Immunological Changes in Tropical Sprue

I. N. ROSS* AND V. I. MATHAN

From the Wellcome Research Unit, Christian Medical College Hospital, Vellore 632004, India

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SUMMARY

Immunological measurements in 85 southern Indian patients with tropical sprue were compared with two groups of controls, namely, 150 patients with gastrointestinal diseases not associated with malabsorption, and 57 normal asymptomatic subjects. A significant increase in intraepithelial lymphocytes in jejunal surface and crypt epithelium, related to the severity of the mucosal morphological abnormality, was the most significant finding in tropical sprue. Measurements such as increased serum concentrations of IgG, IgE, complement component C4, and orosomucoid, gastric parietal cell antibodies and lymphopenia with a low peripheral blood T cell count, which were found in patients with sprue, differed significantly from the healthy control group but not from the group of gastrointestinal controls without malabsorption. The ability to produce functional antibodies was preserved in tropical sprue. The results suggest that the small intestinal enteroocyte abnormality in tropical sprue is not the result of a primary immunological process but that the observed alterations are a sequelae of mucosal damage and compromised integrity of the mucosal barrier.

INTRODUCTION

Tropical sprue is a primary malabsorption syndrome which affects residents of and visitors to the tropics (Baker and Mathan, 1968; Klipstein and Baker, 1970). The aetiology of this syndrome is as yet unknown but the available evidence suggests that it is likely to be multiple and that at least in some individuals, the malabsorption may be the result of an infection (Baker and Mathan, 1971). Electronmicroscopic examination of the ultrastructure of jejunal mucosa in patients with tropical sprue in southern India have clearly established enterocyte damage in crypt and surface epithelium as the basic lesion (Mathan, Mathan, and Baker, 1975). In addition to the damaged enterocytes, a significant lymphocytic infiltration of the epithelial layer, particularly in close proximity to damaged enterocytes is present. Although increase in intraepithelial lymphocytes (IEL) has been noticed by several workers (Swanson and Thomassen, 1965; Klipstein, 1970; Baker and Mathan, 1971; Mathan et al., 1975), they have been counted in only a small number of patients (Montgomery and Shearer, 1974). This increase in IEL suggests...
that immunological alterations at the level of the small intestinal epithelium may play a role in the pathogenesis of the lesion. This view is further supported by the finding of immunological alterations, including increase in IEL in two other gastrointestinal diseases associated with morphological changes, malabsorption and diarrhoea; guttten sensitive enteropathy (coeliac disease) and cow’s milk protein intolerance (Ferguson, 1977; Aasquith and Haeney, 1979; Phillips, Rice, Frank, and Walker, 1979).

There are no reports of detailed immunological investigations in patients with tropical sprue. The limited studies that have been reported (Jarnum, Jejeebhoy, and Singh, 1968; Klipstein and Falaiye, 1969; Maldonado and Sanchez, 1969; Samuel, Singh, and Jarnum, 1970, Misra, Malhotra, and Malaviya, 1973; Montgomery and Shearer, 1974) are not sufficient to decide whether immunological changes are primary events in the pathogenesis or are secondary results of the structural and functional alterations in the small intestinal mucosa. This paper reports the results of a systematic investigation of immune function in a group of patients with tropical sprue in southern India and appropriate controls.

SUBJECTS STUDIED AND METHODS

A group of patients with tropical sprue and two control groups were investigated.

Tropical sprue. This group included fifty consecutive admissions to a metabolic ward from January 1978, in whom the diagnosis of tropical sprue was confirmed by standard investigations (Baker and Mathan, 1971) and stored sera from 35 other patients selected randomly from earlier admissions with tropical sprue. All these patients had diarrhoea for longer than two weeks, in many for several months or years and malabsorption shown by at least two tests. At least two faecal samples were examined for parasites and cultured for enteropathogenic bacteria. Jejunal luminal fluid was also tested for parasites in 41 per cent of patients. A peroral jejunal biopsy was obtained just distal to the ligament of Treitz, using a Crosby–Kugler capsule (Crosby and Kugler, 1957). The tissue was fixed in Bouin’s fixative, paraffin mounted and 4 μm sections cut and stained with haematoxylin and eosin. The biopsies were coded and graded independently as either normal or as showing mild, moderate or severe changes, as previously described (Mathan et al., 1975).

Forty-seven per cent of this group showed malabsorption of fat, xylose and vitamin B₁₂ while the remainder had malabsorption of at least two substances. The mean age was 37 years (range nine to 60 years) and the male to female sex ratio was 1·5 to 1.

