Interleukin-32α expression in human colonic subepithelial myofibroblasts

YUHKI YAGI[1], AKIRA ANDOH[3], HIROTSUGU IMAEDA[1], TOMOKI AOMATSU[1], RIE OHSAKI[1], OSAMU INATOMI[1], SHIGEKI BAMBA[1], TOMOYUKI TSUJIKAWA[1], TOMOHARU SHIMIZU[2] and YOSHIHIDE FUJIYAMA[1]

Departments of [1]Medicine and [2]Surgery and [3]Division of Mucosal Immunology, Graduate School of Medicine, Shiga University of Medical Science, Seta Tukinowa, Otsu 520-2192, Japan

Received August 22, 2010; Accepted October 19, 2010

DOI: 10.3892/ijmm.2010.575

Abstract. Interleukin (IL)-32 is a recently described proinflammatory cytokine, characterized by induction of nuclear factor (NF)-κB activation. We studied IL-32α expression in human colonic subepithelial myofibroblasts (SEMFs). Colonic SEMFs were isolated from normal human colon tissue. IL-32α protein expression was evaluated by Western blot analyses, and IL-32α mRNA expression was analyzed by real-time PCR. IL-32α mRNA was weakly expressed without a stimulus, and its expression was markedly enhanced by IL-1ß and TNF-α. IL-1ß and TNF-α enhanced intracellular accumulation of IL-32α protein, but IL-32α was not detected in supernatants. Each cytokine dose- and time-dependently induced IL-32α mRNA expression. An inhibitor of phosphatidylinositol 3-kinase (LY294002) significantly suppressed IL-1ß- and TNF-α-induced IL-32α mRNA expression, although MAPK inhibitors had no effect. Akt activation in response to these cytokines was confirmed by Western blotting. Blockade of NF-κB activation by an adenovirus expressing a stable mutant form of IκBα markedly suppressed IL-1ß- and TNF-α-induced IL-32α mRNA expression. Human colonic SEMFs expressed IL-32α in response to IL-1ß and TNF-α. IL-32α mRNA expression depends on the phosphatidylinositol 3-kinase and the NF-κB system.

Introduction

Ulcerative colitis (UC) and Crohn's disease (CD), two common forms of idiopathic inflammatory bowel disease (IBD), are chronic, relapsing inflammatory disorders of the gastrointestinal tract. IBD is thought to result from inappropriate and ongoing activation of the mucosal immune system driven by the presence of normal luminal flora (1-3). This aberrant response is most likely facilitated by defects in both the barrier function of the intestinal epithelium and the mucosal immune system. The investigation of the immunological responses in the intestinal mucosa is a fundamental approach to the understanding of the pathophysiology of IBD.

Interleukin (IL)-32 is a recently described cytokine that is produced by T lymphocytes, natural killer cells, monocytes, and epithelial cells (4,5). Although IL-32 was first reported as a transcript in IL-2 activated NK and T cells, it appears that epithelial cells are the dominant and widespread source (6). The gene encoding IL-32 is located on human chromosome 16p13.3 and is organized into eight exons (7). There are four splice variants (IL-32α, IL-32ß, IL-32δ and IL-32γ), and IL-32α is the most abundant transcript. Of particular importance, IL-32 is prominently induced by interferon (IFN)-γ in lung epithelial cells and monocytes (4). IL-32 exhibits several properties typical of proinflammatory cytokines (4,5). For example, it stimulates the secretion of proinflammatory cytokines and chemokines such as IL-1ß, TNF-α, IL-6 and IL-8 by means of the activation of NF-κB and p38 mitogen-activated protein kinases (MAPKs) (4,5).

Netea *et al* recently demonstrated that IL-32 augments the production of IL-1ß and IL-6 induced by muramyl dipeptide (MDP), a peptidoglycan fraction of bacteria, by means of the nucleotide-binding oligomerization domain proteins (NOD1 and NOD2) through a caspase-1-dependent mechanism (5). NODs are a family of intracytoplasmic bacterial sensors, and recognition of bacterial peptidoglycans subsequently induces NF-κB activation (8). Mutations in *NOD2* have been implicated in the pathogenesis of CD (9,10), and CD patients homozygous for the frameshift 3020insC mutated allele have defective responses to MDP in cytokine production (11,12). Recently, it has been shown that the *NOD2* mutation in CD patients potentiates NF-κB activity and IL-1ß processing (13). Thus, these data suggest a pivotal role of IL-32 in the pathophysiology of IBD and in particular of CD.

