Immunologically mediated tissue damage in the gut is associated with increased production of proinflammatory cytokines, which activate the transcription factor NF-κB in a variety of different cell types. The mechanisms/factors that negatively regulate NF-κB in the human gut and the pathways leading to the sustained NF-κB activation in gut inflammation remain to be identified. Pretreatment of normal human intestinal lamina propria mononuclear cells (LPMC) with transforming growth factor-β1 (TGF-β1) resulted in a marked suppression of TNF-α-induced NF-κB p65 accumulation in the nucleus, NF-κB binding DNA activity, and NF-κB-dependent gene activation. TGF-β1 also increased IkBα transcripts and protein in normal LPMC. In marked contrast, treatment of LPMC from patients with inflammatory bowel disease with TGF-β1 did not reduce TNF-α-induced NF-κB activation due to the overexpression of Smad7. Indeed, inhibiting Smad7 by specific antisense oligonucleotides increased IkBα expression and reduced NF-κB p65 accumulation in the nucleus. This effect was due to endogenous TGF-β1. TGF-β1 directly stimulated IkBα promoter transcriptional activity in gut fibroblasts in vitro, and overexpression of Smad7 blocked this effect. These data show that TGF-β1 is a negative regulator of NF-κB activation in the gut and that Smad7 maintains high NF-κB activity in gut inflammation by blocking TGF-β1 signaling.

The excessive mucosal immune responses in gut inflammation are characterized by increased synthesis of proinflammatory cytokines, which activate the NF-κB pathway in the gut wall (1). For example, the activity of the p65 subunit of NF-κB is enhanced in the mucosa of patients with inflammatory bowel disease (IBD), 1 and inhibition of NF-κB with a specific p65 antisense oligonucleotide is effective in preventing/treating experimental models of IBD and efficiently down-regulates cytokine production by intestinal macrophages from Crohn’s disease (CD) patients (2–4).

Many important questions remain regarding the biochemical pathways that lead to the sustained NF-κB activation in gut inflammation. NF-κB is up-regulated by a large number of inflammatory cytokines, including IL-1, IL-18, TNF-α, all of which are produced in excess in inflamed bowel (1). In contrast, little is known about the mechanisms/factors that negatively regulate NF-κB activity in the normal and inflamed human intestine.

Transforming growth factor-β1 (TGF-β1) is a multifunctional cytokine capable of regulating the growth, differentiation, and function of immune and non-immune cells (5). TGF-β1 initiates signaling through the ligand-dependent activation of a complex of heterodimeric transmembrane serine/threonine kinases, consisting of type I (TGF-β1 RI) and type II (TGF-β1 RII) receptors (6). After TGF-β1 binding, there is phosphorylation and activation of TGF-βRI by the constitutively active and auto-phosphorylated TGF-βRII (6). Activated TGF-βRII then directly phosphorylates Smad2 and Smad3. Smad2 and Smad3 associate with Smad4 and translocate to the nucleus where Smad protein complexes participate in transcriptional control of target genes (7). Targeted disruption of the Smad3 gene is associated with diminished responsiveness to TGF-β1 (8). Mutant mice exhibit a massive infiltration of T cells and pyogenic abscess formation in the stomach and intestine (8). Recent studies have identified several molecules that control TGF-β1 signal transduction and activity (6). The antagonistic Smad, Smad7, associates with the ligand-activated TGF-βRI and interferes with the activation of Smad2/Smad3 by preventing interaction with TGF-βRI (9).

Overexpression of Smad7 in the gut of patients with IBD blocks TGF-β1 signaling (10). Studies in animals have shown that defective TGF-β1 activity promotes and/or exacerbates chronic intestinal inflammation (11). Nonetheless, the molecular mechanisms underlying the counter-regulatory effects of TGF-β1 in the gut are not known. Studies using cell lines have demonstrated that TGF-β1 can either promote or inhibit NF-κB activation (12–15) depending on the cell type used.

