

Volume 12, issue 3, September 2001 | Articles originaux

Quantitation of chemokines (MDC, TARC) expression in mucosa from Crohn's disease and ulcerative colitis

Abstract

Chemokines and their receptors are involved in the migration of different mononuclear cells. Among them macrophages-derived chemokines (MDC) and thymus-and activation regulated chemokine (TARC) belong to a new cluster of genes involve in Th2 lymphocytes homing. Cytokines appear to play a significant role in pathogenesis of inflammatory bowel diseases with an excessive Th1 response in chronic lesions of Crohn's disease (CD) and a Th2 pattern in both earlier mucosal CD lesions and in mucosa of ulcerative colitis (UC). Here we

demonstrate that RNAm coding for MDC and TARC are expressed in mucosa from CD and UC patients. Using real-time fluorescent RT-PCR, MDC and TARC mRNA were increased in CD inflamed mucosa. Moreover MDC and TARC transcripts were increased in inflamed CD specimen compared to non-involved CD mucosa. These differences both discriminate CD from UC patients. Additionally, MDC protein was produced in isolated mononuclear cells from peripheral blood (PBMC) or mucosa (LPMC) from UC and CD patients: spontaneously, MDC production from PBMC was increased in CD compared to UC patients. MDC production from CD PBMC was also higher than that found in healthy controls. Together, these data indicate that MDC should be involved in the lymphocytes homing in mucosa from CD patients.

Full text

INTRODUCTION

The pathogenesis of human inflammatory bowel disease (IBD), Crohn's disease (CD), and ulcerative colitis (UC) remains poorly understood. The development of these diseases may be a result of uncontrolled or inadequately down-regulated cellular immune responses in the intestinal mucosa to a hitherto unknown agent, probably a constituent of the luminal contents. This leads to mucosal injury, breakdown of the epithelial barrier function, and increased influx of luminal contents to the lamina propria, which might further exaggerate the uncontrolled immune response. Many reports strongly suggest that enhanced T cell activation is a key component in the pathogenesis of CD [1]. Compared with control cells a significantly higher number of peripheral CD T cells display early activation antigens (Ags), produce increased amounts of soluble IL-2R, and proliferate more in response to microbial Ags. However, lamina propria T cells extracted from inflammatory lesions of patients with either UC or CD show reduced T cell receptor/CD3 pathway-induced proliferation, even in comparison to the already reduced proliferation of control lamina propria T cells compared to peripheral blood T cells, whereas CD2 pathway-induced proliferation is intact (CD) or decreased to only a minor degree (UC) [2, 3]. In addition, purified $CD4^+$ lamina propria T cells from patients with CD stimulated *via* the CD2 pathway produce an increased amount of interferon gamma (IFN-gamma), whereas the same cells from patients with UC stimulated either *via* the CD3 pathway or the CD2 pathway produce an increased amount of IL-5 [4]. These data strongly suggest that lamina propria T cells not only manifest an abnormal activation

state with regard to proliferation but, in addition, appear to be more differentiated T cells with respect to Th1 cytokines production in CD [5]. Moreover increased apoptosis of T cells in controls or UC compared to CD patients is associated with a generally altered activation state characterized by proliferative hyporesponsiveness to T cell receptor/CD3 pathway stimulation and relative intact responsiveness to CD2 pathway stimulation [6]. These results suggest that lamina propria T cells are in a partially anergic state in controls that places severn,al kinds of limitations on the capacity of such cells to undergo clonal expansion, and reflect a break-down in an analogous state of tolerance in CD patients. Among T lymphocytes, $CD4^+$ T cells seem to play an important role in the pathogenesis of IBD since in IBD patients, an expansion of the $CD4^+$ T cell pool is observed in both the mucosa and peripheral blood. Moreover despite the proportion of activated cells is increased, circulating numbers of gut homing lymphocytes characterized by $alpha_4beta_7$ integrin are decreased in colonic mucosal of IBD patients [7]. This may arise due to a dilution effect from recruited naive T cells, or from integrin down regulation. These data suggest that the altered lymphocyte subsets reported in IBD, mask variations specific to gut homing populations.

The control of T cell migration depends on the combined actions of various adhesion molecules and a vast array of chemotactic factors (chemokines) [8, 9]. The role of adhesion molecules in T cell migration is well characterized, but that of chemokines and their receptors at mucosal level is still unclear. The chemokine receptors comprise two major groups; the CC chemokine receptors 1-10 (CCR1-10) that bind CC chemokines and the CXC chemokine receptors 1-4 (CXCR1-4) that bind CXC chemokines. In general, the CC chemokines and their receptors are involved in the migration of monocytes, eosinophils, basophils and T cells, while CXCR1 and CXCR2 are involved in the migration of neutrophils [8-10]. However recent reports have indicated that chemokines produced at the sites of inflammation are likely to play a major role in the recruitment of particular cell types that infiltrate pathological lesions [11-15]: previous data have showed that trafficking of Th1 and Th2 lymphocytes is differentially controlled through the selective expression of adhesion molecules and chemokine receptors [11, 13, 16].

Although these findings suggest that different chemotactic signals are required for extravasation, migration, and tissue homing, the hierarchy and composition of these signals are unknown. Among ten CC and five CXC chemokine receptors

Th1 cells predominantly express CXCR3 and CCR5; Th2 cells selectively express CCR3, CCR4, and CCR8 [15, 16]. Two CC chemokines macrophage-derived chemokine (MDC) [17] and thymus- and activation-regulated chemokine (TARC) [18], which act especially on chronically activated Th2 lymphocytes may play a role in Th2 lymphocyte trafficking or, alternatively, in the development and effector action of Th2 lymphocytes.

As the control of homeostatic circulation of mononuclear cells in the gastrointestinal mucosa is a multistep process, and as chemokines are selectively involved in tissue infiltration by activated lymphocytes and macrophages, MDC and TARC chemokines level of transcription was assessed by a real-time quantitative polymerase chain reaction (PCR) in order to compare inflamed and non-inflamed tissue from patients with CD or UC. The purpose of this study was also to compare chemokine release of freshly isolated lamina propria mononuclear cells (LPMC) and peripheral blood mononuclear cells (PBMC) from CD and UC patients before and after stimulation by antibody (Abs) directed against CD3 or CD2 and CD28 receptors.

MATERIAL AND METHODS

Patients and samples

Twenty-two patients with CD (n = 22: mean age 35 years; range (9-78)) and nine patients with UC (n = 9: mean age 44 years; range (23-53)) were studied: they were compared to nine control subjects and four non specific or acute self limited colitis patients (ASLC) (2 diverticulitis, 2 infectious colitis). Controls undergone endoscopy for irritable bowel syndrome or colon carcinoma: biopsy specimen were sampled on mucosa without lesions where colonic or ileal mucosa was histologically normal. In ASLC patients biopsy specimens were taken in abnormal areas of mucosal inflammation associated to non specific and diffuse polymorphonuclear leucocytes infiltration without architectural or cryptic abnormalities. Among 22 CD and 9 UC patients, respectively 18 and 5 patients were sampled both in inflamed and non inflamed colonic mucosa for paired comparison. As CD could affected small or large bowell, 8 and 5 impaired specimen were respectively sampled from affected and non affected ileum either during endoscopy (n = 5 and 4 respectively) or surgery (n = 3 and 1 respectively). Endoscopic biopsy specimens of patients or controls were placed immediatly in liquid nitrogen and stored at - 80° C until assayed. All biopsy

specimens were performed with a same kind of endoscopic forceps brad a similar size and weight. PBMC were isolated from 7 CD and 9 UC patients to be compared to 6 healthy controls. LPMC were isolated from surgical specimen from 3 CD and 4 UC patients. No patients or controls with history of atopia or allergy were included.

