IL-4 and IL-13 Up-Regulate Intestinal Trefoil Factor Expression: Requirement for STAT6 and De Novo Protein Synthesis

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*J Immunol* 2004; 172:3775-3783; doi: 10.4049/jimmunol.172.6.3775

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IL-4 and IL-13 Up-Regulate Intestinal Trefoil Factor Expression: Requirement for STAT6 and De Novo Protein Synthesis

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The development of intestinal goblet cell hyperplasia/hypertrophy during nematode infection involves the Th2 cytokines IL-4 and IL-13 via STAT6 activation. This is thought to play an important role in host protective immunity against the infection. In this study we demonstrate that IL-4 and IL-13 up-regulate the specific goblet cell product trefoil factor-3 (TFF3) from the mucus-producing HT-29 CL.16E and HT-29 cells selected by adaptation to methotrexate. Up-regulation of TFF3 mRNA and protein levels occurred in a time- and dose-dependent fashion and was accompanied by up-regulation of the goblet cell product mucin 2 (MUC2). Addition of actinomycin D before IL-4/IL-13 stimulation led to decreases in TFF3 mRNA levels similar to those observed in controls without IL-4/IL-13. Furthermore, IL-4-mediated increased TFF3 transcription required de novo protein synthesis. Stable transfection of HT-29 CL.16E cells with a truncated dominant-negative form of STAT6 produced a cell line that was unresponsive to IL-4/IL-13. Although only one consensus STAT6 binding site is contained in the TFF3 gene, located in the intron 1, it did not operate as an enhancer in the context of an SV40 promoter/luciferase construct. Thus, STAT6 activation mediates a transcriptional enhancement of TFF3 by induction of de novo synthesized protein in goblet cells. The Journal of Immunology, 2004, 172: 3775-3783.
cytokine-mediated increase in TFF3 transcription is shown to be STAT6-dependent and to require de novo protein synthesis.

Materials and Methods

Materials

LY294002, cycloheximide (CHX), actinomycin D, and wortmannin were purchased from Sigma-Chemie (St. Quentin Fallavier, France). A 425-bp humane IL-13 DNA was used as specific probe for Northern blot analysis and was provided by S. Ribieras (Institut de Génétique et de Biologie Moléculaire et Cellulaire, Strasbourg, France). The plasmid LA778 containing STAT6 cDNA was provided by W. J. LaRochelle (National Cancer Institute, Bethesda, MD). The plasmid pCEFL-HA containing the neomycin resistance gene was provided by S. Emani (Institut National de la Santé et de la Recherche Médicale, Unité 482, Paris, France). The plasmid named p800 containing the first 800 bp of TFF3 promoter cloned into HindIII and SacI sites of plasmid pGL3-basic (positions −867 to +66), the TFF3.d1 plasmid containing the first 495 bp of TFF3 promoter cloned into the HindIII and KpnI sites of plasmid pGL3-basic, and the pUC 18 10.4 plasmid containing 10 kb of hTFF3 genomic DNA have been previously described (27). All oligonucleotides used for RT-PCR and EMSA analysis were custom-synthesized by Invitrogen (Cergy-Pontoise, France).

The Ab directed against P85α subunit of phosphatidylinositol 3-kinase (PI3–K) was obtained from Upstate Biotechnology (Mundolsheim, France). The rabbit polyclonal IgG anti-STAT6 M200 directed against the N-terminal fragment of the protein was obtained from Santa Cruz Biotechnology (Le Perray en Yvelines, France). The chicken polyclonal IgG anti-STAT6 used in EMSA analysis has been prepared by one of us (M.H.H.). Specific Abs used for flow cytometric analysis were referred to as a mAb-Hil-4.Ra (R&D Systems, Lille, France), mAb-Hil-13.Ra1 (Diaclone, Besançon, France), or mAb-Hil-13.Ra2 (Diaclone). The secondary Ab used for flow cytometric analysis was an FITC-labeled goat anti-mouse IgG (H+L) (Chemicon, Mundolsheim, France). Human rIL-4 was obtained from Chemicon, Mundolsheim, France. Human rIL-13 was a gift from A. Minty (Sano Schering-Plough through the courtesy of F. Brieure (Dardilly, France). Human rIL-4 was obtained from Chemicon, Mundolsheim, France. Human rIL-13 was obtained from Nichirei, Tokyo, Japan. The chicken polyclonal IgG anti-STAT6 was provided by S. Ribieras (Institut de Génétique et de Biologie Moléculaire et Cellulaire, Strasbourg, France). The plasmid pCEFL-HA containing the neomycin resistance gene was provided by S. Emani (Institut National de la Santé et de la Recherche Médicale, Unité 482, Paris, France).

