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Possible involvement of the interleukin-15 and interleukin-15 receptor system in a heightened state of lamina propria B cell activation and differentiation in patients with inflammatory bowel disease

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Background. We investigated the possible roles of the interleukin (IL)-15 and IL-15 receptor (IL-15R) system in a heightened state of B-cell activation and differentiation in intestinal mucosa with inflammatory bowel disease (IBD). ***Methods.*** The expression of IL-15 and IL-15Rα mRNA and protein in inflamed colonic mucosal tissues with IBD, and in control tissues was examined by reverse transcriptase-polymerase chain reaction and immunohistological methods. The effects of recombinant (r)IL-15 on the expression of IL-15Rα on lamina propria B cells and the production of immunoglobulin G (IgG) were analyzed in vitro, using lamina propria mononuclear cells (LPMCs) isolated from control tissues. ***Results.*** The intensity of IL-15 and IL-15Rα mRNA was greater in the mucosal tissues of patients with IBD, especially in those of patients with ulcerative colitis (UC), than in control tissues. Compared to control tissues, mononuclear cells positive for IL-15Rα protein were observed in greater proportions in tissue sections from patients with IBD, especially in those from patients with UC, where IL-15Rα protein was localized to CD20-positive B cells to a significant degree. There were increases in the proportions of IL-15Rα-positive B cells and IgG-producing cells in rIL-15- or rCD40L-stimulated cultures of LPMCs, with stimulatory effects being greater in the presence of their combination. ***Conclusions.*** These data suggest that the IL-15 and IL-15R system may play important roles in the activation and differentiation of lamina propria B cells in patients with IBD, especially in those with UC.

Key words: interleukin-15, interleukin-15 receptor, B cells, ulcerative colitis, Crohn's disease

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Introduction

There is evidence that the deficient or excessive expression of and responses to immunoregulatory cytokines are almost certain to be involved in the pathogenesis of both forms of inflammatory bowel disease (IBD)—ulcerative colitis (UC) and Crohn's disease (CD).[1] Among various immunoregulatory cytokines, interleukin (IL)-2 has attracted much attention because of its central role in immune cells.[2] Although it is well documented that IL-2 receptor (IL-2R)α expression is increased in isolated lamina propria mononuclear cells (LPMCs), and soluble IL-2Rα concentrations in plasma and cultures of LPMCs are elevated in patients with IBD,[3–5] several studies have shown that IL-2 bioactivity is not detected or is present in barely detectable concentrations in their tissue homogenates or unstimulated cultures of LPMCs and mucosal biopsy specimens,[6–8] despite the fact that increased numbers of T cells and B cells with activation markers infiltrate the mucosal lesions of IBD.[3,4] Other immunoregulatory cytokines with similar functional properties are assumed to be involved in immune activation in the inflamed mucosa of patients with IBD.

IL-15, a novel cytokine with wide cellular distribution, in, for example, monocytes, epithelium, endothelium, fibroblasts, and muscle cells, uses β-, and γ-chains of IL-2R for signal transduction.[9] Despite a lack of sequence identity, IL-15 exerts many immunological activities previously described for IL-2 in various types of cells. These include stimulation of the proliferation and activation of T cells, the production of inflammatory cytokines from natural killer cells, and the migration of T cells and natural killer cells.[10–15] IL-15 also causes the induction of proliferation and differentiation in B cells, the expression of cytotoxic programs in T cells, natural killer cells, and lymphokine-activated killer cells, and the regulation of survival of T cells, natural killer cells, and neutrophils.[10,16–24] Because of these inherent mul-

tiple immunological functions, IL-15 is implicated in the pathogenesis and maintenance of several chronic inflammatory diseases with immune activation.[9,25] Indeed, recent studies have indicated the possible contribution of IL-15 to local immune responses in patients with IBD. Kirman and Nielsen[26] reported that IBD patients with moderate or severe disease activity, but not those with slight disease activity or controls, had an increased percentage of IL-15-expressing peripheral blood mononuclear cells. In our previous study, increased expression of IL-15 mRNA and protein was observed in the intestinal mucosa of patients with IBD.[8] The relevance of IL-15 to the local immune response in patients with IBD was further confirmed by Liu et al.,[27] who showed that IL-15 was highly expressed in the inflamed mucosa of patients with IBD, with possible stimulatory effects on mucosal T cells.

