Human leucocyte antigens A, B, C, and DRW in idiopathic "warm" autoimmune haemolytic anaemia

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Summary and conclusions

Twenty patients with idiopathic "warm" autoimmune haemolytic anaemia and 40 controls were typed concurrently for human leucocyte antigens (HLA) A, B, C, and DRW. There was a significantly stronger association of HLA-B8 with the disease ($\chi^2=10.39$; $p=0.018$) than HLA-DRW3 ($\chi^2=3.71$; $p=0.35$), and the patients also showed a significant increase in BW6 homozygosity ($\chi^2=7.13$; $p=0.01$) and a corresponding reduction in BW4 ($\chi^2=7.13$; $p=0.02$). (All p values corrected for number of antigens at each locus.)

These findings suggest that susceptibility to idiopathic autoimmune haemolytic anaemia is associated more closely with the HLA-B locus than with DRW3.

Introduction

Human leucocyte antigens (HLA) expressed on B lymphocytes and macrophages but not on T lymphocytes—that is, the DRW antigens¹⁻³—may have a role in determining the fate of allografts⁴⁻⁶ and be associated with susceptibility to disease.⁶ HLA-B8 is increased in many diseases suspected of being autoimmune.⁶ An increased prevalence of HLA-A1 and B8 was reported in patients with IgG or complement coating on their red cells,⁷ and the few patients with idiopathic autoimmune haemolytic anaemia showed an increase in B8.

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We have tried to determine the possible correlation between HLA-A, B, and DRW and idiopathic autoimmune haemolytic anaemia.

Subjects and methods

We studied 20 patients with "idiopathic" autoimmune haemolytic anaemia of unknown aetiology. Patients with drug-induced disease or autoimmune haemolysis secondary to other conditions were excluded. All patients had "warm" antibodies and had positive titres in direct antiglobulin tests at the time of diagnosis (see table I). All the patients had been treated, and blood samples were taken at follow-up visits. Defibrinated blood (15 ml) was used for HLA typing, and Sequestrene-EDTA and clotted blood specimens for a repeat haemolytic screen. Forty healthy people unrelated to each other and to the patients served as controls. Most were staff of the centre.

Tissue typing—All the patients and controls were typed concurrently for serologically defined histocompatibility antigens of the HLA-A, B, C, and DRW loci. Lymphocytes were separated with Ficoll-paque gradient centrifugation.⁸ B-cell enriched mononuclear cells were used for DRW typing. Plastic or siliconised glassware was used throughout, and the washing media contained 10% autologous serum except when otherwise indicated. Monocytes were removed from the lymphocyte suspension by adding carbonyl iron powder in TC-199 containing 40% autologous serum, incubating at 37°C for 40 minutes, and sedimenting with a magnet. B-cell enriched suspensions were prepared by removing E-rosetting cells (T cells) as follows. Lymphocyte suspensions were mixed with aminoethylisothiuronium-hydrobromide-treated sheep red blood cells,⁹ incubated for five minutes at 37°C, centrifuged at 60 g for eight minutes, and incubated for 30 minutes at 4°C. The cells were gently suspended and layered over Ficoll-paque and spun at 400 g for 10 minutes. The lymphocytes at the interface (B cells) were collected and washed twice in TC-199, yielding a lymphocyte suspension containing 80-90% of viable B cells, the average yield being 75 000 B cells/ml of blood processed. Typing was performed by the standard NIH technique,¹⁰ and 118 sera defining 32 HLA-A, B, and C specificities and 25 sera defining seven DRW specificities were used (see table II). Anti-HLA-DRW sera were preabsorbed with pooled human platelets to eliminate any anti-HLA-A, B, and C activity.

Haemolytic screen—Sera from all the patients were fully screened

TABLE I—HLA types and results of haemolytic screening in idiopathic warm autoimmune haemolytic anaemia

