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J Clin Invest. 1972;51(6):1602-1605. <https://doi.org/10.1172/JCI106958>.

Concise Publication

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Predominance of Histocompatibility Antigen HL-A8 in Patients with Gluten-Sensitive Enteropathy

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ABSTRACT HL-A phenotypes were determined in 24 unrelated patients with gluten-sensitive enteropathy (GSE) using a lymphocyte microcytotoxicity test. 21 of the 24 patients had HL-A8 in the second segregant series, a frequency of 0.875. In contrast, the HL-A8 frequency in 200 normal individuals was 0.215 (difference significant at $P < 0.002$), and in 6 patients with villous atrophy due to tropical sprue or hypogammaglobulinemia the HL-A8 frequency was 0.17 (difference from normal not significant). The HL-A types in the families of three HL-A8 positive patients with GSE indicated that the HL-A8 antigen was inherited as an autosomal dominant. Frequencies of the other HL-A antigens in the GSE group did not differ significantly from that of the normal group. These findings are compatible with the hypothesis that GSE is due to the presence of an abnormal "immune response (Ir) gene," leading to the production of pathogenic antiglutin antibody or, alternatively, to the presence of a particular membrane configuration leading to the binding of gluten to epithelial cells with subsequent tissue damage.

INTRODUCTION

Studies with inbred strains of mice have shown that susceptibility to viral leukemias is genetically determined and at least in part associated with certain antigens of

This work has been presented in part at the Southern Sectional Meeting of the American Federation for Clinical Research, 27 January 1972.

Received for publication 10 January 1972 and in revised form 29 March 1972.

the major mouse histocompatibility locus (H-2) (1, 2). In man there is evidence that acute lymphocytic leukemia (3, 4) and Hodgkin's disease (5-7) are associated with an increased frequency of certain antigens of the major histocompatibility locus (HL-A).¹ Furthermore, glomerulonephritis (8) and systemic lupus erythematosus (SLE) (9), both non-neoplastic diseases, may be associated with a higher incidence of particular HL-A antigens. In the present investigation, we report on the HL-A specificities of a large group of patients with gluten-sensitive enteropathy (GSE), yet another disease in which a particular HL-A antigen occurs with increased frequency.

METHODS

HL-A typing. HL-A antigens were determined by the use of a lymphocyte microcytotoxicity method as previously described (10). Sera used to detect HL-A antigens were obtained from the Serum Bank maintained by the Transplantation and Immunology Branch of the National Institute of Allergy and Infectious Diseases (NIAID), from Doctors M. Jeannet and F. Kissmeyer-Nielsen, or from Hyland Laboratories, Los Angeles, Calif. Antigens typed for included HL-A1, 2, 3, 5, 7, 8, 9, 10, 11, 12, 13, W5, W10, W14, W19, W22, and W28. In all instances except W14, W19, and W28, where only 1 serum was used, at least 2 and as many as 10 sera were used to define each specificity. The following sera were used to identify HL-A8: Daly (Rogentine), Welch 65-9-12-18-01, Willet 51-5-08-05-01, Makepiece 1-16-0-10-09-01, D66-17910 VI 03-7-08-22-01, and Rose 2-16-0-10-30-01. Daly and Welch, and an additional

¹ Abbreviations used in this paper: HL-A, major histocompatibility locus; Ir, immune response; GSE, gluten-sensitive enteropathy; SLE, systemic lupus erythematosus.

TABLE I
*HL-A Antigen Frequencies in Normal Individuals and Patients with Gluten-Sensitive Enteropathy**

HL-A antigen	Normals (200)	GSE (24)
First segregant series		
1	0.350	0.666
2	0.415	0.458
3	0.215	0.166
9	0.265	0.083
10	0.095	0.292
11	0.085	0.083
W19	0.105	0.041
W28	0.045	0
Second segregant series		
5	0.120	0.083
7	0.200	0.083
8	0.215	0.875†
12	0.315	0.375
13	0.10§	0.041
W5	0.205	0
W22	0.07	0.083
W10	0.160	0.041
W14	0.075	0.166

* The number of individuals studied is in parentheses.

† $P < 0.002$.

§ Frequency determined in 50 individuals.

one to four of these sera were employed in typing each patient for HL-A8. (The numbers refer to the code of the serum as listed in the Catalog of Tissue Typing Reagents, NIAID, while the name in parentheses indicates the source of "Daly.") An antigen was assigned to an individual when all or nearly all sera specific for the antigen reacted with his cells. Studies with normals showed that reproducibility of replicate determinations performed on the same individual was 97%.