In this study we investigated functionally if TGF-β1 negatively regulates NF-κB in human intestinal LPMC and if the chronic activation of the NF-κB pathway seen in IBD LPMC is due to impaired negative regulation of the TGF-β1 signaling pathway due to enhanced Smad7 expression.
EXPERIMENTAL PROCEDURES

Patients and Samples—Mucosal samples were taken from resection specimens of 15 patients with moderate-to-severe CD. In nine patients the primary site of disease was the colon, and in six both the terminal ileum and colon were involved. Eleven patients were receiving mesalazine and/or antibiotics, and five were on corticosteroids. The indication for surgery was a chronic disease, unresponsive to medical treatment. Mucosal samples were also taken from four patients with active ulcerative colitis (UC) undergoing colectomy; all were receiving mesalazine and steroids. Age-matched controls included normal colectomy specimens from 17 patients with colon cancer. Local ethical approval was obtained.

Isolation and Culture of Lamina Propria Mononuclear Cell—LPMC were prepared as described previously (16), and an aliquot was immediately used for extracting cytosolic and nuclear proteins. The remaining LPMC were resuspended in RPMI 1640 (Sigma, Dorset, UK) supplemented with a serum replacement reagent HL-1 (Biotwitaker, Wokingham, UK) and cultured in the presence or absence of TGF-β1 (final concentration ranging from 1 to 5 ng/ml, Sigma) for 1–24 h. In other experiments, IBD LPMC were resuspended in RPMI 1640 supplemented with HL-1 and cultured in the presence or absence of a Smad7 antisense or control sense oligonucleotide (both used at a final concentration of 2 μg/ml) for 24 h in the absence of lipofectamine (10). Smad7 antisense and sense oligonucleotide sequences have been reported previously (10). In four separate experiments, IBD LPMC were treated with the Smad7 antisense in the presence or absence of a neutralizing TGF-β1 antibody (5 μg/ml; R & D Systems, Abingdon, UK). After 24 h, an aliquot of LPMC was used for extracting proteins for analysis of Smad7. The remaining cells were extensively washed, resuspended in RPMI 1640 plus HL-1 and cultured in the presence or absence of TGF-β1 (5 ng/ml) for 7–14 h and then stimulated with TNF-α. Finally, to examine the effect of NF-κB activity on Smad7 expression, IBD LPMC were stimulated with N-p-tosyl-lphenylalanine chloromethyl ketone (TPCK, 10 μM, Sigma), an inhibitor of NF-κB activation, or ethanol (vehicle) for 5 up to 24 h.

RNA Extraction, cDNA Preparation, and Reverse Transcriptase-PCR—Total RNA, cDNA preparation, and RT-PCR were carried out as indicated previously (16). PCR primers were as follows: IL-8, 5'-TGCAAGCTCTGTTGAAAGG-3' (forward) and 5'-ATTGCTACTGCTTGCCACTTAC-3' (reverse); ICAM-1, 5'-ATCACCAACCAGCCAGAAAT-3' (forward) and 5'-GCCACCCAGGACACGAAATG-3' (reverse); β-actin, 5'-GCC ACC ACA CCT TCT ACA-3' (forward) and 5'-CAG GTC TTT GCG GAT GTC-3' (reverse). RT-PCR products were electrophoresed in 1% agarose gel containing 0.3 μg/ml ethidium bromide.

Electrophoretic Mobility Shift Assay (EMSA)—Nuclear protein-DNA binding studies were carried out for 20 min at room temperature in a 20-ml reaction volume containing 10 mM Tris, 50 mM KCl, 1 mM dithiothreitol, 2.5% glycerol, 5 mM MgCl₂, 1 mM dithiothreitol, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride (all from Sigma). For the detection of cytosolic and nuclear NF-κB/p65, 10 μg/sample of cytosolic or nuclear proteins were separated on a 10% SDS-PAGE gel. p65 was detected using a rabbit anti-human NF-κB/p65 (1:500 final dilution) antibody (Santa Cruz Biotechnology, Santa Cruz, CA) or control isotype IgG antibody (Dako Ltd., Milan, Italy) (both used at a concentration of 2.5 μg/ml) were incubated with the nuclear proteins for 45 min before adding the DNA probe. A 6% non-denaturing polyacrylamide gel was used for electrophoretic separation. After blotting to a membrane, labeled oligonucleotides were detected with a chemiluminescence EMSA kit (Pierce).

