

Dermatitis Herpetiformis

IMMUNOLOGIC CONCOMITANTS OF SMALL INTESTINAL DISEASE AND RELATIONSHIP TO HISTOCOMPATIBILITY ANTIGEN HL-A8

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ABSTRACT In the present study the relation between the gluten-sensitive intestinal lesion observed in dermatitis herpetiformis (DH) and in gluten-sensitive enteropathy (coeliac sprue) (GSE) was analyzed. Jejunal IgA synthesis in DH was estimated from the extent of incorporation of [¹⁴C]leucine into IgA in jejunal biopsy specimens during short-term in vitro culture. Patients with DH have significantly elevated incorporation values as compared to normal control individuals ($18,880 \pm 13,614$ vs. $5,830 \pm 3,190$ cpm/mg tissue protein/90 min) ($P < 0.02$) and the degree of elevation correlates well with the degree of morphologic abnormality. Thus patients with DH are similar to patients with GSE where elevated local mucosal IgA synthesis has also been observed.

By using both morphologic and immunologic criteria for evaluating intestinal status, patients with DH and intestinal disease were distinguished from patients with DH free of intestinal disease. Of the eight patients in the former group, seven carried HL-A8 (87.5%), an incidence which is strikingly similar to that observed in patients with GSE alone (88.5%). In contrast, of the seven patients in the latter group (without gastrointestinal disease) two had HL-A8, an incidence (27%) not significantly different from that in the normal population (20%) ($P > 0.1$).

Thus, both in respect to local mucosal increase in IgA synthetic rates and in respect to the association with HL-A8, the intestinal lesion of DH is similar to that of GSE.

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INTRODUCTION

The skin disorder dermatitis herpetiformis (DH)¹ is associated with an intestinal abnormality resembling that observed in gluten-sensitive enteropathy (GSE). The association was originally described by Marks, Shuster, and Watson (1), who found villous atrophy in approximately 67% of DH patients. Other workers have confirmed this finding (2-4) although Brow, Parker, Weinstein, and Rubin (5) found villous abnormalities in 21 of 22 DH patients utilizing a multiple biopsy technique. In subsequent studies, it was suggested that the villous atrophy, like that of GSE, is improved by withdrawal of gluten from the diet (6). However, the lesion in DH is different from that in GSE in that it tends to have a patchy distribution and (perhaps as a result) is usually not associated with symptomatic malabsorption (5, 6).

A further association between DH and GSE is that in both diseases there is a high incidence of the histocompatibility antigen, HL-A8 (7, 8). It is as yet unclear how a gene for histocompatibility operates in relation to the development of these diseases but from a variety of evidence, it is felt that immunologic factors may be involved (7, 8).

In the present studies we have carried forward analysis of the relation between DH and GSE in two ways. First, we estimated jejunal IgA synthetic rates in DH patients by measuring incorporation of labeled amino

¹ *Abbreviations used in this paper:* DH, dermatitis herpetiformis; GI, gastrointestinal; GSE, gluten-sensitive enteropathy.

acid into IgA by jejunal mucosal biopsies in vitro; our aim was to establish whether or not DH patients are similar to patients with GSE, who have been previously shown to have increased jejunal IgA production by this technique (9). Second, we analyzed a DH patient group with regard to HL-A type and presence of intestinal lesions as determined by morphologic, enzymatic, and immunologic criteria; our aim was to determine whether gastrointestinal (GI) tract abnormalities in DH was related to the presence or absence of HL-A8.

METHODS

Patients and controls. 15 patients with DH were studied; this included 14 patients who underwent complete studies as described below and one patient who underwent HL-A phenotyping and jejunal biopsy only. These patients ranged in age from 22 to 72 yr and consisted of six females and nine males. The diagnosis of DH was established in each case on the basis of: (a) characteristic history and physical examination marked by episodes of pruriginous vesicles occurring most frequently on extensor surfaces; (b) skin biopsy demonstrating subepidermal vesicles accompanied by a polymorphonuclear cell and eosinophil infiltrate; (c) prompt response of lesions to either diaminodiphenylsulfone (dapsone) or sulfapyridine with relapse upon subsequent withdrawal of medication; and (d) absence of circulating antibodies to basement membrane, thus ruling out the diagnosis of bullous pemphigoid. The average duration of skin disease in this study group was 9 yr. At the time of study, 11 patients were receiving sulfone treatment, three patients were being treated with sulfapyridine, and one patient was receiving no therapy.

