References

- ¹ Pickup JC, Keen H, Parsons JA, Alberti KGMM. Continuous subcutaneous insulin infusion: an approach to achieving normoglycaemia. *Br Med J* 1978;ii:204-7.
- ² Pickup JC, Keen H, Parsons JA, Alberti KGMM. Continuous subcutaneous insulin infusion: good blood glucose control for up to 4 days. *Diabetologia* 1979;16:385-9.
- ³ Pickup JC, Keen H, Parsons JA, Alberti KGMM, Rowe AS. Continuous subcutaneous insulin infusion: improved blood glucose and intermediary metabolite control in diabetics. *Lancet* 1979;i:1255-8.
- ⁴ Pickup JC, White MC, Keen H, Kohner EM, Parsons JA, Alberti KGMM. Long-term continuous subcutaneous insulin infusion in diabetics at home. *Lancet* 1979;iii:870-3.
- ⁵ Pickup JC, Keen H. Continuous subcutaneous insulin infusion: a developing tool in diabetes research. *Diabetologia* 1980;18:1-4.
- ⁶ Pickup JC, Keen H, Viberti GC, et al. Continuous subcutaneous insulin infusion in the treatment of diabetes mellitus. *Diabetes Care* 1980;3:290-300.
- ⁷ Tamborlane WV, Sherwin RS, Genel M, Felig P. Reduction to normal of plasma glucose in juvenile diabetes by subcutaneous administration of insulin with a portable infusion pump. *N Engl J Med* 1979;300:573-8.
- ⁸ Tamborlane WV, Sherwin RS, Genel M, Felig P. Restoration of normal lipid and amino acid metabolism in diabetic patients with a portable insulin-infusion pump. *Lancet* 1979;i:1258-61.
- ⁹ Tamborlane WV, Sherwin RS, Genel M, Felig P. Outpatient treatment of juvenile-onset diabetes with a preprogrammed portable subcutaneous insulin infusion system. *Am J Med* 1980;68:190-6.
- ¹⁰ Champion MC, Shepherd GAA, Rodger NW, Dupré J. Continuous subcutaneous insulin infusion in the management of diabetes mellitus. *Diabetes* 1980;29:206-12.
- ¹¹ Raskin P, Pietri A, Unger R. Changes in glucagon levels after four to five weeks of glucoregulation by portable insulin infusion pump. *Diabetes* 1979;28:1033-5.
- ¹² Pickup JC, Keen H, White MC, Kohner EM, Parsons JA, Alberti KGMM. Perfusions sous-cutanées d'insuline chez les diabétiques: résultats cliniques. *Journ Annu Diabetol Hotel Dieu* 1980;17-22.
- ¹³ Pickup JC, Viberti GC, Bilous RW, et al. Metabolic and hormonal decompensation after deliberate withdrawal of prolonged subcutaneous insulin infusion. *Diabetologia* (in press).

(Accepted 11 December 1980)

HLA-DR patterns in pernicious anaemia

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

Abstract

The pattern of HLA-DR antigens was studied in a group of 66 patients with Addisonian pernicious anaemia, comprising a subgroup of 18 patients with associated endocrine disease and a subgroup of 48 patients with no associated endocrine disease. Compared with a control group of 120 subjects all 66 patients showed an increase in HLA-DR2 and DR4 and a decrease in DR3 ($p < 0.02$). Significant differences were also found between the endocrine and non-endocrine subgroups for patterns of HLA-DR antigens ($p < 0.005$) and for pairwise combinations of HLA-DR antigens ($p < 0.01$). Relative to controls, the endocrine subgroup showed an increase of HLA-DR3/DR4 (relative risk 4.0), contrasting with an increase of HLA-DR2/DR4 (relative risk 6.85) and DR4/DR5 (relative risk 5.38) in the non-endocrine subgroup.

These observations suggest that HLA-DR antigens or closely linked genes may interact to influence susceptibility to pernicious anaemia (or endocrine disease, or both). Thus interactive effects related to HLA-DR2/DR4 and DR4/DR5 may predispose to pernicious anaemia without endocrine disease, whereas interactive effects related to HLA-DR3/DR4 may predispose to pernicious anaemia in association with endocrine disease.