Real-time quantitative PCR procedure [19-21]

Real time quantitative PCRs were performed by fluorescent TaqMan methodology using the ABI Prism 7700 (Perkin Elmer, Foster city, CA, USA) which allows sample and rapid quantification of a target sequence during the exponential phase of PCR amplifications. The Taq Man reaction is based on the 5' nuclease activity of Taq polymerase to cleave a specific dual labeled fluorogeneic hybridization probe during the extension phase of PCR. One fluorescent dye serves as a reporter [FAM (6-carboxyfluorescei ne)] and its emission spectrum is quenched by a second fluorescent dye [TAMRA (6 carboxy-tetramethul-rhodamine)].

During the extension phase of the PCR cycle, the exonuclease activity of the DNA polymerase cleaves the probe and releases the reporter dye, resulting in an increase in the fluorescence signal. Real time quantitative PCRs were performed using cDNA [1 mug of mRNA transformed with superscriptII (Boerhinger)] mixed with 20 mulMaster Mix 2X Buffer (Perkin Elmer), 600 nM of each primer, 200 nM of the labelled probe in a total volume of 40 mul. A first step of 10 min at 95° C was followed by 40 cycles of amplification (95° C for 15 sec and 60° C for 60 sec). For each sample, the ABI Prism 7700 software provided an amplification curve constructed by relating the fluorescence signal intensity (delta Rn) to the cycle number. The delta Rn value corresponded to the variation in the reporter fluorescence intensity before and after PCR, normalized to the fluorescence of an internal passive reference.

Cycle threshold (Ct) was defined as the cycle number at which a significant increase in the fluorescence signal was first detected (the higher the starting copy number, the lower the Ct). Using serial dilutions of a positive control cDNA (PBMC activated by PHA-IL2) a standard curve was generated on the basis of relationship between the Ct value and the logarithm of the starting copy number. The PCR efficiency was deduced from the standard curve by the software and expressed as $E = 10^{1/S} - 1$, where S = the curve slope. The relative

quantification of MDC and TARC gene expression was then calculated after normalization of the total amount of cDNA tested by an active reference. The active reference, defined as a constant RNA sequence, used in our assay was 18 S (PE - Biosystem). Using the comparative Ct method, the amount of target sequence in unknown samples (us), normalized to the 18 S endogenous reference, was expressed relative to a calibrator (cb = normal mucosal tissue samples of control subjects in our assay). This relative amount (R) is given by the formula: $R = (1 + E)^{-\text{deltadelta}^{Ct}}$ where deltadelta^{Ct} = deltaCt(us) - deltaCt(cb) [deltaCt (us) = Ct (target sequence) - Ct (active reference) in the unknown sample and deltaCt(cb) = Ct (target sequence) - Ct (active reference) in the calibrator].

PCR amplification

All PCR reactions were performed in duplicate for each data point as described above. Primers and probes for target genes (MDC, TARC) were choosen with the assistance of the software Primers Express (Perkin-Elmer Applied Biosystems, Foster City, CA). Endogenous reference gene was quantified by using the 18S RNA kit form PE Biosystem. Following primer sequences were used for amplification of MDC and TARC: sens 5' TGTGGCGCTTCAAGCAACT 3' - antisens 5' AAGTGTTTCACCACGCGCA 3' for MDC and sens 5' TTCTCTGCAGCACATCCACG 3' - antisens 5' TCCCTTGAAGTACTCCAGGCA 3' for TARC. Probes for MDC and TARC were respectively 5' CCCCTACGGCGCCAACATGGA 3' and 5' TCGAGGGACCAATGTGGGCCG 3'.

RNA extractions

Total RNA was extracted from mucosa biopsies by using SV total RNA isolation kit (Promega Corp, Madison WI). The quality of the RNA samples was determined by visualization under UV light the 18S and 28S RNA bands after electrophoresis through agarose gels (1%) and staining with ethidium bromide.

cDNA synthesis

Reverse transcription of RNA was done in a final volume of 20 mul containing 2 mug of total RNA. The reverse transcription used Hexaprimers and Superscript II kit (Gibco BRL, Gaithersburg, Mannheim, Germany) according to the manufacter's instructions.

Lamina propria mononuclear cell (LPMC) and peripheral mononuclear cells (PBMC) isolation [22]

LPMC were isolated from freshly resected mucosa using a previously described dithiothreitol-EDTA-collagenase method. Briefly, the intestinal specimen was washed in Hank's balanced salt solution (HBSS) (Life Technologies). The mucosa was dissected away from the underlying layers and minced in small pieces (approximately 3 x 3 mm). They were then freed of mucus by incubation with 2 mM dithiothreitol (Sigma) in HBSS and additives (250 mg/ml gentamycin, 100 U/ml penicillin, 10 mug/ml streptomycin, 2 mM glutamin and 10 mM hepes) (Life Technologies) for 15 min at 37° C. The pieces of mucosa were then incubated three times in HBSS free of calcium and magnesium (HBSS-CMF) (Life Technologies) containing 1 mM EDTA for 45 min at 37° C. After washes in HBSS until the remaining epithelial cells were removed, the mucosa was digested for 16 hours in Dulbeco's modified Eagle's medium (DMEM) (Life Technologies) and additives containing 0.1 mg/ml collagenase D (Roche Diagnostics), 200 U/ml Dnase I (Sigma), and 0.1 mg/ml trypsin inhibitor (Sigma) at 37° C in a shake incubator. After incubation, the cells were collected, washed twice in complete HBSS and then subjected to centrifugation at 960 g for 30 min through a 30-40-60-100% percoll gradient (Sigma). The cells collected at the 40-60% and 30-40% interfaces were washed twice, counted and checked for viability using 0.1% trypan blue. PBMC were isolated by Ficoll-Hypaque density gradient centrifugation (900 g, 20 min) from heparinised blood samples. Cells were then frozen until activation experiments.

Activation experiments

LPMC and PBMC were activated for 24 hours or 48 hours in 24 wells or 96 wells plates at a concentration of 1.10^6 cells/ml in RPMI 1640 supplemented with 10% fetal calf serum (Sigma), 1% L-glutamine, 100 U/ml penicillin, and 100 mug/ml streptomycin (Life Technologies). Cells were cultured with the indicated combinations of the following antibodies and mitogens: anti-CD2 antibody pair $T11_{2,3}$ [23] used at a dilution of 1:500 each, anti-CD3 (clone X35) [24] coated at the concentration of 1mug/ml for 2 hours at 37° C, anti-CD28 (clone CD28.2) at 1 mug/ml and PHA (Sigma) at 5 mug/ml. Supernatants for both activated and non activated cells were harvested and stored at - 20° C.

Detection of cytokine and chemokine secretion

TARC and MDC secreted in the culture supernanants were measured in duplicate by performing specific ELISA. Each recombinant cytokine was used as a standard. The TARC secretion was determined by using ELISA kit from R&D according to the manufacturer's instructions. Briefly 96 well assay plates were coated overnight with 100 mul of 1 mug/ml monoclonal anti-TARC. Samples and standard were added for 24 hours followed by 100 mul of 150 ng/ml biotinylated antibody for 2 hours. Avidin-horseradish peroxydase (HRP) solution was added for 30 min followed by addition of substrate ABTS for 30 min. Plates were read with a Biorad microplate reader at 405 nm. Results under 100 pg/ml and 40 pg/ml should be considered as negative for MDC and TARC respectively. MDC levels were determined in culture supernatants by ELISA as described previously [22].