A fairly consistent mode of alterations in the number and composition of activated B cells and plasma cells has been reported to be present in the lamina propria of intestinal mucosa with IBD. There are increased expressions of cell-surface activation antigens, such as IL-2R, transferrin receptor, and 4F2, on lamina propria B cells in both UC and CD.[3] Increased numbers of immunoglobulin G (IgG) plasma cells have also been shown to be scattered in the inflamed areas, with positive correlations with the degree of histological inflammation, which is in parallel with the observation that cultures of isolated LPMCs from such areas synthesize and secrete IgG to a much greater degree than do the cells from control mucosa.[28–32] Although these phenomena have been thought to represent a general response to a variety of inflammatory stimuli, including immunoregulatory cytokines, it is largely unknown whether IL-15 could contribute to a heightened state of B-cell activation and differentiation in intestinal mucosa with IBD. We therefore evaluated the mucosal expression of IL-15 and its specific α-chain receptor, IL-15Rα, in the inflamed colonic tissues of patients with IBD. We also examined the effects of IL-15 on the activation and differentiation of lamina propria B cells, using LPMCs isolated from control tissues.

Patients, materials, and methods

Study groups

Inflamed colonic mucosal tissues were obtained from colonoscopic biopsies in 15 patients with UC. The group consisted of eight men and seven women, ranging in age from 17 to 58 years (mean, 31 years). Nine patients had total colitis, 4 had left-sided colitis, and 2 had proctitis. The duration of disease ranged from 3 months to 11 years. At the time of study, 5 patients were receiving 5-aminosalicylic acid or sulfasalazine alone, 2 were on prednisolone alone, 6 were being treated with prednisolone plus 5-aminosalicylic acid or sulfasalazine, and 2 were on no specific drug therapy. Inflamed colonic mucosal tissues were also obtained from colonoscopic biopsies in 15 patients with CD. The group included nine men and six women, ranging in age from 21 to 52 years (mean, 27 years). Six patients had disease limited to the colon, and 9 had disease in the colon and small intestine. The disease had been present for 9 months to 13 years. At the time of the study, 10 patients were receiving 5-aminosalicylic acid alone, 2 were being treated with prednisolone plus 5-aminosalicylic acid, and 3 were on no specific drug therapy. Uninflamed normal colonic mucosal tissues were obtained from colonoscopic biopsies in 12 patients with colonic adenoma and from surgical specimens in 8 patients with colonic adenocarcinoma. This control group consisted of 12 men and eight women, ranging in age from 31 to 72 years (mean, 57 years). All samples were obtained with informed consent, in accordance with the Helsinki Declaration, and the presence or absence of inflammation was confirmed by the histological examination of adjacent tissues in all patients.

Reverse transcriptase-polymerase chain reaction (RT-PCR) amplification

The expression of the transcripts for IL-15 and IL-15Rα in the biopsy specimens of colonic mucosal tissues was determined by RT-PCR, as described elsewhere.[33–36] Total RNA was extracted from biopsy tissues by the acid guanidinium thiocyanate-phenol-chloroform method. Single-step amplification of the *IL-15* and *IL-15R*α genes was performed in a 50-μl reaction mixture, containing 5 U of AMV reverse transcriptase, 0.4 mM of the sense and antisense primers for IL-15, IL-15Rα, or β-actin,[33–37] and 5 U of Taq polymerase, according to the manufacturer's instructions (Titan One Tube RT-PCR Kit; Roche Molecular Biochemicals, Mannheim, Germany). Reverse-transcription of total RNA into complementary DNA was conducted at 50°C for 30 min, and the amplification phase consisted of 30 cycles (β-actin) or 35 cycles (IL-15 and IL-15Rα) of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min. As a negative control, the DNA sample was omitted from the reaction mixture. The amplified products were electrophoresed in a 1.5% agarose gel containing ethidium bromide, and an image analysis system was used to quantify the signal intensity of IL-15 and IL-15Rα mRNA with the baseline for normalization being set as the intensity of β-actin.