Protein Extraction and Western Blot Analysis—LPMC were homogenized and cytosolic extracts collected in buffer A containing 10 mM Hepes (pH 7.9), 10 mM KCl, 0.1 mM EDTA, and 0.2 mM EGTA. Nuclear extracts were prepared by solubilizing the remaining nuclei in buffer C containing 20 mM Hepes (pH 7.9), 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, and 10% glycerol. Both buffers were supplemented with 1 mM dithiothreitol, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM phenylmethane sulfonyl fluoride (all from Sigma). Western blot showing NF-κB/p65 in nuclear extracts from normal LPMC. LPMC were either left untreated or pretreated with TGF-β1 for 7, 14, or 28 h and then stimulated with medium (UNST) or TNF-α for 30 min. After detection of p65, the membrane was stripped and incubated with a histone-1 antibody (lower blot) to ascertain equivalent loading of the lanes. B, quantitative analysis of the NF-κB/p65/histone-1 ratio. Each point represents a single subject. TNF-α increased the nuclear p65/histone ratio (p < 0.01), and this was reduced to control levels by pretreatment with TGF-β1. C, TGF-β1 inhibits TNF-α-induced NF-κB binding activity in normal LPMC. Representative EMSA showing NF-κB/DNA binding activity. LPMC were either left untreated or pretreated with TGF-β1 for 7 h and then stimulated with medium or TNF-α for 30 min. Nuclear proteins, extracted from LPMC treated with TNF-α only, were incubated with monoclonal p65 or p50 antibody and then analyzed. The specificity of the NF-κB binding complex was confirmed by incubating nuclear proteins, extracted from LPMC treated with TNF-α only, with excess of specific unlabelled NF-κB probe or nonspecific DNA. One of five representative experiments is shown. D, TGF-β1 inhibits TNF-α-induced IL-8 RNA in normal LPMC. A, representative agarose gel showing IL-8 and β-actin RNA transcripts. LPMC were either left untreated or treated with TGF-β1 for 7 h and then stimulated with medium (UNST) or TNF-α for 5 h. One of four representative experiments is shown.
Primary gut fibroblasts were either left untreated or treated with TGF-β1 for 7 h and then stimulated with medium (UNST) or TNF-α for the indicated time points. The membrane was stripped and incubated with a β-actin antibody (lower blot) to ascertain equivalent loading of the lanes. B, quantitative analysis of the IL-8/β-actin ratio (mean ± 1 S.E.). TNF significantly decreased cytosolic IκBα after 20 min (p < 0.01).

RESULTS

TGF-β1 Inhibits NF-κB Activation in Normal LPMC—Stimulation of cells with TNF-α alone resulted in a marked nuclear translocation of p65 (Fig. 1A). Preincubation with TGF-β1 prevented the TNF-α-induced p65 nuclear translocation (Fig. 1, A and B), with no change in the content of cytosolic NF-κB (not shown). Stimulation of normal LPMC with TNF-α also enhanced specific NF-κB DNA binding activity (Fig. 1C). Super-shift assays showed that TNF-α enhanced both p65/p50 heterodimer and p50 homodimer. Preincubation of LPMC with TGF-β1 reduced the TNF-α-induced NF-κB DNA complexes, with a more marked effect on p65/p50 heterodimer (Fig. 1C).

As TNF-α up-regulates IL-8 transcription through an NF-κB-dependent mechanism (19), we established whether TGF-β1 inhibited IL-8 gene expression. As expected TNF-α-treated LPMC dis-
Fig. 3. A, TGF-β1 enhances IκBα protein in normal LPMC. Upper panel, representative Western blot showing IκBα in cytosolic extracts from normal LPMC cultured in medium (UNST) for 7 h or TGF-β1 for the indicated time points. One of five representative experiments is shown. After detection of IκBα, the membrane was stripped and incubated with a β-actin antibody (middle panel). The membrane was stripped and incubated with a β-actin antibody (lower blot) to ascertain equivalent loading of the lanes. Inset, quantitative analysis of the IκBα/β-actin ratio. TGF-β1 significantly increased IκBα at all times (p < 0.01). B, TGF-β1 induces IκBα transcripts in normal LPMC. Representative agarose gel showing IκBα and β-actin RNA transcripts in normal LPMC. LPMC were either left untreated (UNST) or treated with TGF-β1 for the indicated time points and then analyzed as experimental procedures. One of three representative experiments using cells from different patients is shown. C, pretreatment of normal LPMC with CHX does not affect TGF-β1-induced IκBα. Normal LPMC were cultured with CHX and then stimulated with TGF-β1. Two of four representative experiments using LPMC from different normal controls is shown.

