IL-15 Is Highly Expressed in Inflammatory Bowel Disease and Regulates Local T Cell-Dependent Cytokine Production

Zhanju Liu, Karel Geboes, Stefaan Colpaert, Geert R. D’Haens, Paul Rutgeerts and Jan L. Ceuppens

*J Immunol* 2000; 164:3608-3615; doi: 10.4049/jimmunol.164.7.3608

http://www.jimmunol.org/content/164/7/3608
IL-15 shares biological activities but no significant sequence homology with IL-2. It induces T cell recruitment to sites of inflammation, T cell proliferation, and cytokine production and rescue from apoptosis. The aim of this study was to investigate expression of IL-15 and its effects on proinflammatory cytokine production in inflammatory bowel disease (IBD). Immunohistochemistry demonstrated local IL-15 production by macrophages in inflamed mucosa from IBD patients. Isolated lamina propria mononuclear cells from these patients but not from controls produced IL-15 when stimulated with LPS or IFN-γ. Moreover, lamina propria T cells (LP-T) from IBD patients were more responsive to IL-15 as compared with controls, and IL-15 alone without a primary T cell stimulus induced IFN-γ and TNF production by isolated IBD LP-T cells, especially by LP-T cells from patients with Crohn’s disease. LP-T cells from IBD patients could induce CD40-CD40 ligand (CD40L) interaction-dependent TNF and IL-12 production by monocytes in a coculture system. This capacity of LP-T cells was strongly enhanced by preincubation in IL-15 and was the result of higher CD40L expression after culture in IL-15. These data indicate that IL-15 is overexpressed in the inflamed mucosa in IBD and that IL-15 enhances local T cell activation, proliferation, and proinflammatory cytokine production by both T cells and macrophages, the latter via a CD40-CD40L interaction-dependent mechanism. Treatment directed against IL-15 may have therapeutic potential in IBD. The Journal of Immunology, 2000, 164: 3608–3615.

Recently, IL-15 has been found to be overexpressed in rheumatoid arthritis (RA) and allograft rejection (15–17). IL-15, found in the synovial tissue of RA patients, can recruit and activate T cells in the synovial membrane. RA synovial fluid T cells activated by IL-15 exhibit proinflammatory activities in vitro such as cytokine production and CD69 and adhesion molecule expression, and they up-regulate monocyte TNF production (15, 16). Of note, treatment targeted to components of the IL-15R, including IL-15Rα-chain and the common γ-chain, has been shown to effectively prevent development of some experimental diseases such as collagen-induced arthritis and allograft rejection (18, 19) and to markedly block Ag-specific delayed hypersensitivity and leukocyte infiltration within the delayed-type hypersensitivity sites (20). IL-15 is also highly expressed at the transcriptional and translational levels in leprosy skin lesions and by alveolar macrophages from patients with active sarcoidosis (21, 22). In these patients, IL-15 has been demonstrated not only to augment the local T cell response in microbial immunity (21), but also to trigger the sarcoid T cell growth and to accelerate the development of the T cell avedolitis (22). Taken as a whole, these observations strongly indicate that IL-15 plays a role in a variety of pathological conditions.

Preliminary data have suggested the potential relevance of IL-15 to the immune responses in human inflammatory bowel disease (IBD). Kirman and Nielsen (23) reported that IL-15 could be detected in the sera of patients with ulcerative colitis (UC), but not in patients with Crohn’s disease (CD). Intracellular staining showed that the percentage of IL-15-positive cells was increased in mononuclear cells in both UC and CD patients. Expression of IL-15 mRNA was also found to be significantly increased in inflamed rectal mucosa of IBD (24). In the present study, we intended to gain further insight concerning the potential role of IL-15.
in the pathogenesis of IBD. To this end, we studied local production of IL-15 in inflamed mucosa, as well as the functional effect of IL-15 on mucosal T cells and on T cell-monocyte interactions.

