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# Dose-effect of interleukin-10 and its immunoregulatory role in Crohn's disease



## Abstract

Background: Interleukin-10 (IL-10) is currently being extensively studied in clinical trials for the treatment of Crohn's disease (CD). Only marginal effects have, however, been reported, and the dose-response curve was bell-shaped contrasting with the reported data from in vitro experiments. Aim: To use another in vitro model to analyze the effect of rhIL-10 and rhIL-4 on the spontaneous mucosal TNF-alpha secretion in patients with CD, and to characterize the phenotype of the cells targeted by rhIL-10. Methods: Non-

inflamed colon biopsies from CD patients were cultured for 16 hours in presence of different concentrations of rhIL-10 or rhIL-4. The numbers of TNF-alpha-secreting cells among isolated lamina propria mononuclear cells (LPMNC) were estimated by Elispot. Results: Both rhIL-10 and rhIL-4 down-regulate TNF-alpha secretion by LPMNC from CD patients, with a more pronounced effect with rhIL-10. These effects were closely linked to the cytokine concentrations used, with a bell-shaped dose-response curve. Residual TNF-alpha secretion, in the presence of optimal rhIL-10 concentration was mainly attributable to CD3+ T cells. In contrast, at higher rhIL-10 concentrations, CD3- cells contributed significantly to the TNF-alpha secretion. Conclusions: The in vitro model we used, demonstrates that IL-4, but mostly IL-10, efficiently suppresses TNF-alpha secretion in LPMNC from CD patients, with a dose-response curve similar to results obtained in vivo. Resistance at high rhIL-10 concentrations was associated with a change in the phenotype of TNF-alpha-secreting cells.

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## Full text

### ABBREVIATIONS

CD: Crohn's disease

CHX: cycloheximide

Elispot: enzyme-linked-immunospot

FCS: fetal calf serum

IL-4: interleukin-4

IL-10: interleukin-10

IFN-gamma: interferon gamma

LPMNC: lamina propria mononuclear cells

PWN: pokeweed mitogen

rh: recombinant human

SC: secreting cells

TNF-alpha: tumor necrosing factor alpha

## INTRODUCTION

Crohn's disease (CD) is a chronic relapsing inflammatory disease of unknown origin, associated with a shift in the balance of cytokine production by T cells toward the T helper 1 subclass, and with increased secretion of proinflammatory cytokines [1, 2]. Among these, the key role of tumor necrosing factor-alpha (TNF-alpha) has been demonstrated by prolonged clinical remissions among patients treated with an intravenous infusion of a chimeric monoclonal antibody to TNF-alpha [3, 4]. *In vitro* studies have shown that the production of TNF-alpha is increased in the mucosa of patients with Crohn's disease [2, 5, 6]. The role of TNF-alpha, in the pathogenesis of Crohn's disease has been suggested to be a cofactor for mucosal Th1 responses [7]. Models of experimental colitis in mice have also demonstrated a predominant pathogenic role of TNF-alpha, so that potential immunotherapeutic strategies for CD include recombinant anti-inflammatory cytokines. Among these, interleukin-4 (IL-4) and interleukin-10 (IL-10) appear as logical candidates since they both suppress TNF-alpha secretion by human monocytes [8-10].

Decreased production of IL-4 has been reported in Crohn's diseases, at least in chronic disease [11, 12]. However, compared to healthy controls, intestinal monocytes from patients with Crohn's disease have been reported to have a diminished response to the inhibitory effect of IL-4 on the TNF-alpha secretion [13]. The anti-inflammatory effect of IL-10 in the gut has been more directly appreciated by the appearance of severe colitis in IL-10-knockout mice, as well as by the ability of continuous IL-10 administration to prevent intestinal disease in a variety of animal models of colitis [14, 15]. However, several reports have indicated an increased production of IL-10 within the gut mucosa of patients with IBD, as well as higher concentrations of circulating IL-10 compared to healthy controls [16, 17]. On the other hand, it has been demonstrated that the elevated production of pro-inflammatory cytokines by IBD mononuclear phagocytes can be down-regulated by the addition of IL-10 *in vitro* or by topical treatment with IL-10 enema preparations *in vivo*, indicating that the intrinsic intestinal bioactivity of IL-10 might be insufficient to control local inflammation [9].

Therefore, several therapeutic trials using rhIL-10 have been performed. Unfortunately, subcutaneously administered rhIL-10 to patients with steroid-refractory, mild to moderately active, or chronic active CD, showed only a marginal clinically beneficial effect [18-20]. In addition, studies indicated a bell-shaped dose-response curve, with reduced effects at higher doses, contrasting with the dose-related effect obtained *in vitro* on isolated lamina propria mononuclear cells (LPMNC) stimulated with pokeweed mitogen (PWM) [9].

To shed light on these contradictory observations, and to understand the reasons for the lack of response to high doses of rhIL-10, we develop an *in vitro* model to study the effect of rhIL-10 and rhIL-4 on TNF-alpha secretion, in conditions as close as possible to the *in vivo* situation. This model consisted of a 16 hours organotypic culture system allowing cell interactions, in the presence of the recombinant cytokines but in the absence of any stimulant. The spontaneous TNF-alpha secretion was then analyzed at the single cell level by an Elispot technique on isolated LPMNC.

## MATERIALS AND METHODS

### *Patients*

Twenty-nine patients with CD and 14 control patients were enrolled in this study. Among the 29 CD patients, 15 had ileocolonic resection because of symptomatic ileal strictures. At the time of surgery, 7 of these patients were receiving corticosteroids (n = 5) and/or immunosuppressives (azathioprine) (n = 4), and 4 patients were receiving antibiotics. The other 14 CD patients, were not receiving immunosuppressives or corticosteroids, and they underwent routine ileo-colonoscopy for colon cancer screening. Control patients were referred for routine colonoscopy for cancer screening. All the patients gave informed consent, and the study was approved by the ethical committee of the Université Libre de Bruxelles.

