(2'-5') Oligo adenylate synthetase activity in leucocytes of patients with inflammatory bowel disease

R STALNIKOWICZ, K GODER, F KARMELI, C FIOCCHI,
AND D RACHMILEWITZ

From the Departments of Gastroenterology, Hadassah University Hospital, Jerusalem, Israel, and the Cleveland Clinic Foundation, Cleveland, Ohio, USA.

SUMMARY The activity of an interferon induced enzyme, (2'-5') oligo adenylate synthetase, was determined in peripheral blood mononuclear cells and granulocytes of patients with inflammatory bowel disease and compared with its activity in cells isolated from normal subjects. In spite of the fact that circulating interferon is detected in patients with inflammatory bowel disease, we failed to see any increase in (2'-5') oligo adenylate synthetase activity in these patients. The mean $\pm$ SE enzyme activity given in nmol ATP incorporated into (2'-5') isoadenylate oligomers by extracts of 10^5 peripheral blood mononuclear cells/21 hours was in normal subjects 1.84 ± 0.30 (n=27), in patients with active Crohn's disease 1.38 ± 0.15 (n=20) and in patients with active ulcerative colitis 1.14 ± 0.23 (n=21). (2'-5') oligo adenylate synthetase activity in granulocytes was also similar in normal subjects and in patients with active ulcerative colitis or Crohn's disease. The enzyme activity in patients with active disease was similar both prior to and during steroid therapy. The low (2'-5') oligo adenylate synthetase activity in peripheral blood mononuclear cells and granulocytes of patients with active inflammatory bowel disease may reflect decreased cellular response to interferon or a difference in the type of interferon elevated in viral diseases and in inflammatory bowel disease.

Circulating immune interferon was detected in patients with inflammatory bowel disease.¹ Interferon besides its anti-viral function, is also an immune regulator which can induce or perpetuate some of the immunological observations in inflammatory bowel disease: decreased numbers of suppressor T cells, hypergammaglobulinaemia, and the formation of immune complexes.^{2,3} The natural killing activity of peripheral blood mononuclear cells, which was found to be decreased in inflammatory bowel disease patients, is enhanced and normalised after their exposure to interferon.⁴

(2'-5') oligo adenylate synthetase is one of several enzymes induced by interferon and has been implicated in its antiviral and anti-proliferative effects.⁵ Its increase in peripheral blood leucocytes can serve as a sensitive indicator of their exposure to interferon. (2'-5') oligo adenylate synthetase activity was found to increase after injection of interferon as well as in viral

diseases, chronic viral related diseases, multiple sclerosis, and Burkitt's lymphoma. On the other hand, decrease in this enzyme activity was reported in patients with non-Hodgkins lymphoma and acute lymphoblastic leukaemia.⁶

The aim of the present study was to further elucidate the possible role of interferon in the pathogenesis of inflammatory bowel disease by determination of (2'-5') oligo adenylate synthetase activity in peripheral blood mononuclear cells and granulocytes of these patients during active disease and remission.

Methods

PATIENTS

Heparinised blood was obtained from 44 patients, 24 with ulcerative colitis and from 20 patients with Crohn's disease. For comparison, blood samples were also obtained from 27 normal subjects, and from three patients with shigellosis. The age and sex of patients in the various groups are detailed in Table 1. Ulcerative colitis was diagnosed

Address for correspondence: D Rachmilewitz, MD, Department of Gastroenterology, Hadassah University Hospital, PO Box 12000, Jerusalem, Israel.
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Table 1 Sex and age of patients with inflammatory bowel disease

	No	M/F	Age (yr) (mean $\pm$ SE)
Normal subjects	27	3/4	30.0 $\pm$ 2.5
Shigellosis	3	3/0	20.0 $\pm$ 4.0
Ulcerative colitis			
Active	21	11/10	34.0 $\pm$ 3.4
Remission	3	2/1	32.0 $\pm$ 2.3
Crohn's disease			
Active	20	10/10	28.0 $\pm$ 2.1

sigmoidoscopically and confirmed by biopsy. The extent of the disease was assessed by colonoscopy and/or barium enema. In 12 patients the disease involved only the rectum, all of them had active disease, 11 patients had proctosigmoiditis, nine of them had active disease, and four of the patients had universal colitis, three of whom had active disease. Disease activity was determined according to clinical signs: fever, abdominal pain, number of bowel movements per day, the presence of blood in the stool and erythrocyte sedimentation rate. Blood was obtained from 17 patients with active disease before initiation of steroid therapy and from four patients during steroid treatment. All patients with ulcerative colitis in remission were maintained on sulphasalazine.