Materials and Methods

Patients

Twenty-two tissue specimens were obtained from the inflamed ileum of 14 patients with active CD (10 male and 4 female, mean 36 years) and inflamed colon of 8 patients with active UC (six male and two female, mean 45 years). The preoperative diagnosis of IBD was based upon classical clinical, radiological, and endoscopic features and was confirmed by histological examination of the resection specimens. Indications for bowel resection in CD were the presence of fistulae (n = 5) and stenosis with clinical signs of obstruction (n = 11); while in UC indications for surgery were mostly therapy-resistant inflammatory colitis. Among IBD patients, 14 were receiving sulfasalazine or an oral aminosalicylic acid preparation at the time of operation, and 8 patients were on no treatment. None of them received immunosuppressants such as corticosteroids, cyclosporin A, or azathioprine. Control tissues were obtained from 10 patients (7 men and 3 women, mean age 48 years) undergoing right hemicolectomy for cancer. Ileum was obtained from 7 patients and colon from 10 patients, and all tissues were remote from neoplastic involvement and were macroscopically normal. Additionally, uninvolved ileum (n = 6) from CD and uninvolved colon (n = 5) from UC were also obtained.

Heparinized peripheral blood samples were obtained from outpatients including 10 cases with UC (seven men and three women, mean age 34 years) and 10 cases with CD (seven men and three women, mean 34 years). The diagnosis was established by conventional clinical features and histological criteria. Only eight patients with CD and four patients with UC were treated with sulfasalazine or an oral aminosalicylic acid preparation. None received any immunosuppressants such as corticosteroids and cyclosporin A. In addition, blood samples from 11 healthy volunteers (seven men and four women, mean age 32 years) were also taken for comparison.

Reagents

Purified human rIL-15 was obtained from PeproTech (Rocky Hill, NJ). Anti-IL-15 mAb M112 (mouse IgG1) was purchased from Genzyme (Cambridge, MA). Anti-CD40 ligand (CD40L) mAb M90 (mouse IgG1) was a gift from Immunex (Seattle, WA). FITC- or PE-conjugated anti-CD3 (clone UCHt1, mouse IgG1), FITC-conjugated anti-CD69 (clone FN50, mouse IgG1), PE-conjugated anti-CD40L (clone TRAP1, mouse IgG1), and FITC- or PE-conjugated anti-CD14 (clone MGG-2, mouse IgG1) were purchased from PharMingen (San Diego, CA). RPMI 1640 medium, PBS, and penicillin-streptomycin were purchased from BioWhittaker (Walkersville, MD). Bovine calf serum was obtained from HyClone (Logan, UT), LPS (Escherichia coli serotype 026:B6) (Sigma, St. Louis, MO). Purified human rIL-15 was obtained from Genzyme (Cambridge, MA). Bovine serum albumin, 100 mM HEPES, 100 mM EDTA, 400 mM NaCl, 100 mM NaF, 100 U/ml penicillin, 100 U/ml streptomycin, 10 U/ml amphotericin B, 30% fetal calf serum, and 0.5% BSA were used for the manufacture’s instructions. Briefly, PBMC were magnetically labeled with CD33 MicroBeads (Miltenyi Biotec) at 4°C for 15 min and then passed through a separation column that was placed in the magnetic field of a MACS separator. The magnetically labeled CD33+ cells were retained in the column while the unlabeled CD33- cells ran through. After removal of the column from the magnetic field, the magnetically retained CD33+ cells were eluted in PBS supplemented with 5 mM EDTA and 0.5% BSA. The resultant cell population was >90% CD14+, as determined by flow cytometric analysis (FACSORT, Becton Dickinson, San Jose, CA). All cell populations were cultured in RPMI 1640 medium supplemented with 0.3 mg/ml l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 4 U/ml polybrein B, 5 μg/ml amphotericin B, and 10% iron-supplemented bovine calf serum at 37°C in 5% CO2-humidified atmosphere.

Immunohistochemistry

Tissue samples (n = 10 in CD, n = 7 in UC, n = 7 in normal ileum, and n = 10 in normal colon) were immediately embedded in OCT compound (Tissue-Tek; Miles, Elkhart, IN), frozen in liquid nitrogen, and stored at –70°C. Cryostat sections (5 μm) of intestinal tissue were fixed and stained as previously described (25). Endogenous peroxidase activity in colonic tissue was blocked with 0.3% H2O2, supplemented with 0.1% saponin (Sigma) for 30 min. Serial sections were first incubated with anti-IL-15 mAb M112 (10 μg/ml) for 30 min. After washing, the sections were incubated for 30 min with biotin-labeled rabbit anti-mouse IgG (Dako, Glostrup, Denmark) at 1:400. After additional washes, the sections were incubated for 30 min with avidin-biotin-peroxidase complex (Dako). All procedures were conducted at room temperature. The color reaction developed with 3-amino-ethylcarbazole (Janssen, Beerse, Belgium). The slides were counterstained with hematoxylin. Cells were counted as described (25). Negative control experiments were performed by incubating sections with irrelevant isotype-matched mouse IgG1 or by omitting the primary Ab.

