

Volume 12, issue 1, March 2001 | Articles originaux

Analysis of interleukin-18, interleukin-1 converting enzyme (ICE) and interleukin-18-related cytokines in Crohnís disease lesions.

Abstract

A local increase of interleukin-18 (IL-18) expression has been recently demonstrated in Crohn's disease (CD), suggesting a role for mature IL-18 (cleaved by ICE protease) in the induction of proinflammatory cytokines and Th1 polarization observed in CD lesions. The aim of this study was to investigate IL-18 modulation and its potential immune consequences in CD lesions. We showed

increased IL-18 production in chronic CD lesions and identified epithelial cells and macrophages as IL-18-producing cells. A twofold increase in ICE a, b, and/or g mRNA that encodes for the complete mature peptide required for ICE activity, and a marked increase in IL-18R-positive immune cells was observed in chronic lesions compared to uninvolved areas or normal control samples. Chronic lesions also displayed intense transcription of IL-18-induced cytokines, IFN-g, IL-1b, TNF-a, and IL-8. By contrast, when neither IL-18 nor ICE mRNAs were enhanced (early asymptomatic CD lesions), IL-18-induced cytokines were not up-regulated. These results are in accordance with a putative role of mature IL-18 in the pathogenesis of CD.

Full text

INTRODUCTION

Interleukin-18 (IL-18), a recently discovered cytokine [1, 2] is classified as a member of the IL-1 family of cytokines [3]. Like IL-1beta, the precursor form of IL-18 (proIL-18) lacks a signal peptide, and requires cleavage by the cysteine protease IL-1beta-converting enzyme (ICE/caspase-1) to produce the mature cytokine that is released from the cell [4, 5]. The 24 kDa proIL-18 form can be accumulated within the cell. This could facilitate rapid cleavage and secretion of the 18 kDa mature form when the cell is activated. ProIL-18 can also be secreted [6] and Fantuzzi *et al.* identified an extracellular serine esterase, proteinase-3 (PR-3), which is able to cleave proIL-18 into an active form [7]. ProIL-18 could therefore be processed intracellularly as well as extracellularly.

IL-18 is mainly synthesized by dedicated antigen-presenting cells: dendritic cells, activated macrophages, and Küpffer cells. IL-18 acts in synergy with IL-12 to induce Th1 differentiation and IFN-gamma production and enhances the cytotoxic activity of immune cells mediated by Fas ligand and perforin molecules [8]. IL-18 also has proinflammatory properties by inducing the production of IL-1beta, TNF-alpha, and chemokines, interleukin-8 (IL-8), macrophage inflammatory protein-1alpha (MIP-1alpha), and monocyte chemotactic protein-1 (MCP-1) [9, 10]. The pleiotropic activities of IL-18 suggest an important role for this cytokine in triggering and polarization of the immune response.

In a previous study, we reported IL-18 production in the human intestinal tract [11]. IL-18 was synthesized not only by macrophages and "dendritic-like" cells of

lymphoid structure, but also by epithelial cells of the villi and the top of the crypts. This could be a feature of epithelial barriers, as airway [12] and skin epithelium [13] were recently shown to synthesize IL-18. We also observed the presence of IL-18R-positive immune cells in the lamina propria of normal colonic specimens [14]. Interestingly, IFN-gamma-producing intra-epithelial lymphocytes (IELs) of the normal colonic mucosa were shown to have the same cellular distribution along the surface-crypt axis as IL-18-positive epithelial cells [15]. Altogether, these results are in accordance with a role of IL-18 in mucosal immunity.

IL-18 up-regulation might be implicated in the immune disorders of Crohn's disease (CD), an inflammatory bowel disease associated with a polarized Th1 cytokine profile [16, 17]. IL-18 mRNAs are increased in lesions of dextran sulphate sodium-induced colitis, a murine model of CD, and blocking of IL-18 results in improvement of disease activity [18]. In man, two recent reports have shown an increase in IL-18 mRNA in CD lesions [19, 20] with the presence of the active subunit of ICE (p20) [19] and mature IL-18 protein [19, 20]. As IL-18 promotes the synthesis of proinflammatory cytokines, it can be hypothesized that up-regulation of IL-18 in CD lesions contributes to local inflammation and tissue injury. In this field, Monteleone *et al.* showed that IL-18 produced by lamina propria mononuclear cells has an *in vitro* biological activity [19]. Our study investigated the production of IL-18 and the infiltration of potential IL-18-responsive cells (bearing IL-18 receptors) in lesions, and quantified IL-18, ICE and IL-18-induced cytokine transcripts in lesions at different stages of the disease. The results support the hypothesis that IL-18 may play an important role in the immune disorders of CD.

MATERIALS AND METHODS

Patients and tissue samples

Specimens of normal gut mucosa.

Molecular analysis: specimens of normal mucosa from stomach (n = 3), ileum (n = 4), and large intestine (n = 9) were obtained from normal areas situated away from (> 5 cm) the adenocarcinoma, from surgically excised cancers. Immediately after removal, mucosa specimens were snap-frozen in liquid nitrogen and stored at - 80° C.

Immunohistochemistry: samples from 15 normal small intestines (6 jejunal samples, 9 ileal samples), and 20 normal large intestines (15 colonic samples, 5 rectal samples) were examined. Samples were derived from unaffected areas of surgically excised cancers and endoscopic biopsies taken from the healthy small intestine (n = 3) and colon (n = 4) of seven patients with functional disorders. A specimen of "unprepared" colon (from a colonic segment surgically resected for intestinal obstruction) was also included in the series.

Specimens of mucosa from Crohn's disease patients.

Immunohistochemistry: mucosal specimens from 3 men and 4 women with a mean age of 36.3 years (range: 21 to 66 years) with chronic CD were studied. The diagnosis of CD was established on the basis of conventional clinical and histopathological criteria. Specimens were obtained from surgical resections in 3 cases and biopsies in 4 cases. At the time of analysis, 6 patients were receiving immunosuppressive therapy (corticosteroids in 3 cases, azathioprine in two cases, salicylates in one case), and one patient was not taking any medication. In all patients, mucosal disease activity was graded as severe on the basis of the following histological changes: presence of mucosal destruction and/or ulcers and/or fistulae. Five patients had purely ileal involvement, and two had purely colonic involvement.

