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Regulation of eotaxin-3/CC chemokine ligand 26 expression by T helper type 2 cytokines in human colonic myofibroblasts

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Summary

Eotaxins induce the trafficking of eosinophils to the sites of inflammation via CC chemokine receptor 3 (CCR3). In this study, we investigated eotaxin-3/CC chemokine ligand 26 (CCL26) expression in the inflamed mucosa of patients with inflammatory bowel disease (IBD), and characterized the molecular mechanisms responsible for eotaxin-3 expression in human colonic myofibroblasts. Eotaxin-3 mRNA and protein expression was evaluated by real time-polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA), respectively. Eotaxin-3 mRNA expression was elevated significantly in the active lesions of ulcerative colitis (UC) patients. Significant elevations were also observed in the active lesions of Crohn's disease (CD) patients, but this was significantly lower than that detected in the active UC lesions. There were no significant increases in the inactive lesions of UC or CD patients. Colonic myofibroblasts were identified as a major source of eotaxin-3 in the colonic mucosa, and interleukin (IL)-4 and IL-13 enhanced eotaxin-3 mRNA and protein expression significantly in these cells. There was a significant positive correlation between mucosal eotaxin-3 and IL-4 mRNA expression in the active lesions of IBD patients. The IL-4- and IL-13-induced eotaxin-3 mRNA expression was regulated by the signal transducer and activator of transcription-6 (STAT-6) and suppressor of cytokine signalling (SOCS)1-mediated pathways. Interferon (IFN)-γ acts as a negative regulator on the IL-4- and IL-13-induced eotaxin-3 expression via STAT-1 activation. Eotaxin-3 expression was elevated specifically in the active lesions of IBD, in particular UC. Eotaxin-3 derived from colonic myofibroblasts may play an important role in the pathophysiology of UC.

Keywords: eosinophil, IBD, SOCS, STAT

Introduction

Inflammatory bowel diseases (IBDs), ulcerative colitis (UC) and Crohn's disease (CD) are chronic intestinal disorders of unknown aetiology [1]. The most widely accepted hypothesis on the pathogenesis of IBD is that the mucosal immune system has an aberrant response towards luminal antigens such as dietary factors and/or commensal bacteria in genetically susceptible individuals [1–6]. This may be supported by recent findings that the genes encoding innate immune responses are also responsible for determining susceptibility to IBD [7,8]. In addition, IBD is often characterized by an imbalance between the effector and the regulatory activities of intestinal immunity, with a preponderance of proinflammatory cytokines [9].

Eosinophils participate in a number of biological processes such as wound healing, defence against parasites and allergic inflammation [10]. Eosinophils are characterized by specific cytoplasmic granules containing four major proteins: eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), eosinophil protein X (EPX) and major basic protein (MBP) [11]. Recent studies suggest that eosinophils also play a role in tissue repair through fibroblast stimulation by releasing ECP and transforming growth factor (TGF)-β [10].

A growing number of studies support a functional role for eosinophils in IBD [11–14]. Eosinophil numbers were increased in active disease compared with normal control subjects [10]. Other studies have documented the increased faecal content of ECP in patients with UC, and a

high content of ECP and EPX in the stool of patients with UC and CD [12,15,16]. Eosinophil granule proteins are increased in the whole-gut lavage fluid from patients with CD and UC [17]. Lampinen *et al.* reported that eosinophils are activated in the remission phase rather than the active phase of UC patients, suggesting a role of these cells in tissue repair. Eosinophils are increased and activated during inflammation in IBD, but a clear role for them in the pathogenesis of IBD has not yet been elucidated.

The eotaxin subfamily of CC chemokines consists of eotaxin-1/CCL11, eotaxin-2/CCL24 and eotaxin-3/CCL26 [18]. All eotaxins induce the trafficking of eosinophils to the sites of inflammation via CC chemokine receptor 3 (CCR3), which is also expressed by several different cell types, including basophils, dendritic cells, smooth muscle cells, epithelial cells and fibroblasts [19]. The sequence similarity between the three eotaxins is limited (<40%), but their functional properties are very similar [20]. Eotaxin-1 and -2 are expressed by both haematopoietic and non-haematopoietic cells, but eotaxin-3 expression has been reported to be limited to non-haematopoietic cells [18]. Interleukin (IL)-4 is the main inducer for eotaxin-3 expression, whereas eotaxin-1 is up-regulated by IL-4 and the proinflammatory cytokine tumour necrosis factor (TNF)-α [21]. Eotaxin-3 is expressed in vascular endothelial cells and human dermal fibroblasts after IL-4 and IL-13 stimulation [21], and this is dependent upon the IL-4-/IL-13-specific transcription factor, signal transducers and activator of transcription (STAT)-6 [20,21]. Eotaxin-3 is expressed on the surface of IL-4-stimulated endothelial cells and promotes eosinophil transmigration [22].

