Interleukin-15 and Its Soluble Receptor Mediate the Response to Infliximab in Patients With Crohn’s Disease

GREGORY BOUCHAUD,*‡ ERWAN MORTIER,*‡ MATHURIN FLAMANT,**§ ISABELLE BARBIEUX,*‡ ARIANE PLET,*‡ JEAN-PAUL GALMICHE,**§ YANNICK JACQUES,*‡ and ARNAUD BOURREILLE**§

*INSERM, U892, Équipe Cytokine/Récepteur, Nantes, France; ‡Institut de Recherche Thérapeutique de l’Université de Nantes, Nantes, France; §INSERM, U913, Nantes, France; ¶INSERM, OIC-04, Nantes, France; ¶CHU Nantes, Hôtel Dieu, Institut des Maladies de l’Appareil Digestif, Nantes, France

BACKGROUND & AIMS: Infliximab is a monoclonal antibody against tumor necrosis factor that is used to treat patients with inflammatory bowel disease. We investigated serum levels and cellular expression of interleukin (IL)-15 and its receptor (sIL-15Rα) in patients with Crohn’s disease (CD) treated with infliximab; and the effect on sIL-15Rα secretion by epithelial cells. METH-ODS: CD patients were given infliximab (n = 40; 3 infusions); 37 healthy controls were studied. Serum levels of IL-15, sIL-15Rα, and complex were determined by radioimmunoassay and cytokine levels by enzyme-linked immunosorbent assay. IL-15Rα and A Desintegrin and Metalloproteinase 17 levels were assessed by immunohistochemistry. Epithelial cell lines (HT-29 and Caco-2) were cultured with infliximab, adalimumab, or etanercept. Patients were classified as responders and nonresponders according to their Crohn’s Disease Activity Index and clinical observations. RESULTS: Before infliximab, IL-15 was higher in responders than in controls and nonresponders. After infliximab, IL-15 decreased in responders while remaining stable in nonresponders. sIL-15Rα and IL-15/sIL-15Rα complex levels were higher in CD than in controls and increased only in responders after infliximab. IL-15Rα and A Desintegrin and Metalloproteinase 17 colocalized in epithelial cells and were higher in CD patients. In vitro, infliximab but not adalimumab and etanercept induced sIL-15Rα secretion by epithelial cells. CONCLUSIONS: Serum level of sIL-15Rα and the IL-15/sIL-15Rα complex increased in responder patients and the response was associated with a decrease of IL-15. Infliximab induced the release of the IL-15 receptor α, suggesting a specific modulation of IL-15 and its soluble receptor by reverse signaling through transmembrane tumor necrosis factor α. Keywords: Interleukin-15 Receptor Alpha Subunit; Crohn’s Disease; Infliximab; Epithelial Cell.

Therapies targeted against cytokines are used commonly in inflammatory bowel disease (IBD). Among them, anti–tumor necrosis factor (TNF)α monoclonal antibodies are effective and used in clinical routine. Infliximab (IFX) is an anti-TNFα monoclonal antibody that binds soluble and cell-surface TNFα with high affinity and specificity. However, despite its efficacy for the treatment of active Crohn’s disease (CD), its anti-inflammatory action is not fully understood.

Anti-TNFα agents could reduce inflammation by neutralizing soluble TNFα and its subsequent interaction with its receptors. However, differences in efficacy profiles despite similar affinities for soluble TNFα suggest that neutralization of soluble TNFα may not be the major mechanism of action. Interestingly, IFX binds to transmembrane TNFα (tmTNF), and thereby acts either as an antagonist by blocking tmTNF interaction with its receptors or as an agonist by initiating tmTNF-mediated reverse signaling, leading to cell activation or cytokine suppression. Among the molecular partners involved in reverse signaling are the proteases signal peptide peptidase-like 2a and 2b that participate in tmTNF-mediated induction of interleukin (IL)-12 production. Accordingly, binding of anti-TNF agents to tmTNF could induce the activation of proteases implicated in the secretion of cytokines or in the cleavage of membrane-anchored receptors. Although several studies have shown that all anti-TNFα bind to tmTNF, there is evidence for differential induction of cytokine suppression through reverse signaling. For example, it was shown that IFX but not etanercept could down-regulate IL-1α and TNFα secretions and increase IL-10 secretion. These differences could explain, first, the unequal efficacies of these agents and, second, that primary nonresponder patients to one molecule may respond to another.

