The Journal of Immunology

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J Immunol 2000; 165:7234-7239; ;
doi: 10.4049/jimmunol.165.12.7234
http://www.jimmunol.org/content/165/12/7234

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Up-Regulation of the IL-12 Receptor β2 Chain in Crohn's Disease[1]

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Crohn' s disease (CD) is a chronic intestinal inflammatory disorder characterized by aberrant mucosal Th1 cell activation and production of IL-12, the major Th1-driving factor. The T cell response to IL-12 is dependent on the expression of a specific receptor composed of two subunits, termed IL-12Rβ1 and IL-12Rβ2. The content of IL-12Rβ2, as measured at the mRNA level, is crucial in regulating Th1 differentiation. In this study we therefore investigated IL-12Rβ2 RNA transcripts in CD. IL-12Rβ2 expression was increased in active CD as well as *Helicobacter pylori* (HP)-associated gastritis and *Salmonella* colitis compared with that in inactive CD, ulcerative colitis, noninflammatory controls, and celiac disease. In contrast, IL-12Rβ1 transcripts were expressed at comparable levels in all samples. In CD, IL-12Rβ2 expression strictly correlated with tyrosine phosphorylation of STAT4, a key component of the IL-12-dependent Th1 polarization. This was associated with a pronounced expression of IFN-γ. Transcripts for IL-12/p40 were detected in CD, HP-positive, and *Salmonella* colitis patients, but not in celiac disease, indicating that IL-12Rβ2 up-regulation occurs only in IL-12-associated Th1 gastrointestinal diseases. Finally, we showed that stimulation of lamina propria mononuclear cells with IL-12 enhanced IL-12Rβ2, suggesting that IL-12 regulates IL-12Rβ2 expression in human gastrointestinal mucosa. The data show that the signaling pathway used by IL-12 to induce Th1 differentiation is increased at the site of disease in CD, further supporting the view that IL-12/IL-12R signals contribute to the inflammatory response in this condition. ***The Journal of Immunology,*** **2000, 165: 7234–7239.**

A large body of evidence indicates that activated Th1 cells play a central role in the pathogenesis of tissue damage in Crohn's disease (CD)[3] (1). Data have been also provided showing that IL-12, the major Th1-inducing factor, is up-regulated in CD tissue and that IL-12 contributes to the preferential expansion of IFN-γ-secreting cells in this condition (2–5). Furthermore, studies in murine models have demonstrated that neutralization of IL-12 leads to rapid and complete recovery of experimental colitis resembling CD (6). Signal transduction by IL-12 results in rapid phosphorylation and activation of STAT molecules (7). Although IL-12 can activate both STAT3 and STAT4, the effect of IL-12 on Th1 cell differentiation depends specifically on the expression of STAT4. Indeed, T cells from STAT4-deficient mice manifest impaired IFN-γ production in response to IL-12 and are unable to promote the development of colitis when transferred to immunodeficient mice (8, 9). In contrast, overexpression of STAT4 in transgenic mice results in the induction of Th1-mediated colitis (10). Taken together these observations support the concept that the IL-12/STAT4 signaling pathway is important in promoting Th1 cell activation and causing tissue injury in the intestine (11, 12).

The T cell response to IL-12 is dependent on the expression of high affinity IL-12R composed of two subunits, termed IL-12Rβ1 and IL-12Rβ2 (13). Studies in both humans and experimental models have demonstrated that the responsiveness to IL-12 of Th1 cells and the lack of responsiveness of Th2 cells correlate with the differential expression of the IL-12R chains in these two cell types. Th1 cells express both the IL-12Rβ1 and IL-12Rβ2 subunits, whereas Th2 cells express only the IL-12Rβ1 chain. Although Th1 and Th2 cells can bind IL-12, only Th1 cells are capable of signaling in response to IL-12 (14, 15). These observations indicate that the IL-12Rβ2 chain is the signaling component of the IL-12R. This is in agreement with the demonstration that IL-12Rβ2, in contrast to IL-12Rβ1, contains tyrosine residues in its cytoplasmic domain and directly interacts with STAT4 (13, 16). The level of IL-12Rβ2 can therefore be crucial in determining the balance of Th1/Th2 cytokines during the course of an immune response (17). Consistent with this idea, a preferential expression of IL-12Rβ2 has been documented in human diseases characterized by a typical Th1-type inflammatory response (18, 19).

In this study we investigated IL-12Rβ2 expression in CD tissue. The hypothesis tested was that increased IL-12Rβ2 expression can contribute to the polarization of the Th1-type cytokine profile in CD by promoting IL-12 signaling through the STAT4 pathway. We show here that IL-12Rβ2 is enhanced at the site of disease in CD, and that IL-12Rβ2 expression correlates with the activation of STAT4 proteins and IFN-γ accumulation. In addition, we provide evidence that up-regulation of IL-12Rβ2 occurs in IL-12-associated Th1 gastrointestinal diseases, suggesting that IL-12 enhances

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Received for publication February 7, 2000. Accepted for publication September 6, 2000.

