Department of Cardiology, Royal Melbourne Hospital, Victoria 3050, Australia.

References

[1] Meltzer, L E, and Kitchell, J R, in *Textbook of Coronary Care*, ed L E Meltzer and A J Dunning, p 1. Amsterdam, Excerpta Medica, 1972.
[2] Meltzer, L E, in *Acute Myocardial Infarction*. ed D G Julian and M F Oliver. Edinburgh, Livingstone, 1971.
[3] Hofvendahl, S, *Acta Medica Scandinavia*, 1971, suppl No 519.
[4] MacMillan, R L, and Brown, K W G, *Canadian Medical Association Journal*, 1971, **105**, 1037.
[5] Martin, S P, *et al*, *Annals of Internal Medicine*, 1974, **81**, 289.
[6] *British Medical Journal*, 1975, **1**, 646.
[7] Rose, G, *British Journal of Preventive and Social Medicine*, 1975, **29**, 147.
[8] Mather, H G, *et al*, *British Medical Journal*, 1976, **2**, 925.
[9] Lindholm, J, *et al*, *American Heart Journal*, 1976, **91**, 673.
[10] Leftkovits, R, Hunt, D, and Sloman, J G, in preparation.
[11] Robinson, J S, Sloman, J G, and McRae, C, *Medical Journal of Australia*, 1964, **1**, 427.
[12] Luxton, M, *et al*, *Medical Journal of Australia*, 1975, **1**, 612.
[13] O'Rourke, M F, *et al*, *British Medical Journal*, 1976, **2**, 837.
[14] Szklo, M, *et al*, *Circulation*, 1976, **53** and **54**, suppl No 2, p 129.
[15] Sloman, J G, Stannard, M, and Goble, A J, *American Heart Journal*, 1968, **75**, 140.
[16] Reader, R, *Medical Journal of Australia*, 1972, **2**, Special suppl, p 3.
[17] Christie, D, *Medical Journal of Australia*, 1974, **1**, 390.
[18] Gordon, T, and Thom, T, *Preventive Medicine*, 1975, **4**, 115.
[19] Hunt, D, *et al*, *Australian and New Zealand Journal of Medicine*, 1976, **6**, 433.

(*Accepted 28 January 1977*)

HLA patterns in pernicious anaemia

BERTA UNGAR, J D MATHEWS, B D TAIT, D C COWLING

British Medical Journal, 1977, **1**, 798-800

Summary

The pattern of HLA antigens was studied in 127 patients with Addisonian pernicious anaemia. The pattern in the whole group of patients differed significantly from that in 586 controls. But different subgroups of the patients had different HLA antigens. Among 27 patients with anaemia associated with endocrine disease there was an increased frequency of HLA-B8, B18, and BW15. The remaining 100 patients, who did not have endocrine disease, showed increased frequencies of HLA-B7 and B12. The positive association with HLA-B12 among this subgroup was confined to 62 patients with severely impaired vitamin B_{12} absorption, including 13 patients with vitamin B_{12} neuromyelopathy, who had the highest frequencies of HLA-B7 and B12. The significant heterogeneity in HLA patterns in different clinical subgroups of these patients indicates genetic heterogeneity in pernicious anaemia and explains previous discrepancies in the associations between HLA antigens and pernicious anaemia.

Introduction

Genetic and autoimmune factors may contribute to the pathogenesis of pernicious anaemia, atrophic gastritis, thyroid disease, diabetes, and other autoimmune endocrine disorders.[1-5]

Pernicious anaemia has been associated with HLA-B7 or HLA-A3, or both,[6-9] though Eastmond and Woodrow found no particular association with any antigen.[10] Patients with pernicious anaemia and vitamin B_{12} neuromyelopathy may have an increase in the phenotype A2-B12,[11] while those with thyroid autoantibodies may have an increased frequency of B8.[12]

Royal Melbourne Hospital, Victoria, Australia, 3050
BERTA UNGAR, MD, FRACP, deputy director, department of haematology
J D MATHEWS, MD, NH&MRC fellow, university department of medicine
B D TAIT, PHD, senior scientist, tissue typing laboratory
D C COWLING, MD, FRACP, director, department of haematology

These findings are complex and might have arisen by chance. If these associations do exist, however, they would suggest that pernicious anaemia is a heterogeneous disease and that types of the condition with different manifestations are associated with different HLA antigens. To test this hypothesis, we HLA-typed 127 patients with pernicious anaemia who were independently classified according to the presence or absence of associated endocrine disease, vitamin B_{12} neuromyelopathy, and severe gastric atrophy.

