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HL-A3 AND HL-A7 IN PERNICIOUS ANAEMIA AND AUTOIMMUNE ATROPHIC GASTRITIS

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SUMMARY

Increased frequencies of HL-A7 and the HL-A3,7 haplotype have been demonstrated in pernicious anaemia. There was no significant increase in any HL-A specificity in autoimmune atrophic gastritis. In the whole series of patients with either pernicious anaemia or atrophic gastritis there was a significant increase in the frequency of HL-A3. No association has been found between any HL-A specificity and an increased incidence of either serum gastric parietal cell or intrinsic factor antibody compared with the overall incidence in pernicious anaemia, atrophic gastritis or in the whole series.

INTRODUCTION

Within the last few years a variety of associations between antigens of the major human histocompatibility antigen (HL-A) system and disease have been reported (Bodmer & Bodmer, 1974). In particular, associations have been described between certain HL-A antigens and some of the organ-specific autoimmune diseases (Grumet *et al.*, 1973; Platz *et al.*, 1974; Nerup *et al.*, 1974). Recently Whittingham *et al.* (1975) have reported increased frequencies of HL-A3 and HL-A7 in a series of twenty-seven patients with atrophic gastritis, some of whom had concomitant pernicious anaemia. In this paper we report the results of a larger study of seventy-five patients sub-divided into (a) pernicious anaemia and (b) autoimmune atrophic gastritis without evidence of vitamin B12 malabsorption.

PATIENTS AND METHODS

Patients and controls

(a) *Pernicious anaemia.* Twenty-nine patients (twenty-five females and four males) were studied. This series included both patients who presented with frank pernicious anaemia

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and those who were found to have latent pernicious anaemia, as defined by Callender & Spray (1962), on investigation of patients presenting with endocrine disease or on follow-up of patients known to have achlorydic atrophic gastritis. Vitamin B12 malabsorption was demonstrated either by the Schilling urinary excretion test or by a total body count of ^{58}Co-labelled B12 absorption (Irvine *et al.*, 1970).

(b) *Autoimmune atrophic gastritis.* Forty-six patients (all but one of whom were female) were studied. This series of patients was obtained by carrying out gastric analyses on patients attending the Endocrine Clinic, Royal Infirmary of Edinburgh. Forty-three patients had achlorhydria following histamine or pentagastrin stimulation, while the remaining three had marked hypochlorhydria ($\leqslant$0·5 mEq HCl) in the post-stimulation hour. In some instances the diagnosis was confirmed by gastric biopsy. Most of these patients had presented with autoimmune endocrine disease and most had already been shown to have serum gastric parietal cell or intrinsic factor antibody. The majority of these patients form part of a follow-up study of atrophic gastritis described elsewhere (Irvine, Cullen & Mawhinney, 1974) and most, if not all, correspond to type A or autoimmune gastritis as described by Strickland & Mackay (1973).

(c) *Controls.* Normal HL-A frequencies in south-east Scotland were obtained by typing 400 random blood donors, concurrently with the typing of the patients.

Methods

HL-A typing. Lymphocytes were separated from defibrinated blood by centrifugation on a Ficoll–Triosil gradient (Böyum, 1968) for 15 min at 2000 *g*. Lymphocytes were washed three times in medium 199 before counting. The cell suspensions usually contained 2–10% monocytes, as determined by phase-contrast microscopy. If more than 20% monocytes were present, they were removed by incubating the suspension on a protein-enriched glass bead column for 30 min at 37°C and then eluting the lymphocytes with medium 199. When there was excessive contamination with red blood cells (RBC) these were agglutinated with chicken anti-human RBC serum (25% v/v) and the lymphocyte-rich supernatant was decanted off and washed two more times.

Typing for HL-A specificities was carried out by a modification of the Kissmeyer-Nielsen (Kissmeyer-Nielsen & Thorsby, 1970) lymphocytotoxicity technique. Lymphocytes were suspended in a mixture of three parts of fresh-frozen rabbit serum and one part of fresh-frozen human AB serum to a concentration of approximately 2000 cells/μl. One microlitre of this suspension was added to one microlitre of antiserum under paraffin in a glass tissue-typing tray of our own manufacture and incubated for 1 hr at 37°C. One microlitre of freshly centrifuged 1·25% trypan standard blue was then added and the trays were allowed to stand for 10 min at room temperature before reading by phase contrast microscopy.

