

## *Alimentary tract and pancreas*

# Altered gastrointestinal immune response in sarcoidosis

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**SUMMARY** Because of the possible clinical association between coeliac disease and sarcoidosis, the incidence of humoral sensitivity to dietary proteins was examined in patients with sarcoidosis. Raised concentrations of circulating IgG antibodies to alpha gliadin were found in 41/99 sarcoid patients whereas antibody levels to casein, beta lactoglobulin and ovalbumen were similar to normal controls. Subsequently, a group of 26 sarcoid patients were selected for small intestinal biopsy; 11 had raised and 15 normal alpha gliadin antibody (AGA) levels. One AGA positive patient had villous atrophy consistent with coeliac disease. Intraepithelial lymphocyte (IEL) counts were raised in AGA positive (median 30; 95% confidence limits 22-46) and AGA negative (median 24; 95% confidence limits 19-32) sarcoid patients when compared with a control group (median 13.5; 95% confidence limits 10-18)  $p < 0.01$ . Serum IgG concentrations were raised in 11/52 patients tested but there was no correlation between IgG levels and the presence of IgG anti-gliadin antibodies. HLA Dr typing was done in 21 of the 26 biopsied patients. The coeliac disease associated antigen Dr3 was present in eight of 21 (38%) which is very similar to the prevalence in unselected blood donors (34%). There was no significant difference in IEL counts between Dr3 positive and Dr3 negative sarcoid patients. These findings suggest that in patients with sarcoidosis, there is an altered gastrointestinal mucosal immune response, accompanied in about 40% of patients by specific sensitisation to wheat protein.

Sarcoidosis is a chronic granulomatous disease of unknown aetiology which most commonly affects the lungs. The pathogenesis of the disease is thought to involve an altered immune response to an as yet unidentified antigen or antigens.<sup>1</sup> The gastrointestinal tract, with the exception of the liver is rarely involved.<sup>2</sup> It has recently been reported, however, that coeliac disease and sarcoidosis may be clinically associated.<sup>3</sup> Accordingly, it is possible that abnormalities of the gastrointestinal immune system may contribute to the pathogenesis of sarcoidosis by

mounting an immune response to dietary antigens which normally evoke a state of oral immune tolerance.

The aim of this study was to assess sensitisation of the intestinal immune system to dietary antigens in patients with sarcoidosis by measuring circulating antibody levels to four dietary proteins. These included alpha gliadin, casein, beta lactoglobulin and ovalbumen. In a selected group, the morphology of the small intestine and the HLA status were assessed.

## Methods

### DIETARY PROTEIN ANTIBODIES

Serum was obtained from 99 patients with sarcoidosis (mean age 36.6 range 16-63 years: 56 men, 43

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women). Sarcoidosis was histologically proven in 88/99 patients and 64/99 were treated with steroids at some stage during their illness. The sera were tested for antibodies to alpha gliadin, casein, beta lactoglobulin and ovalbumen using a modification of the enzyme linked immunosorbent assay system (ELISA) as previously described.<sup>4</sup> An ELISA index greater than 2 standard deviations above the laboratory standard for alpha gliadin, corresponding to an ELISA index of greater than 3.5, was said to be raised. Antibody levels for casein, beta lactoglobulin and ovalbumen were compared with a group of 50 normal controls.

#### TOTAL IMMUNOGLOBULIN LEVELS

Levels of IgG, IgA, and IgM were measured in 52/99 samples using laser nephelometry and results expressed in IU/ml.

#### SMALL INTESTINAL HISTOLOGY

In 26 patients four mucosal biopsies were obtained endoscopically from the third part of the duodenum. Thirteen were receiving oral steroids at the time of biopsy; mean dose 10 mg, range 2.5–20 mg. Biopsies were also obtained from 16 control subjects with a similar age and sex distribution to the sarcoidosis patients; five healthy volunteers, two patients with idiopathic recurrent oral ulceration, and nine patients subsequently diagnosed as having the irritable bowel syndrome. Biopsies were assessed by routine histology and intraepithelial lymphocytes (IEL) counted.<sup>5</sup> The slides were coded and evaluated by two pathologists unaware of the clinical details. Between 500–1000 epithelial cells were counted and the number of lymphocytes present expressed as a percentage.