Case No	Sex	Age (years)	HLA types		Result of latest serum red-cell antibody screen	Direct antiglobulin test titres				
						Anti-IgG		Anti-IgM (latest)	Anti-IgA (latest)	Anti-C3 (latest)
			A-C	DRW		At presentation	Latest			
1	F	73	A1, A3, B8, B14, BW6	3, 7	—	800	3200	0	0	0
2	F	32	A2, A29, B8, B12, BW4, BW6	3, 4	—	6200	3200	0	0	0
3	F	31	A1, A25, B8, BW6	2, 3	—	1600	1600	0	0	0
4	F	46	A1, B8, BW6	3	—	+	0	0	0	200
5	F	28	A2, A8, B15, BW6, CW3	3, 4	—	640	0	0	0	0
6	F	70	A2, AW24, B8, B13, BW4, BW6	2, 7	—	6400	25	0	0	0
7	M	73	A1, AW24, B7, B8, BW6	3, 4	+	1280	0	0	0	60
8	M	65	A1, A3, B8, B40, BW6, CW3	3, 6	—	400	200	0	0	100
9	F	76	A3, A11, B7, B40, BW6, CW3	4, 5	—	+	100	0	0	0
10	F	60	A2, A3, B8, B14, BW6	3, 4	—	+	0	0	0	0
11	F	78	A25, A28, B12, B14, BW4, BW6	4	—	+	0	0	0	0
12	F	47	A1, B8, BW6	3	—	+	0	0	0	50
13	F	57	A1, A2, B40, BW4, BW6, CW3	4	—	+	0	0	0	0
14	M	62	A1, A11, B8, BW6	3	—	640	0	0	0	120
15	F	78	A3, AW19, B14, BW16, BW6	6, 7	+	3200	3200	0	0	100
16	M	30	A1, A2, B7, BW4, BW6	2	+	800	400	0	0	0
17	M	55	A1, A2, B8, BW35, BW6	3, 7	+	+	3200	0	0	0
18	F	72	A1, A2, B8, B12, BW4, BW6	3, 4	+	3200	1600	0	0	0
19	F	47	A1, A3, B8, B14, BW6	4	—	400	1600	0	0	60
20	F	64	A1, A2, B8, B40, BW6, CW3	3, 4	+	200	50	0	0	120

* Titre not available.

for red-cell antibodies by standard techniques (saline, enzyme, and indirect antiglobulin tests) and by a direct antiglobulin test using polyvalent antihuman globulin. Patients with a positive result on screening were further tested with monospecific anti-IgG, anti-IgM, anti-IgA, and anti-C3 sera.

Results

Tables I and II list the HLA types found. B8 was significantly more prevalent in the patients (15 cases; 75%) than in the healthy controls (11; 27.5%) and remained so ($\chi^2=10.39$; $p=0.018$) after correction for the number of antigens tested. All the patients were positive for BW6; 14 (70%) of them were homozygous BW6 compared with only 12 (30%) of the controls ($\chi^2=7.13$; corrected $p=0.01$). There was a corresponding decrease in the prevalence of BW4 in the patients (6 cases; 30%) compared with the controls (28; 70%), which was significant after correction ($\chi^2=7.13$; $p=0.02$). HLA-A1, the haplotype A1, B8, and DRW3 were also increased in the patients, but not significantly so after correction (table II). Other HLA types and other markers (ABO and rhesus blood groups) showed no abnormal distribution.

TABLE II—Prevalence of HLA types in 20 patients with idiopathic autoimmune haemolytic anaemia and 40 controls

	No (%) of patients	No (%) of controls	p (corrected for No of antigens at each locus)*
A1	13 (65)	15 (37.5)	>0.5
B8	15 (75)	11 (27.5)	0.018
A1, B8	11 (55)	10 (25)	>0.5
BW4:4	0	9 (22.5)	>0.5
BW6:6	14 (70)	12 (30)	0.01
BW4:6	6 (30)	19 (47.5)	>0.5
DRW1	0	4 (10)	>0.5
DRW2	3 (15)	11 (27.5)	>0.5
DRW3	13 (65)	14 (35)	0.35
DRW4	10 (50)	18 (45)	>0.5
DRW5	1 (5)	4 (10)	>0.5
DRW6	2 (10)	2 (5)	>0.5
DRW7	4 (20)	11 (27.5)	>0.5

* χ^2 test. Antigens not significantly more prevalent in either group were A2, A3, A9, A11, AW24, A25, A26, A28, A29, AW30, and AW32; B5, B7, B12, B13, B14, B15, BW16, B17, B18, BW21, BW22, B27, BW35, B40, and BW41; and CW3 and CW4.

Discussion

HLA-B8 is associated with various diseases suspected of being autoimmune.⁶ Our results confirm the increased prevalence of B8 reported⁷ in patients with various diseases—including a few with idiopathic autoimmune haemolytic anaemia—who had positive titres in direct antiglobulin tests. Clauvel *et al.*,¹¹ however, failed to detect an increase in B8 in patients with idiopathic autoimmune haemolytic anaemia but did find an increase in HLA-A3. Population differences might have contributed to these results.

Since DRW antigens may be analogous to the immune-associated antigens (Ia) in mice and be implicated in susceptibility to the disease, a stronger association with the DRW antigens

than with B8 might be expected. This was shown for dermatitis herpetiformis,¹² in which a stronger association was found with DRW3 than with B8. Conversely, in myasthenia gravis no greater association with DRW3 than with B8 was reported.¹³

The increase in HLA-DRW3 observed in our patients with idiopathic autoimmune haemolytic anaemia was not statistically significant. Thus the association of the disease with B8 was stronger than with DRW3, suggesting that any gene(s) responsible for the disease must be closer to the B than the DRW locus. This is supported by the increased prevalence of BW6 homozygosity found in the patients.