Patients with GSE in exacerbation consisted of ten individuals with a positive history of malabsorption syndrome, documented malabsorption of fat and D-xylose, clinical responses to gluten restriction, and a typical jejunal histologic picture. At the time of determination of IgA incorporation values, these patients had been on a gluten-containing diet for at least 7 days.

Control populations were studied to determine normal IgA incorporation rates (*vide infra*) and to establish the normal frequency of HL-A phenotypes. IgA incorporation values were determined in ten normal volunteers without dermatologic or GI symptoms and with normal jejunal histology. Normal HL-A frequencies were established in 350 blood bank donors as previously reported (7, 8). These values are in essential agreement with previously reported frequencies obtained in other populations.

The purpose of the study was described to all participants and informed consent was obtained in each instance.

Determination of IgA incorporation values. Jejunal biopsies were obtained with a Rubin biopsy tube (10) positioned at the ligament of Treitz. Estimates of mucosal IgA synthetic rates were made by determining the extent of incorporation of [¹⁴C]leucine into IgA by jejunal biopsy specimens during incubation for 90 min (cpm/mg protein incubated/90 min incubation). Incorporation of labeled amino acid was determined by a previously described technique utilizing a solid-phase immunoadsorbent, bromoacetyl cellulose-anti-IgA (9). This technique has previously been shown to be monospecific for IgA and yields appropriately low values in patients with selective IgA deficiency and/or hypogammaglobulinemia.

Brush border enzyme activities. Brush border enzyme activities of biopsy specimens were determined on all specimens except one. Alkaline phosphatase was assayed by a modification (11) of the method of Bessie, Lowry, and Brock (12) with para-nitrophenylphosphate as the substrate. Sucrase and trehalase activities were assayed by a modification (11) of the method of Dahlqvist (13). Enzyme values in specimens were normalized with respect to tissue protein; for this purpose, tissue protein was determined by the method of Lowry, Rosebrough, Farr, and Randall (14).

Absorptive function studies. Patients were evaluated for absorptive function with D-xylose absorption studies, as well as serum carotene and folic acid determinations. D-Xylose absorption was tested by oral administration of 25 g of D-xylose and measurement of subsequent 5-h urinary excretion of the pentose, using the method of Roe and Rice (15); less than 20% excretion during this interval was considered abnormal. Serum carotene was determined by extraction of serum with alcohol and petroleum ether (16) and serum folate was determined by a microbiological assay utilizing the organism *Lactobacillus casei* (17).

Biopsy studies. Interpretation of jejunal morphology after tissue fixation in a formaldehyde-mercuric chloride-sodium acetate solution was made by three independent observers, who had no prior knowledge of HL-A type of the patients under study. Degree of intestinal abnormality was defined as follows: "1+" represents minimal but definite villous blunting and mild infiltration of the lamina propria with plasma cells and lymphocytes; "2+" represents moderate villous blunting and lamina propria infiltration; "3+" represents a villous to crypt height ratio of less than one and heavy cellular infiltration; "4+" represents loss of villi with deep crypts, epithelial cell disarray, and heavy inflammatory cell infiltration of the lamina propria; categories +1 and +2 were considered partial villous atrophy and categories +3 and +4 were considered severe villous atrophy.

HL-A typing. HL-A antigens were determined by the NIH Histocompatibility Typing Center using a lymphocyte microcytotoxicity method as previously described (18). 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 and from the sera collection of the typing center. The reproducibility of the assay when replicate determinations were done in normal individuals was 97%. It has previously been demonstrated that drugs used for therapy of DH do not affect the ability to correctly determine presence or absence of HL-A8 (8).

Statistics. All determinations of statistical significance were made by utilizing Student's *t* test.