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[1] This work was supported by Grants CNR 96.03133.CT04, from the Italian National Research Council and Research Project "Malattie Infiammatorie Croniche Intestinali ed Autoimmuni-Componenti Immunoregolatorie della Mucosa nella Patogenesi e Prevenzione" (art 12 DLgs 502/1992).

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[3] Abbreviations used in this paper: CD, Crohn's disease; CS, corticosteroids; LPMC, lamina propria mononuclear cells; LPL, lamina propria lymphocytes; UC, ulcerative colitis; IBD, inflammatory bowel diseases; HP, *Helicobacter pylori*.

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the expression of its own receptor in human gastrointestinal mucosa. In aggregate, our data support the concept that IL-12/IL-12R-mediated signals contribute to the local immune inflammatory response in CD.

Materials and Methods

Patients and samples

Mucosal samples were taken from freshly obtained intestinal resection specimens of four patients with CD. The primary site of involvement was ileal in one and ileocolonic in three patients. The disease was active in all patients, as defined by a CD activity index >200 (20). At the time of surgery two patients were taking corticosteroids (CS) and two were taking mesalazine plus antibiotics. In all patients indication for surgery was a chronic active course poorly responsive to medical treatment. From patients with ileocolonic involvement, mucosal samples were taken from involved (gross lesions) and spared, ileal and colonic areas.

Additional mucosal samples were taken during endoscopy from 13 CD patients. In these patients the primary site of involvement was ileal in five, ileocolonic in three, and colonic in five. At the time of endoscopy, 11 patients had active disease. Five patients were taking CS, five were taking mesalazine, one was taking mesalazine plus antibiotics, and the remaining two were not receiving treatment.

Controls included colonic mucosal samples taken from involved areas of nine ulcerative colitis (UC) patients undergoing endoscopy, 13 patients with irritable bowel syndrome (IBS) undergoing endoscopy for recurrent abdominal pain, and one patient with diverticular disease. Both IBS and diverticular disease patients were categorized as the non-IBD control group. In addition, biopsy specimens were taken from three patients with infectious (salmonella) colitis. All UC patients had active disease at the time of the study, as defined by clinical criteria (21) supplemented by endoscopic and histopathologic data (22, 23). Disease activity was moderate in two and mild in seven patients. Disease extent was substantial in four and distal in five patients. Two patients were taking CS, five were taking mesalazine, and the remaining two were not receiving treatment.

Autologous PBMC were obtained from three CD and three UC patients, and three non-IBD controls. Biopsy specimens from the distal duodenum of four patients with untreated celiac disease and four normal controls were obtained during upper gastrointestinal endoscopy. Diagnosis of celiac disease was made according to the original or revised European Society for Pediatric Gastroenterology and Nutrition criteria for celiac disease (24, 25). The histopathologic diagnosis was based on typical mucosal lesions with crypt cell hyperplasia, villous atrophy, and increased number of intraepithelial lymphocytes. All celiac disease patients were positive for anti-endomysial and anti-gliadin Abs. Control patients were under investigation for gastrointestinal symptoms, but had normal histology and were anti-endomysial and anti-gliadin Ab negative. Additional samples were taken from the gastric antrum of three patients with documented diagnosis of *Helicobacter pylori* (HP)-associated gastritis and three normal controls (HP-negative subjects). Biopsy specimens were snap-frozen in liquid nitrogen and stored at −80°C until used.

The study was approved by the department ethical committee.

Lamina propria mononuclear cell (LPMC) isolation and culture

LPMC were isolated by the DTT-EDTA-collagenase sequence as previously described (2, 5, 23). The isolated cells were counted and checked for viability using 0.1% trypan blue (viability ranged from 90–94%). PBMC were isolated by density gradient centrifugation (Lymphoprep, Nycomed Pharma, Oslo, Norway) from 10-ml heparinized blood samples.

To analyze which cells express IL-12Rβ2, $CD3^+$ lamina propria lymphocytes (LPL) were isolated from biopsy specimens of three patients with CD and three normal controls. $CD3^+$ LPL were purified incubating LPMC with immunomagnetic beads armed with mAb for CD3 according to the instructions of the manufacturer (Dynal, Oslo, Norway). Purified cells were >93% as determined by FACS analysis.

