Influence of nutritional status on immune functions in patients with Crohn’s disease

A D HARRIES, V A DANIS, AND R V HEATLEY

From the Department of Gastroenterology, University Hospital of Wales, and Departments of Surgery and Medicine, Welsh National School of Medicine, Cardiff

SUMMARY Nutritional status and immune function were correlated with clinical features in 56 patients with Crohn’s disease. These were divided arbitrarily into either undernourished or well nourished groups according to whether their midarm circumference was below or above 90% of ideal standard. Results were also compared with 33 patients with ulcerative colitis and 28 normal subjects. Undernourished patients with Crohn’s disease had significantly reduced total lymphocyte and T lymphocyte counts and a reduced proportion of monocytes that ingested latex particles. Well nourished patients with Crohn’s disease were similar to the two control groups. Twenty one undernourished patients with Crohn’s disease were also followed during the course of two to four months’ nutritional treatment with an enteral supplement. Nutritional therapy was associated with significant anthropometric gains as well as significant rises in total lymphocyte and T lymphocyte counts. Serum orosomucoids were significantly higher in undernourished patients and decreased significantly during nutritional therapy. The results show that undernutrition and disease activity may be associated with reduced immunological competence in patients with Crohn’s disease, but all these measurements can be improved by short term nutritional treatment.

Although the aetiology of Crohn’s disease is still unknown there is considerable evidence to implicate immune mediated mechanisms. Patients with Crohn’s disease have reduced numbers of circulating T cells1–5 and many patients are unable to mount delayed type hypersensitivity responses,6–9 although not all results are consistent.10–15 While T lymphocyte populations are not greatly affected by drug treatment15 16 they can be depressed by long standing disease possibly caused by long term steroid treatment.17 Further evidence for reduced cellular immunity in Crohn’s disease comes from observations of reduced lymphocyte responsiveness to non-specific mitogens,18 19 although once again there are conflicting reports.20–22

Patients with Crohn’s disease represent a heterogeneous population with differing clinical activity, anatomic extent of disease and treatment regimens. This as well as differences in methodology may contribute to the variation in observed data. Another important variable that needs to be considered is nutrition. While malnutrition is common in Crohn’s disease, mainly due to anorexia, acute inflammation and enteric loss of nutrients many patients retain good nutrition.23–26

The immunological abnormalities found in Crohn’s disease resemble those found in protein calorie malnutrition in terms of low circulating T lymphocytes27 28 and impaired cutaneous hypersensitivity reactions.27 29 30 Both of these abnormalities can be improved with nutritional repletion.27 31 32 Reports of reduced lymphocyte responsiveness to non-specific mitogens are, however, inconsistent in patients with protein calorie malnutrition.33–37 In both Crohn’s disease and protein calorie malnutrition serum immunoglobulin concentrations may be normal or raised.30 38–42 Serum IgA concentrations are often high in protein calorie malnutrition, and this is also the case in Crohn’s disease.43

We have studied a number of immune parameters in patients with Crohn’s disease and related these findings to various assessments of nutritional status. Results have been compared with those from patients with ulcerative colitis and normal control subjects.

Address for correspondence: Dr R V Heatley, Senior Lecturer, Department of Medicine, St James’s University Hospital, Leeds LS9 7TF.

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Methods

Patients
Fifty six patients with Crohn's disease and, for control purposes, 33 patients with ulcerative colitis were selected from patients attending a gastrointestinal clinic. An additional 28 hospital personnel served as healthy control subjects. Clinical features of patients with Crohn's disease were documented: disease distribution was assessed by radiographic examination; the extent of small bowel resection was obtained from pathology reports; the activity of disease was assessed clinically by a simple index and a score of five or more was taken to indicate active disease. In many patients, serum orosomucoids (α1 acid glycoprotein) were measured on a Beckman rate reaction nephelometer and this was used to supplement the clinical activity score.