Gastrointestinal controls. One hundred and fifty consecutive admissions with a variety of gastrointestinal illnesses other than tropical sprue were studied to obtain a group which would be of comparable socioeconomic and age composition to the tropical sprue patients. Many of these subjects had tropical enteropathy (Baker and Mathan, 1972) with malabsorption of one substance (usually d-xylose or fat). The mean age of this group was 36 years (range 13–64) and the male to female ratio was 2 to 1.

Normal controls. Fifty-seven healthy southern Indians consisting of laboratory staff, blood donors and volunteers from a village who had been screened for
gastrointestinal disease. The mean age was 37 years (range 18–54) and the male to female sex ratio was 5 to 1.

**IMMUNOLOGICAL METHODS**

**Serum**

All serum samples were stored at -20 °C until tested. A single radial immunodiffusion (SRID) technique (Mancini, Carbonara, and Heremans, 1965) was used to determine the concentration of IgA, IgG and IgM, using the globulin fraction of sheep monospecific heavy chain antisera (Seward Laboratories, London, England) and IgD using goat anti-human IgD serum (Meloy Laboratories Inc., Springfield, Virginia, U.S.A.). IgE concentrations were measured with Phadebas IgE kits (Pharmacia Diagnostics AB, Uppsala, Sweden). Standard curves were constructed using pooled serum standard calibrated against WHO international reference preparations 67/97 for IgA, IgG and IgM, Seward Laboratories serum standard for IgD or by Phadebas IgE kit standard for IgE.

The ability to produce functional antibody was tested by measuring in sera naturally occurring *Escherichia coli* (E. coli) agglutinating antibody, using a haemagglutination technique before and after treatment with 2-mercaptoethanol (2ME) (Webster, Efrat, and Asherson, 1974) and rotavirus antibody with a microscale enzyme linked immunosorbent assay (ELISA) (Ghose, Schnabl, and Holmes, 1978).

Serum complement C3 and C4 concentrations were measured by electroimmuno- diffusion (Laurell, 1966) and Serum orosomucoid by single radial immunodif- fusion (SRID). Antisera to C3, C4 and orosomucoid were obtained from Seward Laboratories. Standard curves were constructed using pooled serum samples calibrated against a serum standard for C3c (c1, A-globulin), C4 (c1, ±-globulin) and orosomucoid (acid α1-glycoprotein) (Behringwerke AG, Marburg, West Germany).

Reticulin antibody (RAB), gastric parietal cell antibody (GPC), smooth muscle antibody (SM), mitochondrial antibody (MA) and antinuclear factor (ANF) were tested for by an indirect immunofluorescent method (Johnson and Dorling, 1977). Three µm cryostat sections of rat liver, kidney and stomach were incubated with the test serum at a starting dilution of 1:10. All positive sera were retested at a dilution of 1:40. Antibodies present were detected using fluorescein conjugated sheep anti-human immunoglobulin (Wellcome Research Laboratories, Beckenham, England). Serum samples known to be positive for RAB (IgG class), GPC and ANF were used as controls.

**Peripheral blood lymphocytes**

Lymphocytes were obtained from defibrinated blood after separation on a Ficoll-hypaque gradient with an initial separation of lymphocytes in anaemic patients using 5 per cent dextran in normal saline (Jondal, Holm, and Wigzell, 1972). Peripheral blood B lymphocytes were identified and quantitated by surface immunoglobulin staining and T lymphocytes by an E rosette technique with locally available sheep red cells (Waller and MacLennan, 1977). An absolute lymphocyte
count was obtained on each patient and all quantitation of B and T lymphocytes was done on a minimum count of 400 cells.

Lymphocyte transformation was performed by a microtiter method (Waller and MacLennan, 1977), stimulating cells at a concentration of $75 \times 10^3$ per well in triplicate with phytohaemaglutinin (PHA) at a final dilution of 1:100 and 1:300 (Difco Laboratories, Detroit, Michigan, U.S.A.) and with pokeweed mitogen (PWM) at final dilutions of 1:20 and 1:60 (Grand Island Biological Co., New York, U.S.A.). After addition of tritiated thymidine (Bhabha Atomic Research Centre, Trombay, India—specific activity 1 Ci/ mmol) on the third day the cells were harvested 16 hours later (Minivent, London, England) on to glass fibre filter strips (Whatman Inc., New Jersey, U.S.A) and counted. The results were expressed as log$_{10}$ mean final counts per cell (c.p.m.) in stimulated culture less c.p.m. in unstimulated culture.