Cell culture

The HT-29 CL.16E cells selected by butyrate treatment of parental cells have been described previously (28). HT-29 cells selected by adaptation to metothrexate (referred to as HT-29 MTX) were provided by T. Lesfueure (Institut National de la Santé et de la Recherche Médicale, Unité 505, Paris, France). Cells were grown in DMEM (Invitrogen) on day 2. Brieﬂy, for each well, 1 µg of reporter plasmids were mixed with 4 µl of Lipofectamine reagent (Qiagen, Courtaboeuf, France) on day 2 with the transfection reagent (pCEFL-HA) for 3 h, according to the manufacturer’s instructions. The 1088-bp PCR product encoded the first 659 N-terminal aa of STAT6, corresponding to a dominant-negative form. The PCR product was purified and subcloned with T4 DNA ligase into BgII/EcoRI-digested pCEFL-HA vector. This plasmid was named DNSTAT6.

Transient and stable transfections

For transient transfections, −2 × 105 HT-29 CL.16E cells/well (six-well plates) were plated on day 1 and transfected by the Lipofectamine reagent (Invitrogen) on day 2. Brieﬂy, for each well, 1 µg of reporter plasmids were mixed with 4 µl of Lipofectamine reagent in 200 µl of serum-free DMEM. In all transfection experiments, a plasmid with a Renilla luciferase reporter gene under the control of a thymidine kinase promoter (pHRL-TK; Promega, Madison, WI) was used as an internal control. Eight hundred microliters of complete medium was added, and cells were incubated for 48 h. Transfection mixture was then replaced with fresh complete medium for an additional 12 h before cytokine treatment. On day 4, cells were left untreated or were transfected with 10 ng/ml IL-4 or IL-13 for 24 h. Cells were then harvested, and the firefly and Renilla luciferase activities were measured in a luminometer (Molecrite; Dynatech Laboratories, Chantilly, VA) using the Dual Luciferase Reporter Assay system (Promega) in accordance with the manufacturer’s instructions.

Stable transfections were performed as follows: −105 HT-29 CL.16E cells/well (9.6-cm2) were plated on day 1 and transfected by the SuperFect transfection reagent (Qiagen, Courtaboeuf, France) on day 2 with the pCEFL-HA or the DNSTAT6 plasmid for 3 h, according to the manufacturer’s protocol. After 4 days, transfected cells were selected for 3 wk of culture in 800 µg/ml G418 (Geneticin; Invitrogen). Resistant colonies were ring-cloned as individual colonies. Cells were then left under selection and were subjected to analysis of ectopic overexpression of the truncated STAT6 form by immunoblot analysis.

RT-PCR analysis

First-strand cDNA was synthesized using Superscript II (Invitrogen). cDNAs were amplified using the following primers (5′–3′): hTFF3 forward (983 bp): gtagctggctgaacagcttggt; reverse, cattgcagcagctgctgcttg; hTFF2 (92 bp): forward, aatggctgctgctgctgcctg; reverse, cattgcagcagctgctgctgc; hIL-4 (200 bp): forward, gcctgcgtgctgctgctgc; reverse, cacacagctgctgctgctgc; hIL-13 (400 bp): forward, cattgcagcagctgctgctgc; reverse, cattgcagcagctgctgctgc. Amplifications were performed in an automated thermal cycler (denaturation: 96°C, 30 s; annealing: 60°C, 30 s; extension: 72°C, 1 min). Amplifications of 25 and 25 cycles were performed for GAPDH and TFF3, respectively, and amplifications of 35 cycles were performed for MUC2 and IL receptor chains. In addition to conventional PCR, real-time PCR
was conducted by rapid cycling using the LightCycler instrument and Fast Start DNA Master SYBR Green I (Roche, Meylan, France) according to the manufacturer’s instructions. Results normalized to \( \beta \)-actin amplified from the same cDNA mix are expressed as attomoles per microgram of RNA.

