Aryl hydrocarbon receptor-driven signals inhibit collagen synthesis in the gut

Ivan Monteleone, Francesca Zorzi, Irene Marafini, Davide Di Fusco, Vincenzo Dinallo, Roberta Caruso, Roberta Izzo, Eleonora Franzè, Alfredo Colantoni, Francesco Pallone and Giovanni Monteleone

Dipartimento di Medicina dei Sistemi, Università Tor Vergata, Rome, Italy

Fibrostrictures (FS) are a major complication of Crohn’s disease (CD). Pathogenesis of FS is not fully understood, but activation of fibroblasts and excessive collagen deposition are crucial in the development of FS. Here, we investigated the role of aryl hydrocarbon receptor (AhR) in intestinal fibrosis. AhR RNA and protein expression were evaluated in intestinal fibroblasts of CD patients and controls. CD fibroblasts were stimulated with TGF-β1 or TNF-α in the presence or absence of the AhR activator Ficz, an AhR antagonist CH223191, or a specific AhR-silencing RNA. In CD fibroblasts, TGF-β1 and TNF-α increased Col1A1, Col3A1 and α-SMA transcripts and collagen secretion and this effect was reduced by Ficz and upregulated by CH223191. TGF-β1 or TNF-α induced activation of p38 and ERK1/2 MAP kinases was decreased by Ficz and increased by CH223191. The inhibitory effect of Ficz on Map kinase activation and collagen induction was abolished by AhR silencing. To assess the role of AhR in vivo, mice with trinitrobenzene-sulfonic-acid induced colonic fibrosis were given Ficz or CH223191. Mice given either Ficz or CH223191 produced less or more collagen respectively as compared with control mice. Our results indicate that AhR is a negative regulator of profibrotic signals in the gut.

Keywords: Aryl hydrocarbon receptor · Collagen · Crohn’s disease · Fibroblasts · Strictures

Introduction

Crohn’s disease (CD), one of the major forms of inflammatory bowel diseases (IBD) in human beings, is characterized by a chronic, segmental, and transmural inflammation that can involve any part of the alimentary tract [1, 2]. At the onset, more than two-thirds of CD patients exhibit inflammatory lesions, which are responsible for symptoms such as diarrhea, abdominal pain, and weight loss [2]. However, over the long-term, CD course can change and culminate in formation of fibrostrictures (FS), which represent one of the major indications for surgery [3, 4]. FS are more frequent in patients with ileal disease and both genetic and environmental factors increase the risk to develop such a complication [3, 5]. While persistent inflammation with injury of epithelial and deep layers of the intestine is considered the major driver for FS formation [6], the sequence of events linking the tissue-damaging immune-inflammatory response with FS development is not entirely known. Accumulation of collagen-rich extracellular matrix is the biological hallmark of FS and there is evidence that cytokines (e.g., TGF-β1, IL-1, IL-13, IL-17A, TNF-α) produced in excess by both immune and nonimmune cells in the gut of CD patients stimulate fibroblasts to produce excessive amounts of collagen, thereby contributing to intestinal fibrosis [7–12]. Indeed, strategies aimed at blocking either profibrotic cytokines or intracellular pathways triggered by such cytokines (e.g., NF-κB) have been employed with success in the prevention...
or cure of colitis-induced fibrosis in mice [11, 13]. Unfortunately, however, no therapeutic medical strategy for preventing/treating FS in CD exists at the moment, but this clinical scenario could greatly improve by identifying targets that control fibrogenesis.