RESULTS

Levels of chemokine transcripts in active lesions of patients with IBD

The amount of MDC and TARC transcripts were determined by real time quantitative PCR in active Crohn's disease (n = 22), ulcerative colitis (n = 9) and acute self-limited colitis (ASLC) lesions (n = 4). All these specimens were sampled in colonic inflamed sites and compared to the specimens in normal colon mucosa from control group (n = 9). Results are expressed as n folds the mean level observed in controls (Table 1) which is considered as the calibrator. A significant increase in MDC ($p < 0.01$) and TARC ($p < 0.001$) RNA transcripts was observed in CD patients (Table 1). Despite a trend for increase amount of MDC and TARC transcript in CD mucosa compared to UC mucosa, these differences were not significant ($p < 0.07$). An increased level of MDC transcript in inflamed colonic mucosa of CD patients was also found in affected CD ileum (7.52 ± 2.66 fold the level in normal colon mucosa from control group). For CD ileitis, when results were expressed as n folds the mean level observed in ileum non inflamed mucosa, no significant difference was found (Table 1). In UC patients transcripts were both present but not significantly different from controls. Finally, only TARC transcripts were increased in ASLC compared to controls ($p < 0.01$).

Level of chemokine transcripts at the site of inflamed and non inflamed mucosa

To evaluate whether the increase in MDC and TARC mRNA occured only in the affected mucosa the amounts of these transcripts were examined in both the

involved and non involved colonic mucosa of the same patient with either segmental colonic CD involvment (n = 18) or left-sided ulcerative colitis (n = 5). Compared to healthy mucosa, the level of MDC and TARC mRNA (Figure 1) was increased in affected paired mucosa only in CD patients ($p < 0.04$ and $p < 0.005$ respectively). A high variability in the amounts of TARC and MDC levels was observed in the patient series. So, analysis was performed according to the treatment. Comparison of patients treated (n = 4) or not (n = 14) by oral steroids shows a decreased of MDC transcripts in patients on steroids either in affected or healthy mucosa (mean level expressed as n fold the calibrator, respectively 2.7 *vs* 14.6 in affected and 0.6 *vs* 4.5 in healthy mucosa). However amount of MDC transcripts remains higher in inflamed compare to non inflamed mucosa when comparisons according to the treatment were done. Despite a trend for increase amount of MDC and TARC transcripts in affected compared to non affected small bowel of CD, these differences were not significant (Table 1): however these samples were paired in one CD patients and their values were respectively equals to 3.20 and 0.21 for MDC mRNA or 1.62 and 0.85 for TARC transcript. Finally, in CD patients (n = 18) MDC and TARC transcripts were above the upper limit of the CI 95% from the control group in 80 and 44% for respectively inflamed and non inflamed mucosa.

Considering UC patients, no difference in the transcripts level was observed between affected and non affected paired samples. Among 5 UC patients only one received oral steroid treatment; in this case MDC transcript expressed as n fold the calibrator was 0.52 *vs* 0.69 in healthy and inflamed mucosa, respectively compared to 2.05 *vs* 2.26 in four UC patients without steroid treatment.

In vitro *chemokines production by PBMC and LPMC after CD2 or CD3 stimulations*

MDC and TARC production in cell culture supernatant were analysed at the PBMC and LPMC levels. There was no difference in TARC production between CD, UC or control PBMC before and after stimulation (data not shown). By contrast PBMC from CD patients produced more frequently before any stimulation significant levels of MDC (5 out of 7 CD patients, 3 out of 9 UC patients and 2 out of 6 controls) (Figure 2). All the experiments in CD, UC patients and controls, whatever MDC or TARC production (data not shown), were associated to MDC and/or TARC production after PHA (5 mug/ml) stimulation showing the ability of cells to produce both chemokines. Oral or

topic steroid therapy did not influence spontaneous MDC production in CD patients (2/3 treated patients *versus* 3/4 untreated patients produced significant level of MDC; in UC patients 1/6 treated patient *versus* 2/3 untreated patients were MDC producers before any stimulation). Moreover the level of MDC production was higher in CD than in UC and controls. It was worthnoting that in one CD patient the level of MDC spontaneous production was upper to 10,000 pg/ml despite steroid treatment: other CD patients produced MDC amounts in a range from 325 to 736 pg/ml compared to 176-384 and 100-150 pg/ml in UC and controls respectively. After stimulation, the percentage of MDC producing subjects was similar in CD, UC patients and controls (extreme values: respectively 120-877, 100-424 and 138-1,000 pg/ml after stimulation by $CD2^+CD28$ and 173-2,000, 144-2,500 and 100-1,440 pg/ml after stimulation by $CD3^+CD28$).

At the LPMC level (Figure 3) neither CD nor UC produced significant level of MDC before stimulation. In CD patients (n = 3), a significant increased production was observed in two patients after $CD3^+CD28$ stimulation and in one patient after $CD2^+CD28$ stimulation whereas the same stimulations enhanced MDC production in only one of 4 UC patients (275 and 734 pg/ml) (Figure 3A). Considering TARC production by LPMC, no difference was observed before and after stimulation with very low levels of chemokine production (Figure 3B).

DISCUSSION

Although the inflammatory response within the intestinal mucosa is complex, cytokines appear to play a significant role in pathogenesis of IBD and some patterns are emerging. In CD, several data suggest that an excessive Th1 response occurs which lead to increased amounts of proinflammatory cytokines as IL-1, IL-6 or TNF-alpha and excessive production of IL-12 and IFN-gamma [25]. However, in contrast with chronic and older lesions, a Th2 pattern with an increased expression of IL-4 and IL-5 was observed in the early ileal lesions of patients with CD taken 3 months after curative ileal resection [26]. Conversely to CD patients, the cytokine profile is consistent with a Th2 T cell response in UC. Whether chemokines are involved in a differential recruitment of key effector subsets of T cells such as Th1 and Th2 is largely debated *in vitro* or in animal models [8, 11, 27-29].

In the present study we analyzed at the RNA and protein levels the production of two chemokines MDC and TARC potentially involved in IBD pathogenesis. These chemokines were chosen in regard to their functional properties and for the location of their encoding genes in a cluster of genes located on the 16 chromosome which was pointed out as a significant contributor to IBD genetics. In a first step a quantitative approach of both chemokines transcription was conducted from a biological material of CD and UC biopsy specimens. The most relevant results were observed in CD patients for which MDC and TARC transcript levels in inflamed sites were higher than those characterized in healthy colonic mucosa from the control group. Such a difference for MDC was not observed neither in UC patients nor in ASLC patients. However, the question remained as to know wether this abnormal chemokine transcription in CD patients was restricted to affected sites. To address this issue, we compared the level of chemokine transcripts in inflamed and non inflamed paired samples of CD patients. A significant difference was observed with a 3 fold increase in inflamed tissue. Such a difference was not observed in UC specimen. In a second step MDC production by PBMC and LPMC from CD, UC and control subjects was measured at the protein level in supernatant of cultured cells. Whereas no or low levels of MDC production were found at the basal state in UC and controls PBMC cultures, CD PBMC clearly produce MDC at a significant level without any stimulation. Considering LPMC, MDC production after stimulation was more frequently found in CD than in UC. TARC analysis did not show any differences between CD, UC and controls at the protein level with no or low levels of chemokine production. This apparent discrepancy with the data observed at the RNA level in CD biopsy specimen may be explained either by an *in situ* activation pathway different from CD2 or CD3 or more likely by a delay in TARC synthesis compared to MDC. Moreover as TARC transcripts were also increased in ASLC, it appears as a secondary and non specific inflammatory event.