Among proinflammatory cytokines, IL-15 plays a key role in the activation of innate and adaptive immune responses and its modulation by anti-TNF agents could deeply regulate the inflammatory process in CD. IL-15 was identified for its IL-2–like ability to stimulate the growth of T cells, but different studies have shown that the 2 cytokines exert complementary effects within the

Abbreviations used in this paper: CRP, C-reactive protein; IFX, infliximab; IL, interleukin; sIL-15R, secreted form of the interleukin-15 receptor; tm, transmembrane; SD, standard deviation; TNF, tumor necrosis factor.

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immune system. IL-15 is produced by monocytes, macrophages, dendritic cells, and also by nonhematopoietic cell types such as enterocytes or stroma cells.6,7

IL-15 binds a tricomolecular IL-15 receptor (IL-15R) complex formed by the β and γ chains of the IL-2R and a specific receptor chain (IL-15Rα). Moreover, membrane-bound IL-15Rα can undergo a proteolytic cleavage involving A Desintegrin and Metalloproteinase 17 (ADAM17) (also known as TNFα converting enzyme), releasing a soluble form of IL-15Rα.8–10 Soluble IL-15Rα retains a high affinity for IL-15 and can act either as a powerful antagonist of IL-15 action through IL-15Rα/β/γ complex8,11 or as an agonist of IL-15 action through the IL-15Rβ/γ complex.12

The role of IL-15 in IBD is not clearly understood. IL-15 expression has been shown in intestinal epithelial cells as well as in the intestinal mucosa.13,14 Moreover, IL-15–positive cells are more abundant in the lamina propria of CD and ulcerative colitis patients compared with uninflected patients.15 The potential role of IL-15 in the physiopathology of IBD was highlighted in the murine dextran sulphate sodium model of colitis. IL-15–deficient mice had significantly better survival rates compared with control mice after induction of acute colitis. Macroscopic and histologic scores of inflammation also were significantly lower in IL-15 knockout mice associated with a reduction of the CD44highCD8+ T-cell infiltrate in the lamina propria of the mice.16 Conversely, it was shown that IL-15 neutralization by sIL-15Rα reduced inflammation and mucosal cell infiltrate in the SCID transfer model.17 The aim of this study is to explore the effect of TNF antagonist on the regulation of IL-15 and its soluble receptor which has never been done. Therefore, the aims of our study were to evaluate secretion of IL-15 and its soluble receptor in active CD patients and to evaluate their modulation after IFX treatment according to the clinical response. We also analyzed, ex vivo, the cellular expression of the transmembrane IL-15Rα (tmIL-15Rα), and, in vitro, the effect of the different anti-TNFα molecules on the secretion of sIL-15Rα.

**Patients and Methods**

**Patients**

Patients with active CD were treated by 3 infusions of IFX (5 mg/kg) at weeks 0, 2, and 6. A clinical remission to IFX was defined by a Crohn’s Disease Activity Index (CDAI) score of less than 150 points after treatment. When the patients were treated for a perineal disease, the clinical remission was assessed by the disappearance of draining fistulas at week 6. Blood samples were taken before the first infusion of IFX (week 0 or before IFX) and after the third infusion of IFX (week 6 or after IFX) and were centrifuged and stored at −80°C. Blood samples also were collected in 37 healthy volunteers. Each patient gave informed and written consent, and the biocollection was declared to the local ethical committee and the Fédération des Biothèques, University Hospital, Nantes, France.