Crohn's disease was diagnosed radiologically and/or endoscopically. In 16 patients the disease affected the terminal ileum, and four patients had ileocolitis. All patients had active disease. Disease activity was determined by the presence of fever, abdominal pain, diarrhoea, and rapid erythrocyte sedimentation rate. Blood was obtained from seven of the patients before steroids were given and from 13 patients while on steroid therapy.

Shigellosis was diagnosed clinically and in all instances the diagnosis was confirmed by positive stool culture.

ENZYME ASSAY

Cell extraction and oligo adenylate synthetase activity were performed according to Merlin *et al.*⁷

For the isolation of granulocytes, 10 ml venous blood were collected in 2 ml ACD solution: (citric acid-citrate-dextrose, anti-coagulant solution). Six millilitres of Dextran 70 (Macrodex) in 0.9% NaCl (Pharmacia, Sweden) were added and the erythrocytes were allowed to settle for 60 minutes at room temperature. The supernatant was centrifuged at 800 rpm (4°C) for 12 min. To lyse residual erythrocytes, the cell pellet was suspended twice in 3.0 ml H₂O for 30 seconds and lysis was stopped by the addition of

1.0 ml KCl 0.6 N. The cells were then centrifuged for 12 min at 800 rpm. The cells were resuspended in 20 ml of phosphate buffered saline and 10 ml of Ficoll-Hypaque were added before a 30 min centrifugation (1400 rpm, 4°C). Cells were counted and suspended at 2×10^7 cells/ml in lysis buffer containing: 20 mM Hepes buffer pH 7.5, 5 mmol MgCl₂, 120 mmol KCl, 7 mmol dithiothreitol, 10% v/v glycerol and 0.5% Nonidet P-40. Lysis was carried out at 4°C and the extract was centrifuged at 8000 g for 6 min. The supernatant was kept frozen at -70°C until assayed for the enzyme activity.

Peripheral blood mononuclears were isolated from 2 ml of heparinised venous blood by means of Ficoll-Hypaque density gradient centrifugation. Cell lysis was carried out in the same way as described for granulocytes. 10^6 cells were suspended in 0.1 ml lysis buffer containing 0.5% Nonidet P-40. After centrifugation, 6 min at 8000 g, the extracts were also frozen at -70°C.

For determination of the enzyme activity 0.01 ml extract were mixed with 0.05 ml poly (rI):(rC) agarose beads and incubated at 30°C for 15 min. The beads were recovered by centrifugation, were washed and resuspended in 0.01 ml reaction mixture containing 10 mmol/l Hepes buffer pH 7.5, 5 mmol/l MgCl₂, 7 mmol/l dithiothreitol, 10% (v/v) glycerol, 2.5 mmol/l (³²P)- α -ATP (0.3 Ci/mmol/l 10 mmol/l creatine phosphate, 3 mg/ml creatine kinase, and 40 μ g/ml poly (rI):(rC). After incubation for 21 hours at 30°C, 1 unit of bacterial alkaline phosphatase in 0.01 ml of 140 mmol/l tris base were added and the mixture was incubated for one hour at 37°C; 0.04 ml water was then added; the beads were removed by centrifugation, and 0.01 ml of the supernatant was added to a 0.6 ml alumina column (Acid WAI, Sigma) in 1 mol/l glycine HCl buffer pH 2. A total of 3 ml buffer were passed through the column for each sample, collected in scintillation vials and counted in the ³H-channel of a Tri-Carb counter. This procedure measures the (A_{2'}) nA nucleotides formed. The enzyme activity of each sample was determined in triplicate. The variability between triplicates was less than -10%. In each assay, the same standard representing pooled extracts of peripheral blood mononuclear cells isolated from five normal subjects was included. The mean oligo adenylate synthetase activity for the standard was 1.70 ± 0.02 (n=10; $\bar{x} \pm$ SE), nmol ATP incorporated into (2'-5') isoadenylate oligomers/ 10^5 cells/21 h.

INTERFERON ASSAY

In several normal subjects and patients plasma, interferon concentration was determined according

to Levin *et al.*⁸ In the assay used, interferon activity is assessed by inhibition of the cytopathic effect of vesicular stomatitis virus on fibroblasts of the MDBK bovine continuous cell line, which have been shown to be sensitive to human interferon α and interferon γ .

Statistical analysis of the data was performed according to the Student's *t* test for paired and unpaired data.