MDC is a novel beta-chemokine first reported by Godiska *et al.* [17] but that has also been characterized as stimulated T cell chemotactic protein (STCP)-1 [13]. While macrophages produce MDC constitutively [17, 26], NK cells, monocytes, and CD4 lymphocytes produce MDC upon appropriate stimulation [13]. Interestingly, increased expression of the genes encoding chemokines, such as TARC or MDC, that selectively chemoattract CCR4 Th2-type lymphocytes, were also observed in monocyte-derived DCs [13, 30, 31]. Whereas MDC mRNA is clearly detectable in resting macrophages, and its levels is further increased on activation of these cells, the MDC gene appears to be completely silent in

monocytes [30-32]. Even when monocytes are activated either by adherence to plastic or by LPS, no MDC mRNA is detectable, whereas the expression of other acute genes is strongly induced. By contrast activation of these cells by TNF-alpha and IL-1beta leads to a further enhancement of MDC mRNA levels [33]. Since we observed a high level of MDC production in peripheral blood cells of CD patients and based on the observation that monocytes generally do not produced MDC we assumed that lymphocytes or DCs are the major source of MDC among CD PBMC. In this regard a high level of MDC mRNA following stimulation of T cells lines by anti-CD3 has been reported [15]. MDC production, by activated T cells, without being a selective property of Th2 cells, is preferentially associated with the production of Th2 rather than Th1 cytokines both *in vitro* and *in vivo*. Naive (RA^+) $CD4^+$ T cells polarized toward the Th2 pattern of cytokine production produced higher MDC amounts than naive $CD4^+$ T cells polarized toward the Th1 profile [14]. Interestingly, MDC production by T cell clones positively correlated with the production of IL-4, IL-5, and IL-6, but not with the production of IL-10 [14]. By contrast, there was an inverse correlation between the production of MDC and the production of IFN-gamma by these clones, thus supporting the concept that IL-4 and IFN-gamma play an opposite regulatory role on MDC production [13]. However after $CD3^+$CD28 stimulation secretion of MDC and TARC appears to be independent of endogenous production of IL-4 and IFN-gamma either in Th1 and Th2 cells [34].

These last finding rise the possibility that in CD, T lymphocytes engaged toward a Th1 pattern may also produce significant levels of MDC upon stimulation. The high level of MDC transcripts in CD lesions not found in UC, may result either from a local recruitment of MDC producing cells and/or from a *in situ* mucosal activation. In this regard, it is well established that lamina propria T cells of CD patients are preferentially stimulated *via* CD2 pathway [2-4] and we showed that CD2 as well as CD3 activation of CD LPMC enhanced MDC production. Nevertheless, the question remains as to know the biological significance of MDC overexpression in CD pathogenesis. MDC has been described as a chemoattractant for monocytes, monocyte-derived dendritic cells, IL-2-activated NK cells, and chronically activated T cells [13, 17, 35-38]. Phenotypic analysis of MDC-responsive cells shows that they are enriched for Th2 lymphocytes [39] or a subset of double-positive cells that express high levels of CD3 and CD4 and that have reduced levels of CD8 [40]. So far, only CCR4 has been identified as a functional receptor for MDC. In addition, the closest related CC chemokine, TARC [18, 41, 42] is also a specific ligand for CCR4 [34]. CCR4 the

receptor for TARC and MDC is expressed on ~ 20% of adult peripheral blood effector/memory T cells [40, 43]. In the inflamed mucosa of IBD, $CD4^+$ T cells and macrophages are the most abondant cells but the number of gut homing ($alpha_4beta_7$) lymphocytes is decreased in IBD [7]. These intestinal ($alpha_4beta_7$) memory and naive T cells respond poorly to MDC or TARC [44]. To determine which chemokine receptors are involved in T lymphocytes localization to the intestinal mucosa, their expression on human intestinal lamina propria (LPL) and intraepithelial (IEL) lymphocytes was assessed. All LPL and IEL expressed CXCR3 and CCR5 receptors while CCR3, CCR4, CCR7, CXCR1 and CXCR2 were not expresed. So LPL from normal or non-inflamed mucosa did not migrate toward the CCR4 ligand [27]. In contrast, Th2 cells preferentially express CCR4 [11] and to a lesser extent, CCR3: this is in agreement with data showing that CCR4 T cells attracted by MDC and TARC selectively gave rise to Th2 type cell lines producting IL-4 and IL-5 [40]. Moreover, the expression of CCR4 and CCR8 is selectively increased on Th2 cells following TCR and CD28 engagement without IL-4 requirement [41].

It is possible that posttranslational modification of either the C-terminus or glycosylation of the MDC may affect its activity on lymphocytes or monocytes. Although no HIV-1 strains have been described that can enter $CD4^+$ cells using CCR4 as coreceptor, a truncated form of MDC missing two NH2-terminal residues, *i.e.* MDC (3-69), was found to inhibit HIV-1 infection [45]. This truncated MDC (3-69) variant has reduced chemotactic potency and increased HIV-1 inhibitory activity compared with intact MDC (1-69). So cells from CD mucosa attrated by MDC need to be further characterized. Finally beside its pivotal role in the primarly response for the long-term recruitment of antigen-specific Th2 cells to target organs, during chronic inflammatory responses with repeated exposure to allergen, which could be of interest in IBD, novel regulatory activities of MDC in innate immunity during sepsis was recently described [46, 47]. Therefore MDC production in CD mucosa need to be explored at earlier stages of CD and it relationship to pro-inflammatory cytokines production specificely determined.

CONCLUSION

To conclude, in the present study we examined at the RNA and protein levels, the possible involvement of MDC and TARC chemokines in IBD pathogenesis. To our knowledge, so far no data were available on the subject. The data reported

strongly suggest a role of MDC which may discriminate CD from UC. Nevertheless, several questions are still opened and remain to be further clarified. Firstly, both MDC producing cells in CD lesions, and effector cells specifically attracted in inflamed mucosa need to be precisely characterized. Secondly, the mechanisms by which MDC production is enhanced in CD are still unclear. Finally, our data by pointing out in CD pathogenesis, a protein encoded on a chromosomic region considered at risk for IBD [48] bring new insight to CD genetics which disserved to be further analyzed at the genomic level.

Acknowledgements. * These authors contributed equally to this work.

This work was supported by grants from Solvay-Pharma fondation d'entreprise (Paris) and the Critt GBM Bretagne (Rennes).

References

1. Powrie F. 1995. T cells in inflammatory bowel disease: protective and pathogenic roles. (Review) *Immunity* 3: 171.

2. Targan S R, Deem R L, Liu M, Wang S, Nel A. 1995. Definition of a lamina propria T cell responsive state. Enhanced cytokine responsiveness of T cells stimulated through the CD2 pathway. *J. Immunol.* 154: 664.

3. Boirivant M, Marini M, Di Felice G, Pronio A M, Montesani C, Tersigni R, Strober W. 1999. Lamina propria T cells in Crohn's disease and other gastrointestinal inflammation show defective CD2 pathway-induced apoptosis. *Gastroenterology* 116: 557.

4. Fuss I J, Neurath M, Boirivant M, Klein J S, de la Motte C, Strong S A, Fiocchi C, Strober W. 1996. Disparate $CD4^+$ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP cells manifest increased secretion of IFN-gamma whereas ulcerative colitis LP cells manifest increased secretion of IL-5. *J. Immunol.* 157: 1261.