There was no apparent relation between the HLA type and the titre, immunoglobulin class, or persistence of the antibody in the 20 patients studied.

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References

- Rood JJ van, Leeuwen A van, Parleviet J, Termijnden A, Keuning JJ. LD typing by serology IV: description of a new locus with three alleles. In: *Histocompatibility testing*. Copenhagen: Munksgaard, 1975:629-36.
- Wernet P, Winchester RJ, Dupont B, Kunkel HG. Serological aspects of a human alloantigen system with restricted tissue distribution, the equivalent of the murine Ia system? In: *Histocompatibility testing*. Copenhagen: Munksgaard, 1975:637-42.
- Solheim BC, Bratlie A, Winther N, Thorsby E. LD typing with antisera produced by planned immunization. In: *Histocompatibility testing*. Copenhagen: Munksgaard, 1975:713-8.
- Ting A, Morris PJ. Matching for B-cell antigens of the HLA-DR series in cadaver renal transplantation. *Lancet* 1978;i:575-7.
- Persijn GG, Leeuwen A van, Hoozeboom J, Gabb BW, Nag Tegabl A, van Rood JJ. Matching for HLA antigens of A, B, and DR loci in renal transplantation by Eurotransplant. *Lancet* 1978;i:1278-81.
- Dausset J, Svejgaard A. *HLA and disease*. Baltimore: Williams and Wilkins, 1977.
- Da Costa JAG, White AG, Parker AC, Grigor GB. Increased incidence of HLA-A1 and B8 in patients showing IgG or complement coating on their red cells. *J Clin Pathol* 1974;27:353-5.
- Bojum A. Separation of leucocytes from blood and bone marrow. *Scand J Clin Lab Invest* 1968;suppl 97.
- Kaplan E, Clark C. An improved rosetting assay for detection of human T lymphocytes. *J Immunol Methods* 1974;5:131-5.
- Terasaki PI, Park MS. Microdroplet lymphocyte cytotoxicity test. In: *NIAID manual of tissue typing techniques 1976-1977*. Bethesda, Maryland: US Department of Health Education and Welfare, 1978:69-80 (DHEW publication No (NIH) 76-545).
- Clauvel JP, Marcelli-Barge A, Coggia Gautier I, Poirier JC, Benajam A, Dausset J. HLA-A antigens and idiopathic autoimmune haemolytic anaemias. *Transplant Proc* 1974;VI:447-8.
- Solheim BG, Albrechtsen D, Thorsby E, Thune P. Strong association between an HLA-DW3 associated B cell alloantigen and dermatitis herpetiformis. *Tissue Antigens* 1977;10:114-8.
- Naeim F, Keesey JC, Herrmann C, Lindstrom J, Zeller E, Walford RL. Association of HLA-B8, DRW3 and anti-acetylcholine receptor antibodies in myasthenia gravis. *Tissue Antigens* 1978;12:381-6.

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ONE HUNDRED YEARS AGO In the current number of the *Archives of Dermatology*, Dr L Duncan Bulkley of New York publishes an account of two cases in which syphilitic disease was probably contracted through the medium of cigars. Both the infected persons were physicians who had closely studied this disease, and every care appears to have been taken to exclude other modes of contagion. In the first case, the initial manifestation was situated on the upper lip. There was an indurated sore on the margin of the lip at the left side, and a second sore towards the right side. No other lesion could be found either on the penis or elsewhere. The patient denied having ever had any venereal ulcer. He had not kissed anyone except members of his own family for a long time. Enlargement of the submaxillary glands, mucous patches of the fauces, and a macular eruption on the skin, followed. The patient was a great smoker of cigars made in

the country. In the second case, the primary sore appeared about the middle of the lower lip at the site of a fissure from which the patient had suffered for some years. Enlargement of the glands beneath the jaw occurred, and a general papulo-erythematous syphilide subsequently appeared. There had been no exposure to venereal contagion for many months. This patient also was a considerable cigar-smoker, and very careful investigation failed to discover any other origin of the syphilis. In connection with these observations, Dr Bulkley remarks that he lately had a cigar-maker, who was suffering from extensive syphilitic ulceration of the mouth, under treatment at the Demilt Dispensary. This man acknowledged using his saliva during his work for moistening the ends of the cigars. Dr Bulkley also states that he has himself seen the workmen in a cigar manufactory moisten the tips of cigars in their mouths. (*British Medical Journal*, 1880.)