Therapy with anti-TNFα Antibody Enhances Number and Function of Foxp3+ Regulatory T Cells in Inflammatory Bowel Diseases

Gilles Boschetti,*† Stéphane Nancey,*† Fatima Sardi,* Xavier Roblin,‡ Bernard Flourié,*† and Dominique Kaiserlian*  

Background: Inflammatory bowel diseases (IBDs) are associated with up-regulation of TNFα, hyperactivation of proinflammatory effector T cells (Teffs) and inefficient control by regulatory CD4+CD25+Foxp3+ T cells (Tregs). The aim of this prospective study was to investigate the short-term impact of treatment of IBD patients with anti-TNFα antibodies (infliximab or adalimumab) on the frequency, phenotype, and suppressive function of Tregs.  

Methods: Active IBD patients including 16 with Crohn’s disease and 9 with ulcerative colitis were treated with anti-TNFα mAb. PBMCs were harvested immediately before and 2 weeks after the first injection. The frequency and phenotype of circulating CD4+CD25+Foxp3+ Tregs were analyzed by flow cytometry, and their suppressive function was assessed by the ability of purified CD4+CD25+CD127− Tregs to inhibit the proliferation of allogenic CD4+CD25− Teffs.

Results: CD4+CD25+Foxp3+ Treg frequency was significantly lower in active IBD patients than in controls (2.8% ± 0.4% vs. 4.6% ± 0.6%, respectively; P = 0.01). On day 14 following the first anti-TNFα infusion, the frequency of circulating Tregs was significantly enhanced in IBD patients (4.0% ± 0.5% vs. 2.8% ± 0.4%, before treatment; P = 0.001), with a 2- to 3-fold increase in the intensity of Foxp3 expression. In addition, infliximab treatment enhanced the suppressive function of circulating Tregs, as shown by inhibition of Teff proliferation at a 1:8 Treg/Teff ratio (28% ± 5% vs. 66% ± 10%, after treatment; P = 0.04).

Conclusions: These data demonstrate that anti-TNFα treatment of active IBD rapidly enhances the frequency of functional Foxp3+ Tregs in blood and potentiates their suppressive function. This indicates that Treg potentiation may represent an unanticipated outcome of anti-TNFα biotherapy in IBD.

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Key Words: anti-TNFα, regulatory T cells, infliximab, adalimumab, Inflammatory bowel diseases

Inflammatory bowel diseases (IBD) encompassing Crohn’s disease (CD) and ulcerative colitis (UC) are supposed to be linked to impaired mucosal tolerance to the gut microbial flora, leading to the development of an intestinal delayed-type hypersensitivity reaction, ultimately responsible for severe and irreversible intestinal lesions. The inflammatory T-cell response is induced via the local release of proinflammatory cytokines including tumor necrosis factor α (TNFα), IL-12/IL-23, and IFNγ, and mediated by both pathogenic Th1- and Tc1-type CD4+ and CD8+ T cells. Anti-TNFα mAbs including both infliximab (IFX), a chimeric mouse antibody, and adalimumab (ADA), a humanized antibody, have demonstrated their efficacy for the induction and maintenance of remission in a significant proportion of patients refractory to conventional therapy. However, some patients failed to respond or lost response with time to the treatment. In vitro studies using normal human cells and ex vivo studies with cells from treated IBD patients indicate that several mechanisms can contribute to the clinical efficacy of anti-TNFα mAb therapy. These include neutralization of soluble TNFα present in serum and mucosal gut lamina propria, binding to the membrane-anchored TNFα precursor or to TNFα bound to its high-affinity receptor, TNFR1. Anti-TNFα mAbs have been shown to promote apoptosis of monocytes and T cells in vitro and to correct the defect in T-cell apoptosis characteristic of IBD. In addition, anti-TNFα mAb treatment promotes restoration of the gut barrier and healing of intestinal lesions.  