The mechanisms responsible for the accumulation of eosinophils in IBD lesions remain unclear, but it is likely that various steps involving several chemotactic factors operate in order to recruit eosinophils from the peripheral blood into the tissues. The purpose of this study was to investigate mucosal eotaxin expression in IBD patients. Moreover, we examined the molecular mechanisms underlying eotaxin induction in the inflamed mucosa of IBD.

Materials and methods

Reagents

Recombinant human cytokines were purchased from R&D Systems (Minneapolis, MN, USA). All other reagents were purchased from Sigma Chemical Co. (St Louis, MO, USA). Human eotaxin enzyme-linked immunosorbent assay (ELISA) kits were purchased from R&D Systems. STAT-1-specific, STAT-6-specific, suppressor of cytokine signalling (SOCS)-specific and control siRNAs were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Tissue samples

The diagnosis of IBD was based on conventional clinical and endoscopic criteria. Biopsied specimens from 42 patients with UC (21 active and 21 inactive patients) and 33 patients with CD (16 active and 17 inactive patients) were used, with informed consent. The disease activity of the samples was determined by endoscopic and histological findings. Normal colorectal tissues ($n = 16$) were obtained by surgical resection of colon cancer at distal tumour sites. The ethics committee of the Shiga University of Medical Science approved this project.

Culture of human colonic myofibroblasts

Primary colonic myofibroblast cultures were prepared according to the method reported by Mahida *et al.* [23]. The cellular characteristics and culture conditions have also been described in our previous report [24]. Samples of the human adult colonic mucosa were obtained from surgical specimens (>5 cm from the tumour margin) from patients undergoing a partial colectomy for carcinoma, with their informed consent. All studies were performed on passages 3–6 of myofibroblasts isolated from six resection specimens. The human colon cancer cell lines HT-29 and Caco-2 were obtained from the American Type Culture Collection (Manassas, VA, USA) [25].

ELISA for the quantification of antigenic eotaxin-3

Eotaxin-3 protein in the samples was quantified by an ELISA kit, purchased from R&D Systems.

Immunohistochemical staining for eotaxin-3 protein

The cells were grown on a culture slide system (BD, Franklin Lakes, NJ, USA). The cells were fixed and reacted with anti-eotaxin-3 antibodies (R&D Systems) and cyanin 2 (Cy2)-labelled secondary antibody (Rockland Immunochemicals, Gilbertsville, PA, USA). Nuclei were visualized using mounting medium with 4-6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Burlingame, CA, USA). A digital confocal laser scanning microscope (Nikon, Tokyo, Japan) was used for analysis.

Real-time polymerase chain reaction

The expression of mRNA in the samples was assessed by real-time PCR analyses. The oligonucleotide primers used in this study were human eotaxin-3 (sense: GACCTGGGT GCGAAGCTATG, anti-sense: TGGGAGGAAACACCCTCT CC), human IL-4 (sense: TGCCTCCAAGAACACAACTG, anti-sense: GTTTCAGGAATCGGATCAGC), human IL-13 (sense: GTGGCCCAGTTTGTAAAGGA, anti-sense: CAG

CACAGGCTGAGGTCTAA) and β-actin (sense: TGACC CAGATCATGTTTGAGACCT, anti-sense: CCACGTCACAC TTCATGATGATGGAG). Real-time PCR was performed using a LightCycler 480 system (Roche Applied Science, Tokyo, Japan). The PCR was performed using a SYBR Premix Ex Taq (Takara, Shiga, Japan). The data were normalized *versus* β-actin for human eotaxin-3.