In this study, we investigated the expression of IL-32α in human colonic subepithelial myofibroblasts (SEMFs). Colonic SEMFs are α-smooth muscle actin (α-SMA)-positive

Correspondence to: Dr Akira Andoh, Division of Mucosal Immunology, Graduate School of Medicine, Shiga University of Medical Science, Seta Tukinowa, Otsu 520-2192, Japan
E-mail: andoh@belle.shiga-med.ac.jp

Key words: cytokine, inflammatory bowel disease, phosphatidylinositol-3 kinase

mesenchymal cells and are located subjacent to the basement membrane of the small and large intestines. These cells belong to a family of α-SMA positive fibroblast-like cells, such as the cells of the hepatic stellate (Ito) renal mesangial/ tubolointerstitial, the lung interstitial contractile cells, and the pancreatic stellate cells (14-16). Colonic SEMFs act to mediate information flow in both directions to and from the intestinal epithelium and the immune and the other mesenchymal and neural elements of the lamina propria, and play an important role in ECM metabolism affecting the growth of the basement membrane in the intestinal mucosa. Current observations suggest a role of colonic SEMFs in the pathophysiology of IBD via IL-32 expression.

Materials and methods

Reagents. Recombinant human IL-1ß, IL-17 and IFN-γ were purchased from R&D Systems (Minneapolis, MN), and other cytokines were obtained from PeproTech (Rocky Hill, NJ). Anti-human IL-32α antibodies were purchased from R & D Systems. All other reagents were purchased from Sigma Chemical Co. (St Louis, MO).

Culturing of human colonic SEMFs and colonic epithelial cell lines. Primary colonic SEMF cultures were prepared according to a method reported by Mahida *et al* (17). The cellular characteristics and culture conditions have been described in our previous report (18).

Real-time-polymerase chain reaction (PCR). Expression of human IL-32α mRNA in samples was assessed by real-time PCR analyses. Real-time PCR was performed using a LightCycler 2.0 system (Roche Applied Science, Tokyo, Japan) with the following primers specific for human IL-32α; 5'AGCTGGAGGACGACTTCAAA (nucleotides 192-212, GenBank accession No. BC018782) (19) and 3'AGGTG GTGTCAGTATCTTCA (642-623). PCR products were ligated into TA cloning vectors (Promega, Madison, WI) and sequenced. PCR was conducted using a SYBR-Green PCR Master mix (Applied Biosystems, Foster City, CA). Data were normalized versus ß-actin for human IL-32.

Western blot analyses. For analysis of IL-32α protein expression, cells were exposed to cytokines for predetermined periods of time. Cells were then washed with PBS and lysed in SDS sample buffer containing 100 μM orthovanadate. For Western blotting, 10 μg of protein from each sample was subjected to SDS-PAGE on a 4-20% gradient gel under reducing conditions (20). Biotinylated anti-human IL-32α antibodies were purchased from R&D Systems and peroxidase-conjugated streptavidine was purchased from Dako Japan (Kyoto, Japan). Subsequently, detection was performed using the enhanced chemiluminescence Western blotting system (Amersham).

For Akt phosphorylation analyses, cells were exposed to cytokines for predetermined periods of time. Antibodies directed against phosphorylated and total Akt were purchased from Cell Signaling Technology (Beverly, MA), and peroxidase-conjugated secondary antibodies were purchased from Amersham (Arlington Heights, IL).

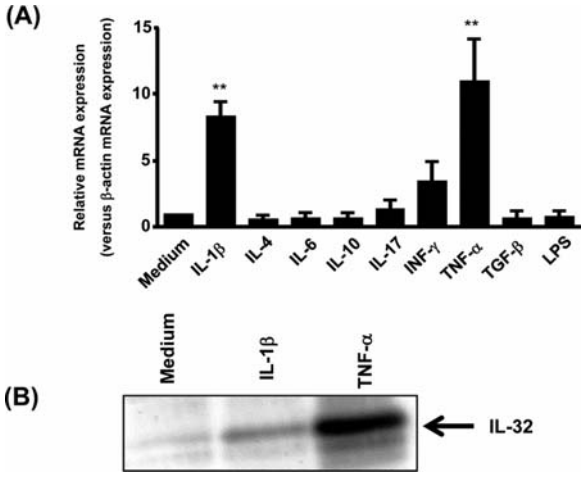

Figure 1. IL-32α mRNA and protein expression in human colonic SEMFs. (A) IL-32α mRNA expression. Cells were stimulated with cytokines [IL-1ß (10 ng/ml) or other cytokines (100 ng/ml)] for 12 h. IL-32α mRNA expression was analyzed by real-time PCR analyses. Data are expressed as IL-32α mRNA relative to ß-actin mRNA expression (mean ± SD of 4 different experiments); $^{**}P<0.01$. (B) Intracellular IL-32α protein expression. Cells were stimulated with IL-1ß (10 ng/ml) or TNF-α (100 ng/ml) for 48 h, and then lysed with lysis buffer. IL-32α protein was analyzed by Western blotting.

