The role of the novel Th17 cytokine IL-26 in intestinal inflammation

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ABSTRACT

Background and aims: Interleukin 26 (IL-26), a novel IL-10-like cytokine without a murine homologue, is expressed in T helper 1 (Th1) and Th17 cells. Currently, its function in human disease is completely unknown. The aim of this study was to analyse its role in intestinal inflammation.

Methods: Expression studies were performed by reverse transcription-PCR (RT-PCR), quantitative PCR, western blot and immunohistochemistry. Signal transduction was analysed by western blot experiments and ELISA. Cell proliferation was measured by MTS (3-(4,5-dimethylthiazol-2-yl)-5-(carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay. IL-26 serum levels were determined by an immunoluminometric assay (ILMA).

Results: All examined intestinal epithelial cell (IEC) lines express both IL-26 receptor subunits IL-20R1 and IL-10R2. IL-26 activates extracellular signal-related kinase (ERK)1/2 and stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) pathways, Akt and signal transducers and activators of transcription (STAT) 1/3. IL-26 stimulation increases the mRNA expression of proinflammatory cytokines but decreases cell proliferation. In inflamed colonic lesions of patients with Crohn’s disease, an elevated IL-26 mRNA expression was found that correlated highly with the IL-8 and IL-22 expression. Immunohistochemical analysis demonstrated IL-26 protein expression in colonic T cells including Th17 cells expressing the orphan nuclear receptor RORγt, with an increased number of colonic IL-26-expressing cells in active Crohn’s disease.

Conclusion: Intestinal cells express the functional IL-26 receptor complex. IL-26 modulates IEC proliferation and proinflammatory gene expression and its expression is upregulated in active Crohn’s disease, indicating a role for this cytokine system in the innate host cell response during intestinal inflammation. For the first time, IL-26 expression is demonstrated in colonic RORγt-expressing Th17 cells in situ, supporting a role for this cell type in the pathogenesis of Crohn’s disease.

Interleukin 26 (IL-26), initially named AK155, was originally identified by subtraction hybridisation coupled with representational difference analysis as a gene upregulated in human T cells following infection with herpesvirus saimiri (HVS), with the capacity to transform these cells in culture.\(^1\) The IL-26 protein has 24.7% amino acid identity and 47% amino acid similarity to human IL-10. IL-26 is a member of the IL-10-like cytokine family composed of cytokines with limited homology to IL-10, including IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28A, IL-28B and IL-29. IL-28A/B and IL-29 are also known as interferon λs (IFNλs). IL-26 signals through a novel receptor complex consisting of the transmembrane proteins IL-20R1 and IL-10R2.\(^2\) In addition to cytokine-specific ligand-binding chains, IL-22, IL-26, IL-28A/B and IL-29 use a common second receptor chain (IL-10R2) for assembling their active receptor complexes and signalling. The IL-26 gene is located on chromosome 12q14 in a cluster where IFNγ and IL-22 are also positioned. IL-26 forms homodimers similarly to IL-10.\(^3\) IL-26 was originally described as a gene upregulated upon HVS infection in T cells but could also be detected in unstimulated fresh peripheral blood cells of healthy donors by reverse transcription-PCR (RT-PCR) analysis.\(^4\) Although the IL-10 transcript was detected in most cell lines of T or B cell lineage, the IL-26 transcript was rather specific for T cells. In another study, basal conditions no IL-26 mRNA could be detected in monocytes, natural killer (NK) cells or B and T cells.\(^5\) Its expression could be induced only in NK cells and T cells upon stimulation with IL-2/IL-12 and anti-CD3 monoclonal antibody, respectively.\(^6\) This induction of IL-26 mRNA was observed specifically in activated memory cells (CD4\(^{+}\); CD45RO\(^{+}\)) and during polarization toward type 1 T helper (Th1) cells.\(^7\) A recent, preliminary study with in vitro differentiated T cells derived from healthy human blood donors suggests that Th17 cells also express IL-26 following stimulation with IL-23.\(^8\) Th17 cells are a newly discovered subset of inflammatory T cells that differentiate in humans under the influence of IL-18, IL-6, IL-21 and IL-23.\(^9\) They are characterised by the expression of the transcription factor RORγt and the IL-23 receptor (IL-23R).\(^9\) They produce a specific pattern of cytokines and chemokines such as IL-17A, IL-17F, IL-21, IL-22 and CCL20.\(^9\) Th17 cells contribute to the pathogenesis of many inflammatory autoimmune diseases such as multiple sclerosis or rheumatoid arthritis (reviewed in Furuwaza-Carballeda et al\(^{10}\)) and inflammatory bowel disease (IBD).\(^11\)\(^12\)\(^13\)\(^14\)

