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Interleukin 2 receptor expression by macrophages in inflammatory bowel disease

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SUMMARY

The expression of interleukin 2 receptor by macrophages from normal and inflamed terminal ileum and colon has been studied by using two monoclonal antibodies. In tissue sections from normal ileum and colon, scattered positive lymphocytes and only occasional weakly positive macrophages were seen. In ileal and colonic Crohn's disease or ulcerative colitis many positive macrophages and lymphocytes were seen in the lamina propria. These findings were confirmed by staining cytospin preparations of isolated intestinal mononuclear cells. The isolated macrophages were able to phagocytose opsonized zymosan and the majority were able to undergo a respiratory burst when triggered with opsonized zymosan or phorbol myristate acetate (PMA), suggesting that they were activated. Stimulation with interferon-γ or lipopolysaccharide did not increase the number of macrophages staining with the antibodies to the interleukin 2 receptor. Therefore we postulate that a large majority of the macrophages expressing interleukin 2 receptor in inflammatory bowel disease are a recently recruited population of cells.

Keywords interleukin 2 receptor macrophages inflammatory bowel disease

INTRODUCTION

Human intestinal macrophages are likely to provide the first line of defence against any micro-organisms or toxins breaching the epithelial barrier (Donnellan, 1965). Properties of macrophages which could provide this defence include the ability to phagocytose, to release oxygen radicals (Nathan *et al.*, 1983) and neutral proteases (Johnson *et al.*, 1982) and to present antigens to T cells (Unanue, 1984). Morphological and immunohistochemical heterogeneity of intestinal macrophages has been demonstrated (Selby *et al.*, 1983; Mahida *et al.*, 1986).

In inflammatory bowel disease there is an increase in the mucosal macrophage population. There is an increase in monocyte turnover (Meuret, Bitzi & Hammer, 1978) and activation (Mee, Szawatakowski & Jewell, 1980; Doe & Forsman, 1982) and it is likely that the increase in mucosal macrophage population is derived mainly from circulating monocytes.

Interleukin 2 (IL-2) is a T cell-derived growth factor which is involved in the regulation of T and B lymphocytes. Activated T cells and B cells express IL-2 receptors and recently they have also been demonstrated on activated, but not resting, monocytes (Waldmann, Goldman & Tsudo, 1987). Alveolar macrophages from patients with pulmonary sarcoidosis have recently been shown to express IL-2 receptors (Hancock, Muller & Cotran, 1987).

Isolated intestinal macrophages in inflammatory bowel disease have an enhanced ability to undergo a respiratory burst and hence appear to be activated (submitted for publication). In this study IL-2 receptor expression by macrophages in normal and inflamed colon and terminal ileum was studied, both in tissue sections and after isolation.

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MATERIALS AND METHODS

Tissue

Macroscopically and histologically normal terminal ileal (6) and colonic mucosa (13), all at least 5 cm from tumour, were obtained from operation resection specimens. Normal mucosa from one patient undergoing colonic resection for severe constipation was also used. Inflamed mucosa was obtained from patients undergoing intestinal resection for inflammatory bowel disease (nine with ulcerative colitis, eight with Crohn's colitis and eight with ileal Crohn's disease).

All patients undergoing intestinal resection usually had similar bowel preparation and they all received intravenous cefuroxime and metronidazole before and after the operation. All the patients with inflammatory bowel disease, except one with colonic Crohn's disease, were receiving intravenous hydrocortisone. For immunohistochemistry tissue was covered with OCT and frozen in liquid nitrogen. Sections (4 μm) were cut at a later date, fixed in acetone, and stored at $-20°C$ until used for staining.