Adenovirus-mediated gene transfers. We used a recombinant adenovirus expressing a stable mutant form of IκBα (Ad-IκBΔN) (21), a recombinant adenovirus expressing a dominant negative mutant of c-Jun (Ad-DN-c-Jun) (22) and a recombinant adenovirus containing bacterial ß-galactosidase cDNA (Ad-LacZ). The stable mutant form of IκBα (IκBΔN) lacks 54 NH_2-terminal amino acids of wild-type IκBα, and is neither phosphorylated nor proteolyzed in response to signal induction, but fully inhibits NF-κB activation. The dominant negative mutant c-Jun (TAM67) lacks the transactivational domain of amino acids 3-122 of wild type c-Jun, but it retains the DNA-binding domain. In preliminary experiments, Ad-LacZ infections of colonic myofibroblasts with a multiplicity of infection (MOI) of 10 showed a maximal expression (85% positive) of ß-galactosidase. The recombinant adenovirus was transferred into the cells, and cells were made quiescent for 48 h before being assessed for the effects of the transferred gene.

Statistical analysis. Statistical significances of differences were determined by the Mann-Whitney U test (StatView Version 4.5). Differences resulting in P-values <0.05 were considered to be statistically significant.

Results

To investigate the regulatory mechanisms underlying IL-32α induction in colonic SEMFs, cells were stimulated with various cytokines for 12 h and IL-32α mRNA expression was assessed by real-time PCR analyses (Fig. 1A). In these cells, IL-32α mRNA was weakly expressed without any stimulus, and IL-1ß and TNF-α markedly enhanced IL-32α mRNA expression. IFN-γ weakly induced IL-32α mRNA expression.

Similar results were observed at the protein level. Cells were stimulated for 24 h with IL-1ß and TNF-α, and IL-32α

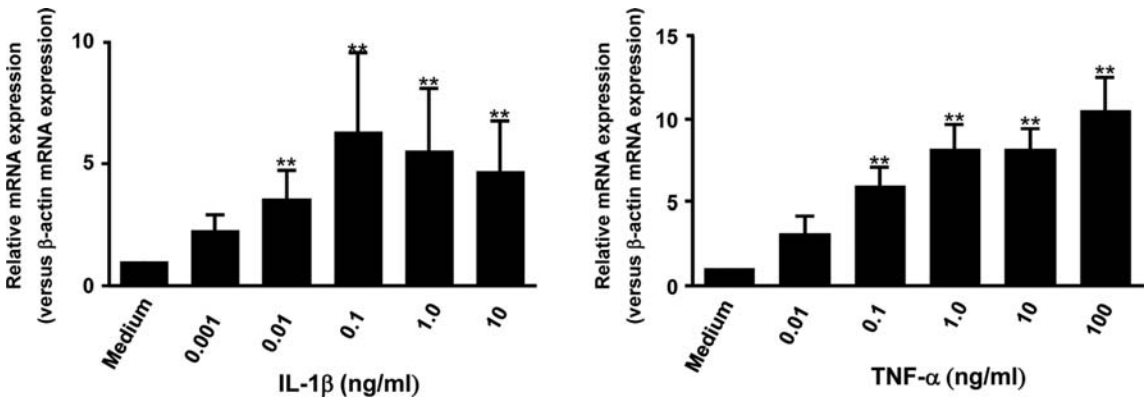

Figure 2. Dose-dependent induction of IL-32α mRNA in colonic SEMFs. Cells were incubated with different doses of each cytokine, and IL-32α mRNA expression was determined by real-time PCR analyses. Data are expressed as IL-32α mRNA expression relative to ß-actin mRNA expression (mean ± SD of 4 different experiments); **P<0.01.