Aryl hydrocarbon receptor (AhR), a member of the Per-ARNT-Sim superfamily of proteins, is a cytosolic transcription factor ubiquitously expressed in vertebrate cells and known for mediating the toxicity of xenobiotic molecules as 2,3,7,8-tetrachlorobenzo-p-dioxin [14, 15]. AhR can be activated by additional ligands including environmental, dietary, and endogenous aromatic compounds as indole-3-carbinol (I3C), 6-formylindolo[3,2-b]carbazole (Ficz), and 6,12-diformylindolo[3,2-b]carbazole [15, 16]. AhR is present in the cytosol in an inactive form, bound to several cochaperones [14]. After binding with ligands, AhR dissociates from the chaperones and translocates into the nucleus, where it binds to its dimerization partner AhR nuclear translocator, and the AhR/AhR nuclear translocator complex initiates transcription of genes with promoters containing a dioxin or xenobiotic consensus sequence [14]. It is widely known that AhR plays a key role in the differentiation, activation, and proliferation of many cell types in various organs [17, 18]. In this context, we have recently shown that Ficz-driven activation of AhR in T cells triggers regulatory signals in the gut, which culminate in the production of IL-22 and suppression of experimental colitis in mice [19]. AhR regulates also the cytokine response of human fibroblasts to inflammatory stimuli as well as expression of several genes that are known to be upregulated during the development of fibrosis in liver and lung [20–23]. Consistently previous studies have shown that AhR-deficient mice are more susceptible to develop fibrosis in liver [24–26]. Altogether these findings prompted us to explore whether AhR is a regulator of collagen synthesis in the gut.

## Results

**AhR activation negatively regulates collagen production by intestinal fibroblasts**

AhR RNA transcripts were constitutively expressed in fibroblasts isolated from the gut of patients with CD, patients with ulcerative colitis (UC), and controls with no significant differences among groups (Fig. 1A and Supporting Information Fig. 1). Flow cytometry analysis showed that nearly 50% of intestinal fibroblasts expressed AhR in both inflammatory bowel diseases (IBD) and controls (Fig. 1B). These data were confirmed by Western blot analysis of total proteins extracted from fibroblasts of patients and controls (Fig. 1C). To determine whether AhR activation regulates collagen production, fibroblasts isolated from FS of CD patients were treated with TGF-β1 or TNF-α, two known inducers of collagen [12], in the presence or absence of Ficz for 24 h. As expected, stimulation of fibroblasts with TGF-β1 or TNF-α induced a significant increase of transcripts for Col1A1, Col3A1, and α-SMA, a marker of fibroblast activation (Fig. 2A and B). In contrast, these two cytokines did not affect AhR expression (Supporting Information Fig. 2). Treatment of fibroblasts with Ficz did not alter the basal RNA expression of Col1A1, Col3A1, and α-SMA, but significantly reduced TGF-β1 or TNF-α driven RNA transcripts for Col1A1, Col3A1, and α-SMA (Fig. 2A and B). To further assess the role of AhR in the control of collagen expression, CD fibroblasts were stimulated with TGF-β1 or TNF-α in the presence or absence of CH223191, a specific inhibitor of interaction of AhR with its ligand. CH223191 significantly enhanced Col1A1 RNA transcripts in unstimulated fibroblasts as well as RNA expression of Col1A1, Col1A3, and α-SMA in fibroblasts stimulated with TGF-β1 or TNF-α (Fig. 3A and B). As expected, preincubation of fibroblasts with CH223191 fully abolished the inhibitory effect of Ficz on TGF-β1 induced collagen (Supporting Information Fig. 3). To further confirm the role of AhR in the control of collagen production, RNA transcripts for Col1A1 and Col1A3 were evaluated in fibroblasts transfected with AhR siRNA. Transfection of fibroblasts with AhR siRNA significantly reduced AhR expression (Fig. 4A and B).
AhR activation inhibits fibroblast collagen expression induced by profibrotic cytokines. (A and B) CD intestinal fibroblasts were stimulated with (A) TGF-β (1 ng/mL) or (B) TNF-α (15 ng/mL) in the presence or absence of Ficz. After 24 h Col1A1 (top), Col3A1 (middle), and α-SMA (bottom) were examined by real-time PCR and normalized to β-actin. Data are shown as mean ± SD of three experiments where one sample was used in each experiment.

Silencing of AhR made cells resistant to the inhibitory effect of Ficz on TGF-β1 and TNF-α driven Col1A1 and Col1A3 RNA induction (Fig. 4A and B).