Nutritional Assessment
Standard anthropometric measurements were performed on all subjects. These included height, weight, midarm circumference (MAC), and skinfold thickness. Midarm circumference was measured at the midpoint between the acromioclavicular joint and olecranon process. Skinfold thickness was measured by Holtain calipers at triceps, biceps, subscapular, and suprailiac regions. The sum of these four measurements was expressed as a total skinfold thickness in millimetres. Midarm muscle circumference (MAMC) was derived from the midarm circumference and triceps skinfold thickness (TSF): MAMC (cm) = MAC (cm) − 0.314 × TSF (mm). Anthropometric measurements, with the exception of skinfold thickness, were related to ideal standards given for men and women and expressed as a percentage of ideal.

Observed weight was expressed as a per cent of minimum desirable weight (ideal) based on tables compiled by the Society of Actuaries. The number of subjects falling below the fifth percentile for triceps skinfold thickness, midarm circumference and midarm muscle circumference was also calculated from tables. Patients with Crohn's disease were divided into two groups, those with midarm circumference <90% ideal (26.4 cm men, 25.7 cm women), undernourished, and those with midarm circumference >90% ideal who were considered well nourished.

Laboratory measurements included haemoglobin and serum albumin, measured on an SMA Plus auto analyser, and serum prealbumin, measured by radial immunodiffusion.

Cell Preparation and Culture
Mononuclear cells were separated from heparinised blood by centrifugation, after 50% dilution in phosphate buffered saline, over Ficoll-hypaque density gradient (specific gravity 1.077), 450 g for 20 minutes at room temperature. Total mononuclear cells were enumerated by vital staining of whole blood with crystal violet in 1% acetic acid. T cells were enumerated by rosetting with 2-amino-ethylisothiouranium bromide (AET) treated sheep red blood cells. Latex ingesting cells were excluded in the AET-E rosette counts. Monocytes were enumerated separately as per cent latex ingesting cells (200 μl cells at 2×10⁶/ml were incubated for 15 minutes at 37°C with 50 μl of a 10⁹ particles/ml suspension of latex particles, centrifuged for five minutes at 50 g, and kept at 4°C until counted). Percentage counts were related to the absolute mononuclear cell count in the blood. T lymphocyte subsets were identified using the OKT series of monoclonal antibodies (Ortho Diagnostic Systems, New Jersey, USA). Peripheral blood lymphocytes were cultured, after adherent cell depletion, in round bottom microculture plates (Sterilin) in RPMI 1640 medium (Gibco) containing 10% fetal calf serum (Tissue Culture Services) and 1% penicillin and streptomycin for eight days in a humidified chamber at 37°C in 5% CO₂ in air. Cells were cultured in three concentrations (2.5, 5, and 10×10⁵/ml) in quadruplicate with half of the culture stimulated with pokeweed mitogen (Gibco) to a final dilution of 1/250. Supernatants were stored at −60°C until assayed.

Mononuclear cells were allowed to adhere to 35 mm Petri dishes at 37°C and at the same time exposed to a suspension of latex particles. After one hour the non-adherent cells were removed, and the proportion of monocytes that ingested latex particles (both in the adherent and non-adherent fractions) was estimated, and the number of adherent cells was counted on an inverted phase contrast microscope.

Radioimmunoassay
Immunoglobulins (G, A, and M) were measured in the supernatant by solid phase radioimmunoassay. Briefly, heavy chain specific rabbit antibody (Dako) was adsorbed to flexible polyvinyl chloride plates (Dynatech) for one hour. After blocking the remaining protein binding sites with bovine serum albumin and Tween 20 for 30 minutes at 37°C, 25 μl of supernatant was placed in duplicate wells and allowed to rest for one hour at 37°C. The plates were then washed and incubated with 25 μl of 125I-labelled purified myeloma protein for a further hour, after which unbound radioactivity was washed off and each well counted separately on an LKB gamma counter. G, A, and M estimations were
Nutrition and immunity in Crohn's disease

added together as a measure of total immunoglobulin production.

Validation of immunological methods
On three separate occasions blood was collected from the same individual and divided into four lots for individual analysis of T lymphocyte and monocyte levels. The mean coefficient of variation (CV%) by one observer was: T cell count CV=7.9%, n=12; monocyte count CV=17.3%, n=12. A control preparation of human immunoglobulins was assayed by radioimmunoassay on 10 different occasions with a coefficient of variation of 13.3% (n=10). When replicate cultures of the same lymphocyte preparation were cultured on the same occasion the coefficient of variation was 22.4% (n=8).