Introduction

We have documented significant heterogeneity in HLA-B patterns in clinical subgroups of patients with Addisonian pernicious anaemia. In patients without endocrine disease

pernicious anaemia was associated with HLA-B7 and B12, whereas in patients with coexistent endocrine disease pernicious anaemia was associated with HLA-B8, B15, and B18.¹

The purpose of the present study was to test for associations between pernicious anaemia and HLA-DR antigens and to determine what bearing these might have on the pathogenesis of pernicious anaemia and its relation to autoimmune endocrine disease.

Patients and methods

We studied 66 unrelated Caucasians with pernicious anaemia who attended this hospital for treatment or review during 1979-80. They comprised 25 men and 41 women aged 20 to 94 years (mean age 66.5 years) who satisfied the standard diagnostic criteria for Addisonian pernicious anaemia.² All had impaired vitamin B₁₂ absorption, shown by a Schilling test result of 0-5%. Serum antibodies to human parietal cells were present in 60 patients and to intrinsic factor in 31.

Of the 66 patients, 48 (17 men and 31 women) were clinically free of endocrine disease ("non-endocrine" subgroup), while 18 (eight men and 10 women) had known endocrine disease ("endocrine" subgroup). Three of these patients (including one diabetic) had thyrotoxicosis due to Graves's disease, five had primary myxoedema, one (a diabetic) had idiopathic Addison's disease of the adrenals, one had myasthenia gravis, and 10 had diabetes mellitus (seven were dependent on insulin).

TABLE 1—Prevalences of HLA-DR antigens in 66 patients with pernicious anaemia with and without associated endocrine disease and 120 healthy controls. Figures are numbers of subjects (percentages in parentheses)

HLA antigen	Patients with pernicious anaemia			Controls (n = 120)
	Total (n = 66)	With endocrine disease (endocrine group: n = 18)	Without endocrine disease (non-endocrine group: n = 48)	
DR1	13 (19.7)	3 (16.7)	10 (20.8)	24 (20.0)
DR2	29 (43.9)	2 (11.1)	27 (56.2)	31 (25.8)
DR3	14 (21.2)	8 (44.4)	6 (12.5)	39 (32.5)
DR4	29 (43.9)	10 (55.5)	19 (39.6)	34 (28.3)
DR5	14 (21.2)	4 (22.2)	10 (20.8)	25 (20.8)
DRW6	5 (7.6)	2 (11.1)	3 (6.2)	22 (18.3)
DR7	14 (21.2)	4 (22.2)	10 (20.8)	25 (20.8)

Royal Melbourne Hospital, Victoria 3050, Australia

BERTA UNGAR, MD, FRACP, deputy director, department of haematology
 J D MATHEWS, MD, PHD, NHMRC (Australia) principal research fellow,
 University of Melbourne department of medicine
 B D TAIT, PHD, senior scientist, tissue-typing laboratory
 D C COWLING, MD, FRACP, director, department of haematology

All patients were typed for HLA-A and B locus antigens as before, and the results were consistent with our other study.¹ HLA-DR antigen typing was carried out by the standard prolonged micro-lymphocytotoxicity dye exclusion assay recommended by the Seventh International Histocompatibility Workshop³ on B lymphocytes separated from peripheral blood by the rabbit antihuman F(ab')₂ technique.^{4,5} Seven DR antigens were determined using 40 antisera, the specificities of which were designated on the basis of their reactivity with cells which had been typed for DR antigens as part of the seventh histocompatibility workshop. HLA-DR types were determined concurrently in the 66 patients with pernicious anaemia

Results

Table I shows the distribution of HLA-DR antigens in the patients and controls, and table II the relative risks and significance levels. For the whole series of patients with pernicious anaemia there was an increase in prevalence of HLA-DR2 (relative risk 2.25; $p=0.02$) and DR4 (relative risk 1.98; $p=0.04$) and a decreased prevalence of DRW6 (relative risk 0.37; $p=0.05$). Heterogeneity over the whole series of antigens was significant ($p=0.02$). The antigenic pattern for patients with associated endocrine disease (table II) was significantly different from that for patients without endocrine disease ($p=0.005$). The