Histology

Immunohistochemistry for the detection of IL-15Rα was performed using the biospy specimens of colonic mucosal tissues in patients with IBD and controls, as described elsewhere.[38–40] In brief, the tissue was immediately frozen in liquid nitrogen with OCT compound (Miles, Elkhart, IN, USA). Sections, 4-μm-thick, were cut from frozen tissue by a cryostat, fixed in acetone at room temperature for 15 min, and treated with 0.3% hydrogen peroxide in absolute methanol to quench endogenous peroxidase activity. After the blocking of background staining with normal goat serum diluted 1:40, the sections were incubated with the anti-human IL-15Rα polyclonal antibody, H-107 (1:50 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C for 2 h. The color was developed using a streptavidin-biotin detection kit, DAKO LSAB-2 System, Peroxidase (Dakopatts, Glostrup, Denmark), with subsequent counterstaining with hematoxylin. As negative controls for each sample, the anti-human IL-15Rα polyclonal antibody was omitted or replaced by control rabbit IgG (Pepro Tech, Rocky Hill, NJ, USA). Lamina propria mononuclear cells positive for IL-15Rα were counted in three random fields and values were expressed as the mean number of cells per mm^2.

An indirect two-color immunofluorescence microscopic study was performed, using the biopsy specimens, to identify the phenotype of IL-15Rα-positive cells in the colonic mucosa according to a method described previously.[35,41] Briefly, 4-μm-thick cryostat sections were fixed in acetone at room temperature for 15 min and incubated with normal goat serum diluted 1:40. The sections were incubated with a combination of the anti-human IL-15Rα polyclonal antibody, H-107 (1:40 dilution) and the monoclonal antibody (1:50 dilution), L-26 (anti-CD20; Dakopatts) or UCHT-1 (anti-CD3; Dakopatts), followed by incubation with the fluorescein isothiocyanate (FITC)-conjugated Fab fraction of goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) and lissamine rhodamine-conjugated Fab fraction of goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories). As negative controls, the rabbit serum was used instead of the anti-human IL-15Rα polyclonal antibody, or the primary antibody was omitted. Then the tissue sections were covered with a medium containing *p*-phenylenediamine and observed with a MRC-1024 confocal laser scanning fluorescence microscope (BioRad Laboratories, Hercules, CA, USA).

Isolation of LPMCs

LPMCs were isolated from the surgical specimens of uninflamed control colonic mucosal tissues by an enzymatic method, using collagenase and deoxyribonuclease, followed by purification with a Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradient, as described elsewhere.[8,39,42] Cell viability of isolated LPMCs was more than 95%, as determined by trypan blue exclusion.

Flow cytometric analysis

Freshly isolated LPMCs were cultured, at 2×10^6 cells/ml, in RPMI-1640 medium with 5% heat-inactivated fetal calf serum (FCS), hydroxyethylpiperazine ethanesulfonic acid buffer, 100 units/ml of penicillin G, and 100 μg/ml of streptomycin (culture medium) in the presence or absence of 100 ng/ml of recombinant (r)IL-15 (R&D Systems, Minneapolis, MN, USA), 100 ng/ml of rCD40L (R&D Systems), or 10 μg/ml of phytohemagglutinin-P (PHA; Sigma Chemical, St. Louis, MO, USA) for 24 h in a 5% CO_2 incubator. At the end of the incubation period, the cells were suspended in Hank's balanced salt solution containing 1% FCS and 0.1% NaN_3. They were then incubated with adequate amounts of the PC-5-conjugated anti-CD20 antibody (Immunotech, Marseille, France), phycoerythrin (PE)-conjugated anti-HLA-DR antibody (BD Biosciences, San Diego, CA USA), and the anti-human IL-15Rα polyclonal antibody, H-107, at 4°C for 30 min, followed by incubation with the FITC-conjugated goat anti-rabbit IgG (BD Biosciences). The stained cells were analyzed with the flow cytometer FACScaliber (BD Biosciences).[43]