Patients. 24 unrelated patients were studied. These included 8 males and 16 females. 23 patients were Caucasian and 1 was Negro. Before inclusion in the study the diagnosis of GSE was established by the presence of each of the following criteria: (a) a history of unequivocal improvement of diarrhea after gluten restriction; (b) malabsorption of fat (steatorrhea greater than 5 g/day on a 100 g fat intake), and carbohydrate (D-xylose excretion less than 5 g/5 hr after a 25 g oral dose; (c) villous blunting associated with submucosal infiltration of plasma cells in jejunal biopsy specimens.

Family studies. HL-A phenotypes were determined in the relatives of three patients.

Control group. The control group comprised 200 normal male and female Caucasian blood bank donors of the NIH Clinical Center. An additional group of 50 normal blood bank donors were phenotyped concurrently with the patient group. Six other patients with malabsorption and villous blunting of the jejunal mucosa were studied. These patients who were not gluten sensitive included two with tropical sprue and four with hypogammaglobulinemia.

Statistical analysis. The individual HL-A antigen frequencies in the GSE group were compared with the an-

tigen frequencies in the control group. P values of differences in frequencies were ascertained by multiple normal deviate tests (11) which adjust the probability levels by correcting for the number of antigens in each segregant series. Significance was assigned to frequency differences for which P values were less than 0.01.

RESULTS

HL-A antigens occurring in patients with GSE. HL-A antigen frequencies of 200 normal individuals comprising the control population are shown in Table I. These frequencies are similar to those published by others for normal Caucasians (12, 13) and were also observed in 50 blood bank donors tested simultaneously with the GSE patients. HL-A antigen frequencies of the patients are also shown in Table I, while their HL-A phenotypes are shown in Table II. The most striking finding was that in the second segregant series HL-A8 was found in 21 of 24 patients corresponding to a frequency of 0.875. In contrast, HL-A8 was found at a 0.215 frequency in the group of control individuals. This difference is significant at the $P < 0.002$ level. Each of the three patients

TABLE II
*HL-A Phenotypes in 24 Patients with Gluten-Sensitive Enteropathy**

Patient	Sex	First segregant series	Second segregant series
G. K.	M	9/10	8/12
M. N.	F	1	8/12
L. M.	F	1/2	7/8
W. G.	M	1/11	8/W22
H. C.	F	2/10	8
F. F.	F	2/10	5/8
L. H.	F	1/10	8/12
D. R.	F	1	8/W10
T. B.	M	1/W19	8/W14
N. G.	M	2	7/8
B. W.	F	2/3	8/12
E. B.	F	1/2	8/12
B. B.	F	1/3	8/W14
E. D.	M	1/2	8/12
S. L.	F	1	8
J. R.	M	1/9	8
R. W.	F	1/2	8/W14
F. B.	M	1/10	8/13
D. R.	F	2/3	5/8
S. C.	F	1/10	8/W22
H. B.	F	1/11	8/W14
B. N.	F	2/10	12
L. H.	F	2/3	12
S. Mc.	M	1	12

* A single antigen reported in a segregant series indicates either a double dose of the antigen or the presence of an undefined antigen.

TABLE III
HL-A Genotypes of Three Patients with Gluten-Sensitive Enteropathy and their Families

Patient	Parents	Normal sibling(s)
G. K. (M) 9, 12/10, 8	D. K. (M) 9, 12/2, W10 J. K. (F) 1, ?*/10, 8	
L. M. (F) 1, 8/2, 7	R. M. (M) W28, W5/1, 8 B. M. (F) 2, 7/? , ?	D. M. (F) 1, 8/2, 7
B. B. (F) 3, W14/1, 8	H. B. (F) 2, W5/1, 8	

* ? = undefined antigen; M = male; F = female.

in the GSE group not having HL-A8 had an identical phenotype in the second segregant series, namely HL-A12 (B. N., L. H., S. Mc., Table II).

The frequency of other HL-A antigens in the GSE group was compared with that in the normal group, and no other significant differences were found (including HL-A1, 7, 9, 10 W5, W10). The HL-A1,8 and HL-A2,8 combinations were found frequently in the 21 patients who had HL-A8, but not significantly more frequently than in the 43 control individuals who had HL-A8 (0.75 vs. 0.84 frequency for 1,8, and 0.42 vs. 0.28 for 2,8 in patients and normals respectively). W5 was not found in any GSE patient but only occurred in 2 of 43 control individuals with HL-A8.