Lymphocytes from patients and controls were tested for the following twenty HL-A antigens: HL-A1, 2, 3, 9, 10, 11 and W29 at the first locus, and HL-A 5, 7, 9, 10, 12, 13, 14, 17, 27, W10, 15 and 22 at the second locus. The more common HL-A specificities (including HL-A3 and HL-A7) were represented on the tray by at least three different antisera. All readings were carried out in duplicate,

Autoantibodies. Serum gastric parietal cell antibody was demonstrated by the indirect immunofluorescent technique (Irvine, 1963) and serum type I intrinsic factor antibody by the charcoal absorption method as described by Irvine (1966).

Statistical methods

The observed frequencies of HL-A specificities in the patient groups were compared with the frequencies in the normal population by calculating Chi-square with Yates' correction. *P* values were not corrected for the number of specificities tested (Bonferroni correction) because the significant associations found in our series confirm previously recorded associations (Bodmer & Bodmer, 1974).

RESULTS

The frequencies of the various HL-A specificities in the control population did not differ significantly from the HL-A frequencies in the United Kingdom (Midlands) as described by Stokes *et al.* (1972) or from those in southern Scotland described in an earlier report (White *et al.*, 1973).

The frequencies of the various HL-A specificities in the control population, pernicious anaemia and atrophic gastritis are shown in Table 1. It can be seen that in pernicious anaemia there was a significant increase in the frequency of HL-A7 ($\chi^2 = 8{\cdot}29$; $0{\cdot}001 < P < 0{\cdot}005$)

TABLE 1. Frequencies of HL-A specificities in pernicious anaemia and atrophic gastritis

HL-A specificity	Normal (n = 400) population frequency (%)	Pernicious anaemia (n = 29)		Atrophic gastritis (n = 46)	
		Number positive	Frequency (%)	Number positive	Frequency (%)
HL-A1	42·5	13	44·8	16	34·8
HL-A2	45·8	12	41·4	24	52·9
HL-A3	26·5	12	41·4*	17	37·0*
HL-A9	11·8	4	13·8	8	17·4
HL-A10	11·0	2	6·9	1	2·2
HL-A11	8·0	1	3·5	3	6·5
W29	3·3	1	3·5	0	0
HL-A5	8·0	0	0	1	2·2
HL-A7	26·8	15	51·7†	13	28·3
HL-A8	29·5	9	31·0	13	28·3
HL-A12	29·0	9	31·0	15	32·6
HL-A13	2·5	0	0	2	7·3
HL-A14	7·5	2	6·9	5	10·9
HL-A17	11·0	1	3·5	3	6·5
HL-A27	7·0	0	0	1	2·2
W10	13·5	2	6·9	5	10·9
W15	7·5	3	10·3	4	8·7
W18	7·0	1	3·5	4	8·7
W22	1·3	1	3·5	0	0
HL-A3,7	14·3	9	31·0‡	7	15·2

* Increases in HL-A3 not significant at the 5% level.
† $\chi^2 = 8{\cdot}29$; $0{\cdot}005 < P < 0{\cdot}001$.
‡ $\chi^2 = 5{\cdot}85$; $0{\cdot}025 < P < 0{\cdot}01$.