To investigate whether IL-12 enhances IL-12Rβ2 expression, normal LPMC were resuspended in complete medium (RPMI 1640 supplemented with 10% FCS, 1% L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin; all from Sigma, St. Louis, MO) at a concentration of 2×10^6 cells/ml and cultured in 24-well plates in the presence of PHA (1 μg/ml; Sigma) for 12 h. After that, nonadherent LPMC were collected, washed twice with 1× PBS, and cultured in the absence or the presence of graded doses of recombinant human IL-12 (Sigma; final concentration ranging from 0.1–10 ng/ml) for 6 h. To parallel LPMC cultures were added 10 ng/ml IL-12 and either a neutralizing IL-12 or IFN-γ Ab (both used at a final concentration of 1 μg/ml; both purchased from Sigma). To verify the efficiency of the anti-IFN-γ Ab, nonadherent LPMC cultures were also stimulated with 10 ng/ml IFN-γ in the presence or the absence of anti-IFN-γ (1 μg/ml). In additional experiments LPMC were isolated from the inflamed colon of four patients with CD and cultured (2×10^6 cells/ml) in the presence of a neutralizing IL-12 Ab or a nonrelevant control Ab (rabbit IgG; 1 μg/ml; both purchased from Sigma) for 24 h. At the end of the culture, LPMC were collected and used for RNA extraction.

Tissue homogenate preparation

Biopsy or surgical mucosal samples taken from all patients enrolled in this study were used for both RNA and protein analysis on freshly obtained whole tissue. Mucosal samples were separately placed in sterile tubes containing 1–2 ml of cold guanidine thiocyanate buffer (for RNA extraction) or 0.5 ml of lysis buffer (for protein extraction). The latter contained 0.0625 mol/L Tris (pH 6.8), 2% SDS, 3% 2-ME, 10% glycerol, 100 mmol/L sodium fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mmol/L PMSF (all from Sigma). Tissue samples were homogenized using a tissue homogenizer (Ystral, Dottingen, Germany).

RNA and cDNA preparation and semiquantitative RT-PCR

Total RNA extracted from freshly obtained mucosal samples and (both unstimulated and stimulated) LPMC, $CD3^+$ LPL, and $CD3^+$-depleted LPMC were used to prepare cDNA as previously described (2, 5). In preliminary experiments we established the optimal number of cycles for obtaining a PCR product within the linear portion of the curve. For this purpose an equivalent amount of cDNA per sample (2 μl/reaction) was amplified using specific primers for β-actin (for 16, 18, 22, 24, and 26 cycles) or for each IL-12R subunit, IFN-γ, IL-4, or IL-5 (for 18, 20, 23, 26, and 28 cycles). PCR were performed in a total volume of 50 μl as previously described (2, 26). For Southern blotting experiments cDNA samples were incubated for 18 cycles with β-actin primers or for 23 cycles with primers for each IL-12R subunit, IL-12/p40, IFN-γ, IL-4, and IL-5 and were detected using specific cDNA probes (26). The cDNA probes were DNA fragments encoding the full-length PCR product. RT-PCR products were used as probes only after each product was cloned and its sequence verified. PCR primers (Genosys, Cambridge, U.K.) were as follows: IL-12Rβ1, 5′-CTTCCAGAAGGCTGTCAAG-3′ and 3′-CTGTGATTCAATGCAATACG-5′; IL-12Rβ2, 5′-GGATGCTCATTGGCATTTAT-3′ and 3′-CAGGCCAGTTTGCAGACAA-5′; IL-12/p40, 5′-CATTCGCTCCTGCTGCTTCAC-3′ and 3′-TACTCCTTGTTGTCCCCTCTG-5′; IFN-γ, 5′-AATGCAGGTCATTCAGATG-3′ and 3′-TTGGACATTCAAGTCAGTT-5′; IL-4, 5′-GCTAGCATGTGCCGGCAACTT-3′ and 3′-CAACGTACTCTGGTTGGCTTC-5′; IL-5, 5′-GAGGATTCCTGTTCCTGT-3′ and 3′-GCGCAACAAACCAGTTTAG-5′; and β-actin, 5′-CGAGGCCCAGAGCAAGAGA-3′ and 3′-CGTGACATTAAGGAGAAGCTGTG-5′. The level of RNA transcripts was measured by laser densitometry and expressed as arbitrary units.