Patients and methods

We studied 127 unrelated Caucasians with pernicious anaemia who attended the Royal Melbourne Hospital for treatment or review during 1976. They comprised 46 men and 81 women who were selected because they satisfied standard diagnostic criteria for Addisonian pernicious anaemia.[3]

All the patients had impaired vitamin B_{12} absorption, shown by a Schilling test result of 0-5% (mean $2{\cdot}0 \pm 0{\cdot}1$) in the absence of gastric surgery or intestinal malabsorption. One hundred and twenty-four patients had serum parietal cell antibodies against human gastric mucosa, and 65 (51·2%) had serum intrinsic factor antibodies. The records of the patients were reviewed before HLA-typing and showed that 13 patients had vitamin B_{12} neuromyelopathy (subacute combined degeneration of the spinal cord) and that 27 (five men and 22 women) had overt endocrine disease. Six of these patients (including one diabetic) had thyrotoxicosis due to Graves's disease, eight (including one diabetic) had primary myxoedema, one had idiopathic Addison's disease of the adrenals, and 14 had diabetes mellitus (10 were dependent on insulin).

HLA types were determined concurrently in the 127 patients with pernicious anaemia and in 586 unrelated Caucasian subjects (blood donors, people attending a health screening centre, and laboratory staff) using the standard lymphocytotoxicity assay to detect 8 A-locus and 14 B-locus antigens.[13 14]

To evaluate the significance of heterogeneity for HLA type, a modified χ^2 statistic on two degrees of freedom[15] was calculated from the 2×2 table for each antigen, and χ^2 was summed over all antigens at either the A locus or the B locus, or both. The expected distribution of the summary χ^2 was derived by using a computer to repeatedly draw random samples of notional cases from the pooled group of cases and controls and repeatedly recalculate the summary χ^2. The probability that the observed heterogeneity over all antigens could have arisen by chance was obtained by comparing the observed value of the summary χ^2 with its empirical distribution.[15] All the P values were calculated to correspond to two-tailed tests of statistical significance.

Results

Table I compares the prevalence of HLA antigens in 586 controls and 127 PA patients. Both HLA B12 and B18 were more common among all the patients with pernicious anaemia than among the controls. Heterogeneity over all antigens was significant for the A locus (P=0·05), the B locus (P=0·01), and for both loci combined (P=0·003). Thus the overall pattern of HLA types in patients with pernicious anaemia differed significantly from that in controls. The relative risks[16] for the B locus comparisons are shown in table II. The differences at the A locus were generally less, probably reflecting linkage disequilibrium between the A and B loci.

TABLE I—*Prevalence of HLA antigens in 127 patients with pernicious anaemia. Results are numbers (and percentages)*

HLA antigen	Controls (n=586)	Total series with anaemia (n=127)	Patients with anaemia only and no endocrine disease: Schilling test 0-2% Gastric atrophy (n=62)	Patients with anaemia only and no endocrine disease: Schilling test 2-5% Atrophic gastritis (n=38)	Patients with anaemia only and no endocrine disease: Anaemia only (n=100)	Patients with anaemia and endocrine disease (n=27)
A locus						
1	188 (32·0)	36 (28)	14 (23)	12 (32)	26	10 (37)
2	276 (47·1)	71 (56)	37 (60)	21 (55)	58	13 (48)
3	146 (24·9)	24 (19)	13 (21)	6 (16)	19	5 (19)
9	113 (19·3)	30 (24)	16 (26)	10 (26)	26	5 (19)
10	70 (11·9)	22 (17)	10 (16)	5 (13)	15	6 (22)
11	72 (12·3)	6 (5)	3 (5)	3 (8)	6	0
28	33 (5·6)	11 (9)	4 (6)	3 (8)	7	4 (15)
W19	34 (5·8)	9 (7)	3 (5)	5 (13)	8	1 (4)
B locus						
5	52 (8·9)	8 (6)	6 (10)	1 (3)	7	0
7	148 (25·3)	39 (31)	22 (35)	12 (32)	34	5 (19)
8	155 (26·5)	29 (23)	9 (15)	8 (21)	17	12 (44)
12	151 (25·8)	46 (36)	32 (52)	8 (21)	40	6 (22)
13	31 (5·3)	5 (4)	2 (3)	1 (3)	3	2 (7)
14	47 (8·0)	10 (8)	5 (8)	4 (11)	9	1 (4)
18	18 (3·1)	13 (10)	2 (3)	5 (13)	7	6 (22)
27	48 (8·2)	8 (6)	3 (5)	1 (3)	4	3 (11)
W15	34 (5·8)	11 (9)	4 (6)	4 (11)	8	3 (11)
W16	13 (2·2)	4 (3)	2 (3)	1 (3)	3	1 (4)
W17	42 (7·2)	10 (8)	3 (5)	5 (13)	8	2 (7)
W22	31 (5·3)	8 (6)	1 (2)	4 (11)	5	2 (7)
W35	95 (16·2)	11 (9)	5 (8)	2 (5)	7	4 (15)
W40	70 (11·9)	15 (12)	5 (8)	7 (18)	12	3 (11)