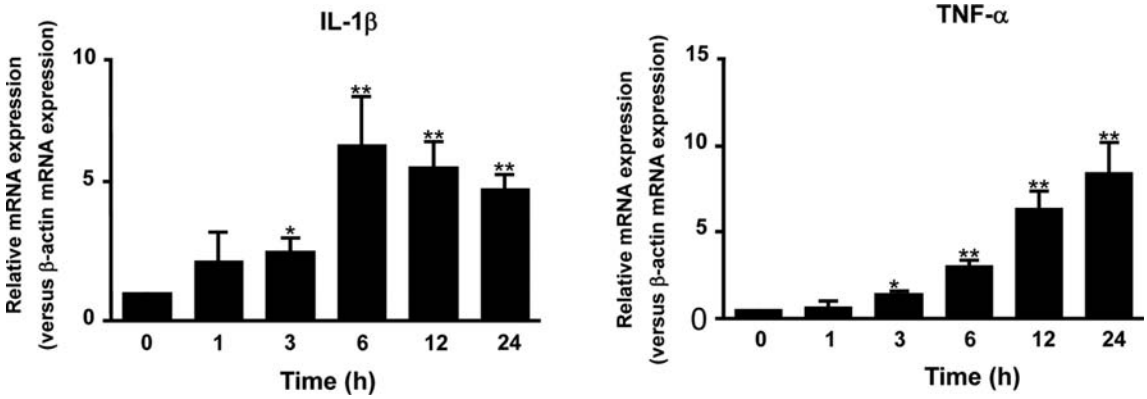

Figure 3. Kinetics of IL-32α mRNA expression in human pancreatic myofibroblasts. Cells were stimulated with IL-1ß (10 ng/ml) or TNF-α (100 ng/ml) for predetermined times, and IL-32α mRNA expression was sequentially analyzed by real-time PCR analyses. Data are expressed as IL-32α mRNA expression relative to ß-actin mRNA expression (mean ± SD of 4 different experiments;. **P<0.01.

protein expression was analyzed by Western blots. IL-32α was detected as a molecular weight protein of 25 kDa, which is comparable with a previous report (5). Stimulation with IL-1ß and TNF-α enhanced intracellular accumulation of IL-32α protein (Fig. 1B). However, we could not detect the secreted forms of IL-32α in supernatants.

The effects of IL-1ß and TNF-α on IL-32α mRNA expression were more closely examined. Colonic SEMFs were incubated for 12 h with increasing concentrations of IL-1ß and TNF-α, and the IL-32α mRNA expression was analyzed by real-time PCR. As shown in Fig. 2, these cytokines dose-dependently up-regulated IL-32α mRNA expression. The IL-1ß effect was detected at a concentration as low as 0.01 ng/ml, and reached a maximum at 1.0 ng/ml. The TNF-α effect was observed at a concentration as low as 0.1 ng/ml, and reached a maximum at 100 ng/ml.

The kinetics of IL-1ß- and TNF-α-induced IL-32α mRNA expression were evaluated (Fig. 3). Cells were stimulated with IL-1ß (10 ng/ml) or TNF-α (100 ng/ml) and sequential changes in IL-32α mRNA expression were determined. IL-1ß induced an increase in the accumulation of IL-32α mRNA, and this reached a maximum 6 h after stimulation. Thereafter, the induced-IL-32α mRNA levels decreased. TNF-α also induced a gradual but continuous increase in the accumulation of IL-32α mRNA for 24 h.

The MAP-kinase and phosphatidylinositol 3-kinase (PI3K)/Akt pathways are implicated in cytokine signaling in various cell types. To investigate the molecular mechanisms underlying IL-32α induction in colonic SEMFs, we evaluated the effects of the following inhibitors: p42/44 MAP kinase inhibitors (PD98059 and U0126) (23,24), a p38 MAPK inhibitor (SB203580) (25) and a PI3K inhibitor (LY294002) (26). Real-time PCR demonstrated that treatment with the MEK inhibitors (PD98059 and U0126) or a p38 MAPK inhibitor (SB203580) had no effect on IL-1ß- and TNF-α-induced IL-32α mRNA (Fig. 4). Contrary to these findings, a PI3K inhibitor, LY294002 (27) significantly blocked the effect of IL-1ß and TNF-α on IL-32α mRNA expression (Fig. 4). These results suggest that PI3K activation is involved in IL-1ß- and TNF-α-induced IL-32α mRNA expression in these cells.

In colonic myofibroblasts, the induction of Akt phosphorylation by IL-1ß and TNF-α was evaluated by Western blotting. As shown in Fig. 5, IL-1ß and TNF-α induced the Akt phosphorylation as early as 5 min after the stimulation. These data indicate that Akt, a protein kinase recruited by

