Deficient interleukin 2 dependent proliferation pathway in T lymphocytes from active and inactive ulcerative colitis patients


Abstract

There is increasing evidence that ulcerative colitis is associated with an abnormality of the immune system. Although the aetiology remains unknown, it has been suggested that the immune system of these patients is implicated in the pathogenesis of their disease. T cell function was investigated in ulcerative colitis patients and defective phytohaemagglutinin induced T cell mitogenesis was found. The DNA synthesis induced by stimulation with phorbol esters plus ionophore (ionomycin), however, was normal. These changes cannot be ascribed to either decreased interleukin 2 synthesis or to a defective interleukin 2 receptor expression after cellular activation. Moreover, this defective proliferative response of the T lymphocytes was observed even in the presence of saturated concentrations of exogenous interleukin 2. These results emphasise that the interleukin 2 dependent proliferation pathway is deficient in T lymphocytes from ulcerative colitis patients.

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Methods

PATIENT POPULATION
Fifty two patients (31 men and 21 women with a mean (SD) age of 40 (15) years) with ulcerative colitis and 31 age and sex matched healthy controls were studied. Each gave his consent of the experimental protocol. The diagnosis of ulcerative colitis was established on the basis of clinical symptoms and on the endoscopic and pathological demonstration of abnormal, inflamed colonic mucosa. Disease activity in each patient was analysed according to Truelove’s criteria. Twenty five patients (14 men and 11 women, aged 14–54 years) had active disease (14 mild, nine moderate, and two severe). There were 27 patients with inactive disease (17 men and 10 women, aged 23–76 years). When the experimental study was performed, 17 patients had been taking steroids (14 plus sulphasalazine) for at least three weeks. The remaining 35 patients had not taken steroids. Of these, 27 were taking sulphasalazine and eight were receiving no specific treatment.

Exclusion criteria for this study were: (1) the existence of clinical, serological, microbiological or pathological proof of inflammatory
intestinal disease other than ulcerative colitis and (2) the presence of intercurrent diseases or drugs, excepting steroids, that could change the results of the immunological studies performed.

REAGENTS
Several monoclonal antibodies (MoAb) that recognise specific molecules on the surface of certain cellular subpopulations were used in the studies of phenotypic characterisation and activation: OKT11 (anti-CD2), OKT3 (anti-CD3), OKT4 (anti-CD4), OKT8 (anti-CD8), OKT9 (anti-CD71) (Ortho-mune, Orthodiagnostic Systems, NJ, USA); B1 (anti-CD20), Mo2 (anti-CD14), TAC (anti-CD25) (Coulter Clone, Coulter Immunology, FL, USA); and Leu-11B (anti-CD16), Anti-HLA-DR (Becton Dickinson, CA, USA).

CULTURE MEDIUM
RPMI 1640 (Flow Lab, CA, USA) supplemented with 1% L-glutamine (Flow Lab), 0.5% HEPES (Flow Lab), and 1% penicillin-streptomycin was used for cultures. This will be referred to as 'complete medium'.

ISOLATION OF LYMPHOID CELLS
Peripheral blood mononuclear cells (PBMNC) were obtained from the heparinised venous blood of the subjects by Ficoll-Hypaque density gradient centrifugation (Nyegaard Co, Oslo, Norway). T cells were purified by double rosetting as previously described, the purity being greater than 95% in every case. After counting, cells were resuspended in complete medium supplemented with 10% heat inactivated fetal calf serum (FCS; Gibco, NY, USA), and checked for viability by trypan blue exclusion. In other experiments, supplementation with autologous or pooled AB serum (10% and 20% v/v) was performed.