Nutritional treatment
Twenty one undernourished Crohn’s disease patients were followed during the course of nutritional therapy. Thirteen patients were on prednisolone (mean daily dose 11 mg) at initial assessment, and 14 patients on a mean daily dose of 11 mg at the end of nutritional therapy. Ten of these patients had their dose of prednisolone unaltered. Nutritional therapy was given with a liquid enteral supplement (Ensure Plus) and was carried out on an outpatient basis. The patients recorded on diary cards the quantity of Ensure Plus consumed per day. The calories provided by their ordinary diet alone was assessed by an experienced dietician using a semi weighed method over a period of three days. The initial daily calorie intake (mean ± SD) was 2510±700 Kcal and the calorie intake provided by the ordinary diet at the end of the treatment period was 2670±680 Kcal. The enteral supplement provided a mean extra calorie intake of 550 Kcal per day, which increased the overall mean daily calorie intake to 3220 Kcal. Baseline measurements were compared with those made at the end of nutritional therapy, which was two to four months. Additional measurements performed on these patients included erythrocyte sedimentation rate, platelet count, and creatinine height index.49

Statistical analysis
Student’s t test for unpaired data was used to compare results between the four groups. Student’s t test for paired data was used to compare results during nutritional therapy. Data in Tables were expressed as mean ± standard deviation and standard error bars were used in Figures.

Results
Clinical features and nutrition
There were 28 patients in each of the undernourished and well nourished Crohn’s disease groups. Their mean ages (±SD) were 35±13 years and 42±14 years respectively. There were 17 men and 11 women in the undernourished Crohn’s disease group and 12 men and 16 women in the well nourished Crohn’s disease group. The age of the ulcerative colitis group was 49±15 (16 were men and 17 women) and that of the normal subject group was 46±11 years (13 were men and 15 women). Thus each group had a similar age and sex distribution with the colitis group being slightly older.

The mean clinical activity index score was 4.0 in the undernourished Crohn’s disease group compared with 2.9 in the well nourished Crohn’s disease group. Twelve undernourished patients had clinically active disease (a score of five or more) compared with 11 of the well nourished Crohn’s disease patients. The two Crohn’s disease groups did not differ significantly in their clinical disease activity scores. Serum orosomucoid levels (mean ± SD), however, were significantly higher in the undernourished Crohn’s disease group (1.39±0.53 g/l, n=25) compared with the well nourished Crohn’s disease group (0.80±0.36 g/l, n=22) p<0.001. This was not taken into account in analysis, because there were no differences in the clinical activity scores.

In other respects the two Crohn’s disease groups were very similar. The mean duration of disease was eight years for the undernourished group and 10 years for the well nourished group. The distribution of disease in the undernourished Crohn’s disease group was: ileocaecal ileocolon (16), diffuse small bowel (six), and colon (six). The distribution of disease in the well nourished Crohn’s disease group was: ileocaecal ileocolon (17), diffuse small bowel (three), colon (five), and anorectal (three). Surgical resection had been carried out on 13 undernourished Crohn’s disease patients (mean length of small bowel resection 70 cm) and on 16 well nourished Crohn’s disease patients (mean length 55 cm). Fifteen undernourished Crohn’s disease patients were on prednisolone (mean dose 9 mg/day) compared with 12 in the well nourished Crohn’s disease patients. Eleven undernourished Crohn’s disease patients were on Salazopyrin compared with eight of the well nourished Crohn’s disease patients.

All anthropometric measurements, as well as haemoglobin, albumin, and prealbumin values were significantly reduced in undernourished Crohn’s disease patients compared with well nourished
Crohn's disease and ulcerative colitis patients and normal controls (Table 1).