Flow cytometric analysis

Single-cell suspensions of HT-29 CL.16E cells obtained after trypsinization were cultured in petri dishes with constant rocking at 37°C in 5% CO\(_2\) for 48 h to allow re-expression of receptors lost during the treatment with trypsin. Approximately \( 5 \times 10^5 \) cells were incubated in PBS/10 mM EDTA for 15 min at 4°C. After washing with PBS, 0.1% BSA, and 0.05% NaN\(_4\), the cells were incubated with 4 \( \mu \)g of specific Abs anti-hIL-4R, anti-hIL-13R\(_\alpha\), or anti-hIL-13R\(_\beta\) in PBS/5% BSA for 1 h at room temperature. After washing, cells were incubated with 2 \( \mu \)g of FITC-labeled secondary Ab. Cells were then fixed in PBS/0.8% paraformaldehyde, and \( 5 \times 10^3 \) to \( 10^4 \) cells/condition were analyzed for fluorescence by single-color flow cytometry using a FACSscan and FloMax software (DAKO, Trappes, France).

EMSA

After treatment with cytokines, cells were lysed in low salt buffer (20 mM HEPES (pH 7.9), 10 mM KCl, 1 mM EDTA, 1 mM EGTA, 0.2% Nonidet P-40, 10% glycerol, 0.1 mM Na\(_2\)VO\(_4\), 1 mM PMSF, 1 mM DTT, 2 \( \mu \)g/ml aprotinin, 1 \( \mu \)g/ml leupeptin, and 1 \( \mu \)g/ml pepstatin) at 4°C for 10 min. After centrifugation for 5 min at 3,000 \( \times \) g, pellets were extracted with high salt buffer (low salt buffer supplemented with 420 mM NaCl and 20% glycerol) for 30 min at 4°C. Samples were cleared by centrifugation at 12,000 \( \times \) g at 4°C. Nuclear extracts (10 \( \mu \)g of total proteins) were then incubated for 20 min at room temperature in a mixture containing 20 mM HEPES (pH 7.9), 4% Ficoll, 1 mM Mg\(_2\)Cl\(_2\), 40 mM KCl, 0.1 mM EGTA, 0.5 mM DTT, and 160 \( \mu \)g/ml poly(dI-dC) with 1 ng of \(^{32}\)P-labeled oligonucleotides. Samples were separated on a 4.5% nondenaturing polyacrylamide gel at 400 V for 3 h at 4°C. Gels were then dried and exposed to BioMax film (Kodak) overnight. The following oligonucleotides corresponding to STAT6 response element sequences were used: C(\( \mu \), 5'-CACCT

**FIGURE 1.** MUC2 and TFF3 expression in relation to growth and differentiation of HT-29 CL.16E cells. A, RT-PCR analysis of the expression of TFF3, MUC2, and GAPDH. D3 and D34 are the days after seeding the cells: for TFF3 expression, 25 cycles of amplification; for MUC2, 35 cycles; for GAPDH, 23 cycles. B, TFF3 and \( \beta \)-actin mRNA levels revealed by Northern blot analysis. C, TFF3 cell content visualized by immunoblotting. The results shown are representative of three separate experiments.

**FIGURE 2.** Analysis of IL-4R/IL-13Rs in the human epithelial tumoral cell line HT-29 CL.16E. Left panel, RT-PCR analysis of IL-4R\(_\alpha\), IL-13R\(_\alpha\), IL-13R\(_\beta\), and GAPDH transcripts. The sizes of the amplification products are given. Right panel, Flow cytometric analysis of IL-4R\(_\alpha\), IL-13R\(_\alpha\), and IL-13R\(_\beta\) chain expression (dark lines). Controls were performed with an irrelevant IgG (gray lines). Mean fluorescence intensity was analyzed with FloMax software (DAKO). Each tracing is representative of at least three different experiments.
TCCCAAGAACAGA-3′; and TFF3 (intron 1), 5′-ATCACCTTCTCGGGAAGTCACA-3′. For supershift experiments, 2 μl of anti-STAT6 was added to the samples before or after the addition of labeled oligonucleotides. Competition experiments were performed in the presence of 100-fold molar excesses of the cold STAT6 consensus oligonucleotides.