Forty patients with active CD were included retrospectively in the study (12 males, 28 females; mean age, 37.8 y). After IFX, 32 patients were in remission and 8 had no benefit of the treatment and remained in an active phase of their disease. Four of them underwent an endoscopic evaluation before or at the beginning of IFX. Mucosal lesions have been observed in all cases. In one additional patient, a perineal examination revealed active lesions. After infliximab, mucosal lesions still were observed by endoscopy in 5 patients during the following 6 months. IFX was interrupted in 5 patients and steroids were increased or re-introduced in 2 patients, methotrexate was introduced in 1 patient, and surgery was performed in 2 patients. IFX was first increased either alone or in combination with parental nutrition, then interrupted and replaced unsuccessfully by adalimumab in 3 patients.

According to the Montreal classification, the disease was located predominantly in the colon (L2) or the ileocolon (L3) in 62.5% and 32.5%, respectively, and the disease was mostly nonstricturing and nonpenetrating (B1). Fourteen patients (35.0%) had a perineal disease, 7 were treated for fistulas alone, and the others for a combination of fistulas and a nonstricturing and nonpenetrating disease (B1, perineal). Six patients were treated by a combination of steroids and immunosuppressors, 3 patients received only steroids, and 31 received only immunosuppressors. Treatment was not modified except for the steroids, which were always stopped between the first and third infusions. Clinical and demographic characteristics were compared between responders and nonresponders. The mean age at diagnosis was significantly lower in responders: 28 (standard deviation [SD], 10.8) vs 40 (SD, 13.9) years (P = .03), and the duration of disease tended to be shorter 4.3 (SD, 3.5) vs 9.8 (SD, 7.8) years (P = .06). The clinical and demographic characteristics of the patients are summarized in Table 1.

**Quantification of Cytokine Serum Level**

The quantification of sIL-15Rα, IL-15, and IL-15/sIL-15Rα complex was determined as previously described.10 Sandwich radioimmunoassays were set up in which the goat anti-human IL-15Rα polyclonal antibody AF247 or the mouse monoclonal anti-human IL-15 Mab247 (R&D, Abingdon, UK) was used as capture, antibody and radio-iodinated neutralizing monoclonal anti-human IL-15Rα antibody M161 which were kindly provided by Gemmbab (Utrecht, The Netherlands) or radio-iodinated mouse monoclonal anti-human IL-15 BE-29 (Diaclone; Besançon, France) which was used as tracer. Both radioimmunoassay was specific for sIL-15Rα or IL-15 noncomplexed or IL-15/sIL-15Rα complex. A purified recombinant sIL-15Rα protein18 and recombinant
IL-15 (Peprotech, Neuilly sur Seine, France) were used as standards. Capture antibodies were coated (5 µg/mL; 50 µL/well) to high-adsorption Nunc maxisorp plates (Fisher Bioblock Scientific, Illkirch, France). Wells were saturated with phosphate-buffered saline (PBS)–bovine serum albumin 0.5% for 15 minutes, and samples (50 µL/well) were incubated for 1 hour at 4°C. The M161 or BE-29 monoclonal antibodies were iodinated using the iiodination reagent method and then added (1 nmol/L, 50 µL/well) for 1 hour at 4°C. Supernatants of each well were collected and the wells were washed twice with PBS. The radioactivity associated with the wells (bound fraction) and contained in the supernatants and washes (unbound fraction) was determined. The upper normal value of IL-15 (6.1 pmol/L) was calculated by the mean ± 2 SD of the values measured in healthy volunteers. The quantitative serum determination of IL-1β, IL-17, IL-6, and TNFα was realized using commercially available, solid-phase, enzyme-linked immunosorbent assay kits (R&D Systems). The minimum detectable doses of IL-1β, TNFα, and IL-6 were 1 pg/mL, 1.6 pg/mL, and 0.7 pg/mL, respectively. All samples were assayed in duplicate.