**Immune Parameters: Relation to Nutrition**
Peripheral blood lymphocyte numbers were significantly reduced in undernourished Crohn's disease patients, and absolute T cell numbers as well as percentages of total lymphocyte numbers were also significantly reduced (Table 2). Values in well nourished Crohn's disease patients were similar to the control groups. Circulating monocyte numbers were only marginally increased compared with the other groups. The proportion of adherent monocytes was reduced in Crohn's disease but was not affected by nutritional variables. The proportion of monocytes ingesting latex, however, was significantly reduced in malnourished Crohn's disease patients compared with nourished Crohn's disease patients (p<0.01). In all patients with Crohn's disease there was a significant correlation between weight (per cent of ideal) and peripheral lymphocyte numbers (r=0.446, p<0.001) or T lymphocyte numbers (r=0.513, p<0.001, n=56). There was also a significant correlation between serum albumin concentrations and total lymphocytes (r=0.333, p<0.05) or T lymphocytes (r=0.368, p<0.05, n=56). Furthermore, there was a significant reduction in OKT4⁺ cells (well nourished 35.9±9.5%, undernourished 24.5±6.4%) and OKT8⁺ (well nourished 28.2±5.8%, undernourished 18.1±6.4%) (p<0.05, n=10 in each group). The ratios OKT4/OKT8 were not significantly different, however (undernourished 1.42±0.59, well nourished 1.24±0.41).

Immunoglobulin production at low lymphocyte concentration (2.5x10⁹/ml) was significantly reduced in Crohn's disease patients compared with the control groups (p<0.01), and although the undernourished group tended to produce less immunoglobulins than the nourished group, these differences were not significant (Fig. 1).

Results for IgA, IgM, and IgG production analysed separately were similar to those analysed for total immunoglobulin production. There was no significant correlation between immunoglobulin production and nutritional variables.

**Immune Parameters: Relation to Clinical Features**
Different clinical features had no significant effect upon white cell subpopulations nor upon functional characteristics of monocyte ingestion or immunoglobulin production from lymphocytes. There were significant although weak negative correlations, however, between serum orosomucoids and peripheral lymphocyte counts (r=0.355, p<0.05) and T lymphocyte counts (r=−0.364, p<0.05) n=47.

**Nutritional Therapy in Crohn's Disease**
Table 3 shows the changes in anthropometric, haematological, and biochemical indices after nutritional therapy. Anthropometric gains as well as gain in creatinine height index were significant. There was also an improvement in disease activity: the activity index (mean ± SEM) decreased from 4.7±0.7 to 3.7±0.6, the ESR decreased from 31±5 mm/h to 29±4 and the platelet count decreased from 500±34x10⁹/l to 460±20, none of these changes being significant. Seromucoids (mean ± SEM) decreased significantly from 1.39±0.11 g/l to 1.12±0.10 g/l (p<0.01).

Nutritional improvement was also associated with a rise in peripheral blood lymphocytes and T cells in particular and a fall in circulating monocytes (Table 4). Monocyte adherence function did not change but

### Table 1 Nutritional status in Crohn's disease patients and controls

<table>
<thead>
<tr>
<th></th>
<th>MAC &lt;90% (n=28)</th>
<th>MAC &gt;90% (n=28)</th>
<th>Ulcerative colitis (n=33)</th>
<th>Normal (n=28)</th>
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<tr>
<td><strong>Anthropometry</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Weight (% ideal)</td>
<td>85.4±7.0*</td>
<td>108.4±15.5</td>
<td>120.0±19.0</td>
<td>113.7±12.4</td>
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<tr>
<td>MAMC (% ideal)</td>
<td>83.3±7.6*</td>
<td>102.0±9.6</td>
<td>103.6±9.6</td>
<td>102.5±8.5</td>
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<tr>
<td>Total skinfold (mm)</td>
<td>30.5±9.8*</td>
<td>57.6±22.9</td>
<td>64.0±21.0</td>
<td>56.0±17.3</td>
</tr>
<tr>
<td><strong>Laboratory measurements</strong></td>
<td></td>
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<tr>
<td>Albumin (g/l)</td>
<td>36.3±5.7*</td>
<td>43.9±3.5</td>
<td>42.6±3.1</td>
<td>46.0±3.3</td>
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<tr>
<td>Prealbumin (mg/dl)</td>
<td>21.0±6.7*</td>
<td>28.7±6.6</td>
<td>28.1±5.7</td>
<td>30.1±4.3</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>11.5±1.4*</td>
<td>13.4±1.4</td>
<td>13.5±1.8</td>
<td>13.9±1.5</td>
</tr>
</tbody>
</table>