RESULTS

IgA incorporation values in patients with DH. The mean value for incorporation of [¹⁴C]leucine into IgA by jejunal biopsy specimens in normal individuals was $5,830 \pm 3,190$ cpm/mg tissue protein/90 min (mean \pm SD) (Fig. 1). This value was associated with normal jejunal morphology and normal absorptive function. In contrast, the mean incorporation values for DH patients was increased to $18,880 \pm 13,614$ cpm/mg/90 min. This level of incorporation is significantly greater than the normal level ($P < 0.02$) and is in the range

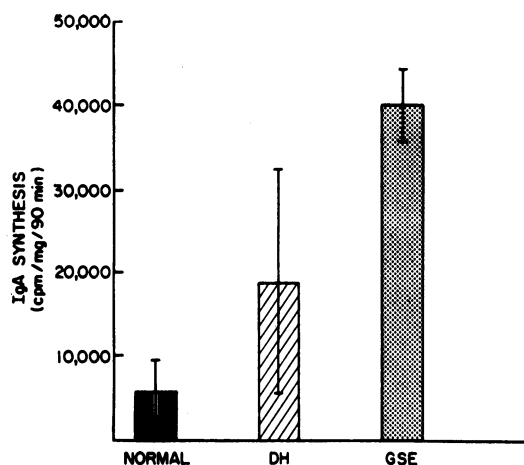


FIGURE 1 IgA synthesis in normal individuals, patients with DH, and patients with active GSE. The bars indicate mean \pm SD.

of values observed in patients with GSE (Fig. 1) previously and concurrently studied.

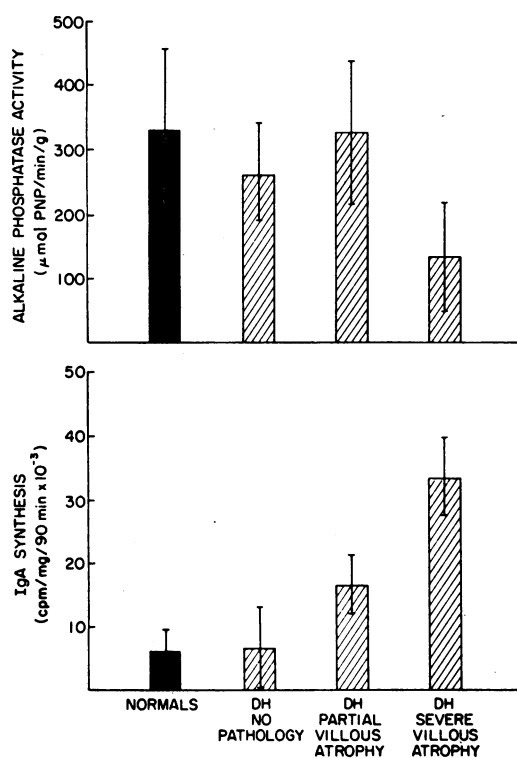


FIGURE 2 IgA synthesis and alkaline phosphatase values of normal individuals and 14 patients with DH in various histologic categories (see text). The DH-no pathology group has six patients including one patient with normal histology studied 5 mo after treatment for intestinal giardiasis. The partial villous atrophy and severe villous atrophy groups include five and three patients, respectively. PNP, para-nitrophenylphosphate.

Although the mean incorporation value of the DH patient group was increased above normal, not all the DH patients had abnormal values. In particular, if degree of histologic abnormality was correlated with IgA incorporation value, it became evident that greater degrees of histologic abnormality were associated with greater incorporation values. Thus, in Fig. 2, it is seen that incorporation values in various categories of histologic abnormality increase progressively as the morphological changes of villous atrophy and inflammatory cell infiltration of the lamina propria become more marked. One apparent exception to this pattern is a patient (J. M.) with normal histology associated with an incorporation value of 17,722 cpm/mg/90 min. This patient was biopsied 5 mo after treatment of intestinal giardiasis and the elevated IgA incorporation value may be a late manifestation of this extraneous disease.

No correlation between therapeutic drug type or dosage and villous abnormality or IgA incorporation value was apparent. The single patient on no therapy displayed a normal biopsy and incorporation value.

Brush border enzyme activities and absorptive function of DH patients. As indicated in Fig. 2 and Table I, mean brush border alkaline phosphatase values as well as mean disaccharidase values were not significantly different from normal ($P > 0.3$) in the patient groups with normal or mild histologic changes but were significantly less than normal ($P < 0.05$) in the patient group with severe histologic changes and the highest IgA incorporation values. D-Xylose absorption and serum carotene levels were normal in all patient groups (mean carotene value 136 ± 82 vs. normal > 60 μ g/100 ml) and serum folate values tended to be borderline low (6.4 ± 4.2 vs. normal > 5.0 ng/ml), perhaps because of drug therapy (3).