**Intraepithelial lymphocytes**

IEL were counted in well-oriented sections of jejunal biopsies in the surface and the crypt epithelium and expressed as IEL per 100 epithelial cell nuclei (Ferguson, 1977). The crypt villous junction was identified in well-oriented sections independently by two observers by the presence of a shelf and by noting the maturation of the epithelial cells. Counts were started at least 10 epithelial nuclei away from the junction on the surface epithelial side and five nuclei away on the crypt side. A minimum of 100 epithelial cell nuclei were counted in five different sections separated from each other by at least 12 μm (fourth section on serial ribbons).

**Jejunal luminal fluid**

Jejunal luminal fluid samples were collected on ice by siphonage from just distal to the ligament of Treitz. The fluid was centrifuged at 400 g for 10 minutes and stored at $-20^\circ$C after the addition of 500 i.u. of Aprotinin (Trasylol, Bayer, West Germany) per ml of fluid.

Secretory IgA and free secretory component (SC) were tested for independently by an immunodiffusion technique (Ouchterlony, 1953) using antisecretory IgA (Seward Laboratories) and anti free secretory piece (Behringwerke AG). Rotavirus antibodies in the jejunal luminal fluid were detected by the enzyme linked immunosorbent assay (ELISA) as described above after concentrating the fluid 10 fold by ultrafiltration.

**Statistical analysis**

Data on immunoglobulins, complement, orosomucoid, peripheral blood lymphocytes, B and T lymphocytes and IEL were found to have a Gaussian distribution only after logarithmic transformation (Stein, Warl, and Woods, 1977). Quantitative data were compared by one-way analysis of variance and, when significant, probability values were calculated by the least significant difference method (Snedcoor and Cochrant, 1963). When two groups only were being compared Student's $t$ test was used. When variances were found by Fisher's $F$ test
to be unequal, a modified Student's t test was performed. Qualitative data were compared using the chi squared test.

RESULTS

Serum immunoglobulins

The mean serum concentration of IgA, IgD and IgM was not significantly different in any of the three groups of subjects (Table 1). The mean IgG in tropical sprue was significantly higher (p < 0.05) than in normal controls but was not significantly different from that in gastrointestinal controls. The IgE concentration in tropical sprue and gastrointestinal controls was higher than that seen in the normal control group but the ANOVA F value was not significant. Gastrointestinal parasites (G. lamblia, hookworm, S. stercoralis or E. histolytica) were present in the faeces and/or jejunal luminal fluid of 12 of 25 tropical sprue and 11 of 28 gastrointestinal controls in whom IgE was determined. When the patients with parasites were compared to normal controls (mean ± 2 S.D. IgE concentrations tropical sprue 2645, 403–1732, gastrointestinal controls 2330, 355–1536) the mean concentrations were significantly higher (p < 0.01). There was also a positive correlation between IgE concentrations and peripheral blood eosinophil numbers (r 0.33 p < 0.05). The prevalence of gastrointestinal parasites in tropical sprue and gastrointestinal controls was not significantly different.

Functional antibodies

E. coli antibody titre was log_{10} 1.51 (1:32) or higher in all subjects. Mean log_{10} titres were not significantly different between normal controls and tropical sprue when either untreated or 2 ME treated serum was tested (Table 2). After 2 ME treatment 15 of 28 normal controls and 29 of 40 tropical sprue had titres higher than log_{10} 0.9 (1:8). The mean rotavirus antibody titres were also not significantly different between tropical sprue and age matched normal controls.

