Enhanced production of interleukin 1- β by mononuclear cells isolated from mucosa with active ulcerative colitis of Crohn's disease

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SUMMARY IL1- β production by mononuclear cells isolated from normal and active inflammatory bowel disease mucosa was studied. Significantly more IL1- β was produced spontaneously by mononuclear cells from the inflamed mucosa compared with those from normal colonic mucosa (median 190 pg/ml (range 45-700) v 20 pg/ml (0-165)). Stimulation with lipopolysaccharide enhanced IL1- β production by mononuclear cells from active inflammatory bowel disease mucosa but not those from normal mucosa. Depleting the mononuclear cells of macrophages, by panning with monoclonal antibody 3C10, reduced the amount of IL1- β produced. Enhanced IL1- β production from the inflamed mucosa may play an important role in the mediation of many inflammatory responses. The enhanced production appears to be the result of a recruited population of cells.

Interleukin 1 (IL1) is a polypeptide produced predominantly by stimulated macrophages and monocytes. The structurally distinct forms of IL1, IL1- α and IL1- β have been identified. They are coded by two separate genes on chromosome 2 and messenger RNA for IL1- β predominates over that coding for IL1- α .¹ Although they share only 26% sequence homology, their biological activities are, for the most part, identical.² This could be explained by identical cell surface receptors for the two forms of IL1.³ Interleukin 1 exerts an influence over a wide range of biological functions. It acts as an endogenous pyrogen and induces hepatocytes to synthesise acute phase proteins - for example, serum amyloid A, C-reactive protein, fibrinogen.^{1,2,4} It activates T lymphocytes,⁵ increases antibody synthesis by B cells⁶ and induces granulocyte release from the bone marrow.⁷ It enhances fibroblast collagen production⁸ and alters prostaglandin production.¹ Thus IL1 appears to play a major role in inflammatory responses.

There is little current information on IL-1 production by the intestinal mucosa in ulcerative colitis or Crohn's disease. The aim of this study was to

investigate IL1- β production by mononuclear cells isolated from normal and inflamed mucosa (with or without stimulation with lipopolysaccharide (LPS) using ELISA.

Methods

TISSUE

Normal colonic mucosa was obtained from colon resected for carcinoma (six) or for severe idiopathic constipation (one). The mucosa used was macroscopically and histologically normal and that from colon resected for carcinoma was obtained at least 5 cm from the tumour.

Inflamed colonic and ileal mucosa was obtained from intestine resected for active inflammatory bowel disease. Nine patients had ulcerative colitis, two Crohn's colitis and five ileal Crohn's disease. All the patients were on intravenous corticosteroids at the time of the operation.

ISOLATION AND CULTURE OF INTESTINAL MONONUCLEAR CELLS

Mononuclear cells (MNC) were isolated from normal and inflamed mucosa using a modified EDTA-collagenase technique.⁹ In brief, epithelial cells were removed by shaking strips of mucosa with 5 mmol

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EDTA in three, half hour steps. After washing, the mucosa was digested with collagenase (from *Clostridium histolyticum*) at a concentration of 1 mg/ml in 10% fetal calf serum/RPM1 (Gibco), for three hours at 37°C. Mononuclear cells were obtained by centrifugation on Ficoll-Paque (Pharmacia).

In some studies, the mononuclear cells were depleted of macrophages by a panning technique¹⁰ using a macrophage specific monoclonal antibody 3C10¹¹ (gift from Dr Steinman, New York).

Cytospin preparations of unfractionated and 3C10 depleted mononuclear cells were made and stained with monoclonal antibodies Y1/82A (macrophage specific, obtained from Dr D Y Mason, Oxford), TO15 (B cell specific, obtained from Dr D Y Mason, Oxford), and T910 (T cell specific; Dakopatts). For Y1/82A, the peroxidase technique¹² was used for staining and for the other antibodies, the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique¹³ was used.