**PI3-K activity assay**

Cells were lysed at 4°C in 20 mM Tris-HCl (pH 7.4), 140 mM NaCl, 10 mM EDTA, 4 mM NaVO₄, 100 mM NaF, 10 mM pyrophosphate, and 1% Nonidet P-40. After centrifugation, 100 μg of supernatant proteins were used for immunoprecipitation with a specific Ab directed against human P85α PI3-K. PI3-K activity was measured in the immunoprecipitates as previously described (30, 31). Labeled phosphoinositides were visualized and quantified using a PhosphorImager SI and ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

**Statistics**

Statistical analysis was conducted using Student’s t test, and significance was assigned at p ≤ 0.05.

**Results**

**Expression of TFF3 in the MUC-secreting cell line HT-29 CL.16E**

The hallmarks of the differentiation of intestinal goblet cells are the early expression of TFF3, followed by the expression of MUCs (26). The subclone 16E of the human colonic epithelial cell line HT29 undergoes differentiation to the goblet cell-like phenotype after reaching confluence (28). We examined the relative expressions of TFF3 and MUC2 in HT-29 CL.16E cells during their differentiation into the MUC-secreting phenotype. The appearance of TFF3 and MUC2 transcripts was analyzed by RT-PCR in the course of the culture from a subconfluent (day 3) up to a late postconfluent (day 34) state. Transcripts for these genes became strongly expressed after confluence (Fig. 1A). Real-time PCR indicated a dramatic increase in TFF3 expression between day 3 (56 ± 7 attomol/μg total RNA; n = 5) and day 34 (1756 ± 136 attomol/μg total RNA; n = 5). Northern blot analysis revealed that the TFF3 transcripts extracted from the HT-29 CL.16E cells were similar in size to those found in intestinal goblet cells (0.4 kb; Fig. 1B). In a Western blot analysis, a single species (~7 kDa), corresponding closely to the molecular mass of the TFF3 peptide, was revealed in cell extracts, and the intensity of the band strongly increased when cells were committed to differentiation (Fig. 1C).

**Presence of IL-4 and IL-13 receptors in the HT-29 CL.16E cell line**

As illustrated in Fig. 2, the specific transcripts for IL-4Rα (335 bp), IL-13Rα1 (509 bp), and IL-13Rα2 (1088 bp) were detected in HT-29 CL.16E cells by RT-PCR. The presence of these chains was further evidenced by flow cytometry, as the fluorescence intensity was shifted on the right when cells were incubated with the antisera raised against the IL-4Rα, IL-13Rα1, or IL-13Rα2 chain (Fig. 2). IL-2Rγ chain transcripts were also detected (data not shown). We could not demonstrate a differential expression pattern for the IL-4 and IL-13 receptors in the course of differentiation.

**IL-4 and IL-13 stimulate TFF3 expression in HT-29 CL.16E cells**

The expression of TFF3 in HT-29 CL.16E cells was first investigated by Northern blot analysis of total RNA after 24 h of stimulation with graded concentrations of IL-13 ranging from 0.1–100

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**FIGURE 3.** TFF3 gene expression in HT-29 CL.16E cells stimulated with increasing amounts of Th2 cytokines for 24 h. Upper panel, Northern blot analysis of TFF3 and β-actin transcripts isolated from IL-13-stimulated cells. Lower panel, TFF3 immunoreactivity (IR-TFF3) in IL-4- and IL-13-treated cells. Data (mean ± SE of three separate experiments) were expressed as a percentage of the control. *, p < 0.05 vs the control group.

**FIGURE 4.** Time course of TFF3 gene expression by the intestinal goblet cell line HT-29 CL.16E stimulated with IL-4 or IL-13. Upper panel, Analysis of TFF3 and GAPDH expression in IL-13-treated cells by RT-PCR. The PCR products were size-fractionated and visualized by ethidium bromide staining in agarose gels. Lower panel, TFF3 cell content was quantified by RIA. Data (mean ± SE of three separate experiments) were expressed as a percentage of the control. *, p < 0.05 vs the control group.
ng/ml. As shown in Fig. 3, HT-29 CL.16E cells constitutively expressed low levels of TFF3 mRNA, and IL-13 induced a concentration-dependent increase in TFF3 mRNA expression, first detectable at 1 ng/ml of IL-13 and maximal at 10 ng/ml. The real-time PCR revealed 8- and 13-fold increases in TFF3 mRNA levels upon treatment with IL-4 (10 ng/ml) and IL-13 (10 ng/ml), respectively. Cell extracts from similar experiments were also quantitated by RIA for TFF3 protein contents. Both cytokines similarly increased the synthesis of TFF3 protein in the HT-29 CL.16E cell line in a dose-dependent fashion (Fig. 3). Comparable results were obtained in HT-29 MTX cells, which harbor a goblet cell-like phenotype (data not shown). Fig. 4 illustrates the kinetics of up-regulation of TFF3 mRNA expression by IL-13, with a 2-fold increase at 6 h and a 7-fold increase at 24 h of treatment, as measured by real-time PCR. Changes in TFF3 mRNA levels were paralleled by an increase in TFF3 cellular content, as assessed by RIA. As illustrated in Fig. 4, both IL-4 and IL-13 markedly enhanced TFF3 peptide synthesis. The effect was detectable after 24 h and was maximal after 48 h of stimulation with the cytokines. Incubation of the cells with IL-13 (10 ng/ml) also resulted in an increase in MUC2 mRNA levels, as evaluated by semiquantitative RT-PCR (data not shown).