TABLE 2. Frequencies of HL-A specificities in combined series of atrophic gastritis and pernicious anaemia

HL-A specificity	Normal (n = 400) population frequency (%)	Pernicious anaemia and atrophic gastritis (n = 75)		
		Number positive	Frequency (%)	Significance
HL-A3	26·5	29	38·7	$0{\cdot}025 < P < 0{\cdot}05$
HL-A7	26·8	28	37·3	$0{\cdot}05 < P < 0{\cdot}1$
HL-A3,7	14·3	16	21·3	$0{\cdot}1 < P < 0{\cdot}2$

and of the HL-A3,7 haplotype ($\chi^2 = 5{\cdot}85$; $0{\cdot}01 < P < 0{\cdot}02$) compared with the control group. A smaller increase in the frequency of HL-A3 was not statistically significant ($\chi^2 = 3{\cdot}00$; $0{\cdot}05 < P < 0{\cdot}1$). In atrophic gastritis there was a similar increase in the frequency of HL-A3 but this also was not statistically significant ($\chi^2 = 2{\cdot}26$; $0{\cdot}1 < P < 0{\cdot}2$). There was no tendency for the frequency of HL-A7 or of the HL-A3,7 haplotype to be increased in this group.

The frequencies of HL-A3, HL-A7 and the HL-A3, 7 haplotype in the whole series of patients with either pernicious anaemia or atrophic gastritis are shown in Table 2. When the data were pooled for the whole series of seventy-five patients, there was a significant increase in the frequency of HL-A3 ($\chi^2 = 4{\cdot}60$; $0{\cdot}02 < P < 0{\cdot}05$) but no significant increase in HL-A7 ($\chi^2 = 3{\cdot}48$; $0{\cdot}05 < P < 0{\cdot}1$) or the HL-A3, 7 haplotype ($\chi^2 = 2{\cdot}44$; $0{\cdot}1 < P < 0{\cdot}2$).

There was no change increase in the frequency of any other HL-A specificity in either group of patients or in the series as a whole.

The incidences of serum gastric parietal cell antibody or intrinsic factor antibody in both groups and in the whole series are shown in Table 3. The incidences of gastric parietal cell antibody and intrinsic factor antibody in pernicious anaemia correspond to those normally found (Irvine, 1965). Because of the bias in the selection of patients for gastric analysis the incidence of both antibodies in atrophic gastritis is considerably higher than usual (Fisher & Taylor, 1965). No HL-A specificity was found to be associated with an increased incidence of either autoantibody compared with the overall incidence in either patient group or in the series as a whole.

TABLE 3. The incidence of organ-specific antibodies in the patient groups

Patient group	Serum gastric parietal cell antibody			Serum intrinsic factor antibody		
	Number tested	Number positive	Percentage positive	Number tested	Number positive	Percentage positive
Pernicious anaemia	27	27	100	27	14	51·9
Atrophic gastritis	46	39	84·8	46	10	21·7
Whole series	73	66	90·4	73	24	32·8

DISCUSSION

The increased frequency of HL-A3 in the whole series of patients with pernicious anaemia or uncomplicated atrophic gastritis demonstrated in the present study confirms the association of HL-A3 with gastric autoimmune disease recorded by Whittingham *et al.* (1975). However, it is of considerable interest that in our series the patients with pernicious anaemia differ from those with atrophic gastritis in showing increased frequencies of both HL-A7 and the HL-A3,7 haplotype. This may correlate with the finding of an increased frequency of HL-A7 in the series reported by Whittingham *et al.* (1975). These authors did not, however, subdivide their series into those with and without pernicious anaemia, so it is uncertain if the preponderance of patients with pernicious anaemia (nineteen out of twenty-seven) was responsible for the increased frequency of HL-A7. This difference in the frequency of certain HL-A specificities in pernicious anaemia and atrophic gastritis is surprising, since there is considerable evidence that immunologically mediated injury to the gastric parietal cell mass results in a spectrum of disease ranging from atrophic gastritis with diminished acid secretion to pernicious anaemia. Thus the histological appearance of the gastric mucosa is identical in atrophic gastritis and pernicious anaemia, intrinsic factor secretion following histamine or pentagastrin stimulation in patients with atrophic gastritis is intermediate between normal and patients with pernicious anaemia (Irvine *et al.*, 1970), and the natural history of autoimmune atrophic gastritis is of a steadily increasing incidence of pernicious anaemia with each year of follow-up (Irvine *et al.*, 1974; Strickland & Mackay, 1973). The association of pernicious anaemia, but not autoimmune atrophic gastritis, with HL-A7 and the HL-A3,7 haplotype would therefore suggest that the progression from autoimmune atrophic gastritis to pernicious anaemia is under the influence of an HL-A-linked gene.

