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Role of interleukin 1 in inflammatory bowel disease – enhanced production during active disease

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Abstract

Interleukin 1 is a polypeptide cytokine produced by various cell types and has been shown to have a major role in inflammatory and immunological responses. In experimental colitis it proved to be a dominant mediator and a reliable marker of inflammation. The aim of the present study was to determine in vitro the extent of production and release of interleukin 1 from colonic mucosa of patients with active untreated inflammatory bowel disease. Colonic mucosal biopsy specimens were obtained during colonoscopy from 17 patients with ulcerative colitis, eight patients with Crohn's disease of the colon, and 16 normal control subjects. Interleukin 1 content was determined in fresh and 24 hour organ cultured mucosa as well as in cultured medium. Interleukin 1 content and release were significantly higher in the inflamed mucosa compared with that of control subjects. Prednisolone inhibited interleukin 1 release in a dose dependent fashion. We conclude that colonic mucosal interleukin 1 content and production is significantly raised in active inflammatory bowel disease and may have a role in the pathogenesis of the inflammatory response. Pharmacological suppression of tissue interleukin 1 production may have a beneficial therapeutic effect.

Interleukin 1 is a polypeptide cytokine produced by various tissue cells[1] and has a variety of biological properties.[2] It is a key mediator that is released by monocyte macrophages in inflammatory and immunological responses.[3 4] Interleukin 1 acts locally by releasing prostaglandins, thromboxane, and platelet activating factor from the inflammatory cells, and systemically as a circulating hormone, it induces fever and the production of acute phase reactants by the liver.[3 5]

Since infiltration of inflammatory cells in the gut wall is a feature of inflammatory bowel disease, interleukin 1 may have a role in its pathogenesis. Recently, peripheral blood mononuclear cells obtained from patients with Crohn's disease were shown to produce in vitro high quantities of interleukin 1 compared with normal control cells,[6] and enhanced production of interleukin 1-beta was shown in colonic mononuclear cells isolated from patients with inflammatory bowel disease.[7] Moreover, mucosal interleukin 1 values were reported by us to be increased in two models of experimental colitis – in trinitrobenzene sulfonic acid colitis induced in rats[8] and in a rabbit model of acute colitis induced by enteropathogenic *Escherichia coli*.[9] In both models, mucosal interleukin 1 was found to be the most sensitive marker of colonic inflammation.

The aim of the present study was to determine the interleukin 1 content in fresh and cultured inflamed colonic mucosa obtained from patients with active ulcerative colitis and Crohn's disease of the colon and to assess the effect of drugs on its release during 24 hours of culture.

Materials and methods

STUDIES WITH MUCOSAL SPECIMENS

Mucosal tissue specimens were obtained during fibreoptic colonoscopy from inflamed sites in the recto-sigmoid colon of patients with untreated active ulcerative colitis and Crohn's colitis, as well as from normal control subjects without any abnormalities in their colon. The major reasons for colonoscopy in the control group were non-specific abdominal complaints, bleeding, haemorrhoids, and occult blood in the stool. Biopsy specimens obtained from the control groups did not show any histological abnormality. The diagnosis of ulcerative and Crohn's colitis was established according to clinical, endoscopic, pathological, and radiological criteria. In all patients with ulcerative colitis clinical activity was manifested by bloody diarrhoea and verified histologically by the presence of mucosal ulceration, crypt abscesses, and infiltration with inflammatory cells. The mean (SE) clinical activity index in patients with Crohn's colitis was 230 (48). Histological examinations in these patients showed mucosal ulcerations and mononuclear infiltration of the mucosa. No granuloma were found in any biopsy specimens examined. No subjects, controls, or patients, had received any medication for at least two weeks before the biopsy specimens had been obtained. The age and sex of the subjects examined are presented in Table I. The study protocol was approved by the local hospital's Helsinki committee. Tissue specimens were cultured (37°C, 5% CO_2, 95% air) for 24 hours, as described earlier.[10] In brief, the tissue was placed on a metal grid over the central well of the culture dish (Falcon) containing the culture medium which consisted of 0·7 ml RPMI 1640 (BioLab, Israel) containing penicillin (100 U/ml) and streptomycin (100 μg/ml). In some experiments mucosal biopsy specimens obtained from the same patient were also incubated for one, two, three, and four hours. Each culture dish contained three specimens. Fresh and cultured specimens, average weight 10 mg, were homogenised with a polytron homogeniser (Kinematic, Kriens-Lu, Switzerland) for 20 seconds at a speed grade of 6 in 0·5 ml 50 mM Tris HCl buffer, pH 7·4, containing 100 mM

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NaCl, 1 mM $CaCl_2$, and dextrose (1 mg/1 ml). These samples, as well as the samples of the cultured medium, were kept at −70°C until assayed for their interleukin 1 value.

In several experiments with ulcerative colitis mucosa, four to five tissue samples obtained from the same subject were incubated immediately after excision in the absence or presence of prednisolone (Sigma, St Louis, MO, USA) 1·56–100 μg/ml.