#### HLA TYPING

Typing for HLA A, B, and Dr antigens was done in 21 of the 26 patients biopsied using the technique of Terasaki *et al.*<sup>6</sup>

This study was approved by the hospital ethics committee.

#### STATISTICAL ANALYSIS

Intraepithelial lymphocyte counts were expressed as medians, ranges and 95% confidence limits of the median.<sup>7</sup> The data were analysed using the Wilcoxon's rank-sum test for unpaired non-parametric data and Kendall's rank correlation.

## Results

#### SERUM ANTIBODIES

Antigliadin antibody results are shown in Figure 1. Raised antigliadin antibody titres (AGA >2 SD

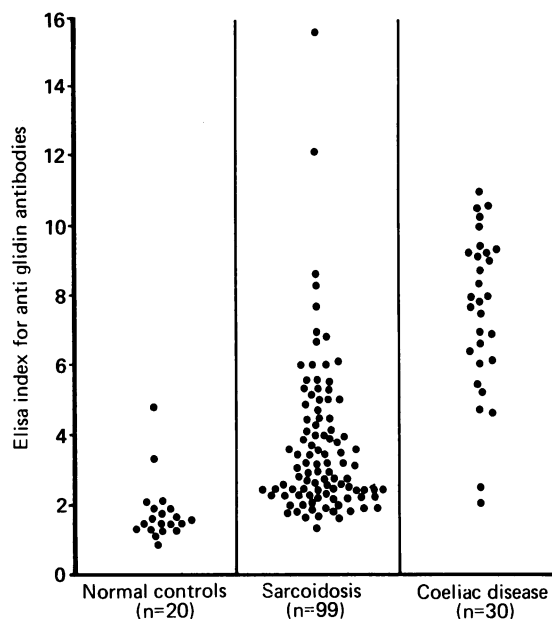


Fig. 1 Antigliadin antibody titres in sarcoidosis patients, normal controls and patients with biopsy proven coeliac disease. An ELISA index of 3.5 is the upper limit of normal for our laboratory. AGA titres above this level are present in 5% of the normal controls, 41% of the sarcoidosis patients and 93% of the patients with coeliac disease.

above laboratory standard) were present in 41/99 (41%) of sarcoidosis patients with titres being markedly raised (titres >2.56 SD above the laboratory standard) in 32/99 (32%). For ease of comparison AGA results in 20 normal laboratory controls (mean age 33; range 17–70) and 30 patients with biopsy proven coeliac disease (mean age 25.9; range 23–31 years) are also shown in Figure 1. Alpha gliadin antibody levels were raised in 5% of the normal subjects, 41% of the sarcoidosis patients and 93% of the patients with coeliac disease. Antibody levels to casein, beta lactoglobulin and ovalbumen were similar to the normal controls.

#### IMMUNOGLOBULIN CONCENTRATIONS

Mean immunoglobulin concentrations in the sarcoidosis patients were: IgG  $178.1 \pm 116.5$  IU/ml, IgA  $170.1 \pm 107.4$  IU/ml and IgM  $211.1 \pm 97.1$  IU/ml. Eleven patients had raised IgG concentrations, seven raised IgA concentrations, and four raised IgM concentrations. There was no relationship between abnormally raised IgG, IgA, or IgM and raised alpha gliadin antibody concentrations. Specifically there was no significant correlation between IgG anti-gliadin antibody and total IgG concentrations ( $p > 0.05$ ).