| Table 1. Serum immunoglobulins in normal controls, tropical sprue and gastrointestinal controls groups |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | IgA             | IgG             | IgM             | IgD             | IgE U/ml         |
| Normal controls |                 |                 |                 |                 |                 |
| n = 57          | 2.57            | 12.38           | 0.07            | 0.01            | 668             |
| (1.22–5.42)     | (7.56–20.27)    | (0.58–1.97)     | (0.00–0.15)**   | (25–17584)      |
| Tropical sprue  |                 |                 |                 |                 |                 |
| n = 56          | 2.66            | 14.01*          | 0.94            | 0.01            | 1715            |
| (1.15–6.17)     | (6.76–29.14)    | (0.38–23.3)     | (0.00–0.25)     | (189–15570)     |
| Gastrointestinal controls |                 |                 |                 |                 |                 |
| n = 82          | 2.41            | 13.21           | 0.98            | 0.01            | 946             |
| (1.08–5.36)     | (6.31–22.75)    | (0.36–2.68)     | (0.00–0.28)     | (51–17462)      |
| n = 111         | 2.41            | 13.21           | 0.98            | 0.01            | 946             |
|                 |                 |                 |                 |                 | (51–17462)      |
|                 |                 |                 |                 |                 |                 |
| Results show geometric mean in g/l (excepting IgE) and 2 S.D. range in parentheses.
* Significant difference on comparison with normal controls group (p = 0.05), but not with gastrointestinal controls group.
** Indicates undetectable by S/RID, lower limit of detection for IgD 0.003 g/l.
Table 2. Serum E. coli and rota virus antibody titres in normal controls and tropical sprue groups

<table>
<thead>
<tr>
<th></th>
<th>Mean E. coli log_{10} a.h. titre</th>
<th>Mean E. coli log_{10} a.h. titre, 2 ME treated</th>
<th>Mean rota virus log_{10} a.h. titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>2.06 (1:32–1:1024)*</td>
<td>0.98 (1:8–1:64)</td>
<td>3.23 (1:256–1:16 384)</td>
</tr>
<tr>
<td></td>
<td>n = 28</td>
<td>n = 28</td>
<td>n = 50</td>
</tr>
<tr>
<td>Tropical sprue</td>
<td>2.16</td>
<td>1.10</td>
<td>2.99 (1:256–1:4096)</td>
</tr>
<tr>
<td></td>
<td>(1:32–1:1024)</td>
<td>(1:8–1:128)</td>
<td>n = 40</td>
</tr>
<tr>
<td></td>
<td>n = 40</td>
<td></td>
<td>n = 50</td>
</tr>
</tbody>
</table>

* Figures in parentheses represent titre range in doubling dilutions. There was no significant difference between the two groups for either E. coli or rota virus antibody titres.

Auto antibodies

GPC antibody was detected in 17 of 73 tropical sprue and six of 52 gastrointestinal controls but in none of the normal controls. The prevalence of GPC in tropical sprue was significantly higher when compared to normal controls (p < 0.005) but not to gastrointestinal controls. Fifteen of the 17 GPC antibody positive tropical sprue patients had malabsorption of vitamin B₁₂, but prevalence of vitamin B₁₂ malabsorption in tropical sprue without the antibody was not significantly different. Three of the gastrointestinal controls with GPC antibody had B₁₂ malabsorption and two others had ulcerative colitis. The only other autoantibody detected was SMA in one and ANF in two gastrointestinal controls who had ulcerative colitis.

Complement and orosomucoid

The mean serum concentrations of complement fraction C4 and orosomucoid were significantly increased in tropical sprue and gastrointestinal control groups compared to normal controls (p < 0.001), but there was no difference between the two patient groups for either of these two proteins (Table 3). Mean serum complement C3 concentrations did not differ significantly between any group.

Table 3. Serum C3, C4 and orosomucoid concentrations in normal controls, tropical sprue and gastrointestinal controls groups

<table>
<thead>
<tr>
<th></th>
<th>C3</th>
<th>C4</th>
<th>Orosomucoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>0.90 (0.53–1.53)</td>
<td>0.28 (0.11–0.70)</td>
<td>0.82 (0.34–1.99)</td>
</tr>
<tr>
<td></td>
<td>n = 50</td>
<td>n = 33</td>
<td>n = 28</td>
</tr>
<tr>
<td>Tropical sprue</td>
<td>0.87 (0.21–3.36)</td>
<td>0.45 (0.15–1.36)*</td>
<td>1.46 (0.65–3.20)*</td>
</tr>
<tr>
<td></td>
<td>n = 33</td>
<td>n = 32</td>
<td>n = 46</td>
</tr>
<tr>
<td>Gastrointestinal controls</td>
<td>0.93 (0.29–3.03)</td>
<td>0.46 (0.14–1.48)*</td>
<td>1.22 (0.35–4.31)*</td>
</tr>
<tr>
<td></td>
<td>n = 69</td>
<td>n = 59</td>
<td>n = 80</td>
</tr>
</tbody>
</table>

Results show geometric mean concentration in g/l and 2 S.D. range in parentheses.
* Significant difference on comparison with normal controls group (p < 0.001). There was no difference between the two patient groups for any of these proteins.
**Peripheral blood lymphocytes (PBL)**

The mean total lymphocyte count in tropical sprue and gastrointestinal controls was significantly lower than normal controls (Table 4). There was no significant differences between the PBL counts in tropical sprue and gastrointestinal controls.