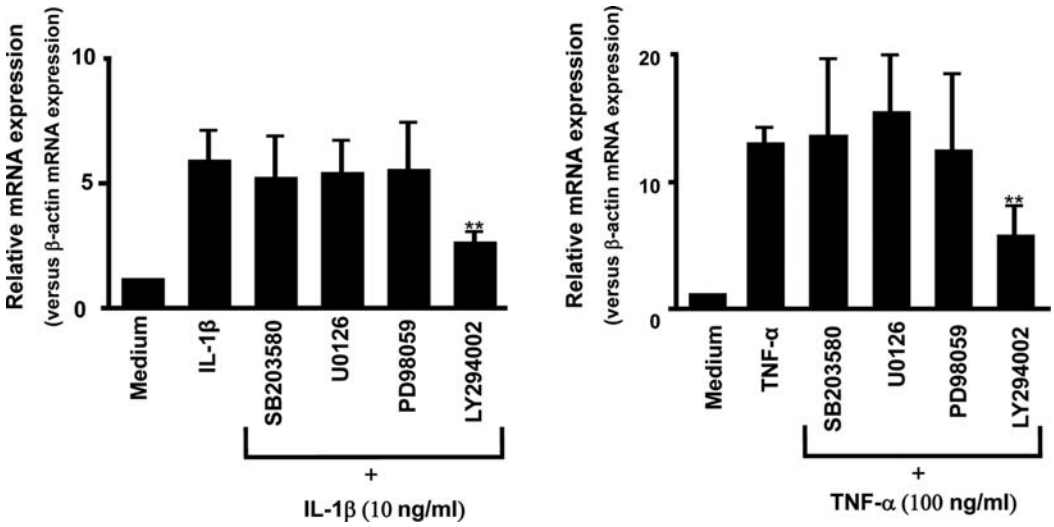

Figure 4. Effects of MAPK inhibitors and a PI3K inhibitor on IL-32α mRNA expression. Cells were stimulated with each cytokine [IL-1ß (10 ng/ml) or TNF-α (100 ng/ml)] in the presence or absence of MEK inhibitors [PD98059 (20 μM) and U0126 (12.5 μM)], a p38 inhibitor [SB203580 (25 μM)], and a PI3K inhibitor [LY294002 (25 μM)] for 12 h, and then IL-32α mRNA expression was determined by real-time PCR. Data are expressed as IL-32α mRNA expression relative to ß-actin mRNA expression (mean ± SD of 4 different experiments); **P<0.01.

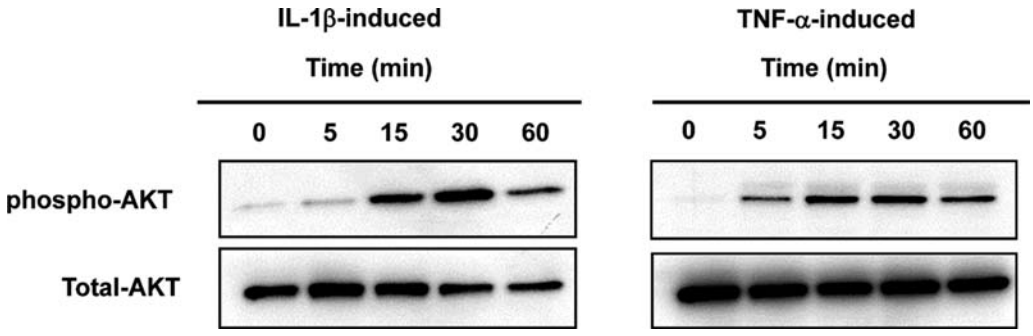

Figure 5. Kinetics of Akt activation in human colonic SEMFs. Cells were stimulated with cytokines [IL-1ß (10 ng/ml) or TNF-α (100 ng/ml)], and phosphorylated (phospho-) and total Akt were sequentially detected by Western blotting.