played a strong increase in IL-8 RNA transcripts (Fig. 1D). However TNF-induced IL-8 transcripts were markedly inhibited by pretreatment with TGF-β1 (Fig. 1D).

TGF-β1 Inhibits NF-κB/p65 Nuclear Translocation and Induces IκBα in Normal LPMC—One potential mechanism by which TGF-β1 suppresses NF-κB activation could be related to its ability to prevent nuclear translocation of p65. IκBα protein was analyzed in cytosolic extracts isolated from LPMC precultured with TGF-β1 for 7 h and then treated with TNF-α for various times. In untreated LPMC, the cytoplasmic IκBα signal almost completely disappeared after 20 min of treatment with TNF-α and returned to the original level after 40 min of stimulation (Fig. 2A). Pretreatment of LPMC with TGF-β1 for 7 h increased IκBα expression and subsequent stimulation with TNF-α led to no reduction in IκBα (Fig. 2A). The key change in LPMC pretreated with TGF-β1 (compared with cells pretreated with medium) prior to TNF-α was a substantially higher IκBα/β-actin ratio at all time points after treatment (Fig. 2B). Cytosolic extracts were prepared from normal LPMC treated with TGF-β1 for various times and analyzed by Western blotting. TGF-β1 treatment resulted in enhanced IκBα protein expression for up to 28 h (Fig. 3A). TGF-β1 also increased IκBα transcripts in normal LPMC (Fig. 3B). The effect of TGF-β1 on IκBα transcripts did not depend on the synthesis of new proteins as TGF-β1-mediated IκBα induction was not inhibited by CHX (Fig. 3C). In contrast TGF-β1 did not affect IκBβ protein expression (Fig. 3A).

LPMC from Patients with IBD Exhibit High NF-κB/p65 Nuclear Accumulation and Low Cytosolic IκBα—Abundant immunoreactivity for NF-κB/p65 was seen in nuclear extracts from IBD LPMC in comparison with control samples (Fig. 4A). Importantly, the high p65 nuclear accumulation documented in IBD LPMC was associated with a reduced cytoplasmic IκBα protein expression compared with normal LPMC (Fig. 4B). Two bands, likely corresponding to the basal and phosphorylated form of IκBα, were seen in IBD LPMC samples (Fig. 4B). We next determined if TGF-β1 could inhibit NF-κB activation in cells from IBD patients. The already high nuclear NF-κB/p65 increased modestly after stimulation with TNFα (Fig. 5A). However pretreatment with TGF-β1 had no effect (Fig. 5A). Likewise TGF-β1 did not reduce DNA binding activity in extracts of IBD LPMC (Fig. 5B).

Ablation of Smad7 in IBD LPMC Allovs TGF-β1 to Down-regulate NF-κB Activation—IBD LPMC were treated with a Smad7 sense or antisense and then analyzed for both nuclear NF-κB/p65 and cytosolic IκBα. Treatment with Smad7 antisense inhibited Smad7 protein expression (Fig. 6A). Both unstimulated and Smad7 sense-treated LPMC exhibited low cytosolic IκBα and high levels of nuclear p65 (Fig. 6, B and C). In antisense treated LPMC, however, there was a marked in-
Smad7 Sustains High NF-κB in IBD

![Image](http://www.jbc.org/)