Analysis of soluble forms of collagen in fibroblast culture supernatants confirmed that, in unstimulated cells, CH223191 but not Ficz significantly upregulated collagen secretion (Fig. 5). Moreover, Ficz dose-dependently inhibited TGF-β1 and TNF-α induced collagen secretion while CH223191 increased such a synthesis (Fig. 5). Neither Ficz nor CH223191 changed fibroblast viability or proliferation (Supporting Information Fig. 4). AhR activation/inhibition exerted either negative or positive effects on collagen production even in intestinal fibroblasts isolated from controls (Supporting Information Fig. 5).

AhR controls MAP kinase activation in CD fibroblasts

Activation of p38 and ERK1/2 MAP kinases play a role in the TGF-β1 and TNF-α driven collagen production [27–29]. Therefore, we next investigated whether the AhR-mediated control of collagen synthesis was associated with changes of this intracellular pathway. To this end, we monitored p38 and ERK1/2 activation by flow cytometry and by Western blotting using specific antibodies that recognize the phosphorylated/active forms of these proteins.

In unstimulated conditions, the fractions of cells expressing p-p38 or p-ERK1/2 were not affected by Ficz while were significantly increased by CH223191 (Fig. 6A and B). TGF-β1 and TNF-α significantly increase the percentages of fibroblasts expressing p-p38 and p-ERK1/2, and this effect was either decreased or increased by Ficz or CH223191, respectively (Fig. 6A and B). TGF-β1 and TNF-α enhanced also the fractions of cells expressing p-Smad2/3 or p-NF-κB/p65, respectively, but neither Ficz nor CH223191 changed such percentages (Fig. 6A and B). Western blotting analysis of total proteins extracted from the above-stimulated fibroblasts confirmed that Ficz reduced TGF-β1 induced ERK, p38 and TNF-α induced ERK, p38, whereas did not alter Smad2/3 or NF-κB/p65 phosphorylation (Supporting Information Fig. 6A and B).
AhR controls TNBS-induced intestinal fibrosis in mice

To translate these data in vivo, we used an experimental model of intestinal fibrosis induced in Balb/c mice by repeated, rectal administration of low doses of trinitrobenzene-sulfonic acid (TNBS) [13]. To determine whether AhR activation interferes with collagen synthesis and fibrosis development, mice were given intraperitoneally either Ficz or CH223191 after the fifth week of TNBS administration (Fig. 7A). This time point was selected on the basis of previous studies showing that deposition of collagen begins at week 4 after the first TNBS administration [13]. Extent and severity of inflammation and fibrosis were assessed in animals sacrificed on week 8. As expected, mice treated with repeated doses of TNBS exhibited minimal intestinal inflammation but marked thickening of the colon wall. Masson’s trichrome staining of colonic sections and collagen RNA and protein analysis using whole colonic samples confirmed the increased collagen induction in TNBS-treated mice as compared to controls. Mice given Ficz exhibited a significant reduction of collagen and profibrogenic cytokines, such as TNF-α and IL-17A, while those receiving CH223191 produced more collagen, TNF-α and IL-17A as compared to TNBS-treated mice (Fig. 7B and C). Neither Ficz nor CH223191 affected expression of IL-13 and TGF-β1 two fibrogenic cytokines [30, 31] (Fig. 7C). To exclude the possibility that the anti-fibrotic effect of Ficz is on T cells and innate lymphoid cells (ILC), mice were depleted of these two cell types using a specific anti-CD90.2 antibody 12 h before each Ficz injection. Depletion of T cells and ILC did not alter the anti-fibrotic effect of Ficz (Supporting Information Fig. 7).