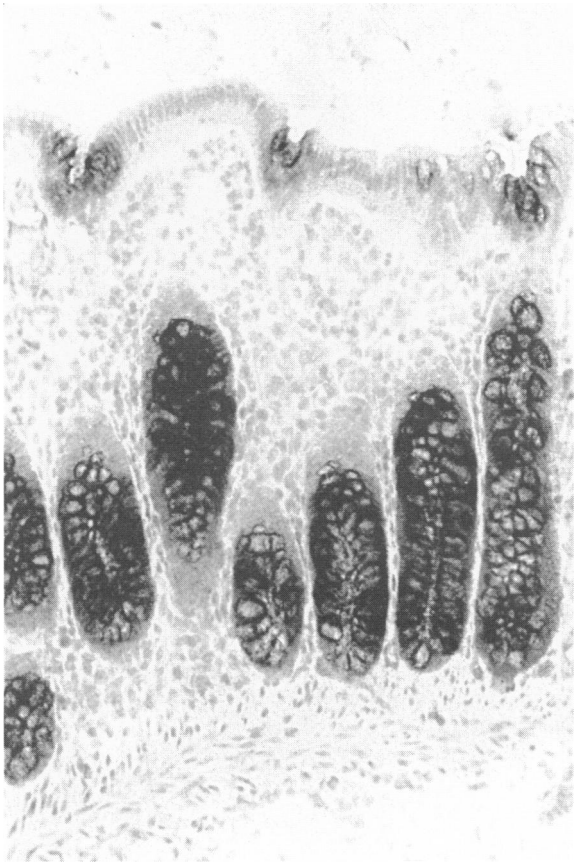

Fig. 1. Section of normal colon stained with anti-Tac antibody. No positive cell is present in the lamina propria. Staining of epithelial cells is due to endogenous alkaline phosphatase activity and was ignored. Original magnification × 40.

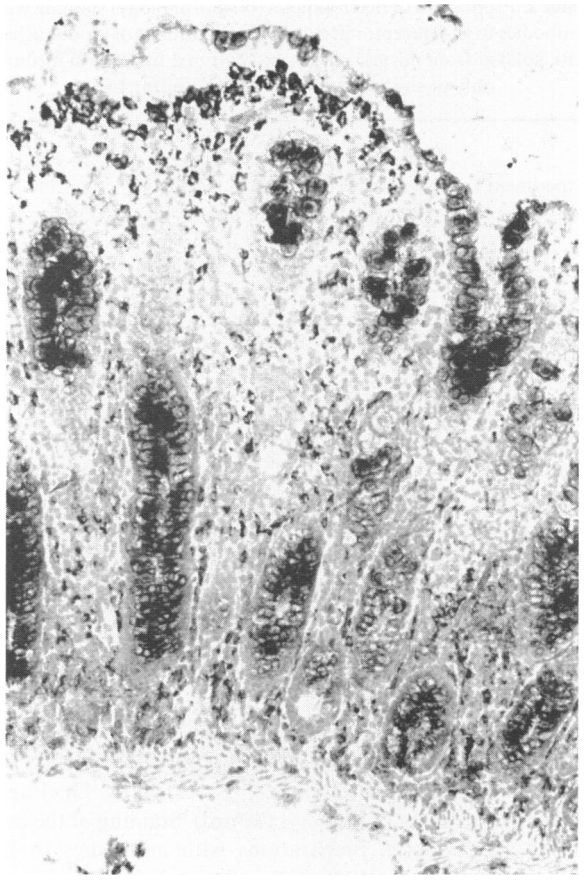

Fig. 2. Section of colonic Crohn's disease stained with anti-Tac antibody. Positive macrophages and lymphocytes are seen throughout the lamina propria. Staining of epithelial cells is due to endogenous alkaline phosphatase activity and was ignored. Original magnification × 87.

Cell isolation

Mononuclear cells were isolated from normal and inflamed colonic mucosa using a modified EDTA-collagenase technique of Bull & Bookman (1977). Epithelial cells were removed by shaking pieces of mucosa with 5 mmol EDTA in three half-hour steps. This was followed by digestion with collagenase (from *Clostridium histolyticum*; Boehringer, Mannheim) at a concentration of 100 mg/100 ml of culture medium (10% fetal calf serum in RPMI; from Gibco) for 3 h. Mononuclear cells were obtained by centrifugation on Ficoll-Paque (Pharmacia). Cytocentrifuge preparations of isolated intestinal mononuclear cells were made, air dried, fixed in acetone and stored at −20°C until used for immunohistochemistry.