**Mechanism of the IL-4- and IL-13-mediated up-regulation of TFF3 expression.**

In a variety of cell types, activation of gene expression by Th2 cytokines often relies on phosphorylation and activation of the transcription factor STAT6. To identify DNA elements that could be involved in IL-4/IL-13-induced up-regulation of TFF3, we constructed luciferase reporters containing putative regulatory sequences of the human TFF3 gene. The proximal part of the TFF3 promoter does not contain canonical STAT6 binding elements (TTCnnnnGAA), but five noncanonical binding sites (TTnmmmnnAA) were identified. Luciferase reporter constructs containing up to 800 bp of TFF3 promoter have been described previously by one of us (27). They were transiently transfected into intestinal cells. No apparent modification of the reporter activity was observed upon IL-4/IL-13 treatment (data not shown). Intron 1 of TFF3 contains one canonical STAT6 binding site (Fig. 5). Thus, the construct BB containing the 1.7-kb intron 1 was subcloned upstream of the SV40 promoter of the pGL3 promoter vector. Other constructs containing shorter segments of intron 1, including the putative STAT6 DNA binding element, were also prepared (Fig. 5). Transient transfections of these constructs were performed on HT-29 CL.16E cells treated with IL-13 for 24 h. The activity of the intron 1-containing reporter was not modified by IL-13 treatment (Fig. 5). Similarly to the TFF3 gene, the polymeric IgR (pIgR) gene contains a unique STAT6 binding site localized in intron 1. This element was shown to be involved in the IL-4-induced up-regulation of pIgR (32). We therefore used the construct p12 containing a 554-bp segment of pIgR intron 1, including the STAT6 recognition sequence upstream of the SV40 promoter of the pGL3 promoter vector as an internal control (32). We observed a 3-fold increase in luciferase enzymatic activity upon stimulation with IL-13 (Fig. 5). In HT-29 cells, the IL-4-mediated up-regulation of endogenous pIgR has been shown to depend on de novo protein synthesis that cooperates with STAT6 itself bound to its cognate DNA element in the noncoding part of the gene (32). We tested whether it was true for IL-4/IL-13-mediated up-regulation of TFF3 in HT-29 CL.16E cells. Cells were left untreated or were stimulated with IL-13 for 24 h in either the presence or the absence of CHX. Total RNA was isolated, and the levels of TFF3 transcripts were measured by semiquantitative RT-PCR. For each sample, we analyzed mRNA for the housekeeping gene GAPDH. We found that the mRNA level for TFF3 was increased ~5-fold after IL-13 treatment, whereas simultaneous treatment with CHX abolished this up-regulation (Fig. 6). Otherwise, experiments with actinomycin D revealed that the stability of TFF3 mRNA was not modified in IL-4/IL-13-treated cells (Fig. 7).

The up-regulation of TFF3 by IL-4/IL-13 does not seem to be mediated through the STAT6 binding element located in intron 1. To determine whether IL-13 stimulation affected protein-DNA interactions, we isolated nuclear extracts from HT-29 CL.16E cells treated with IL-4 or IL-13 for 20 min and performed in vitro