Discussion

This study was undertaken to investigate the role of AhR in the control of intestinal fibrosis. We initially showed that AhR was constitutively expressed in intestinal fibroblasts isolated from FS of CD patients as well as in intestinal fibroblasts of UC patients and controls. Although treatment of CD fibroblasts with Ficz did not modify the basal expression of collagen, inhibition of AhR with CH223191 led to increased collagen production, thus suggesting that constitutive AhR expression in these cell types is essential to keep in check collagen synthesis. Fibroblasts isolated from sites of FS in CD have enhanced capacity to respond to profibrotic cytokines by producing collagen [9, 10, 32]. Since studies in other systems have shown that AhR negatively regulates intracellular pathways activated by profibrotic cytokines [33, 34], we
next evaluated whether AhR activation is involved in the TGF-β1 and TNF-α induced collagen production. CD fibroblasts displayed a different capacity to synthesize collagen when treated with the AhR ligand, Ficz, or with the inhibitor of AhR activity, CH223191. In particular, Ficz dose dependently reduced collagen RNA and protein expression while inhibition of AhR was followed by enhanced collagen production in response to TGF-β1 and TNF-α. Interestingly however, even at the greatest doses used in our system, Ficz did not completely abolish cytokine-induced collagen synthesis, raising the possibility that AhR does not control all the cytokine-driven intracellular pathways that lead to collagen production. Indeed, analysis of such signals revealed that Ficz abrogated activation of both p38 and ERK1/2 without affecting activation of Smad2/3 and NF-κB in fibroblasts stimulated with TGF-β or TNF-α respectively. The fact that Ficz-mediated abrogation of p38 and ERK1/2 activation was accompanied by a 60% reduction of cytokine-driven collagen synthesis advocates a major role for MAP kinases in the control of collagen production in CD fibroblasts. This is in line with other reports showing the involvement of MAP kinases in the chemokine and profibrotic factor response of intestinal fibroblasts to inflammatory cytokines [35]. The lack of effect of AhR activation or inhibition on Smad2/3 and NF-κB activation in response to TGF-β or TNF-α is noteworthy as these findings indicate that AhR activation does not induce a state of global unresponsiveness in intestinal fibroblasts, perhaps explaining why proliferation and survival of these cells were not affected by Ficz or CH223191.

The AhR-mediated negative regulation of collagen production was supported by our in vivo studies in mice showing that Ficz was effective for minimizing fibrosis associated with chronic long-term inflammation. In contrast, mice receiving CH223191 exhibited a more intense collagen deposition as compared to control mice. In these studies, treatment with both Ficz and CH223191 started at a time point (week 5) that is characterized by pathological...
AhR controls collagen secretion. (A–D) CD fibroblasts were stimulated with (A and C) TGF-β or (B and D) TNF-α in the presence or absence of (A and B) Ficz or (C and D) CH223191 (a-AhR, 10 μM) for 48 h. Total collagen in supernatants was analyzed by colorimetric assay. Data are shown as mean ±SD of three experiments where one sample was used in each experiment.

Figure 5. AhR controls collagen secretion. (A–D) CD fibroblasts were stimulated with (A and C) TGF-β or (B and D) TNF-α in the presence or absence of (A and B) Ficz or (C and D) CH223191 (a-AhR, 10 μM) for 48 h. Total collagen in supernatants was analyzed by colorimetric assay. Data are shown as mean ±SD of three experiments where one sample was used in each experiment.

Materials and methods

Patients and samples

Resected intestinal tissue were obtained from surgical specimens of 15 patients with ileal fibrostenosing CD (median age, 36 years; range: 25–56 years); 9 of these 15 patients were receiving corticosteroids, and 6 of 15 were on corticosteroids and azathioprine. Further intestinal specimens were also taken from five patients with UC undergoing colectomy for a chronic disease and nine UC patients undergoing endoscopy for recent flare-ups (median age, 39 years; range 29–57 years). Five UC patients were receiving corticosteroids while others were treated with mesalazine. Controls are ileal biopsies taken from four patients with irritable bowel syndrome and from macroscopically and microscopically unaffected zones of nine patients undergoing colectomy for colon cancer (median age, 48 years; range 32–68 years).