For double staining for IL-15 and CD68 (activated macrophages) expression, a combination of the peroxidase technique and the alkaline phosphatase antialkaline phosphatase technique was used (25). Endogenous alkaline phosphatase was blocked by levamisole. An anti-CD68 mAb KPI1 (Dako) was used for the identification of activated macrophages, and M112 was applied for the identification of IL-15.

IL-15 production by LPMC

LPMC from IBD patients and controls were cultured at the concentration of 1.0 × 10^6/ml in the absence or presence of LPS (5 μg/ml) or IFN-γ (1000 U/ml). After 48 h of culture, supernatants were aspirated and stored at −70°C for IL-15 assay.

T cell activation by IL-15

For proliferation studies, PB-T or LP-T cells (1.0 × 10^6/ml) were plated in 96-well U-bottom plates (Nunc, Roskilde, Denmark) in triplicate in a total volume of 200 μl per well in the presence of IL-15 (1, 10, 100 ng/ml). After 72 h of culture, supernatants were harvested and assayed for IFN-γ and TNF.

Cell preparations and culture conditions

Peripheral blood T cells (PB-T), lamina propria mononuclear cells (LPMC), and lamina propria T cells (LP-T) from all groups were isolated using the method as described (25). Monocytes used in this study were obtained from a single healthy donor by positive selection using the magnetic activated cell sorter (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacture’s instructions. Briefly, PBMC were magnetically labeled with CD33 MicroBeads (Miltenyi Biotec) at 4°C for 15 min and then passed through a separation column that was placed in the magnetic field of a MACS separator. The magnetically labeled CD33+ cells were retained in the column while the unlabeled CD33- cells ran through. After removal of the column from the magnetic field, the magnetically retained CD33+ cells were eluted in PBS supplemented with 5 mM EDTA and 0.5% BSA. The resultant cell population was >90% CD14+, as determined by flow cytometric analysis (FACSort, Becton Dickinson, San Jose, CA). All cell populations were cultured in RPMI 1640 medium supplemented with 0.3 mg/ml l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 4 U/ml polybrein B, 5 μg/ml amphotericin B, and 10% iron-supplemented bovine calf serum at 37°C in 5% CO2-humidified atmosphere.

Monocyte cytokine production induced by IL-15-activated LP-T cells

Monocytes from a single healthy donor were isolated as described above. Isolated LP-T cells (2.0 × 10^6/ml) from either involved mucosa of IBD patients or uninvolved mucosa of IBD patients and controls were incubated in the presence of either culture medium alone or IL-15 (100 ng/ml) for 12 h. These LP-T cells were collected, washed with PBS three times, and then fixed in 1.0% paraformaldehyde in PBS for 30 min at 4°C. Following fixation, the cells were stained with 0.5 ml 1% paraformaldehyde in saline and analyzed on a FACSort.

Measurement of cytokines

TNF and IL-12 p70 were assayed by sandwich ELISA as described (25). IL-15 was measured by sandwich ELISA using matched Ab pairs and following the manufacturer’s instructions (R&D Systems, Minneapolis, MN). IFN-γ was measured by ELISA using matched Ab pairs according to the manufacture’s instructions (BioSource International, Nivelles, Belgium). The sensitivity of each assay was 10 pg/ml.
**Statistical analysis**

Data were expressed as mean ± SEM. Statistical analysis was performed using the Wilcoxon test for paired samples and Mann-Whitney U test for unpaired data. A value of $p < 0.05$ was considered statistically significant.