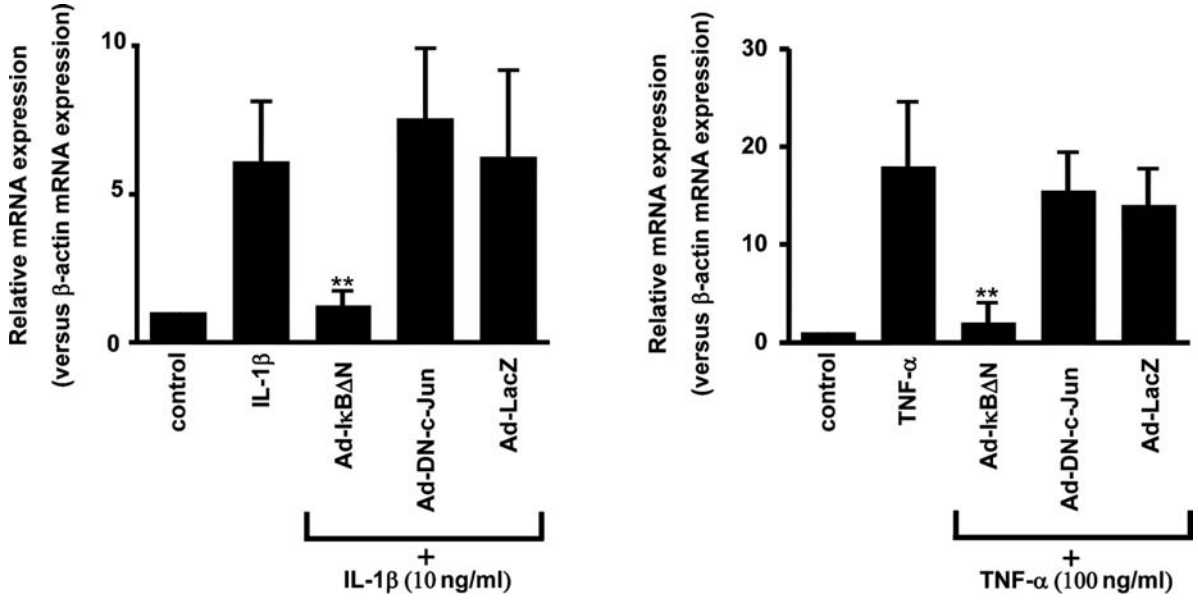

Figure 6. Effects of NF-κB and/or AP-1 inhibition on IL-32α mRNA expression. Cells were infected with an adenovirus expressing the IκBΔN or DN-c-Jun, and after 48 h of infection the cells were stimulated with IL-1ß (10 ng/ml) or TNF-α (100 ng/ml) for 12 h. IL-32α mRNA expression was determined by real-time PCR analyses. Data are expressed as IL-32α mRNA expression relative to ß-actin mRNA expression (mean ± SD of 4 different experiments); **P<0.01. Adenovirus expressing LacZ was used as a negative control.

PI3K activation, is rapidly activated by IL-1ß and TNF-α in colonic SEMFs.

To assess the role of the transcription factors NF-κB and AP-1, we evaluated the effects of a recombinant adenovirus containing a stable mutant form of IκBα (Ad-IκBΔN) and a dominant negative mutant of c-Jun (Ad-DN-c-Jun) on cytokine-induced IL-32α mRNA expression. As shown in Fig. 6, cells were infected with a recombinant adenovirus, cultured for 48 h and stimulated for 12 h with IL-1ß (10 ng/ml) or TNF-α (100 ng/ml), and the expression of IL-32α mRNAs was determined by real-time PCR. Ad-IκBΔN inhibited the effects of both IL-1ß and TNF-α on IL-32α mRNA expression, but Ad-DN-c-Jun did not suppress the effects of IL-1ß or TNF-α. Inhibitory effects were not induced by the Ad-LacZ gene, which was used as a negative control. These results suggest that NF-κB plays a role in IL-1ß- and TNF-α-induced IL-32α mRNA expression.

Discussion

A previous study showed that intestinal epithelial cells are a local site for IL-32α expression (5,28), and that IL-32α expression is enhanced in the inflamed mucosa of patients with IBD (28). In this study, we evaluated the IL-32α expression in colonic SEMFs. This suggests that colonic SEMFs also contribute to the elevation of IL-32α expression in the IBD mucosa. Since IL-32 acts as a proinflammatory cytokine, which is characterized by induction of the release of proinflammatory cytokines (TNF-α, IL-1ß, IL-6 and chemokines) through the NF-κB- and p38 MAPK-activation pathways (4,6), IL-32α derived from colonic SEMFs stimulates infiltrating immune cells to secrete proinflammatory cytokines and contributes to the deterioration of mucosal inflammation.

The molecular mechanisms underlying IL-32α induction remain unclear. Previous studies have demonstrated that proinflammatory cytokines such as IL-1ß, IL-12, IL-18 and IFN-γ are stimulators for IL-32 expression (4,28). In the present study, we demonstrated that IL-1ß and TNF-α are potent inducers of IL-32 mRNA expression in human colonic SEMFs. IL-1ß- and TNF-α-induced IL-32α mRNA expression was suppressed by a PI3K-inhibitor (LY294002), but not by p42/44 MAPK-inhibitors (PD98059 and U0126) or a p38 MAPK-inhibitor (SB203580). Furthermore, in these cells IL-1ß and TNF-α induced the phosphorylation of Akt, a protein kinase immediately recruited by PI3K activation (29). These observations indicate that the PI3K/Akt pathway contributes to IL-1ß- and TNF-α-mediated IL-32α mRNA induction in colonic SEMFs.