**Immunofluorescence Staining**

Colonic biopsy specimens were obtained from 3 CD patients before the first and third IFX infusions and 2 healthy control patients. All CD patients were in clinical remission after the treatment and, moreover, mucosal healing was obtained in 2 patients. Colonic biopsy specimens were immediately frozen and stored at -80°C. Specimens were sectioned at 4–6 µm with a cryostat, placed on slides, air dried, and fixed for 10 minutes with 100% acetone. Before incubation with primary antibodies, the slides were saturated with PBS/bovine serum albumin 0.5%. Primary antibodies were goat anti–IL-15Rα and rabbit anti-ADAM17 (R&D) or anticytokeratin (Santa-Cruz Biotechnology, Santa Cruz, CA) used at concentrations of 15 µg/mL and 10 µg/mL, respectively. Secondary antibodies were rabbit anti-goat IgG Alexa 488 and chicken anti-rabbit IgG fluorescein isothiocyanate used at a concentration of 2 µg/mL. Isotype-matched antibodies were used as negative controls. In the double-immunofluorescence experiments, we checked that each secondary antibody did not cross-react with the primary antibody of the other immunoglobulin species. Fluorescent images were analyzed with an epifluorescent microscope (DMR; Leica Microsystems, Nanterre, France).

**Statistical Analysis**

Quantitative data were expressed as mean ± SD or median (Interquartile range, 25–75). The normality of the distribution was analyzed by the Kolmogorov–Smirnov test. Dichotomized variables were compared using the chi-square test or the Fisher 2-tailed test. Differences between quantitative data were assessed by the paired or unpaired t test and the Mann–Whitney U test or the Wilcoxon signed-rank test for non-normally distributed data. A P value of less than .05 was considered statistically significant.

**Results**

**Clinical and Biological Markers of Activity**

During IFX treatment, sera were collected and clinical and biological markers were analyzed in responder and nonresponder patients. The mean ± SD of the CDAI score was not different between responder and
nonresponder patients before IFX (245.1 ± 108.2 vs 245.4 ± 143.2). After IFX, the mean CDAI score decreased significantly in responder patients (58.9 ± 35.0 vs 245.1 ± 108.2; P < .001), whereas no difference was found in nonresponder patients (198.3 ± 41.1 vs 245.4 ± 143.2; P = .8) (Figure 1A and B). Circulating TNFα levels were higher than normal values in 87% (35 of 40) of patients before the first infusion of IFX. The mean TNFα level was significantly higher in nonresponder patients (22.1 ± 5.2 pmol/L) and in responder patients (20.8 ± 15.8 pmol/L) than in controls (1.6 ± 2.3 pmol/L) (P < .001), but no significant difference was found between the 2 groups of patients (Figure 1C and D). At week 6 after IFX, TNFα decreased in 84% (27 of 32) of responder patients, with individual values dropping to control ranges in 59% of patients (19 of 32). Conversely, TNF remained above the control value in 87% (7 of 8) of nonresponder patients. The mean of TNF levels significantly decreased in responder patients (7.5 ± 10.9 vs 20.8 ± 15.8 pmol/L; P < .001) whereas no difference was found in nonresponder patients (22.1 ± 5.2 vs 20.1 ± 8.6) (Figure 1C and D). The mean of the C-reactive protein (CRP) level was not significantly different between responder and nonresponder patients before IFX (41.6 ± 55.9 vs 32.1 ± 32.0 mg/dL; P = .9) (Figure 1E and F). After IFX, CRP level decreased significantly in responder patients (10.5 ± 13.4 vs 41.6 ± 55.9 mg/dL; P < .001), whereas no difference was found in nonresponder pa-