**Results**

**In situ expression of IL-15 in intestinal tissue**

As shown in Fig. 1, immunohistological analysis demonstrated the presence of numerous IL-15-positive cells with strong cytoplasmic staining in inflamed mucosa from IBD patients. The percentage of IL-15-positive cells in lamina propria of inflamed ileum from CD and of inflamed colon from UC was significantly higher than that in the ileum and colon from control patients (29.5 ± 4.2 in CD, 27.6 ± 4.5 in UC, 5.2 ± 1.7 in normal ileum, and 3.9 ± 1.1 in normal colon, $p < 0.001$). In two patients with infectious colitis (Samonella species), the number of IL-15-positive cells was similar to controls. The majority of IL-15-positive cells in lamina propria of inflamed ileum from CD and of inflamed colon from UC was significantly higher than that in the ileum and colon from control patients (29.5 ± 4.2 in CD, 27.6 ± 4.5 in UC, 5.2 ± 1.7 in normal ileum, and 3.9 ± 1.1 in normal colon, $p < 0.001$). In two patients with infectious colitis (Samonella species), the number of IL-15-positive cells was similar to controls. The majority of IL-15-positive cells in lamina propria of inflamed ileum from CD and of inflamed colon from UC was significantly higher than that in the ileum and colon from control patients (29.5 ± 4.2 in CD, 27.6 ± 4.5 in UC, 5.2 ± 1.7 in normal ileum, and 3.9 ± 1.1 in normal colon, $p < 0.001$). In two patients with infectious colitis (Samonella species), the number of IL-15-positive cells was similar to controls. The majority of IL-15-positive cells in lamina propria of inflamed ileum from CD and of inflamed colon from UC was significantly higher than that in the ileum and colon from control patients (29.5 ± 4.2 in CD, 27.6 ± 4.5 in UC, 5.2 ± 1.7 in normal ileum, and 3.9 ± 1.1 in normal colon, $p < 0.001$). In two patients with infectious colitis (Samonella species), the number of IL-15-positive cells was similar to controls. The majority of IL-15-positive cells in lamina propria of inflamed ileum from CD and of inflamed colon from UC was significantly higher than that in the ileum and colon from control patients (29.5 ± 4.2 in CD, 27.6 ± 4.5 in UC, 5.2 ± 1.7 in normal ileum, and 3.9 ± 1.1 in normal colon, $p < 0.001$). In two patients with infectious colitis (Samonella species), the number of IL-15-positive cells was similar to controls. The majority of IL-15-positive cells in lamina propria of inflamed ileum from CD and of inflamed colon from UC was significantly higher than that in the ileum and colon from control patients (29.5 ± 4.2 in CD, 27.6 ± 4.5 in UC, 5.2 ± 1.7 in normal ileum, and 3.9 ± 1.1 in normal colon, $p < 0.001$). In two patients with infectious colitis (Samonella species), the number of IL-15-positive cells was similar to controls. The majority of IL-15-positive cells in lamina propria of inflamed ileum from CD and of inflamed colon from UC was significantly higher than that in the ileum and colon from control patients (29.5 ± 4.2 in CD, 27.6 ± 4.5 in UC, 5.2 ± 1.7 in normal ileum, and 3.9 ± 1.1 in normal colon, $p < 0.001$). In two patients with infectious colitis (Samonella species), the number of IL-15-positive cells was similar to controls. The majority of IL-15-positive cells in lamina propria of inflamed ileum from CD and of inflamed colon from UC was significantly higher than that in the ileum and colon from control patients (29.5 ± 4.2 in CD, 27.6 ± 4.5 in UC, 5.2 ± 1.7 in normal ileum, and 3.9 ± 1.1 in normal colon, $p < 0.001$). In two patients with infectious colitis (Samonella species), the number of IL-15-positive cells was similar to controls. The majority of IL-15-positive cells in lamina propria of inflamed ileum from CD and of inflamed colon from UC was significantly higher than that in the ileum and colon from control patients (29.5 ± 4.2 in CD, 27.6 ± 4.5 in UC, 5.2 ± 1.7 in normal ileum, and 3.9 ± 1.1 in normal colon, $p < 0.001$). In two patients with infectious colitis (Samonella species), the number of IL-15-positive cells was similar to controls. The majority of IL-15-positive cells in lamina propria of inflamed ileum from CD and of inflamed colon from UC was significantly higher than that in the ileum and colon from control patients (29.5 ± 4.2 in CD, 27.6 ± 4.5 in UC, 5.2 ± 1.7 in normal ileum, and 3.9 ± 1.1 in normal colon, $p < 0.001$). In two patients with infectious colitis (Samonella species), the number of IL-15-positive cells was similar to controls. The majority of IL-15-positive cells in lamina propria of inflamed ileum from CD and of inflamed colon from UC was significantly higher than that in the ileum and colon from control patients (29.5 ± 4.2 in CD, 27.6 ± 4.5 in UC, 5.2 ± 1.7 in normal ileum, and 3.9 ± 1.1 in normal colon, $p < 0.001$).