Determination of phosphorylated STAT4 level

Total proteins were extracted from freshly obtained mucosal samples by using the above-mentioned lysis buffer. After cell lysis the supernatant was collected, run at 4000 × *g* for 40 min (4°C), and stored at −80°C until assay. Total proteins (500 μg/sample) were incubated with anti-STAT4 (C-20; Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C for 2 h. Immune complexes were collected by incubation with protein A/G agarose (Santa Cruz Biotechnology), washed three times with lysis buffer, and heated for 5 min in a boiling water bath in sample buffer for SDS-PAGE. Immunoprecipitates from extracts containing the same amount of protein were analyzed by Western blotting with Ab against phosphotyrosine (p-Tyr; Santa Cruz Biotechnology) and subsequent incubation with HRP-conjugated goat anti-mouse IgG mAb (Santa Cruz Biotechnology). The Ab reaction was detected with a chemiluminescence detection kit (Amersham International, Arlington Heights, IL). After p-Tyr analysis, blots were stripped by incubation for 30 min at 50°C in stripping medium (2% SDS, 0.05 M Tris, and 0.1 M 2-ME) and then incubated with Ab against STAT4 (H-119; Santa Cruz Biotechnology).

Results

IL-12Rβ2 expression is enhanced at the site of disease in CD

Transcripts for IL-12Rβ2 were detected in tissue homogenates from both affected and unaffected intestinal mucosal areas of CD patients. Similarly, transcripts for IL-12Rβ2 were found in mucosal samples taken from patients with UC and non-IBD controls. In addition, IL-12Rβ2 was expressed in freshly isolated LPMC from CD and UC patients as well as in those from non-IBD controls.

When the IL-12Rβ2 content was examined by a semiquantitative RT-PCR, an increased accumulation was seen in whole mucosal samples from CD compared with UC and non-IBD controls (Fig. 1). In CD, IL-12Rβ2 expression was more abundant in mucosal samples taken from affected areas compared with that in spared mucosa (Fig. 1). No difference in terms of IL-12Rβ2 RNA transcripts was observed between spared mucosa of CD patients and UC or normal controls. IL-12Rβ2 RNA level in active UC did not differ from that in non-IBD controls (Fig. 1). To establish which cells express IL-12Rβ2, total RNA was extracted from both $CD3^+$ LPL and $CD3^+$-depleted LPMC and analyzed by Southern blotting after PCR amplification. As shown in Fig. 2, both $CD3^+$ LPL and $CD3^+$-depleted LPMC contained transcripts for IL-12Rβ2. However, an enhanced accumulation of IL-12Rβ2 RNA transcripts was observed only in $CD3^+$ LPL from CD compared with controls (Fig. 2).

Transcripts for IL-12Rβ1 were expressed at comparable levels in mucosal samples from CD and UC patients and non-IBD controls (Fig. 1). Both IL-12Rβ1 and IL-12Rβ2 were barely detectable in PBMC (data not shown).

In CD, IL-12Rβ2 expression strictly correlates with tyrosine phosphorylation of STAT4 and IFN-γ expression

STAT4 is an essential component of the IL-12-dependent pathway for IFN-γ production (7, 8). There is good evidence that the phosphorylation and activation of STAT4 in response to IL-12 are dependent on the presence of IL-12Rβ2 (14, 16, 27, 28). To examine whether up-regulation of IL-12Rβ2 was associated with a pronounced expression of active STAT4, protein extracts from whole mucosal samples were immunoprecipitated with anti-STAT4 Ab and immunoblotted with anti-phosphotyrosine Ab. The level of STAT4 phosphorylation in affected mucosal areas of CD was greater than that in UC and non-IBD controls, although all these groups expressed comparable amounts of the STAT4 proteins (Fig. 3). In paired mucosal biopsy specimens available from three CD patients, three UC patients, and three non-IBD controls, the profile of regulatory cytokines was analyzed by Southern blotting. As shown in Fig. 4, a strong accumulation of transcripts for IFN-γ was detected in CD mucosal samples. Transcripts for IL-4 were expressed at very low levels in both CD and UC as well as non-IBD control mucosa (Fig. 4). IL-5 RNA transcripts were detected only in UC (Fig. 4).

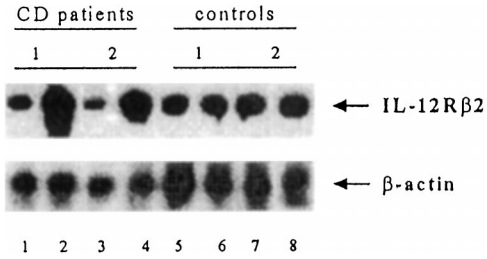

FIGURE 2. RNA transcripts for IL-12Rβ2 and β-actin in $CD3^+$ LPL (*lanes 2, 4, 6,* and *8*) and $CD3^+$-depleted LPMC (*lanes 1, 3, 5,* and *7*) from two patients with CD (*lanes 1–4*) and two non-IBD controls (*lanes 5–8*). cDNA (1.5 and 3 μl for β-actin and IL-12Rβ2, respectively) was incubated with specific primers for β-actin and IL-12Rβ2 for 18 and 23 cycles. Southern blot analysis of RT-PCR products was performed as indicated in *Materials and Methods*. In CD mucosa, IL-12Rβ2 is expressed at a high level in $CD3^+$ LPL. One of two representative experiments is shown.