Molecular analysis: samples of ileal mucosa from chronic CD lesions and uninvolved areas were obtained from ileocolectomy specimens for severe chronic CD. Each sample was examined macroscopically and histologically. The mean age of the 6 patients (2 men, 4 women) was 35.7 years (range: 24-65 years). Three patients received immunosuppressive therapy (corticosteroids). Five patients had purely ileal involvement, and one had ileocolonic disease. Early asymptomatic ileal CD lesions were endoscopic ileal recurrences (aphthoid lesions) diagnosed three months after ileocolectomy for CD. Patients did not receive any treatment at the time of analysis. Specimens were obtained from a previous series published by Desreumaux *et al.* [17] (informed consent was obtained from all patients with approval from the local ethics committee).

Determination of mRNA level using real-time PCR

RNA extraction and cDNA preparation: Total RNA was isolated using the acid-phenol guaninidium method. The extraction yield was quantified by

spectrophotometry. The quality of RNA samples was determined by electrophoresis trough denaturing agarose gels and staining with ethidium bromide, and the 18s and 28s RNA were visualized under UV illumination.

Reverse transcription: One mug of total RNA for each sample was reverse transcribed in a 20 mul volume reaction using 50 U of MMLV reverse transcriptase, 20 U of RNAse inhibitor (Perkin-Elmer Biosystems Inc., Foster City, USA), 1 mM dA/T/C/G (Amersham-Pharmacia, Uppsala, Sweden), 5 mM $MgCl_2$, 10 mM Tris HCl pH 8.3, 100 mM KCl and 50 pM random hexamers. cDNA was diluted to 1:20 in nuclease-free H_2O (Promega Corporation, Madison; WI, USA).

PCR reagents, protocols and controls: IL-18, and ICE primers and probes were designed using Primer Express (Perkin-Elmer) and Oligo 4 (National Biosciences, Plymouth, MN, USA) software purchased from Perkin-Elmer. The sense and antisense primer and probe sequences used were as follows: IL-18 sense CCA AGG AAA TCG GCC TCT ATT; IL-18 antisense CCA TAC CTC TAG GCT GGC TAT CTT T; IL-18 probe FAM-TGA CTG TAG AGA TAA TGC ACC CCG GAC C-TAMRA. ICE sense TGA ATA CCA AGA ACT GCC CAA GT; ICE anti-sense AGC GAT AAA ATC CTT CTC TAT GTG G; ICE probe FAM-TGC CGT GGT GAC AGC CCT GGT-TAMRA. The design of the primers was performed to amplify only three splices (alpha, beta, and gamma) of ICE mRNA but not the gamma- and delta-isoforms. 18s RNA primers and probe reagents, as well as cytokine plate I were purchased from Perkin-Elmer. PCR amplifications were performed using the Taqman core kit (ABI) or Universal master mix (for cytokine plate) under standard conditions according to the manufacturer's instructions. Briefly, reactions were performed in 50 mul reaction volumes containing the cDNA equivalent of 25 ng of total RNA, 1X Taqman Buffer A, 5 mM $MgCl_2$, 200 mM dATP, dCTP, dGTP and 400 mM dUTP, 1.25 U AmpliTaq Gold, 0.5 U UNG (AmpErase uracyl N glycosylase, 250 nM of each primer and 100 nM of the probe). The ABI 7700 Sequence Detector System was set up under the manufacturer's standard thermal cycling conditions. The SDS software analysed fluorescent signals and calculated the cycle threshold. Careful monitoring of negative controls showed the absence of carry-over for each target. One of each type of amplicon, corresponding to IL-18 and ICE was also migrated on agar gel showing a single band at the expected position. Direct sequencing of PCR products confirmed the specificity of PCR reactions. Each reaction was performed in duplicate. Only duplicates with a coefficient of variation less than 10% were taken into account.

The levels of IL-18 and ICE mRNAs in normal gut mucosa and CD specimens were compared using a relative quantification approach based on the fluorescent Taqman methodology, with the standard curve method. Amplification of the housekeeping gene 18S was chosen to normalize results. This method of quantification was chosen because the slope of 18S amplification (3.35) was not strictly similar to that of the target genes (3.7-4), according to the PE Applied Biosystems procedure. A specimen of gut mucosa was first chosen to construct the standard curves. Fivefold serial dilutions of the total cDNA of this sample were prepared. An arbitrary value was assigned to each dilution. Each diluted cDNA was submitted to amplification for IL-18, ICE and 18S genes to measure the C_T values (which estimates the amplification yield of each reaction). Standard curves were obtained by plotting the observed C_T values and the arbitrary value of each diluted cDNA. All specimens of the series were then submitted to IL-18, ICE and 18S gene amplification. The relative amounts of IL-18, ICE and 18S were calculated for each specimen by plotting the C_T value on the appropriate standard curve. This amount was divided by the corresponding relative of amount 18S to obtain a normalized value. Two specimens were chosen as calibrators for ICE and IL-18 analysis, (because of their low level of expression of the target). All normalized values were divided by the "calibrator" to generate the relative expression levels, thereby eliminating the arbitrary units from the standard curve.

Cytokine plate results were interpreted using the comparative threshold cycle method (ddCt) according to the manufacturer's instructions. A sample of normal ileum mucosa was used as the calibrator and all cytokine levels found in early and chronic CD specimens were expressed as an x-fold ratio relative to cytokine levels found in the normal ileal mucosa.

Immunohistochemistry

Five-mum sections were air dried, deparaffinized, and heated three times for 5 min in a microwave oven (in buffered citrate, pH 6.0). The sections were first incubated for 5 min with 3% hydrogen peroxide aqueous solution to quench endogenous peroxidase activity. Goat polyclonal IgG anti-IL-18 was used for IL-18 detection (R&D Systems, Abingdon, United Kingdom). This antibody recognized the inactive precursor of IL-18 (24 kDa) and the mature form (18 kDa). Sections were incubated for 60 min with anti-IL-18 antibodies at a dilution of 1:200 (final concentration of 0.5 mug/ml). The biotinylated conjugate and streptavidin