Phenotypic analysis of IL-15-positive cells was performed in the sections from three patients with active CD showing high scores for the expression of IL-15. Double staining for IL-15 and CD68 demonstrated the presence of IL-15 in CD68$^+$ macrophages, yet a positive staining for IL-15 was found in only half of CD68$^+$ cells (Fig. ID). Double positive cells were mainly seen in the mucosa, and to a lesser extent also in the submucosa, muscularis propria, and serosa.

**IL-15 production by LPMC**

IL-15 is mainly induced by microbial Ags such as LPS, Mycobacterium leprae, and Staphylococcus aureus Cowan strain 1 (5, 6, 8, 21, 27). We studied IL-15 secretion by lamina propria macrophages in vitro. LPMC were isolated and cultured with LPS (5 μg/ml) or IFN-γ (1000 U/ml) for 48 h. As shown in Fig. 2, LPMC from inflamed areas of IBD patients, when stimulated with either LPS or IFN-γ, indeed released IL-15. The levels in the supernatants of IBD LPMC were significantly higher compared with those in the supernatants of control LPMC ($p < 0.005$). Additionally, LPMC were isolated from uninvolved ileum of six patients with CD, uninvolved colon from five patients with UC, and colon of one patient with diverticulitis, and four patients with infectious colitis (Samonella species). These LPMC, similar to control LPMC, could produce only low levels of IL-15 when stimulated with LPS (31 ± 6.5 pg/ml) or IFN-γ (25.4 ± 5 pg/ml), and these levels were significantly lower than those produced by LPMC from inflamed areas of IBD patients (data not shown). We further cocultured LPMC from involved mucosa of eight patients with CD and six patients with UC with CD40L-transfected 3T6 cells, but no IL-15 protein was found in the supernatants. These results, consistent with earlier report (27), indicate that CD40L expressed on...
FIGURE 2. IL-15 production by LPMC. LPMC (1.0 × 10⁶/ml) from control (n = 7), CD (n = 8), and UC (n = 6) were incubated with LPS (5 µg/ml) or IFN-γ (1000 U/ml). After 48 h of culture, supernatants were aspirated and assayed for IL-15 by ELISA. The horizontal bars represent mean values. *p < 0.005 vs medium alone in the same group or vs control at the same culture condition.

LP-T cells (25) does not trigger monocytes/macrophages to produce IL-15.

LP-T cells are activated by IL-15

Experiments on synovial fluid T cells in RA patients have demonstrated that these cells exhibit enhanced responsiveness to IL-15 and that IL-15 in the absence of a primary stimulus can enhance several T cell activities (15, 16). Therefore, the proliferation-inducing effect of IL-15 on highly purified T cells from both IBD patients and controls was compared. IBD LP-T cells, when stimulated with 1, 10, and 100 ng/ml of IL-15, demonstrated a significantly higher proliferative stimulation index than those from controls (Fig. 3A, p < 0.005). Of note, PB-T cells from IBD patients also proliferated more strongly in response to 10 and 100 ng/ml of IL-15 (Fig. 3B, p < 0.005). These data indicate that IL-15, in the absence of a primary stimulus, is a strong inducer of proliferation of T cells in IBD patients, and suggest that these cells are primed to become more responsive to IL-15.

Activation of human T cells with IL-15 also results in cytokine production (10, 16, 28). IFN-γ and TNF have been considered as important proinflammatory cytokines in IBD, especially in CD (29, 30). Therefore, we studied the effect of IL-15 on proinflammatory cytokine production by IBD T cells. Data in Fig. 4A and B demonstrate that the production of IFN-γ and TNF was significantly increased in the IBD LP-T cell cultures in the presence of IL-15, especially in CD. Moreover, IFN-γ but not TNF was also significantly induced in the supernatants of IBD PB-T cells incubated with IL-15 (Fig. 4C and D). These data indicate that IL-15 can directly induce IBD T cells to produce proinflammatory mediators that participate in tissue damage.

IL-15 has been shown to enhance T cell viability (12, 31). We found this effect also to be relevant to mucosal cells. We cultured LPMC from one patient with active CD and one patient with active UC in the presence and absence of IL-15 (100 ng/ml). The viability was determined in a hemocytometer on the basis of trypan blue exclusion. For both samples, the percent of viable cells was maintained at higher levels in the presence of IL-15 after 24 h of culture (90 and 88%), while in the absence of IL-15 the percent of viable cells decreased to 72 and 69%.