### *Tissue sampling*

Mucosal biopsies from endoscopically normal colon mucosa were either obtained by colonoscopy (8 French forceps biopsies) or from surgical specimens after ileo-colectomy (8 French forceps biopsies). Biopsies were immediately transported to the laboratory in Hank's balanced solution containing penicillin

and streptomycin. Biopsies were either used for immediate LPMNC isolation for further analysis or they were used for organotypic cultures (see below).

## EXPERIMENTS ON IMMEDIATELY ISOLATED LPMNC

### *Lamina propria mononuclear cell isolation*

LPMNC were isolated from biopsies by enzymatic treatment as previously described [21]. Briefly, the biopsies were washed and incubated for 1 hour at 37° C in 1 mM EDTA (Sigma Chemical Co-Bornem-Belgium), containing Iscove's medium, to remove intra-epithelial lymphocytes and epithelial cells. The remaining biopsy fragments were cut into small pieces, reincubated under stirring for 1 hour at 37° C, in medium containing 1 mg/ml collagenase-dispase (Boehringer, Mannheim, Germany), and LPMNC were collected in the supernatant. The numbers of LPMNC were counted using a Bürker chamber and the percentage of CD3<sup>+</sup> lymphocytes was estimated by flow cytometry.

### *T cell phenotyping by flow cytometry*

The phenotype of T lymphocytes present among LPMNC was analysed by flow cytometry (FACScan, Becton Dickinson, San José, California, USA) after staining the cells with anti-CD3 PerCP, anti-CD4 FITC and anti-CD8 PE monoclonal antibodies (mAbs) (Becton Dickinson). Lymphocytes populations were gated by forward-sideward scatter light and by gating for CD3<sup>+</sup> cells.

### *Analysis of spontaneous IL-4, IL-10 and TNF-alpha secretions by enzyme-linked immunospot assay (Elispot)*

After LPMNC isolation, cell suspensions were analyzed for numbers of IL-4-, IL-10- and TNF-alpha-secreting cells (SC), by two-site reverse-enzyme-linked immunospot assays as previously described, using epitope-specific mouse anti-human cytokine mAbs as capture and developing reagents [21]. Briefly, individual wells of nitrocellulose-bottomed 96-well Millititer HA plates (MAHAN 4550; Millipore, Eschborn, Germany) were coated with the following anti-cytokine mAbs: anti-IL-4: clone 82-2 (10 mug/ml Mabtech AB, Stockholm, Sweden), anti-IL-10: clone JES3-9D7 (10 mug/ml; Pharmingen, San Diego, CA, USA), and TNF-alpha: clone B7238 (10 mug/ml; Genzyme Diagnostics, England). After blocking the plates (10% FCS and 2% (wt/vol.) milk powder in PBS), 100 µl

of different dilutions of cell suspensions were dispensed into the coated wells. The plates were incubated in 5% CO<sub>2</sub> for 20 hours at 37° C, and after extensive washing, the following biotinylated anti-cytokine mAbs were added: anti-IL-4: clone 12.1 (1 mug/ml, Mabtech); anti-IL-10: clone JES3-12G8 (5 mug/ml, Pharmingen); and anti-TNF-alpha: clone B7373 (1 mug/ml; Genzyme Diagnostics, England). Spots were developed by the addition of streptavidin-horseradish peroxidase (2 mug/ml; Sigma) followed by the substrate solution (3-amino-9-ethylcarbazole, AEC; Sigma). They were then counted under low magnification (x 50). Two-fold serial dilutions were tested for each sample, and the spots were counted in all wells that contained fewer than 200 spots. The spots in these wells were counted for each sample and then reported as the numbers of cytokine-SC per 10<sup>6</sup> total numbers of vital cells. To avoid inter-observer variations, the same investigator read all the plates.

## EXPERIMENTS AFTER ORGANOTYPIC INTESTINAL BIOPSY CULTURE

### *Organotypic culture system*

After washing, the biopsies were incubated in a 24-well plate containing 1 ml of Iscove's medium supplemented with 40 mug/ml gentamycin, 10% fetal calf serum (FCS), 50 mM of 2-mercaptoethanol, 1 mM EDTA (Sigma), and 10 UI/ml of rhIL-2 (clone B 60257; Genzyme Diagnostics). Either rhIL-10 (batch n° 5-LTP-509, Schering-Plough) or rhIL-4 (Genzyme Diagnostics) was added to the culture wells at different concentrations before incubating the plates for 16 hours at 37° C under 5% CO<sub>2</sub>. To confirm that cytokine secretion after organotypic culturing was the result of a new *in vitro* synthesis, experiments were performed in some wells in the presence of 100 mug/ml of cycloheximide (CHX).

### *Analysis of TNF-alpha and IFN-gamma secretions by enzyme-linked immunospot assay (Elispot) after organotypic culture*

After organotypic culture, the biopsies were processed for LPMNC isolation and assayed for the numbers of TNF-alpha- and IFN-gamma-secreting cells (ELISPOT). The same technique was used as described before (see T-cell phenotyping by flow cytometry). Capture antibodies for IFN-gamma (clone 1/D1K (10 mug/ml; Chromogenix, Stockholm, Sweden)) and biotinylated antibodies for IFN-gamma (anti-IFN-gamma: clone 7/B6/1-3 mug/ml-Chromogenix) were used.