IL-12Rβ2 is enhanced in IL-12-associated Th1 gastrointestinal diseases

To investigate whether up-regulation of IL-12Rβ2 is a feature of Crohn's disease or occurs in other Th1-mediated gastrointestinal diseases, the content of IL-12Rβ2 RNA was measured in mucosal samples taken from the distal duodenum of patients with celiac disease and the gastric antrum of patients with HP-associated gastritis. In addition, IL-12Rβ2 RNA transcripts were analyzed in mucosal samples of patients with infectious colitis. For all these groups of patients appropriate controls were included. As shown in Fig. 5, the amount of transcripts for IL-12Rβ2 in the gastric mucosa of HP-infected patients was greater than that detected in the mucosa of HP-negative subjects. Similarly, the level of IL-12Rβ2 RNA was greater in patients with infectious colitis than in normal controls (Fig. 5). In contrast, the IL-12Rβ2 RNA level in celiac disease duodenum did not differ from that in normal duodenal mucosa (Fig. 5).

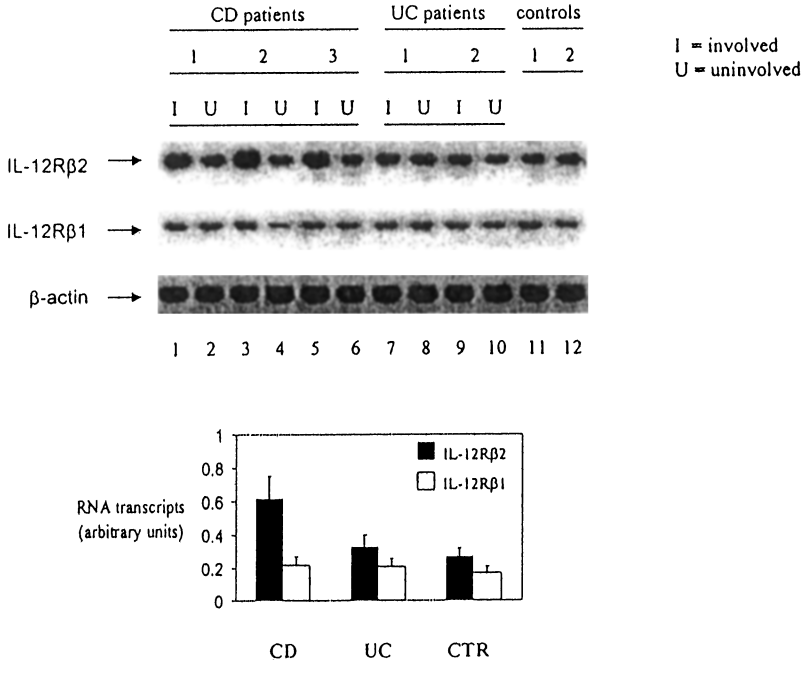

FIGURE 1. The expression of IL-12Rβ2, but not IL-12Rβ1, is increased in the affected mucosal areas from CD compared with that in ulcerative colitis and non-IBD controls. *Upper panel*, RNA transcripts for IL-12Rβ2, IL-12Rβ1, and β-actin in mucosal tissue homogenates from three CD (*lanes 1–6*) and two UC (*lanes 7–10*) patients and two non-IBD controls (*lanes 11* and *12*). In patients with CD and those with UC, mucosal samples were taken from involved and uninvolved tissue. Southern blot analysis of RT-PCR products was performed as indicated in *Materials and Methods*. One representative experiment of four independent experiments is shown. *Lower panel*, Mean $\pm$ SEM of the ratio between IL-12Rβ2 and β-actin or between IL-12Rβ1 and β-actin in mucosal samples taken from involved areas of eight CD patients, eight UC patients, and eight normal controls. The level of RNA transcripts was measured by laser densitometry, and the relative values are expressed in arbitrary units.