Western blot analyses

The stimulated cells were lysed in a sodium dodecyl sulphide (SDS) sample buffer containing orthovanadate. Western blots were then performed according to a method described previously [26]. Detection was performed using the enhanced chemiluminescence Western blotting system (GE Healthcare, Little Chalfont, UK).

STATs and SOCSs mRNA interference (RNAi) experiments

The siRNA for STAT-1, STAT-6, SOCS1, SOCS3, SOCS5 and a control siRNA were used. Human colonic subepithelial myofibroblasts (SEMFs) were cultured for 4 days in complete medium that did not contain antibiotics. The cells were then seeded onto a six-well plate 1 day prior to the transfection, and cultured to 60–70% confluence on the following day. For the RNAi experiments, Lipofectamine™ RNAi MAX Reagent (Invitrogen, Carlsbad, CA, USA) was used.

Statistical analysis

The statistical significance of the differences was determined by Student's *t*-test. Differences resulting in *P*-values less than 0·05 were considered to be statistically significant.

Results

Eotaxin-3 expression in IBD mucosa

Eotaxin-3 mRNA expression was analysed by real-time PCR in biopsied samples from IBD patients. As shown in Fig. 1, eotaxin-3 mRNA was detected very weakly in the samples from the normal mucosa. A remarkable and significant increase of eotaxin-3 mRNA expression was observed in the samples from the active lesions of UC patients. Significant elevation was also observed in the samples from the active lesions of CD patients, but this was significantly lower than that detected in the active lesions of UC patients. There was no significant increase in the inactive lesions of UC and CD patients.

Regulation of eotaxin-3 expression in colonic myofibroblasts and IBD mucosa

Based on the *in-vivo* expression of eotaxin-3 in the inflamed IBD mucosa, we examined eotaxin-3 expression in isolated

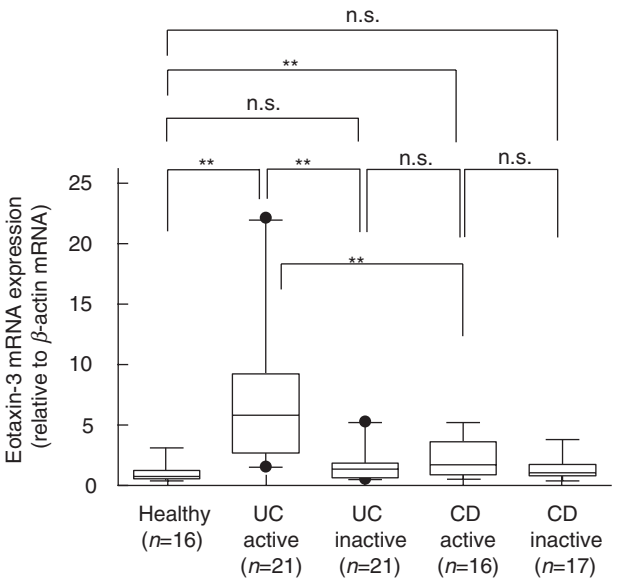

Fig. 1. Eotaxin-3 mRNA expression in the inflammatory bowel disease (IBD) mucosa. Total RNA was extracted from biopsied samples, and eotaxin-3 mRNA expression was evaluated by real-time polymerase chain reaction (PCR) analyses. The eotaxin-3 mRNA expression was expressed relative to the β-actin mRNA expression. $^{**}P < 0{\cdot}01$; n.s.: not significant.

human colonic myofibroblasts. Colonic myofibroblasts were stimulated with various cytokines and lipopolysaccharide (LPS) for 24 h, and eotaxin-3 mRNA expression was then determined by real-time PCR (Fig. 2a). Very weak eotaxin-3 mRNA expression was detected in the unstimulated colonic myofibroblasts, and IL-4 and IL-13 stimulation enhanced eotaxin-3 mRNA expression significantly. Immunohistochemical analyses showed cytoplasmic expression of eotaxin-3 protein in the IL-4- and IL-13-stimulated colonic myofibroblasts (Fig. 2b).

In contrast, in the colonic epithelial cell lines (HT-29 and Caco-2), eotaxin-3 mRNA was not detected in the unstimulated cells. Both IL-4 and IL-13 stimulated eotaxin-3 mRNA expression weakly, but these were somewhat modest compared to the responses of colonic myofibroblasts (Fig. 2c).