There was a significant improvement in PBL counts in tropical sprue \((p < 0.005)\) and gastrointestinal controls \((p < 0.02)\) after a variable period of one to six months in hospital and clinical improvement. There was no difference in the total white cell, polymorphonuclear neutrophil leucocyte or eosinophil counts before or after treatment.

There was no difference between any of the groups for B cell counts or proportions. The mean T cell count was significantly \((p < 0.05)\) reduced in tropical sprue compared to normal controls but was not significantly different from that in gastrointestinal controls (Table 4). Although the T cell count in gastrointestinal controls was lower than that in normal controls the difference was not statistically significant. The proportion of T cells detected did not differ in any of the groups. *In vitro* PBL transformation (Table 5) was significantly reduced only in gastrointestinal controls at both stimulating doses of PHA. PWM stimulation produced a significantly reduced response in both tropical sprue and gastrointestinal controls but the differences between these two groups were not significant. There was considerable differences in the responses of individual patients in both these groups.

**Table 4. PBL count, B and T cell quantitation in normal controls, tropical sprue and gastrointestinal controls groups**

<table>
<thead>
<tr>
<th></th>
<th>Lymphocyte count before treatment</th>
<th>Lymphocyte count after treatment</th>
<th>B lymphocyte count</th>
<th>T lymphocyte count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>2.68 (1.52–4.72)</td>
<td></td>
<td>0.18 (0.09–0.37)</td>
<td>1.91 (1.1–3.26)</td>
</tr>
<tr>
<td></td>
<td>(n = 30)</td>
<td></td>
<td>(6%)</td>
<td>(67%)</td>
</tr>
<tr>
<td></td>
<td>((0.47–5.50))</td>
<td></td>
<td>(3–12%)</td>
<td>(49–92%)</td>
</tr>
<tr>
<td>Tropical sprue</td>
<td>1.60 (1.14–4.86)</td>
<td>2.35 (0.04–0.62)</td>
<td>0.16 (0.04–0.62)</td>
<td>1.52 (0.78–2.97)</td>
</tr>
<tr>
<td>(n = 35)</td>
<td></td>
<td>(6%)</td>
<td>(65%)</td>
<td>(49–86%)</td>
</tr>
<tr>
<td>(p &lt; 0.001)</td>
<td>N.S.</td>
<td>(2–17%)</td>
<td>(n = 12)</td>
<td>(n = 25)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(49–86%)</td>
<td>(n = 25)</td>
<td>(p &lt; 0.05)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>1.89 (0.84–4.22)</td>
<td>2.35 (0.05–0.57)</td>
<td>0.16 (0.05–0.57)</td>
<td>1.74 (0.70–4.34)</td>
</tr>
<tr>
<td>controls</td>
<td>(n = 35)</td>
<td></td>
<td>(64%)</td>
<td>(46–88%)</td>
</tr>
<tr>
<td>(p &lt; 0.001)</td>
<td>N.S.</td>
<td>(2–21%)</td>
<td>(n = 26)</td>
<td>(n = 38)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(46–88%)</td>
<td>(n = 38)</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Results are expressed as the geometric mean lymphocyte count \(\times 10^9/l\) and the percentage representation of B and T cells 2 S.D. range in parentheses.

The \(p\) values indicate the level of significance for difference between the corresponding mean lymphocyte count and the normal controls mean lymphocyte count for that test. N.S. indicates that the difference was not significant.

There was no significant difference between the percentage representation of B and T lymphocytes in any group nor between the two patient groups for B and T lymphocyte counts.
<table>
<thead>
<tr>
<th></th>
<th>PHA 1:100</th>
<th>PHA 1:300</th>
<th>PWM 1:20</th>
<th>PWM 1:40</th>
<th>PWM 1:60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>19.724</td>
<td>10.280</td>
<td>74.04</td>
<td>549.5</td>
<td>4312</td>
</tr>
<tr>
<td>n</td>
<td>13</td>
<td>9</td>
<td>12</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Tropical sprue</td>
<td>12.503</td>
<td>5.105</td>
<td>2.541</td>
<td>2.790</td>
<td>13.38</td>
</tr>
<tr>
<td>n</td>
<td>13</td>
<td>12</td>
<td>13</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Gastrointestinal controls</td>
<td>88.72</td>
<td>32.06</td>
<td>3.381</td>
<td>3.396</td>
<td>12.37</td>
</tr>
<tr>
<td>n</td>
<td>17</td>
<td>15</td>
<td>15</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.02</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>ANOVA</td>
<td>F = 5.52</td>
<td>F = 3.41</td>
<td>F = 6.66</td>
<td>F = 6.66</td>
<td>F = 3.07</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.005</td>
<td>p &lt; 0.005</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