IFN-γ and LPS stimulation

In three experiments mononuclear cells isolated from three normal colons were stimulated with interferon-γ (IFN-γ; from supernatant of Chinese hamster ovary cell line, donated by Dr Scott, Wellcome Biotech), 680 U/ml and/or lipopolysaccharide (LPS; Sigma), 10 μg/ml. Cells were incubated for 42 h after which cytospin preparations were made.

Respiratory burst activity

In some experiments respiratory burst activity of the isolated mononuclear cells was determined. Cells ($1{\cdot}5 \times 10^6$) in 5 ml

Table 1. Percentage (median (range)) of total positive cells or positive macrophages and lymphocytes (on morphology) in cytospin preparations of isolated mononuclear cells stained with anti-IL-2 receptor antibodies

	Normal colon ($n=14$)	IBD colon ($n=11$)
Total positive cells	9 (1–18)	19 (8–34)
Positive macrophages	4 (0–9)	9 (3–18)
Positive lymphocytes	4 (0–10)	10 (2–22)

For all, normal colon vs IBD colon: $P<0{\cdot}01$.

Table 2. Proportion of macrophages (on morphology) staining with antibodies to IL-2 receptor in cytospin preparations of mononuclear cells isolated from normal colons and cultured for 42 h in medium only or in the presence of IFN-γ and/or LPS

Experiment	Culture medium only	IFN-γ (680 U/ml)	LPS (10 μg/ml)	IFN-γ and/or LPS
1	4·0	4·0	4·5	3·5
2	5·0	4·5	6·5	7·5
3	3·0	4·0	3·0	4·5

Results are expressed as percentages.

culture medium were incubated at 37°C with 1 mg of nitroblue tetrazolium (NBT; Sigma) and either 75 μg of opsonized zymosan for 1 h or phorbol myristate acetate, to a final concentration of 200 ng/ml, for 30 min. Cytocentrifuge preparations were then made, air dried, fixed, and stored at −20°C until used. Respiratory burst activity of macrophages was determined by their ability to reduce NBT to a deep blue-black formazan reaction product (Ezekowitz *et al.*, 1985).

Immunohistochemistry

Monoclonal antibodies used were anti-Tac (kindly provided by Dr T. Waldmann, Bethesda), anti-IL-2r (Dakopatts) and NDS21 (anti-HLA pan-D, kindly provided by Dr Fuggle, Nuffield Department of Surgery, Oxford). Staining of the tissue sections and cytospin preparations with antibodies to IL-2 receptors was performed using the enhanced alkaline phosphatase anti-alkaline phosphatase technique (Cordell *et al.*, 1984). Staining with the antibody NDS21 was performed using the peroxidase technique (Gatter, Falini & Mason, 1982). Controls included omitting the primary (monoclonal) antibody or using an irrelevant monoclonal antibody (OX7 kindly provided by Dr A. Williams, Sir William Dunn School of Pathology, Oxford).

Statistical analysis

Statistical analysis was performed using one-way analysis of variance and Wilcoxon's rank sum test (paired or unpaired as indicated).

RESULTS

Control staining

In control sections (stained after omitting the primary antibody or using the irrelevant monoclonal antibody OX7) only epithelial cells were stained because of endogenous alkaline phosphatase activity, despite attempts to block it with levamisole, and they were ignored in subsequent studies. There was usually no staining of cells in the lamina propria.

In control cytospin preparations, occasional positive cells (probably contaminating epithelial cells) were seen.

Studies with antibodies to IL-2 receptor

Staining by the two anti-IL-2 receptor antibodies (of tissue sections as well as cytospin preparations) was largely similar and no distinction is made between these two antibodies in the results.