Recent findings demonstrate that a heterodimer consisting of IL-20R1 and IL-10R2 functions as the IL-26 receptor.\(^1\)\(^4\)\(^15\) This receptor complex is expressed particularly in epithelial cells such as keratinocytes and colonic epithelial cells.\(^14\) However, there is currently very limited information on the biological functions of IL-26. Interestingly, both IL-22 and IL-26 have been identified outside of mammals, and their organization and synteny demonstrate that this cluster of cytokines is well conserved during evolution.\(^15\) However, in contrast to IL-22, no murine IL-26 homologue has been described so far, limiting the experimental opportunities to study the phenotypic

Gut 2009;58:1207–1217. doi:10.1136/gut.2007.130112 1207
consequences of IL-26 gene knockout and IL-26-mediated functions in murine models in vivo. Moreover, there are no studies on the effects of IL-26 in human disease published so far.

Given the IL-26 receptor expression in colonic epithelial cells\(^{19}\) and our recent findings on the important role of other IL-10-like cytokines such as IL-22, IL-28A and IL-29 in the regulation of intestinal epithelial cells (IECs)\(^{14,17}\) and hepatic cells,\(^{18-20}\) we aimed to analyse in this study signal transduction pathways and specific biological functions mediated by IL-26 in IECs. Recently, we demonstrated that the IL-10-related cytokine IL-22, another Th1 and Th17 cytokine sharing the IL-10R2 receptor, is crucial for inflammation and mucosal barrier function.\(^{18,19}\) Therefore, we focused in this study on the role of IL-26 in IECs and in IBD.

**MATERIALS AND METHODS**

**Reagents**

The following antibodies were used in this study: anti-IL-26 and anti-IL-20R1 (R&D Systems, Minneapolis, Minnesota, USA), anti-IL-10R2 (Acros Antibodies, Herford, Germany), anti-ROR\(^{\gamma}\)t (Abcam, Cambridge, UK), anti-pSTAT1 (BD Transduction Laboratories, Franklin Lakes, New York, USA), anti-pSTAT5 (Upstate Biotechnology, Lake Placid, New York, USA), anti-STAT1 and anti-STAT3 (Santa Cruz Biotechnology, Santa Cruz, California, USA). Antibodies against phosphorylated extracellular signal-regulated kinase (ERK)-1/2 (Thr183/Tyr185), phosphorylated stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK; Thr185/Tyr185) and phospho-Akt (Ser473) were purchased from Cell Signaling (Beverly, Massachusetts, USA). Anti-ERK-1/2, anti-SAPK/JNK and anti-Akt antibodies were also from Cell Signaling. Horseradish peroxidase (HRP)-linked antimouse and antirabbit secondary antibodies were purchased from Amersham (Arlington Heights, Illinois, USA) and Santa Cruz Biotechnology. Secondary antibodies for immunohistochemistry (AlexaFluor 488 or AlexaFluor 546 coupled and peroxidase coupled) were from Invitrogen (Karlsruhe, Germany) and Dako (Hamburg, Germany). Fluorescein isothiocyanate (FITC)-conjugated antirabbit antibody was from Sigma-Aldrich (Taufkirchen, Germany) and antirabbit Cy-2 antibody was from Jackson Immuno Research (Suffolk, UK). Recombinant human IL-26 was obtained from R&D Systems.

**Cell culture**

The human colorectal cancer-derived IEC lines SW480, SW620, Caco-2, HT-29, HCT116, T84 and DLD-1 were obtained from the American Type Culture Collection (Rockville, Maryland, USA). Cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) containing 100 IU/ml penicillin, 100 μg/ml streptomycin and 10% heat-inactivated fetal calf serum (FCS) from PAA (Pasching, Austria) in a humidified 5% CO\(_2\) atmosphere at 37°C.

