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HLA antigens in coeliac disease associated with malignancy

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SUMMARY Coeliac patients are at greater risk than the general population of developing malignant neoplasms, particularly lymphomas. The establishment at the Clinical Research Centre of a national collaborative study of coeliac patients with malignancy provided the opportunity to carry out HLA typing for 55 HLA-A, B and C and the 10 recognised DR antigens on a group of coeliac patients with malignancy. Study of a sample of 44 patients with biopsy proven coeliac disease and histologically confirmed malignancy, including 12 with malignant histiocytosis, and 57 coeliac patients without malignancy, failed to show any significant differences in antigen frequencies between patients with and without malignancy. These results indicate that there are no HLA genetic markers associated specifically with the development of malignancy in coeliac disease.

Patients with coeliac disease are at greater risk than the general population of developing neoplasms, principally malignant lymphomas, but also gastrointestinal malignancies.[1 2] In November 1978, in order to establish which non-lymphomatous malignancies complicate coeliac disease and to determine the histological nature of the associated lymphoma, a national collaborative study of coeliac patients with malignancy was set up at the Clinical Research Centre.

The results, based on analysis of 235 patients, showed that the predominant type of malignant lymphoma was a malignant histiocytosis, and that amongst the gastrointestinal malignancies, small intestinal adenocarcinomas and oesophageal carcinomas were the most frequent.[3]

Although previous studies of HLA associations with malignancies have yielded essentially negative results,[4] coeliac disease is strongly associated with certain HLA antigens, primarily HLA DR 3, and in some populations HLA DR 7.[5] The establishment of the register provided the opportunity to carry out HLA-A, B, C and DR antigen typing on a group of coeliac patients with malignancy.

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Methods

PATIENTS

Coeliac patients with malignancy

Coeliac patients with malignancy were those 44 from whom blood samples could be obtained among the 235 individuals included in the national collaborative study who are the subjects of a detailed report elsewhere.[3] All had biopsy proven coeliac disease and histologically confirmed malignancy.

Pathological diagnoses were established by a panel of nine pathologists* or by the chairman of the panel. Malignant lymphomas were classified using the classification adopted by the British National Lymphoma Investigation of non-Hodgkin's lymphomas.[6] Histiocytic cell tumours were further classified as malignant histiocytosis[7] or solid histiocytic tumours.

Forty seven histologically confirmed malignancies occurred in the 44 individuals in the present study. Seventeen (36·2%) were malignant lymphomas. Of the 15 malignant lymphomas which could be adequately classified on the material available, 12 (80·0%) were classified morphologically as

* Drs M H Bennett, A J Blackshaw, D R Davies, P Isaacson, F D Lee, D J Pollock, G Slavin (chairman), H Thompson, D G D Wight.

histiocytic cell tumours (Table 1). All 12 were further classified as malignant histiocytosis. Among the 30 other malignancies, 28 (93·3%) were invasive tumours (Table 2) and two squamous carcinoma *in situ*, one of skin and one of cervix. The most frequent individual gastrointestinal tumour was small intestinal adenocarcinoma, which occurred in four cases. Among the other gastrointestinal malignancies, three arose from rectum, two from caecum and one each from tongue, oesophagus, stomach, appendix, and pancreas. Three individuals each had two malignancies. One had malignant histiocytosis of the small intestine and a small intestinal adenocarcinoma, one squamous carcinomas of tongue and lung, and one squamous carcinomas of lung and skin.

Coeliac patients without malignancy

Coeliac patients without malignancy were 57 unrelated healthy adult patients with biopsy proven coeliac disease attending the gastroenterological clinic at Northwick Park Hospital. The mean age of these individuals when the sample was obtained was similar to the mean age at which malignancy developed among the patients with malignancy.

HANDLING OF SAMPLES

Venous blood samples were received in heparin from coeliac patients with malignancy within 24 hours of collection and the lymphocytes separated over Ficoll-Triosil. Venous blood samples from coeliac patients without malignancy were defibrinated on glass beads immediately after collection and the lymphocytes separated in a similar manner. From both groups cells in 10% dimethylsulphoxide were cryopreserved in liquid nitrogen by the two step cooling procedure and subsequently recovered for testing.[8]

TISSUE TYPING

Subjects were HLA typed for 17 of the A, 30 of the B and eight of the C antigens with a panel of 179 established antisera using a two-colour fluoro-

Table 1 *Morphological classification of malignant lymphomas*

Histological type	*No. (%)**
Well differentiated lymphocytic	1 (6·7)
Undifferentiated large cell	2 (13·3)
Histiocytic cell	12 (80·0)
Unclassified	2
Total	17

* % of malignant lymphomas which could be adequately classified on the material available.