Family studies. Blood was available for typing from family members of patients G. K., L. M., and B. B. The genotypes of the families and patients are shown in Table III. HL-A8 is inherited from either parent in autosomal dominant fashion, as expected (12). It is therefore unlikely that HL-A8 is an acquired specificity or that it is a cross-reacting antigen found more frequently in patients with GSE.

Examination of the genotypes in the family of patient L. M. discloses that D. M., a young sibling (age 6) of L. M., had an identical HL-A genotype with the patient. D. M. does not have clinical evidence of GSE.

Other patients. The HL-A8 frequency in six other patients with malabsorption and blunting of the intestinal villi not due to GSE was 0.17, not significantly different from the control group.

DISCUSSION

In the present study we demonstrate that there is a pronounced increase in the occurrence of the histocompatibility antigen HL-A8 in patients with gluten-sensitive enteropathy. This antigen occurred at a 0.875 frequency in patients with GSE, whereas in a large group of normal individuals it occurred at a 0.215 frequency. This represents a higher degree of association between a disease state and a particular HL-A antigen than any previously reported (3-9).

HL-A antigens may be associated with disease by any of several mechanisms: (a) the antigens may cross-react with viral antigens leading to immunologic tolerance to the virus; this would result in increased susceptibility of individuals with certain HL-A antigens to particular viral infections; (b) the genes controlling the HL-A antigen may be fortuitously linked to, or be identical with, genes determining susceptibility to disease. In one example of this possibility HL-A genes may be linked to an "immune response (Ir) gene" which determines whether or not an individual is capable of making an immune response to certain antigens; such Ir genes have been extensively studied in mice and guinea pigs wherein the ability to make antibody responses to synthetic antigens appears to be linked to histocompatibility types (14); (c) HL-A antigens may serve as cell surface receptors for the attachment of infectious agents or other materials of pathologic significance.

The first of these possibilities is not likely to be relevant to GSE. The latter is clearly related to the ingestion of a food protein, gluten, and is alleviated or cured by elimination of this material from the diet. Furthermore, the disease does not behave epidemiologically as an infectious disease.

The latter two possibilities have greater likelihood of being relevant to GSE and require closer scrutiny. It is possible that the HL-A locus is, in some manner, linked to an Ir gene in GSE since a number of immunologic abnormalities have been demonstrated in this disease. Patients with GSE in relapse have increased serum IgA levels (15) associated with increased mucosal synthesis of IgA (16). More particularly, we have recently shown that patients with GSE but not normal individuals, respond to gluten challenge with an increase in mucosal IgA and IgM immunoglobulin synthesis; furthermore this increase is in large measure due to the synthesis of antigluten antibodies (17). These studies suggest that the local antigluten antibody response may mediate the mucosal injury seen in GSE patients. In parallel with studies in experimental animals (14), the ability of patients with GSE to mount this mucosal antibody response

may be determined by an Ir gene which may in turn be linked to an HL-A gene. This would be the first tangible example of an Ir gene in humans, which in this case would regulate production of IgA or IgM antibodies rather than only IgG immunoglobulins as has been demonstrated in animal systems (18). If this Ir gene hypothesis is correct, GSE could be thought of as a genetically determined disease characterized by an abnormal tendency to respond to the ingestion of gluten with the local synthesis of antigluten antibodies.

The possibility that HL-A8 represents a surface receptor site at which antigen (gluten) may bind is an equally plausible explanation for the association between GSE and particular HL-A antigens. In this regard, Rubin, Fauci, Sleisenger, and Jeffries (19) have demonstrated by immunofluorescence that intestinal epithelial cells of patients with GSE, but not normal individuals, bind gluten. Thus, one may postulate that the pathologic process in GSE involves the attachment of gluten to epithelial surfaces; as a result of such attachment tissue injury occurs or, alternatively, a local antigluten antibody response is elicited which actually mediates the tissue injury. If this receptor hypothesis is correct, GSE could be thought of as a disease characterized by an abnormal tendency to bind gluten at epithelial surfaces which, in turn, leads to local antibody production and tissue injury.

Even if one of these possibilities proves correct we must recognize that the gene determining the HL-A8 type cannot be the sole factor responsible for the occurrence of GSE, since not every patient with GSE has HL-A8. Furthermore, the vast majority of individuals with HL-A8 (20% of the Caucasian population) do not have GSE, and a sibling of patient L. M. had an identical HL-A genotype but no evidence of GSE. Nevertheless, the presence of an increased incidence of a particular HL-A antigen in patients with GSE is compatible with the concept that there is a genetic predisposition for the acquisition of gluten-sensitive enteropathy and that immunological responses are primarily involved in its pathogenesis.