peroxidase were applied for 15 min, and DAB chromogen was used as a peroxidase substrate complex (all from DAKO LSAB$^+$ kit peroxidase, DAKO Copenhagen, Denmark). All incubations were performed at room temperature. Tissue sections were then counterstained with Harris's haematoxylin, and mounted with aqueous mounting medium (Dako). Intrinsic positive controls for immunoreactivity in each section were IL-18 stained cells in lymphoid follicles. Each series of sections contained negative controls without primary antibodies. In addition, a control using IgG from non-immunized goats (R&D systems) at the same concentration as that of anti-IL-18 IgG was carried out in a number of tissue sections, that excluded nonspecific binding. A murine monoclonal IgG1 kappa anti-CD68 (Dako) was used (dilution 1:200; final concentration of 1.8 mug/ml) as primary antibody to label the macrophage lineage. Irrelevant isotype-matched MAb (Dako) was used as negative control and excluded nonspecific binding. CD68 and IL-18 were co-localized on the same sections by double-label immunohistochemistry. The three-step method for IL-18 staining was performed first, as described above. Slides were then incubated with anti-CD68 for 30 min (final concentration of 1.8 mug/ml), followed by 10 min with the labelled polymer alkaline phosphatase (DAKO EnVision System). One drop of levamisole solution (Dako) was added per ml of substrate solution to reduce endogenous alkaline phosphatase. The colour reaction was developed using the Fast Red Substrate System (Dako). Negative controls with isotype-matched antibodies excluded nonspecific binding and cross-reactivity of the Envision system with anti-IL-18 IgG. IL-18R staining was performed with a murine anti-IL-18R monoclonal IgG1 (R&D Systems, Abingdon, United Kingdom) at a final concentration of 0.25 mug/ml, on frozen tissues using the LSAB$^+$ kit peroxidase (Dako). Irrelevant murine IgG were used at the same final concentration as the negative control and excluded nonspecific binding.

Statistical analysis

The significance of differences of ICE transcript levels between chronic ileitis and normal ileal mucosa was assessed by Student's *t* test.

RESULTS

IL-18 production is increased in CD lesions

IL-18 production was first assessed by immunohistochemistry in normal small intestinal mucosa (n = 15) and colo-rectal mucosa (n = 20) from surgically resected specimens and biopsies. In all specimens tested, lymphoid structures showed very strongly labelled cells with a morphology compatible with macrophages and dendritic cells. IL-18-positive cells were also localized in the lamina propria, in the close vicinity of the epithelium, and between lymphoid follicles and epithelial cells. By contrast, IL-18 immunostaining observed in surface epithelium differed between the small intestine and the large intestine. Epithelial cell staining in the small intestine varied in extent and density. Most of the epithelial cells covering the finger-like villi were labelled, whereas the basal part of the crypts were almost completely IL-18-negative (Figure 1a). Columnar cells were uniformly labelled, and a faint IL-18 signal was observed at the basal pole of goblet cells (goblet cell mucus was nonreactive) (Figure 1b). Some strongly IL-18-positive foci were sometimes observed in the crypts of Lieberkühn (Figure 1c). The epithelium of colonic mucosa was strongly labelled from the lumen to the upper part of the crypts (Figure 1d). A similar pattern of IL-18 staining was observed in rectal mucosa specimens (data not shown). Other layers of the intestinal wall did not contain IL-18-producing cells. IL-18 expression was never observed in endothelial cells, fibroblasts, or muscle cells. The mucosa of the small intestine and large intestine is therefore able to synthesize IL-18.

Samples of macroscopically and histologically inflamed intestinal mucosa from 7 patients with severe chronic CD were then analysed. All specimens of Crohn's ileitis (5 cases) and colitis (2 cases) displayed increased IL-18 production compared to the normal specimens described above. In particular, (i) the epithelium was more extensively and more densely labelled, with staining extending into the crypts of Lieberkühn, (ii) the LP was infiltrated by numerous IL-18-producing cells, (iii) epithelioid giant cells of granulomas were strongly labelled, (iv) a large number of IL-18-labelled cells were found in other layers of the gastrointestinal tract (muscularis mucosae and submucosa). Clusters of IL-18-positive cells were also observed in the close vicinity of epithelial erosions or destruction. An example of IL-18 staining in severe chronic Crohn's ileitis is shown in Figure 2a. CD68 staining (which identifies the monocyte/macrophage lineage) revealed massive infiltration by macrophages (Figure 2b). A double IL-18 and CD68 staining showed that most of the IL-18-producing cells from the LP belonged to the macrophage lineage (Figure 2c).

ICE transcripts are increased in CD lesions

IL-18 functionality in CD lesions was first investigated by analysis of IL-18 and ICE transcripts (involved in IL-18 maturation) in lesions, compared to normal mucosal specimens (Table 1).

IL-18 and ICE (alpha, beta and gamma) transcripts were observed in normal ileal mucosa. The IL-18 level (mean: 1.33; SD: 0.19) was similar to that observed in the large intestine (mean: 1.42; SD: 0.36), whereas ICE mRNAs were much more abundant in the small intestine (mean: 7.82; SD: 0.49) than in the large intestine. Six surgically resected specimens from patients with severe chronic Crohn's ileitis and samples of unaffected mucosa from the same patients (available in 4 cases) were then assessed. Ileitis samples showed slightly increased levels of IL-18 mRNAs (mean: 1.83; SD: 0.81) compared to unaffected mucosa (mean: 1.59; SD: 0.60) or normal ileum (mean: 1.33; SD: 0.19), although the difference was not statistically significant. By contrast, ICE protease transcripts were significantly increased in ileitis mucosal samples (mean: 14.46; SD: 5.78) compared to unaffected mucosa (mean: 7.26; SD: 1.98) or normal control cases (mean: 7.82; SD: 0.49) (Student's t test $p = 0.05$).

CD lesions are infiltrated by IL-18 receptor-positive cells

The existence of IL-18R-positive immune cells in lesions is a prerequisite to the induction of IL-18-induced cytokines. We therefore performed immuno-staining for IL-18R on frozen sections of chronic CD lesions and distant uninvolved areas obtained from two patients, and compared the density of IL-18R-positive cells with that of four normal control cases. Lamina propria of normal intestinal mucosa (Figure 3a) was infiltrated by a few IL-18R-positive cells (brown), of a size compatible with that of lymphocytes. By contrast, intense infiltration by IL-18R-positive cells was observed in CD lesions (Figure 3c), with decreased levels in distant uninvolved mucosa (Figure 3b).

IL-18-induced cytokines are increased in chronic CD lesions

The implication of IL-18 modulation in the induction of IL-1beta, TNF-alpha, IFN-gamma, and IL-8 was evaluated by comparing IL-18 and ICE (alpha, beta and gamma) mRNA levels with those of IL-18-induced cytokines in normal specimens and chronic CD lesions and asymptomatic aphthoid CD lesions (early lesions), as IFN-gamma production is not increased at this early stage of the disease [17].