TABLE II—*Relative risks for HLA B locus antigens in pernicious anaemia patients relative to controls (n=586)*

HLA antigen	Total series with anaemia (n=127)	Patients with anaemia only and no endocrine disease: Gastric atrophy (n=62)	Patients with anaemia only and no endocrine disease: Atrophic gastritis (n=38)	Patients with anaemia only and no endocrine disease: Anaemia only (n=100)	Patients with anaemia and endocrine disease (n=27)
B5	0·6	1·1	0·3	0·8	0·0
B7	1·3	1·6	1·4	1·5	0·7
B8	0·8	0·5	0·7	0·6	2·2
B12	1·6	3·1	0·8	1·9	0·8
B13	0·7	0·6	0·5	0·6	1·4
B14	1·0	1·0	1·4	1·3	0·4
B18	3·6	1·1	4·8	2·4	9·0
B27	0·7	0·6	0·3	0·5	1·4
BW15	1·5	1·1	1·9	1·4	2·0
BW16	1·4	1·5	1·2	1·4	1·7
BW17	1·1	0·7	2·0	1·1	1·0
BW22	1·0	0·3	2·1	0·9	1·4
BW35	0·5	0·5	0·3	0·4	0·9
BW40	1·0	0·7	1·7	1·0	0·9
Probability of such a distribution of relative risks arising by chance	P=0·01	P=0·03	P=0·04	P=0·01	P=0·05

The 27 patients with concomitant endocrine diseases (table I) had an increased prevalence of HLA-B8 (12/27; 44%), B18 (6/27; 22%), and BW15 (3/27; 11%) but a smaller proportion than in the total series had B12 (6/27; 22%). This contrasted with an increase in the prevalence of HLA-B7 (34%) and B12 (40%) among the 100 patients without endocrine disease (tables I and II). The difference between the endocrine and non-endocrine patterns of B-locus antigens in pernicious anaemia was significant (P=0·01), and both these patterns were significantly different from that in controls (P=0·01).

The 100 patients with pernicious anaemia but without endocrine disease were subdivided according to the functional severity of the gastric lesion as assessed by Schilling test results.[3] Sixty-two patients with Schilling values below 2% were categorised as having "gastric atrophy" and 38 patients with values of 2-5% were considered to have "atrophic gastritis." HLA-B12 was more prevalent among those with gastric atrophy (32/62; 52%) than among those with atrophic gastritis (8/38; 21%), and the heterogeneity over all B-locus antigens was significant (P=0·01).

In 13 patients with vitamin B_{12} neuromyelopathy there was an increased frequency of B7 (8/13; 62%) and B12 (8/13; 62%), corresponding to the severity of the gastric atrophy.

The non-endocrine type of HLA pattern in those with gastric atrophy (high prevalence of B12, moderately increased prevalence of B7, and low prevalence of B8 and B18) was complementary to the HLA pattern in patients with endocrine disease (high prevalence of B8 and B18, decreased prevalence of B7, and low prevalence of B12). The difference between these two was highly significant (P=0·003 over all B-locus antigens). The non-endocrine type of HLA pattern seen in patients with atrophic gastritis was generally intermediate between these two extremes.