ACKNOWLEDGMENTS

We extend our thanks to Doctors Arthur Cocco, Sidney Malawer, and Irving Brick for referring to us 10 of the patients in this study, and for making available the data establishing the diagnoses; to Dr. John Gart for assistance with the statistical analysis; and to Mrs. Blanche Fors for secretarial assistance with the manuscript.

REFERENCES

- Lilly, F., E. A. Boyse, and L. J. Old. 1964. Genetic basis of susceptibility to viral leukaemogenesis. *Lancet*. 2: 1207.
- Tennant, J. R. 1965. Susceptibility and resistance to viral leukemogenesis in the mouse. II. Response to the virus relative to histocompatibility factors carried by the prospective host. *J. Nat. Cancer Inst.* 34: 633.
- Walford, R. L., S. Finkelstein, R. Neerhout, P. Konrad, and E. Shanbrom. 1970. Acute childhood leukaemia in relation to the HL-A human transplantation genes. *Nature (London)*. 225: 461.
- Rogentine, N. G., R. A. Yankee, J. J. Gart, J. Nam, and R. J. Trapani. 1972. HL-A antigens and disease. Acute lymphocytic leukemia. *J. Clin. Invest.* In press.
- Amiel, J. L. 1967. Study of leukocyte phenotypes in Hodgkin's disease. In *Histocompatibility Testing 1967*. E. S. Curtoni, P. L. Mattiuz, and M. R. Tosi, editors. Munksgaard, A/S, Copenhagen, Denmark. 79.
- Zervas, J. D., I. W. Delamore, and M. C. G. Israëls. 1970. Leucocyte phenotypes in Hodgkin's disease. *Lancet*. 2: 634.
- Forbes, J. F., and P. J. Morris. 1970. Leucocyte antigens in Hodgkin's disease. *Lancet*. 2: 849.
- Patel, R., M. R. Mickey, and P. I. Terasaki. 1969. Leucocyte antigens and disease: I. Association of HL-A2 and chronic glomerulonephritis. *Brit. Med. J.* 2: 424.
- Grumet, F. C., A. Coukell, J. G. Bodmer, W. F. Bodmer, and H. O. McDevitt. 1971. Histocompatibility (HL-A) antigens associated with systemic lupus erythematosus. A possible genetic predisposition to disease. *N. Engl. J. Med.* 285: 193.
- Mittal, K. K., M. R. Mickey, O. D. Singal, and P. I. Terasaki. 1968. Serotyping for homotransplantation. XVIII. Refinement of microdroplet lymphocyte cytotoxicity test. *Transplantation*. 6: 913.
- Wilks, S. S. 1962. *Mathematical Statistics*. John Wiley & Sons, Inc., New York. 290.
- Dausset, J., J. Columbani, L. Legrand, and M. Fellous. 1970. Genetics of the HL-A system. Deduction of 480 haplotypes. In *Histocompatibility Testing 1970*. P. I. Terasaki, editor. Munksgaard, A/S, Copenhagen, Denmark. 53.
- Albert, E. D., M. R. Mickey, A. C. McNicholas, and P. I. Terasaki. 1970. Seven new HL-A specificities and their distribution in three races. In *Histocompatibility Testing 1970*. P. I. Terasaki, editor. Munksgaard, A/S, Copenhagen, Denmark. 221.
- McDevitt, H. O., and B. Benacerraf. 1969. Genetic control of specific immune responses. *Advan. Immunol.* 11: 31.
- Asquith, P., R. A. Thompson, and W. T. Cooke. 1969. Serum-immunoglobulins in adult coeliac disease. *Lancet*. 2: 129.
- Loeb, P. M., W. Strober, Z. M. Falchuk, and L. Laster. 1971. Incorporation of L-leucine-¹⁴C into immunoglobulins by jejunal biopsies of patients with celiac disease and other gastrointestinal diseases. *J. Clin. Invest.* 50: 559.
- Falchuk, Z. M., L. Laster, and W. Strober. 1971. Gluten-sensitive enteropathy: Intestinal synthesis of anti-gluten antibody *in vitro*. *Clin. Res.* 19: 390. (Abstr.)
- Bechtol, K. B., F. C. Grumet, G. F. Mitchell, and T. G. Wegmann. 1971. Genetic control of the immune response to branched synthetic polypeptide antigens in inbred mice. In *Progress in Immunology*. First International Congress of Immunology. Bernard Amos, editor. Academic Press, Inc., New York. 495.
- Rubin, W., A. S. Fauci, M. H. Sleisenger, and G. H. Jeffries. 1965. Immunofluorescent studies in adult celiac disease. *J. Clin. Invest.* 44: 475.