As previously observed in Table 1, a marked enhancement of ICE transcripts was detected in chronic CD lesions, ranging from 1.7- to 2.5-fold higher than in normal control cases, with no significant enhancement of IL-18 transcripts in lesions (range: 1.4-1.6/mean of control cases: 1.3; SD: 0.2) (Figure 4a). The IL-18-induced cytokine pattern was then analysed for the same patients, and expressed as a proportion of the levels found in normal ileal mucosa (Figure 4b). The cytokine transcripts of IL-1beta, TNF-alpha, IFN-gamma, and IL-8 were very strongly induced in chronic lesions, as TNF-alpha, IFN-gamma, IL-1beta, and IL-8 were about 8-, 32-, 1,750-, and 4,000-fold increased. Interestingly, neither IL-18 nor ICE were up-regulated in early CD lesions (Figure 4c) and the pattern of IL-18-induced cytokines in early lesions was quite similar to that of normal tissues (Figure 4d). TNF-alpha, IFN-gamma and IL-8 were inconstantly and moderately increased (range: 1.6- to 3.4-fold induction). Additionally, a weak amplification signal, above the detectable threshold was observed for IL-2, IL-12p35 and IL-12p40 mRNAs in all four specimens tested with no enhancement in lesions (data not shown).

DISCUSSION

We confirmed the presence of increased IL-18 production in chronic CD lesions, and identified epithelial cells and macrophages to be the major source of the IL-18 production. In chronic lesions, ICE transcripts (alpha-, beta-, and/or gamma-isoforms) are twofold increased compared to normal distant areas or control cases. We also showed massive infiltration of chronic lesions by IL-18R-positive immune cells and increased levels of transcripts for IL-18-induced cytokines: IL-1beta, TNF-alpha, IL-8 and IFN-gamma.

We assessed the potential bias of IL-18 induction in mucosal specimens secondary to ischemic stress, cold stress [21], or preoperative osmotic stress [22], by including biopsies and a sample of "unprepared" intestine in the series of surgically resected specimens. Only a slight variability of IL-18 staining was observed between specimens. We can therefore confirm the constitutional production of IL-18 in gut mucosa.

The polyclonal anti-IL-18 antibody used for immunostaining does not discriminate between mature, immature or degraded forms of IL-18. Most of the IL-18 signal observed in normal gut mucosa was probably related to detection of the proIL-18 form, as proIL-18 is the sole form detected by the Western blot

method [19, 20]. The constitutional presence of at least the inactive form of IL-18 in normal mucosa (Figure 1) and the detection of transcripts for ICE splices (alpha, beta, and gamma) that encodes for the complete mature peptide required for ICE activity, in all segments of the digestive tract (Table 1), indicate that large amounts of mature IL-18 might be rapidly processed in response to immune stimulation.

We showed that the extent and density of IL-18 labelling were markedly increased in the epithelium of CD lesions, as previously observed by Pizzaro *et al.* [20]. These authors also reported the presence of numerous scattered IL-18-positive cells within the lamina propria. Double CD68/IL-18 immunostaining showed that most of these cells belonged to the macrophage lineage (Figure 2c).

Evaluation of IL-18 and ICE transcript levels in lesions, unaffected areas or control cases was performed using a semi-quantitative RT-PCR Taqman technology. We observed a minor increase of IL-18 transcripts in lesions (Table 1), whereas a marked increase in IL-18 protein signal was demonstrated by immunostaining (Figure 2a). When IL-18 mRNA was quantified on isolated intestinal epithelial cells and lamina propria mononuclear cells, a twofold increase was only observed in CD specimens *versus* noninflamed controls [20]. Modulation of IL-18 mRNA in lesions probably does not reflect the increase of IL-18 protein secretion. A constitutive promoter activity is observed upstream to the murine IL-18 gene, and IL-18 mRNA does not contain RNA destabilising elements, implying a long half-life for IL-18 mRNA [23]. Unlike that of most other cytokines, regulation of IL-18 gene expression may therefore predominantly occur at the level of processing rather than at the transcriptional and translational level.

Processing of proIL-18 into an active molecule involves ICE protease. Recent reports have shown the presence of the active subunit of ICE (p20) in CD lesions, while only ICE precursor molecules (p45) are found in control samples from normal colonic mucosa [19]. We further demonstrated a twofold increase of ICE alpha, beta, and or gamma splices in lesions compared to distant noninflamed tissues or normal control samples (Table 1). Altogether, these data suggest quantitative and qualitative modifications of ICE in lesions, which are associated with the presence of the mature form of IL-18 [19].

The biological activity of mature IL-18 in lesions was suggested by the capacity of lamina propria mononuclear cells to induce the *in vitro* production of IFN-gamma, which was down-regulated by IL-18 antisense oligonucleotides [19]. To further investigate the biological activity of IL-18 in lesions and the related immune consequences, we assessed infiltration by IL-18R-positive cells and the levels of IL-18-induced cytokine mRNAs in lesions. We first showed an intense infiltration of CD lesions by IL-18R-positive cells compared with distant noninvolved mucosa or control samples (Figure 2), indicating the presence of potential IL-18 sensitive immune cells in lesions that is a prerequisite for the downstream effects of IL-18. We then analyzed the levels of IL-18 and ICE mRNAs and the associated local cytokine profile at different stages of CD, as divergent cytokine patterns have been previously observed in early and chronic lesions [17]. Chronic lesions presented an increased level of ICE mRNA (Figure 4a) and displayed intense synthesis of IFN-gamma, IL-1beta, TNF-alpha, and IL-8 mRNAs (Figure 4b). By contrast, in early lesions, IL-18 and ICE were not increased and the level of IL-18-induced cytokines was low (Figures 4c and 4d).

Despite the limited number of cases studied, and that these cytokines may also be induced by mediators other than IL-18, these findings are consistent with the implication of IL-18 modulation in the immune disorders of CD. In this field, a recent report showed a role of IL-18 in the development of intestinal inflammation in a murine model of dextran sulfate sodium-induced murine colitis [18].

IL-18 overproduction in CD lesions probably does not constitute a causal event in the pathogenesis of CD, but reflects uncontrolled activation of the immune system. In addition to inducing the inflammatory cascade *via* IL-1beta and TNF-alpha, IL-18, *via* the chemokines IL-8 and MCP1 and MIP-1, is also probably involved in the infiltration and activation of neutrophils and macrophages in CD lesions. Finally, the contribution of IL-18 to mucosal injury is suggested by its capacity to induce TNF-alpha and to promote inducible nitric oxide synthetase (iNOS) [24], an enzyme responsible for the production of nitric oxide (NO).