Many cytokine-inducible responses are mediated by DNA binding proteins, such as NF-κB and AP-1. The promoter region of the human IL-32α gene has consensus binding sites for NF-κB (at bp -638 to -649), suggesting an involvement of NF-κB activation in IL-1ß- and TNF-α-induced IL-32α mRNA expression. To confirm this possibility, we used a recombinant adenovirus expressing a stable mutant form of IκBα (Ad-IκBΔN) (21). As shown in Fig. 6, pretreatment with Ad-IκBΔN blocked IL-1ß- and TNF-α-induced IL-32α mRNA expression. These data indicate that NF-κB activation plays a role in IL-32α mRNA induction in colonic SEMFs. In addition, recent studies indicate that the PI3K/Akt pathway regulates activation of transcription factors, such as NF-κB (27,30-33), suggesting cross-talk between the PI3K/Akt pathway and NF-κB activation in cytokine-induced IL-32 mRNA expression.

Whether IL-32 exerts its biological effects as a secretory cytokine remains unclear, since the IL-32 protein does not possess a typical hydrophobic signal peptide in its N-terminus which is a typical feature of secreted cytokines (7). In Cos7 cells transfected with IL-32α cDNA, intracellular IL-32α levels were approximately 7-fold higher compared to secreted IL-32α levels (4). In contrast, in Cos7 cells transfected with IL-32α cDNA, the abundance of IL-32α was comparable in supernatants and lysates (4). In this study, IL-32α was detected in cell lysate, but was not detected in supernatants. Although it is unclear which of the IL-32 isoform is effectively secreted from particular cell types, it may be that IL-32α plays a role as a cytoplasmic protein. Recently, Goda *et al* demonstrated that overexpression of intracellular IL-32α induced apoptosis in HeLa cells (34). These data suggest a role for cytoplasmic IL-32 in cell turnover. Damaged cells are deleted and tissue architecture is restored through apoptosis, and IL-32α may induce apoptosis in damaged cells at inflammatory sites such as IBD.

In conclusion, we demonstrated that IL-32α is expressed in human colonic SEMFs. IL-32α was induced by IL-1ß and TNF-α, and was mediated by interactions between the PI3K/Akt-pathway and the NF-κB system. Interestingly, IL-32α was not secreted by human colonic SEMFs. The role of the cytoplasmic accumulation of IL-32α in colonic SEMFs should be further investigated.

References

1. Mayer L: Evolving paradigms in the pathogenesis of IBD. J Gastroenterol 45: 9-16, 2010.
2. Podolsky DK: Inflammatory bowel disease. N Engl J Med 347: 417-429, 2002.
3. Hibi T and Ogata H: Novel pathophysiological concepts of inflammatory bowel disease. J Gastroenterol 41: 10-16, 2006.
4. Kim SH, Han SY, Azam T, Yoon DY and Dinarello CA: Interleukin-32: a cytokine and inducer of TNFalpha. Immunity 22: 131-142, 2005.
5. Netea MG, Azam T, Ferwerda G, *et al*: IL-32 synergizes with nucleotide oligomerization domain (NOD) 1 and NOD2 ligands for IL-1beta and IL-6 production through a caspase 1-dependent mechanism. Proc Natl Acad Sci USA 102: 16309-16314, 2005.
6. Dinarello CA and Kim SH: IL-32, a novel cytokine with a possible role in disease. Ann Rheum Dis 65 (Suppl 3): iii61-iii64, 2006.
7. Chen Q, Carroll HP and Gadina M: The newest interleukins: recent additions to the ever-growing cytokine family. Vitam Horm 74: 207-228, 2006.
8. Peyrin-Biroulet L, Vignal C, Dessein R, Simonet M, Desreumaux P and Chamaillard M: NODs in defence: from vulnerable antimicrobial peptides to chronic inflammation. Trends Microbiol 14: 432-438, 2006.
9. Hugot JP, Chamaillard M, Zouali H, *et al*: Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 411: 599-603, 2001.
10. Hugot JP: CARD15/NOD2 mutations in Crohn's disease. Ann NY Acad Sci 1072: 9-18, 2006.
11. Netea MG, Ferwerda G, de Jong DJ, *et al*: Nucleotide-binding oligomerization domain-2 modulates specific TLR pathways for the induction of cytokine release. J Immunol 174: 6518-6523, 2005.
12. Netea MG, Kullberg BJ, de Jong DJ, *et al*: NOD2 mediates anti-inflammatory signals induced by TLR2 ligands: implications for Crohn's disease. Eur J Immunol 34: 2052-2059, 2004.