In comparison with an expected random assortment of B-locus phenotypes over the entire series of patients with pernicious anaemia, we found a slight deficiency of homozygous phenotypes B7/-, B12/-, B8/-, B18/-, BW15/- (observed 15; expected 20·8). This contrasted with an excess of the heterozygous phenotypes B8/B18, B8/BW15, and B18/BW15 (observed 8; expected 3·6), which corresponded to the endocrine HLA pattern, and of B7/B12 (observed 14; expected 8·6), which corresponded to the gastric atrophy HLA pattern ($\chi^2_3=11·2$; P<0·02).

The various HLA patterns in clinical subgroups of patients with pernicious anaemia were not attributable to differences in sex, age of onset of anaemia, age at HLA testing, or presence of serum parietal cell or intrinsic factor antibodies.

Discussion

The HLA pattern in our complete series of 127 patients with pernicious anaemia differed from that among normal controls, with relative risks in those with pernicious anaemia of 1·6 for HLA-B12 and 3·6 for HLA-B18.

The overall HLA pattern in this series was composite, comprising three distinct patterns that corresponded to clinical subgroups of PA patients. Firstly, 62 patients with gastric atrophy and without endocrine disease had an increased frequency of HLA-B12 and B7 (relative risks 3·1 and 1·6 respectively). Secondly, 27 patients with endocrine disease had increased frequencies of HLA-B8, B18, and BW15 (relative risks 2·2, 9·0, and 2·0 respectively). Thirdly, 38 patients without endocrine disease but with Schilling test values of 2-5% (atrophic gastritis) had increased frequencies of HLA-B7, B18 and BW15 (relative risks 1·4, 4·8, and 1·9 respectively). The HLA patterns in patients with gastric atrophy and in those with endocrine disease differed significantly (P=0·003).

Although an increased prevalence of HLA-B7 was found in the 100 patients without endocrine disease, the increase in HLA-B12 was confined to the 62 patients with the most severely impaired vitamin B_{12} absorption. The particularly high frequencies of HLA-B7 and B12 (relative risks 4·7 and 4·6 respectively) among the 13 patients with neuromyelopathy presumably reflected the severity of the gastric lesion in these patients. The HLA pattern in 27 patients with endocrine disease (increased HLA-B8, B18, and BW15) was comparable with the HLA pattern in patients with endocrine disease but without pernicious anaemia.[4,5] In our patients with pernicious anaemia and endocrine disease there was no increase in the prevalence of HLA-B7 or B12.

The intermediate pattern of an increased prevalence of HLA-B7, B18, and BW15 included some of the HLA antigens associated with gastric atrophy and some associated with endocrine disease. This suggests that the group of 38 patients with atrophic gastritis but without endocrine disease may have included patients with a latent tendency to either gastric atrophy

or endocrine disease. Such patients are thought to lack one or more of the factors implicated in the full expression of gastric atrophy or endocrine disease.

Our findings are consistent with several reports of associations between HLA antigens and pernicious anaemia.[6-12] We attribute the discrepancies between other reports to the differing proportions of patients with the three HLA patterns.

The relative risks for HLA-B-locus antigens in pernicious anaemia were small. This suggests that there are other important determinants of the condition, possibly including non-HLA-linked genes. Our observations suggest that susceptibility to pernicious anaemia might be partly determined by genes in linkage disequilibrium with the HLA-B-locus,[17] but they might also indicate an effect of HLA-linked genes on the length of survival in patients with pernicious anaemia. The excess of heterozygotes for certain HLA-B-locus antigens suggests that these genes show overdominance or additive effects similar to those in diabetes mellitus.[16]

The contrasting HLA patterns in clinically defined subgroups of patients with pernicious anaemia might indicate heterogeneity in the pathogenesis of pernicious anaemia, and we suggest that pernicious anaemia is determined by the action of autoimmune and other factors on a susceptible gastric mucosa.[18]

We thank the medical staff of the Royal Melbourne Hospital; the patients and their medical practitioners; Sister E Fraser and her staff for most valuable help with the patients; and Mr C M Francis, Mrs P Krupinska, Miss B J Booker, and Miss J L Perkin for technical help.

JDM was supported by the National Health and Medical Research Council of Australia.