Results

(2'-5') oligo adenylate synthetase activity in peripheral blood mononuclear cells isolated from 27 normal subjects, expressed as nmol ATP incorporated into (2'-5') isoadenylate oligomers by extracts of 10^5 cells/21 h was 1.84 ± 0.30 ($\bar{x} \pm \text{SE}$). The enzyme activity in peripheral blood mononuclear cells isolated from patients with active ulcerative colitis or Crohn's disease was similar and not significantly different from the enzyme activity in normal subjects (Fig. 1). In patients with active ulcerative colitis or Crohn's disease, the enzyme activity was not affected by

steroid therapy (Table 2). (2'-5') oligo adenylate synthetase activity in peripheral blood mononuclears of patients with ulcerative colitis in remission, all of whom were maintained on sulphasalazine was also similar to the enzyme activity in normal subjects: 1.56 ± 1.20 ($n=3$; $\bar{x} \pm \text{SE}$). Patients with shigellosis served as a control for another inflammatory disease of the bowel. (2'-5') oligo adenylate synthetase activity in peripheral blood mononuclears isolated from these patients was also similar to that detected in normal subjects: 0.67 ± 0.44 ($n=3$; $\bar{x} \pm \text{SE}$).

The enzyme activity in granulocytes isolated from normal subjects was significantly lower ($p<0.05$) than its activity in normal peripheral blood mononuclear cells. (2'-5') oligo adenylate synthetase activity in granulocytes isolated from patients with active ulcerative colitis and Crohn's disease without therapy was also similar and not different from the enzyme activity in controls (Fig. 2). In patients with active ulcerative colitis there was no correlation between the disease extent and (2'-5') oligo adenylate synthetase activity. In Crohn's disease there was also no correlation between the enzyme activity and site of disease involvement.

In several of the subjects studied plasma interferon concentration was also determined (Table 3). In patients with ulcerative colitis and Crohn's disease, plasma interferon concentration was significantly raised, whereas (2'-5') oligo adenylate synthetase activity was not different from the enzyme activity in normal subjects.

Discussion

Inflammatory bowel disease is characterised in addition to the inflammatory response by symptoms and signs which reflect disturbances in

Fig. 1 Peripheral blood mononuclear cells were isolated from normal subjects, and from patients with active ulcerative colitis and Crohn's disease either before treatment or while on steroids.

Table 2 Effect of steroid treatment on (2'-5') oligo adenylate synthetase activity

	Treatment	
	None	Steroids
Ulcerative colitis	1.18 $\pm$ 0.28 (17)*	0.95 $\pm$ 0.22 (4)
Crohn's disease	1.61 $\pm$ 0.25 (7)	1.26 $\pm$ 0.19 (13)

Peripheral blood mononuclear cells were isolated from patients with active ulcerative colitis and Crohn's disease either before initiation of therapy or while on steroid therapy: prednisone 25-35 mg/d. (2'-5') oligo adenylate synthetase was determined as described in **Methods**, expressed as nmol ATP incorporated into (2'-5') isoadenylate oligomers by extracts of 10^5 cells/21 h. Results are mean $\pm$ SE.

* Number of subjects.

Fig. 2 Granulocytes were isolated from normal subjects and from patients with active ulcerative colitis and Crohn's disease, all of whom were not receiving therapy.

the immune mechanism: immunologically mediated conditions such as uveitis, arthritis, autoimmune haemolytic anaemia, and the favourable therapeutic responses to adrenal corticosteroids. The presence of anticolon antibodies in ulcerative colitis,² lymphocyte cytotoxicity against intestinal epithelial cells, diminished lymphocyte responsiveness to various mitogens,¹⁰ circulating antigen-antibody complexes,⁵ and depressed natural killing activity of peripheral blood mononuclear cells⁴ also characterise these diseases.

Interferon, besides its antiviral properties is also involved in several aspects of the immune system:

interferon enhances antibody production by inhibition of B cells.¹¹ Interferon affects also cellular immunity. One of its characteristic features is enhancement of natural killing activity, either by triggering differentiation of precursor cells or by activating existing natural killer cells.¹² In spite of the fact that viral agents have not been isolated in inflammatory bowel disease, the many immunological abnormalities noted in these patients could suggest some causal relation to interferon.