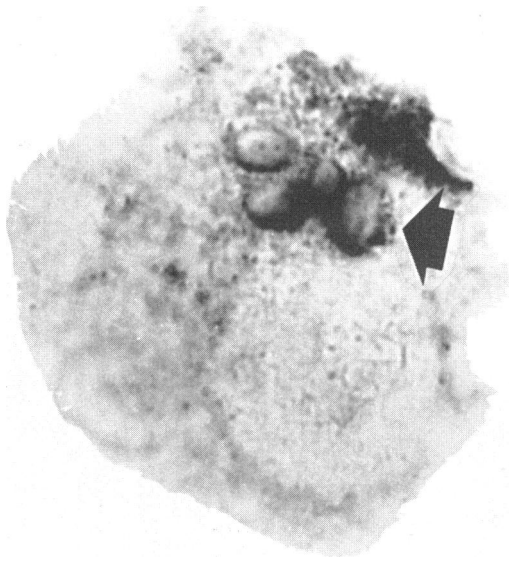

Figure 3. Isolated intestinal macrophage which has phagocytosed zymosan (arrow) and is stained with anti-Tac antibody. Original magnification × 1,300.

Table 3. Proportion of macrophages staining with IL-2 receptor and anti-HLA-D (201521) antibodies, with ingested opsonized zymosan, demonstrating release of oxygen radicals as shown by reduction of NBT to blue-black reaction product

Experiment	IL-2 receptor-positive macrophages (%)	HLA-D-positive macrophages (%)
1	55	13
2	50	25
3	61	26
4	45	29
5	49	9
6	73	5
7	54	11
8	52	17
9	72	22

All experiments were performed on macrophages isolated from inflamed mucosa from colons with inflammatory bowel disease.

Studies on tissue sections

In sections of normal colon scattered positive lymphocytes and only occasional weakly positive macrophages (determined by morphology) were seen in the lamina propria. The weakly positive macrophages were found predominantly in the superficial lamina propria. However, in many areas of the lamina propria no positive cells were seen (Fig. 1).

In the normal terminal ileum positive cells were only rarely seen. Positive cells were, however, present in the Peyer's patches (mainly the dome region and germinal centre).

In inflammatory bowel disease affecting the colon (five ulcerative colitis, eight Crohn's disease), strong to moderate staining of macrophages was seen in 10 and light staining in three (Fig. 2). Many positive lymphocytes were also seen in all sections. In 10, positive macrophages and lymphocytes were present throughout the lamina propria but in three they were confined mainly to the superficial lamina propria.

In ileal Crohn's disease (eight) positive macrophages and lymphocytes were seen throughout the lamina propria in three but in five, positive cells were confined mainly to the superficial lamina propria.

Studies on isolated cells

Viability of the isolated mononuclear cells was always 90% or more. Yields of mononuclear cells per gram of mucosa (median (range)) were: normal colon: $10{\cdot}6 \times 10^6$ ($2{\cdot}2$–$17{\cdot}6 \times 10^6$); inflammatory bowel disease mucosa: $22{\cdot}5 \times 10^6$ ($7{\cdot}6$–$36{\cdot}0 \times 10^6$). The difference was statistically significant ($P < 0{\cdot}01$); Wilcoxon rank sum test).

In cytospin preparations of mononuclear cells isolated from normal and inflamed colon the percentage of positive cells was determined (Table 1). There was a significantly greater proportion of positive cells in preparations isolated from inflammatory bowel disease colons than from normal colons. Of the positive cells the proportion of cells that appeared to be lymphocytes or macrophages, on morphology, was determined. For both the cell types a greater proportion of positive cells were present in preparations of mononuclear cells isolated from inflamed compared to normal colons.

Studies with IFN-γ and/or LPS

In cytospin preparations of mononuclear cells from normal colons stimulated with IFN-γ and/or LPS, the proportion of positive macrophages (on morphology) was determined (Table 2). Stimulation with these molecules did not significantly change the proportion of macrophages staining with the IL-2 receptor antibodies.