**Reverse transcription-PCR**

RT-PCR was performed as previously described.\(^{21}\) Briefly, total RNA was isolated using Trizol reagent (Invitrogen). A 2 μg aliquot of DNase-treated total RNA was reverse transcribed using the Qiagen Omniscript RT kit (Hilden, Germany). To control for genomic contamination, an identical parallel PCR was performed containing starting material that had not been reverse transcribed. The primers for the PCRs are shown in table 1. The specificity of the PCR products was verified by sequencing.

**Table 1** PCR primers used for RT-PCR analysis

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tr>
<td>IL-10R2 reverse</td>
<td>5′-gaagacgccccgctatgagg-3′</td>
</tr>
<tr>
<td>IL-20R1 forward</td>
<td>5′-taaccccttcaccttcaagact-3′</td>
</tr>
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<td>IL-20R1 reverse</td>
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<td>SOCS-3 reverse</td>
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<tr>
<td>β-Actin forward</td>
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</tr>
<tr>
<td>β-Actin reverse</td>
<td>5′-catcagagcaggaagatgga-3′</td>
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**Quantitative PCR**

Real-time PCR was performed with a Rotorgene RG-3000 cycler (Corbett Research, Sydney, Australia) using the QuantiTect SYBR Green PCR Kit from Qiagen following the manufacturer’s guidelines. Oligonucleotide primers (table 1) were designed to amplify genomic DNA, according to the published sequences. The expression of each mRNA was normalised to β-actin expression in the respective cDNA preparation.

**Immunohistochemistry and immunocytochemistry**

 Peroxidase staining of paraffin-embedded tissue slides was performed using standard protocols. Briefly, after deparaffinisation and demasking of antigens, endogenous peroxidases were blocked with H\(_2\)O\(_2\). Slides were blocked with 10% normal serum and were incubated with avidin and biotin. Following incubation with the primary antibody overnight at 4°C, slides were incubated with the secondary, biotin-conjugated antibody. Next, they were incubated with HRP–streptavidin, followed by incubation with the peroxidase substrate 3'-diaminobenzidine (DAB). Slides were counterstained with haematoxylin.

 Double immunofluorescent staining (anti-ROR\(^{\gamma}\)t and anti-IL-26) of colonic tissue was performed on paraffin-embedded tissue. Following deparaffinisation, tissue was fixed for 1 min in a mixture of ice-cold acetic acid and methanol (1:1). Slides were washed with Tris–TWEEN and were blocked for 1 h with normal serum. The first primary antibody (anti-ROR\(^{\gamma}\)t) was incubated overnight at 4°C and the secondary, fluorochrome-coupled antibody was incubated for 1 h at room temperature. After washing, incubation with the second primary antibody (anti-IL-26) and secondary antibody was performed in the same way.

 Immunofluorescence staining of HT-29 cells was performed as described previously.\(^{22}\)

**Signal transduction experiments, protein isolation, gel electrophoresis and immunoblotting**

The signal transduction experiments were performed in overnight serum-starved IEC lines as indicated. Cells were stimulated with 100 ng/ml IL-26, unless indicated otherwise. This concentration was based on pilot experiments demonstrating a significantly greater effect of 100 ng/ml IL-26 for the activation of certain kinases than lower concentrations. Cells were solubilised in lysis buffer\(^{24}\) for 20 min and lysates were cleared by centrifugation at 10 000 g for 20 min. The protein concentration...
of each sample was quantified by the Bradford method. Immunoblotting was performed as previously described.23

Enzyme-linked immunosorbent assay
The BD OptEIA Human IL-8 Elisa Kit II (BD Biosciences, Bedford, Massachusetts, USA) was used for quantification of IL-8 release according to the manufacturer’s instructions.