CELL CULTURE

Cultures were done in flat bottom, tissue culture multiwell plates (Flow laboratories). Intestinal mononuclear cells were cultured at a concentration of 1×10^6 /ml in 10% fetal calf serum/RPM1, in the presence or absence of lipopolysaccharide (LPS; 10 µg/ml; Sigma) for 45 hours. Culture supernatant was collected by centrifugation at 600 g for 10 minutes followed by filtration with a 0.2 µ filter. The supernatant was stored at -70°C before analysis.

Peripheral blood mononuclear cells (isolated by

centrifugation on Ficoll-Paque) from three healthy individuals were also studied as described above.

MEASUREMENT OF IL1-β

The amount of IL1-β present in the supernatants was measured using ELISA (Cistron Biotechnology). It consisted of microtitration wells coated with monoclonal antibody specific for IL1-β (solid phase). Polyclonal rabbit anti-IL1-β was used to detect IL1-β bound to the solid phase. Horseradish peroxidase conjugated anti-rabbit-IgG was then added to the test wells. After development of the substrate, the colour intensity was measured using a microtitration plate reader. The tests were performed in duplicate and the amount of IL1-β in each sample was derived from the optical density curve of known standards. Previous studies¹⁴ have shown the specificity of this assay for IL1-β, with no cross-reactivity with tumour necrosis factor-α, interleukin 2 or interferon-gamma. The sensitivity of this assay has been shown to be equivalent or better than available bioassays.¹⁴ Reproducibility was within 10%.

STATISTICAL ANALYSIS

Statistical analysis was performed using the Wilcoxon's rank-sum test for non-parametric data, paired or unpaired. As data were not normally distributed, results are expressed as a median and range.

Results

There was no significant difference in the proportion of macrophages in mononuclear cells isolated from normal and inflamed colonic mucosa. This was shown by staining cytospin preparations of MNC with the macrophage specific monoclonal antibody Y1/82A. Mononuclear cells from normal colonic mucosa contained median 13% (range 9–19) of macrophages and cells from inflamed colonic mucosa, median 14.5% (range 8–22).

Preliminary studies showed that the amount of IL1-β in supernatants of 1×10^6 /ml MNC, from inflamed mucosa, was usually in the range detected by the assay.

Figure 1 shows the amount of IL1-β produced by MNC isolated from inflamed inflammatory bowel disease mucosa and from normal mucosa, with and without stimulation with LPS. There was significantly more IL1-β produced by MNC isolated from ulcerative colitis or Crohn's colitis mucosa, compared with MNC from normal mucosa (median 190 pg/ml (range 45–700) and 20 pg/ml (0–165) respectively). Lipopolysaccharide stimulation enhanced IL1-β production by MNC from inflammatory bowel disease colons but not from normal colons (Fig. 1).

Fig. 1 IL1-β production by MNC isolated from normal (seven) and inflamed (11) colonic mucosa, either spontaneously or in the presence of LPS. Normal colonic MNC only v IBD colonic MNC only: $p < 0.01$; normal colonic MNC + LPS v IBD colonic MNC + LPS: $p < 0.01$; normal colonic MNC only v normal colonic MNC + LPS: $p = NS$; IBD colonic MNC only v IBD colonic MNC + LPS: $p < 0.01$.

Fig. 2 IL1-β production by MNC (in the presence or absence of LPS) from inflamed and non-inflamed mucosa of four colons with distal ulcerative colitis.

Mononuclear cells isolated from five specimens with ileal Crohn's disease also produced high levels of IL1-β (spontaneously – median 220 pg/ml (range 100–370); after stimulation with LPS – median 235 pg/ml (range 150–470)).

In four colons resected for ulcerative colitis (with distal inflammation) it was possible to study MNC isolated from inflamed as well as non, or, minimally-inflamed areas of the same colon. In three out of four, more IL1-β was produced by mononuclear cells from inflamed areas compared with those from non, or, minimally-inflamed areas (Fig. 2).