Table 2 *Non-lymphomatous invasive malignancies*

*Site**	*No. (%)*
Gastrointestinal tract	14 (50·0)
Lung	3 (10·7)
Breast	1 (3·6)
Skin	1 (3·6)
Ovary	1 (3·6)
CNS	1 (3·6)
Testis	4 (14·3)
Other	3 (10·7)
Total	28 (100)

* Listed in the order in which they occur most frequently in the general population.

chromatic microlymphocytotoxicity procedure[9 10] based on the standard two stage NIH technique. Typing for the 10 recognised DR antigens was carried out on unfractionated B cells by the two colour fluorescence method[11] using a well validated panel of 60 selected antisera standardised against 7th International Histocompatibility Workshop reference cells and our own cell panel. This typing panel included 20 sera which were used in the 8th Workshop.

STATISTICAL ANALYSES

The strength of association with malignancy was estimated for each specificity by the relative risk calculated as Woolf's cross-product ratio with Haldane's correction applied whenever one of the compared values was null.[12] The significance of association was evaluated by Fisher's exact test, one sided. Probability values were not corrected for the number of comparisons made, but because of the number of such comparisons it was decided to regard as significant only values of $p<0·01$.

Results

No significant differences in antigen frequencies were found between the patients with and without malignancy (Table 3). Similarly comparison between the 12 patients with malignant histiocytosis and those without malignancy failed to reveal any significant differences.

Discussion

The coeliac patients with malignancy in the present study were those still alive in 1979 or who were diagnosed between 1979 and the end of March 1981. This has resulted in the sample containing proportionately fewer individuals with malignant lymphomas and proportionately more with gastrointestinal or other malignancies than in the entire

Table 3 *Distribution of HLA-A, B, C and DR antigens in coeliac patients with and without malignancy*

Antigen	Coeliacs with malignancy No. (%) positive N=42 for A, B and C antigens, 44 for DR antigens	Coeliacs without malignancy No. (%) positive N=57	Relative* risk
A 1	32 (76·2)	38 (66·7)	1·60
A 2	14 (33·3)	20 (35·1)	0·93
A 3	3 (7·1)	14 (24·6)	0·24
A 11	2 (4·8)	4 (7·0)	0·66
Aw 23	0 (0·0)	1 (1·8)	0·44
Aw 24	5 (11·9)	6 (10·5)	1·15
A 25	2 (4·8)	3 (5·3)	0·90
A 26	1 (2·4)	2 (3·5)	0·67
A 28	1 (2·4)	1 (1·8)	1·37
A 29	4 (9·5)	8 (14·0)	0·64
Aw 30	3 (7·1)	2 (3·5)	2·12
Aw 31	3 (7·1)	2 (3·5)	2·12
Aw 32	3 (7·1)	3 (5·3)	1·38
Aw 33	2 (4·8)	0 (0·0)	7·10
Aw 34	0 (0·0)	0 (0·0)	
Aw 36	0 (0·0)	0 (0·0)	
Aw 43	0 (0·0)	0 (0·0)	
B 7	2 (4·8)	9 (15·8)	0·27
B 8	38 (90·5)	45 (78·9)	2·53
B 13	2 (4·8)	2 (3·5)	1·38
B 14	6 (14·3)	4 (7·0)	2·21
B 18	3 (7·1)	3 (5·3)	1·38
B 27	1 (2·4)	3 (5·3)	0·44
Bw 35	1 (2·4)	6 (10·5)	0·21
B 37	0 (0·0)	0 (0·0)	
Bw 38	0 (0·0)	1 (1·8)	0·44
Bw 39	1 (2·4)	0 (0·0)	4·16
Bw 41	0 (0·0)	0 (0·0)	
Bw 42	0 (0·0)	0 (0·0)	
Bw 44	8 (19·0)	15 (26·3)	0·66
Bw 45	0 (0·0)	1 (1·8)	0·44
Bw 47	0 (0·0)	0 (0·0)	
Bw 49	2 (4·8)	0 (0·0)	7·10
Bw 50	2 (4·8)	1 (1·8)	2·80
Bw 51	2 (4·8)	2 (3·5)	1·38
Bw 52	0 (0·0)	0 (0·0)	
Bw 53	0 (0·0)	2 (3·5)	0·26
Bw 54	0 (0·0)	0 (0·0)	
Bw 55	0 (0·0)	0 (0·0)	
Bw 56	0 (0·0)	0 (0·0)	
Bw 57	0 (0·0)	0 (0·0)	
Bw 58	0 (0·0)	0 (0·0)	
Bw 60	2 (4·8)	2 (3·5)	1·38
Bw 61	0 (0·0)	1 (1·8)	0·44
Bw 62	3 (7·1)	2 (3·5)	2·12
Bw 63	0 (0·0)	0 (0·0)	
Bu/SV	0 (0·0)	0 (0·0)	
Cw 1	1 (2·4)	1 (1·8)	1·37
Cw 2	1 (2·4)	2 (3·5)	0·67
Cw 3	5 (11·9)	7 (12·3)	0·97
Cw 4	0 (0·0)	5 (8·8)	0·11
Cw 5	3 (7·1)	6 (10·5)	0·65
Cw 6	4 (9·5)	4 (7·0)	1·39
Cw 7	36 (85·7)	47 (82·5)	1·28
Cw 8	0 (0·0)	0 (0·0)	
DR 1	6 (13·6)	4 (7·0)	2·09
DR 2	4 (9·1)	4 (7·0)	1·33
DR 3	42 (95·5)	52 (91·2)	2·02
DR 4	3 (6·8)	10 (17·5)	0·34
DR 5	1 (2·3)	3 (5·3)	0·42
DRw 6	1 (2·3)	3 (5·3)	0·42
DR 7	16 (36·4)	22 (38·6)	0·91
DRw 8	0 (0·0)	0 (0·0)	
DRw 9	1 (2·3)	0 (0·0)	3·97
DRw 10	0 (0·0)	0 (0·0)	

