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Adenovirus 12 and Dermatitis Herpetiformis

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Previous studies have shown that A-gliadin, a major gluten-derived peptide known to activate the gluten-sensitive enteropathy (GSE) of coeliac disease (CD), and the non-structural E1B peptide produced during early lytic gastrointestinal infection with human adenovirus type 12 (AD12) share an identical twelve amino acid sequence. It is suggested that immunological cross reactivity between these two peptides may play an important role in the pathogenesis of CD. As a milder but histologically identical GSE is found in the majority of patients with dermatitis herpetiformis (DH), a condition also thought to be caused by dietary gluten, we postulated that AD12 may also be involved in the pathogenesis of DH. To test this we assayed sera from 40 patients with DH and 18 healthy controls for AD12-neutralizing antibodies as evidence of past viral exposure. Detectable AD12 antibodies (titre of 13 or higher) were found in only 15% (6/40) of DH patients, compared with 27.8% (5/18) controls (not significant). These findings do not support a causative role for AD12 in the pathogenesis of DH.

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The GSE in CD is thought to be caused by a localized cell-mediated immune response against dietary gluten-derived peptides including A-gliadin (1). AD12 is a double-stranded DNA virus which has been isolated from the faeces of children (2). The E1B protein is a non-structural protein encoded by the AD12 genome and expressed early during lytic viral infection (3). A region of sequence homology spanning twelve amino acids has been shown between this AD12 E1B protein and A-gliadin (4), and antibodies directed against synthetic AD12 E1B peptides (containing this twelve amino acid sequence) cross-react with A-gliadin (4). Kagnoff et al. showed that patients with CD have a high level of serum AD12-neutralizing antibodies, suggesting that these individuals have a high incidence of exposure to this virus (5). Patients with CD also show cellular hypersensitivity to synthetic AD12 peptides which contain this twelve amino acid sequence (6, 7). Taken together, these studies provide circumstantial evidence to suggest that immunogenic cross reactivity between the AD12 E1B protein and A-gliadin may play an important role in the pathogenesis of CD. However, Howdle et al. (8) found no evidence of serum antibodies against an AD12 EIB-58kDa peptide in CD patients and questioned the validity of these assumptions. As DH and CD share a number of features, namely a histologically identical GSE and immunogenetic profile, and are both thought to be caused by dietary gluten (9–11), we hypothesized that AD12 may play a pathogenic role in DH.

The primary aim of this study was to determine if patients with DH have a high incidence of exposure to AD12 by comparing the prevalence of AD12-neutralizing antibodies in the sera of patients with DH and healthy controls. Secondarily we hoped to determine if prevalence of serum AD12-neutralizing antibodies was related to the duration of disease.

MATERIALS AND METHODS

This was a case control study. The study population consisted of patients in N. Ireland with DH diagnosed between January 1, 1971 and December 31, 1991. The following were the criteria for diagnosis: (a) clinical and histological features in keeping with a diagnosis of DH and (b) granular deposits of IgA in the dermal papillae or along the basement membrane on direct immunofluorescence of lesional or perilesional skin. The study population was divided into three groups: (a) patients with long-standing DH diagnosed between January 1, 1971 and December 31, 1988 (old DH); (b) patients with recently diagnosed DH between January 1, 1989 and December 31, 1991 (new DH); and (c) age and sex-matched controls. Groups (a) and (c) were recruited between February 1, 1988 and July 31, 1988 and group (b) between January 1, 1991 and December 31, 1991. The control group was recruited from healthy staff in the dermatology and genito-urinary medicine clinics, Royal Victoria Hospital, Belfast.

All patients and controls were seen by one of the authors (JH). They answered a standard questionnaire and had samples of venous

Table I. *Demographic data of patients with DH and controls*

STVA = subtotal villous atrophy.

	New DH	Old DH	Control	*p*
Total no.	22	18	18	
No. males	13	10	9	
No. females	9	8	9	
Mean age (yrs)	43.9	53.9	51.4	
Mean duration of disease (mths)	13.2	89.3		<0.001
No. patients having jejunal biopsy	12	10		
No. patients with STVA (% of those biopsied)	10 (83)	8 (80)		
No. patients on gluten-free diet (%)	16 (73)	11 (61)		
No. on dapsone (%)	14 (64)	7 (39)		
No. patients totally symptomatic (%)	8 (36)	17 (94)		<0.001
No. patients with serum gliadin antibodies (IgG or IgA) (>1/10) (%)	12 (55)	12 (67)	0	<0.05