Increasing evidence has shown the protective role of Foxp3+ CD4+ regulatory T cells (Tregs) in autoimmune and inflammatory diseases and their critical role as...
regulators of peripheral self-tolerance. Tregs contribute to oral tolerance, maintenance of gut homeostasis, and protection against intestinal inflammation. Alterations in the number, survival, or suppressive function of Tregs in mice with genetic disruption of IL-2, MHC class II, TGFβ, or IL-10 induce spontaneous development of chronic inflammatory intestinal disorders resembling IBD. Constitutive CD4+ Tregs express Foxp3, a member of the forkhead-winged helix family of transcription factor that plays a critical role in the development and suppressive function of Tregs. That Foxp3 is a key molecule in Treg protection from intestinal inflammation is supported by the observation that scurfy mice with a mutated Foxp3 gene, as well as Foxp3-deficient mice, develop gut lesions mimicking IBD. Along these lines, mutation of Foxp3 in human is linked to a selective defect in Tregs and is responsible for an immunodeficiency syndrome called IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked). This disorder is characterized by early in life of severe and often lethal enterocolitis, demonstrating the importance of Tregs in maintenance of gut homeostasis and self-tolerance.

Of the few studies on the status of Tregs in active IBD patients, study documented a slight decrease in circulating Foxp3+ T cells of mesenteric lymph nodes from IBD patients during active disease. This prompted us to investigate whether anti-TNFα therapy in active IBD patients could have an impact on the frequency and/or function of peripheral Tregs. We show here that a single administration of anti-TNFα mAb is associated with enhancement of the proportion of Foxp3+ Tregs and of the expression of the Foxp3 protein and the suppressive function of Foxp3+ Tregs.

SUBJECTS AND METHODS

Patients and Healthy Volunteers

Twenty-five consecutive IBD patients (age 36 ± 3 years, mean ± SEM, range 19–73 years) who should be treated by anti-TNFα according to the recommended inductive protocol were prospectively included. They had been previously diagnosed for IBD (16 with CD and 9 with UC) based on clinical, biological, endoscopic, and histological signs of the disease, according to the Lennard-Jones criteria. The baseline characteristics are summarized in Table 1. At inclusion, all patients had active IBD, as determined by a Crohn’s disease activity index (CDAI) > 150 for CD and a Lichtiger index > 6 for UC. All patients were unresponsive to or dependent on conventional treatment (i.e., steroids, immunosuppressors), and none of them had received prior anti-TNFα biotherapy. Clinical response to anti-TNFα treatment was defined as a decrease in the CDAI of at least 70 points or a decrease in the Lichtiger’s score of at least 3 points; clinical remission was defined as a CDAI < 150 points or a Lichtiger score < 3. Clinical response or remission was evaluated 2 and 6 weeks after the first anti-TNFα infusion. Blood samples from 11 healthy volunteers paired for sex and age (6 females and 5 males, range 18–55 years) were used as controls. All IBD patients and healthy volunteers gave their written informed consent to the protocol, which has been previously accepted by the local Ethical Committee of Lyon.

Anti-TNFα Treatment

The anti-TNFα mAb, infliximab (IFX; Remicade, Schering-Plough, Kenilworth, NJ, USA) was administered intravenously at a dose of 5 mg/kg to 10 of 16 CD patients and all 9 UC patients. The anti-TNFα mAb, adalimumab (ADA; Humira, Abbott, Abbott Park, IL, USA) was injected subcutaneously at a dose of 160 mg to 6 of 16 CD patients. Concomitant treatments (including oral steroids, immunosuppressors, and 5-ASA) were maintained stably...