References

1 Whittingham, S, *et al*, *Lancet*, 1969, **1**, 951.
2 Strickland, R G, and Mackay, I R, *American Journal of Digestive Diseases*, 1973, **18**, 426.
3 Ungar, B, *et al*, *Lancet*, 1968, **2**, 415.
4 Nerup, J, *et al*, *Lancet*, 1974, **2**, 864.
5 Cudworth, A G, and Woodrow, J C, *British Medical Journal*, 1976, **2**, 846.
6 Whittingham, S, *et al*, *Clinical and Experimental Immunology*, 1975, **19**, 289.
7 Mawhinney, H, *et al*, *Clinical and Experimental Immunology*, 1975, **22**, 47.
8 Zittoun, R, *et al*, *New England Journal of Medicine*, 1975, **293**, 1324.
9 Goldstone, A H, *et al*, *Clinical and Experimental Immunology*, 1976, **25**, 352.
10 Eastmond, C J, and Woodrow, J D, *British Journal of Haematology*, 1976, **33**, 113.
11 Horton, M A, and Oliver, R T D, *HLA and Disease*, Abstracts. Paris, Editions INSERM, 1976.
12 Horton, M A, and Oliver, R T D, *Tissue Antigens*, 1976, **7**, 239.
13 Terasaki, P I, Vredevoe, D L, and Mickey, M R, *Transplantation*, 1967, **5**, 1057.
14 Mittal, K K, *et al*, *Transplantation*, 1968, **6**, 913.
15 Mathews, J D, *HLA and Disease*, Abstracts, p 276. Paris, Editions INSERM, 1976.
16 Svejgaard, A, *et al*, *Transplantation Reviews*, 1975, **22**, 3.
17 McDevitt, H O, and Bodmer, W F, *Lancet*, 1974, **1**, 1269.
18 Ungar, B, Francis, C M, and Cowling, D C, *Medical Journal of Australia*, 1976, **2**, 900.

(*Accepted 13 January 1977*)

Partial antibiotic decontamination

HENRI F L GUIOT, RALPH VAN FURTH

British Medical Journal, 1977, **1**, 800-802

Summary

Partial antibiotic decontamination and reverse isolation were carried out in nine patients undergoing bone marrow transplantation. The aim of this approach was to eradicate the patient's endogenous potentially pathogenic bacteria while preserving the anaerobic flora of the gut, which help to prevent recolonisation. No exogenous infections developed, and only one patient developed an infection associated with endogenous recolonisation. Colonisation resistance seemed normal in patients during partial antibiotic decontamination. This form of decontamination deserves further study in patients with immunodepression.

Introduction

The micro-organisms that most often cause infections in patients with impaired resistance are *Escherichia coli*, *Klebsiella* spp, and other Enterobacteriaceae, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*.[1] Attempts to prevent infection by total antibiotic decontamination eliminate not only these potential pathogens but also the normal anaerobic gut flora. Animal studies have shown that these anaerobic bacteria strongly inhibit exogenous or endogenous bacterial colonisation.[2 3] This bacteriostatic ability, or colonisation resistance, is severely reduced in decontaminated patients who have been deprived of their anerobic flora. If reverse isolation or total antibiotic decontamination is not completely successful in these patients micro-organisms can easily colonise or recolonise the gut in abnormally high numbers, sometimes causing diarrhoea or infection.[4 5]

Other effects of total antibiotic decontamination are nausea, changed gut motility, atrophy of the villi, and malabsorption. Moreover, the colon tends to become distended, and diarrhoea-like stools are formed.[6 7] To overcome some of these problems in nine patients undergoing bone marrow transplantation we tried to eradicate most of the common aerobic potential pathogens while preserving the anaerobic flora of the gut (partial decontamination), since we considered that these patients had no special predisposition to anaerobic infection.[8]

Isolation Ward, J A Cohen Institute of Radiopathology and Radiation Protection, Leiden, Netherlands
HENRI F L GUIOT, DBIOL, microbiologist

Department of Infectious Diseases, University Hospital, Leiden, Netherlands
RALPH VAN FURTH, MD, professor of internal medicine and infectious diseases

Patients and methods

The three women and six men (mean age 21 (range 16-42) years) were suffering from acute myeloid leukaemia, acute lymphatic leukaemia, or severe aplastic anaemia. They were all treated with immunosuppressive and cytostatic drugs before undergoing bone marrow transplantation.

Most of the patients had had severe granulocytopenia for several weeks ($<0{\cdot}5\times10^6$/l for an average of 45 days; $<0{\cdot}1\times10^6$/l for an