Large patient series analysing IL-18 production, in relation to stage of the disease and medical treatment, are required to confirm and extend these results and to investigate whether IL-18 could provide a new marker which would be predictive of relapse in patients in remission, as recently demonstrated for the downstream cytokines IL-1beta and TNF-alpha [25].

ABBREVIATIONS	
APC: Antigen-presenting cell	IL-18: Interleukin-18
CD: Crohn's disease	NK: Natural killer
ICE: Interleukin-1beta-converting enzyme	Th: T helper
IFN-gamma: Interferon-gamma	TNF-alpha: Tumour necrosis factor-alpha.

CONCLUSION

Acknowledgements. We thank Dr. Claire Mathiot, Dr. Véronique Mosseri, and Dr. Ludovic Lacroix for their valuable contribution to this study, with a particular mention for Christine and Pierre Pagès.

This work was supported by INSERM and the Comité de Paris de la Ligue Nationale Contre le Cancer (LA n° 2).

References

1. Okamura H, Tsutsi H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, Torigoe K, Okura T, Nukada Y, Hattori K, Akita K, Namba M, Tanabe F, Konishi K, Fukuda S, Kurimoto M. 1995. Cloning of a new cytokine that induces IFN-gamma production by T cells. *Nature* 378: 88.

2. Okamura H, Nagata K, Komatsu T, Tanimoto T, Nukata Y, Tanabe F, Akita K, Torigoe K, Okura T, Fukuda S, *et al.* 1995. A novel costimulatory factor for gamma interferon induction found in the livers of mice causes endotoxic shock. *Infect. Immun.* 63: 3966.

3. Ushio S, Namba M, Okura T, Hattori K, Nukada Y, Akita K, Tanabe F, Konishi K, Micallef M, Fujii M, Torigoe K, Tanimoto T, Fukuda S, Ikeda M, Okamura H, Kurimoto M. 1996. Cloning of the cDNA for human IFN-gamma-inducing factor, expression

in *Escherichia coli*, and studies on the biologic activities of the protein. *J. Immunol.* 156: 4274.

4. Gu Y, Kuida K, Tsutsui H, Ku G, Hsiao K, Fleming M A, Hayashi N, Higashino K, Okamura H, Nakanishi K, Kurimoto M, Tanimoto T, Flavell R A, Sato V, Harding M W, Livingston D J, Su M S. 1997. Activation of interferon-gamma inducing factor mediated by interleukin-1beta converting enzyme. *Science* 275: 206.

5. Ghayur T, Banerjee S, Hugunin M, Butler D, Herzog L, Carter A, Quintal L, Sekut L, Talanian R, Paskind M, Wong W, Kamen R, Tracey D, Allen H. 1997. Caspase-1 processes IFN-gamma-inducing factor and regulates LPS-induced IFN-gamma production. *Nature* 386: 619.

6. Puren A J, Fantuzzi G, Dinarello C A. 1999. Gene expression, synthesis, and secretion of interleukin-18 and interleukin-1beta are differentially regulated in human blood mononuclear cells and mouse spleen cells. *Proc. Natl. Acad. Sci. USA* 96: 2256.

7. Fantuzzi G, Karasek J A, Reznikov L L. 1998. A role for neutrophil proteinase-3 (PR-3) in the generation of active IL-18. *Eur. Cytokine Netw.* 9: 378 (abstract).

8. Lebel-Binay S, Berger A, Zinzindohoue F, Cugnenc P, Thiounn N, Fridman W H, Pages F. 2000. Interleukin-18: biological properties and clinical implications. (Review) *Eur. Cytokine Netw.* 11: 15.

9. Puren A J, Razeghi P, Fantuzzi G, Dinarello C A. 1998. Interleukin-18 enhances lipopolysaccharide-induced interferon-gamma production in human whole blood cultures. *J. Infect. Dis.* 178: 1830.

10. Puren A J, Fantuzzi G, Gu Y, Su M S, Dinarello C A. 1998. Interleukin-18 (IFN-gamma-inducing factor) induces IL-8 and IL-1beta *via* TNF-alpha production from non-CD14^{+} human blood mononuclear cells. *J. Clin. Invest.* 101: 711.

11. Pages F, Berger A, Henglein B, Piqueras B, Danel C, Zinzindohoue F, Thiounn N, Cugnenc P H, Fridman W H. 1999. Modulation of IL-18 expression in human colon carcinoma, consequences for tumor immune surveillance. *Int. J. Cancer* 84 : 326.

12. Cameron L A, Taha R A, Tsicopoulos A, Kurimoto M, Olivenstein R, Wallaert B, Minshall E M, Hamid Q A. 1999. Airway epithelium expresses interleukin-18. *Eur. Respir. J.* 14: 553.

13. Naik S M, Cannon G, Burbach G J, Singh S R, Swerlick R A, Wilcox J N, Ansel J C, Caughman S W. 1999. Human keratinocytes constitutively express interleukin-18 and secrete biologically active interleukin-18 after treatment with pro-inflammatory mediators and dinitrochlorobenzene. *J. Invest. Dermatol.* 113: 766.

14. Pages F, Berger A, Lebel-Binay S, Zinzindohoue F, Danel C, Piqueras B, Carriere O, Thiounn N, Cugnenc P H, Fridman W H. 2000. Proinflammatory and antitumor properties of interleukin-18 in the gastrointestinal tract. *Immunol. Letters* 75: 9.

15. Lundqvist C, Melgar S, Yeung M M, Hammarstrom S, Hammarstrom M L. 1996. Intraepithelial lymphocytes in human gut have lytic potential and a cytokine profile that suggest T helper 1 and cytotoxic functions. *J. Immunol.* 157: 1926.

16. Reimund J M, Wittersheim C, Dumont S, Muller C D, Baumann R, Poindron P, Duclos B. 1996. Mucosal inflammatory cytokine production by intestinal biopsies in patients with ulcerative colitis and Crohn's disease. *J. Clin. Immunol.* 16: 144.

17. Desreumaux P, Brandt E, Gambiez L, Emilie D, Geboes K, Klein O, Ectors N, Cortot A, Capron M, Colombel J F. 1997. Distinct cytokine patterns in early and chronic ileal lesions of Crohn's disease. *Gastroenterology* 113: 118.

18. Mitsuyama K, Ide M, Nishiyama T, Tomiyasu N, Shirachi A, Saiki T, Tateishi H, Toyonaga A, Kurimoto M, Okamura H, Tanikawa K. 1998. Role of interleukin-18 in a murine model of dextran sulfate sodium-induced colitis. *Gastroenterology* 114: G4258 (abstract).