Results are expressed as the geometric mean counts per minute, 1 S.D. range in parentheses. Where the F value for ANOVA attains significance, the level of significance, if any, for the difference between the normal controls group mean and the mean of tropical sprue or gastrointestinal controls groups is indicated by a p value or N.S.—not significant.

There was no significant difference between the two patient groups for any mitogen dilution.
some individuals with severe malabsorption having near normal responses while others showed marked reduction. There was no correlation between PBL numbers and lymphocyte transformation.

**Intraepithelial lymphocytes (IEL)**

Jejunal mucosal biopsies from 37 tropical sprue and 15 control subjects were available for counting IEL in the villous surface epithelium. Crypt IEL counts were done in 28 tropical sprue and 14 controls. Histological abnormalities from patients with tropical sprue were graded into mild (10), moderate (15) and severe (12) as described previously (Mathan et al., 1975). The controls were from the groups normal controls and gastrointestinal controls, with no evidence of malabsorption and mainly diagnosed as suffering from the irritable bowel syndrome. The villous

![Graph showing IEL counts in tropical sprue vs control subjects](image)

**Fig. 1.** Intraepithelial lymphocyte counts in normal jejunal mucosal biopsies and biopsies from patients with tropical sprue in the surface (•) and crypt (○) epithelium. (The bars represent geometric means and 95 per cent confidence interval.)
surface IEL geometric mean counts increased steadily from the normal biopsies through the increasing grades of severity of the mucosal lesion (Fig. 1). IEL counts in mucosa with severe morphological lesion were significantly higher than that in the normal, mild or moderate groups \((p < 0.001)\). In biopsies with a moderate lesion the surface IEL counts were significantly higher than in the normal group \((p < 0.05)\). There was no significant differences between mild changes and the normal or moderate group.

Crypt IEL counts in the normal, mild or moderate change group were not significantly different. The geometric mean crypt IEL counts in the group with the severe morphological change was significantly higher than that in all the other groups \((p < 0.001)\) (Fig. 1).

Follow-up biopsies were available in eight patients with tropical sprue. In three of them initially severe biopsy changes gradually improved to mild changes over three to eight months with clinical improvement and correction of malabsorption which was accompanied by a reduction in IEL. In the other five individuals, initially with moderate or mild changes, there was no improvement in the mucosa over periods ranging from three to 15 months and there were no changes detectable in the surface or crypt IEL counts.

**Jejunal luminal fluid**

Secretory component was detectable in all jejunal fluids tested (13 tropical sprue, 22 gastrointestinal controls), while secretory IgA (sIgA) was detected in 11 of 15 tropical sprue patients and 21 of 21 gastrointestinal control patients. Rotavirus antibody in the concentrated jejunal luminal fluid samples were detectable in eight of eight patients with tropical sprue and three of five gastrointestinal controls, titres varying from 1:2 to 1:64.

**Discussion**

Damaged enterocytes in the surface as well as the crypt epithelium of the small bowel is the basic lesion in tropical sprue (Mathan *et al.*, 1975). A significant increase in IEL in the small bowel mucosa was the most consistent immunological abnormality in tropical sprue in the present study. In addition, in tropical sprue serum concentrations of IgG, IgE, complement component C4 and orosomucoid were elevated, gastric parietal cell antibodies were present and there was lymphopaenia with low peripheral blood T cell counts and reduced response to some mitogens. The ability to produce functional antibodies as well as intestinal secretory antibodies was preserved in tropical sprue.

In any consideration of gastrointestinal immunology in developing countries the role of tropical enteropathy, an asymptomatic but widely prevalent alteration in the structure and function of the intestine has to be taken into consideration (Baker and Mathan, 1972). In southern India, structural alterations including changes in the ultrastructure of the enterocyte are present in nearly all individuals. Functional changes such as xylose or fat malabsorption can be demonstrated in about 50 per cent of apparently healthy individuals especially in rural areas. The available
evidence suggests that these alterations are more widely prevalent in the rural areas and in people from the lower socioeconomic strata. These factors prompted the selection of two control groups in the present study; the normal controls came from urban areas and a higher socioeconomic group with better sanitation; the gastrointestinal controls were from a background similar to that of the patients with tropical sprue and in addition had symptomatic gastrointestinal illness with possibly compromised integrity of the mucosal lining of the gastrointestinal tract.