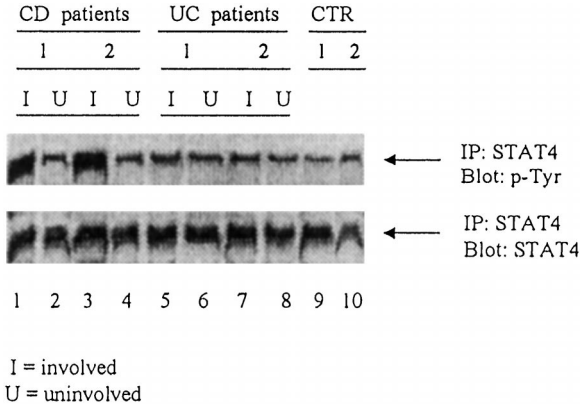

FIGURE 3. Expression of both active (phosphorylated) and inactive STAT4 proteins in mucosal samples from two CD (*lanes 1–4*) and two UC (*lanes 5–8*) patients and two non-IBD controls (*lanes 9* and *10*). In both patients with CD and UC, mucosal samples were taken from involved and uninvolved tissue. Total proteins were immunoprecipitated (IP) with STAT4 Ab, run on 10% SDS-PAGE under reducing conditions, and immunoblotted with anti-phosphotyrosine Ab (p-Tyr). After detection of p-Tyr-STAT4 proteins, the membrane was stripped and reblotted with another STAT4 Ab to ascertain equivalent loading of the lanes. A marked phosphorylation of STAT4 is seen in affected mucosal samples from CD compared with UC or non-IBD controls. The example is representative of three separate experiments.

Transcripts for IL-12/p40 were detected in mucosal samples of patients with CD, HP-associated gastritis, and infectious colitis, but not in celiac disease (Fig. 6). In addition, transcripts for IL-12/p40 were barely detectable in patients with UC (Fig. 6) and non-IBD controls, confirming previous observations (2–4). Together, these data indicate that up-regulation of the IL-12Rβ2 chain occurs only in IL-12-associated Th1 gastrointestinal diseases.

IL-12 enhances IL-12Rβ2 expression in normal LPMC

Recent reports have demonstrated that IL-12 can regulate the expression of IL-12Rβ2 on T cells (15, 29). These data together with the documented observation that IL-12Rβ2 is enhanced in IL-12-associated Th1 gastrointestinal diseases prompted us to investigate whether IL-12 can enhance IL-12Rβ2 expression in human gastrointestinal mucosa. To address this issue, nonadherent LPMC isolated from normal controls were cultured with exogenous IL-12,

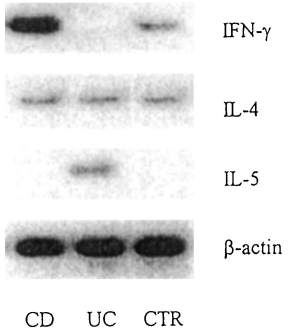

FIGURE 4. Transcripts for IFN-γ, IL-4, IL-5, and β-actin in mucosal tissue homogenates from CD and UC patients and non-IBD controls. Southern blot analysis of RT-PCR products was performed as indicated in *Materials and Methods*. A strong accumulation of transcripts for IFN-γ is documented in CD. IL-4 is expressed at a low level in all samples tested, whereas IL-5 is detectable only in UC. One representative experiment of three independent experiments is shown.

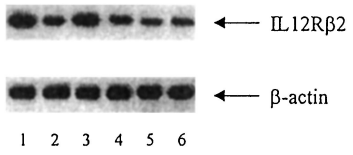

FIGURE 5. Southern blot analysis of RT-PCR products for IL-12Rβ2 and β-actin in mucosal tissue homogenates from an HP-infected patient (*lane 1*), an HP-negative control (*lane 2*), an infectious colitis patient (*lane 3*), a non-IBD control (*lane 4*), a celiac disease patient (*lane 5*), and a normal subject (*lane 6*). Southern blot analysis of RT-PCR products was performed as indicated in *Materials and Methods*. The amount of transcripts for IL-12Rβ2 in patients with HP infection or infectious colitis is greater than that in their respective controls. In contrast, the level of IL-12Rβ2 in the duodenal mucosa of patients with celiac disease is not different from that in normal duodenum. The example is representative of three separate experiments.

and the amount of IL-12Rβ2 RNA transcripts was examined by Southern blotting after PCR amplification. As shown in Fig. 7 (*upper panel*), IL-12 enhanced LPMC IL-12Rβ2, and this effect was inhibited by the addition of a neutralizing anti-IL-12 Ab. As IL-12 is a potent inducer of IFN-γ, and IFN-γ has the ability to enhance IL-12Rβ2, we next examined whether the effect of IL-12 on IL-12Rβ2 was dependent by the induction of IFN-γ. For this purpose, in parallel experiments nonadherent LPMC were cultured with exogenous IL-12 in the presence of a neutralizing anti-IFN-γ Ab, and the amount of IL-12Rβ2 RNA transcripts was examined by Southern blotting after PCR amplification. Interestingly, the anti-IFN-γ reduced, but did not completely abrogate, the induction of IL-12Rβ2 RNA by IL-12 (Fig. 7, *upper panel*). Finally, we showed that addition of anti-IL-12 Ab to the CD LPMC cultures resulted in a marked inhibition of IL-12Rβ2 (Fig. 7, *lower panel*).