TABLE I
Disaccharidase Activity in Jejunal Biopsy Specimens
of Patients with DH*

	Sucrase	Trehalase
Group I (Normal Histology)	35.5 ± 7.7 (6)†	11.5 ± 6.0 (6)
Group II (Partial villous atrophy)	40.2 ± 10.2 (5)	12.1 ± 3.3 (5)
Group III (Severe villous atrophy)	30.0 ± 15.1 (3)	4.6 ± 4.2 (3)
Normal values	52.9 ± 17.9 (12)	14.9 ± 8.6 (12)

* Mean \pm SD. Units = micromoles glucose liberated per gram tissue protein per minute.

† Numbers in parentheses indicate number of patients studied.

Correlation of intestinal disease and HL-A type. By using both intestinal morphology and IgA incorporation values to assess the presence of intestinal disease, it was possible to determine HL-A8 frequency in patients with jejunal disease and in patients without jejunal disease. In the population of 15 DH patients studied, eight or 53% were HL-A8-positive. The HL-A8 frequency in this population does not necessarily reflect the true frequency of HL-A8 in DH because we specifically sought out HL-A8-negative patients for study.

In those patients with intestinal disease as defined by the presence of both 1+ or greater histologic abnormalities and IgA incorporation values greater than 1 SD above the normal mean, it was found that seven out of eight patients (87.5%) were of the HL-A8 phenotype (Fig. 3). This value is strikingly similar to that found in patients with GSE unassociated with DH in two separate studies (7, 19). In contrast, the frequency of HL-A8 in patients with DH unassociated with morphologic and immunologic evidence of intestinal disease was only 28% (two out of seven patients). This frequency is not significantly different from the HL-A8 frequency in normal persons (20%) (7, 8).

DISCUSSION

The association of gluten-sensitive villous atrophy and DH is now well established (1-6). It remains to be demonstrated, however, that the intestinal lesion of DH is identical to or different from that found in GSE and that the pathophysiology of the intestinal lesion of DH is or is not related to that of the skin lesion of DH. In the present study we provide two interrelated pieces of data relevant to these questions. First, we demonstrate that jejunal IgA synthesis, as reflected by incorporation of [¹⁴C]leucine into IgA by jejunal biopsy specimens *in vitro*, is increased in jejunal tissue from patients with DH and that this increase correlates with the degree of morphologic change. Second, we demonstrate that, just as in GSE, intestinal abnormalities are highly correlated with the presence of HL-A8.

With regard to the increased mucosal IgA synthesis, a similar abnormality in mucosal IgA synthesis has previously been demonstrated in patients with GSE and, in both diseases, this increased IgA synthesis is associated with elevated circulating IgA levels (20, 21). Thus, at least with regard to IgA immunoglobulin production, the intestinal lesions of GSE and DH are similar. We have previously shown that in GSE, a substantial portion of the newly labeled immunoglobulin had anti-gliadin specificity (22). In evaluating the identity of the intestinal abnormalities of GSE and DH it will be important to determine if the increased mucosal IgA synthesis is, in DH, also composed largely of antibodies with anti-gliadin specificity.

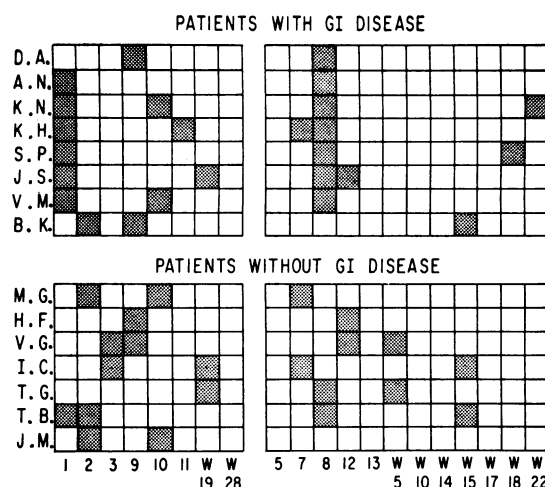


FIGURE 3 HL-A phenotypes of 15 patients with DH. The left-hand blocks include the antigens in the first segregant (LA) series; the right-hand blocks include the antigens in the second segregant (four) series.