**Figure 1.** Clinical and biological markers of activity. The CDAI scores were calculated before and after IFX in CD patients. Total TNFα and CRP levels were measured in the sera of 40 CD patients before and after 3 infusions of IFX and in 37 healthy controls. In responder patients, (A) CDAI scores, (C) TNF levels, and (E) CRP levels were significantly higher compared with controls and decreased after 3 infusions of IFX. In contrast, in nonresponder patients, (B) CDAI scores, (D) TNF levels, and (F) CRP levels, which were significantly higher before the treatment compared with controls, remained increased after the treatment. ***P < .001.
tients (16.7 ± 12.8 vs 32.1 ± 32.0 mg/dL; P = .2) (Figure 1E and F). Significantly increased levels of proinflammatory cytokine IL-6 (24.3 ± 20 pg/mL) and IL-1β (10.4 ± 14 pg/mL) were found in CD patients before IFX treatment vs healthy controls (0.25 ± 1 pg/mL; P < .001) (Figure 2). IL-17 also was measured in the sera of CD patients but no detectable amount was found before or after IFX infusions (data not shown). Before IFX, no significant differences were observed between responders and nonresponders for IL-6 (20.7 ± 23 vs 39.1 ± 28.0 pg/mL; P = .2) or IL-1β (9.93 ± 14.8 vs 13.62 ± 14.0 pg/mL; P = .1). After IFX, the mean IL-6 and IL-1β levels decreased significantly in responder patients (20.7 ± 23 vs 1.9 ± 5.3 pg/mL; P < .001 and 9.93 ± 14.8 vs 0.5 ± 2.1 pg/mL; P < .0001, respectively), whereas they were not changed significantly in nonresponder patients (39.1 ± 28.0 vs 72.3 ± 60.2 pg/mL; P = .8 and 13.62 ± 14 vs 8.3 ± 5.2 pg/mL; P = .8, respectively) (Figure 2). No correlation was found between CRP, TNF, IL-1, and IL-6 levels before and after IFX.

**IL-15, Secreted Form of IL-15Rα, and IL-15/Secreted Form of IL-15Rα Complex in CD Patients During IFX**

Previous studies showing IL-15 overexpression in CD patients prompted us to evaluate IL-15, the secreted form of IL-15Rα (sIL-15Rα), and IL-15/sIL-15Rα complex in the serum of patients. IL-15 was detectable in the serum of 25% (2 of 8) of nonresponder patients and in 81% (26 of 32) of responder patients (Figure 3A). On average for the 40 patients before treatment, IL-15 levels (mean ± SD) were significantly higher in CD patients before treatment than in healthy subjects (9.2 ± 15.4 vs 1.3 ± 2.4 pmol/L; P < .02) (data not shown). IL-15 levels also were significantly higher in IFX responder than in nonresponder patients (11.1 ± 16.5 vs 0.6 ± 1.3; P = .01) than in healthy controls (11.1 ± 16.5 vs 1.3 ± 2.4 pmol/L; P = .002). Conversely, IL-15 levels were not different between nonresponder patients and healthy subjects (P < .001 and P = .003, respectively). Although sIL-15Rα levels were higher in responder than in nonresponder patients, the difference was not significant (12.5 ± 23.4 vs 6.0 ± 7.2 pmol/L). The level of the IL-15/sIL-15Rα complex also was measured (Figure 3C). It was significantly higher in re-
sponder than in nonresponder patients before IFX (20.6 ± 78 vs 1.7 ± 2.9 pmol/L; P = .03), and in responder patients vs healthy subjects (20.6 ± 78 vs 1.3 ± 7 pmol/L; P = .002).

When patients responded to IFX, the median of the IL-15 levels significantly decreased from 2.7 pmol/L (IQ, 0.1–16.0) to 0.6 pmol/L (IQ, 0.0–2.4) (P = .02) (Figure 4A). The number of patients in whom IL-15 was undetectable increased from 22% (7 of 32) before IFX to 47% (15 of 32) after IFX, and IL-15 dropped below the control value in 93% of these patients (30 of 32). In contrast, in nonresponder patients, the median of the IL-15 levels was not modified after IFX (Figure 4D). In responder patients, the median of the sIL-15Rα levels significantly increased after IFX from 2.2 pmol/L (IQ, 3.0–12.8) to 12 pmol/L (IQ, 0.5–34.6) (P = .002) and the proportion of patients with detectable sIL-15Rα increased by 34% after IFX (Figure 4B). In contrast, in nonresponder patients, sIL-15Rα IQ levels did not change significantly (5.99 ± 7.3 vs 3.5 ± 6.4 pmol/L; P = .3) (Figure 4E).

**Figure 3.** IL-15, sIL-15Rα, and IL-15/sIL-15Rα complex levels in the serum of CD patients before and after IFX. Before IFX, IL-15 levels were significantly higher than in healthy controls in responder patients. (A) In contrast, in nonresponder patients, IL-15 levels were comparable with those of controls. (B and C) sIL-15Rα and IL-15/sIL-15Rα complex levels in the serum of CD patients before IFX were significantly higher than in healthy controls in responder patients and comparable with those of the controls in nonresponder patients.