Discussion

This study shows that IL-12Rβ2 expression is enhanced in the intestinal mucosa of patients with CD. IL-12Rβ2 RNA transcripts were mainly detected in areas with gross lesions, with no difference between ileal and colonic samples.

One important finding of the present study is that CD LPMC, but not autologous PBMC, express high levels of IL-12Rβ2. CD is a chronic inflammatory disorder of the gastrointestinal tract characterized by an infiltration of the inflamed regions with $CD4^+$ T cells and macrophages (1). These cells are functionally activated and produce high levels of IFN-γ and TNF-α, indicative of a Th1

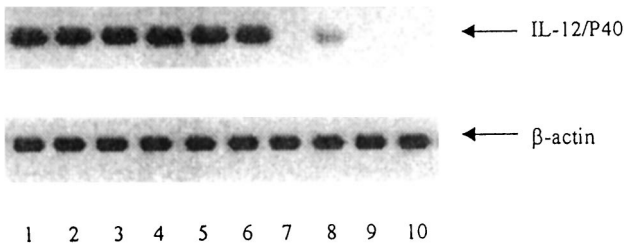

FIGURE 6. Transcripts for IL-12/p40 and β-actin in mucosal tissue homogenates from patients with CD (*lanes 1* and *2*), infectious colitis (*lanes 3* and *4*), HP infection (*lanes 5* and *6*), UC (*lanes 7* and *8*), and celiac disease (*lanes 9* and *10*). Southern blot analysis of RT-PCR products was performed as indicated in *Materials and Methods*. Transcripts for IL-12/p40 are expressed at a high level in the mucosa of patients with CD, infectious colitis, and HP infection. The example is representative of two separate experiments.

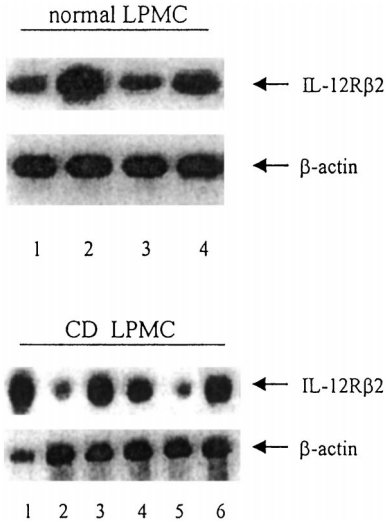

FIGURE 7. IL-12 enhances IL-12Rβ2 in the human intestinal mucosa. *Upper panel*, Effect of rIL-12 on LPMC IL-12Rβ2 expression. LPMC, isolated from normal colonic mucosa, were cultured as described in *Materials and Methods* in the presence of medium alone (*lane 1*) or with IL-12 (10 ng/ml; *lane 2*), IL-12 plus anti-IL-12 Ab (1 μg/ml; *lane 3*), or IL-12 plus anti-IFN-γ Ab (1 μg/ml; *lane 4*). At the end of culture, total RNA was extracted from the LPMC and tested for IL-12Rβ2 expression by Southern blotting. The addition of IL-12 to the LPMC cultures results in an increase in IL-12RB2. This effect is inhibited by the addition of a neutralizing anti-IL-12 Ab. Induction of IL-12Rβ2 by IL-12 is reduced, but is not completely abrogated, by coincubation with a neutralizing anti-IFN-γ Ab. One of two representative experiments is shown. *Lower panel*, Effect of a neutralizing anti-IL-12 Ab on the expression of IL-12Rβ2 in CD LPMC. LPMC, isolated from the inflamed colon of two patients with CD, were cultured in the presence of medium alone (*lanes 1* and *4*) or a neutralizing IL-12 Ab (1 μg/ml; *lanes 2* and *5*) or a nonrelevant control Ab (1 μg/ml; *lanes 3* and *6*) for 24 h. At the end of culture, total RNA was extracted from the LPMC and tested for IL-12Rβ2 expression by Southern blotting. Addition of a neutralizing anti-IL-12, but not a control Ab (rabbit IgG), inhibits CD LPMC IL-12Rβ2. One of two representative experiments is shown.

cytokine profile (30–33). Consistently, we and others have recently found elevated levels of IL-12, the major Th1 inducing factor, in CD mucosa and have shown that boosting lamina propria Th1 cell responses with IL-12 produces severe mucosal degradation (2–4, 12). These observations together with the demonstration that activation of the IL-12/STAT4 pathway in murine models results in CD-like colitis support the pathogenic role of IL-12 in CD (6, 9–11). Data from the present study confirm and expand these observations showing that in CD mucosa, up-regulation of the IL-12Rβ2 chain is tightly correlated with the accumulation of both active STAT4 and IFN-γ. Because IL-12Rβ2 directly interacts with STAT4, it is conceivable that the increased expression of IL-12Rβ2 contributes to stabilize the polarized Th1 phenotype in CD by maintaining mucosal T cells in a state of functional responsiveness to IL-12 and thereby promoting IL-12 signaling through the STAT4 pathway (13–17).