Enzyme-linked immunospot (ELISPOT) assay

An ELISPOT assay was used to enumerate the numbers of IgG-producing cells among LPMCs, as described elsewhere.[42] In brief, microwell plates with a nitrocellulose membrane (Millititer HA; Millipore, Bedford, MA, USA) were coated overnight at 4°C with 1 mg/ml of polyclonal antibody against human IgG Fc (Sigma Chemical). All wells were blocked with the culture medium at room temperature for 60 min before use. Freshly isolated LPMCs were cultured, at 5×10^5 cells/ml, in the culture medium in the presence or absence of 100 ng/ml of rIL-15, 100 ng/ml of rCD40L, or 10 μg/ml of PHA, for 36 h in a 5% CO_2 incubator. At the end of incubation period, the cells were harvested from the culture, transferred to the above microwell plates, and incubated for an additional 96 h in a 5% CO_2 incubator. The plates were then washed thoroughly and incubated with 1 μg/ml of biotin-conjugated polyclonal antibody against human IgG γ-chain (Sigma Chemical) at 4°C for 2 h. The spots of single IgG-producing cells were developed by diaminobenzidine solution contain-

Table 1. Intensity of interleukin (IL)-15 and IL-15 receptor α (IL-15Rα) mRNA in the mucosal tissues of patients with IBD and in control tissues

		IL-15Rα mRNA	
	IL-15 mRNA	531 bp	432 bp
UC (n = 15)	0.82 ± 0.10*	0.53 ± 0.08**	0.36 ± 0.03**
CD (n = 15)	0.55 ± 0.07	0.32 ± 0.03**	0.28 ± 0.02**
Control (n = 12)	0.42 ± 0.04	0.16 ± 0.01	0.14 ± 0.01

* $P < 0.01$; ** $P < 0.005$ vs control
The signal intensity of IL-15 and IL-15Rα mRNA was quantified, with the baseline for normalization being set as the intensity of β-actin
Data values are shown as means ± SE

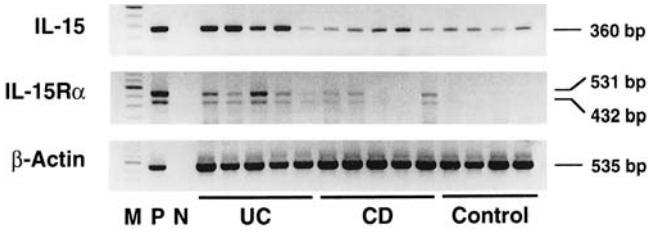

Fig. 1. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis for interleukin (IL)-15, IL-15 receptor α (*IL-15Rα*), and β-actin mRNA in inflamed colonic mucosal tissues with ulcerative colitis (*UC*) or Crohn's disease (*CD*) and uninflamed control tissues. The figure is representative of three separate experiments (*UC*, n = 15; *CD*, n = 15; *control*, n = 12). *M*, 100-bp marker; *P*, positive control (monocytes and phytohemagglutinin-P [PHA]-stimulated peripheral blood mononuclear cells for IL-15 and IL-15Rα mRNA, respectively); *N*, negative control (no cDNA sample)

ing 0.01% H_2O_2. The number of spots was counted using a dissecting microscope.

Statistical analysis

The *t*- and paired *t*-tests were used for statistical analysis, and all data values are shown as means ± SE.

Results

Mucosal expression of IL-15 and IL-15Rα mRNA

Because our previous study with organ cultures and in situ hybridization showed that IL-15 was overexpressed in the inflamed mucosa of patients with IBD,[8] we investigated whether the mucosal IL-15R system was upregulated in such patients. On RT-PCR analysis (Table 1), the intensity of IL-15 and IL-15Rα mRNA was greater in the mucosal tissues of patients with IBD, especially in those of patients with UC, than in control tissues. Representative results obtained on RT-PCR analysis are shown in Fig. 1.

Mucosal expression of IL-15Rα protein

Our immunohistochemical study (UC, n = 8; CD, n = 8; control, n = 8) detected IL-15Rα immunoreactivity in mononuclear cells infiltrating the lamina propria of the colonic mucosa. As shown in Fig. 2, mononuclear cells positive for IL-15Rα protein were observed in greater proportions in the sections from patients with IBD, especially in those from patients with UC (UC, 603 ± 47/mm^2; $P < 0.005$; CD, 429 ± 17/mm^2; $P < 0.01$) than in the control specimens (265 ± 43/mm^2). However, colonic epithelial cells did not express IL-15Rα protein in either IBD or control specimens. A double-immunofluorescence microscopic study was conducted in the tissue sections of patients with IBD showing increased numbers of IL-15Rα-positive cells in the lamina propria (UC, n = 6; CD, n = 6). It showed higher percentages of IL-15Rα positivity in CD20-positive B cells (48.2 ± 4.1%; $P < 0.05$, Fig. 3) than in CD3-positive T cells (30.1 ± 6.0%).