PROLIFERATION STUDIES
T lymphocytes (250 000 cells/ml) were cultured on 96 flat bottom culture plates. Soluble phytohaemagglutinin (PHA; 10 μg/ml; Difco Lab, Detroit, MI, USA), or phorbol-myristate-acetate (PMA; 10 ng/ml; Sigma, St Louis, MO, USA) plus ionomycin (5 μg/ml, Sigma) in the presence or absence of recombinant interleukin 2 (rIL-2; 100 IU/ml; provided by Dr J Farrar and Dr P Sorter; Hoffman-La Roche, NJ, USA) were added at the beginning of proliferation studies. Each reagent was tested in dose/response titrations before use. Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO₂ for three, five, and seven days. Eighteen hours before the end of incubation, 1 μCi of tritiated thymidine (Radiochemical Centre, Amersham, UK) was added. The cells were harvested and the results expressed as mean (SD) counts per minute (cpm) of triplicate cultures.

LYMPHOKINE PRODUCTION
Lymphokine enriched supernatants were obtained by culturing T cells at 37°C at a density of 5 x 10⁶/ml in complete medium supplemented with 10% FCS. Cultures were incubated in the presence or absence of PHA (10 μg/ml) and supernatants were harvested after 24 and 72 hours of incubation, sterilised by filtration through a 0.22 μm filter (Millipore Company, Bedford, CA, USA), and stored at -70°C until use.

IL-2 activity was determined in the supernatants of T cell suspensions by evaluating the dose dependent proliferation induced in the cytotoxic murine line CTTL-2 by the lymphokine present in the cultures.

IMMUNOFLUORESCENCE ANALYSIS
Immunofluorescence staining of cell surface antigens was performed using a standard method as previously described. The fluorescence of 10 000 viable lymphoid cells stained for each different MoAb was quantified using an EPICS C flow cytometer (Coulter).

STATISTICAL ANALYSIS
The data from the groups were compared using the Mann-Whitney U test. A p value of less than 0.05 was considered statistically significant.

Results
PROLIFERATIVE RESPONSE BY T LYMPHOCYTES FROM ULCERATIVE COLITIS PATIENTS IS DEFECTIVE TO MITOGENIC SIGNALS THAT INTERACT WITH MEMBRANE RECEPTORS BUT NORMAL TO INTRACYTOSPLASMIC MITOGENIC SIGNALS
The blastogenic response of PBMNC from ulcerative colitis patients and healthy controls to the stimulation signal given by PHA was analysed first. As can be seen in Figure 1A, PBMNC from ulcerative colitis patients showed a significantly reduced mitogenic response to PHA in respect of the response in healthy controls, after either three or five days of culture (p<0.05). After seven days of culture in the presence of PHA, however, there were no significant differences between the proliferative response of PBMNC from ulcerative colitis patients and healthy controls.

The T lymphocyte population in the PBMNC from ulcerative colitis patients was purified to rule out a possible inhibitory effect of the non-T cells present in the PBMNC from these patients upon the proliferative response of the T lymphocytes. The PHA induced tritiated thymidine uptake by purified T cells from ulcerative colitis patients was also considerably impaired in respect of that in purified T lymphocytes from healthy controls after three and five days of culture (p<0.05) (Fig 1B). The optimum mitogenic dose of PHA for T lymphocytes from controls as well as from patients was the same (10 μg/ml). Since the maximal mitogenic response was
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Figure 1: Kinetics of the blastogenic response of (A) peripheral blood mononuclear cells (PBMNC) and (B) T lymphocytes from 18 ulcerative colitis patients and 12 healthy controls to stimulation with phytohaemagglutinin for three, five, and seven days. Results are expressed as mean (SD) counts per minute (cpm). Statistical differences between both groups (p<0.05) were found at three and five days of culture.

detected on day five in T lymphocyte cultures from both ulcerative colitis patients and healthy controls, the proliferative response was analysed on this day of culture in subsequent experiments. It was found that the blastogenic response to PHA of the purified T lymphocytes from the ulcerative colitis patients was significantly less than that observed in the healthy control group (p<0.05) (Fig 2). This hypoproliferative response cannot be ascribed to a change in the distribution of the mononuclear population or in the T cell subsets studied (Table).

Figure 2: Tritiated thymidine uptake by T cells from ulcerative colitis patients and healthy controls in response to phytohaemagglutinin (PHA) and phorbol-myristate-acetate (PMA) + ionomycin for five days. Results are expressed as mean (SD) counts per minute (cpm). Statistically significant differences were found between the mitogenic response of patients and controls in the presence of PHA (p<0.05). However, there were no statistically significant differences (p>0.05) between patients and controls when the mitogenic association PMA + ionomycin (Ion) was used.