Expression of T cell activation molecules after IL-15 challenge

CD69 is a marker of activation on T cells (32). We studied the kinetics of CD69 expression on IBD T cells after IL-15 priming. Very low to absent levels of CD69 expression were found on freshly isolated PB-T cells from all groups (Fig. 5A). After IL-15 stimulation, its expression was found to be enhanced in all groups, but it was significantly higher on IBD PB-T cells as compared with healthy controls (p < 0.005 at 24 or 48 h of culture). Using two-color immunofluorescence, we analyzed CD69 expression on PB-CD4⁺ and PB-CD8⁺ T cells from one patient with active CD and one patient with active UC in the presence of IL-15 (100 ng/ml). After 24 h of culture, 14 and 15% of the CD4⁺ T cells and 41 and 33% of the CD8⁺ T cells were CD69⁺. Thus, PB-CD8⁺ T cells from IBD patients are more responsive than CD4⁺ T cells (data not shown). In contrast, high levels of CD69 expression were found on freshly isolated LP-T cells from both IBD patients and controls, with no difference between the groups (p > 0.05). CD69 expression on LP-T cells did not change by >5% in the presence of IL-15 (Fig. 5B).

Monocyte IL-12 and TNF production induced by IL-15-activated IBD LP-T cells depends on CD40L signaling

In a previous study, we have shown that LP-T cells from IBD patients induce cytokine secretion by normal monocytes (25). Moreover, IL-15 has been shown to regulate synovial T cell-dependent cytokine secretion by synovial macrophages in RA (16). After demonstrating that IL-15 plays an important role in inducing IBD LP-T cell activation, we wanted to investigate whether IL-15 could enhance T cell-dependent IL-12 and TNF production by monocytes in vitro by an effect on T cells. LP-T cells from all groups were either used immediately after isolation or cultured with or without IL-15 for 12 h. Fixation of T cells was accomplished by incubation in 1% paraformaldehyde for 30 min at 4°C. This procedure is known to prevent T cell cytokine secretion but to
maintain cell-membrane integrity, providing an experimental system for study of cell contact-mediated effects. Paraformaldehyde-fixed LP-T cells were then cocultured with healthy monocytes repeatedly isolated from a single donor. Fig. 6 shows that elevated levels of IL-12 and TNF were found in the supernatants of monocytes cocultured with LP-T cells from IBD patients. When control LP-T cells were cultured in IL-15, they were also able to induce IL-12 and TNF production by monocytes, similar to untreated IBD LP-T cells. Importantly, IL-12 and TNF production was significantly higher in the supernatants of monocytes cocultured with IL-15-activated IBD LP-T cells, demonstrating that IL-15 increases the ability of IBD LP-T cells to induce monocyte cytokine production. We know from our previous study that CD40L on IBD LP-T cells is responsible for monocyte cytokine production in these cocultures. CD40L is a 33-kDa glycoprotein that is transiently expressed on the surface of activated T cells, predominantly the CD4⁺ T cells (33, 34). Expression of CD40L is induced by Th cell activation. Interestingly, inclusion of anti-CD40L mAb in this coculture system of IL-15-activated LP-T cells and normal monocytes significantly down-regulated monocyte IL-12 and TNF production (Fig. 6). Fixed T cells from all groups or monocytes cultured alone or incubated with IL-15 did not produce measurable amounts of cytokines (data not shown).

In the next series of experiments, we explored the possibility that IL-15 could enhance T cell CD40L expression. Fig. 7 demonstrates that IL-15 induced low expression of CD40L on control LP-T cells, while its expression was more strongly induced and also maintained at higher levels on IBD LP-T cells after 24 and 48 h of IL-15 stimulation (p < 0.005 vs controls at 24 or 48 h of culture). On the contrary, its expression was not significantly changed on PB-T cells from all groups (data not shown). From these data, it is proposed that IL-15 alone without a primary T cell stimulus triggers IBD LP-T cell CD40L expression and that IL-15-activated LP-T cells further promote monocyte IL-12 and TNF secretion following engagement by CD40 on monocytes.