Table II. *Serum AD12-neutralizing antibodies in patients with DH and controls*

	New DH	Old DH	All DH	Controls
No. (%) with AD12 antibodies (>1/13)	0	6 (33.3)	6 (15)	5 (27.8)
Total number of subjects	22	18	40	18

blood taken for measurement of the following: (a) AD12-neutralizing antibodies and (b) gliadin antibodies. AD12-neutralizing antibodies are against adenovirus-type specific antigens on the outer surface of the virus particle which are associated with the hexon protein and gamma determinant of the fibre protein (12). Measurement of serum AD12-neutralizing antibodies was as follows. AD12 was obtained from the Central Public Health Laboratory, Colindale, London. Patients' sera were inactivated at 56°C for 30 min, then neutralized with 100 TCD50 (tissue culture doses 50% endpoint) of AD12 for 1 h at room temperature. Virus/serum mixtures were inoculated in duplicate into flat bottomed microwell plates containing HEp2 cell monolayers, and the cytopathic effects were read daily. Antibody titres were expressed as reciprocals of 50% endpoint dilutions. Indirect immunofluorescence was used to detect serum IgA and IgG gliadin antibodies (13).

Statistical analysis

Student's *t*-test, Mann-Whitney U-test and chi squared test with Yate's correction were used to a significance of $p < 0.05$.

RESULTS

Demographic details of both patients and controls are summarized in Table I. There were no significant differences in age or sex between patients with DH, both recent onset and long-standing, and controls. Notable was: (a) duration since initial diagnosis was significantly longer in patients with long-standing DH (mean 89.3 months) than in patients with recently diagnosed DH (mean 13.2 months) ($p < 0.001$); (b) more long-standing than recently diagnosed DH patients were completely asymptomatic (17/18 v. 8/22, $p < 0.001$); (c) gliadin antibodies were detected in 24/40 patients with DH but not in any of the controls ($p < 0.05$); and (d) over 80% of patients with DH who were biopsied had evidence of GSE.

Table II compares the prevalence of serum AD12-neutralizing antibodies between patients with DH and controls. Overall the serum antibody prevalence did not differ significantly between patients and controls. However, both patients with long-standing DH and controls had a higher prevalence of serum AD12 antibodies than patients with recently diagnosed DH ($p < 0.02$, $p < 0.05$ respectively).

DISCUSSION

The prevalence of serum AD12-neutralizing antibodies was both low and did not differ significantly between patients with DH (15.7% had a detectable titre) and controls (27.8%), suggesting a similarly low incidence of past exposure to this virus in both these groups. Significantly more patients with long-standing DH (6/18) and controls (5/18) had detectable serum AD12 antibodies compared with patients with recently diagnosed DH (0/22). In our opinion this was most likely due to the older age (and hence increased likelihood of exposure to AD12) of both patients with long-standing DH (mean 53.9 years) and controls (mean 51.4 years), compared with patients with recently diagnosed disease (mean 43.9 years), although this age difference did not reach statistical significance.

The findings of this study do not support the concept that AD12 infection of the intestinal mucosa initiates DH by triggering an immune response in susceptible individuals directed against the AD12 E-1-B peptide, which becomes chronic due to immunogenic cross reactivity between this E-1-B peptide, and homologous peptide sequences in dietary A-gliadin. They do not however preclude such a possibility for the following reasons: (a) productive AD12 infection of the intestinal mucosa (i.e. in which viral replication occurs with production of both early region peptides such as E-1-B and late region structural peptides such as the hexon protein) might trigger the disease process in a "hit and run" fashion. In such a scenario any humoral immune response against AD12 hexon protein may be short-lived and have remained undetected in the present study, as even patients with recent onset disease had blood samples taken a mean of 13.2 months after initial diagnosis; (b) transient or persistent non-productive AD12 infection of the intestinal mucosa might only produce early region proteins and thus fail to stimulate any humoral response against viral structural proteins. This is a theoretical possibility only as such infection of the intestinal mucosa has yet to be demonstrated in humans with either CD (14) or DH, although latent tonsillar adenovirus has been shown to occur (15); and (c) because of small study group numbers the chance of type two error was relatively high, i.e. a significant difference in serum AD12 antibody prevalence between patients and controls could well have been missed.