**Figure 4.** During IFX treatment, IL-15 level (A) increased in responder patients but (D) remained stable in nonresponder patients. (B and C) sIL-15Rα and IL-15/sIL-15Rα complex levels increased significantly after 3 infusions of IFX in responder patients. (E and F) In contrast, in nonresponder patients, sIL-15Rα and complex levels were not modified after IFX. *P < .05. **P < .01.
The IL-15/sIL-15Rα complex level significantly increased after IFX in 90% (36 of 40) of responder patients, and the median increased from 20 pmol/L (IQ, 0–99) to 26 pmol/L (IQ, 0.5–102) (P = .04) (Figure 4C). Conversely, in nonresponder patients, the IL-15/sIL-15Rα complex level did not change (1.7 ± 2.9 vs 0.37 ± 1.2 pmol/L; P = .02) (Figure 4F). By using a nonparametric (Kruskal–Wallis) test, it also was shown that the IL-15 and sIL-15Rα levels before and after treatment by IFX were not affected significantly by the additional treatments (steroids, immunosuppressors) received by the patients.

**Cellular Expression of IL-15Rα and the Metalloproteinase ADAM17 in Colonic Mucosa**

To localize which cell type expresses IL-15Rα, we next analyzed the expression of IL-15Rα by immunofluorescence staining on colonic biopsy slices. Before IFX treatment, the cellular expression of IL-15Rα (in red) was higher in CD patients (Figure 5D) than in healthy controls (Figure 5C), and minor changes were observed after IFX treatment (not shown). Double-labeling studies in CD patients then showed a co-localization of IL-15Rα with cytokeratin, a specific marker of epithelial cells, suggesting that IL-15Rα was expressed mainly by epithelial cells (Figure 5F). Such a co-localization was not observed in healthy controls (Figure 5E). We then looked for the presence of the TNFα converting enzyme (ADAM17), which has been shown to be involved in the shedding of sIL-15Rα from membrane-bound IL-15Rα. ADAM17 (in green) was found to be overexpressed in CD patients (Figure 5B) when compared with healthy subjects (Figure 5A). Furthermore, IL-15Rα and ADAM17 were co-localized in colonic epithelial cells in CD patients (Figure 5H) but not in healthy controls (Figure 5G), suggesting that ADAM17 might be responsible for the higher sIL-15Rα serum levels found in CD patients. Moreover, in agreement with the fact that serum sIL-15Rα was found higher in responders than in nonresponders, the cellular expression of ADAM17 and IL-15Rα before IFX was higher for the 2 patients in whom IFX induced a mucosal healing and a histologic improvement compared with the patient who had only a clinical response without mucosal healing (not shown).

**IFX Induced sIL-15Rα Secretion**

Having identified epithelial cells as the major source of sIL-15Rα, we next investigated whether IFX could modulate sIL-15Rα secretion by 2 human colonic epithelial cell lines (HT-29 and Caco2) that expressed very low levels of IL-15Rα and did not secrete detectable amounts of sIL-15Rα. The results are shown in Figure 6A for HT-29 cells and similar results were obtained with Caco2 cells (not shown). As shown in Figure 6A, IFX markedly increased the secretion of sIL-15Rα in a dose-dependent manner from 0.01 to 100 μg/mL, with a plateau level obtained at 1 μg/mL (25 ± 3 vs 0.3 ± 1.5 pmol/L; P < .0001). This inducing effect could be reversed almost completely by preincubated epithelial cells with 0.2 nmol/L of GM-6001, an inhibitor of metalloproteases, but not by its negative control, GM-6001neg (Figure 6B). sIL-15Rα was 67 ± 10 pmol/L in the presence of 1 μg/mL IFX and 84 ± 7 pmol/L in the presence of IFX and GM-6001neg vs 15 ± 3 pmol/L in the presence of IFX plus GM-6001 (P < .0001). In contrast, the 2 other anti-TNFα compounds, etanercept and adalimumab, when used at a similar concentration (1 μg/mL) (Figure 6C and D), and at even higher doses (100 μg/mL) (data not shown), did not significantly affect sIL-15Rα secretion.