IL-26 immunoluminometric assay (ILMA)
Ninety-six-well plates (Nunc Maxisorb, Nunc, Wiesbaden, Germany) were incubated with a 1:1000 dilution of a monoclonal anti-IL-26-antibody for 7 days at 4°C. After blocking with 2% non-fat milk for 1 h at room temperature, the plates were incubated with serum samples and different dilutions of recombinant human IL-26 overnight at 4°C. Following incubation with a biotinylated polyclonal IL-26 antibody, neutravidin–HRP was added. Femtoglow (Michigan Diagnostics, Troy, Michigan, USA) was used as a chemoluminescent substrate and luminescence was measured in a microplate luminometer (Orion II, Berthold Detection Systems, Pforzheim, Germany).

Cell proliferation assay
HT-29 cells were seeded onto 96-well plates at a density of 5000 cells/well and grown for 1 day. After starvation in serum-free medium overnight, the cells were incubated with or without IL-26 (10 and 100 ng/ml) in medium containing 0.1% FCS for 48 h. Cell proliferation was determined using the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, Wisconsin, USA) according to the manufacturer’s instructions. Relative means (SEM) from three independent experiments are shown.

Apoptosis assays
Apoptosis assays were performed as described previously.24 For induction of TRAIL (tumour necrosis factor-related apoptosis-inducing ligand)-mediated apoptosis, TRAIL at a concentration of 100 ng/ml was used, and for CD95-mediated cell death, ligand-specific anti-APO-1 monoclonal antibody at concentrations of 500 and 1000 ng/ml was used. Cells were incubated for 24 h with the respective ligands and with or without IL-26 (10 or 100 ng/ml). Cells were harvested and were lysed in hypotonic lysis buffer (0.1% sodium citrate and 0.1% Triton X-100) containing 50 μg/ml propidium iodide, and were incubated at 4°C overnight. The nuclei were then analysed for DNA content by flow cytometry.

Colonic biopsies
All participating patients with IBD were recruited by the IBD center of the University-Hospital Munich-Grosshadern (Germany) and gave written, informed consent prior to biopsy.
sampling. The study was approved by the Ethics Committee of the Medical Faculty of the University of Munich. Biopsies were taken from patients with Crohn’s disease and ulcerative colitis undergoing diagnostic colonoscopy. Patients with indeterminate colitis were excluded from the study. Demographic and clinical data were recorded by analysis of patient charts and a detailed questionnaire including an interview at the time of enrolment. The demographic data are summarised in table 2. The diagnosis of Crohn’s disease or ulcerative colitis was determined according to established guidelines based on endoscopic, histopathological and radiological criteria.

From each patient, four biopsies were collected: two from macroscopically non-inflamed sites and two from macroscopically inflamed mucosa. IL-26 and IL-8 mRNA levels were measured in each individual biopsy. For quantification, the average IL-26 and IL-8 mRNA expression of the two non-inflamed biopsies was compared with the average expression in the two inflamed biopsies (all normalised to \( b\)-actin expression). In addition, IL-22 mRNA levels of these biopsies were available from a previous study\(^\text{16}\) and were included in the correlation analysis.

**Statistical analysis**

Statistical analysis was performed using two-tailed Student t test. A \( p\) values <0.05 was considered as significant. Measured values that differed from the means by more than four times the standard deviation were considered as outliers and were not included in the statistical analysis.

**RESULTS**

**IECs express the IL-26 receptor complex**

To utilise a cell system to study this ligand–receptor system, we first analysed if the IL-26 receptor complex consisting of IL-10R2 and IL-20R1 is expressed in IEC lines. RT-PCR for IL-10R2 and IL-20R1 mRNA was performed in several IEC cell lines (HT-29, SW480, SW620, HCT116, Caco-2, T84 and DLD-1). RT-PCR analysis demonstrated IL-10R2 and IL-20R1 mRNA expression in all IEC lines tested (fig 1A), while IL-26 was not expressed (data not shown). The hepatic cell line HepG2 does not express IL-20R1 (data not shown) and served as a negative control cell line in the following signal transduction experiments. The expression of both IL-26 receptor subunits on the protein level was confirmed by western blot analysis of total protein extracts of HT-29 and SW480 cells (fig 1A, C; left panels) and by indirect immunofluorescence staining (fig 1B, C; middle panels) using specific antibodies against IL-20R1 and IL-10R2.