* Assessing significance by Fisher's extract test, one sided, none of these values was significant at the 1% probability level uncorrected for the number of comparisons made.

national study. Nevertheless, in patients in the sample the predominant type of malignant lymphoma, malignant histiocytosis (Table 1) was the same as among patients from whom blood samples were not obtained, and patients with small intestinal adenocarcinoma and oesophageal carcinoma were represented. Thus patients in this study are reasonably representative of coeliac patients with malignancy as indicated by those included in the national collaborative study.

The principal findings were that there were no significant differences in antigen frequencies between coeliac patients with and without malignancy or between the subgroup of patients with malignant histiocytosis and those without malignancy. This agrees with the results of a recently published study from the west of Ireland involving a smaller number of patients, not all of whom were typed for DR antigens,[13] and suggests there are no specific HLA genetic factors associated with the development of non-lymphomatous malignancy or malignant histiocytosis in coeliac disease. The numbers of patients with small intestinal adenocarcinoma or oesophageal carcinoma were too small to compare with those without malignancy. The failure to find any specific HLA genetic factors associated with the development of malignancy could even in the present study be owing to the relatively small number of patients studied, but the corollary is that if there are any HLA factors associated specifically with the development of malignancy in coeliac disease, they are unlikely to enable identification of the patient particularly at risk.

The negative results of this study do not exclude the involvement of HLA factors in the development of malignancy in coeliac patients. In the present study 94 (93·1%) of the 101 coeliac patients were positive for HLA DR 3 showing that the strong association with this antigen found in other coeliac populations studied is also found in Britain. Further, 38 (37·6%) were positive for HLA DR 7, although the significance of this is uncertain without a sufficiently large and comparable group of healthy individuals without coeliac disease for comparison. Perhaps it is the possession of one or both of these antigens or of a genetic factor closely linked to those determining them which determines the response of a coeliac patient to an environmental factor, perhaps even gluten itself, and results in the development of malignancy.

We are greatly indebted to the many clinicians throughout the country who sent blood samples from patients under their care, to the UK Transplant Service, Bristol, which generously supplied most of the HLA-A, B and C antisera used in the study, and to the many centres which kindly donated DR antisera, particularly the University Hospital, Leiden. We are also grateful to Mr S Burman for help with the analyses, and Dr A J Levi for allowing us to collect control samples from coeliac patients under his care.

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