5. Plevy S E, Landers C J, Prehn J, Carramanzana N M, Deem R L, Shealy D, Targan S R. 1997. A role for TNF-alpha and mucosal T helper-1 cytokines in the pathogenesis of Crohn's disease. *J. Immunol.* 159: 6276.

6. Ina K, Itoh J, Fukushima K, Kusugami K, Yamaguchi T, Kyokane K, Imada A, Binion D G, Musso A, West G A, Dobrea G M, McCormick T S, Lapetina E G, Levine A D, Ottaway C A, Fiocchi C. 1999. Resistance of Crohn's disease T cells to multiple apoptotic signals is associated with a Bcl-2/Bax mucosal imbalance. *J. Immunol.* 163: 1081.

7. Meenan J, Spaans J, Grool T A, Pals S T, Tytgat G N, van Deventer S J. 1997. Altered expression of alpha$_4$beta$_7$, a gut homing integrin, by circulating and mucosal T cells in colonic mucosal inflammation. *Gut* 40: 241.

8. Zlotnik A, Morales J, Hedrick J A. 1999. Recent advances in chemokines and chemokine receptors. (Review) *Crit. Rev. Immunol.* 19: 1.

9. Luster A D. 1998. Chemokines. Chemotactic cytokines that mediate inflammation. *N. Engl. J. Med.* 338: 436.

10. Sallusto F, Kremmer E, Palermo B, Hoy A, Ponath P, Qin S, Förster R, Lipp M, Lanzavecchia A. 1999. Switch in chemokine receptor expression upon TCR stimulation reveals novel homing potential for recently activated T cells. *Eur. J. Immunol.* 29: 2037.

11. Bonecchi R, Bianchi G, Bordignon P P, d'Ambrosio D, Lang R, Borsatti A, Sozzani S, Allavena P, Gray P A, Mantovani A, Sinigaglia F. 1998. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J. Exp. Med.* 187: 129.

12. Bernardini G, Hedrick J, Sozzani S, Luini W, Spinetti G, Weiss M, Menon S, Zlotnik A, Mantovani A, Santoni A, Napolitano M. 1998. Identification of the CC chemokines TARC and macrophage inflammatory protein-1beta as novel functional ligands for the CCR8 receptor. *Eur. J. Immunol.* 28: 582.

13. Andrew D P, Chang M S, McNinch J, Wathen S T, Rihanek M, Tseng J, Spellberg J P, Elias C G. 1998. STCP-1 (MDC) CC chemokine acts specifically on chronically activated Th2 lymphocytes and is produced by monocytes on stimulation with Th2 cytokines IL-4 and IL-13. *J. Immunol.* 161: 5027.

14. Galli G, Chantry D, Annunziato F, Romagnani P, Cosmi L, Lazzeri E, Manetti R, Maggi E, Gray P W, Romagnani S. 2000. Macrophage-derived chemokine

production by activated human T cells *in vitro* and *in vivo*: preferential association with the production of type 2 cytokines. *Eur. J. Immunol.* 30: 204.

15. Sallusto F, Schaerli P, Loetscher P, Schaniel C, Lenig D, Mackay C R, Qin S, Lanzavecchia A. 1998. Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. *Eur. J. Immunol.* 28: 2760.

16. Sallusto F, Lenig D, Mackay C R, Lanzavecchia A. 1998. Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. *J. Exp. Med.* 187: 875.

17. Godiska R, Chantry D, Raport C J, Sozzani S, Allavena P, Leviten D, Mantovani A, Gray P W. 1997. Human macrophage-derived chemokine (MDC), a novel chemoattractant for monocytes, monocyte-derived dendritic cells, and natural killer cells. *J. Exp. Med.* 185: 1595.

18. Imai T, Yoshida T, Baba M, Nishimura M, Kakizaki M, Yoshie O. 1996. Molecular cloning of a novel T cell-directed CC chemokine expressed in thymus by signal sequence trap using Epstein-Barr virus vector. *J. Biol. Chem.* 271: 21514.

19. Heid C A, Stevens J, Livak K J, Williams P M. 1996. Real time quantitative PCR. *Genome Res.* 6: 986.

20. Gibson U E, Heid C A, Williams P M. 1996. A novel method for real time quantitative RT-PCR. *Genome Res.* 6: 995.

21. 1997 . Relative quantitation of gene expression. User Bulletin # 2 ABI PRISM 7700 Sequence Detection System.

22. Bull D M, Bookman M A. 1977. Isolation and functional characterization of human intestinal mucosal lymphoid cells. *J. Clin. Invest.* 59: 966.

23. McMichael A J, Gotch F M. 1987. T cell antigens: new and previously defined clusters. In: McMichael A J, eds. *Leucocytes typing III.* Oxford University Press, Oxford, 34.

24. Bourel D, Genetet N, Merdrignac G, Genetet B. 1987. Anti-CD3 monoclonal antibodies. Characterization and function. *Pathol. Biol.* (Paris), 35: 1285.

25. McClane S J, Rombeau J L. 1999. Cytokines and inflammatory bowel disease: a review. *J. Parenter Eriteral Nutr.* 23: S20.

26. 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.

27. Agace W W, Roberts A I, Wu L, Greineder C, Ebert E C, Parker C M. 2000. Human intestinal lamina propria and intraepithelial lymphocytes express receptors specific for chemokines induced by inflammation. *Eur. J. Immunol.* 30: 819.

28. Vecchi A, Massimiliano L, Ramponi S, Luini W, Bernasconi S, Bonecchi R, Allavena P, Parmentier M, Mantovani A, Sozzani S. 1999. Differential responsiveness to constitutive *vs* Inducible chemokines of immature and mature mouse dendritic cells. *J. Leukoc. Biol.* 66: 489.

29. Tang H L, Cyster J G. 1999. Chemokine up-regulation and activated T cell attraction by maturing dendritic cells. *Science* 284: 819.

30. Hashimoto S, Suzuki T, Dong H Y, Yamazaki N, Matsushima K. 1999. Serial analysis of gene expression in human monocytes and macrophages. *Blood* 94: 837.

31. Hashimoto S, Suzuki T, Dong H Y, Nagai S, Yamazaki N, Matsushima K. 1999. Serial analysis of gene expression in human monocyte-derived dendritic cells. *Blood* 94: 845.

32. Schaniel C, Sallusto F, Ruedl C, Sideras P, Melchers F, Rolink A G. 1999. Three chemokines with potential functions in T lymphocyte-independent and dependent B lymphocyte stimulation. *Eur. J. Immunol.* 29: 2934.

33. Rodenburg R J, Brinkhuis R F, Peek R, Westphal J R, Van Den Hoogen F H, van Venrooij W J, van de Putte L B. 1998. Expression of macrophage-derived

chemokine (MDC) mRNA in macrophages is enhanced by interleukin-1beta, tumor necrosis factor, and lipopolysaccharide. *J. Leukoc. Biol.* 63: 606.

34. Iellem A, Colantonio L, Bhakta S, Sozzani S, Mantovani A, Sinigaglia F, d'Ambrosio D. 2000. Inhibition by IL-12 and IFN-gamma of I-309 and macrophage-derived chemokine production upon TCR triggering of human Th1 cells. *Eur. J. Immunol.* 30: 1030.

35. Lloyd C M, Delaney T, Nguyen T, Tian J, Martinez A C, Coyle A J, Gutierrez-Ramos J C. 2000. CC chemokine receptor (CCR)3/eotaxin is followed by CCR4/monocyte-derived chemokine in mediating pulmonary T helper lymphocyte type 2 recruitment after serial antigen challenge *in vivo*. *J. Exp. Med.* 191: 265.