13. Maeda S, Hsu LC, Liu H, *et al*: Nod2 mutation in Crohn's disease potentiates NF-kappaB activity and IL-1beta processing. Science 307: 734-738, 2005.
14. Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI and West AB: Myofibroblasts. II. Intestinal subepithelial myofibroblasts. Am J Physiol 277: C183-C201, 1999.
15. Powell DW, Adegboyega PA, Di Mari JF and Mifflin RC: Epithelial cells and their neighbors I. Role of intestinal myofibroblasts in development, repair, and cancer. Am J Physiol Gastrointest Liver Physiol 289: G2-G7, 2005.
16. Andoh A, Bamba S, Brittan M, Fujiyama Y and Wright NA: Role of intestinal subepithelial myofibroblasts in inflammation and regenerative response in the gut. Pharmacol Ther 114: 94-106, 2007.
17. Mahida YR, Beltinger J, Makh S, *et al*: Adult human colonic subepithelial myofibroblasts express extracellular matrix proteins and cyclooxygenase-1 and -2. Am J Physiol 273: G1341-G1348, 1997.
18. Okuno T, Andoh A, Bamba S, *et al*: Interleukin-1beta and tumor necrosis factor-alpha induce chemokine and matrix metalloproteinase gene expression in human colonic subepithelial myofibroblasts. Scand J Gastroenterol 37: 317-324, 2002.
19. Strausberg RL, Feingold EA, Grouse LH, *et al*: Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. Proc Natl Acad Sci USA 99: 16899-16903, 2002.
20. Shimada M, Andoh A, Hata K, *et al*: IL-6 secretion by human pancreatic periacinar myofibroblasts in response to inflammatory mediators. J Immunol 168: 861-868, 2002.
21. Obara H, Takayanagi A, Hirahashi J, *et al*: Overexpression of truncated IkappaBalpha induces TNF-alpha-dependent apoptosis in human vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 20: 2198-2204, 2000.
22. Yasumoto H, Kim S, Zhan Y, *et al*: Dominant negative c-jun gene transfer inhibits vascular smooth muscle cell proliferation and neointimal hyperplasia in rats. Gene Ther 8: 1682-1689, 2001.
23. Favata MF, Horiuchi KY, Manos EJ, *et al*: Identification of a novel inhibitor of mitogen-activated protein kinase kinase. J Biol Chem 273: 18623-18632, 1998.
24. Alessi DR, Cuenda A, Cohen P, Dudley DT and Saltiel AR: PD 098059 is a specific inhibitor of the activation of mitogen-activated protein kinase kinase in vitro and in vivo. J Biol Chem 270: 27489-27494, 1995.
25. Cuenda A, Rouse J, Doza YN, *et al*: SB 203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1. FEBS Lett 364: 229-233, 1995.
26. Fang J, Ding M, Yang L, Liu LZ and Jiang BH: PI3K/PTEN/AKT signaling regulates prostate tumor angiogenesis. Cell Signal 19: 2487-2497, 2007.
27. Tang CH, Lu DY, Yang RS, *et al*: Leptin-induced IL-6 production is mediated by leptin receptor, insulin receptor substrate-1, phosphatidylinositol 3-kinase, Akt, NF-kappaB, and p300 pathway in microglia. J Immunol 179: 1292-1302, 2007.
28. Shioya M, Nishida A, Yagi Y, *et al*: Epithelial overexpression of interleukin-32alpha in inflammatory bowel disease. Clin Exp Immunol 149: 480-486, 2007.
29. Cantley LC: The phosphoinositide 3-kinase pathway. Science 296: 1655-1657, 2002.
30. Julien S, Puig I, Caretti E, *et al*: Activation of NF-kappaB by Akt upregulates Snail expression and induces epithelium mesenchyme transition. Oncogene 26: 7445-7456, 2007.
31. Rajaram MV, Ganesan LP, Parsa KV, Butchar JP, Gunn JS and Tridandapani S: Akt/Protein kinase B modulates macrophage inflammatory response to *Francisella* infection and confers a survival advantage in mice. J Immunol 177: 6317-6324, 2006.
32. Peloponese JM Jr and Jeang KT: Role for Akt/protein kinase B and activator protein-1 in cellular proliferation induced by the human T-cell leukemia virus type 1 tax oncoprotein. J Biol Chem 281: 8927-8938, 2006.
33. Li J, Chen H, Tang MS, *et al*: PI-3K and Akt are mediators of AP-1 induction by 5-MCDE in mouse epidermal Cl41 cells. J Cell Biol 165: 77-86, 2004.
34. Goda C, Kanaji T, Kanaji S, *et al*: Involvement of IL-32 in activation-induced cell death in T cells. Int Immunol 18: 233-240, 2006.