### ***Determination of TNF-alpha and IFN-gamma concentrations***

Supernatants from organotypic cell cultures were collected, and TNF-alpha and IFN-gamma concentrations were determined by a very sensitive flow cytometry-based immunofluorescence assay (ImmunoFlow-IFA, BioErgonomics Inc., White Bear Lake, Minnesota, USA) [22]. Samples to be analyzed and the different standards were incubated successively with paramagnetic beads coated with capture anti-TNF-alpha or anti-IFN-gamma mAbs, and with fluorescence labelled secondary antibodies. The mean channel of fluorescence was then determined for each sample and standard on a flow cytometer (FACScan), by drawing a gating bitmap around the main population of beads. The cytokine concentrations in the unknown samples were determined by comparing the mean channel fluorescence of the samples with those of the standard curve. The standard curves are designed to measure cytokine concentrations in the range of 0.5-5,000 pg/ml.

### ***Phenotyping of TNF-alpha-secreting cells***

CD3<sup>+</sup> cell suspensions were obtained from LPMNC after organotypic culture by positive selection with anti-CD3-covered beads (MACS system, Miltenyi Biotec, Bergisch Gladbach, Germany). Twelve biopsies were incubated per experimental condition (0, 40 and 1,000 ng/ml of IL-10), to obtain at least 10 million LPMNC. Twenty  $\mu$ l of MACS CD3 microbeads per 10<sup>7</sup> cells were added and incubated for 15 min at 6° C. The cells were then washed and resuspended in Iscove's medium in order to proceed to magnetic separation by positive selection columns; 90 to 95% purification (CD3<sup>+</sup>-depleted cell suspensions) was obtained after one separation procedure, as confirmed by flow cytometry (anti-CD3 PerCP, Becton Dickinson, FACScan).

## **STATISTICAL ANALYSIS**

The non-parametric, paired, two-tailed Wilcoxon U-test was used to evaluate comparative statistical significance.

## **RESULTS**

### ***Spontaneous ex-vivo cytokine secretion***

LPMNC isolated from colon biopsies obtained from CD patients during routine endoscopy were analyzed for their phenotype and their TNF-alpha, IL-10 and IL-4 secretions, without any additional *in vitro* stimulation, to simulate as closely as possible the *in vivo* situation. Compared to the controls, CD patients had a higher proportion of CD4<sup>+</sup> T cells ( $58.2 \pm 4.7\%$  *versus*  $39.5\% \pm 13.1\%$ ;  $p < 0.01$ ) and a lower proportion of CD8<sup>+</sup> T cells ( $29.1 \pm 10.9\%$  *versus*  $47.3 \pm 9.2\%$ ;  $p < 0.01$ ) among CD3<sup>+</sup> lamina propria T lymphocytes.

The numbers of cells which spontaneously secreted TNF-alpha, were significantly higher among LPMNC from CD patients compared to those of the controls (Figure 1,  $p < 0.001$ ). These numbers were not influenced by the addition of CHX during the Elispot procedure (Table 1), indicating that TNF-alpha synthesis had already occurred *in vivo*, and that the number of TNF-alpha SC reflected the *in vivo* situation. The proportion of IL-10-secreting LPMNC was also higher in CD patients (Figure 1,  $p < 0.05$ ), with only 3 out of 12 patients having a low number of IL-10-SC. These low IL-10 producers were not those characterized by the highest number of TNF-alpha-SC. In contrast, although some CD patients had normal numbers of IL-4-SC, the median obtained for CD patients was more than ten-fold lower than for the controls (Figure 1,  $p < 0.05$ ).

### ***Down-regulation of in vitro intestinal TNF-alpha secretion by rhIL-10***

Non-inflamed colon biopsies obtained from CD patients were cultured for 16 hours, in the presence of increasing concentrations of rhIL-10. LPMNC were then isolated, and the numbers of TNF-alpha-SC were counted by Elispot. In contrast to results obtained for freshly isolated LPMNC, CHX strongly down-regulated TNF-alpha secretion in this organotypic culture of biopsies, indicating that cytokine synthesis occurred during this short culture time (*Table 1*).

As shown in Figure 2 (upper part), the numbers of TNF-alpha-SC were significantly lower when the biopsies were cultured in the presence of rhIL-10. Although a concentration of 5 ng/ml of rhIL-10 already had a suppressive effect on the TNF-alpha secretion, the maximum inhibition was achieved at a dose of 40 ng/ml, with a mean percentage inhibition of  $77.5 \pm 13\%$ ;  $p < 0.01$ ). Higher doses of IL-10 were significantly less effective ( $p < 0.01$  between 40 and 1,000 ng/ml rhIL-10) (Figure 2, upper part). As the number of TNF-alpha-SC did not necessarily reflect the total amount of TNF-alpha secreted, TNF-alpha concentrations were further measured in the culture supernatants of biopsies

collected from 3 different CD patients and incubated with rhIL-10. TNF-alpha secretion was inhibited by 65, 64, and 45% respectively for the three different patients, by the addition of 40 ng/ml of rhIL-10, when compared to results obtained in the absence of rhIL-10.

Data for rhIL-10 inhibition of TNF-alpha secretion could not be compared to those obtained in controls (4 patients tested). The TNF-alpha secretion detected among LPMNC directly isolated from the control colon biopsies was indeed no longer detectable after 16 hours of organotypic culture, even when no exogenous cytokines were added.

### ***Down-regulation of in vitro intestinal TNF-alpha secretion by rhIL-4***

When the non-inflamed colon biopsies obtained from the same patients with Crohn's disease were cultured for 16 hours, in the presence of rhIL-4, a significant reduction in the numbers of TNF-alpha-SC was also observed ( $p < 0.01$ ). However, the residual TNF-alpha secretion remained higher than after incubation with rhIL-10. As shown in Figure 2 (lower part), a maximum inhibition of TNF-alpha secretion was obtained with 80 U/ml of rhIL-4. At higher rhIL-4 concentrations (up to 500 U/ml), no significant difference was observed compared to the TNF-alpha secretion at 80 U/ml of rhIL-4.