**IL-26 induces STAT1/3, ERK-1/2, SAPK/JNK-1/2 and Akt phosphorylation**

Next, we studied if the IL-26 receptor complex expressed by IECs is functional by analysing major intracellular signalling pathways. Since previous studies reported activation of STAT signalling by IL-26,\(^\text{17}\)\(^\text{18}\) we performed western blot analysis and
investigated the influence of IL-26 on the phosphorylation levels of STAT1 and STAT3 in IECs. In comparison with basal STAT1 phosphorylation in unstimulated cells, a strong STAT1 tyrosine phosphorylation was observed upon IL-26 stimulation starting 5 min after cytokine stimulation (fig 2A). IL-26 also induced tyrosine phosphorylation of STAT3 which was strongest after 10 min of stimulation (fig 2B). Densitometric analysis of the phosphorylation blots demonstrated a 14.5- and 22.8-fold increase in phosphorylation for STAT1 and STAT3, respectively (p < 0.005), reaching a maximum increase of 15.8-fold after 24 h. Next, we analysed the role of IL-26 on the transcriptional regulation of the proinflammatory cytokines tumour necrosis factor α (TNFα), IL-8 and IL-6, three major inflammatory mediators in IECs, as downstream readout of IL-26-mediated gene expression. While IL-6 mRNA expression did not change significantly after IL-26 stimulation (data not shown), IL-26 enhanced TNFα and IL-8 mRNA expression 35.1- and 18.0-fold, respectively (p < 0.05), as measured by quantitative PCR (fig 4B). Similarly, we measured a 5.7-fold increased IL-8 protein release by ELISA following IL-26 stimulation (fig 4C).

IL-26 decreases proliferation but does not influence apoptosis in IECs

Recently, we demonstrated that activation of ERK MAP kinases, Akt and STAT proteins following stimulation with chemokines or IL-22 mediates IEC proliferation, while STAT activation following IL-28A and IL-29 stimulation mediates antiproliferative signals in IECs. Therefore, we analysed the effect of IL-26 on IEC proliferation in the following experiments. Similar to IL-28A and IL-29, IL-26 had a modest antiproliferative effect on IECs. Compared with cytokine-free medium-stimulated controls, IEC proliferation was diminished by 22% following stimulation with 10 and 100 ng/ml IL-26 (fig 4A, p = 0.05). However, IL-26 did not significantly influence apoptosis induced by TRAIL or by anti-APO-1 treatment in HT-29 cells (data not shown).

IL-26 mRNA expression is increased in the inflamed colonic mucosa of patients with Crohn’s disease

Since we established that IL-26 upregulates the expression of proinflammatory cytokines in IECs, we next analysed by real-time PCR its expression in intestinal inflammation in vivo. This analysis included a total of 88 biopsy samples taken from 22 different patients with IBD (Crohn’s disease: n = 12; ulcerative colitis: n = 10). The demographics of these patients and the anatomic segment of the biopsy sampling are summarised in table 2.

Ileal and colonic biopsies were taken from sites with endoscopically (macroscopic) inflamed colonic mucosa and were compared with those of endoscopically non-inflamed colonic mucosa taken from the same 22 patients. In patients with Crohn’s disease, the colonic IL-8 mRNA expression, which was determined as a standard inflammatory marker, was 5.9-fold higher in the inflamed biopsy samples compared with uninfamed biopsies (p < 0.05; table 2).

Similarly, we measured increased IL-26 mRNA expression in inflamed colonic biopsies compared with non-inflamed colonic tissue (increase between 1.1- and 15.0-fold; table 2). The increases in IL-8 and IL-26 expression in inflamed biopsies were less significant than in Crohn’s disease samples (data not shown).
IL-26 protein expression in the sera of 40 patients with Crohn’s disease is mirrored by an increased IL-26 serum expression, we analysed the IL-26 mRNA expression in patients with Crohn’s disease compared with controls. To analyse further expression of IL-26 in colonic tissue in situ and to clarify which cells produce IL-26, we performed a detailed immunohistochemical analysis for IL-26 in inflamed and non-inflamed colonic tissue of patients with Crohn’s disease. As shown in the representative images of this analysis in fig 6A and B, IL-26 is expressed in infiltrating immune cells but not in epithelial cells. Importantly, the number of IL-26-expressing cells in inflamed colonic tissue of patients with Crohn’s disease (fig 6D,E) was higher than in uninflamed tissue (fig 6A,B) which is consistent with our quantitative PCR analysis of colonic IL-26 mRNA expression (table 2).