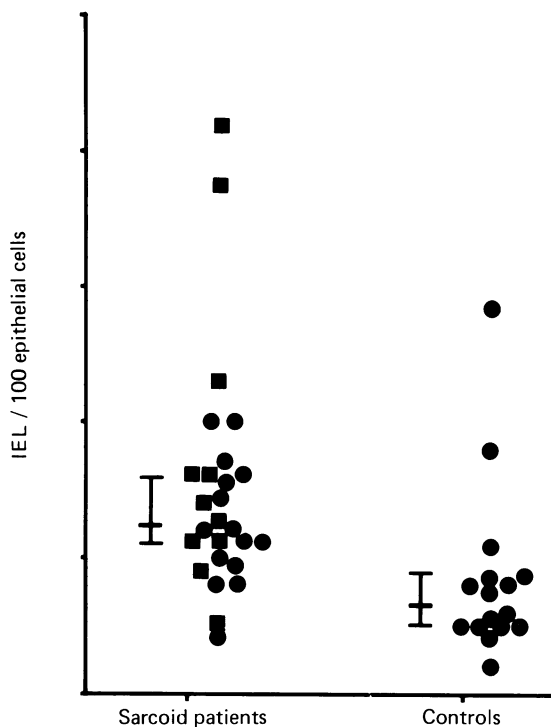


Fig. 2 Sarcoidosis patients with and without antigliadin antibodies had significantly increased intraepithelial lymphocyte counts in duodenal biopsies compared with controls ( $p < 0.01$ ). Black squares refer to sarcoidosis patients with positive antigliadin antibodies. Vertical bars represent medians and 95% confidence limits of the medians. The patient with the highest IEL count (84) also had subtotal villous atrophy.

**SMALL INTESTINAL HISTOLOGY AND INTRAEPITHELIAL LYMPHOCYTE (IEL) COUNTS**  
 Intraepithelial lymphocyte counts are shown in Figure 2. The patient with the highest IEL count was also AGA positive and had subtotal villous atrophy. Interestingly, he was moderately obese and had no symptoms or signs of malabsorption. Nevertheless he reported feeling much better on a gluten free diet and a repeat duodenal biopsy one year later showed marked histological improvement. None of the other biopsies showed villous atrophy. Intraepithelial lymphocyte counts were median 24.5 (range 8–84; 95% confidence limits 22–46) in the sarcoid patients as a group, 30 (10–84; 95% confidence limits 22–46) in the patients with raised AGA's and 24 (8–40; 95% confidence limits 19–32) in the AGA negative patients. All three values were significantly raised compared with IEL counts in 16 control patients, median 13.5 (4–57; 95% confidence limits 10–18);  $p < 0.01$ . There was no significant difference between

IEL counts in the sarcoid patients with and without raised AGA's and no significant difference between those patients receiving and not receiving steroids.

#### HLA TYPING

HLA Dr3 was present in eight of 21 (38%) sarcoid patients, four AGA positive and four AGA negative. This compares with a prevalence of 34% for Dr3 in Irish blood donors (A Finch: unpublished data). There was no significant difference in IEL counts between Dr3 positive (median 22; range 10–40) and Dr3 negative (median 25; range 8–84) sarcoid patients.

#### Discussion

Our results show that intraepithelial lymphocyte (IEL) counts are significantly raised in patients with sarcoidosis compared with controls and that 41% of patients with this condition have raised levels of circulating antibodies to alpha-gliadin (AGA), a wheat protein fraction.

Both raised AGAs and raised IEL counts are typically found in coeliac disease. Up to 93% of patients with coeliac disease have raised AGAs<sup>8</sup> and antibodies to wheat protein are sufficiently specific for this disease to be used successfully as a screening test in selected populations.<sup>9,10</sup> IELs are predominantly suppressor/cytotoxic T lymphocytes which lie between the surface enterocytes of the small intestine.<sup>11</sup> Animal studies have shown that raised IEL counts are indicative of a stimulated local cell mediated immune response<sup>12</sup> to dietary antigens. Our results therefore show evidence of activated cell mediated immunity in the small intestine in sarcoidosis and humoral sensitisation to wheat protein in 42% of these patients.

The significance of these findings is unclear. As coeliac disease and sarcoidosis are both common in Ireland<sup>13,14</sup> and may be clinically associated,<sup>3</sup> the raised AGAs and raised IEL counts may indicate a subpopulation which is genetically predisposed to having coeliac disease. This is unlikely, however, as the prevalence of HLA Dr3, which is found in 90% of coeliac patients,<sup>8</sup> was not increased in our sarcoid patients compared with Irish blood donors. Furthermore there was no association between Dr3 and either raised AGAs or raised IELs in the test group.