### ***Cellular source of TNF-alpha***

One reason for the observed dose-effect of rhIL-10 on TNF-alpha secretion may be that the cellular source of TNF-alpha changes with increasing concentrations of rhIL-10. For two different patients, we therefore tested the effect of 2 different rhIL-10 concentrations on the TNF-alpha secretion by either total LPMNC suspensions or CD3-depleted cell suspensions compared to the results obtained without rhIL-10. Results illustrated in Figure 3 indicate that in the absence of rhIL-10, TNF-alpha secretion could mostly be attributed to CD3<sup>+</sup> T lymphocytes, as only 20% of the TNF-alpha secretion in patient A, and 8% in patient B could be detected in the CD3-depleted cell suspensions.

In contrast, at high rhIL-10 concentration (1,000 ng/ml), TNF-alpha secretion could be attributed to both CD3<sup>+</sup> and CD3<sup>-</sup> cells (39 or 37% of the secretion detected in the CD3-depleted cell suspension respectively for patients A and B), suggesting that the cellular source of TNF-alpha had changed under the

influence of rhIL-10. Optimal concentrations of rhIL-10 suppressed TNF-alpha secretion by both cell suspensions.

### ***Effect of rhIL-10 on intestinal IFN-gamma secretion***

As the inhibitory effect of IL-10 on TNF-alpha secretion was mostly attributable to an effect on T cells, we further analyzed the IFN-gamma, in the presence of 40 or 1,000 ng/ml of rhIL-10, in 3 patients. As shown in Figure 4, both the numbers of IFN-gamma-SC and the IFN-gamma concentrations released in the culture supernatants were inhibited in the presence of rhIL-10. Although both the numbers of IFN-gamma-SC and the IFN-gamma concentrations decreased at 40 ng/ml of rhIL-10, IFN-gamma secretion rose again in the presence of 1,000 ng/ml of rhIL-10, suggesting that T cells partially escape from the inhibitory effect of IL-10 at high concentrations.

## **DISCUSSION**

From both experimental and clinical studies, accumulating evidence indicates that TNF-alpha is a key pro-inflammatory mediator in IBD and especially in CD. Down-regulation of TNF-alpha may therefore constitute a therapeutic goal and can be achieved by administration of either an anti-TNF-alpha monoclonal antibody or an antagonist of this pro-inflammatory cytokine. The first option has proven to be very efficient in patients with severe intractable disease or in those who suffer from entero-cutaneous fistulae, which are often very difficult to treat [4, 23]. As an alternative, IL-4 and IL-10 were identified as potentially more physiological therapeutic molecules to treat inflammatory diseases involving T helper 1 (Th1)-type responses because of their ability to down-regulate several important activities of Th1 cells and macrophages, especially TNF-alpha secretion [8, 9, 24].

In this study, by using a sensitive Elispot technique, we confirmed the increased release of TNF-alpha by LPMNC in CD patients, in the absence of any *in vitro* stimulation of the cells [2, 5, 6]. These results reflected the *in vivo* situation, as the number of spots was not influenced by the addition of CHX. We also demonstrated that the *in vivo* secretion of IL-4 by LPMNC was diminished in CD patients, as previously reported for *in vitro* activated LPMNC isolated from the colon mucosa from CD patients [12]. In contrast, the numbers of IL-10-SC were generally higher among LPMNC from CD patients compared to controls. These

data are in accordance with those of Autschbach *et al.*, reinforcing the hypothesis that the local production of IL-10 by mucosal LPMNC could remain insufficient to down-regulate pro-inflammatory cytokines [17]. As recently reported, only some patients presented a low production of IL-10 [25] which could be explained by the genetic polymorphism of IL-10 [26-27].

Following the demonstration by Schreiber *et al.* of an impaired response of LPMNC to the inhibitory effect of IL-4, contrasting with a dose-dependent inhibition by IL-10 of pokeweed mitogen-induced TNF-alpha secretion by LPMNC, clinical trials were performed using intravenous or subcutaneous administration of rhIL-10, in an attempt to treat CD [9, 13, 18-20]. However, these studies showed only marginal effects of rhIL-10 administration on clinical remission. Surprisingly, these studies indicated a bell-shaped dose-response curve, with less efficacy at higher doses, contrasting with the dose-related effect previously observed *in vitro* [9]. This loss of anti-inflammatory effect at high doses of IL-10 was also observed in patients with rheumatoid arthritis [28].

To resolve this apparent paradox, we investigated the anti-inflammatory effect of various concentrations of rhIL-10 and rhIL-4 by their capacity to decrease TNF-alpha secretion within LPMNC in organotypic cultures of non-inflamed colon biopsies. In contrast to the study of Schreiber *et al.*, who stimulated isolated LPMNC with PWM, our *in vitro* organotypic culture system enables better interactions with neighbouring cells and with extracellular matrix. As no exogenous stimulation was necessary, the cytokine secretion is considered to be a spontaneous one. Furthermore, the cytokine secretion observed in this system was the result of new *in vitro* synthesis as demonstrated by the strong inhibition observed in the presence of CHX.

When increasing concentrations of rhIL-10 or rhIL-4 were added to the organotypic cultures, significant inhibition of TNF-alpha secretion was observed. The effects obtained with IL-10 were stronger than those obtained with IL-4. No synergistic effect could be obtained by the simultaneous addition of both IL-10 and IL-4 (data not shown), contrasting with results obtained for stimulated peripheral blood monocytes from CD patients [29]. The intracellular molecular mechanisms of this anti-inflammatory effect of IL-10 have been previously investigated, but the results remain conflicting. Whereas IL-10 has been proposed to reduce cytokine mRNA stability by Bogdan *et al.*, other work indicates that IL-10 inhibits cytokine gene transcription [30, 31]. IL-10 may also

act on TNF-alpha secretion by inhibition of the activation of I $\kappa$ B kinase or by suppressing NF $\kappa$ B DNA binding activity [32, 33]. IL-10 has also recently been shown to markedly upregulate expression of the SOCS3 gene, which is known to suppress cytokine activation [34, 35].