Proportions of IL-15Rα-positive B cells in rIL-15- or rCD40L-stimulated cultures of LPMCs

Parallel cultures of LPMCs were incubated with and without rIL-15, rCD40L, or PHA for 24 h, and three-color flow cytometric analysis was performed to determine the percentages of lamina propia B cells with the expression of IL-15Rα and the activation marker, HLA-DR (Fig. 4). As shown in Table 2, there were increases in the proportions of IL-15Rα-positive cells in rIL-15- or rCD40L-stimulated cultures, with greater stimulatory effects in the presence of both rIL-15 and rCD40L, albeit at a less significant degree as compared with the response induced by PHA. Notably, most IL-

Table 2. Positivity of IL-15Rα and HLA-DR expression in CD20-gated B-cell fraction in cultures of lamina propria mononuclear cells (LPMCs) incubated with or without rIL-15, rCD40L, or phytohemagglutinin (PHA)

	IL-15Rα$^+$ (%)	HLA-DR$^-$, IL-15Rα$^+$ (%)	HLA-DR$^+$, IL-15Rα$^+$ (%)
Medium	32.7 ± 4.8	6.1 ± 2.4	26.6 ± 5.5
rIL-15[a]	40.1 ± 6.0	5.8 ± 2.3	34.3 ± 7.0
rCD40L[a]	42.0 ± 5.3	8.9 ± 2.4	33.1 ± 6.8
rIL-15[a] + rCD40L[a]	51.2 ± 6.4*	9.1 ± 2.8	42.0 ± 8.2*
PHA[b]	69.1 ± 7.5*	7.6 ± 3.6	61.6 ± 9.5*

* $P < 0.01$ vs medium
Data values are shown as means ± SE in the experiments using control LPMCs ($n = 7$)
The proportions of IL-15Rα$^+$ cells, HLA-DR$^-$, IL-15Rα$^+$ cells, and HLA-DR$^+$, IL-15Rα$^+$ cells in freshly isolated LPMCs were 32.4 ± 9.5%, 10.3 ± 3.5%, and 22.2 ± 6.4%, respectively
[a] 100 ng/ml
[b] 10 μg/ml

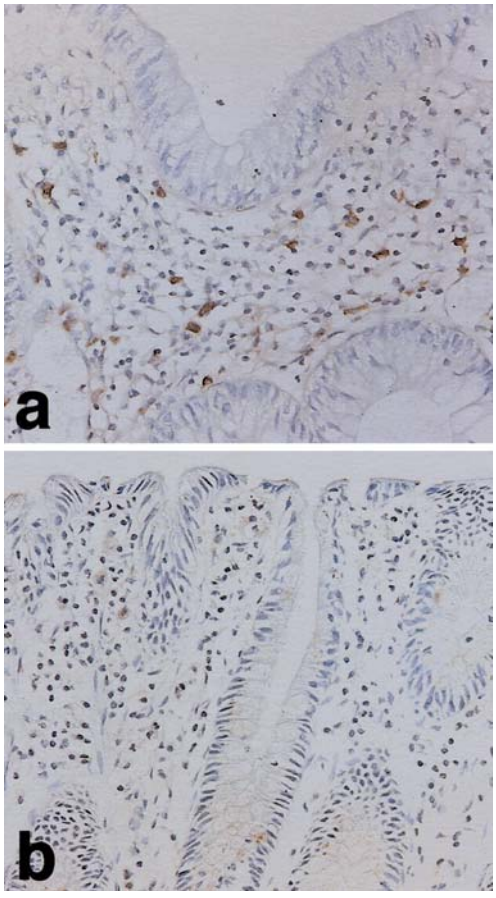

Fig. 2a,b. Immunohistochemistry, detecting IL-15Rα in infiltrating cells in sections of inflamed colonic mucosal tissues with UC (**a**) and uninflamed control tissues (**b**). Formation of *brown signals* indicates the localization of IL-15Rα protein. The figures are representative of four separate experiments (UC, $n = 8$; CD, $n = 8$; control, $n = 8$). **a,b** ×200

15Rα-positive cells were detected in the cell fraction with HLA-DR, suggesting that the expression of IL-15Rα might be associated with the activation process of lamina propria B cells.