**Figure 5.** Cellular expression of ADAM17 (in green) in (A) healthy controls and on (B) colonic biopsy specimens obtained from CD patients before IFX. (A and B) The cellular expression of ADAM17 was higher in CD patients compared with healthy controls. Cellular expression of IL-15Rα (in red) in (C) healthy controls and on (D) colonic biopsy specimens obtained from CD patients before IFX. (C and D) The cellular expression of IL-15Rα was higher in CD patients compared with healthy controls. IL-15Rα also is co-localized with cytokeratin in (E) CD patients and in (D) healthy controls. ADAM17 also is co-localized with IL-15Rα in (H) CD patients and not in (G) healthy controls. Immunostaining was specific for IL-15Rα and ADAM-17.
Discussion

This study shows the in vivo expression in human serum of a naturally secreted sIL-15Rα and IL-15/sIL-15Rα complex in CD that the IL-15/IL-15Rα system could be owing to a direct effect of IFX on cellular tmTNF and not only a consequence of the decreased inflammation. Moreover, the downstream mechanisms involved appeared to be specific to IFX because etanercept and adalimumab did not induce the release of sIL-15Rα by epithelial cells.

IL-15 serum levels significantly decreased in patients who experienced a clinical remission after IFX. This could be owing to a general benefice linked to a reduced inflammation or to the release of sIL-15Rα from IL-15Rα-bearing cells. sIL-15Rα, which retains a high affinity for IL-15, subsequently could decrease circulating free IL-15 levels by forming IL-15/sIL-15Rα complexes. Our in vitro data indeed showed that IFX was able to induce the secretion of significantly higher amounts of sIL-15Rα by epithelial cells, whereas etanercept or adalimumab did not affect sIL-15Rα secretion. These results suggest that the specific release of sIL-15Rα was induced by IFX and not by etanercept or adalimumab.
sIL-15Rα after IFX binding on tmTNF, rather than the general reduced inflammation, is responsible for the decrease of IL-15 serum levels.

Different studies have shown the implication of ADAM17 in the shedding of sIL-15Rα. Cleavage of membrane-bound IL-15Rα (tmIL-15Rα) by metalloproteinases is supported by our immunohistochemical data showing the following: (1) IL-15Rα and ADAM17 are co-localized on epithelial cell membranes in CD colonic biopsy specimens; (2) expression level of both proteins were increased after IFX treatment; and (3) release of sIL-15Rα by IFX is decreased markedly by GM6001, which inhibits ADAM17 action.

In a genetic analysis of a cohort of CD patients, it was shown that one haplotype of the ADAM17 gene was associated with a good response to IFX, suggesting that the ADAM17 gene may be involved in the response to IFX as part of a multigenetic mechanism. Such IFX-induced specific release of sIL-15Rα could explain correlated sIL-15Rα increase and IL-15 decrease in the sera of responder patients. A similar observation has been observed in rheumatoid arthritis patients treated with IFX in whom the IL-15 levels decreased after treatment. It would be interesting to test in the sera of such rheumatoid arthritis patients whether the sIL-15Rα is expressed and increased in correlation with the IL-15 decrease during IFX treatment.

In nonresponder patients, IL-15 levels were comparable with those obtained in controls and significantly lower than those measured in responder patients. This could suggest that our population of nonresponders included nonflamed patients with irritable bowel symptoms. This is very unlikely because the TNFα, CRP, IL-1β, and IL-6 levels in these patients before IFX were comparable with those shown by responder patients and their decrease of cytokine levels after IFX was not correlated, therefore allowing them to be considered as nonresponders to IFX. The inefficacy of IFX was not explained by an imbalance between TNFα and TNFβ because their proportions were similar in the sera of responder and nonresponder patients (data not shown). The discrepancy between the IL-15 levels could not be explained by differences in the demographic, clinical, or therapeutic characteristics of the responder vs nonresponder patients.