19. Monteleone G, Trapasso F, Parrello T, Biancone L, Stella A, Iuliano R, Luzza F, Fusco A, Pallone F. 1999. Bioactive IL-18 expression is up-regulated in Crohn's disease. *J. Immunol.* 163: 143.

20. Pizarro T T, Michie M H, Bentz M, Woraratanadharm J, Smith M F Jr, Foley E, Moskaluk C A, Bickston S J, Cominelli F. 1999. IL-18, a novel immunoregulatory cytokine, is up-regulated in Crohn's disease: expression and localization in intestinal mucosal cells. *J. Immunol.* 162: 6829.

21. Conti B, Jahng J W, Tinti C, Son J H, Joh T H. 1997. Induction of interferon-gamma inducing factor in the adrenal cortex. *J. Biol. Chem.* 272: 2035.

22. Takeuchi M, Okura T, Mori T, Akita K, Ohta T, Ikeda M, Ikegami H, Kurimoto M. 1999. Intracellular production of interleukin-18 in human epithelial-like cell lines is enhanced by hyperosmotic stress *in vitro. Cell Tissue Res.* 297: 467.

23. Tone M, Thompson S A, Tone Y, Fairchild P J, Waldmann H. 1997. Regulation of IL-18 (IFN-gamma-inducing factor) gene expression. *J. Immunol.* 159: 6156.

24. Olee T, Hashimoto S, Quach J, Lotz M. 1999. IL-18 is produced by articular chondrocytes and induces proinflammatory and catabolic responses. *J. Immunol.* 162: 1096.

25. Schreiber S, Nikolaus S, Hampe J, Hamling J, Koop I, Groessner B, Lochs H, Raedler A. 1999. Tumour necrosis factor alpha and interleukin-1beta in relapse of Crohn's disease. *Lancet* 353: 459.

Figures

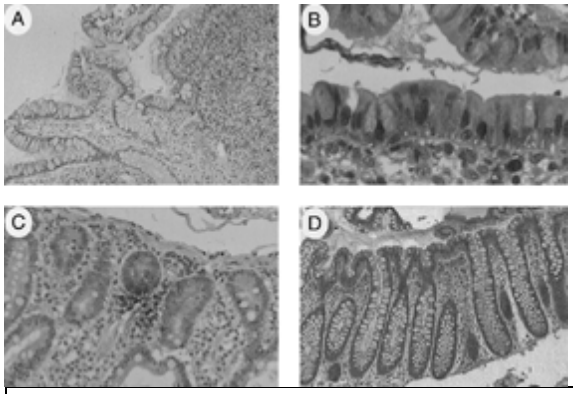


Figure 1

Immunohistochemical detection of IL-18 protein in normal mucosa of the gastrointestinal tract (original magnification x 200).

(A) Terminal ileal mucosa. **(B)** High power magnification (x 1,000) of the ileal epithelium. **(C)** IL-18 expression

observed in a few crypts of Lieberkühn. **(D)** Large intestinal mucosa.

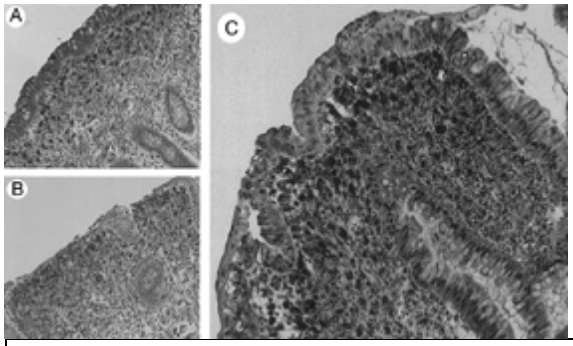


Figure 2

IL-18-producing macrophages infiltrate colonic CD lesions.

(A) IL-18 immunostaining (original magnification x 200). **(B)** CD68 immunostaining (original magnification x 200). **(C)** Double staining of IL-18-expressing cells (brown) with anti-CD68 Ab (red). Cells stained purple-brown are due to expression of IL-18 by CD68-positive cells (macrophages) (original magnification x 400).

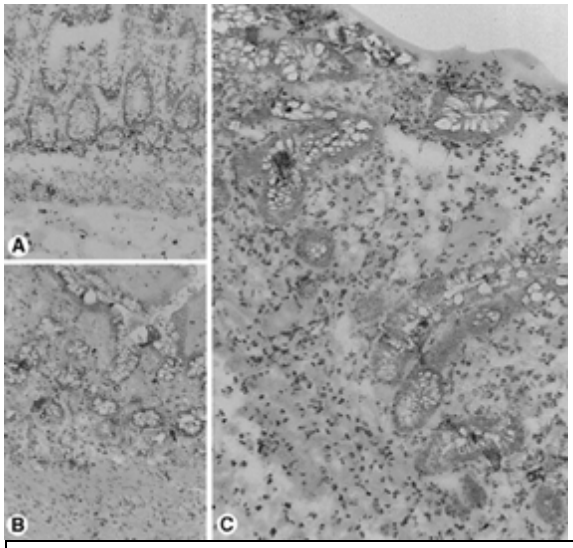


Figure 3

Immunohistochemical detection of IL-18R-positive cells (brown) in (A) normal intestinal mucosa (B) distant uninvolved mucosa (C) Crohn's disease lesion (A and B: original magnification: x 200; C: x 400).