36. Lieberam I, Förster I. 1999. The murine beta-chemokine TARC is expressed by subsets of dendritic cells and attracts primed $CD4^+$ T cells. *Eur. J. Immunol.* 29: 2684.

37. Bonecchi R, Polentarutti N, Luini W, Borsatti A, Bernasconi S, Locati M, Power C, Proudfoot A, Wells T N, Mackay C, Mantovani A, Sozzani S. 1999. Up-regulation of CCR1 and CCR3 and induction of chemotaxis to CC chemokines by IFN-gamma in human neutrophils. *J. Immunol.* 162: 474.

38. Bonnecchi R, Sozzani S, Stine J T, Luini W, d'Amico G, Allavena P, Chantry D, Mantovani A. 1998. Divergent effects of interleukin-4 and interferon-gamma on macrophage-derived chemokine production: an amplification circuit of polarized T helper 2 responses. *Blood* 92: 2668.

39. Gonzalo J A, Pan Y, Lloyd C M, Jia G Q, Yu G, Dussault B, Powers C A, Proudfoot A E, Coyle A J, Gearing D, Gutierrez- Ramos J C. 1999. Mouse monocyte-derived chemokine is involved in airway hyperactivity and lung inflammation. *J. Immunol.* 163: 403.

40. Chantry D, Romagnani P, Raport C J, Wood C L, Epp A, Romagnani S, Gray P W. 1999. Macrophage-derived chemokine is localized to thymic medullary epithelial cells and is a chemoattractant for $CD3^+$, $CD4^+$, $CD8^{low}$ thymocytes. *Blood* 94: 1890.

41. Imai T, Baba M, Nishimura M, Kakizaki M, Takagi S, Ioshie O. 1997. The T cell-directed CC chemokine TARC is a highly specific biological ligand for CC chemokine receptor 4. *J. Biol. Chem.* 272: 15036.

42. Imai T, Nagira M, Takagi S, Kakizaki M, Nishimura M, Wang J, Gray P W, Matsushima K, Yoshie O. 1999. Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. *Int. Immunol.* 11: 81-88.

43. D'ambrosio D, Iellem A, Bonecchi R, Mazzeo D, Sozzani S, Mantovani A, Sinigaglia F. 1998. Selective up-regulation of chemokine receptors CCR4 and CCR8 upon activation of poloarized human type 2 Th cells. *J. Immunol.* 161: 5111.

44. Campbell J J, Haraildsen G, Pan J, Rottman J, Qin S, Ponath P, Andrew D P, Warnke R, Ruffing N, Kassam N, Wu L, Butcher E C. 1999. The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells. *Nature* 400: 776.

45. Struyf S, Proost P, Sozzani S, Mantovani A, Wuyts A, de Clercq E, Schols D, Van Damme J. 1998. Enhanced anti-HIV-1 activity and altered chemotactic potency of NH_2-terminally processed macrophage-derived chemokine (MDC) imply an additional MDC receptor. *J. Immunol.* 161: 2672.

46. Yoneyama H, Harada A, Imai T, Baba M, Yoshie O, Zhang Y, Higashi H, Murai M, Asakura H, Matsushima K. 1998. Pivotal role of TARC, a CC chemokine, in bacteria-induced fulminant hepatic failure in mice. *J. Clin. Invest.* 102: 1933.

47. Matsukawa A, Hogaboam C M, Lukacs N W, Lincoln P M, Evanoff H L, Kunkel S L. 2000. Pivotal role of the CC chemokine, macrophage-derived chemokine, in the innate immune response. *J. Immunol.* 164: 5362.

48. Nomiyama H, Imai T, Kusuda J, Miura R, Callen D F, Yoshie O. 1998. Human chemokines fractalkine (SCYD1), MDC (SCYA22) and TARC (SCYA17) are clustered on chromosome 16q13. *Cytogenet. Cell Genet.* 81: 10.

Figures

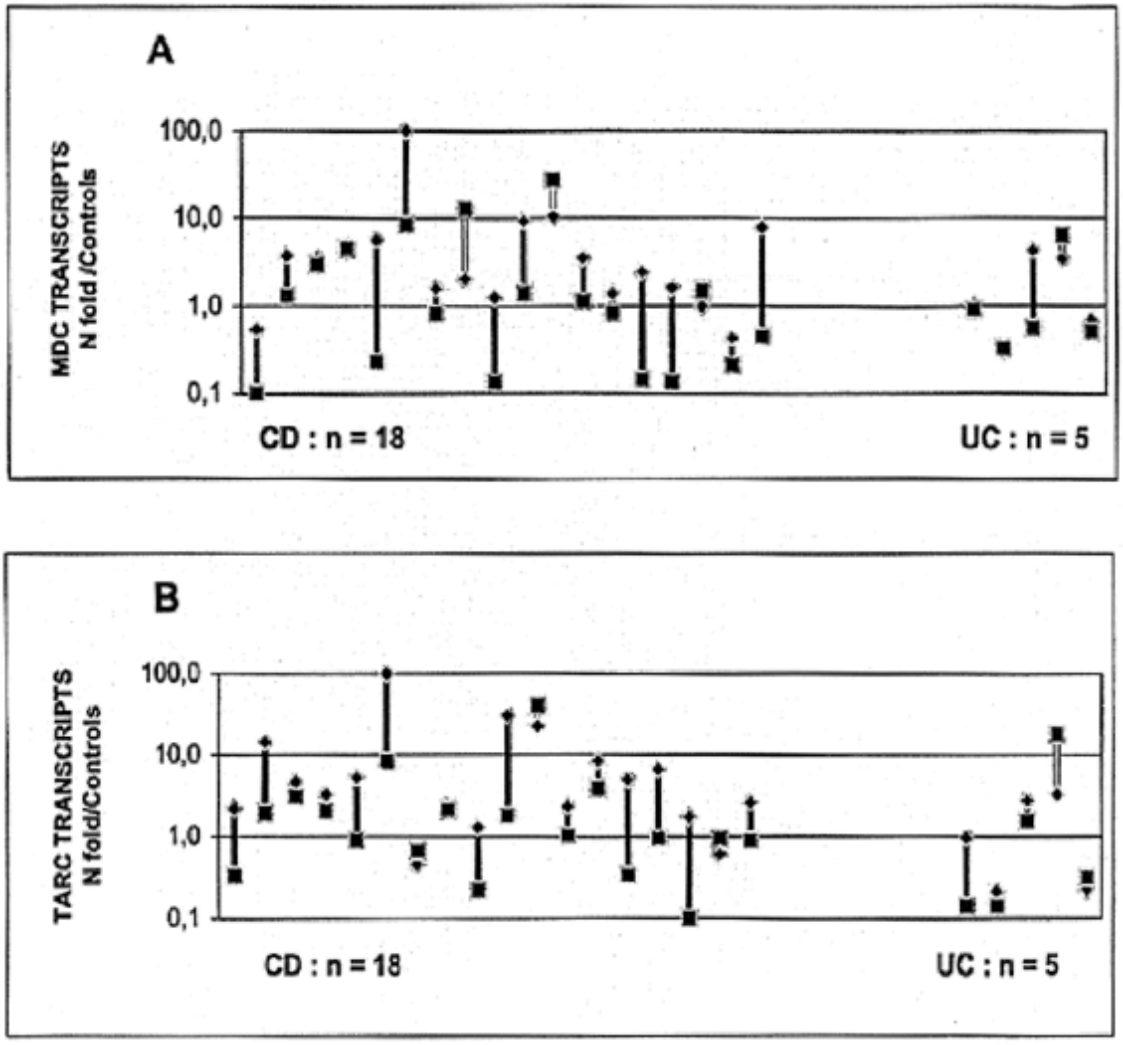


Figure 1
Relative and normalized mRNA levels of MDC (A) and TARC (B) expressed as n-fold the calibrator in paired inflamed and non inflamed CD (n = 18) or UC (n = 5) specimen.

