | 11     | 29  | 8.5   | 54  | 11.9  |
| 40          | 20 | 10    | 116 | 11.2   | 30  | 8.8   | 39  | 8.6   |

Listed below are the chi-squared values greater than 4 when the HLA antigen frequency of Northern Ireland donors is compared with that of Bristol, Glasgow and Eire. P values in brackets have been corrected for the number of antigen frequencies compared.

- a. $\chi^2 = 6.108$  \hspace{1cm} $p = 0.0129$ (0.27)
- b. $\chi^2 = 4.958$  \hspace{1cm} $p = 0.0246$ (0.516)
- c. $\chi^2 = 7.115$  \hspace{1cm} $p = 0.0075$ (0.158)
- d. $\chi^2 = 13.305$  \hspace{1cm} $p = 0.0004$ (0.0084)
- e. $\chi^2 = 7.921$  \hspace{1cm} $p = 0.0050$ (0.105)
- f. $\chi^2 = 5.067$  \hspace{1cm} $p = 0.0231$ (0.485)
- g. $\chi^2 = 4.648$  \hspace{1cm} $p = 0.0294$ (0.617)
In Table 2, which shows the gene frequencies of HLA antigens in Northern Ireland blood donors, $X_1$ and $X_2$ indicate the gene frequencies of antigens which we have not been able to detect.

No significant difference in antigen frequency was found between male and female donors. The frequency of HLA -A10 differs significantly with the age of donor. Seventeen blood donors (14.3 per cent) aged 16-35 were HLA -A10 positive compared to three blood donors (3.7 per cent) aged 36-65 ($p=0.0257$). There were seven blood donors from Co. Antrim (14.6 per cent) who were HLA -A10 positive whereas all of the Belfast donors were HA -A10 negative ($p=0.0275$). However these differences are no longer significant after correction for the number of comparisons made. Detailed tables of the analysis of the Northern Ireland blood donors on the basis of place of birth, age and sex are available from the authors.

### Table 2

**Gene frequencies of HLA antigens in 200 Northern Ireland Blood donors**

| HLA Antigen | Gene frequency |
|-------------|----------------|
| A1          | .2450          |
| A2          | .2618          |
| A3          | .1427          |
| A9          | .0808          |
| A10         | .0513          |
| A11         | .0726          |
| A28         | .0253          |
| A29         | .0408          |
| $X_1$       | 0.797          |
|             | 1.0000         |
| B5          | .0330          |
| B7          | .1845          |
| B8          | .1907          |
| B12         | .1456          |
| B13         | .0126          |
| B14         | .0780          |
| B15         | .0228          |
| B17         | .0461          |
| B18         | .0408          |
| BW22        | .0202          |
| B27         | .0279          |
| BW35        | .0408          |
| B40         | .0513          |
| $X_2$       | 1.057          |
|             | 1.0000         |
DISCUSSION

We have compared the frequency of 21 HLA antigens in Northern Ireland blood donors with that of blood donors from Bristol, Glasgow and Eire. In the latter two comparisons only one antigen frequency in each, HLA -A11 and -B12 respectively, was significant at the 5 per cent level. By definition, out of 20 comparisons, on the average, we would expect one of them to be significant at the 5 per cent level. When we multiplied the initial P value for the two significant comparisons by a factor of 21 (the number of comparisons made) we found that in neither case was it still significant. However in the comparison of HLA antigen frequency in donors from Northern Ireland with those of Bristol we found that five antigen frequencies HLA -A1,B7,-B8,-B14 and -B18 differed significantly. One of these, HLA -B14, was still significant after correction for the number of comparisons made. These differences are unlikely to be due to the sera which have been well defined both here and at the National Tissue Typing Reference Laboratory, Bristol. Our number of blood donors tested (200) is much smaller than the number tested in Bristol (1,036) and this could be one of the reasons for the difference in frequencies between the two centres.

Our finding that a blood donor population from Northern Ireland has a very similar HLA antigen frequency to a blood donor population from Glasgow and from Eire but varies significantly in five antigens from a Bristol blood donor population, agrees with the ABO distribution of blood donors from these areas (see Table 3). Also, although the population of Northern Ireland at the moment is stable it is made up of two distinct origins – the Irish Gaelic and the Ulster Scot.