**A**

IBD LPMC  Normal LPMC

|     | 1 | 2 | 3 | 4 | 5 |
|-----|---|---|---|---|---|
| NF-κB/p65 | ![Image](http://www.jbc.org/) | ![Image](http://www.jbc.org/) | ![Image](http://www.jbc.org/) | ![Image](http://www.jbc.org/) | ![Image](http://www.jbc.org/) |
| Histone-1 | ![Image](http://www.jbc.org/) | ![Image](http://www.jbc.org/) | ![Image](http://www.jbc.org/) | ![Image](http://www.jbc.org/) | ![Image](http://www.jbc.org/) |

**B**

IBD LPMC  Normal LPMC

|     | 1 | 2 | 3 | 4 | 5 |
|-----|---|---|---|---|---|
| IKKα | ![Image](http://www.jbc.org/) | ![Image](http://www.jbc.org/) | ![Image](http://www.jbc.org/) | ![Image](http://www.jbc.org/) | ![Image](http://www.jbc.org/) |
| β-actin | ![Image](http://www.jbc.org/) | ![Image](http://www.jbc.org/) | ![Image](http://www.jbc.org/) | ![Image](http://www.jbc.org/) | ![Image](http://www.jbc.org/) |

![Image](http://www.jbc.org/)

Fig. 4. A, representative Western blot showing NF-κB/p65 in nuclear extracts from LPMC isolated from the colon of five patients with IBD and five normals. After detection of NF-κB/p65, the membrane was stripped and incubated with a histone-1 antibody (lower blot). One of two representative experiments analyzing cells from five patients with CD and three with UC and eight normal LPMC samples is shown. Inset, quantitative analysis of the p65/histone-1 ratio (n = 8 patients/group). The ratio was significantly higher in IBD patients (p < 0.01). B, representative Western blot showing IκBα in cytosolic extracts from LPMC isolated from the colon of five patients with IBD (three CD and two UC) and five normals. One of two representative experiments analyzing using cells from five CD, three UC and eight normal samples is shown. Note that the blot shows IκBα in the same LPMC samples analyzed for nuclear NF-κB (A). Inset, quantitative analysis of the IκBα/β-actin ratio (n = 8 patients/group). The ratio was significantly higher in normal patients (p < 0.01).

increase in cytosolic IκBα and reduced nuclear p65 immunoreactivity (Fig. 6, B and C), which was prevented by a neutralizing TGF-β1 antibody, suggesting that inhibition of Smad7 allows endogenous TGF-β1 to inhibit NF-κB. Addition of a control IgG to the antisense treated IBD LPMC did not reduce cytoplasmic IκBα or increase nuclear p65 (not shown).

NF-κB DNA binding activity was used to verify the effects of antisense on Smad7. IBD LPMC pretreated with medium or sense oligonucleotide and then cultured in medium alone showed high NF-κB binding activity (Fig. 6D), which was slightly enhanced by treatment with TNF-α and only modestly decreased by pre-treatment with TGF-β1. IBD LPMC treated with Smad7 antisense showed reduced NF-κB binding activity when cultured with medium alone. In these cells, stimulation with TNF-α resulted in a high NF-κB binding, which was prevented by TGF-β1 (Fig. 6D).

Smad7 Controls TGF-β1 Induction of IκBα—In immune-mediated gut damage, tissue degradation is mediated by matrix metalloproteinases produced by local fibroblasts (20), which also participate in the pro-inflammatory cytokine cascade (21). In addition gut fibroblasts can be grown in culture, making them ideal for transfection studies. Primary gut myofibroblasts were therefore transfected with a construct carrying the luciferase reporter gene under the transcriptional control of the human IκBα promoter (pIκBα-Luc) or with a control vector pRLTK. Sixteen hours after transfection, cells were incubated with TGF-β1 for 2, 4, or 24 h. TGF-β1 increased transcriptional activity by ~2.5- and 3.5-fold after 2 and 4 h (Fig. 7). Cotransfection with a plasmid encoding Smad7 abolished this effect (Fig. 7).

**Inhibition of NF-κB Does Not Change Smad7 Expression in IBD LPMC**—Signaling through NF-κB induces Smad7 in fibroblast cell lines (22), so we analyzed whether the sustained NF-κB activity seen in IBD LPMC could contribute to Smad7 induction. IBD LPMC were treated with TPCK, an inhibitor of NF-κB activity, and then analyzed for Smad7. As expected 5-h treatment of IBD LPMC with TPCK inhibited NF-κB/p65 nuclear translocation, but there was no change in Smad7 (Fig. 8), indicating that regulation of Smad7 in IBD LPMC is different from that documented in fibroblast cell lines (22).