### TABLE 1. Baseline Characteristics of IBD Patients and Controls

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<tr>
<th></th>
<th>CD</th>
<th>UC</th>
<th>Healthy volunteers</th>
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<tr>
<td>Number</td>
<td>16</td>
<td>9</td>
<td>11</td>
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<tr>
<td>F/M</td>
<td>9/7</td>
<td>6/3</td>
<td>6/5</td>
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<tr>
<td>Mean age, years (range)</td>
<td>35 (19–73)</td>
<td>38 (21–67)</td>
<td>30 (18–55)</td>
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<tr>
<td>Duration of disease, years</td>
<td>10</td>
<td>9</td>
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<td>CD location, n (%)</td>
<td></td>
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<tr>
<td>Ileocolonic</td>
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<tr>
<td>Ileal</td>
<td>4 (25)</td>
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<tr>
<td>Colonic</td>
<td>1 (6)</td>
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<tr>
<td>Anorectal lesions, n (%)</td>
<td>11 (69)</td>
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<td>Concomitant medication, n (%)</td>
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<tr>
<td>Aminosalicylates</td>
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<td>Corticosteroids</td>
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<td>—Azathioprine/6-MP</td>
<td>10 (63)</td>
<td>3 (33)</td>
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<tr>
<td>—Methotrexate</td>
<td>1 (6)</td>
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<td>Disease activity</td>
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<tr>
<td>CDAI</td>
<td>198 ± 20</td>
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<td>Lichtiger’s score</td>
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<td>Anti-TNFα treatment, n (%)</td>
<td>10 (62)</td>
<td>9 (100)</td>
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<td>Adalimumab (ADA)</td>
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until the end of the study, that is, 6 weeks after the first anti-TNFα infusion. Patient follow-up during the protocol period included clinical examination and routine biological analysis (hematocrit and leukocyte count).

**Blood Samples**

Blood samples were harvested from the patients immediately before and 2 weeks after the first anti-TNFα mAb injection, that is, just before the planned second injection with IFX or ADA. Blood samples from 11 healthy volunteers were harvested only once.

**Flow-Cytometry Assay**

Human peripheral blood mononuclear cells (PBMCs), freshly isolated from blood by density gradient centrifugation (lymphocyte separation medium, Eurobio, Les Ulis, France) were surface-stained with FITC-conjugated CD4, CD62L, lymphocyte-activation gene-3 (LAG-3), PE-conjugated CD8, CD4, inducible costimulator (ICOS), PECy5-conjugated CD3, CD25, and PeCy7-conjugated CD4. An intracellular Foxp3 staining was performed according to the manufacturer’s instructions (eBiosciences, San Diego, CA). Briefly, cells were permeabilized, fixed, and then stained with APC-conjugated Foxp3 (clone PCH 101). For each surface and intracellular staining, the relevant isotype control was used. Immunostaining was analyzed by flow cytometry on a LSRII analyzer with FACSDiva software (Becton Dickinson, San Jose, CA).

**Treg Purification**

CD4⁺CD25⁺ Tregs were purified from PBMC by magnetic cell separation (MACS) using a CD4⁺CD25⁺CD127⁻ regulatory T-cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany). CD4⁺CD127⁻ T cells were first isolated by MACS-negative selection using a cocktail of lineage-negative biotinylated mAb and antibiotin microbeads. CD4⁺CD25⁺CD127⁻ T cells were subsequently isolated by positive selection using anti-CD25 mAb-coated microbeads. The resulting CD4⁺CD25⁺CD127⁻ Tregs eluted from the column routinely contained > 90% Foxp3⁺ cells assessed by flow cytometry.

**FACS Sorting of Naïve T Cells**

Naïve CD4⁺CD25⁻ T cells (Teffs) were FACS-sorted from PBMC of a single healthy human blood donor. CD4⁺ T cells were preenriched by MACS using a CD4⁺ T-cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany), and CD4⁺CD25⁻ T cells were then FACS-sorted (FACSAria cell sorter; Becton Dickinson, San Jose, CA) using FITC-conjugated CD4 and PE-conjugated CD25. The purity of CD4⁺CD25⁻ T cells with this method was routinely > 96%. Sorted CD4⁺CD25⁻ Teff cells were frozen in liquid nitrogen until use as Teffs in suppression assays.

**In Vitro Suppression Assays**

Because expression of Foxp3 is not an absolute marker of Tregs, we investigated the impact of treatment with anti-TNFα on Treg suppressive function. Graded numbers of CD4⁺CD25⁺CD127⁻ T cells, containing > 90% Foxp3⁺ cells (Tregs), were cocultured for 5 days at 37°C in 5% CO₂ in triplicate wells in the presence of allogenic CD4⁺CD25⁻ T cells (2.10⁷ cells) and soluble anti-CD3/CD28 beads (1 bead/10 Teffs; Dynal Biotech, Oslo, Norway) in complete RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum and antibiotics. Tregs and Teffs were cocultured at 1:2 to 1:64 Treg/Teff ratios. [³H]-thymidine (1 μCi/well) was added during the last 18 hours of culture. Proliferation was assessed on day 5 of culture by [³H]-thymidine incorporation, using a β-plate liquid scintillation counter Topcount NXT (Packard instrument, Rungis, France). Cultures containing anti-CD3/CD28-stimulated Tregs alone or Teffs alone were used as negative and positive controls, respectively. The Treg suppression assay was performed in 4 IBD patients, before and after IFX, and in 4 healthy donors as controls. Percentage suppression of proliferation was determined as 1 – (cpm coculture Treg-Teff/cpm Teff population alone) x 100%.