Comparisons by Wilcoxon paired test of inflamed [diamond] and non inflamed [square] specimen show significant differences both for MDC ($p < 0.04$) and TARC ($p < 0.005$) in CD but not in UC. Target chemokine expression was increased (filed bars) or decreased (open bars) in inflamed samples compared to paired non inflamed mucosa.

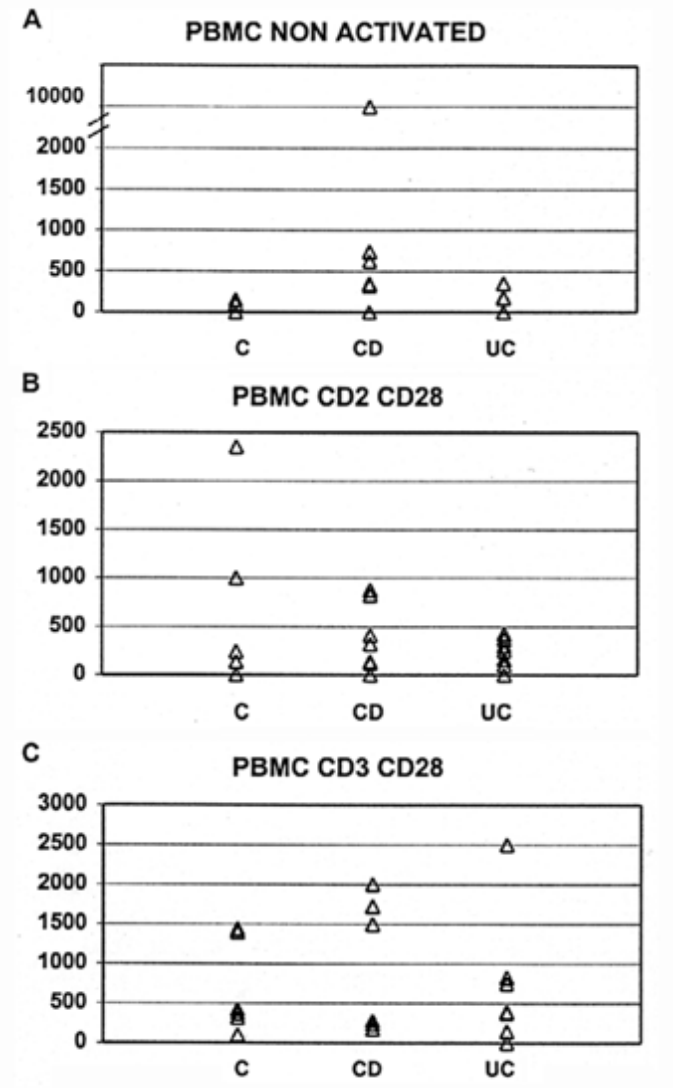


Figure 2
MDC production (pg/ml) by peripheral blood mononuclear cells (PBMC) before (A) and after $CD2^+CD28$ (B) or $CD3^+CD28$ (C) stimulation in control (C, n = 6), Crohn's disease (CD, n = 7), and Ulcerative colitis (UC, n = 9) subjects.

Value under 100 pg/ml and 40 pg/ml for MDC and TARC respectively should be considered as negative.

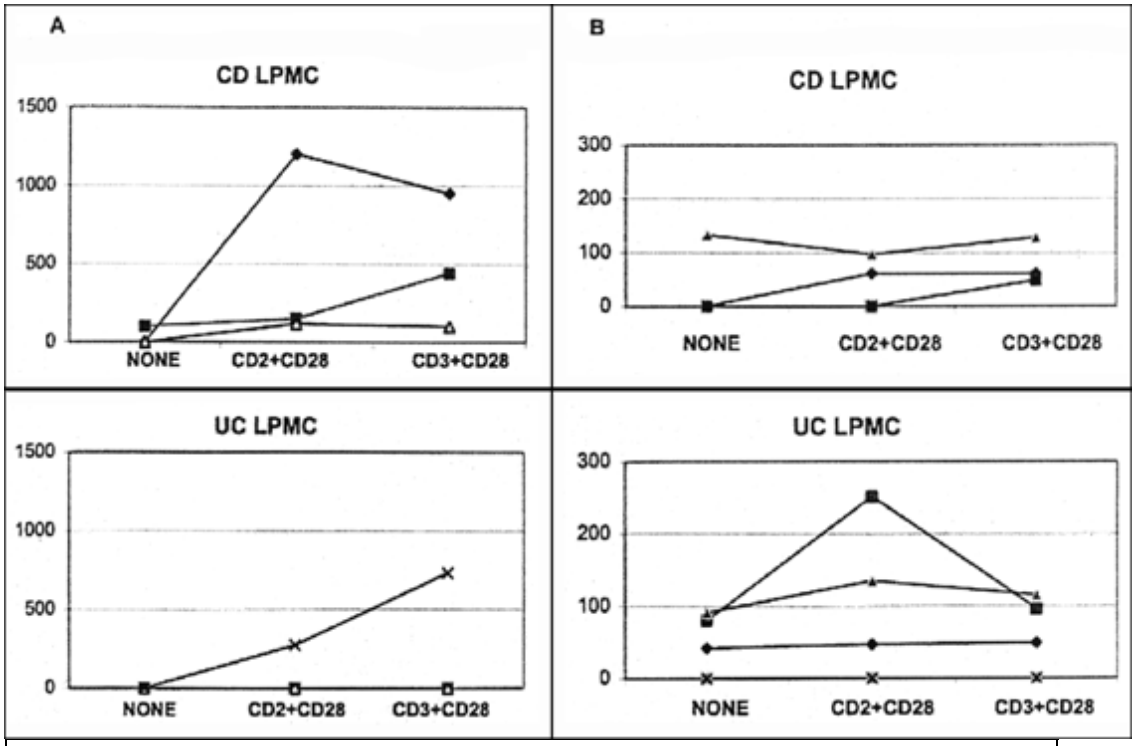


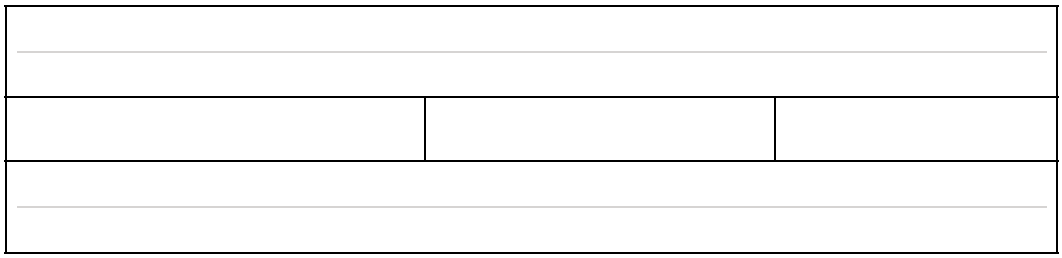
Figure 3
MDC (A) and TARC (B) production (pg/ml) by lamina propria mononuclear cells (LPMC) before and after $CD2^+CD28$ or $CD3^+CD28$ stimulation in Crohn's disease (CD, n = 3) and Ulcerative colitis (UC, n = 4) patients.

Value under 100 pg/ml and 40 pg/ml for MDC and TARC respectively should be considered as negative.