**DISCUSSION**

In this work we show that TGF-β1 is a powerful negative regulator of the transcription factor NF-κB in the human intestine. We first demonstrated that pretreatment of normal LPMC with TGF-β1 blocked the TNFα induced nuclear translocation of p65, and this was associated with induction of IκBα. Both NF-κB binding activity and expression of the NF-κB-dependent IL-8 gene by TNF-α were inhibited by preincubation of LPMC with TGF-β1. In marked contrast, TGF-β1 was unable to inhibit NF-κB activation in IBD LPMC. It is highly likely that the high Smad7 seen in the inflamed mucosa of IBD patients (10) is the major contributor to this failure of negative regulation. Indeed decreasing Smad7 with an antisense oligonucleotide allowed endogenous TGF-β1 to up-regulate IκBα and lower NF-κB accumulation in the nucleus.

In response to NF-κB activating stimuli, IκBα is phosphorylated by upstream kinases, ubiquitinated and finally degraded by the proteasome pathway. After degradation, IκBα protein is,
however, rapidly re-synthesized through a mechanism, which is partly dependent on NF-κB activity (19). Since LPMC treated with TGF-β1 show a level of IκBα higher than that seen in both untreated or TNF-α-stimulated cells, and previous studies have shown that TGF-β1 stimulates IκBα in epithelial and B cell lines (14, 15), we focused our experiments on the possibility that TGF-β1 could induce IκBα synthesis in LPMC. Indeed, treatment of LPMC with TGF-β1 increased IκBα protein and mRNA. Furthermore, we showed that TGF-β1 enhanced IκBα promoter transcriptional activity in primary gut fibroblasts, clearly indicating that TGF-β1 controls IκBα gene expression. The mechanism by which TGF-β1 stimulates IκBα transcription is not known; however, sequence analysis of the human IκBα promoter revealed the presence of 4 potential TGF-β/activin-inducible CAGA box elements (data not shown). The presence of multiple CAGA box elements has been described for several TGF-β-inducible promoters and in the plasminogen activator inhibitor-type gene promoter have been demonstrated to interact with Smad3/Smad4 and to be required for TGF-β1 responsiveness (23). The fact that overexpression of Smad7 in gut myofibroblasts blocked the TGF-β1-induced IκBα promoter activity supports the role of Smad3 pathway in regulating IκBα expression.

Another critical question remains as whether as TGF-β1 influences NF-κB activation directly or via other cytokines. Indeed, it is known that TGF-β1 can stimulate IL-10 production in some cell systems and that IL-10 inhibits TNF-α-induced IκBα degradation in both monocytic and intestinal epithelial cell lines (24, 25). We think this is unlikely. First, treatment of normal LPMC with TGF-β1 did not enhance IL-10 RNA transcripts (not shown). Second, an increase in IκBα RNA induction in response to TGF-β1 occurs after a very short time (i.e. 1 h of stimulation), thus making improbable the involvement of other TGF-β1-induced cytokines.

TGF-β1 is one of the most widely distributed cytokines that acts on virtually all cell types and their function. Particularly, TGF-β1 plays an important role in the control of immune homeostasis and prevention of mucosal inflammation (11). TGF-β1 knock-out mice develop a severe multiple organ inflammatory disease, in which the lymphocytic infiltration of the affected organs is associated with increased production of inflammatory cytokines (26). Abrogation of TGF-β1 signaling in T cells alone is sufficient to break T and B cell homeostasis and induce T cell-mediated inflammatory lesions in various organs, including the intestine (27). Finally, it has been demonstrated that the loss of TGF-β1 activity results in the induction and/or amplification of pathogenic responses responsible for the development of experimental colitis resembling either CD or UC (11, 28). Consistent with this we have recently shown that in both CD and UC there is diminished TGF-β1/Smad3 signaling, due to high Smad7 (10). The data in the present study confirm and expand on the major role of Smad7 in IBD.