**Statistical Analysis**

Statistical analysis was performed using the nonparametric Mann–Whitney U test (unpaired comparisons), the Wilcoxon signed rank test, or the paired t test (paired data) as appropriate. Results were considered statistically significant at a 95% level of confidence (P < 0.05, 2-sided). Data are expressed as means ± SEMs.

**RESULTS**

**Active IBD Patients had Low-Frequency of Tregs**

The frequency of peripheral blood Tregs was analyzed in 25 IBD patients prior to anti-TNFα treatment and 11 age-matched healthy volunteers. FACS analysis of PBMC showed a lower proportion of circulating CD4⁺CD25high Tregs in active IBD patients, compared with healthy volunteers (2.3% ± 0.2% vs. 4.3% ± 1.3%, respectively; P = 0.03; Fig. 1A,B). The frequency of Foxp3⁺-expressing CD4⁺CD25⁺ Tregs among CD4⁺ T cells was also significantly lower in active IBD patients (both CD and UC) compared with the controls (2.8% ± 0.4% vs. 4.6% ± 0.6%, respectively; P = 0.01; Fig. 1C,D). Tregs were similarly decreased in the blood of CD patients (3.1% ± 0.4%) and UC patients (2.5% ± 0.6%; P = 0.3; Fig. 1D). No significant correlation was found between Foxp3⁺ Treg frequency and IBD activity, location of intestinal lesions, or drug intake (i.e., steroids,
azathioprine, 6-mercaptopurine, methotrexate, or 5-ASA; data not shown).

Anti-TNFα mAb Treatment Was Associated with Restoration of Normal Frequency and Absolute Number of Tregs

The effect of anti-TNFα mAb treatment on Tregs was analyzed by comparing the frequency, absolute number, and phenotype of Tregs 2 weeks after the first injection, as compared to before infusion. A single exposure to anti-TNFα mAb was associated with a significant increase in the percentage of CD4⁺CD25⁺Foxp3⁺ cells in 20 of 25 IBD patients on day 14 (mean 4.0% ± 0.5%) compared with the pretreatment value (2.8% ± 0.4%; \( P = 0.001 \); Fig. 2A). Likewise, an increase in Treg proportion among CD4⁺ T cells was observed in 14 of 16 CD patients (mean 4.4% ± 0.6% after vs. 2.9% ± 0.5% prior to treatment; \( P = 0.003 \)) and 6 of 9 UC patients (3.7% ± 0.7% after vs. 2.6% ± 0.7% prior to treatment; \( P = 0.05 \); Fig. 2B). In CD, enhancement of Tregs was observed in 9 of 10 and 5 of 6 patients who received IFX and ADA, respectively (Fig. 2C).

Anti-TNFα mAb treatment enhanced the ratios of Tregs to either naive Teffs (CD4⁺CD25⁻Foxp3⁻; 0.05 ± 0.01 vs. 0.07 ± 0.01; \( P = 0.01 \); Fig. 3A) or activated Teffs (CD4⁺CD25⁺Foxp3⁻; 0.14 ± 0.03 vs. 0.27 ± 0.05; \( P = 0.001 \); Fig. 3B). This was a result of the rise in absolute numbers of Tregs after treatment (19.8 ± 3.7 × 10⁶/L vs. 32.9 ± 6.2 × 10⁶/L; \( P = 0.004 \); Fig. 3C), whereas the proportions of

**FIGURE 1.** Tregs are diminished in patients with active IBD prior to anti-TNFα treatment. Representative dot plot analysis of Tregs from peripheral blood of healthy volunteers (\( n = 11; \) A, C, left) and active IBD patients (\( n = 25; \) A, C, right). The histograms (B, D) represent mean ± SEM proportion of the percent CD4⁺CD25high T cells in total leukocytes (B) and percent CD4⁺CD25⁺Foxp3⁺ T cells in gated CD4⁺ T cells (D) from either healthy controls (B, D, black bars), total IBD patients (B, D, white bars), CD patients (\( n = 16; \) D, hatched bar), and UC patients (\( n = 9; \) D, gray bar).
neither naive nor activated Teffs were affected (Fig. 3D). Thus, anti-TNFα mAb therapy selectively enhanced the frequency and absolute number of Tregs, without affecting the proportion of conventional Teffs.