Significantly more patients with recently diagnosed DH than patients with long-standing disease had persisting clinical symptoms (see Table I). In our opinion this was likely due to a combination of factors, including: (a) optimum drug dosage (i.e. minimal dose to control symptoms) was less likely to have been established in recently diagnosed patients; (b) the therapeutic effects of a gluten-free diet are delayed (11, 16, 17) and may not yet have been manifest in patients with recently diagnosed disease; and (c) spontaneous disease remission was more likely in patients with long-standing DH. Over 80% of patients with DH who had a jejunal biopsy had histological evidence of GSE in this study. Others have reported similar findings (18–20).

In conclusion the findings of this study provide no evidence to support, but do not preclude, a role for AD12 in the pathogenesis of DH.

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REFERENCES

1. Kagnoff MF. Coeliac disease: genetic, immunological and environmental factors in disease pathogenesis. Scand J Gastroenterol 1985; 20 (suppl 114): 44–55.
2. Middleton PJ. Role of viruses in pediatric gastrointestinal disease and epidemiological factors. In: Tyrell DA, Kapikian AZ, eds. Virus infections of the gastrointestinal tract. New York: Marcell Dekker, 1982: 211.
3. Flint SJ. Structure and genomic organisation of adenoviruses. In: Tooze J, ed. DNA tumor viruses. Cold Spring Harbor. 1980: 383.
4. Kagnoff MF, Austin RK, Hubert JJ, Bernardin JE, Kasarda DD. Possible role for a human adenovirus in the pathogenesis of coeliac disease. J Exp Med 1984; 160: 1544–1557.
5. Kagnoff MF, Paterson YJ, Kumar PJ, et al. Evidence for the role of a human intestinal adenovirus in the pathogenesis of CD. Gut 1987; 28: 995–1001.
6. Karagiannis JA, Priddle JD, Jewell DP. Cell mediated immunity to a synthetic gliadin peptide resembling a sequence from adenovirus 12. Lancet 1987; 1 (8538): 884–886.
7. Mantzaris GJ, Karagiannis JA, Priddle JD, Jewell DP. Cellular hypersensitivity to a synthetic dodecapeptide derived from human adenovirus 12 which resembles a sequence from A gliadin in patients with coeliac disease. Gut 1990; 31 (6): 668–673.
8. Howdle PD, Blair Zajdel ME, Smart CJ, Trejdosiewicz LK, Blair GE, Losowky MS. Lack of serological response to an E-1-B protein of AD12 in coelic disease. Scand J Gastroenterol 1989; 24 (3): 282–286.
9. Katz SI, Strober W. The pathogenesis of dermatitis herpetiformis. J Invest Dermatol 1978; 70 (2): 63–75.
10. Otley C, Hall RP. Dermatitis herpetiformis. Dermatol Clinics 1990; 8 (4): 759–769.
11. Hall RP. Dermatitis herpetiformis. J Invest Dermatol 1992; 99 (6): 873–81.
12. Horwitz MS. Adenoviridae and their replication. In: Fields BN, Knipe DM, eds. Field's Virology, 2nd edn, vol 2. New York: Raven Press 1990: 1679–1721.
13. Unsworth D, Manuel PD, Walker-Smith JA, Campbell JA, Johnson GD, Holbrow EJ. New immunofluorescent blood test for gluten sensitivity. Arch Dis Child 1981: 56: 864–868.
14. Carter MJ, Willcocks MM, Mitchison HC, Record CO, Madeley CR. Is a persistent adenovirus infection involved in coeliac disease? Gut 1989; 30 (11): 1563–1567.
15. Neumann R, Genersch E, Eggers HJ. Detection of adenovirus nucleic acid sequences in human tonsils in the absence of infectious virus. Virus Res 1987; 7: 93–97.
16. Fry L, McMinn RMH, Cowan J, Hoffbrand AV. Effect of gluten free diet on dermatological, intestinal and haematological manifestations of dermatitis herpetiformis. Lancet 1968; 1: 557–561.
17. Fry L, Seah PP, Riches DJ, Hoffbrand AV. Clearance of the skin lesions in dermatitis herpetiformis after gluten withdrawal. Lancet 1973; 1: 82–91.
18. Marks J, Schuster S, Watson AJ. Small bowel changes in dermatitis herpetiformis. Lancet 1966; 2: 1280–1282.
19. Lawley TJ, Strober W, Yaoita H, Katz SI. Small intestinal biopsies and HLA types in dermatitis herpetiformis patients with granular and linear IgA skin deposits. J Invest Dermatol 1980; 74: 9–12.
20. Strober W. Intestinal abnormalities. In: Katz SI (moderator). Dermatitis herpetiformis. The skin and the gut. Ann Intern Med 1980; 93: 857–874.