To analyse the hypothesis that an inhibitory factor present in the ulcerative colitis patients’ serum could be involved in the defective proliferation found in the T lymphocytes, T lymphocyte cultures were performed simultaneously in the presence of either fetal calf, heterologous AB, or autologous serum. Neither ulcerative colitis patients nor healthy controls showed a significant difference in the PHA induced T cell mitogenic response, regardless of the serum used (p>0.05). In all the different experimental conditions analysed, the T cell blastogenesis in ulcerative colitis patients (FCS 62 358 (15 312) cpm, heterologous AB serum 65 563 (14 834) cpm, autologous serum 69 421 (16 513) cpm) was found to be defective in respect of those in healthy controls (FCS 116 671 (16 324) cpm, heterologous AB serum 120 514 (15 410) cpm, autologous serum 125 412 (17 211)) (p<0.05). We also tested the possibility that T cells isolated from ulcerative colitis patients will recover a normal proliferative response to PHA after a period of rest in culture before stimulation. In three cases, we did not find that the decreased proliferative response of T lymphocytes from ulcerative colitis patients was restored when they were rested after 48 and 72 hours in culture (data not shown).

Finally, the T cell proliferative response was analysed after stimulus with mitogenic molecules that directly interact with protein kinase C and intracellular Ca++: PMA and ionomycin. The proliferative response of T cells from ulcerative colitis patients and healthy controls was similar in these experimental conditions (p>0.05) (Fig 2).

Possible variations in the proliferative response of T cells from ulcerative colitis patients to stimulation with PHA were also investigated with regard to clinical parameters. There were no significant differences between the PHA induced proliferative response of T lymphocytes from patients with active and inactive disease (p>0.05). Likewise, there were no significant differences between the PHA induced proliferative response of T lymphocytes from patients with proctitis versus those with total colon involvement, nor between those patients studied within the first

Phenotypic study of the peripheral blood mononuclear cells (PBMNC) and purified T cells from ulcerative colitis patients and healthy controls

<table>
<thead>
<tr>
<th>Patients (Mean (SD))</th>
<th>Controls (Mean (SD))</th>
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<tbody>
<tr>
<td>T lymphocytes (CD3+)</td>
<td>60.6 (9.1)</td>
</tr>
<tr>
<td>B lymphocytes (CD20+)</td>
<td>142.2 (4.7)</td>
</tr>
<tr>
<td>Monocytes (CD14+)</td>
<td>21.7 (8.6)</td>
</tr>
<tr>
<td>Natural killer (CD16+)</td>
<td>14.0 (3.2)</td>
</tr>
<tr>
<td>T lymphocytes:</td>
<td></td>
</tr>
<tr>
<td>CD4+</td>
<td>95.5 (2.8)</td>
</tr>
<tr>
<td>CD8+</td>
<td>45.2 (5.7)</td>
</tr>
<tr>
<td>CD4+/CD8+</td>
<td>33.4 (6.7)</td>
</tr>
<tr>
<td>CD4+/(CD8+</td>
<td>1.46 (0.3)</td>
</tr>
</tbody>
</table>

PBMNC were studied by flow cytometry with OKT3 (anti-CD3), OKT4 (anti-CD4), OKT8 (anti-CD8), OKT11 (anti-CD2), B1 (anti-CD20), Mo2 (anti-CD14), and Leu-11b (anti-CD16) Moab, and a second step fluoresceinated reagent, as indicated in the methods section. Table gives the percentage of cells staining above the second step reagent background.

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five years of diagnosis versus those with disease of more than five years' duration. Nor were differences observed between patients with the chronic continuous evolutive type of the disease and those with the intermittent form. The hypoproliferative response of T lymphocytes to PHA stimulation was similar in ulcerative colitis patients with and without colectomy (p > 0.05), and the blastogenic response of T cells from patients taking steroids was similar to that in patients who were not taking these drugs (p > 0.05) (Fig 3).