These responses were investigated in normal and inflamed mucosa of IBD patients [8 normal mucosa, UC (11 active and 8 inactive lesions) and CD (10 active and eight inactive lesions), total $n = 45$]. A significant positive correlation was found between eotaxin-3 and IL-4 mRNA expression (Pearson's correlation $R = 0.69$, $P < 0{\cdot}0001$, $n = 45$) (Fig. 3a). However, there was no correlation between eotaxin-3 and IL-13 mRNA expression (Fig. 3b).

Eotaxin-3 induction by IL-4 and IL-13

The effects of IL-4 and IL-13 were analysed precisely. As shown in Fig. 4a,b, IL-4 and IL-13 induced eotaxin-3

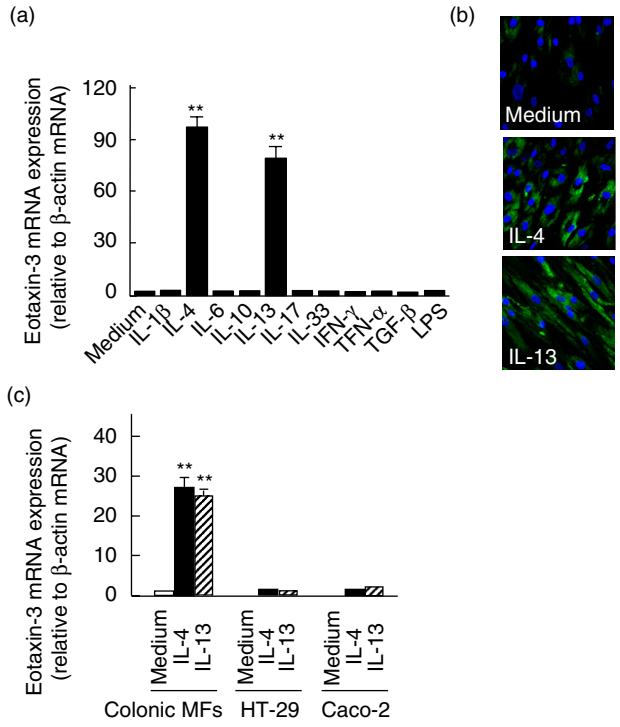

Fig. 2. Eotaxin-3 mRNA expression in human colonic myofibroblasts. (a) Colonic myofibroblasts, derived from normal mucosa, were stimulated with cytokines (100 ng/ml) for 24 h. Eotaxin-3 mRNA expression was then analysed by real-time polymerase chain reaction (PCR). (b) Eotaxin-3 protein expression in interleukin (IL)-4 or IL-13 stimulated cells. The cells were grown on culture slides and stimulated with cytokines (100 ng/ml) for 24 h. Eotaxin-3 was stained with anti-eotaxin-3 antibodies. (c) Comparison of eotaxin-3 mRNA expression in intestinal epithelial cell lines (HT-29 and Caco-2 cells) and colonic myofibroblasts. The cells were stimulated with cytokines (100 ng/ml) for 24 h. Eotaxin-3 mRNA expression was expressed relative to the β-actin mRNA expression (mean ± standard deviation from three different experiments). MFs: myofibroblasts. $^{**}P < 0{\cdot}01$ *versus* medium only.

mRNA expression dose- and time-dependently. Similarly, IL-4 and IL-13 induced eotaxin-3 protein secretion in a dose- and time-dependent manner (Fig. 4c,d).

Role of STAT-6 activation in IL-4-/IL-13-induced eotaxin-3 expression

It has been reported that IL-4 and IL-13 activate STAT-6, which is a transcription factor required for many biological functions [27]. To confirm this response in our system, we evaluated the effects of IL-4 and IL-13 on the activation of STAT-6 in colonic myofibroblasts. As shown in Fig. 5a, IL-4 and IL-13 induced phosphorylation of STAT-6 as early as 15 min after stimulation. In the STAT-6 siRNA-transfected cells, this IL-4- and IL-13-induced eotaxin-3 expression was reduced significantly (Fig. 5b). These findings indicate that IL-4- and IL-13-induced eotaxin-3 expression is dependent upon STAT-6 activation in human colonic myofibroblasts.