One explanation could be linked to the mechanisms involved in cell secretion of IL-15 itself. Indeed, it recently was shown that IL-15 secretion is highly dependent on its intracellular pre-association with IL-15Rα. IL-15Rα binds tightly to IL-15 and stabilizes the protein in the endoplasmic reticulum/Golgi apparatus. The IL-15/IL-15R complex and excess uncomplexed IL-15Rα are translocated to the plasma membrane where they can be cleaved by matrix metalloproteinases, resulting in the shedding of sIL-15Rα and soluble IL-15/sIL-15Rα complexes. Thus, differences between responder and nonresponder patients regarding IL-15 concentrations might be explained either by default in the signaling mechanisms downstream of tmTNF activation or in those involved in the translocation and cleavage of the complex.

Modulation of IL-15 and sIL-15Rα secretions by anti-TNF agents may be of particular interest to control the inflammatory response during IBD. Different studies using IL-15–transgenic mice and IL-15–deficient mice have shown that IL-15 plays a significant role in inflammatory diseases such as rheumatoid arthritis, in which the inflammatory response leading to joint destruction is quite similar to CD. IL-15–deficient mice were more resistant to dextran sulphate sodium colitis compared with control wild-type mice. In a murine collagen-induced arthritis model, IL-15 induced IL-17 production and IL-23R expression in T cells and IL-15 synergized with IL-23 to induce production of IL-17. Similar mechanisms implicating IL-15 overexpression therefore could operate in other inflammatory diseases in which Th17 responses have been described. It has been shown that blocking IL-15 action by its recombinant soluble receptor, sIL17Rα, helps to prevent collagen-induced arthritis in mice, thus reinforcing the beneficial action of soluble receptor in inflammatory disease.

Moreover, the IFX-induced release of sIL-15Rα also could participate in the restoration of cell apoptosis, which was described in patients with active CD treated by IFX. IL-15 is a well-known potent inhibitor of apoptosis by blocking adaptor protein recruitment to the TNF receptor 1. Intracellular domains of ligand-stimulated TNF receptor 1 and tmIL-15Rα compete for binding TNF receptor associated factor 2 (TRAF)2, with tmIL-15Rα showing a higher affinity for TRAF2 than TNF receptor 1. Then, upon activation with IL-15, tmIL-15Rα rapidly depletes TRAF2, which becomes unavailable for assembly with the TNF–TNF receptor 1 complex. We could speculate that in responder patients, the IFX-induced shedding of sIL-15Rα could release TRAF2 and restore its availability for the TNF–TNF receptor 1 complex, which in turn induces cell apoptosis.

In conclusion, our results show that IL-15 and its soluble receptor are implicated in the response to IFX in CD, and suggest that the mechanism of action of IFX through tmTNF reverse signaling involves activation of ADAM17, leading to the release of sIL-15Rα by colonic epithelial cells and subsequent decrease of circulating IL-15. They also suggest that measurement of IL-15 and sIL-15Rα in CD patients could represent useful prognostic parameters for the response to IFX. Further studies using greater cohorts of patients, however, are required to address this issue. Of further interest also will be the analysis of IL-15 and sIL-15Rα in CD patients treated with other anti-TNF as well as other classes of therapeutic agents. Adalimumab, another anti-TNF therapeutic antibody used to treat CD, is unable to induce such a release in vitro, suggesting that its mode of action is different from that of IFX. In line with this observation is the fact that a majority of patients who were intolerant or did not respond to infliximab appeared to be responders to adalimumab. It therefore will be interesting...
to compare the evolution of the IL-15 and sIL-15Rα parameters in patients treated with adalimumab vs IFX. Such studies could reinforce the interest of these parameters as prognostic tools for the choice of anti-TNF treatment.

References

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Reprint requests
Address requests for reprints to: Grégory Bouchaud, PhD, INSERM, U892 CRONA, IRITUN, Equipe Cytokine/Récepteur, 8, Quai Moncousu, F-44007 Nantes. e-mail: bouchaud-g@univ-nantes.fr; fax: (33) (0) 240-35-66-97.

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