Peripheral Treg Frequency and Clinical Response or Remission to anti-TNFα mAb Treatment

A single administration of anti-TNFα mAb resulted within 2 weeks in clinical improvement in 22 of 25 IBD patients (88%), which persisted for up to 6 weeks. Among the 22 responder patients, 15 were improved, and 7 achieved remission; peripheral Treg frequency was increased in 13 and 6 of those patients, respectively. Among the 3 nonresponder patients, peripheral Treg frequency was not affected by treatment in 2 patients but was increased in the last patient (1.1% prior vs. 4.4% after anti-TNFα treatment). Conversely, 3 of 5 patients in whom peripheral Treg proportion did not change after anti-TNFα mAb had an excellent clinical response (improvement n = 2, remission n = 1) to treatment after 2 and 6 weeks. Overall, no correlation was found between the change in the frequency of peripheral Tregs and the percentage change in the clinical score (CDAI or Lichtiger) before and after anti-TNFα mAb ($r = 0.19; P = 0.37$).
Treg Phenotype and Up-Regulation of Foxp3 Expression after Anti-TNFα Therapy

Flow cytometry analysis of Foxp3 expression level in total CD4+ T cells revealed a robust increase (i.e., 2- to 3-fold) in mean fluorescence intensity (MFI) following the treatment with anti-TNFα (203 ± 51 before vs. 498 ± 159 after; \( P = 0.02 \); Fig 4). Phenotypic analysis of Tregs was also carried out in patients before and after anti-TNFα mAb therapy. CD62-L expression on CD4+CD25+ cells decreased at a nonsignificant statistical level after anti-TNFα treatment (MFI: 1135 ± 138 before vs. 1404 ± 275 before; \( P = 0.5 \); Fig. 5A,B), without changes in the percentage of CD62L+ Tregs (data not shown). In contrast, anti-TNFα treatment resulted in a moderate increase in the frequency of ICOS+ Tregs (8.3% ± 2.5% before vs. 12.3% ± 4.4% after, respectively; \( P = 0.42 \); Fig. 5C,D) and up-regulated ICOS expression on CD4+CD25+Foxp3+ Tregs (81 ± 19 before vs. 142 ± 45 after treatment, respectively; \( P = 0.33 \); Fig. 5E). There was no detectable change in Treg expression of LAG-3 after anti-TNFα treatment (data not shown).

Anti-TNFα mAb Treatment Potentiated Treg Function

Because expression of Foxp3 was not an absolute marker of Tregs, we investigated the impact of treatment with anti-TNFα on Treg suppressive function. We analyzed the suppressive function of purified CD4+CD25+CD127− T cells33 highly enriched (>90%) in Foxp3+ cells, using

FIGURE 3. Relative impact of anti-TNFα mAb treatment on Tregs and Teffs in IBD. Histograms of FACS analysis (mean ± SEM) of Tregs/naive Teffs (A) and Tregs/activated Teffs (B) before (A–D, white bars) and after (A–D, gray bars) anti-TNFα mAb injection and in healthy controls (A and D, black bars). Number of peripheral CD4+CD25+Foxp3+ Tregs in CD (C, left), UC (C, middle), and total IBD patients (C, right) prior to and after anti-TNFα mAb. Histogram represents the mean ± SEM proportion of naive Teffs (CD4+CD25-; D, left) and activated Teffs (CD4+CD25-Foxp3+; D, right) in gated CD4+ T cells of IBD patients prior to and after anti-TNFα and in healthy volunteers.
allogeneic CD4⁺CD25⁻ T cells (sorted from a single healthy donor) as responder CD4⁺ T cells (Teffs). A comparable dose-dependent suppression was observed with Tregs from IBD patients and with those from healthy volunteers (data not shown). However, IFX treatment induced a significant increase in the suppressive function of Tregs, as shown by 50% inhibition of Teff proliferation achieved by Tregs at a Treg/Teff ratio of 1:4 prior treatment compared with 1:12 after treatment (Fig. 6A). The suppressive potential of Tregs calculated at the 1:8 Treg/Teff ratio was significantly higher after (66% ± 10%), compared with before (28% ± 5%) IFX administration (P = 0.04; Fig. 6B). Representative analysis of the suppressive efficacy of Tregs from 1 CD patient of the 4 IBD patients tested is illustrated in Figure 6C.