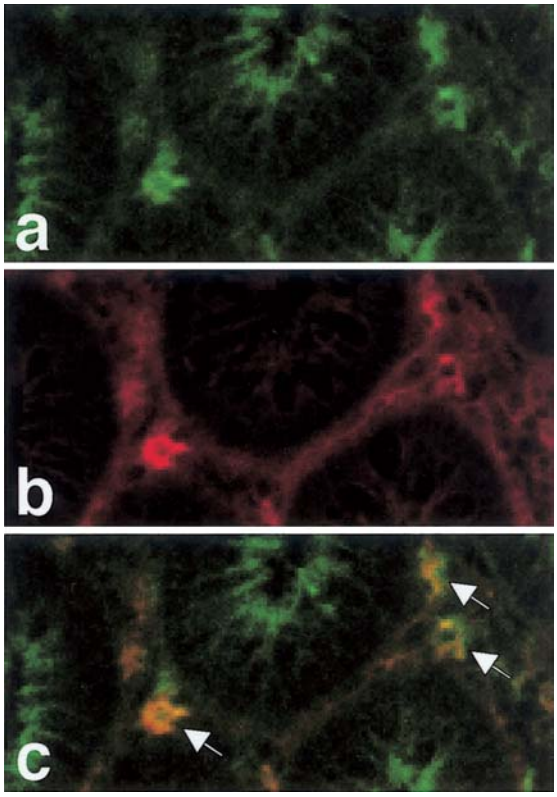

Fig. 3a–c. Confocal laser scanning microscopy, showing immunofluorescence staining of CD20 (**a**, *green*) plus IL-15Rα (**b**, *red*) in infiltrating cells in section of inflamed colonic mucosal tissues with UC. Double-positive cells are stained *yellow* (**c**, *arrows*). The figures are representative of three separate experiments (UC, $n = 6$; CD, $n = 6$). **a–c** ×200

Numbers of IgG-producing cells in rIL-15- or rCD40L-stimulated cultures of LPMCs

Separate cultures of LPMCs were incubated with and without rIL-15, rCD40L, or PHA for 36 h, and after an additional period of 96 h, the numbers of IgG-producing cells were examined by an ELISPOT assay. In parallel with the results in the experiments for IL-15Rα expression on lamina propria B cells, increases in the numbers