In this study the expression of IL-12Rβ2 was analyzed at the mRNA level only, because no commercially Ab capable of specifically and selectively recognizing the distinct subunit of IL-12R is as yet available. The possibility that the content of transcripts for IL-12Rβ2 is not associated with a pronounced induction of the IL-12Rβ2 protein should therefore be considered. However, it is worth noting that in other systems IL-12-dependent signaling correlates with the selective expression of the transcripts encoding the IL-12Rβ2 chain (14, 15).

Although the mechanism by which IL-12Rβ2 expression is regulated in the human gastrointestinal mucosa remains to be clarified, some observations made in the present study suggest that bacteria and bacterial products/components may contribute to modulate IL-12Rβ2. Indeed, IL-12Rβ2 was increased in HP-associated gastritis and *Salmonella* colitis, two infectious entities, and in CD, a disease characterized by an abnormal mucosal immune response to microbial agents derived from the intestinal luminal flora (34). In contrast, no increase in IL-12Rβ2 was seen in UC. These data fit into the concept that the immune response in CD differs from that in UC (1) and suggest that UC may be the atypical response to infectious etiologies, whereas the response in CD is the same as that in infections.

Studies in several experimental models of bacterial infections and in human diseases support the role of the IL-12/IL-12R pathway in the anti-microbial immune defense (35). Evidence indicates that IL-12 is required both for early control of infection, through the stimulation of NK cell IFN-γ production, and for generation and perhaps maintenance of acquired memory response, directed by Th1 cells (35–37). Importantly, in diseases due to intracellular pathogens, such as *Mycobaterium tuberculosis* and *Salmonella*, reduced expression of IL-12R has been associated with severe manifestations of the diseases due to an impaired ability to mount a protective IL-12-dependent Th1 response (36). It can thus be assumed that during *Salmonella* colitis, up-regulation of IL-12Rβ2 is an essential requirement to maintain cell responsiveness to IL-12 and to establish a protective immunity against the pathogen (38).

The fact that IL-12Rβ2 is increased in CD, HP-associated gastritis, and *Salmonella* colitis is consistent with previous studies showing that IL-12Rβ2 is highly expressed in Th1 cell lines and established Th1-mediated diseases (14, 15, 18, 19). However, we were able to detect no increase in IL-12Rβ2 in duodenal samples from patients with celiac disease, a gluten-sensitive Th1-mediated enteropathy (39, 40). Our data also indicate that in the gastrointestinal mucosa up-regulation of IL-12Rβ2 occurs only in IL-12-associated diseases and suggest that IL-12 may directly influence the level of IL-12Rβ2 on T cells. First, we detected transcripts for IL-12 in CD, HP-associated gastritis, and *Salmonella* colitis, but not in celiac disease, confirming the recent observation that IL-12 is undetectable in celiac disease mucosa (39). Second, we showed that a neutralizing IL-12 Ab dramatically down-regulated IL-12Rβ2 expression in CD LPMC. Finally, we demonstrated that the addition of IL-12 to normal LPMC cultures resulted in an increase in IL-12Rβ2. The effect of IL-12 on LPMC IL-12Rβ2 expression was reduced, but was not completely abrogated, by a neutralizing IFN-γ Ab. This supports results from studies of other systems showing that IL-12 can enhance IL-12Rβ2 through a mechanism that is only in part dependent on IFN-γ (14, 15, 17, 29).

Transcripts for IL-12Rβ1 were detected in all intestinal mucosal samples analyzed. This result apparently contrasts with our previous negative finding of IL-12Rβ1 RNA in purified normal lamina propria T lymphocytes (5). Differences in the samples and the amount of RNA analyzed as well as in the methods used might well account for such discrepancy. In contrast to IL-12Rβ2, the content of IL-12Rβ1 in CD mucosa did not differ from that in UC or non-IBD controls, clearly indicating that the expression of the IL-12R subunit is differently regulated in the human intestine during chronic inflammation.

In conclusion, our data show that the signal pathway used by IL-12 to promote Th1 cell development is enhanced at the mucosal level in CD, further supporting the idea that IL-12/IL-12R signals contribute to the local inflammatory response in CD. Studies are

now in progress to explore whether molecules inhibiting the binding of IL-12Rβ2 with STAT4 can dampen IL-12-driven Th1 responses in the intestinal mucosal microenvironment.

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