Isolation and culture of intestinal fibroblasts

All the reagents were purchased from Sigma-Aldrich (Milan, Italy) unless specified. Intestinal fibroblasts were isolated and phenotypically characterized as described elsewhere [36]. In all the experiments, fibroblasts were used freshly or between passages 2 and...
Figure 6. AhR activation leads to inactivation of p38 and ERK1/2 in CD fibroblasts. (A) CD intestinal fibroblasts were either left unstimulated (unst) or stimulated with TGF-β or TNF-α in the presence or absence of Ficz (final concentration 200 nM) or CH223191 (a-AhR, 10 μM) for 24 h. p-P38(pT180/pY182), p-ERK1/2(pT202/pY204)(pT184/pY186), p-NF-kBp65, and p-Smad2/3 were assessed by flow cytometry. Numbers indicate the percentages of p-p38+, p-ERK1/2+, p-NF-kBp65+, or p-Smad2/3+ cells in the designated gates. Isotype control stain is also indicated. One of three representative experiments in which cells of three patients were used is shown. (B) Percentages of p-p38+, p-ERK1/2+, p-NF-kBp65+, or p-Smad2/3+ cells isolated from three CD patients and stimulated with TGF-β or TNF-α in the presence or absence of Ficz (200 nM) or CH223191 (a-AhR, 10 μM) for 24 h. Data are shown as the mean ± SD of three experiments where one sample was used in each experiment. *p < 0.04 versus unstimulated; **p < 0.001 versus unstimulated; #p < 0.03 versus TGF-β; +p < 0.02 versus TNF-α.
Figure 7. Ficz-treated mice are largely protected from TNBS-induced intestinal fibrosis. (A) Schematic view of TNBS-induced intestinal fibrosis model. Balb/c mice were given weekly TNBS treatments and Ficz or CH223191 (a-AhR) were administered starting after the fifth TNBS administration. (B) Representative colon cross-sections of CTR mice and TNBS-treated mice receiving Ficz or CH223191 (a-AhR) stained with Masson’s trichrome. Percentages of animals harboring mild, moderate, and severe fibrosis are also indicated. (C and D) Colonic samples were taken from CTR mice and TNBS-treated mice injected with Ficz or CH223191 (a-AhR) and analyzed (C) for Col1A2, TGF-β, TNF-α, IL-17A, and IL-13 RNA by real-time PCR and normalized for β-actin or (D) for total collagen by colorimetric assay. Data are shown as mean ± SD of three separate experiments (n = 12 mice total per group).
8 and were detached using Trypsin/EDTA solution. To examine whether AhR regulates collagen production, fibroblasts isolated from CD patients were starved overnight and then stimulated with TGF-β1 (1 ng/mL; Peprotech EC, London, UK) or TNF-α (15 ng/mL; R&D Systems, Abingdon, UK) in the presence or absence of Ficz (final concentration, 100–400 nM; Alexis, Milan, Italy) or 2-methyl-2H-pyrazole-3-carboxylic acid (CH223191), an AhR antagonist (final concentration 10 μM; Calbiochem, Nottingham, England) for 24–48 h. To control the specific effects of Ficz, fibroblasts were preincubated for 24 h with a silencer select siRNA for AhR (s1200, Life Technologies, Monza, final dilution 100 pM) or control siRNA (final concentration 100 pM) before the stimulation with TGF-β1, TNF-α or Ficz. At the end, cells were used to extract RNA and total proteins and cell-free supernatants were analyzed for collagen content.