In contrast to a previous *in vitro* study, but consistent with clinical studies, a bell-shaped curve was obtained in our study by adding increasing concentrations of IL-10 [9, 19, 20]. This bell-shaped dose-response curve gives evidence for a complicated regulation network within the intestinal mucosa. Focusing on the cellular source of TNF-alpha, we demonstrated that resistance to IL-10 at high concentrations was associated with a modification of the predominant cell type involved in TNF-alpha secretion. In the absence of IL-10 or in the presence of low to intermediate IL-10 concentrations (up to 40 ng/ml), TNF-alpha was mainly secreted by T cells, with a minor contribution by other cells. In contrast, at higher IL-10 concentrations (1,000 ng/ml), non-T cells secreted a substantial proportion of the TNF-alpha, associated with an increased secretion of IFN-gamma. Increased IFN-gamma secretion by T and/or NK cells induced by IL-10 at high concentrations has already been reported in humans receiving iv injection of LPS in combination with either placebo or rhIL-10 [36]. IFN-gamma released at high IL-10 concentrations could then activate macrophages to secrete TNF-alpha, which, in our system, appears in this condition to be mainly secreted by non-T cells.

## CONCLUSION

In conclusion, *in vitro* studies, as well as animal models of colitis, have generated great enthusiasm for rhIL-10 as a candidate therapy for human IBD. Reported clinical studies are however deceiving in terms of response rates and in terms of the dose-response curve. In this study, we provide more evidence that patients with Crohn's disease are not deficient in IL-10, but that immunomodulation with exogenous IL-10 is, however, efficient. By mimicking the *in vivo* situation, the *in vitro* organotypic culture system we used indicates that best responses are achieved with intermediate doses giving some explanation for the bell-shaped dose-response curve observed in clinical studies. The rebound of TNF-alpha secretion at high rhIL-10 concentrations appears to be associated with a change of the TNF-alpha-secreting cell phenotype, with an increasing role of non-T cells.

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Figures

Cytokine-secreting cells / 10<sup>6</sup> LPMNC

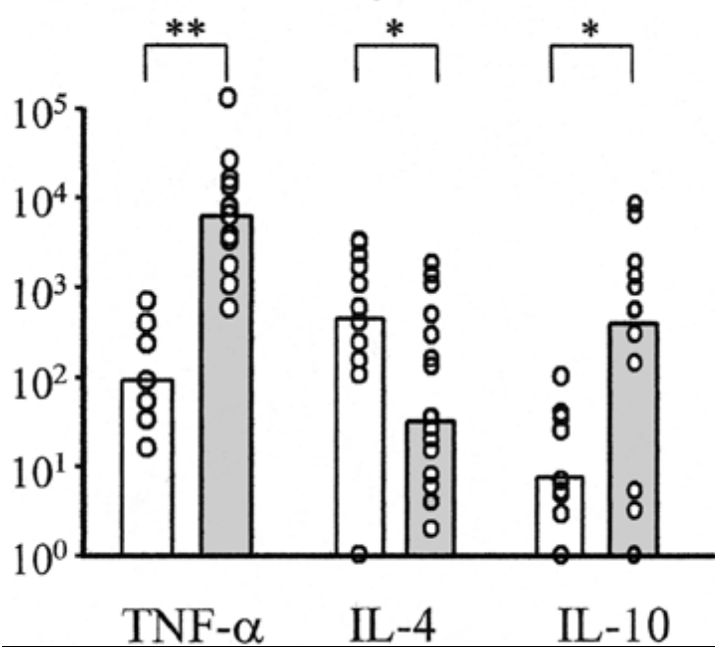


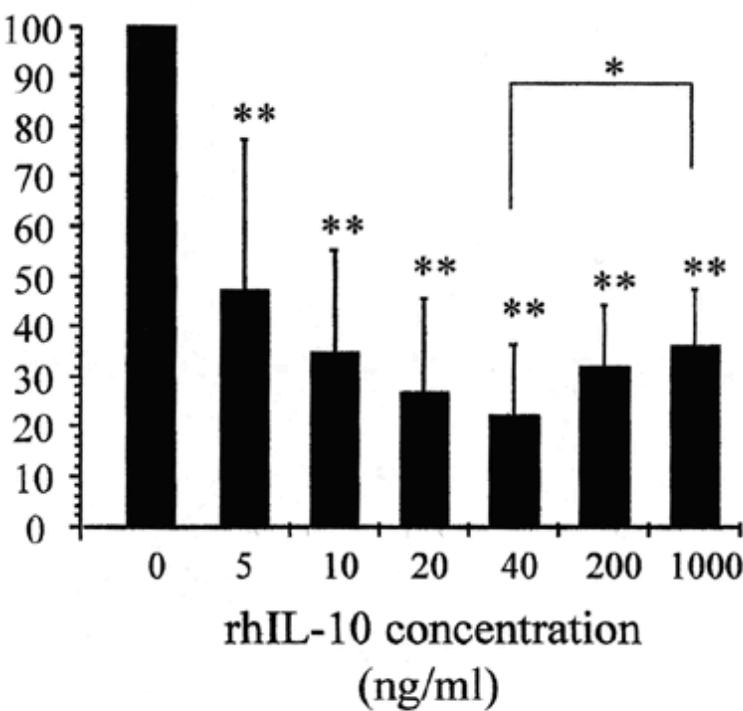
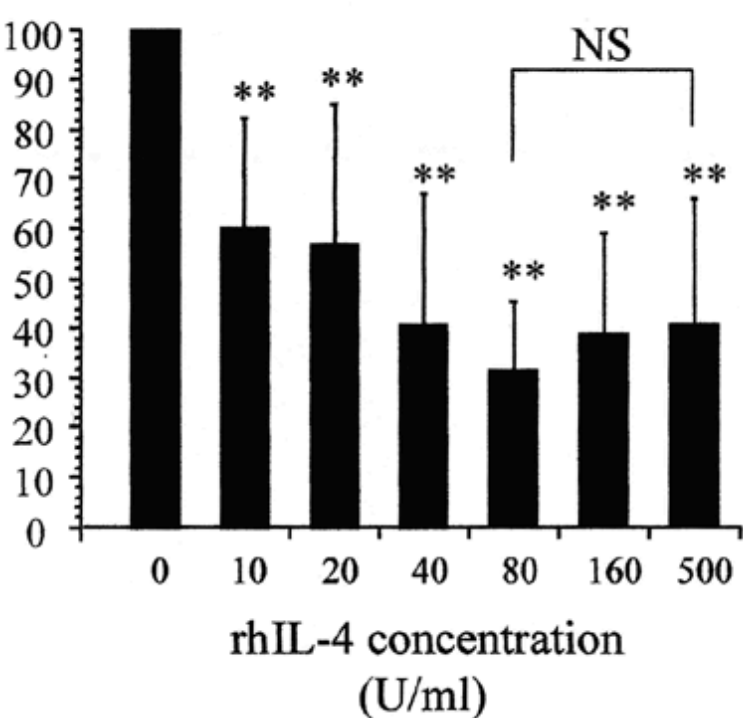
Figure 1