TABLE II—Relative risks (RR) for HLA-DR antigens in patients with pernicious anaemia (with and without associated endocrine disease) relative to controls. Significance of differences between total series, subgroups, and controls (p value)

HLA antigen	Total series with anaemia (n = 66) v controls (n = 120)			Patients with anaemia and endocrine disease (n = 18) v controls (n = 120)			Patients with anaemia and no endocrine disease (n = 48) v controls (n = 120)			Patients with anaemia and endocrine disease (n = 18) v patients with anaemia and no endocrine disease (n = 48)		
	RR	χ^2_1	p	RR	χ^2_1	p	RR	χ^2_1	p	RR	χ^2_1	p
DR1	0.98	0.00	NS	0.80	0.11	NS	1.05	0.02	NS	0.76	0.14	NS
DR2	2.25	6.39	0.02	0.36	1.87	NS	3.70	14.03	0.0005	0.10	10.83	0.001
DR3	0.56	2.66	NS	1.66	0.99	NS	0.30	6.99	0.01	5.62	7.99	0.005
DR4	1.98	4.63	0.04	3.16	5.34	0.02	1.66	2.01	NS	1.91	1.36	NS
DR5	1.02	0.00	NS	1.09	0.02	NS	1.00	0.00	NS	1.09	0.02	NS
DRW6	0.37	3.97	0.05	0.56	0.57	NS	0.30	3.95	0.05	1.88	0.44	NS
DR7	1.02	0.00	NS	1.09	0.02	NS	1.00	0.00	NS	1.09	0.02	NS
Total		17.66 (df = 7)	0.02		8.92 (df = 7)	NS		27.00 (df = 7)	0.001		20.80 (df = 7)	0.005

NS = Not significant. df = Degrees of freedom.

TABLE III—Prevalences of HLA-DR antigen combinations in patients with pernicious anaemia (with and without associated endocrine disease) and in controls. Relative risks for antigen combinations for pernicious anaemia between these subgroups and relative to controls. Significance of differences between subgroups and between non-endocrine subgroup and controls (p value)

HLA antigen combination	No of subjects			Relative risks (RR) and significance levels					
	Patients with anaemia and endocrine disease (n = 18)	Patients with anaemia and no endocrine disease (n = 48)	Controls (n = 120)	Patients with anaemia and endocrine disease v patients with anaemia and no endocrine disease			Patients with anaemia and no endocrine disease v controls		
				RR	χ^2	p	RR	χ^2	p
DR2/DR3	0	5	3	0.00	2.03	NS	4.55	4.74	0.04
DR2/DR4	0	11	5	0.00	4.95	0.03	6.85	13.99	0.0005
DR3/DR4	4	0	8	*	11.36	0.001	0.00	3.36	NS
DR4/DR5	2	4	2	1.38	0.12	NS	5.38	4.43	0.04
.	.	.	.	.	.	.	.	.	.
Total	18	48	120		35.38 (df = 17)	0.01		45.82 (df = 21)	0.003

*Relative risk was large but undefined because DR3/DR4 genotype was absent from non-endocrine subgroup of patients.

and in 120 unrelated healthy Caucasian control subjects of similar ethnic origin.

Statistical evaluation was carried out for HLA-DR associations. For each 2×2 table χ^2 on one degree of freedom was calculated without Yates's correction, and the relative risk was calculated from the cross-product ratio, as described.⁶ For individual antigens we quote p values which were "uncorrected" for the total number of comparisons made.⁶ Generally the p values derived from the χ^2 test agreed closely with the p values calculated by Fisher's exact test, but we preferred to use the χ^2 statistic because it was simpler to calculate and has additive properties.