**FIGURE 5.** Intron 1 of the human TFF3 gene contains a putative STAT6 binding site. HT-29 CL.16E cells were transiently transfected with the indicated luciferase reporter constructs and were left untreated or were treated with IL-13 (10 ng/ml) for 24 h before harvesting and measurement of luciferase activity. Designations and diagrams of the reporter constructs are given on the left, and fold induction after IL-13 stimulation is on the right. Indicated on the diagram are the complete intron 1 (1.7 kb); the positions of exon I, exon II, and exon III; and the restriction enzyme sites used for internal deletions. P12, containing a 554-bp segment of pIgR gene intron 1 including the STAT6 recognition sequence upstream of the SV40 promoter of pGL3 promoter vector, was used as a control.
EMSA experiments with a probe spanning the canonical STAT6 site (Fig. 8). We identified one major IL-4- or IL-13-inducible nucleoprotein complex. Competitor experiments and treatment with Ab to STAT6 demonstrated that this factor was present in the complex. These data indicate that type 2 cytokines may induce activation of STAT6 and its binding to this DNA element from intron 1.

Expression of dominant-negative STAT6 in HT-29 CL.16E cells blocks IL-13-mediated TFF3 gene induction

To evaluate further the function of STAT6 in IL-4/IL-13-mediated TFF3 gene induction, we established stable HT-29 CL.16E cells expressing a dominant-negative STAT6 protein. As shown in Fig. 9A, clone M expressed high amounts of the truncated STAT6 protein. We then asked whether TFF3 gene induction was affected by dominant-negative STAT6. Cells were treated with or without IL-13 at 10 ng/ml for 24 h. Total RNA was prepared, and TFF3 induction was analyzed by real-time PCR. As shown in Fig. 9B, TFF3 mRNA was profoundly induced by IL-13 in the parental HT-29 CL.16E cells and in the control clone D, which was obtained after transfection with an empty vector. In contrast, TFF3 gene expression was not activated in clone M by IL-13 treatment. In the same set of experiments, quantification of TFF3 in cell extracts revealed 4- and 2-fold increases in immunoreactive material in IL-13-treated parental cells or clone D cells, respectively, whereas TFF3 immunoreactivity was not increased in clone M (Fig. 9C). Similar results were observed using transfected HT-29 MTX cells (data not shown). Taken together, these results indicate that STAT6 plays a crucial role in TFF3 expression.

PI3-kinase is not involved in IL-13-induced TFF3 gene induction

Several investigations showed that IL-4 and IL-13 also signal along the insulin receptor substrate-1/-2, PI3-K pathway; for example, to increase paracellular permeability in the intestinal wall (22). We therefore tested the hypothesis that PI3-K could be involved in the IL-4/IL-13-mediated activation of TFF3 gene expression in HT-29 CL.16E cells. As shown in Fig. 10A, IL-13 increased PI3-K activity in HT-29 CL.16E cells. To block PI3-K signaling, we used the specific inhibitor wortmannin at a concentration of 1 μM, which has been reported to be optimal for PI3-K inhibition in several intestinal cell lines (33). Wortmannin treatment of the cells did not modify the IL-13-induced TFF3 gene expression (Fig. 10, B and C). Similar results were obtained with LY294002, another inhibitor of PI3-K (data not shown).

Discussion

Infection with nematode parasites typically induces a Th2-type response, which contributes to the expulsion of worms (4, 34). IL-4 has been considered to play a key role in the host’s protective immunity, and recently, the importance of IL-13, a related cyto-
kine, has been highlighted in Th2 development and host protection against nematode infection (11). The Th2-type response is characterized by mucosal changes, including goblet cell hyperplasia (35, 36). Goblet cells contained in the intestinal epithelium are the main source of MUCs and TFF3 in the gut. TFF3 is thought to associate with MUC to increase the viscoelasticity of the mucus gel (37). These high molecular mass structures may play an important role in the trapping and removal of intestinal nematodes from the gut (38). Recently, it has been reported that IL-13- or STAT6-deficient mice infected with *N. brasiliensis* or *Trichinella spiralis*, respectively, did not exhibit goblet cell hyperplasia (6, 39). Additionally, a study performed with SCID or TCR−/− mice showed that dysregulated Th2 cells cause goblet cell metaplasia in the intestine through IL-4 release (40). Nevertheless, little is known about the mechanism that regulates goblet cell proliferation and differentiation in the gut. In the present study we hypothesized...
that IL-4 or IL-13 cindirectly induce intestinal epithelial cells to engage in the goblet cell differentiation program and express TFF3 and MUCs.