* p<0.001 compared with Crohn's disease MAC >90% and controls.
Crohn's disease patients are in two groups. Undernourished with midarm circumference less than 90% ideal (MAC <90%) and well nourished with midarm circumference greater than 90% ideal (MAC >90%).
Nutrition and immunity in Crohn's disease

Table 2  Peripheral blood cells and monocyte function in Crohn's disease patients and controls

<table>
<thead>
<tr>
<th>Crohn's disease</th>
<th>MAC &lt;90% (n=28)</th>
<th>MAC &gt;90% (n=28)</th>
<th>Ulcerative colitis (n=33)</th>
<th>Normal (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocyte number (×10^9/ml)</td>
<td>17.3±6.3*</td>
<td>25.2±8.3</td>
<td>23.7±10.2</td>
<td>24.2±6.6</td>
</tr>
<tr>
<td>T cell number (×10^9/ml)</td>
<td>8.6±4.6*</td>
<td>16.3±6.3</td>
<td>15.9±6.6</td>
<td>16.0±4.9</td>
</tr>
<tr>
<td>T cell (%)</td>
<td>49±12*</td>
<td>64±10</td>
<td>68±10</td>
<td>67±11</td>
</tr>
<tr>
<td>Monocyte number (×10^9/ml)</td>
<td>4.3±1.4</td>
<td>3.6±2.2</td>
<td>3.5±2.0</td>
<td>3.9±1.2</td>
</tr>
<tr>
<td>Monocyte adherence (%)</td>
<td>33±18</td>
<td>33±18</td>
<td>55±29</td>
<td>67±10</td>
</tr>
<tr>
<td>Monocyte latex ingestion (%)</td>
<td>63±16*</td>
<td>72±14</td>
<td>64±18</td>
<td>68±9</td>
</tr>
</tbody>
</table>

* p<0.001, † p<0.05 compared with Crohn's disease MAC >90%.

Crohn's disease patients are in two groups. Undernourished with midarm circumference less than 90% ideal (MAC <90%) and well nourished with midarm circumference greater than 90% ideal (MAC >90%).

latex ingestion improved although not significantly. In vitro production of immunoglobulins at lymphocyte concentrations of 2.5×10^9/ml increased significantly (p<0.03) but did not reach control levels (Fig. 2).

There was no significant correlation between change in nutritional variables and change in immune measurements. In 12 patients, who all showed a decrease in serum orosomucoids, T cell lymphocytes (mean change ±SEM) increased significantly by 5.1±1.07×10^9/ml (p<0.001, paired Student's t test). Seven patients showed an increase in serum orosomucoids, and T cell lymphocytes showed a non-significant increase of 2.3±1.6×10^9/ml. The other two patients had no change in serum orosomucoids.

Discussion

In this study we have shown that patients with Crohn's disease can be divided into two groups according to nutritional assessment and that there are significant differences both in circulating immune cells and in in vitro immune functions. Well nourished Crohn's disease patients were shown to resemble healthy control subjects and patients with ulcerative colitis who are considered to have normal parameters of cell mediated immunity and normal nutritional measurements.

Although the separation of the Crohn's disease patients into two nutritional categories using midarm circumference was done on an arbitrary basis, it has previously been found to be useful with respect to biochemical measurements. Midarm circumference has also been used in the tropics as a simple and reliable means of objectively assessing nutritional status in children. The two Crohn's disease groups were very different with regard to weight and other anthropometric measurements expressed as per cent of ideal standards. The ideal standards rest on the assumption that triceps skinfold thickness, midarm circumference, and midarm muscle circumference change little with age once adulthood is reached. Frisancho has shown that while this is essentially true for men, women tend to increase these measurements with age. The

Fig. 1  In vitro immunoglobulin production by peripheral blood lymphocytes from undernourished Crohn's disease patients (MAC <90%) compared with well nourished Crohn's disease patients (MAC >90%) and controls.
groups in our study, however, were not only similar in age and sex but the anthropometric measurements were also expressed as percentiles. Those falling below the fifth percentile were judged to have abnormally low values. The majority of patients in our undernourished Crohn’s disease group fell into this category in marked contrast to the other three groups.