SOCS1 plays a central negative role in IL-4-/IL-13-induced eotaxin-3 expression

SOCS1–3 act as negative regulators of cytokine signalling [20]. A previous study reported negative regulatory actions of SOCS1 and SOCS3 for the IL-4- and IL-13-induced eotaxin-3 gene expression [20]. In addition, SOCS5 has been reported to play a role in eosinophilic inflammation in mice [28]. Based on these notions, we evaluated the effects of siRNAs specific for SOCS1, SOCS3 and SOCS5 on IL-4- and IL-13-induced eotaxin-3 mRNA expression in colonic myofibroblasts. As shown in Fig. 5c, silencing SOCS1 induced a significant and marked enhancement of IL-4-induced eotaxin-3 mRNA expression. Silencing SOCS3 and SOCS5 also induced an increase in eotaxin-3 mRNA

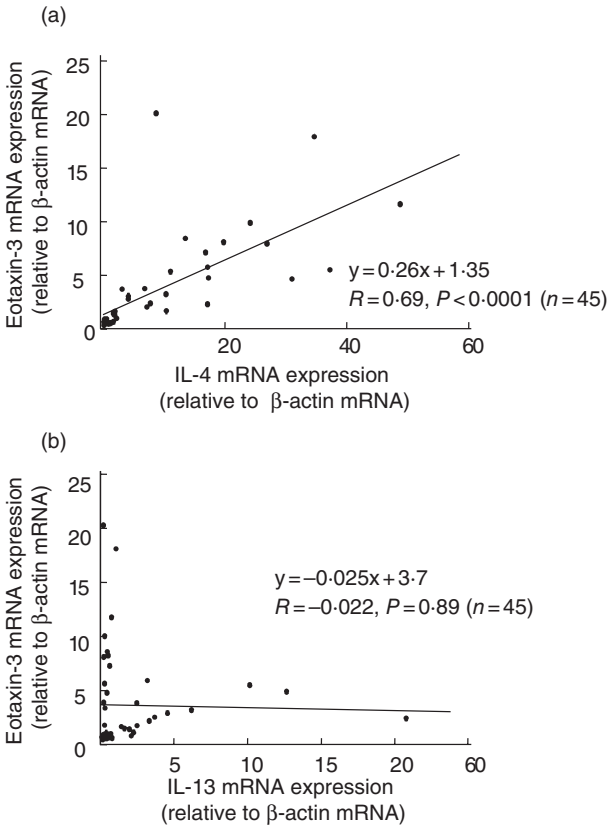

Fig. 3. Correlation between mucosal eotaxin3 and interleukin (IL)-4/IL-13 mRNA expression in the mucosa. Eotaxin-3 mRNA expression was expressed relative to the β-actin mRNA expression. (a) A significant positive correlation was observed between eotaxin-3 and IL-4 mRNA levels (Pearson's correlation $R = 0{\cdot}69$, $P < 0{\cdot}0001$, $n = 45$). (b) There was no correlation between eotaxin-3 and IL-13 mRNA expression ($n = 45$).