INTERLEUKIN I DETERMINATION

Interleukin 1 activity was determined by its induction of interleukin 2 production by murine EL-4 cells as described previously.[11] Briefly, 0·25 ml cultures of 2×10^5 EL-4 cells in a 96 well flat bottom plate are cocultured with the sample and 2×10^{-7} M calcium ionophore A23187 for 24 hours. The culture fluids are then tested for interleukin 2 activity using the CTLL-20 interleukin 2 dependent cell line. The interleukin 2 activity is directly proportional to the input of interleukin 1. Units of interleukin 1 activity were calculated relative to a standard of pure recombinant human interleukin 1 beta prepared as described previously[12] by a computer program described by Davis *et al.*[13] All tissue extracts were centrifuged at 10 000 *g* for three minutes and filter sterilised before assay. In several experiments tissue homogenates and medium after the culture were assayed for their interleukin 1 activity also by ELISA. The ELISA assay kit for human interleukin 1 alpha was obtained from Endogen, Boston, MA, USA. The ELISA assay kit for human interleukin 1 beta was obtained from Cistron Biotechnology, Pine Brook, NJ, USA. Both assays were performed according to the manufacturers' instructions. Statistical evaluation was performed according to the paired and unpaired Student's *t* test.

Results

MUCOSAL SPECIMEN STUDIES

Time course: interleukin 1 tissue content and release was linear with time (tissue: r=0·71, y=5·6+0·48x, p<0·01; medium: r=0·713, y=5·37+0·039x, p<0·01). Fresh colonic mucosa obtained from patients with active ulcerative colitis contained significantly higher values of interleukin 1 compared with control mucosa (p<0·01). Colonic mucosa of patients with Crohn's colitis contained even higher values than in ulcerative colitis (fig 1; p<0·02). After 24 hours of culture the interleukin 1 content of the normal mucosa increased by 94 times compared with its concentration in the fresh uncultured mucosa. The content in the cultured mucosa of ulcerative colitis and Crohn's colitis patients also increased and reached significantly higher concentration compared with the cultured normal mucosa (Fig 1).

Release of interleukin 1 into the medium by mucosa obtained from ulcerative colitis patients during 24 hours increased significantly compared with its release by the normal mucosa. Interleukin 1 release by Crohn's mucosa was also significantly increased and was even higher than

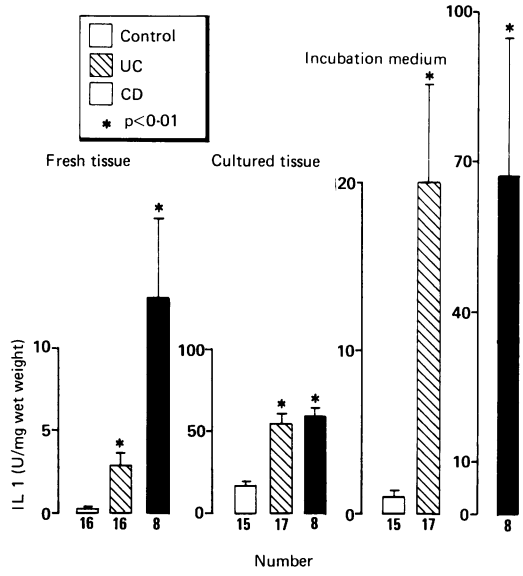

Figure 1: interleukin 1 (IL 1) values in fresh and 24 hour cultured colonic mucosa and incubation medium obtained from patients with ulcerative colitis (UC) and Crohn's disease (CD) and from normal control subjects.

Fresh and incubated mucosal specimens were processed and prepared for IL 1 determination, as described in Materials and methods. IL 1 was also determined in the incubation medium. Results are mean (SE).

**Significantly different from control (p<0·01).*

the release by the ulcerative colitis mucosa (p<0·01). Comparison of interleukin 1 determination in the cultured tissue and medium by bioassay (EL-4 assay) and ELISA assay is presented in Table II. The pg/ml values determined by the bioassay were based on the specific activity or recombinant interleukin 1 as performed using this assay, which is 5×10 U/mg.[12] The results clearly show good correlation between the two methods of assay and show that substances derived from the homogenisation did not interfere with the bioassay. Prednisolone significantly and decreased interleukin 1 content and release in a dose dependent manner during 24 hours of culture (tissue: r=0·596, y=83·1−0·53x, p<0·05; medium: r=0·515, y=49·3

TABLE I *Age and sex of the subject groups*

	Ulcerative colitis	*Crohn's disease*	*Control*
No	17	8	16
Women	5	5	7
Men	12	3	9
Age (yr):			
Range	19–72	13–68	30–86
Mean (SE)	43 (3·4)	34 (5·3)	51·6 (4·0)

TABLE II *Comparison between tissue and medium interleukin 1 determination by bioassay and ELISA assay. Results, mean (SE)*

Group (no)		*Bioassay (pg/ml)*	*ELISA assay (pg/ml)*
Control (3)	Cultured tissue	539 (211)	673 (256)
	Medium	67 (41)	80 (25)
Ulcerative colitis (4)	Cultured tissue	2113 (157)	1863 (195)
	Medium	247 (58)	205 (67)
Crohn's disease (4)	Cultured tissue	3898 (863)	2385 (664)
	Medium	2183 (861)	1300 (637)

Homogenates of cultured tissue and medium were assayed for IL 1 activity by bioassay and ELISA assay as described in Materials and methods.