IL-2 PRODUCTION OF T CELLS FROM ULCERATIVE COLITIS PATIENTS IS NORMAL

Three different steps can be defined in the activation and proliferation of T cells. Firstly, IL-2 is produced; secondly, IL-2R is expressed; and thirdly, upon binding to its specific receptors, IL-2 promotes T cell mitogenesis.

There were no statistical differences in the low rates of spontaneous production of IL-2 by T cells from ulcerative colitis patients (mean (SD) 1210 (213) cpm) and healthy controls (1514 (312) cpm) (p > 0.05). PHA stimulated T cells from the two populations also showed similar levels of IL-2 production (patients at 24 hours 24619 (5985) cpm; 72 hours 21304 (7703) cpm) (controls at 24 hours 26316 (5324) cpm; 72 hours 24638 (6334) cpm) (p < 0.05).

T LYMPHOCYTES FROM ULCERATIVE COLITIS PATIENTS SHOW NORMAL IL-2R EXPRESSION, BUT DEFICIENT BLASTOGENIC RESPONSE TO THIS LYMPHOKINE

The simultaneous findings of a hypoproliferative response and normal IL-2 production after PHA stimulation of T cells from ulcerative colitis patients could be attributed to defective expression of the IL-2R or some change in the mitogenic response induced by the interaction of IL-2 with its receptor, or both. To investigate the first suggestion, quantitative flow cytometric studies were performed after T cell staining with monoclonal antibodies specific for the IL-2R (anti-CD25). Antibodies to CD71 structure and DR molecules were also employed for a broader analysis of the T cell activation process. In basal conditions and after PHA stimulation for one, three, or five days, the proportion of stained cells and the intensity of the expression of CD25 antigen were similar in the T cell cultures from ulcerative colitis patients and healthy controls (p > 0.05) (Fig 4). Similar patterns were also found in the T cell membrane expression of CD71 and DR molecules in both ulcerative colitis patients and healthy controls.

Finally, we investigated the proliferative response of T cells from ulcerative colitis patients in the presence of saturating concentrations of exogenous IL-2. In the absence of stimulation with PHA, similar low mitogenic responses to exogenous IL-2 were found in T lymphocyte cultures from ulcerative colitis patients and controls (p > 0.05). Moreover, exogenous addition of saturant concentrations of IL-2 to the PHA stimulated T cell cultures from ulcerative colitis patients neither returned to normal nor significantly increased their blastogenic response (p > 0.05) (Fig 5).

Discussion

We have demonstrated that T lymphocytes from ulcerative colitis patients show a defective proliferative response to the mitogenic signals given by lectins that interact with surface molecules, but a normal blastogenic response to intracytoplasmatic protein kinase C activators. This T lymphocyte deficiency is independent of the clinical activity of the ulcerative colitis and cannot be ascribed to defective IL-2 production but rather to an impaired proliferative response by T lymphocytes to this lymphokine.
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The functional change shown in T lymphocytes from ulcerative colitis patients is not related to the activity of the disease, since it is also present in asymptomatic patients without clinical evidence of disease activity. Moreover, colectomised patients presented a similar defective proliferative response to the mitogenic signals.

These findings show that ulcerative colitis is not only associated with a local immune abnormality in the intestine but also with a systemic change in the immune system. We have recently shown that this immunological change is not restricted to the T lymphocyte population, since natural killer cells are also impaired in symptomatic ulcerative colitis patients. However, the T cell deficiency, unlike the change in natural killer cells, remains independently of the clinical activity in these patients. This T cell immunodeficiency has been found previously in other autoimmune diseases and may be implicated in the development of the impaired immune system regulation that plays a part in the pathogenesis of the intestinal and extraintestinal lesions observed in these patients.

We thank Ms G Perale and Ms C Lorenze for their skilful technical assistance.

19 MacDermott RP, Bridgeon MJ, Thurmond RD. Peripheral blood mononuclear cells from patients with inflammatory bowel disease exhibit normal function in the alllogeneic
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