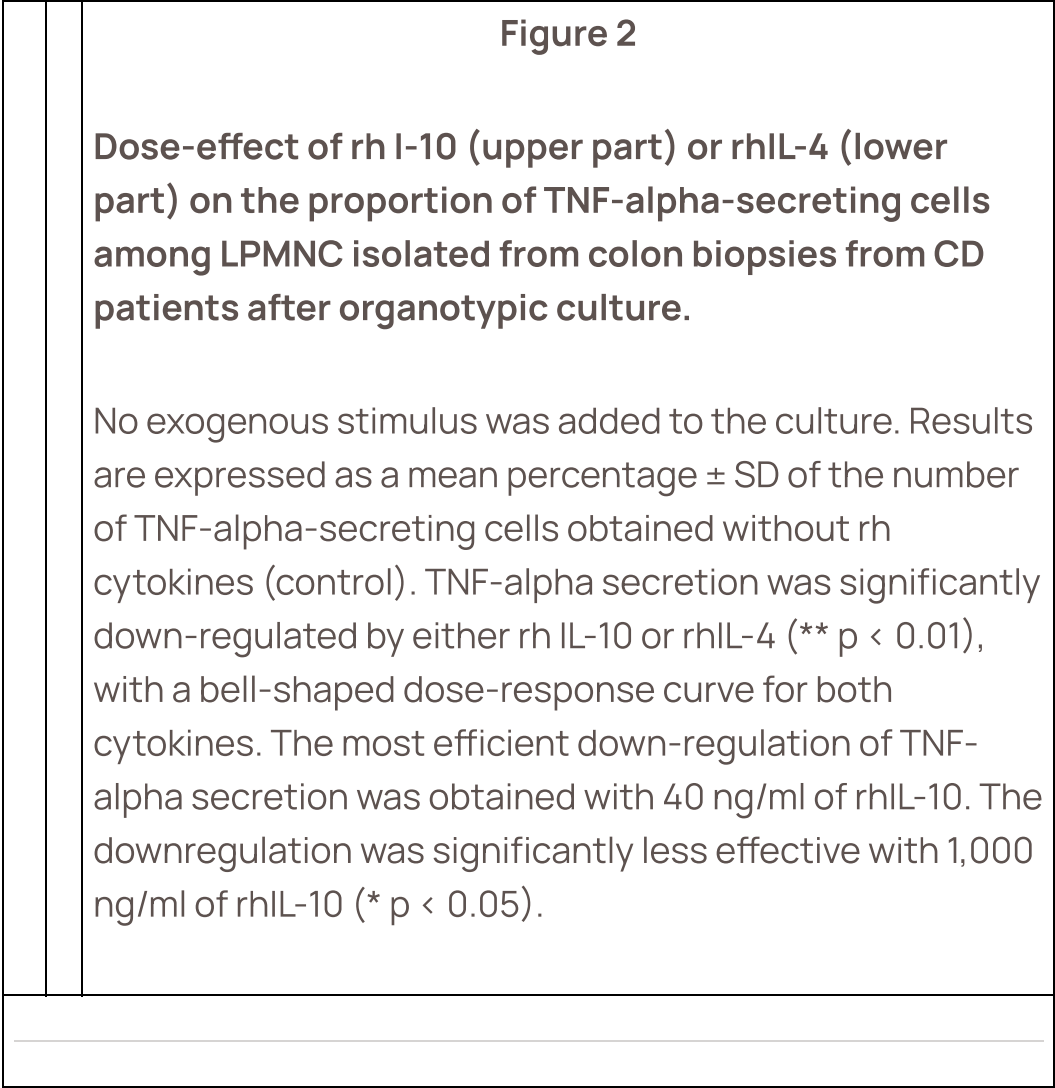
**Spontaneous cytokine secretion after LPMNC isolation from non-inflamed colon mucosa assessed by Elispot.**

Circles represent the individual results of the numbers of cytokine secreting cells/10<sup>6</sup> LPMNC. Bars represent the medians of the results, *gray* bars for Crohn's disease patients, and *white* bars for controls. Compared to controls, the proportion of TNF-alpha and IL-10 secreting cells was significantly increased in patients with Crohn's disease (\*\*  $p < 0.001$ ; \*  $p < 0.05$ ). The proportion of IL-4-

secreting cells was, in contrast, decreased in patients with Crohn's disease (\* p < 0.05).