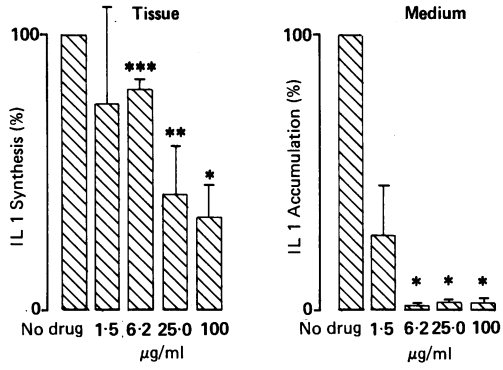

*Figure 2: Effect of prednisolone on 24 hour mucosal content and release of interleukin 1 (IL 1). Several mucosal specimens were obtained from each patient with active ulcerative colitis. Prednisolone (1·5–100 μg/ml) was added to the culture medium. Samples incubated without prednisolone served as controls. Results are mean (SE) of experiments performed with tissue obtained from three patients and expressed as a per centage of control. *p=0·01; **p=0·05; ***p=0·02.*

−0·53x, p<0·02) (Fig 2). The inhibitory effect of prednisolone on interleukin 1 release was more pronounced than its effect on tissue content. Table III shows a representative set of results of both the interleukin 1 bioassay and ELISA assays of tissue samples taken from a patient with ulcerative colitis and cultured for 24 hours. The data show a clear dose dependent inhibition by prednisolone of both interleukin 1 biological activity and immunoreactive interleukin 1 protein in the tissue homogenate. Furthermore, the ELISA data confirm that the observed inhibition was caused by an inhibition of interleukin 1 protein release and not by the carryover effects of the drug into the bioassay.

Discussion

Interleukin 1 has been implicated as a major mediator in inflammatory and immunological responses.[3-5] Since immune as well as other tissue injury mechanisms may play a part in the pathogenesis of inflammatory bowel disease, the role of interleukin 1 as a key mediator may be of importance.[14] Recently, Satsangi *et al*[6] found enhanced spontaneous interleukin 1 production by blood mononuclear cells in Crohn's disease and increased LPS-stimulated production of blood mononuclear interleukin 1 in Crohn's disease and in ulcerative colitis.

The present study shows that normal colonic mucosa in culture is capable of synthesising and releasing interleukin 1. Moreover, inflamed mucosa of patients with inflammatory bowel disease produce and release significantly higher amounts of interleukin 1, as reflected by its content in the culture of tissue and medium. Its enhanced generation in active inflammatory bowel disease is probably derived from the presence of increased number of cells capable of synthesising interleukin 1 in the inflamed mucosa. Mucosal interleukin 1 was found to be associated with the colonic inflammatory response in two models of experimental colitis.[8 9] In the trinitrobenzene sulfonic acid induced chronic colitis in rats and in acute colitis induced in rabbits by enteropathogenic *E coli*, mucosal interleukin 1 was found to be the most sensitive marker of colonic inflammation, much more so than myeloperoxidase activity or mucosal eicosanoids. In these two models an excellent correlation was found between mucosa interleukin 1 and myeloperoxidase activity.[9]

Corticosteroids were reported to block interleukin 1 production by macrophages,[15] probably by decreasing the availability of arachidonic acid and arachidonate metabolites. Inhibitors of lipoxygenase activity were also shown to inhibit interleukin 1 production,[16] suggesting a role for leukotrienes in its production. On the other hand, it was shown that prostaglandin E_2 inhibits interleukin 1 production[16] and may have a role in a negative feedback control of its production or release.

In the present work prednisolone was found to inhibit significantly interleukin 1 release from the inflamed mucosa. Interestingly, prednisolone was more effective in inhibiting interleukin 1 release than it was in reducing its tissue content, suggesting that steroids may have a dual inhibitory effect on interleukin 1 activity in the inflamed tissue.

The results of the present study suggest that interleukin 1 may have an important role in the pathogenesis and propagation of the inflammatory response in ulcerative colitis and in Crohn's disease and that its inhibition by specific agents may bear a potential therapeutic benefit.

TABLE III *Effect of prednisolone on interleukin 1 values in cultured colonic mucosa of an ulcerative colitis patient*

Prednisolone (μg/ml)	*Bioassay (pg/mg)*	*ELISA assay (pg/ml)*
None	41·6	32·6
6	32·8	27·2
12	24·5	27·7
25	15·9	15·1
50	13·6	24·2
100	4·3	4·8

Six biopsy specimens were obtained from a patient with active ulcerative colitis. Each was cultured for 24 hours in the absence or presence of prednisolone. Following the culture, the tissue was homogenized and assayed for IL 1 activity by bioassay and ELISA assay (IL 1 alpha and beta), as described in Materials and methods.

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