The sums of all χ^2 values for individual antigens and combinations of antigens were referred to χ^2 tables on the appropriate number of degrees of freedom to assess the significance of the heterogeneity observed over all possible comparisons. Extensive simulation studies showed that this procedure does not give rise to misleading results. For comparison, by Fisher's exact test for the DR2/DR4 combination in non-endocrine disease the exact p value was 0.00063; after allowing for the 22 antigen combinations compared this corresponds to a corrected p value of 0.014.

pattern in patients without endocrine disease was significantly different from that in control subjects ($p=0.001$), whereas the overall pattern for patients with endocrine disease was not significantly different from that of controls, despite an association of HLA-DR4 with the endocrine subgroup. In the non-endocrine subgroup there was a highly significant excess of HLA-DR2 (relative risk 3.70; $p<0.0005$), a non-significant excess of DR4 (relative risk 1.66; $p=0.16$), and a deficiency of DR3 (relative risk 0.30; $p=0.01$) and DRW6 (relative risk 0.30; $p=0.05$). On inspection, it appeared that the effects related to HLA-B locus antigens, which were similar to those described before,¹ would be best explained as being secondary to the linkage disequilibrium between HLA-B and HLA-D locus alleles.

Table III summarises the distributions of combinations of HLA-DR antigens according to subject group. There was a highly significant excess of certain combinations in the non-endocrine group compared with control subjects ($p=0.003$), with major contributions from DR2/DR4 (relative risk 6.85; $p=0.0005$), DR2/DR3 (relative risk 4.55; $p=0.04$), and DR4/DR5 (relative risk 5.38; $p=0.04$). Patients with endocrine disease differed from the non-endocrine group

($p=0.01$), notably because of an excess of DR3/DR4 (relative risk was large but undefined because the DR3/DR4 genotype was absent from the non-endocrine subgroup; $p=0.03$).

Table IV shows the relation of the DR2/DR4 effect to the separate effects of DR2 and DR4 in the non-endocrine subgroup of patients. Relative to subjects with neither DR2 nor DR4, the risks were 10.15 for DR2+/DR4+, 2.84 for DR2+/DR4-, and 1.27 for DR2-/DR4+. Hence the separate effects of DR2 and DR4 may be less important than the interactive effect of DR2/DR4.

TABLE IV—Prevalences of HLA-DR2, DR4, and DR2/DR4 in subgroup of 48 patients with pernicious anaemia without associated endocrine disease and in controls. Relation of risk of pernicious anaemia to presence of HLA-DR antigens (singly or in combination) relative to their absence

HLA genotype		No of subjects		Risk of disease relative to low-risk group
DR2	DR4	Patients with anaemia and no endocrine disease (n = 48)	Controls (n = 120)	
+	+	11	5	10.15
+	-	16	26	2.84
-	+	8	29	1.27
-	-	13	60	1.00

Discussion

Genetic heterogeneity in Addisonian pernicious anaemia¹ is suggested by its familial aggregation⁷ in the absence of a simple pattern of inheritance and by its association with autoimmune endocrine diseases⁸. The HLA associations are likewise complex, with evidence that different HLA-B-linked genes may interact to influence susceptibility or survival.¹

In the absence of autoimmune endocrine disease there is an increased risk of pernicious anaemia in subjects who are HLA-DR2 and in subjects who are DR4, but the risk is greatest in subjects with DR2/DR4 and those with DR4/DR5. There is a decreased risk of pernicious anaemia in subjects who are DR3, unless this occurs with DR2; in such patients with pernicious anaemia DR2 may decrease the risk of endocrine disease.⁹

There is also an increased risk of pernicious anaemia in subjects who have autoimmune endocrine disease.⁸ Our findings indicate that the risk is increased for subjects with DR3 or DR4, and may be greatest in subjects with both DR3 and DR4.

Probably the risk of pernicious anaemia is most closely related to specific combinations of pairs of HLA-DR antigens; for example, DR2/DR4 is most strongly associated with non-endocrine disease, and DR3/DR4 is most strongly associated with endocrine disease. This suggests that the effects of the

different HLA-DR haplotypes are interactive and non-additive and that the effects of combinations of DR haplotypes are not merely the sum of the average effect of each haplotype. The effect of HLA-DR2 in attenuating the association of DR3 with endocrine disease¹⁰ (table III) further exemplifies this concept. Non-additive genetic interactions with MHC haplotypes have been described in diabetes mellitus⁹ and in other conditions, including an immune response system in mice.¹¹

Our observations confirm that there is genetic heterogeneity in pernicious anaemia and that some of this heterogeneity is related to the presence or absence of associated endocrine disease. The complexity of HLA-DR patterns between and within the clinical subgroups suggests that HLA-DR-linked alleles may interact in a complex way to influence susceptibility or resistance to the gastric-endocrine group of autoimmune disorders.