Our results show the necessity of using people born in Northern Ireland as a control group when examining the HLA antigen frequency of people with a particular disease. For example, to have used the Bristol blood donor HLA frequency as controls in our study on patients with a Plummer-Vinson stricture would have led us to believe that HLA -B8 was significantly more frequent in the patients than in the controls, whereas this is not the case when we use controls from Northern Ireland (Middleton et al. 1978).

| Area         | Group O | Group A | Group B | Group AB |
|--------------|---------|---------|---------|----------|
| N. Ireland* | 54.74   | 32.97   | 9.65    | 2.63     |
| Bristol*     | 43.54   | 45.22   | 8.37    | 2.86     |
| Glasgow*     | 53.08   | 32.90   | 10.87   | 3.15     |
| Eire†        | 55.89   | 30.60   | 10.89   | 2.60     |

* Taken from Kopéc.
† Taken from Dawson.
We have compared the variation in HLA antigen frequency in two age groups. There are no significant differences after correction in the groups suggesting that although some HLA antigens are associated with disease these effects neutralise each other in the population. Previous findings have shown that older people have a larger number of HLA specificities than young people (Gerkin et al. 1974, Bender et al 1973). Like ourselves other recent studies have failed to confirm this (Albert et al. 1974, Murcurová et al. 1975). In our study the average number of HLA specificities per person below 36 was 3.309 and the average in the 36–65 age group was 3.387.

Requests to us for tissue typing relatives of prospective platelet recipients have recently increased. It is very difficult to obtain a full house match for these recipients from their relatives. Having a panel of potential platelet donors should make it easier to obtain a perfect match for these platelet recipients.

SUMMARY

Histocompatibility (HLA) antigen testing of 200 normal donors was performed to provide a baseline for studies on the HLA antigen frequency of patients with diseases. The results were compared with similar results from Bristol, Eire and Glasgow. The Belfast HLA antigen frequencies closely resembled the results from Eire and Glasgow but showed marked differences from Bristol. This finding corresponds to ABO blood group distribution. Our tissue typed blood donors can now be used as platelet donors if required.

ACKNOWLEDGEMENTS

We would like to thank Col. T. E. Field and Mr. K. McLaughlin of the Northern Ireland Blood Transfusion Service for obtaining the blood donor samples. The HLA antigen frequency of blood donors from Bristol, Eire and Glasgow were obtained by the kind co-operation of Dr. C. C. Entwistle, National Tissue Typing Reference Laboratory, Bristol, Mrs. S. Spencer, Tissue Typing Laboratory, Jervis Street Hospital, Dublin, and Mr. B. Creighton, Clinical Immunology Laboratory, Royal Infirmary, Glasgow.

REFERENCES

Albert, E. D., Mickey, M. R., Ting, A. and Terasaki, P. I. (1974). Z. Immun. – Allergie – Forsch., 147, 1.
Bender, K., Rutier, G., Mayerova, A. and Hiller, C. (1973). International Symposium on HLA reagents, ed. Ragsmey, R. H. and Sprack, J. V., Karger, Basel.
Dawson, G. W. P. (1964). Ann. Hum. Genet., 28, 49.
Gerkins, V. R., Ting, A., Menck, H. T., Casagrande, J. T., Terasaki, P. I., Pike, M. C. and Henderson, B. E. (1974). J. Nat. Cancer Inst., 52, 1909.
Grumet, F. C., Coukell, A., Bodmer, J. G., Bodmer, W. F. and McDevitt, H. O. (1971). New Engl. J. Med., 285, 4, 193.
Kopec, A. C. (1970). The Distribution of the Blood Groups in the United Kingdom. University Press, Oxford.
Middleton, D., Logan, J. S., Magennis, B. P. and Nelson, S. D. To be published.
Mucurova, H., Ivanyi, P., Sajdlova, H. and Trojan, J. (1975). Tissue Antigens, 6, 269.
Nelson, S. D. and Middleton, D., (1975). Ulster med. J., 44, 48.