**Induction of colonic fibrosis**

TNBS was dissolved in 45% ethanol and administered intrarectally to 8-week-old female balb/c mice for 7 weeks as previously described [13]. Ficz (1 μg/mouse) was dissolved in dimethyl sulfoxide at a final concentration of 0.1 μg/μL, and 10 μL of this solution were mixed with 140 μL PBS and given intraperitoneally to mice every 48 h after the fifth week of TNBS administration. AhR antagonist (CH223191) (10 μg/mouse) were dissolved in PBS and given intraperitoneally every 48 h after the fifth week of TNBS administration. Control mice were given PBS alone. To evaluate whether the anti-fibrotic effect of Ficz was dependent on T cells and ILC, mice were treated with anti-CD90.2 depleting antibody (1 mg/mouse; Biolegend, San Diego, CA, USA) or control Rat IgG (1 mg/mouse; Biolegend) 12 h before each Ficz injection. Mice were examined three times a week for signs of colitis including weight loss and killed at week 8; afterward tissues were collected for histology, RNA analysis, and collagen analysis. Colonic sections were stained with H&E and with Masson’s trichrome to detect connective deposition. Fibrosis was scored as mild, moderate, or severe, depending on the density and extent of trichrome-positive connective tissue staining and disruption of tissue architecture, compared with the water control mice [13].

**Protein extraction and Western blot analysis**

Total proteins were extracted from intestinal fibroblast using a buffer containing 10 mmol/L HEPES (pH 7.9), 10 mmol/L KCl, 0.4 mol/L NaCl, 1 mmol/L ethylenediaminetetraacetic acid, 1 mmol/L ethylene glycol-bis-(aminoethyl ether)-N,N,N,N-tetraacetic acid, and 10% glycerol, 1 mmol/L dithiothreitol, 10 mg/mL aprotinin, 10 μg/mL leupeptin, and 1 mmol/L phenylmethanesulphonyl fluoride (all from Sigma). Total proteins (100 μg/sample) were separated on a 10% SDS-PAGE and incubated with rat anti-human monoclonal AhR antibody (1 μg/mL; Abcam, Cambridge, England), mouse monoclonal p-Smad2/3 antibody (Cell signaling, Danvers, MA, USA) (final dilution 1:500), mouse monoclonal p-NF-κB/p65 antibody (Cell signaling) (final dilution 1:500), mouse monoclonal p-p38 antibody (Millipore, Billerica, MA, USA) (final dilution 1:500), mouse monoclonal p-ERK1/2 antibody (Santa Cruz Biotechnology, Inc.) (final dilution 1:500), followed by horseradish peroxidase conjugated secondary antibodies (Dako SpA, Milan, Italy). After detection blots were stripped and incubated with a mouse anti-human β-actin antibody (Sigma; final dilution 1:5000) followed by a goat anti-mouse antibody conjugated to horseradish peroxidase (Dako; final dilution, 1:20 000). All the reactions were detected with a Super Signal West DURA chemiluminescence kit (Pierce Biotechnology, Rockford, IL, USA).

**RNA extraction, complementary DNA preparation, and real-time polymerase chain reaction**

RNA isolation, reverse transcription of the RNA, and real-time PCR were carried out as previously described [19]. RNA was extracted by using TRizol reagent according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA, USA). A constant amount of RNA (1 μg/sample) was reverse transcribed into complementary DNA, and this was amplified using the following conditions: denaturation for 1 min at 95°C; annealing for 30 s at 60°C for human collagen I (Col1A1), human Col3A1, human alpha smooth muscle actin (α-SMA), and human and mouse β-actin, at 58°C for human AhR and mouse Col1A2, followed by 30 s of extension at 72°C. All real-time PCR data were normalized to β-actin. Primer sequences are indicated in Table 1. Gene expression was calculated using the ΔΔCt algorithm.