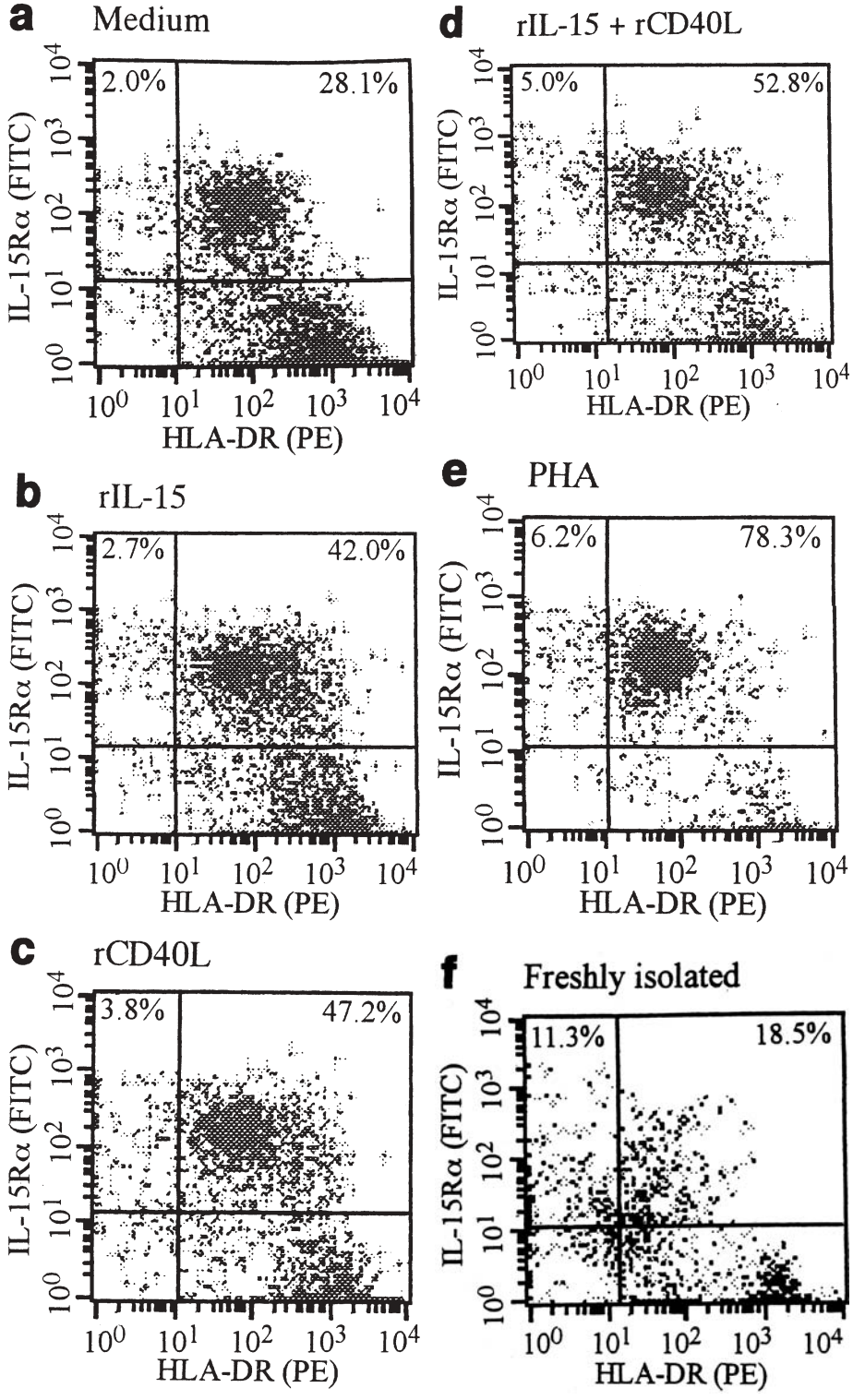

Fig. 4a–f. Flow cytometric analysis, detecting positivity of *IL-15Rα* and *HLA-DR* expression in CD20-gated B-cell fraction in cultures of lamina propria mononuclear cells (LPMCs) incubated with or without recombinant IL-15 (*rIL-15*), rCD40L, or PHA (**a**, medium; **b**, rIL-15; **c**, rCD40L; **d**, rIL-15 + rCD40L; **e**, PHA; **f**, freshly isolated cells). The figures are representative of seven separate experiments using control LPMCs. *FITC*, fluorescein isothiocyanate; *PE*, phycoerythrin

of IgG-producing cells were observed in rIL-15- or rCD40L-stimulated cultures, with additive effects in the presence of their combination, although these effects were lower than those seen when the cells were cultured with PHA (Table 3).

Discussion

Recent evidence indicates that the expression of IL-15Rα chain that is induced by cell activation is both necessary and sufficient for IL-15 binding on immune

Table 3. Number of IgG-producing cells in cultures of LPMCs incubated with or without rIL-15, rCD40L, or PHA

	IgG-producing cells (per 10^5 cells)
Medium	73.8 ± 13.8
rIL-15[a]	96.7 ± 19.8
rCD40L[a]	109.9 ± 10.5
rIL-15[a] + rCD40L[a]	122.7 ± 19.2*
PHA[b]	209.3 ± 47.4*

* $P < 0.01$ vs medium
Data values are shown as means ± SE in the experiments using control LPMCs ($n = 8$)
[a] 100 ng/ml
[b] 10 μg/ml

cells, suggesting that the regulation of IL-15Rα expression may be an important process for subsequent IL-15-related immune responses.[44] In this study, we investigated, simultaneously, the mucosal expression of IL-15 and IL-15Rα by RT-PCR analysis, using the inflamed tissues of patients with IBD and uninflamed control tissues. The intensity of IL-15 and IL-15Rα mRNA was greater in the mucosal tissues of patients with IBD, especially in those of patients with UC, than in control tissues. We previously found that increased mucosal expression of IL-15 protein was detected in the intestinal mucosa of patients with IBD, and this abnormality was found more frequently in patients with UC than in those with CD.[8] Our subsequent studies showed that IL-15 was capable of inducing a proliferative response and lymphokine-activated killer activity, expression of cell-surface IL-2Rα, release of soluble IL-2Rα, and the production of tumor necrosis factor-α and interferon-γ in LPMCs, as well as the prevention of apoptosis in mucosal T-cell lines.[8,45] These functional roles of IL-15 in mucosal immune cells were also confirmed by a recent study showing that this cytokine could enhance proliferation and activation in local T cells and proinflammatory cytokine production by monocytes in a coculture system.[27] Taken as a whole, it is strongly suggested that the IL-15 and IL-15R system may be deeply involved in the activation and maintenance of mucosal immune cells in IBD, especially in UC.