The immunological measurements, increased concentrations of IgG, IgE, C4 and orosomucoid and the changes in lymphocytes which were abnormal in tropical sprue compared to normal controls did not differ significantly from those in gastrointestinal controls. The concentrations of IgG in normal controls and gastrointestinal controls were higher than reported in studies from temperate zones and supports earlier observations that IgG concentrations in developing countries are higher than in people living in protected environments (Samuels et al., 1970; Stuart, 1978). Earlier immunological studies in tropical sprue concentrated on serum immunoglobulin concentrations as well as the immunoglobulin class of lamina propria plasma cells and did not show any consistent pattern. Some authors have reported elevated IgG concentrations and others have reported increased lamina propria plasma cells. An increased IgG concentration has been reported in large bowel disease rather than small bowel disease and is presumed to be due to the greater antigenic load in the distal bowel (Hodgson and Jewell, 1978). The development of immunoglobulins especially IgG, in germ-free mice has been shown to be related to the presence of intestinal microflora (Nielsen and Friis, 1980). The higher IgG concentrations in populations in India and among Australian Aboriginals (Stuart, 1980) may be related to increased small bowel bacterial colonization (Bhat, Shantakumari, Rajan, Mathan, Kapadia, Swarnabai, and Baker, 1972) and the greater exposure to intestinal parasites (Chandra and Newberne, 1977).

The higher IgG concentrations in gastrointestinal controls and tropical sprue may, in addition, reflect an increased permeability of the gut to antigens compared to normal controls. The increase in IgE concentration correlated directly with the presence of intestinal parasites and indicates that the ability to produce this class of antibody is preserved in tropical sprue. In addition the normal production of antibodies to E. coli and rotavirus including rotavirus antibodies in small intestinal luminal fluid, as well as the preservation of the conversion from IgM class antibodies to IgA and/or IgG class antibodies, show that there is no defect in the production of functional antibodies in tropical sprue.

Low concentrations of serum C3 and C4 have been described in untreated gluten sensitive enteropathy with a return to normal or near normal concentrations on withdrawal of gluten (Rooth, Peters, and Doe, 1977) suggesting complement consumption in an Arthus type reaction in the small intestine. In contrast, inflammatory bowel disease concentration of serum complement components are usually increased even in the presence of circulating immune complexes (Ross, Thompson, Montgomery, and Asquith, 1979). This is thought to reflect an acute phase protein response to nonspecific inflammation. The pattern of serum concentrations of C3, C4 and orosomucoid seen in tropical sprue and gastroin-
testinal controls was different from that seen in patients with inflammatory bowel disease and presumably represents another type of acute phase protein response. The prevalence of auto-antibodies in tropical sprue, in addition to demonstrating similarities to gluten sensitive enteropathy (Magalhaes, Peters, and Doe, 1974) could also act as markers of certain immune response genes RAB was not detected in any of the patients, confirming earlier observations in tropical sprue in the western hemisphere (Seah, Fry, and Holborow, 1973). The prevalence of GPC antibody (23 per cent) in tropical sprue was similar to that seen in several other conditions (Jewell and Hodgson, 1976) and was not significantly different from that in gastrointestinal controls. A gastric lesion with gastric atrophy has been documented in over 80 per cent of tropical sprue patients (Baker and Mathan, 1971) and appears to be part of the primary mucosal lesion of the gastrointestinal tract in tropical sprue. An earlier study showed some degree of atrophic gastritis (20 per cent) in healthy southern Indian controls (Vaish et al., 1965). While the factors leading to atrophic gastritis in this population are not clear it is probable that the development of GPC antibody is secondary to damaged parietal cells and is not a unique feature of tropical sprue.