Discussion

CD and UC are characterized by infiltration of activated leukocytes in inflamed intestinal mucosa, i.e., CD25⁺ T cells, B cells, and RFD9⁺ CD68⁺ macrophages and by local proinflammatory cytokine production (29, 30). However, no Ag has been identified

![FIGURE 4. IFN-γ and TNF production by T cells in response to IL-15. LP-T cells (A and B) or PB-T cells (C and D) (1 × 10⁶/ml) from normal donors (Nor), control patients (Con), and IBD patients were cultured in the absence or presence of IL-15 (100 ng/ml). After 72 h of culture, supernatants were harvested and assayed for IFN-γ (A and C) and TNF (B and D) by ELISA. The horizontal bars represent mean values. *, p < 0.05 vs controls; **, p < 0.005 vs controls; +, p < 0.05 vs UC LP-T cells under the same culture conditions.

![FIGURE 5. Kinetics of CD69 expression on T cells after IL-15 stimulation. PB-T (A) or LP-T (B) (1.0 × 10⁶/ml) from healthy donors, control patients (Con), and IBD patients were cultured and stimulated with IL-15 (100 ng/ml) for 24 and 48 h. The resting and activated cells were stained with either PE-conjugated anti-CD3 mAb and FITC-conjugated anti-CD69 mAb or FITC- and PE-conjugated isotype-matched mouse IgG, and analyzed on a FACSort. Data were expressed as mean percentage of CD69⁺ CD3⁺ T cells ± SEM in each group at each time point. *, p < 0.005 vs controls at the same time point or vs fresh T cells; +, p < 0.05 vs fresh T cells (t = 0).
so far to trigger this chain of events. The results presented in this study suggest the involvement of IL-15 in the pathogenesis of IBD. First, we confirm that IL-15 is overexpressed in inflamed mucosa of IBD patients and show that activated macrophages are the source of IL-15. Further, we found that IL-15, in the absence of a primary stimulus, induces IBD LP-T cells to proliferate and to produce proinflammatory cytokines such as TNF and IFN-γ. Finally, our results also show that IL-15 facilitates monocyte IL-12 and TNF production in a T cell-macrophage contact-dependent way, in which CD40-CD40L interactions play an essential role.

IL-15 mRNA is expressed in a variety of tissues and cells (1, 4), while its protein is mainly produced by activated monocytes/macrophages and monocyte-derived dendritic cells, especially on stimulation with microbial products such as LPS, *M. leprae*, and *S. aureus* Cowan strain 1 (5, 6, 8, 15, 21, 27). Findings on expression of IL-15 mRNA without protein suggest that production of IL-15 mRNA and IL-15 protein are differentially regulated. Our results show local production of IL-15 in the inflamed mucosa in IBD as evidenced by immunohistochemistry but also by culture of LPMC with LPS or IFN-γ, allowing us to demonstrate the actual secretion of this cytokine. Similar findings have been reported in patients with RA and leprosy (15, 21). Although CD40 ligation of monocytes or macrophages strongly induces proinflammatory cytokine production such as IL-6, IL-8, IL-12, and TNF (33, 34), our present results demonstrate that IL-15 is not secreted by monocytes when stimulated with IBD LP-T cells, previously shown to express functional CD40L (25). Moreover, CD40L transfectants were unable to induce IL-15 production by lamina propia macrophages, although some IL-12 and TNF were found in the same coculture system. We also cocultured purified monocytes from four healthy donors with CD40L-transfected 3T6 cells for 48 h. IL-15 protein was not measured in the supernatants (data not shown). These findings suggest that IL-15 secretion by mucosal macrophages is not induced by T cell-dependent CD40 engagement but that it may rather be induced by microbial Ags. Our results are in accordance with earlier reports (27, 35). IL-15 protein is not detected in the supernatants of PBMC when stimulated with irradiated CD40L-transfected L cells, but it can be measured...
in *S. aureus* Cowan strain-stimulated cell cultures (27). Monocyte-derived dendritic cells produce IL-15 when infected by influenza virus, but not when stimulated with CD40L or LPS (35). To date, luminal bacteria and their products have been proposed to be involved in the initiation and perpetuation of IBD (29, 30). One mechanism potentially involved might be through induction of IL-15. A leaky intestinal barrier with increased intestinal permeability in IBD intensifies luminal Ag absorption (29, 30), which, in turn, may lead to an exaggerated local IL-15 production in the mucosa.