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 Miss S H Solty, Mrs B J Melny, Mr P M Joseph, and Mr B Paspaliaris for technical help.

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

References

- Ungar B, Mathews JD, Tait BD, Cowling DC. HLA patterns in pernicious anaemia. *Br Med J* 1977;i:798-800.
- Ungar B, Stocks AE, Martin FIR, Whittingham S, Mackay IR. Intrinsic-factor antibody, parietal-cell antibody and latent pernicious anaemia in diabetes mellitus. *Lancet* 1968;ii:415-8.
- Bodmer WF, Batchelor JR, Bodmer JG, Festenstein H, Morris PJ, eds. *Histocompatibility testing 1977*. Copenhagen: Munksgaard, 1978.
- Nelson DL, Strober W, Abelson LD, Bundy BM, Mann DL. Distribution of alloantigens on human Fc receptor-bearing lymphocytes: the presence of B cell alloantigens on sIg-positive but not sIg-negative lymphocytes. *J Immunol* 1977;118:943-6.
- Grier JO, Abelson LA, Mann DL, Amos DB, Johnson AH. Enrichment of B lymphocytes using goat antihuman F(ab')₂. *Tissue Antigens* 1977;10:236.
- Svejgaard A, Jersild C, Staub Nielsen L, Bodmer WF. HLA antigens and disease. Statistical and genetical consideration. *Tissue Antigens* 1974;4:95-105.
- Whittingham S, Mackay IR, Ungar B, Mathews JD. The genetic factor in pernicious anaemia. *Lancet* 1969;i:951-4.
- Chanarin I. *The megaloblastic anaemias*. Oxford: Blackwell Scientific, 1979.
- Cudworth AG, Festenstein H. HLA heterogeneity in diabetes mellitus. *Br Med Bull* 1978;34:285-9.
- Bodmer WF, Bodmer JG. Evolution and function of the HLA system. *Br Med Bull* 1978;34:309-16.
- Dorf ME, Stimpfling JH. Coupled complementation of immune response genes controlling responsiveness to H2.2 alloantigen. *J Exp Med* 1977;146:571-8.

(Accepted 11 December 1980)

ONE HUNDRED YEARS AGO SIR,—About eight months ago, you were good enough to allow me to point out that, in cases of deaths from chloroform, it transpired in evidence that little of the drug was used; that it was given on one thickness of lint, or flannel; also, that the patient was quickly placed under its full influence, where death took place during or after the anaesthetised state. I pointed out how unfair it was for a section of the profession to pour pure chloroform vapour into patients' lungs, and, in cases of death, attribute the death to the use of the drug.

As Dr Jacob then attacked my views, I now use his statistics to show him and the profession I was right. In every case he has recorded, direct evidence is given that chloroform vapour was poured into the patients' lungs from lint, flannel, or inhalers (*vide* his table). In Cases No 32 and 88, no mention is made of how the towel and napkin were used; that is, held over the face or not.

Is it not true that chloroform vapour is a cardiac depressant? That the prospect of an operation is also a cardiac depressant? That, in ordinary stethoscopic examinations, patients often feel faint, and ask for water; and, if standing, ask to sit down? Again: is the vapour of

chloroform heavier than air? Can air circulate as freely when a meshed thing like lint, flannel, or even a lady's veil, is held over the face, as if no such impediment were there? Does the air breathed during chloroform administration require mixing with only a small percentage of chloroform vapour (5 per cent.), and no more?

But, some say, patients die after they have come out of the chloroform. To this I reply, that such patients as much "come out" of the chloroform because they are seen to recover some of their senses, as the partridge, fatally struck with a shot, may be said to come out of it, because it is seen to fly as straight after the shot as before it for half a mile, then rise perpendicularly into the air ("tour," as sportsmen call it), and drop dead.

The first death from the administration of good respirable air, properly charged with chloroform vapour, has yet to happen; and I challenge Dr Jacob, or any member of the profession, to record a well-authenticated case, when the vapour has been administered on rational principles, and the patient's heart and pulse have been let alone.—I am, sir, yours truly, WILLIAM FEARNLEY. (*British Medical Journal*, 1881.)