It is important also to mention the differences between the GI abnormalities in DH and GSE. Specifically, the lesion in DH usually has a patchy distribution and, perhaps as a result of this, is usually not associated with clinically significant malabsorption (5). Furthermore, McClelland et al. (23) has shown that the IgA concentration in jejunal aspirates of patients with DH was increased, in keeping with the findings presented here; in contrast, the IgA concentration in jejunal aspirates from "coeliac" patients was essentially normal. Finally, in as yet unpublished studies, we have found that gluten peptides do not affect alkaline phosphatase increases in epithelial cells during organ culture of jejunal tissue obtained from patients with DH and normal individuals whereas inhibition of such increases is regularly produced in jejunal tissue from patients with GSE.^a These various differences between DH and GSE are consistent with the view that even if the basic mechanisms of tissue injury in DH and GSE are similar, pathologic processes specific to each of the two diseases can be, and probably are, present.

Regarding the correlation of HL-A8 and intestinal disease, in this study and a companion study of 28 unselected English DH patients, we have shown that the incidence of HL-A8 in DH patients with jejunal disease is identical to the incidence of HL-A8 in GSE patients (24). In contrast, the incidence of HL-A8 in DH patients without jejunal disease is similar to the incidence in normal individuals (24). The present study represents an independent analysis of the incidence of

^aR. L. Gebhard, H. Kjaeveman, G. Sessoms, and W. Strober. Behavior of tissue from patients with dermatitis herpetiformis in organ culture. Manuscript in preparation.

HL-A8 in DH and is perhaps more rigorous than the previous analysis in that the presence or absence of intestinal disease was evaluated by two criteria: morphology and local mucosal IgA synthesis. Taken together, the two studies demonstrate that HL-A8 is related to the gluten-sensitive intestinal disease in both DH and GSE. One may also draw the conclusion that there are two forms of DH, one form associated with HL-A8 and intestinal disease and one form not so associated.

The nature of the relationship between HL-A8 and gluten-sensitive intestinal disease is not understood. One possibility is that the HL-A8 gene may be linked in DH and GSE patients with a pathologic immune response gene which, in both cases, predisposes to the synthesis of destructive antibodies. A precedent for such a phenomenon is found in inbred animal systems where histocompatibility genes are linked to the ability to respond to particular polypeptide antigens (25). It is therefore possible that because of the presence of abnormal immune response genes, both DH and GSE patients respond to polypeptides released during digestion of gluten by producing pathogenic antibody at mucosal sites. The finding of increased jejunal IgA production in both DH patients and GSE patients is consistent with this idea.

Alternatively, since the HL-A8 antigen is a cell surface protein, it may be part of an epithelial cell surface configuration which is capable of binding gluten peptides in patients with GSE and DH. This binding phenomenon may lead to the local production of antibodies directed toward gluten peptides and subsequent epithelial cell injury form antibodies affixing to cells at the site of antigens (gluten peptides) already bound to cells. This hypothesis has the advantage that it can be tested experimentally, and, in fact, Rubin, Fauci, Sleisenger, and Jeffries (26) have already demonstrated by immunofluorescence that gliadin binds to epithelial cells of GSE patients but not of normal individuals.

Whichever of these alternatives prove true, the question remains unanswered as to why gluten-sensitivity makes its appearance in only certain individuals with HL-A8 and particularly in individuals with DH and HL-A8. The answer is likely to lie in the necessity for additional genes which interact with the gene responsible for HL-A8 (or a gene closely linked with the HL-A8 gene) to produce disease. For instance, one may hypothesize that there exists several genes, A, B, and C which can interact in various combinations to produce disease. Arbitrarily, B is identical to the gene responsible for HL-A8 (or a gene closely linked with the HL-A8 gene); gene A may act in concert with B to produce both DH and gluten-sensitivity (conceivably by separate pathologic mechanisms). Gene C may

also act in concert with B to produce GSE; in this case C is identifiable only through its interaction with B and, unlike A, does not produce a second disease when present with B.

If this scheme is correct we might expect that the gluten-sensitivity due to the A and B interaction might differ, however subtly, from that due to the B and C interaction; this appears to be true in that the GI lesion in DH is patchy and mild whereas that in ordinary GSE is diffuse and usually more severe. Referring back to the explanation of GSE that has, as its basic feature, an abnormal attachment of gliadin to the gut epithelial cell, one might conceive of genes A, B, and C as determinants of surface proteins which must be present in close juxtaposition in order to result in binding; thus, the combination of genes A and B or the combination of genes B and C, but not any of these genes alone, result in the abnormal binding phenomenon and only in the presence of these gene pairs is disease seen.

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