Table 1
Relative and normalized mRNA levels in inflamed mucosa expressed as n-fold the calibrator

	CD (colitis) (n = 22)	UC (colitis) (n = 9)	ASLC (colitis) (n = 4)	CD (ileitis) (n = 8)
MDC	10.5 (6.9)*	5.4 (2.3)	3.2 (1.3)	1.9 (0.7)
TARC	11.54 (5.3)**	5.0 (3.4)	5.0 (2.4)*	1.8 (0.9)

Results expressed as mean (SEM).
* $p < 0.01$, ** $p < 0.001$, *vs* calibrator by Mann-Whitney test.



Authors

F. Jugde, M. Alizadeh, C. Boissier, D. Chantry, L. Siproudhis, S. Corbinais, E. Quelvennec, F. Dyard, J.-P. Campion, M. Gosselin, J.-F. Bretagne, G. Sémana, D. Heresbach

Service des Maladies de l'Appareil Digestif, CHU Pontchaillou, 35033 Rennes.

In this issue

Virus-cell interactions: impact on cytokine production, immune evasion and tumor growth

T. H. Mogensen, S. R. Paludan
virus, cytokines, signal transduction, immune evasion, tumor growth.

Safety, pharmacokinetics and pharmacodynamics of recombinant human tumour necrosis factor-binding protein-1 (Onercept) injected by intravenous, intramuscular and subcutaneous routes into healthy volunteers

I. Trinchard-Lugan, Q. Ho-Nguyen, W.M. Bilham, M. Buraglio, A. Ythier, A. Munafo
recombinant human tumour necrosis factor-binding protein-1 (r-hTBP-1), safety, pharmacokinetics, pharmacodynamics.

Direct effects of tumor necrosis factor alpha (TNF-alpha) on murine skeletal muscle cell lines. Bimodal effects on protein metabolism

B. Alvarez, L. S. Quinn, S. Busquets, F. J. López-Soriano, J. M. Argilés
cytokines, myotubes, cachexia, protein turnover.

Receptor-like properties of the 26 kDa transmembrane form of TNF

A. Domonkos, A. Udvardy, L. László, T. Nagy, E. Duda
cytokine, TNF family, receptor, signaling, reverse-signaling.

Local administration of interleukin-1beta for the treatment of lung abscesses induces neutrophil activation and changes in proinflammation cytokine production

A. Simbirtsev, E. Variouchina, V. Konusova, A. Kotov, S. Ketlinsky, A. Salamatov, L. Bisenkov
recombinant human interleukin-1beta, lung inflammation, abscess, neutrophils, cytokines.

Effects of interleukin-2 therapy on the proliferation and differentiation of CD4/CD25 positive and CD4/CD25 negative cells in HIV+ patients

L. Caggiari, S. Zanussi, M. D'Andrea, M. T. Bortolin, C. Crepaldi, C. Caffau, P. de Paoli
cytokines, Ki67, intracellular IFN-gamma.

Tumor necrosis factor-alpha and nitric oxide in vertically HIV-1-infected children: implications for pathogenesis

J. González-Nicolás, S. Resino, J. L. Jiménez, S. Alvarez, M. Fresno, M. A. Muñoz-Fernández
tumor necrosis factor, nitric oxide, HIV-infection, immunological and virological markers.

Serum levels of angiogenic cytokines in systemic lupus erythematosus and their correlation with disease activity

E. Robak, A. Wozniacka, A. Sysa-Jedrzejowska, H. Stepien, T. Robak
systemic lupus erythematosus, disease activity, angiogenesis, platelets, hepatocyte growth factor, vascular endothelial growth factor, basic fibroblast growth factor, transforming growth factor beta.

Modulation of the allergen-induced human IgE response in Hu-SCID mice: inhibitory effect of human recombinant IFN-gamma and allergen-derived lipopeptide

C. Duez, H. Gras-Masse, H. Hammad, H. Akoum, A. Didierlaurent, C. André, A.-B. Tonnel, J. Pestel
SCID mouse, IgE, IFN-gamma, lipopeptide, house dust mite.

Cytokine-inducing activity of a proline-rich polypeptide complex (PRP) from ovine colostrum and its active nonapeptide fragment analogs

A. Zablocka, M. Janusz, K. Rybka, I. Wirkus-Romanowska, G. Kupryszewski, J. Lisowski
ovine colostrum, proline-rich polypeptide complex (PRP), nonapeptide (NP) and hexapeptide (HP) from PRP, oligomers of NP and HP, cytokines.

Quantitation of chemokines (MDC, TARC) expression in mucosa from Crohn's disease and ulcerative colitis

F. Jugde, M. Alizadeh, C. Boissier, D. Chantry, L. Siproudhis, S. Corbinais, E. Quelvennec, F. Dyard, J.-P. Campion, M. Gosselin, J.-F. Bretagne, G. Sémana, D. Heresbach
real-time TR-PCR, chemokines, Crohn’s disease, ulcerative colitis.

Activation of caspase-3 by interferon alpha causes interleukin-16 secretion but fails to modulate activation induced cell death

O. Ludwiczek, A. Kaser, R. O. Koch, W. Vogel, W. W. Cruikshank, H. Tilg
interleukin-16, interferon-alpha, caspase-3.

Cytokine deviation induced by intrathymic injection of staphylococcal enterotoxin B (SEB)

P. Goettelfinger, F. Lecerf, S. Berrih-Aknin, M. German-Fattal
SEB, intrathymic injection, anergy, cytokine deviation, quantitative RT-PCR.

Strain-dependent difference in inducible nitric oxide synthesis (iNOS) expression in rat pancreatic islets correlates with interferon regulating factor 1 (IRF-1) and heat shock protein 70 (HSP70) expression

J. Johannesen, F. Pociot, A. E. Karlsen, T. Mandrup-Poulsen, J. Nerup
type 1 diabetes, islets of Langerhans, beta-cells, cytokines, interleukin-1beta, interleukin-1beta receptor type I, nitric oxide, inducible nitric oxide synthase, heat shock protein 70, interferon regulating factor 1.

Neutrophil apoptosis in autoimmune Fas-defective MRL lpr/lpr mice

E. Del Giudice, A. Ciaramella, N. Balestro, D. Neumann, P. G. Romano, M. P. Cesaroni, G. Maurizi, P. Ruggiero, D. Boraschi, P. Bossù
neutrophils, apoptosis, autoimmunity.

A role for interferon-gamma in the hypermetabolic response to murine toxoplasmosis

D. Arsenijevic, F. de Bilbao, P. Giannakopoulos, L. Girardier, S. Samec, D. Richard
toxoplasma, hypermetabolism, reactive oxygen species, lipid peroxidation, UCP1, interferon-gamma.

Protection of mice from malaria after co-administration of recombinant mouse granulocyte-macrophage colony-stimulating factor and methionine-enkephalin

P. Pal Singh, S. Singh
Plasmodium berghei, protection, rmGM-CSF, met-enkephalin.

Semiautomated and simultaneous analysis of the interleukin-10 gene microsatellites IL-10G and IL-10R by fluorescence-based polymerase chain reaction reveals significant differences in allele distributions between Caucasians (Germany) and Africans (Gabon)

D. Kube, D. Schmidt, M. Mörmann, A.C. Uhlemann, J. Tomiuk, H. Tesch, P.G. Kremsner
microsatellites, interleukin-10 promoter, multiplex-PCR, Caucasian, African.

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



John Libbey Eurotext Arnette doin Pradel
Lavoisier Hermes TEC & DOC Médecine SCIENCES