Several studies have demonstrated effects of IL-15 on resting T cells. IL-15 signals its receptor on the surface of T cells and induces the activation of Janus kinase 1 and 2 as well as the tyrosine phosphorylation and nuclear translocation of STAT3 and STAT5 (36). IL-15 selectively induces memory CD4+⁸⁻, CD8+ T cell, and naive CD8+ T cell proliferation, but not naive CD4+ T cell proliferation (9). Thus, it is considered as important for T cell activation in a TCR/CD3 complex-independent fashion, and it may preferentially facilitate the activation of memory T cells which in inflammatory tissues represent the majority of T cells (37). IL-15 has also been shown to induce cytokine secretion by T cells (10, 16, 28). The present studies demonstrate that IBD LP-T cells are sensitive to IL-15. This finding was different from an earlier report (31) in which IL-15 was unable to induce CD40L expression on resting naive CD4+ T cells from healthy controls. In fact, we also did not observe CD40L neutralizing mAb in these cocultures. Down-regulation of IL-15 production by macrophages and IL-15-activated T cells has been shown to lead to enhanced production of TNF and IL-12 by monocytes/macrophages (16, 31, 39). Inclusion of anti-CD40L mAb effectively prevents mucusal inflammation in CD (38). Therefore, our findings on IL-15 effects might be relevant to the pathogenesis of IBD, as IL-15 stimulates mucusal T cell activation and proinflammatory cytokine production. Moreover, IL-15 may be an effecter cytokine for strong bystander stimulation of T cells and up-regulating their survival (12, 31).

In a previous study, we have demonstrated that IBD LP-T cells express CD40L and that they can induce IL-12 and TNF production by monocytes in a coculture system. This monocyte cytokine production is initiated by CD40 ligation (25). In contrast, cell contact between monocytes/macrophages and IL-15-activated T cells has been shown to lead to enhanced production of TNF and IL-12 by monocytes/macrophages (16, 31, 39). Inclusion of anti-CD40L neutralizing mAb in these cocultures was shown by Avie et al. to down-regulate IL-12 production, indicating that CD40-CD40L interactions play a critical role in the IL-15 effects (31). Our results demonstrated that IL-15 could stimulate IBD LP-T cells to express CD40L and that IL-15-activated IBD LP-T cells more strongly (as compared with freshly isolated IBD LP-T cells) induced monocyte TNF and IL-12 secretion in a CD40-CD40L interaction-dependent manner. Because the monocytes in our coculture system have not been in contact with IL-15, we think that the effect of IL-15 on monocyte cytokine secretion can be predominantly attributed to the enhanced CD40L expression on T cells after incubation with IL-15. This finding was different from an earlier report (31) in which IL-15 was unable to induce CD40L expression on resting CD4+ T cells from healthy controls. In fact, we also did not observe an effect of IL-15 on PB-T cell CD40L expression, but only on LP-T cells. Thus, IL-15 seems to enhance CD40L expression on activated T cells only. To further substantiate this point, we have stimulated PB-T cells from IBD patients and normal controls with immobilized anti-CD3. CD40L expression was found to be markedly induced on these T cells after 12–16 h of culture. Importantly, in the presence of IL-15, CD40L expression was further enhanced (our unpublished results).

The observations made in the present studies provide a potential mechanism of T cell responses in inflamed mucosa of IBD. A model system can be envisaged in which exposure to inflammatory stimuli in vivo such as LPS or mycobacterial heat-shock proteins causes mucosal macrophages to secrete proinflammatory mediators such as IL-15 and TNF. Both function to recruit leukocytes into inflamed mucosa. IL-15 induces T cell proliferation, favors T cell/macrophage interactions and proinflammatory cytokine production, and promotes B cell isotype switching in the lamina propria. IL-15 not only directly triggers T cell activation and proinflammatory cytokine production, but it also may synergize with other cytokines (e.g., IL-12) to mediate immune injury in the mucosa (40). IL-15 indeed enhances expression of the IL-12Rβ1 chain on CD4+ T cells and synergizes with IL-12 to strongly induce CD4+ T cell IFN-γ production (31). Additionally, IL-15 may also induce intraepithelial lymphocyte proliferation and enhance their cytotoxic activity against intestinal epithelial cells (41). All these events, which can potentially be initiated and/or maintained by IL-15, may result in chronic inflammation, an abnormal immune response, and tissue damage. Therefore, treatment targeted against IL-15 may be beneficial for these patients.

Acknowledgments

We thank Dr. R. Armitage (InnuneX, Seattle, WA) for providing us with the anti-CD40L mAb M90 and M. Adé for expert technical assistance in performing the ELISA.

References