Although the two Crohn’s disease groups were similar in clinical features, serum orosomucoid concentrations were significantly higher in the undernourished group: orosomucoids are thought to be one of the best laboratory indices of disease activity in Crohn’s disease, and this suggests that intestinal inflammation was higher in undernourished patients.

With nutritional therapy, the significant improvements in weight, anthropometric indices, and creatinine height index were matched by improvement in lymphocyte and T cell lymphocyte counts, immunoglobulin production, and monocyte ingestion of latex. Disease activity appeared to improve, however, with a significant, although small, reduction in serum orosomucoids. Furthermore, the increase in T cell lymphocytes was particularly marked in those patients who showed a reduction in orosomucoid concentrations.

Disease activity is an important determinant of malnutrition in Crohn’s disease and the present study emphasises the difficulties encountered in trying to study the two variables independently and in trying to explain the results. In active disease hypoalbuminaemia is mainly a consequence of protein loss from the inflamed gut and reduction of lymphocytes and T cell lymphocytes may also be a result of the same mechanism. Malnutrition has been shown to be associated with low thymic hormone activity, however, and it is possible that undernutrition in Crohn’s disease results in impaired differentiation of T cell lymphocytes. A recent study raises the possibility that there is a thymosin dependent immunodeficiency state in Crohn’s disease. Specific nutritional defects, in particular iron, zinc, and vitamin C deficiency, may also be associated with impaired immune competence, and these occur not uncommonly in patients with Crohn’s disease.

It is not possible in this study to state whether nutrition or disease activity is the most important determinant of immune competence. It clearly shows, however, that before any comments can be made about the immunological status of Crohn’s disease patients, and indeed any disease group

| Table 3 Improvement of nutritional status after nutritional treatment in 21 undernourished Crohn’s disease patients |
|---------------------------------|-----------------|-----------------|
| Weight (% ideal)                | Before treatment | After treatment | p < 0.01 |
| Midarm muscle circumference (% ideal) | 83.7 ± 8.5     | 89.3 ± 8.4     | p < 0.01 |
| Total skinfold (mm)             | 20.0 ± 8.5     | 26.0 ± 12.8    | p < 0.01 |
| Albumin (g/l)                   | 36 ± 6.7       | 39.1 ± 5.8     | NS |
| Prealbumin (mg/dl)              | 23.7 ± 6.6     | 25.6 ± 6.5     | NS |
| Haemoglobin (g/dl)              | 11.7 ± 1.7     | 12.0 ± 1.7     | NS |
| Creatinine height index         | 0.73 ± 0.19    | 0.85 ± 0.24    | p < 0.05 |

NS = not significant.

| Table 4 Changes in immune parameters after nutritional treatment in 21 undernourished Crohn’s disease patients |
|---------------------------------|-----------------|-----------------|
| Lymphocyte number (×10⁵/ml)     | Before treatment | After treatment |
|                                 | 18.3 ± 6.2      | 23.3 ± 8.3      | p < 0.05 |
| T cell lymphocytes number (×10⁵/ml) | 9.2 ± 4.9      | 12.6 ± 5.9     | p < 0.01 |
| T cell (%)                      | 50 ± 14         | 56 ± 14         | NS |
| Monocyte number (×10⁵/ml)       | 4.3 ± 1.4       | 3.6 ± 1.6       | p < 0.05 |
| Monocyte adherence (%)          | 41 ± 27         | 32 ± 18         | NS |
| Monocyte latex ingestion (%)    | 63 ± 25         | 75 ± 21         | NS |

NS = not significant.
which is at risk nutritionally, there must be an assessment of the patient’s nutritional status.

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References

Harries, Danis, and Heatley

472

Influence of nutritional status on immune functions in patients with Crohn's disease.

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