Efficacy of the LPS used in these studies was confirmed in experiments on peripheral blood MNC from three healthy individuals. Mononuclear cells

Table 1 Interleukin 1-β (pg/ml) in supernatant of 10⁶/ml of unfractionated MNC and MNC depleted of macrophages with antibody 3C10, isolated from three colons with active ulcerative colitis

	Unfractionated MNC	3C10 depleted MNC
(1)	85	25
(2)	95	0
(3)	320	80

Table 2 Median (range) percentage of macrophages, T cells and B cells (identified by monoclonal antibodies) in cytopsin preparations of unfractionated MNC and MNC after panning with antibody 3C10. The MNC were isolated from mucosa of three colons with active ulcerative colitis

	Macrophages	T cells	B cells
Unfractionated MNC	18 (15–23)	44 (40–48)	16 (10–18)
3C10 depleted MNC	5 (4–7)	41 (40–46)	17 (10–23)

(1×10⁶/ml; cultured under the same conditions as intestinal MNC) cultured without LPS produced median 85 pg/ml (range 60–130) of IL1-β but in the presence of LPS (10 μg/ml) produced median 2280 pg/ml (range 1840–3100) of the cytokine.

Interleukin 1-β production by unfractionated MNC and MNC depleted of macrophages by panning with monoclonal antibody 3C10, was also compared (Table 1). In all three colons, there was less IL1-β in supernatants of mononuclear cells depleted of macrophages. Depletion of macrophages by panning was confirmed by staining cytopsin preparations of the mononuclear cells with macrophage specific monoclonal antibody Y1/82A (Table 2).

Discussion

This study shows that mononuclear cells isolated from mucosa of colons with active inflammatory bowel disease, cultured *in vitro*, produced much greater amounts of IL1-β than mononuclear cells isolated from normal colonic mucosa. This increase appears to be the result of enhanced production of the cytokine on a per cell basis as there was no significant difference in the proportion of macrophages present in MNC from normal or inflamed colonic mucosa. Stimulation with LPS enhanced production of this cytokine by mononuclear cells isolated from inflamed mucosa but not by those cells isolated from normal colonic mucosa.

Although other cells have been shown to produce IL1, macrophages are likely to be the major source of this cytokine.¹ This was confirmed in our experiments where IL1-β production was considerably reduced after depletion of macrophages by panning with the monoclonal antibody 3C10.

Enhanced production of IL1-β by cells isolated from inflamed colons is likely to be caused by the presence of activated macrophages.¹⁵ Peripheral blood monocytes stimulated with endotoxin or phagocytosis produce IL1. The lack of such a response to LPS by mononuclear cells isolated from normal colons suggests that normal intestinal macrophages are 'desensitised' to LPS with respect to this function. Thus the activated macrophages which produce IL1-β in the mucosa of inflammatory bowel disease are likely to be derived from circulating monocytes (an elicited population of cells). Potency of the LPS used in these studies was confirmed by experiments on peripheral blood MNC. Increased monocyte turnover has been shown in inflammatory bowel disease.¹⁶ They have also been shown to be activated.^{17,18} Using mouse thymocyte proliferation assay, spontaneous IL1 production by peripheral blood mononuclear cells in patients with Crohn's disease has been reported.¹⁹

The normal intestinal macrophage is located predominantly below the epithelium and is likely to come in contact with a wide variety of antigens and bacterial products. Thus its inability to be stimulated by LPS may protect against an inflammatory response being mounted unnecessarily under normal circumstances. It is likely that during an inflammatory response, monocytes are recruited into the mucosa. These may then mediate responses like the production of IL1 and generation of oxygen radicals, triggers for the development of an even greater inflammatory response.

All the patients with inflammatory bowel studied were receiving corticosteroids. It is unlikely that the enhanced production of IL1- β is due to the effect of this drug. On the contrary, corticosteroids have been shown to inhibit the production of this cytokine.²⁰ Therefore, this drug may actually be diminishing the production of IL1- β by MNC from inflamed mucosa.

In addition to being an important mediator of inflammatory and immunological reactions,^{1,21} in inflammatory bowel disease, IL1 is likely to be involved in the repair of damaged connective tissue by synthesising and remodelling components of the matrix like collagen, fibronectin, and proteoglycans^{8,23} and may contribute to fibrosis. It may also be involved in the induction of mucus secretion.²²

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