It is possible that the humoral response to a dietary protein and increased IEL counts are epiphenomena of sarcoidosis which reflect general activation of the mucosal immune system. The recent observations, however, that patients with Crohn's disease have sarcoid like lymphocytosis of the lower respiratory tract<sup>15</sup> and that patients with coeliac disease frequently suffer from pulmonary disease,<sup>16</sup> in conjunc-

tion with our results, suggests that immunological events in the gastrointestinal mucosa may play a primary pathogenic role in the development of sarcoidosis. This is supported by recent evidence of a common mucosal immune system in man<sup>17</sup> and by evidence that the antigen in pulmonary sarcoidosis may reach the lungs *via* the blood stream rather than through the airways.<sup>1</sup> Moreover, specific sensitisation to wheat protein in the absence of raised antibody levels to other dietary proteins suggests that gluten may have a specific aetiological role in a proportion of sarcoid patients. If this is the case a gluten free diet will be of therapeutic value in this group and controlled clinical trials to evaluate this therapy are needed.

#### References

- 1 Semenzato G, James DG. The immunological approach to the enigma of sarcoidosis. *Sarcoidosis* 1984; **1**: 24–35.
- 2 Scadding JG, Mitchell DN. *Sarcoidosis*. London: Chapman and Hall, 1985: 366–78.
- 3 Douglas JD, Gillon J, Logan RFA, Grand IWB, Crompton GK. Sarcoidosis and coeliac disease: an association. *Lancet* 1984; **ii**: 13–4.
- 4 O'Farrelly C, Kelly J, Hekkens W, *et al*. Alpha-Gliadin antibody levels: a serological test for coeliac disease. *Br Med J* 1983; **286**: 1007–10.
- 5 Ferguson A, Murray D. Quantitation of intraepithelial lymphocytes in human jejunum. *Gut* 1971; **12**: 988–94.
- 6 Terasaki PI, Bernoco D, Park MS, Ozturk G, Iwaki Y. Microdroplet testing for HLA A, B, C, and Dr antigens. *Am J Clin Pathol* 1978; **69**: 103–20.
- 7 Diem K, Lentner C, eds. *Scientific Tables; Documenta Geigy*. Basle: JR Geigy SA, 1970: 161.
- 8 O'Farrelly C, Feighery C, O'Briain DS, *et al*. Humoral response to wheat protein in patients with coeliac disease and enteropathy associated T cell lymphoma. *Br Med J* 1986; **293**: 908–10.
- 9 Friis SU, Gudmand-Hoyer E. Screening for coeliac disease in adults by simultaneous determination of IgA and IgG gliadin antibodies. *Scand J Gastroenterol* 1986; **21**: 1059–62.
- 10 Cacciari E, Salardi S, Volta U, *et al*. Can antigliadin antibody detect symptomless coeliac disease in children with short stature? *Lancet* 1985; **i**: 1469–71.
- 11 Dobbins WO. Human intestinal intraepithelial lymphocytes. *Gut* 1986; **27**: 972–85.
- 12 Strobel S, Ferguson A. Modulation of intestinal and systemic immune responses to a fed protein antigen, in mice. *Gut* 1986; **27**: 829–37.
- 13 Mylotte M, Egan-Mitchell B, McCarthy CF, McNicholl B. Incidence of coeliac disease in the West of Ireland. *Br Med J* 1973; **i**: 703–5.
- 14 Logan J. Prevalence of sarcoidosis in the Irish Republic. *Acta Med Scand [suppl]* 1964; **425**: 126.
- 15 Smiejan JM, Cosnes J, Chollet-Martin S, *et al*. Sarcoid-like lymphocytosis of the lower respiratory tract in patients with active Crohn's disease. *Ann Intern Med* 1986; **104**: 17–21.
- 16 Edwards C, Williams A, Asquith P. Bronchopulmonary disease in coeliac patients. *J Clin Pathol* 1985; **38**: 361–7.
- 17 Czerkinsky C, Prince SJ, Michalek SM, *et al*. IgA antibody-producing cells in peripheral blood after antigen ingestion: Evidence for a common mucosal immune system in humans. *Proc Natl Acad Sci USA* 1987; **84**: 2449–53.