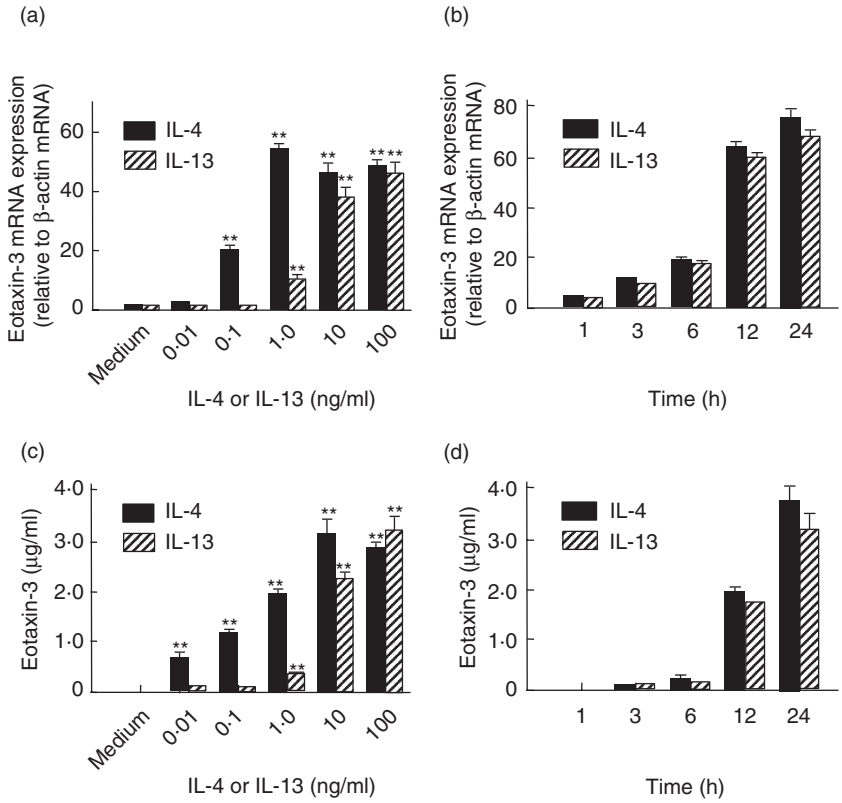

Fig. 4. Effects of interleukin (IL)-4 and IL-13 on eotaxin-3 mRNA expression in myofibroblasts derived from normal colonic mucosa. (a) Dose-dependent effects of IL-4 and IL-13 on eotaxin-3 mRNA expression. The cells were incubated for 24 h with increasing concentrations of IL-4 or IL-13. Eotaxin-3 mRNA expression was expressed relative to the β-actin mRNA expression [mean ± standard deviation (s.d.) from three different experiments]. $^{**}P < 0{\cdot}01$ *versus* medium only. (b) Time-dependent effects of IL-4 and IL-13 on eotaxin-3 mRNA expression. The cells were stimulated with IL-4 (100 ng/ml) or IL-13 (100 ng/ml) for the predetermined times. Eotaxin-3 mRNA expression was expressed relative to β-actin mRNA expression (mean ± s.d. from three different experiments). (c) Dose-dependent effects of IL-4 and IL-13 on eotaxin-3 protein secretion. The cells were incubated for 24 h with increasing concentrations of IL-4 or IL-13, and eotaxin-3 levels were determined by enzyme-linked immunosorbent assay (ELISA). All values are expressed as means ± s.d. ($n = 3$). $^{**}P < 0{\cdot}01$ *versus* medium only. (d) Time-dependent effects of IL-4 and IL-13 on eotaxin-3 protein secretion. The cells were stimulated with IL-4 (100 ng/ml) or IL-13 (100 ng/ml) for predetermined times, and eotaxin-3 levels were determined by ELISA. All values are expressed as mean ± s.d. ($n = 3$).

expression, but these effects were much weaker than the effects of the SOCS1 siRNA. Similar effects of siRNAs specific for SOCS1, SOCS3 and SOCS5 were also observed for IL-13-induced eotaxin-3 mRNA expression (Fig. 5d). Thus, it became clear that SOCS1 is a major negative regulator for the IL-4- and IL-13-induced activation of eotaxin-3 gene expression in human colonic myofibroblasts.

Effects of interferon (IFN)-γ on IL-4-/IL-13-induced eotaxin-3 expression

A T helper type 1 (Th1)/Th2 cytokine imbalance with a predominance of Th1 cytokines has been suggested to be of pathogenic importance in IBD [29]. This led us to investigate the effects of IFN-γ, a major Th1 cytokine, on IL-4-/IL-13-induced eotaxin-3 expression in human colonic myofibroblasts. As shown in Fig 6a, IFN-γ suppressed IL-4-induced eotaxin-3 mRNA expression dose-dependently. Similarly, IFN-γ suppressed IL-13-induced eotaxin-3 mRNA expression dose-dependently (Fig. 6b). Thus, the Th1 cytokine IFN-γ had a significant inhibitory effect on Th2 cytokine (IL-4- and IL-13)-induced eotaxin-3 mRNA expression in human colonic myofibroblasts.

In order to define the molecular mechanisms underlying the inhibitory effects of IFN-γ, we evaluated the effects of IFN-γ on STAT-6 activation. As shown in Fig. 6c, IFN-γ induced inhibition of STAT-6 activation in colonic myofibroblasts.