TNF-α-secreting cells  
(% of control)





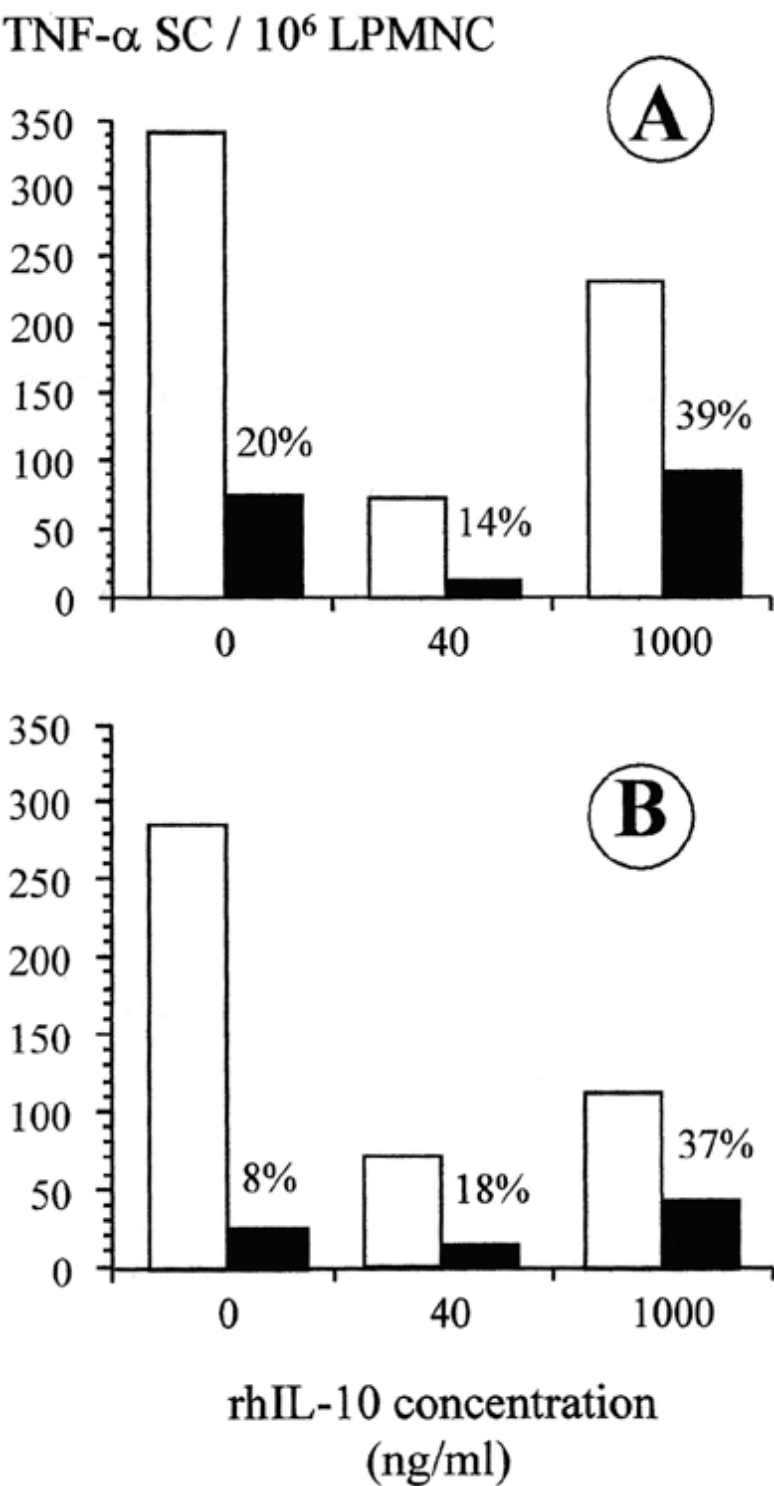


**Figure 3**

**Influence of rhIL-10 and its concentration on the cellular source of TNF-alpha secreted by colon biopsies from two different CD patients, A and B.**

TNF-alpha-secreting cells were detected by Elispot, and their numbers were expressed per 10<sup>6</sup> LPMNC. Their

numbers were compared between total LPMNC (white columns) and CD3-depleted cell suspensions (black columns), both of which were obtained after organotypic culture in the presence of rhIL-10. Percentages above the black columns indicate the proportion of TNF-alpha-secreting cells within CD3-depleted cell suspensions. In the absence of rh IL-10, TNF-alpha secretion was mostly attributable to CD3<sup>+</sup> cells but the contribution of CD3<sup>-</sup> cells became more important at high concentration of rhIL-10, suggesting that relative resistance to high IL-10 concentrations could be associated with a change in the phenotype of the TNF-alpha-secreting cells.

IFN- $\gamma$  SC (●)

IFN- $\gamma$  concentration (o)  
(% of control)