DISCUSSION

This prospective study provides, to our knowledge, the first clear-cut evidence that therapy with anti-TNFα mAb rapidly enhances the number and suppressive function of peripheral blood Foxp3⁺ Tregs in patients with active IBD. This effect observed in both CD and UC patients was induced with comparable efficacy with IFX and ADA and was detectable as early as 2 weeks following the first antibody injection. The impact of anti-TNFα mAb therapy appeared selective for Tregs, as shown by (1) the increased...
FIGURE 5. Surface expression of CD62L and ICOS on peripheral Tregs after anti-TNFα mAb. CD4⁺CD25⁺ regulatory T cells from IBD patients before and after anti-TNFα treatment. A: Histograms were gated on CD4⁺CD25⁺ cells (as indicated); the MFI of CD62L is shown. B: MFI of CD62L in the CD4⁺CD25⁺ Treg cells prior to and after treatment is shown in the charts. C: Representative FACS plot of ICOS expression on the CD4⁺CD25⁺ cells is shown. D: Frequency of ICOS from all patients before and after TNFα mAb. E: MFI of ICOS from all patients before and after TNFα mAb is shown in the chart.
We first observed that IBD patients (both CD and UC) exhibited a lower frequency of total and Foxp3-expressing CD4+CD25high T cells, compared with that in age-matched normal individuals. This is in agreement with previous studies showing that active IBD feature a selective defect in circulating Foxp3+CD4+CD25+ T cells that is only partly compensated for by a rise in Foxp3+ cells in the inflamed gut lamina propria. As previously described in patients with rheumatoid arthritis (RA), anti-TNFα mAb treatment caused an increase in circulating Foxp3+ Tregs up to levels close to those of healthy individuals. Moreover, Foxp3+ Tregs from treated patients expressed a 2- to 3-fold increase in Foxp3 protein expression. Finally, purified Foxp3+CD4+CD25CD127/C0 Tregs exhibited increased in vitro suppressive function toward allogeneic CD4+CD25/C0 responder T cells. Given that Foxp3 can be expressed at a low level by conventional CD4+ T cells in humans, our data strongly support that anti-TNFα treatment resulted in a selective enhancement of functional Tregs.

Several mechanisms may have contributed to the potentiation of Tregs by anti-TNFα mAb therapy. First, anti-TNFα mAb treatment could have promoted the survival of Tregs, for example, by reducing the rate of apoptosis, as observed after IFX treatment in RA. Second, the quantitative increase in Tregs expressing elevated levels of Foxp3+ and suppressive function could be a result of activation and proliferation of preexisting Foxp3+ Tregs or of their differentiation by conversion from Foxp3-/C0 precursors. Third, although TNFα at a high dose inhibits Treg function, exposure to a low dose of TNFα rather promotes survival, activation, and expansion of Tregs, by signaling via its high-affinity receptor TNFR II. It may be speculated that whereas anti-TNFα mAb may neutralize serum TNFα efficiently, its more limited access into the inflamed mucosa might allow binding of residual cytokine to Tregs compatible with their enhanced survival and functionality.