**Fig. 5.** TGF-β1 fails to inhibit NF-κB activation in IBD LPMC. A, representative Western blots showing NF-κB/p65 in nuclear extracts from IBD LPMC. LPMC were either cultured with medium or TGF-β1 for 7 h and then stimulated with medium or TNF-α for 30 min. After detection of p65, the membrane was stripped and incubated with a histone-1 antibody (lower blot). Two of five representative experiments analyzing LPMC isolated from three patients with CD and two with UC are shown. Inset, quantitative analysis of the p65/histone-1 ratio (n = 8 patients/group). B, two representative EMSA blots showing NF-κB-DNA binding activity in IBD LPMC. LPMC were either cultured with medium or TGF-β1 for 7 h and then stimulated with medium or TNF-α for 30 min. Two of five representative experiments analyzing LPMC isolated from three patients with CD and two with UC are shown.
FIG. 6. A, treatment of IBD LPMC with a specific Smad7 antisense but not sense oligonucleotide inhibits Smad7 expression. IBD LPMC were cultured in the absence (control) or presence of a specific Smad7 antisense (AS) or control sense (S) oligonucleotide or antisense plus a neutralizing TGF-β1 antibody (aTGF) for 24 h. The example is representative of three separate experiments analyzing in total LPMC from four IBD patients (three CD and one UC), quantitative data for which are shown in the inset. Each point represents a single subject. Antisense significantly reduced Smad7 \( (p < 0.01) \). B, inhibition of Smad7 enhances IκBα expression in IBD LPMC. The inset shows quantitative analysis of IκBα/β-actin ratio in LPMC from four patients with IBD (three CD and one UC). Each point represents a single subject. Antisense significantly increased the ratio \( (p < 0.01) \), and this increase was abolished by anti-TGF-β1. C, inhibition of Smad7 inhibits NF-κB/p65 nuclear accumulation in IBD LPMC. The inset shows quantitative analysis of p65/histone-1 ratio in LPMC from four patients with IBD (three CD and one UC). Each point is a single subject. Antisense significantly decreased nuclear p65 \( (p < 0.01) \), and this decrease was abolished by anti-TGF-β1. D, antisense to Smad 7 allows TGF-β1 to inhibit TNF-α-induced NF-κB activation in IBD LPMC. IBD LPMC were cultured in the absence or presence of a specific Smad7 antisense or sense oligonucleotide for 24 h. The cells were washed and cultured with or without TGF-β1 for 7 h and then stimulated with or without TNF-α for 30 min. In IBD LPMC pretreated with the Smad7 antisense, basal NF-κB binding activity was reduced compared with cells pretreated with medium (control) or Smad7 sense. In LPMC precultured with medium or sense Smad7 oligonucleotide, only a modest increase in NF-κB binding was seen in response to TNF-α. In these cells, no inhibition was observed when cells were pretreated with TGF-β1. In LPMC preincubated with Smad7 antisense, TGF-β1 completely inhibited the TNF-α-induced NF-κB binding activity. One of four representative experiments analyzing LPMC isolated from two patients with CD and two with UC is shown.
endogenous TGF-β1. Indeed, treatment of IBD LPMC with a neutralizing TGF-β1 antibody prevented the Smad7 antisense-mediated inhibition of NF-κB activation. Furthermore, abrogation of Smad7 by specific antisense DNA resulted in a complete inhibition by TGF-β1 of the TNF-α-induced NF-κB binding DNA activity.

Activation of NF-κB/p65 seems to play a key role in the pathogenesis of colitis (2–4). In keeping with this, we showed that IBD LPMC exhibited high p65 nuclear accumulation, which was associated with reduced expression of cytosolic IκBα. In addition, the demonstration that TGF-β1 negatively regulated NF-κB activation in normal LPMC and that treatment of IBD LPMC with a specific Smad7 antisense resulted in an inhibition of NF-κB activation allowed to delineate a scenario in which defective TGF-β1 signaling due to exaggerated Smad7 helps maintain high NF-κB activity, thereby expanding the local inflammatory response in IBD. Blocking Smad7 might be a useful way to dampen mucosal inflammation in IBD.

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