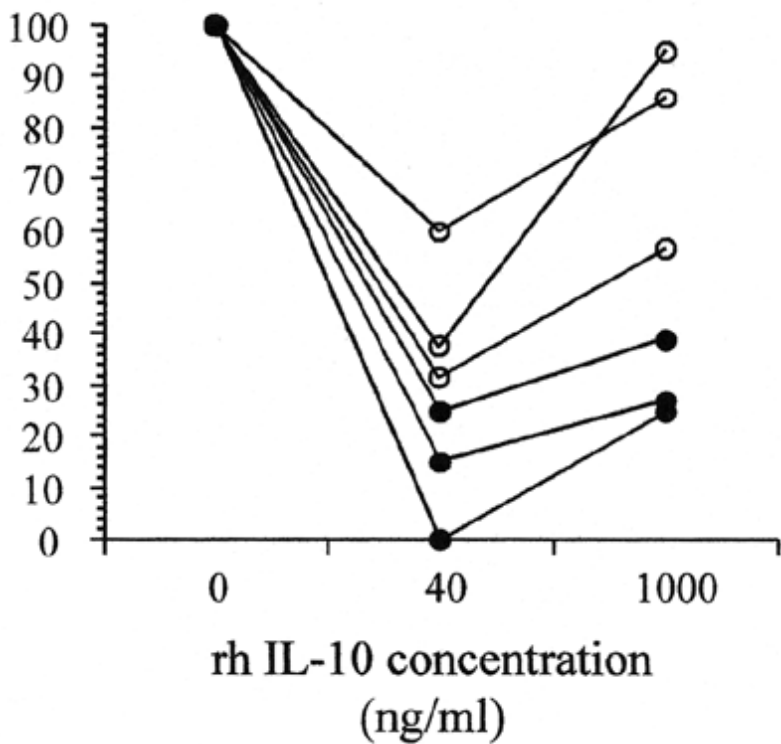


Figure 4

Effect of rhIL-10 on IFN-gamma secretion.

After organotypic culture of colon biopsies in the presence of two different concentrations of rhIL-10, IFN-gamma -secretion was estimated by the number of IFN-gamma-secreting cells measured by Elispot (black circles) and by the IFN-gamma concentration measured by a flow cytometry-based immunofluorescence assay (open circles). Results are expressed as the percentage of those obtained in the absence of rhIL-10. The IFN-gamma secretion was down-regulated by 40 ng/ml of rhIL-10, whereas a higher concentration of rhIL-10 was less efficient.

Table 1  
Effect of cycloheximide on the numbers  
of TNF- $\alpha$ -secreting cells

|      | % of TNF- $\alpha$ -secreting cells |                                              |
|------|-------------------------------------|----------------------------------------------|
|      | Immediate isolation<br>of LPMNC     | LPMNC isolation<br>after organotypic culture |
| CHX- | 100%                                | 80%                                          |
| CHX+ | 91%                                 | 3%                                           |

Left part of the table: LPMNC were isolated and cells were immediately incubated for TNF- $\alpha$  detection by Elispot in the presence (CHX+; CHX: 100  $\mu$ g/ml) or absence of CHX (CHX-). Results indicate that CHX has no significant effect on TNF- $\alpha$  secretion indicating that the cytokine was synthesised *in vivo*.  
Right part of the table: LPMNC were isolated and incubated in an organotypic culture before TNF- $\alpha$  detection by Elispot in the presence (CHX+; CHX: 100  $\mu$ g/ml) or absence of CHX (CHX-). In this case, CHX profoundly decreased TNF- $\alpha$  secretion, indicating that the cytokine was synthesised *in vitro*.

|  |  |  |
|--|--|--|
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|  |  |  |
|  |  |  |

Authors

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## In this issue

### ○ Methods to map protein interactions in mammalian cells: different tools to address different questions

Sven Eyckerman, Jan Tavernier

two-hybrid, enzymatic complementation, FRET/BRET, MAPPIT, protein splicing.

### ○ Absence of endogenous interleukin-10 enhances the evolution of acute lung injury

Salvatore Cuzzocrea, Emanuela Mazzon, Laura Dugo, Ivana Serraino, Rosanna Di Paola, Tiziana Genovese, Angela De Sarro, Achille P. Caputi

carrageenan, inflammation, nitric oxide, peroxynitrite, superoxide dismutase, IL-10.

### ○ Dose-effect of interleukin-10 and its immunoregulatory role in Crohn's disease

Alain Schmit, Monica Carol, Fabienne Robert, Patrick Bontems, Jean-Jacques Houben, André Van Gossum, Michel Goldman, Françoise Mascart

Crohn's disease, interleukin-10, tumor necrosis factor- $\alpha$ .

### ○ Expression of genes for cytokines and cytokine-related functions in leukocytes infected with Herpes simplex virus: comparison between resistant and susceptible mouse strains

Søren R. Paludan, Jesper Melchjorsen, Lene Malmgaard, Søren C. Mogensen

leukocytes, cytokines, cDNA arrays, Herpes simplex virus.

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lymphocytes, apoptosis, whole blood, activation markers, drug effects, immunosuppression.

## ○ Cytokine pattern in cystic fibrosis patients during antibiotic therapy and gene therapy using adenoviral vector

Philippe Reix, Gabriel Bellon, Jacques Bienvenu, Andrea Pavirani, H  l  ne Levrey-Hadden

cytokine, cystic fibrosis, adenoviral vector, antibiotic.

## ○ Induction of interleukin-2 receptor alpha (IL-2Ralpha) expression by interleukin-2: important role of the interleukin-2 receptor beta chain region between the two Stat5 docking sites

V  ronique Imbert, Roger Rezzonico, Patrick Reichenbach, Markus Nabholz

Stat5, IL-2 receptor, T lymphocytes, CD25.

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bronchopulmonary hyperreactivity, eosinophilia, IL-5, TRFK-5.

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*A. castellanii*, cytokine, MGC.

## ○ The mechanism by which interleukin-1 beta reduces net fluid absorption from the rat colon

Sawsan Ibrahim Kreydiyyeh, Rana Al-Sadi

Interleukin-1 beta, Na<sup>+</sup>K<sup>+</sup> ATPase, NaKCl2, Cl<sup>-</sup> secretion, Na<sup>+</sup> absorption, colon.

## ○ Proinflammatory interferon-gamma - inducing monokines (interleukin-12, interleukin-18, interleukin-15) - serum profile in patients with systemic lupus erythematosus

Ewa Robak, Tadeusz Robak, Anna Wozniacka, Malgorzata Zak-Prelich, Anna Sysa-Jedrzejowska, Henryk Stepien

SLE, Interferon gamma, IL-12, IL-15, IL-18, disease activity.

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