Both peripheral blood lymphocytes and T cells by E rosetting were reduced in tropical sprue and gastrointestinal controls compared to normal controls. This reduction was more marked in tropical sprue, but in both the groups, after a period in hospital with clinical improvement the PBL counts increased significantly. B cells and null cells were not reduced. PBL may be reduced, normal or increased in gluten sensitive enteropathy and in inflammatory bowel disease (Ferguson, 1977; Kraft, 1979). In gluten sensitive enteropathy, the reduction in PBL is associated with a reduction in the number and proportion of circulating T cells with preservation of B cells and increase in null cells (O'Donoghue, Lancaster-Smith, and Laviriere, 1976; Bullen and Slosowsky, 1978). There is a significant inverse correlation between circulating T cells and IEL in this condition. In tropical sprue while T cells were reduced there was no increase in B or null cells and there was no correlation between T cells and IEL counts. The reduction in circulating lymphocytes may be due to reduced production, trapping in damaged small intestine or excessive loss or destruction. The latter two mechanisms may occur in tropical sprue since the lack of correlation between IEL counts and peripheral blood T cells would suggest a loss of T cells into the lumen from the epithelium as has been shown in various conditions (Douglas, Weetman, and Haggis, 1976).

All the immunologic measurements considered so far were found to be abnormal in tropical sprue and gastrointestinal controls compared with normal controls. The gastrointestinal tract in normal controls would show minimal lesions associated with nonspecific tropical enteropathy, in gastrointestinal controls the integrity of the gastrointestinal mucosa is further compromised by the presence of a variety of pathological lesions other than those producing malabsorption while in tropical sprue there is a specific lesion which damages the integrity of the mucosa significantly. It can therefore be assumed that the immunological changes are a secondary phenomena consequent to alterations in the gastrointestinal mucosal barrier and a greater exposure to antigens present in the lumen of the gut.
IEL count was the single abnormality in tropical sprue and the degree of abnormality paralleled the severity of the mucosal lesion (Fig. 1). In contrast to reports in patients with gluten sensitive enteropathy there was a significant increase in IEL in the crypt epithelium also. The severity of the mucosal morphological abnormality in tropical sprue correlates well with the severity of malabsorption (Baker and Mathan, 1971). Further the enterocyte damage in tropical sprue is not confined to the surface epithelium but is detectable also in the crypts (Mathan et al., 1975). Significant clustering of IEL around the damaged enterocytes is often observed and it is known that damaged enterocytes are extruded from the sides of the villi long before they reach the normal zone of extrusion at the villous tip (Mathan et al., 1975).

The quantitation of IEL is difficult. In gluten sensitive enteropathy using the method in the present study (IEL per 100 epithelial cells) (Ferguson, 1977) or by counting IEL per unit length of surface epithelium, an increase in IEL has been shown (Holmes, Asquith, and Stokes, 1974). However, when counting IEL by using a unit length of the muscularis mucosa as the reference, no increase was noted by Guix, Skinner, and Whitehead (1979). It is unlikely that in gluten sensitive enteropathy the increased IEL is only a factor of reduced surface epithelial cells since epithelial area may be reduced fivefold or more with only a twofold increase in IEL (Ferguson, 1976) and in dermatitis herpetiformis the only histologically detectable alteration in the small bowel may be an increase in IEL in the villous epithelium (Chiu and Watson, 1978).

IEL are T cells, but the mechanism by which they are localized to the gut epithelium are not fully understood (Ferguson, 1977). What is the significance of IEL increase in tropical sprue? It has been suggested that in gluten sensitive enteropathy, IEL are acting as killer (K) cells (Ezeoke, Ferguson, and Fakhri, 1974) and that the epithelial cell basement membrane may also be a target (Chiu and Watson, 1978). Virus-like particles have been noted in damaged enterocytes in tropical sprue (Mathan and Mathan, 1978) and clustering of IEL around damaged enterocytes has been noted (Mathan et al., 1975). Are these cells involved in the elimination of damaged enterocytes either by direct cellular cytotoxicity or by antibody dependent cellular cytotoxicity? Experimentally cell mediated immune reaction in the small intestine can lead to villous atrophy and crypt hyperplasia (Ferguson, 1977). Is it possible that such a mechanism is operative in tropical sprue?

This investigation in patients with tropical sprue has shown that humoral immune functions including the capacity to produce secretory antibodies are preserved in the presence of intestinal mucosal damage. The majority of abnormalities including the reduction in PBL appear to be secondary to compromised intestinal mucosal integrity. The significant elevation in IEL parallels the degree of mucosal damage, the basic lesion in tropical sprue. The aetiology of the mucosal damage is not yet known although, from epidemiological studies, at least some cases are likely to be the result of an infectious agent (Mathan and Baker, 1971). A further understanding of the increased IEL awaits the identification of an aetiological agent.
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REFERENCES