Respiratory burst activity

In some experiments the ability of the macrophages from inflamed colons to undergo a respiratory burst was determined. Cells triggered to release oxygen radicals by opsonized zymosan or PMA, in the presence of NBT, were stained with the IL-2 receptor antibodies. Mononuclear cells that had phagocytosed zymosan were readily identified by microscopy (Fig. 3). The proportion of IL-2 receptor-positive mononuclear cells, with ingested zymosan, that had undergone a respiratory burst (as shown by blue-black formazan reaction product) was determined. There was a significantly greater proportion of IL-2 receptor-positive macrophages that showed evidence of release of oxygen radicals compared to HLA-D positive macrophages (with ingested zymosan particles) isolated from the same colons (Table 3). In mononuclear cells isolated from seven inflamed colons the ability of IL-2 receptor-positive macrophages to undergo a respiratory burst in response to PMA showed that a median 80% (range 43–83%) showed evidence of release of oxygen radicals.

DISCUSSION

Using enhanced APAAP staining we have shown that in tissue sections of normal terminal ileum and colon only a few lymphocytes and occasional macrophages stain with antibodies to IL-2 receptors. In contrast, in sections of inflamed ileum and colon many lymphocytes and macrophages were positive. Previous studies (Selby *et al.*, 1984; Pallone *et al.*, 1987) using similar antibodies have concentrated on staining of lymphocytes only. Studies on isolated mononuclear cells supported the findings in tissue sections although the number of positive cells, in preparations isolated from normal colons, were a little higher than expected. This may be due to the inclusion of lymphoid aggregates (usually not identifiable despite using a dissecting microscope) which contain a number of positive lymphocytes and some macrophages (unpublished observations). Alternatively it may be due to contamination by epithelial cells, which have endogenous alkaline phosphatase activity.

Studies with opsonized zymosan confirmed that many of the large mononuclear cells were macrophages, as shown by their ability to phagocytose these particles. Using opsonized zymosan and PMA as triggers we have also shown that the majority of macrophages staining with anti-IL-2 receptor antibodies are able to undergo a respiratory burst. An enhanced or up-regulated respiratory burst is one of the characteristics of an activated macrophage (Adams & Hamilton, 1984). Thus the intestinal macrophages staining with these antibodies appear to be activated. In normal colon and ileum, therefore, there appear to be very few activated macrophages. This has been confirmed by assessing respiratory burst activity of isolated intestinal macrophages using 'pan-macrophage' markers (submitted for publication).

The studies reported here were performed on patients with a severe relapse of inflammatory bowel disease which required resection and high-dose steroid treatment before this. Effect of corticosteroids on the expression of IL-2 receptors on macrophages is not known. However, studies on rectal biopsies of patients with active inflammation (on no treatment) have shown many IL-2 receptor-positive mononuclear cells in the lamina propria (unpublished observations).

Whether the anti-IL-2 receptor antibodies are binding functional IL-2 receptors on the intestinal macrophages remains to be determined. Studies on monocytes activated by IFN-γ or LPS have shown that functional IL-2 receptors are induced and that the majority of these are of the low affinity type (Holter *et al.*, 1987). However, some of the high affinity receptors were also demonstrated (about 0·5).

The studies with IFN-γ and LPS on mononuclear cells from normal colonic mucosa suggest that the majority of macrophages staining with anti-IL-2 receptor antibodies are derived from the circulating monocytes and are an elicited population of cells. The functional significance of expression of IL-2 receptor on macrophages is unknown. Incubating activated monocytes with recombinant IL-2 has been shown to lead to increased

production of hydrogen peroxide (Holter *et al.*, 1987). Thus it can be postulated that IL-2 secreted by activated T cells may induce the release of oxygen radicals which may have a role in anti-microbial responses by macrophages. Another suggestion is that the low affinity receptors are able to bring high levels of IL-2 in close proximity to lymphocytes which may then be induced to proliferate (Dr G. MacPherson, personal comunication).

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