Circulating immune interferon was detected in 12 out of 20 patients with active inflammatory bowel disease by Strickland *et al.*¹ We have also recently shown activation of the interferon system in the serum of all Crohn's disease and most ulcerative colitis patients tested.¹³

In several of the patients with inflammatory bowel disease included in the present study, plasma interferon concentration was determined and found also to be significantly raised when compared with its concentration in normal subjects. Decreased natural killing activity of peripheral blood mononuclear cells in active inflammatory bowel disease was also reported⁴ and confirmed by us.¹² Ginsburg *et al* has also reported normalisation of the decreased natural killing activity of mononuclears in a small number of patients with inflammatory bowel disease upon their exposure to interferon.⁴

(2'-5') oligo adenylate synthetase is an enzyme whose activity in various cells is increased following their exposure to interferon. An increase in this enzyme activity was reported in peripheral blood mononuclear cells isolated from patients with viral diseases.⁶ It is highly suggestive that (2'-5') oligo adenylate synthetase has an important role in the antiviral effects of interferon. In the present study we explored whether in inflammatory bowel disease patients, this enzyme activity is increased.

The results reported in the present study indicate that in inflammatory bowel disease, in spite of the presence of circulating interferon^{1 12} (2'-5') oligo adenylate synthetase activity in peripheral blood mononuclear cells was not increased in all the patients tested, irrespective whether the disease was active or not.

As basal (2'-5') oligo adenylate synthetase activity in granulocytes is lower than in peripheral blood mononuclear cells, its determination in patients with inflammatory bowel disease is expected to be more sensitive for the detection of stimulated enzyme activity. In the present study, however, this enzyme activity in granulocytes isolated from patients with active ulcerative colitis

Table 3 Plasma interferon concentration and (2'-5') oligo adenylate synthetase activity in peripheral blood mononuclear cells

	No	Interferon (units/ml)	(2'-5') oligo adenylate synthetase activity (nmol ATP)
Normal subjects	7	4±3	1.20±0.22
Ulcerative colitis			
Active	7	15.9±2.1	1.22±0.18
Remission	3	21.4±5.6	1.56±1.20
Crohn's disease			
Active	3	21.3±3.6	1.34±0.25

Plasma interferon and (2'-5') oligo adenylate synthetase activity in peripheral blood mononuclear cells were determined in normal subjects and patients as described in **Methods**. Results are mean±SE.

and Crohn's disease was also not enhanced.

(2'-5') oligo adenylate synthetase activity is affected *in vitro* by steroids,¹⁴ a medicine which several of the patients with active inflammatory bowel disease included in the present study were receiving. In the present study, however, the enzyme activity in patients with active disease was similar whether or not they were on steroid therapy. Similarly, the depressed natural killer cell activity in patients with inflammatory bowel disease was found not to be affected by treatment with steroids.¹⁵

All patients with ulcerative colitis in remission included in this study were maintained with sulphasalazine. In all of them plasma interferon concentration was raised whereas (2'-5') oligo adenylate synthetase activity was not different from the enzyme activity in normal subjects. It, therefore, seems that steroids and sulphasalazine have no effect both on plasma interferon concentrations and on (2'-5') oligo adenylate synthetase activity in these patients.

In a preliminary report,¹⁶ the enzyme activity in peripheral blood mononuclear cells of 12 patients with active ulcerative colitis was found to be significantly decreased when compared with its activity in seven normal subjects. In the present completed study, relating to 21 patients with active ulcerative colitis and 27 normal subjects, this decrease in the enzyme activity did not have statistical significance. The difference between the preliminary finding reported in the initial abstract and those herewith reported are probably related to the different number of subjects in the preliminary and the present final report.

The observation herewith reported, that there is no increase in (2'-5') oligo adenylate synthetase activity in inflammatory bowel disease, suggests that there is a difference in the nature of interferon effects in viral diseases and inflammatory bowel disease. The fact that the enzyme activity was not increased in patients with ulcerative colitis and Crohn's disease could, therefore, be because of the decreased responsiveness of peripheral blood mononuclear cells and granulocytes to interferon production. It is also conceivable that the raised interferon detected in these patients is of the immune type (interferon γ) which is not a potent inducer of (2'-5') oligo adenylate synthetase activity.¹⁷

The results reported in the present study neither prove nor rule out possible viral aetiology in inflammatory bowel disease. The cumulative data suggest that in inflammatory bowel disease raised circulating interferon concentration in blood results from its synthesis by leucocytes stimulated

by viral or any other immune antigen. The lack of increase in (2'-5') oligo adenylate synthetase activity in peripheral blood mononuclear cells of patients may reflect their decreased responsiveness. Further studies establishing this enzyme activity in tissue inflammatory cells and the effect of the various types of interferon on this enzyme activity will further clarify this issue.

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