Next, we compared IL-26 expression with the expression of IL-22, another IL-10-like cytokine expressed in Th17 cells. IL-22 expression data were available from a previous study. Although there was also a high correlation between IL-8 and IL-26 mRNA expression in patients with ulcerative colitis (r = 0.79; fig 5B) and IL-26 expression was significantly increased up to 8.1-fold in the inflamed tissue (p = 0.02; table 2), the range 0.56–4.17; p < 0.01 vs Crohn’s disease).

IL-26 serum protein expression is higher in patients with Crohn’s disease compared with controls

In addition, we developed an ILMA as described in the Materials and methods section for the detection of IL-26 protein using a monoclonal IL-26 antibody for capturing and a polyclonal IL-26 specific antibody for detection. To determine if the increased colonic IL-26 mRNA expression in patients with Crohn’s disease is mirrored by an increased IL-26 serum expression, we analysed the IL-26 protein expression in the sera of 40 patients with Crohn’s disease and 39 unrelated controls by ILMA. Analysis revealed significantly higher IL-26 serum levels in patients with Crohn’s disease (mean concentration 2.42 ng/ml, range 0.79–17.56) in comparison with control patients (mean concentration 1.17 ng/ml, range 0.56–4.17; p < 0.01 vs Crohn’s disease).

Intestinal RORγt-expressing Th17 cells produce IL-26 and their number is increased in active Crohn’s disease

To analyse further expression of IL-26 in colonic tissue in situ and to clarify which cells produce IL-26, we performed a detailed immunohistochemical analysis for IL-26 in inflamed and non-inflamed colonic tissue of patients with Crohn’s disease. As shown in the representative images of this analysis in fig 6A and B, IL-26 is expressed in infiltrating immune cells but not in epithelial cells. Importantly, the number of IL-26-expressing cells in inflamed colonic tissue of patients with Crohn’s disease (fig 6D,E) was higher than in uninflamed tissue (fig 6A,B) which is consistent with our quantitative PCR analysis of colonic IL-26 mRNA expression (table 2).

A preliminary study of Th17 cells from healthy donors differentiated from peripheral T cells in vitro suggested Th17 cells as the source of IL-26 production. To clarify if colonic Th17 cells express IL-26 in situ, we performed co-staining using specific antibodies directed against IL-26 and RORγt, a transcription factor that is specifically expressed in Th17 cells. Examples of this immunohistochemical analysis shown in fig 7.
**Figure 5** Intestinal interleukin 26 (IL-26) mRNA expression is upregulated in inflammatory bowel disease and correlates with IL-8 and IL-22 mRNA expression. (A, B) The increase of IL-26 mRNA expression in inflamed colonic and ileal biopsies (in comparison with uninflamed tissue) correlated highly with the respective IL-8 mRNA expression in Crohn’s disease (CD) (A, r = 0.96) and ulcerative colitis (UC) (B, r = 0.79). (C, D) High correlation between the increase of mRNA expression of IL-26 and IL-22, another IL-10-related T helper 17 (Th17) cytokine, in inflamed intestinal biopsies from patients with (C) CD and (D) UC (r = 0.81 and 0.89, respectively).

**Figure 6** The number of infiltrating interleukin 26 (IL-26)-positive immune cells is increased in active Crohn’s disease. Representative immunoperoxidase analysis of IL-26 expression of uninflamed colonic tissue (A–C) and inflamed colonic tissue (D–F) taken from a patient with Crohn’s disease. In the negative controls, the secondary antibody was omitted. Original magnification was ×20 (A, C, D, F), (B, E) Detail from A and D, respectively.