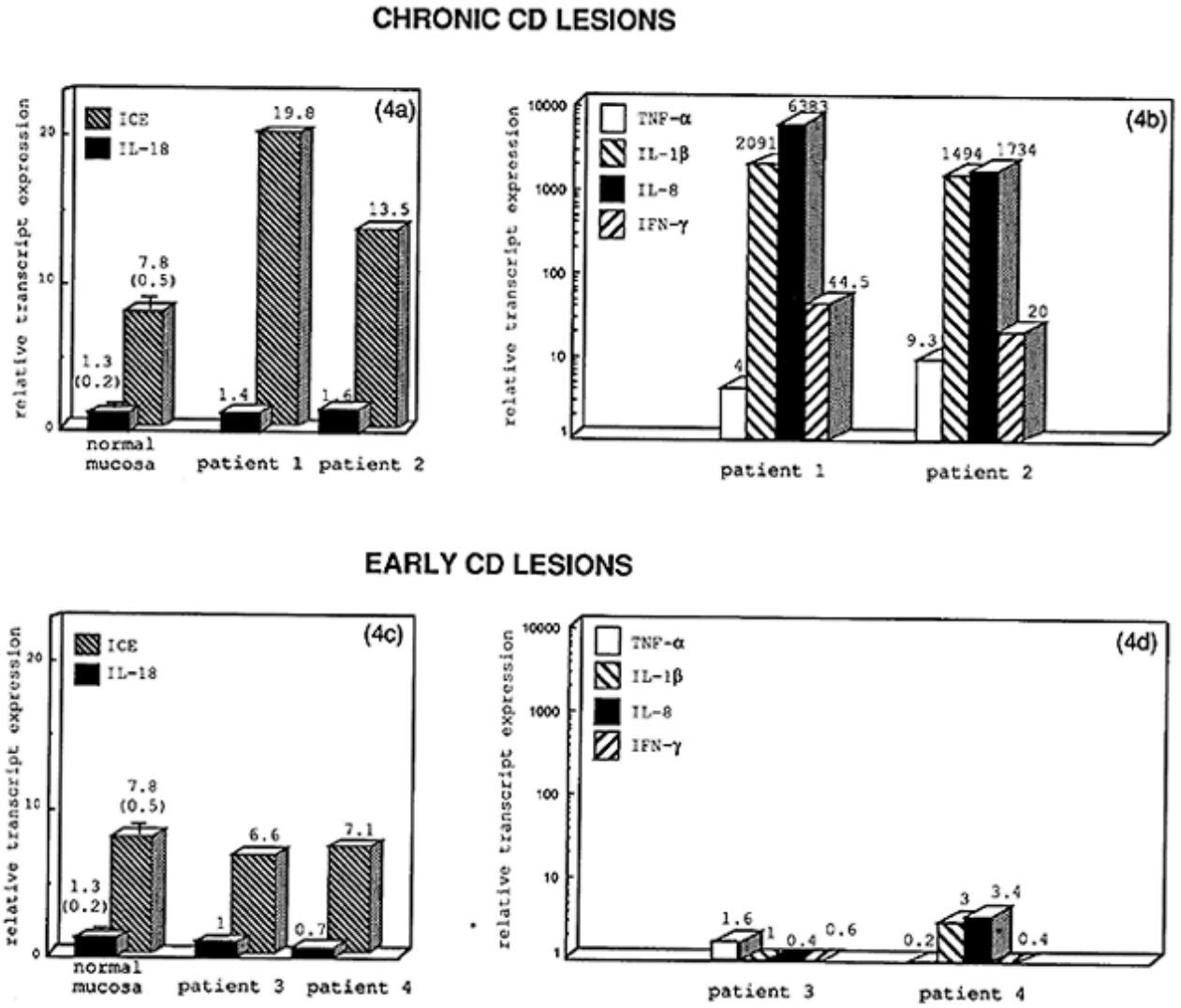


Figure 4

Quantification of IL-18 (solid bars) and ICE transcripts (hatched bars) normalized for the 18S gene, in two patients with chronic ileal CD lesions (4a) and two patients with early ileal CD lesions (4c).

The mean and SD of IL-18 and ICE transcripts in ileal mucosa of normal control cases are shown for comparison. TNF-alpha, IL-1beta, IL-8 and IFN- mRNAs are determined for the same patients by quantitative PCR normalized for 18S and are expressed as an x-fold ratio relative to cytokine levels found in normal ileal mucosa (4b, 4d).

Table 1
Relative quantification of IL-18 and ICE transcripts in normal mucosa as compared with unaffected mucosa and chronic CD ileitis

	Samples *(No.)*	IL-18 transcripts *mean (SD)*	ICE transcripts *mean (SD)*
Normal ileal mucosa	(4)	1.33 (0.19)	7.82 (0.49)
Large intestine	(9)	1.42 (0.36)	2.93 (1.10)
Chronic CD ileitis	(6)	1.83 (0.81)	14.46 (5.78)[2]
Unaffected mucosa[1]	(4)	1.59 (0.60)	7.26 (1.98)

[1] Unaffected areas distant from chronic CD ileitis were available for 4 cases.
[2] Comparison between chronic ileitis and normal ileum mucosa estimated by Student's *t* test ($p = 0.05$).

Authors

F. Pagès, V. Lazar, A. Berger, C. Danel, S. Lebel-Binay, F. Zinzindohoué, P. Desreumaux, C. Cellier, N. Thiounn, D. Bellet, P.H. Cugnenc, W.H. Fridman

Laboratoire de Recherche Chirurgicale, Pavillon Gustave-Roussy, Escalier A. 3e étage, Hôpital Cochin, 27, rue du Faubourg-Saint-Jacques, 75674 Paris Cedex

In this issue

◯ Regulation of interferon-g receptor (INF-gR) chains: a peculiar way to rule the life and death of human lymphocytes.

P. Bernabei, A. Allione, L. Rigamonti, M. Bosticardo, G. Losana, I. Borghi, G. Forni, F. Novelli

human, IFN-g, IFN-g receptor, signal transduction, cellular proliferation, apoptosis.

The interleukin-6 cytokine system in embryonic development, embryo-maternal interactions and cardiogenesis.

P. Seiler, G. Plenz, M.C. Deng
interleukin-6, embryo, blastocyst, endometrium, heart, gp130.

Role of cytokines in the modulation of nitric oxide production by cyclic AMP.

Z. Zidek
cyclic AMP, prostaglandins, nitric oxide, interleukin-10, tumor necrosis factor-a, interferon-g.

The 3’ untranslated region of tumor necrosis factor-a is highly conserved in idiopathic pulmonary fibrosis (IPF).

R.W. Freeburn, H. Kendall, L. Dobson, J. Egan, N.J. Simler, A.B. Millar
TNF-a, idiopathic pulmonary fibrosis, polymorphism.

Endogenous interferon-g impairs bacterial clearance from lungs during Pseudomonas aeruginosa pneumonia.

M.J. Schultz, A.W. Rijneveld, P. Speelman, S.J.H. van Deventer, T. van der Poll
interferon-g, cytokines, host defense, Pseudomonas aeruginosa, pneumonia.

Development of allergic contact dermatitis requires activation of both tumor necrosis factor-receptors.

F.M. Becke, T. Hehlgans, G. Brockhoff, D.N. Männel
allergic contact dermatitis, p75TNF receptor, p55TNF receptor, Langerhans cells, dendritic cells.

Transforming growth factor beta-1 and interferon-alpha in the aids dementia complex: possible relationship with cerebral viral load?

O. Perrella, P.B. Carreiri, A. Perrella, C. Sbreglia, F. Gorga, D. Guarnaccia, G. Tarantino
transforming growth factor-beta1, HIV-RNA, AIDS-dementia complex, interferon-alpha.