Because IL-4Rs are known to be present on intestinal epithelial cells (19), we first investigated the presence of IL-4Rs on cultured HT-29 CL.16E cells. These cells harbor a goblet-cell like phenotype in the early postconfluence (28), and we show a marked increase in TFF3 and MUC2 expression in cells committed to differentiation. We also demonstrated the presence of IL-4R α- and IL-13R α-chains, which are subunits of the functional IL-4Rs. These cells can therefore respond to IL-4 or IL-13 and constitute a valid system to study the direct effects of IL-4 and IL-13 on epithelial cell differentiation. As one hallmark of the differentiation of intestinal goblet cells is the early expression of TFF3, we first investigated the effects of IL-4/IL-13 on TFF3 expression, as evaluated by Northern blot, real-time PCR, and RIA. These two prototypic Th2 cytokines up-regulate the expression of TFF3 in a goblet cell line. This up-regulation presumably involves indirect trans-activation of the TFF3 gene and is associated with enhanced TFF3 peptide synthesis.

We found that IL-13 induced TFF3 gene expression from 6 h onward and that this gene induction was dependent on de novo protein synthesis. It is likely that de novo synthesis of a transcription factor(s) is required to elicit TFF3 gene expression. Alternatively, it might be an enzyme or some other protein that modulates transcription through its effect on DNA-bound factors. Whatever the precise underlying mechanism, STAT6 is an essential component of the TFF3 gene induction by IL-13, because in our study the expression of a dominant-negative STAT6 in HT-29 CL.16E cells abolished the cytokine-mediated TFF3 expression.

The downstream molecular events associated with the STAT6 pathway upon IL-4/IL-13 activation and leading to goblet cell metaplasia are difficult to assess because the identification of differentiation factors for goblet cell lineage has remained elusive. Kruppel-like transcription factor 4 (formerly gut-enriched Kruppel-like factor) is a zinc finger transcription factor expressed in the epithelia of skin, lungs, gastrointestinal tract, and several other organs (41). A recent study provided the first evidence that Kruppel-like transcription factor 4 is a goblet cell-specific differentiation factor in the intestine (42). Otherwise, a goblet cell silencer inhibitor element interacting with a goblet cell silencer inhibitor element binding protein has been shown to be essential for goblet cell-specific expression of TFF3 upon HT-29 subclone exposure to keratinocyte growth factor (43). An attractive hypothesis would be that transcription factors are up-regulated in the course of IL-4/IL-13-induced goblet cell metaplasia according to a STAT6-dependent pathway.

Previous studies using airway hypersensitivity models showed that IL-4/IL-13 were key elements in allergen-induced goblet cell metaplasia (7, 8, 18, 44). Inhalation of IL-4 led to up-regulation of the MUC gel and to goblet cell metaplasia and IL-4-induced MUC2 gene expression in cultured airway epithelial cells (45). Although the expression of MUC2 approtein was not specifically evaluated in our experiments, our data show that TFF3 and MUC2 genes are coexpressed upon IL-4/IL-13 treatment of the cells.

PI3-K, a ubiquitous lipid kinase involved in receptor signal transduction by tyrosine kinase receptors, has been shown to stimulate adipocyte and myogenic differentiation (46, 47). Two distinct studies performed with different cell lines show that PI3-K may either stimulate or inhibit enterocyte differentiation (33, 48). We observed that PI3-K inhibition in HT-29 CL.16E cells significantly reduced the spontaneous goblet cell-like differentiation, as several investigations showed that IL-4/IL-13 may also signal along the insulin receptor substrate-1/-2, PI3-K pathway in intestinal cells (22), we tested the hypothesis that the PI3-K and STAT6 pathways could both be activated upon IL-4/IL-13 treatment of cells, leading to TFF3 gene induction. As wortmannin or LY294002 did not modify cytokine-evoked TFF3 gene induction, it seems likely that distinct pathways govern TFF3 expression in the course of spontaneous differentiation of goblet-like cells and in type 2 cytokine-induced goblet cell metaplasia.

In summary, our study is the first to show that IL-4 or IL-13 induces TFF3 expression via a direct effect on intestinal epithelial cells, through a STAT6- and de novo protein synthesis-dependent pathway. These results do not rule out other important effects of IL-4/IL-13 on goblet cell growth, for example, that may occur concurrently to the activation of goblet cell-specific transcriptional machinery.

Acknowledgments

We thank Dr. Hilde Schjerven for the gift of the construct p12, and Drs. François Brière and Adrian Minty for the gifts of human IL-4 and IL-13, respectively.

References