Figure 7 Interleukin 26 (IL-26) is expressed in colonic RORγt-expressing T helper 17 (Th17) cells in active Crohn’s disease. Immunofluorescence analysis of colonic IL-26 (A, E) and RORγt expression (B, F) in colonic tissue in situ from patients with Crohn’s disease. (C) (merged image of A and B) and (G) (merged image of E and F) demonstrate that a number of RORγt-expressing Th17 cells also express IL-26. D and H are images from C and D overlaid with the phase contrast microscopic image from the same section, demonstrating the lack of IL-26 expression in colonic intestinal epithelial cells.

Intestinal inflammation

![Figure 7](https://source.unsplash.com/random/800x600)

**DISCUSSION**

This study represents the first report demonstrating a role for IL-26 in human disease. IL-26 is a novel member of the IL-10-like cytokine family and shares with IL-10 the IL-10R2 subunit for signalling. While IL-10 has anti-inflammatory properties, we here demonstrate that IL-26 has proinflammatory functions in IECs and is upregulated in IBD. Recently, our group also demonstrated proinflammatory functions for IL-22, another IL-10-like cytokine in IECs. Similarly, IL-26 upregulates the gene expression of proinflammatory cytokines in IECs. In addition, we demonstrate functional IL-26 receptor expression in IECs. IL-26 activates ERK and SAPK/JNK MAP kinases, Akt and STAT proteins in IECs, which are the major signalling pathways of other proinflammatory cytokines with increased expression in IBD such as IL-22 and IL-31. In contrast, cell lines such as HepG2, which do not express the IL-20R1 subunit, are not responsive to IL-26 treatment.

It has been shown that activated T cells are a major source of IL-26 production. Depending on the T cell source of cytokine production, cytokines have been differentiated in Th1 and Th2 cytokines. Previous studies demonstrated an induction of IL-26 production particularly in Th1 cells. Crohn’s disease is considered to represent a Th1- and Th17-mediated intestinal inflammation, while ulcerative colitis has, at least to some degree, features of Th2-mediated colitis. Consistent with this observation, in our study the relative upregulation of IL-26 expression (compared with uninflamed tissue) was more pronounced in inflamed lesions of patients with Crohn’s disease compared with patients with ulcerative colitis. Moreover, colonic IL-26 mRNA expression correlated strongly with IL-8 and IL-22 mRNA expression, confirming IL-26 as a marker of intestinal inflammation.

Our detailed immunohistochemical analysis revealed for the first time IL-26 protein expression in situ. There was an increased number of IL-26-expressing cells in active Crohn’s disease which correlated with the results of our quantitative PCR analysis of colonic biopsy tissue taken from inflamed and uninflamed mucosa affected by Crohn’s disease. IL-26 expression was found in infiltrating intestinal T cells including RORγt-expressing Th17 cells, but not in primary IECs. This is of particular interest, since very recent evidence suggests that Th17 cells contribute to the pathogenesis of IBD, particularly Crohn’s disease. This is also supported by a recent genome-wide association study demonstrating IL23R, which is expressed by Th17 cells, to be a Crohn’s disease susceptibility gene. We recently confirmed IL23R as an IBD susceptibility gene in the German population. Interestingly, we demonstrated that the genetic risk for Crohn’s disease mediated by IL23R is directly linked to the expression of the proinflammatory cytokine IL-22 which is also expressed in Th17 cells. Moreover, we and others demonstrate that a considerable number of RORγt-expressing Th17 cells also express IL-26, while, however, not all Th17 cells are producers of IL-26. One difficulty of this analysis is the fact that RORγt and IL-26 are expressed in two different cell compartments (RORγt in the nucleus and IL-26 in the cytosol). Therefore, RORγt-expressing Th17 cells, which are imaged only on a cytosolic cross-section, will not appear RORγt-positive on such a cross-sectional image. To demonstrate the expression of RORγt and IL-26 in different cell compartments, we performed reconstructational analysis of cross-sectional images of single cells. A video sequence of such sequential cross-sections of a single cell imaged by confocal microscopy can be found in the Supplemental materials online (Video file S1). Frames of this video sequence are shown in fig. 8A–F, demonstrating the nuclear expression of RORγt and the cytosolic expression of IL-26. Interestingly, cross-sections taken outside of the nucleus (fig 8E,F) appear not to be stained for RORγt. Only in Th17 cells with very strong IL-26 and RORγt expression did IL-26 and RORγt staining appeared to “co-localise” in the perinuclear region (fig 8F–I).
demonstrated an increased expression of Th17 cytokines and chemokines such as IL-17A, IL-17F, IL-22 and CCL20 in active Crohn’s disease.16 32–35 Here, we show significantly increased IL-26 serum levels in patients with Crohn’s disease compared with healthy controls. However, overall IL-26 serum levels were rather low, which is probably related to the fact that only less than 1% of cells in the peripheral blood are Th17 cells.36 In contrast, the high number of infiltrating RORγt-expressing intestinal Th17 cells in active Crohn’s disease suggests an important role for this cell type in local tissue inflammation. IL-26-producing Th17 cells are likely to sustain intestinal inflammation since we demonstrate here that IL-26 upregulates the gene transcription of several proinflammatory cytokines such as IL-8 and TNFα in IECs, while its own expression is upregulated in IBD. A recent study demonstrated that peripheral T cells, which differentiated under the influence of IL-23 and IL-1β to Th17 cells, produce IL-26, supporting Th17 cells as a major source of IL-26 production.1 In contrast, IL-23 is mainly produced in ileal dendritic cells.37 We and others recently demonstrated that ileal dendritic cells play a