The precise mechanisms responsible for the enhanced suppressive function of Tregs in anti-TNFα-treated IBD patients remain elusive. Numerous studies have well established that the function of Tregs is governed by the transcription factor Foxp3, which leads to autoimmune and chronic inflammatory disorders in humans and animals (and 2) T cells transfected with Foxp3 acquire regulatory abilities. In the present study, we documented a significant enhancement of Foxp3 expression (MFH) in IBD patients’ Tregs after anti-TNFα therapy, which is consistent with potentiation of Treg suppressive function. Several Treg cell-surface molecules, such as ICOS, LAG3, and CD62L, have been associated with Treg’s suppressive potential. We observed that CD62L (i.e., L-selectin), which dictates T-cell entry into

![FIGURE 6. IFX treatment potentiates CD4+CD25CD127 regulatory T-cell ability to suppress the proliferation of CD4+CD25 effector T cells. Regulatory T cells were isolated from peripheral blood mononuclear cells from 4 IBD patients by MACS selection. Effector T cells were FACs- sorted from a single healthy volunteer. CD4+CD25 Teffs (2 × 10⁶ cells/well) were stimulated with anti-CD3/CD28 beads and cocultured in the presence of CD4+CD25 CD127 Treg cells at different responder/suppressor ratios. Proliferation (triplicate cultures) was measured by [³H]-thymidine incorporation. A: Results (percent inhibition of proliferation) represent data from 4 patients with IBD prior to and after IFX. Dotted lines show 50% of inhibition of proliferation. B: Percent inhibition of proliferation at a ratio of 1:8 regulator/suppressor cells from IBD patients (n = 4) prior to and after IFX. C: Representative thymidine incorporation (CPM) at a ratio of 1:8 from a CD patient before and after IFX.](image-url)
lymph nodes, was constitutively expressed on IBD Tregs, and down-regulated after anti-TNFα mAb treatment. This observation is in accordance with a recent study in RA showing that IFX induces a distinct subset of Foxp3⁺CD62L⁻ Tregs with a more robust suppressive function.⁴² It may be proposed that this effect reflects a tendency of blood Tregs to reach peripheral tissues, particularly the inflamed gut, as previously reported for L-selectin-negative Tregs up-regulating skin or gut homing integrins.⁴³ In addition, we observed that anti-TNFα mAb treatment tended to enhance ICOS expression on Tregs. Previous studies have shown that ICOS is up-regulated on Ag-induced Tregs with enhanced proliferation and suppressive properties.⁴⁴,⁴⁵ Thus, it is possible that anti-TNFα mAb induced an increase in more potent Ag-specific Tregs. Alternatively, treatment did not affect expression of LAG-3, a CD4-related Treg molecule that binds MHC class II and modulates both in vitro and in vivo suppressive Treg function.⁴⁶

It should be emphasized that potentiation of circulating Tregs by anti-TNFα therapy described in this study (1) may not necessarily concern Tregs present in the intestinal mucosa and (2) could be the consequence rather than the cause of clinical improvement. Indeed, a marked clinical response was seen within 2 weeks after the first injection of IFX or ADA, concurrent with Treg potentiation. It is thus equally possible (1) that Tregs contribute to the efficacy of anti-TNFα mAb and/or (2) that the restoration of a normal Treg compartment is consecutive to anti-TNFα therapy-induced recovery of intestinal homeostasis. Further kinetic studies will be necessary to determine whether Treg potentiation is the cause or consequence (or both) of anti-TNFα treatment’s efficacy in IBD. Indeed, we cannot exclude that Treg number and function potentiation are more the consequence of anti-TNFα mAb–induced clinical improvement than that of anti-TNFα treatment by itself. Even if the observation that 1 IBD patient who failed to achieve a clinical response to anti-TNFα has enhanced Treg number in peripheral blood supports a specific effect of the anti-TNFα therapy, we acknowledge that our study was not designed to and powerful enough to definitely conclude this. In addition, as some IBD patients (n = 3) achieved a clinical response (and even a remission in 1 case) to anti-TNFα treatment without a rise in Treg frequency, other ways by which active IBD might be improved by anti-TNFα merit further considerations. These data are in accordance with those found by Ehrenstein et al.⁴⁶ in rheumatoid arthritis patients; they failed to detect a systematic concordance between response to anti-TNF (infliximab and adalimumab) therapy and Treg changes.

In any case, this study documents a novel potential mechanism that could contribute to the beneficial effect of anti-TNFα therapy in IBD. Tregs might thus represent an important target for optimization of existing therapies against TNFα or its receptor, or for the development of novel biotherapies focusing on other proinflammatory cytokines relevant in IBD.

REFERENCES