**Flow cytometry**

To assess the intracellular expression of AhR and the phosphorylated (p) form of p38, Erk1/2, NF-κB/p65 and Smad2/3, cells were fixed with 1% formaldehyde for 20 min and subsequently permeabilized with 0.5% saponin in 1% bovine serum albumin and stained with anti-AhR (AB84833, 1:50, final dilution; Abcam, Cambridge, UK), anti-p-p38 (36/p38, pT180/pY182)-PE (final dilution 1:50; BD Biosciences, San Jose, CA, USA), anti-p-ERK1/2 (25/MEK1, pT202/pY204;pT184/pY186)-PE (final dilution 1:50; BD Biosciences), anti-p-NF-κBp65-IFITC (k10-895.121.50, 1:50 final dilution; ebioscience, San Diego, CA, USA), and anti-p-Smad2/3(D27F4, 1:50 final dilution; Cell Signaling). Appropriate secondary antibodies and isotype-matched controls (BD Biosciences) were included in all experiments and 100 000 events were collected for each sample. Lamina propria mononuclear cells, isolated from colitic mice treated with Ficz and anti-CDC90.2 depleting antibody or control Rat IgG, were stained with CD45-APCCy7 (BD Biosciences) and real-time polymerase chain reaction.
Table 1. Primer sequence of both human and mouse genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>human Col1A1</td>
<td>5'-GGACACAGAGGTTCAGTGG-3', 3'-GGTGACTTTGGAGACACAGG-5'</td>
</tr>
<tr>
<td>human Col3A1</td>
<td>5'-GGGAAATGGGTACGTTTGC-3', 3'-CTTGGTAGTTGTTGAAAGG-5'</td>
</tr>
<tr>
<td>human α-SMA</td>
<td>5'-TCTGGAGATGTCAGTCACCACA-3', 3'-ACCCACCTGTGGAGACCTCC-5'</td>
</tr>
<tr>
<td>human AhR</td>
<td>5'-GGGACAAATCAGAGAGCTTG-3', 3'-TGAGGAAGCGATAGAAGACC-5'</td>
</tr>
<tr>
<td>mouse Col1A2</td>
<td>5'-ACACAGTGATGATGGAGGCAC-3', 3'-ACAGCTACATGCGGAAGCG-5'</td>
</tr>
<tr>
<td>mouse TNF-α</td>
<td>5'-ACCTCTACACTCAGATCATC-3', 3'-GAGTACGAGAGGCTCACCC-5'</td>
</tr>
<tr>
<td>mouse IL-17A</td>
<td>5'-TCAGACTACCTCAACCCGTC-3', 3'-TTCAGGACCCAGAGTTCTTC-5'</td>
</tr>
<tr>
<td>mouse TGF-β</td>
<td>5'-GTGGAATACCAACGGGATCAG-3', 3'-AGCAGTCTCTCTGTTGAG-5'</td>
</tr>
<tr>
<td>mouse IL-13</td>
<td>5'-GAGCAACATCGACAGAGACC-3', 3'-AATCCAAGGCTACACAAGAC-5'</td>
</tr>
<tr>
<td>β-actin</td>
<td>5'-AAAGTGACCCAGATCTGTGGAGAC-3', 5'-AGCAGTCCAGCGGAGGAT-3'</td>
</tr>
</tbody>
</table>

30°C. Annexin V/PI-negative cells were considered as viable cells. Cells were analyzed using a FACSVersus flow cytometer and FACSSuite software (BD Biosciences).

Collagen assay

Total collagen was measured in fibroblasts-free supernatants and mouse tissue samples by Sircol Collagen Assay Kit (BioColor Ltd, Belfast, UK) in accordance with the manufacturer's instructions.

Statistical analysis

Differences between groups were compared using Student's t-test and Mann–Whitney test.

Acknowledgments: No specific funding has been received for this study.

Conflict of interest: The authors declare no financial or commercial conflict of interest.

References


**Abbreviations:** AhR: Aryl hydrocarbon receptor · CD: Crohn’s disease · FS: Fibrostrictures · ILC: Innate lymphoid cells · TNBS: Trinitrobenzene sulfonic acid · UC: Ulcerative colitis

**Full correspondence:** Dr. Ivan Monteleone, Dipartimento di Medicina dei Sistemi, Università Tor Vergata di Roma, Via Montpellier 1, 00133 Rome, Italy
Fax: +39-06-72596158
e-mail: ivan.monteleone@uniroma2.it

Received: 1/10/2014
Revised: 10/12/2015
Accepted: 14/1/2016
Accepted article online: 19/1/2016
学霸图书馆

www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：
图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具