Figure 8  Interleukin 26 (IL-26) and RORγt expression are confined to different cellular compartments. (A–F) Sequential single cell images derived from cross-sectional confocal microscopic imaging using an anti-IL-26 antibody (secondary antibody AlexaFluor 488 labelled) and anti-RORγt antibody (secondary antibody AlexaFluor 546 coupled) demonstrating nuclear expression of RORγt (red) and cytosolic expression of IL-26 (green). The corresponding video file can be found in the Supplementary data (Video file S1) online. (G–I) Only in cells with very high IL-26 (G) and RORγt expression (H) was an impression of “co-localisation” in the perinuclear region visualised in the merged images (I).
major role in the luminal sampling of bacterial pathogens, thereby potentially linking defects in the antibacterial defence with the pathogenesis of Crohn’s disease.

In addition, we demonstrate that IL-26 is functional in IECs. IL-26-induced STAT1/5 activation results in increased SOCS-3 mRNA expression. This confirms our previous studies demonstrating that SOCS-3 is an immediate early transcriptional target of STAT1/5. Interestingly, in Crohn’s disease, increased colonic STAT1 phosphorylation and SOCS-3 protein levels were found, supporting our findings of a proinflammatory regulatory role for IL-26 in Crohn’s disease. Another study demonstrated in a murine colitis model that SOCS-3 plays a negative regulatory role for IL-26 in Crohn’s disease. IL-26 is probably an important effector cytokine by which Th17 cells develop via a lineage distinct from the T helper type 1 and 2 lineages.

In conclusion, our results correlate highly with the expression of IL-22, another Th17 gene.
What is the diagnosis?

**CLINICAL PRESENTATION**

A 68-year-old man with a history of dyspepsia and hiatus hernia presented with 15 kg weight loss with occasional nausea and vomiting episodes over the previous year. He was not taking any regular medication. He underwent gastroscopy, colonoscopy and barium swallow 1 year earlier to investigate change in bowel habit and dyspepsia. These tests revealed hiatus hernia and a non-specific motility disorder of the distal oesophageal segment. Clinical examination was unremarkable.

Blood tests were all normal. An ultrasound scan of the abdomen was normal and repeat gastroscopy again showed only hiatus hernia. A chest x-ray, which was performed in view of weight loss and smoking history, was normal.

Symptoms initially improved with regular domperidone; however, vomiting became more frequent and occurred in association with abdominal pain.

A repeat ultrasound scan of the abdomen was arranged (fig 1).

**QUESTION**

What is the diagnosis?

See page 1288 for the answer.

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The role of the novel Th17 cytokine IL-26 in intestinal inflammation

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_Gut_ 2009 58: 1207-1217 originally published online May 15, 2008
doi: 10.1136/gut.2007.130112

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