Interferon-beta (INF-b) antibodies in interferon-b1a- and interferon-b1b-treated multiple sclerosis patients.

Prevalence, kinetics, cross-reactivity, and factors enhancing interferon-b immunogenicity in vivo.

P. Perini, A. Facchinetti, P. Bulian, A.R. Massaro, D. De Pascalis, A. Bertolotto, G. Biasi, P. Gallo
interferon-beta, natural autoantibodies, multiple sclerosis.

The promoter polymorphism of the interleukin-6 gene regulates interleukin-6 production in neonates but not in adults.

S. Kilpinen, J. Hulkkonen, X.Y. Wang, M. Hurme
interleukin-6, cord blood, gene polymorphism, inflammatory mediators.

The hepatitis C virus (HCV) induces a long-term increase in interleukin-10 production by human CD4+ T cells (H9).

O. Delpuech, D. Buffello-Le Guillou, E. Rubinstein, C. Féray, M.A. Petit
HCV-RNA, interleukin-10, H9 T cells, CD81, capsid mutation, protein kinase C.

Retrovirus-mediated gene transfer of the cytokine genes interleukin-1b and tumor necrosis factor-a into human neuroblastoma cells: consequences for cell line behavior and immunomodulatory properties.

C. Coze, T. Leimig, M.T. Jimeno, P. Mannoni
neuroblastoma, immunotherapy, gene therapy, cytokine, IL-1b, TNF-a.

Characterisation of cytokine mRNA expression in tumour-infiltrating mononuclear cells and tumour cells freshly isolated from human colorectal carcinomas.

A. Csiszar, T. Szentes, B. Haraszti, W. Zou, D. Emilie, G. Petranyi, E. Pocsik
colorectal cancer, tumour-infiltrating lymphocytes, cytokines, gene expression, RT-PCR.

Analysis of interleukin-18, interleukin-1 converting enzyme (ICE) and interleukin-18-related cytokines in Crohnís disease lesions.

F. Pagès, V. Lazar, A. Berger, C. Danel, S. Lebel-Binay, F. Zinzindohoué, P. Desreumaux, C. Cellier, N. Thiounn, D. Bellet, P.H. Cugnenc, W.H. Fridman
intestinal mucosa, Crohn's disease, interleukin-18, caspase-1/ICE, immunohistochemistry, reverse transcription polymerase chain reaction.

Daily production of human tumor necrosis factor in lipopolysaccharide (LPS)-stimulated ex vivo blood culture assays.

J.C. Aguillon, A. Escobar, V. Ferreira, A. Aguirre, L. Ferreira, M.C. Molina, A. Ferreira
TNF, cytokine, LPS.

Inducible nitric oxide synthase attenuates chronic colitis in human histocompatibility antigen HLA-B27/human b2 microglobulin transgenic rats.

H.S. Blanchard, E. Dernis-Labous, D. Lamarque, J. Tran van Nhieu, Z. Szepes, J.F. Fléjou, E. Wollman, B.J.R. Whittle, M. Breban
rodent, inflammation, nitric oxide, cytokines, in vivo animal models.

Up-regulation of mucin secretion in HT29-MTX cells by the pro-inflammatory cytokines tumor necrosis factor-a and interleukin-6.

M.G. Smirnova, S.L. Kiselev, J.P. Birchall, J.P. Pearson
MUC5AC, MUC5B, mucus-secreting cell line HT29-MTX.

A Jurkat T cell variant resistant to death receptor-induced apoptosis. Correlation with heat shock protein 27 and 70 levels.

J.E. Ricci, L. Maulon, V. Battaglione-Hofman, C. Bertolotto, F. Luciano, B. Mari, P. Hofman, P. Auberger
apoptosis, caspases, Hsp27, Hsp70, T lymphocytes.

Increased cell surface expression of histocompatibility class II IAd in c-FOS transfected clones of the P388D1 murine macrophage cell line.

Y. Le Garrec, E. Rouer, F. Conti, P. Michel, A. Pompidou
MHC class II antigens, murine macrophages, c-Fos.

Transforming growth factor-b inhibits interleukin-10 synthesis by human monocytic cells.

S. Suberville, A. Bellocq, I. Peguillet, O. Lantz, P. Stordeur, B. Fouqueray, L. Baud
transforming growth factor-b1, interleukin-10, U. 937 cells.

Evidence for tumor necrosis factor receptors (TNFRs) in human MRC5 fibroblast cells.

D. Derouich-Guergour, H. Pelloux, D. Aldebert, P. Demenge, P. Ambroise-Thomas
MRC5, fibroblasts, TNF receptor, RT-PCR, binding, flow cytometry.

Interleukin-9 induces 24P3 lipocalin gene expression in murine T cell lymphomas.

C. Orabona, L. Dumoutier, J.C. Renauld
cytokines, lipocalin, IL-9, 24P3.

Contrasting effect of interleukin-4 on the expression of cytosolic phospholipase A2, 5-lipoxygenase and 5-lipoxygenase-activating protein in peritoneal macrophages from control and ovalbumin-sensitized rats.

L. Escoubet-Lozach, C.F. M'Rini, A. Rey, M. Béraud, J.C. Lepert, J. Bernard, J.R. Mathieu, B. Pipy
cytokines, lipid mediators, macrophages, rat.

Fibrinogen cooperates with cytokines to induce interleukin-6 receptor mRNA expression in human hematopoietic CD34+ progenitors.

A. Hatzfeld, B. Panterne, N.O. Fortunel, M.N. Monier, J.A. Hatzfeld
human CD34+ cells, fibrinogen, competence factor, IL-6 receptor.

Balance between autocrine interleukin-1b and caspases defines life versus death of polymorphonuclear cells.

P. Bossù, E. del Grosso, M.P. Cesaroni, G. Maurizi, N. Balestro, A. Stoppacciaro, E. del Giudice, P. Ruggiero, D. Boraschi
apoptosis, interleukin-1, caspases, polymorphonuclear leukocytes, ICE.

Rat interleukin-10: production and characterisation of biologically active protein in a recombinant bacterial expression system.

V. Ball, S. Vigues, C.K. Gee, S. Poole, A.F. Bristow
IL-10, recombinant, rat, cytokines.

Les éditions JLE

30 rue Berthollet, Bât. A,
94110 Arcueil, France

+ 33 (0)1 46 73 06 60

contact@jle.com

Publishing Policy

Books and e-books

Want to publish ?

Become a partner