A number of other organ-specific autoimmune diseases have been shown to have associations with certain HL-A antigens. Thus HL-A8 has been shown to be associated with both Graves' disease (Grumet *et al.*, 1973) and idiopathic Addison's disease (Platz *et al.*, 1974). In addition HL-A8 and W15 have been shown to be associated with diabetes mellitus, particularly insulin-dependent diabetes (Nerup *et al.*, 1974). The recent demonstration of antibody to pancreatic islet cells in diabetes mellitus (Bottazzo, Florin-Christensen & Doniach, 1974; MacCuish *et al.*, 1974) provides support for the concept of an autoimmune form of diabetes mellitus; and permits the inclusion of diabetes mellitus in the spectrum of organ-specific autoimmune diseases. These associations of autoimmune endocrine disease with HL-A8 led Nerup *et al.* (1974) to suggest that HL-A8 or an HL-A8-associated immune response gene might be the common denominator for the development of endocrine autoimmunity. However, the findings of this present study and that of Whittingham *et al.* (1975), the frequency with which autoimmune atrophic gastritis and pernicious anaemia occur in association with autoimmune endocrine disease (Irvine & Barnes, 1974; Irvine, 1975), and the lack of an association of an HL-A specificity with Hashimoto's thyroiditis (Barnes & White, unpublished data) argue against this concept. The most attractive of the possible explanations for associations between HL-A antigens and disease is that immune response genes are closely linked to the major histocompatibility complex (McDevitt & Bodmer, 1974). In autoimmune thyroiditis in the mouse the presence of a histocompatibility-linked immune response gene having a role in the pathogenesis of the disease has been demonstrated by the relationship between a particular histocompatibility antigen, an antibody response to thyroglobulin and the pathological severity of disease (Vladutiu & Rose,

1971). Indeed, McDevitt & Bodmer (1974) consider that demonstrable association of these three factors is a prerequisite for concluding that a histocompatibility-linked immune response gene is involved in the pathogenesis of a disease. Thus in myasthenia gravis HL-A2 has been correlated with antibody to skeletal muscle (Feltkamp *et al.*, 1974), in multiple sclerosis high titre measles antibody has been correlated with HL-A3,7 and W18 (Jersild *et al.*, 1973) and in patients with coeliac disease and in control subjects an increased frequency of high titre antibody to gluten has been demonstrated in HL-A8-positive individuals compared with HL-A8-negative individuals (Scott *et al.*, 1974). We therefore considered the possibility that there might be an association between an HL-A specificity and the presence of an organ-specific serum autoantibody in pernicious anaemia and atrophic gastritis. However, in the present study no HL-A specificity was associated with an increased incidence of either serum parietal cell antibody or intrinsic factor antibody compared with the overall incidence in pernicious anaemia, atrophic gastritis or the series as a whole. This is in contrast to the association of HL-A3 with serum intrinsic factor antibody recently reported in patients with pernicious anaemia (Workshop on pernicious anaemia, diabetes mellitus and Addison's disease (Workshop, 1974). In addition to the report of the association of multiple sclerosis with HL-A3 and 7, the only other significant association reported with these antigens is that of paralytic poliomyelitis (Pietsch & Morris, 1974).

Since associations between HL-A antigens and disease may be investigated not only by population studies, but also by family studies, it is of interest that Mackay *et al.* (1974) have recently reported a sibship in which the HL-A3,7 haplotype is associated with gastric autoimmune disease. In population studies linkage disequilibrium between HL-A genes and closely linked loci, such as putative immune response genes, is required before HL-A typing can be used to detect associations between immune response genes and specific diseases (McDevitt & Bodmer, 1974) and the strength of the association between an HL-A specificity and a disease depends on the degree of this linkage disequilibrium. In family studies however, such linkage disequilibrium is not necessary and therefore further family studies are indicated to establish the strength of the relationship between HL-A3- and HL-A7-linked immune response genes and autoimmune atrophic gastritis and pernicious anaemia.

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