In keeping with a previous observation that IL-15Rα was distributed on activated peripheral blood mononuclear cells, but not on resting cells,[44] we were interested in localizing IL-15Rα protein in the intestinal mucosa, where resident mononuclear cells are in a heightened state of activation because of continuous exposure to a myriad of antigens. Immunohistochemical analysis in the present study showed that the localization of IL-15Rα protein was essentially restricted to mononuclear cells infiltrating within the lamina propria. As expected from the results of RT-PCR analysis, mononuclear cells positive for IL-15Rα protein were observed in greater proportions in the tissue sections from patients with IBD, especially in those from patients with UC, as compared with controls. Double-immunofluorescence microscopic study showed higher percentages of IL-15Rα positivity in CD20-positive B cells than in CD3-positive T cells in the tissue sections of patients with IBD showing increased numbers of IL-15Rα-positive cells. These findings not only confirm a heightened state of activation in lamina propria B cells in patients with IBD, especially in those with UC, but also suggest that these cells are a primary target for the IL-15 protein that is overexpressed in situ in such patients.[8,27,45]

It is generally accepted that the induction of B-cell activation is mediated largely through the interaction between CD40L and immunoregulatory cytokines, including IL-15.[17,46–48] Because the above immunohistological results suggested that the expression of IL-15Rα in lamina propria B cells might be associated with the cell-activation process, we examined the percentages of IL-15Rα-positive B cells in LPMCs after they were cultured in the presence of rIL-15 and/or rCD40L. There were increases in the proportions of IL-15Rα-positive B cells in rIL-15- or rCD40L-stimulated cultures, with greater stimulatory effects in the presence of both rIL-15 and rCD40L, albeit at a less significant degree as compared with the response induced by PHA (which acts as a polyclonal mitogenic activator). These findings imply that IL-15 itself and CD40L may represent the relevant soluble factors for the induction of IL-15Rα on lamina propria B cells in situ. In this respect, our preliminary experiments have shown that LPMCs could produce soluble CD40L when they were cultured in the presence of rIL-15 (unpublished observation). Taken together, these results suggest that the IL-15 and IL-15R system may play important roles in the activation of lamina propria B cells under costimulation by the release of soluble CD40L from activated T cells or the induction of membrane-bound CD40L on activated T cells.[17,46–48]

It has been shown that IL-15 has stimulatory activity for the induction of IgG secretion in B cells costimulated with CD40L.[17] To address whether the IL-15 and the IL-15R system is involved in the differentiation of lamina propria B cells, we examined the numbers of IgG-producing cells after LPMCs were cultured with rIL-15 and/or rCD40L. In parallel with the results obtained in the experiments for IL-15Rα expression on lamina propria B cells, increases in the number of IgG-producing cells were observed in rIL-15- or rCD40L-stimulated cultures, with additive effects in the presence of the combination, although the effects were lower than those seen when the cells were cultured with PHA. The role of the IL-15 and IL-15R system in the differentiation of lamina propria B cells was also re-

cently shown by Hiroi et al.,[49] who have demonstrated that IL-15 is a critically important cytokine for the differentiation of $sIgM^+$, $sIgA^-$ and $sIgM^-$, and $sIgA^+$ B-1 cells expressing IL-15R into IgA-producing cells in mucosal tissues. These findings strongly suggest that the IL-15 and IL-15R system could contribute to a heightened state of B-cell differentiation in the intestinal mucosal lesions of IBD, especially in those with UC, where increased expression of both IL-15 and IL-15Rα is observed.

In summary, the data presented here suggest that the IL-15 and IL-15R system may play important roles in the activation and differentiation of lamina propria B cells in patients with IBD, especially in those with UC. Further studies will be required to clarify the molecular mechanisms involved in this phenomenon.

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