Furthermore, we evaluated the effects of a STAT-1-specific siRNA on IFN-γ-induced inhibitory effects on eotaxin-3 mRNA expression, as STAT-1 is a master transcription factor of IFN-γ signalling [30]. As shown in Fig. 6d, the STAT-1 siRNA attenuated completely the IFN-γ-induced inhibitory effects on eotaxin-3 mRNA expression.

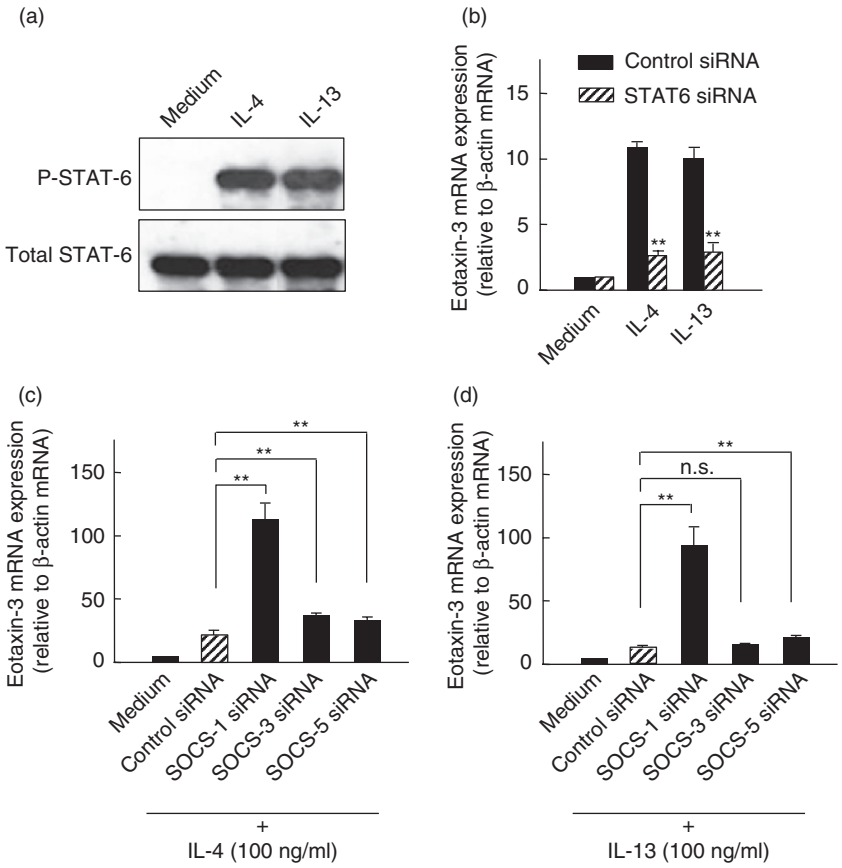

Fig. 5. Cellular signalling for interleukin (IL)-4- and IL-13-mediated eotaxin-3 induction. (a) IL-4- and IL-13-induced signal transducer and activator of transcription-6 (STAT6) activation. Colonic myofibroblasts, derived from normal mucosa, were stimulated with cytokines [IL-4 (100 ng/ml) and IL-13 (100 ng/ml)] for 15 min, and the phosphorylated (p-) and total STAT-6 were detected by Western blotting. (b) Effects of STAT-6 silencing on eotaxin-3 expression. The cells were transfected with the siRNA specific for STAT-6 and incubated for 24 h. Eotaxin-3 mRNA expression was expressed relative to β-actin mRNA expression [mean ± standard deviation (s.d.) from three different experiments]. $^{**}P < 0{\cdot}01$ *versus* control siRNA. (c) Effects of suppressor of cytokine signalling (SOCS) silencing on IL-4-induced eotaxin-3 expression. The cells were transfected with siRNAs specific for SOCS1, SOCS3 and SOCS5, and incubated for 24 h with IL-4 (100 ng/ml). Eotaxin-3 mRNA expression was expressed relative to β-actin mRNA expression (mean ± s.d. from three different experiments). (d) Effects of SOCS silencing on IL-13-induced eotaxin-3 expression. The cells were transfected with siRNAs specific for SOCS1, SOCS3 and SOCS5 and incubated for 24 h with IL-13 (100 ng/ml). Eotaxin-3 mRNA expression was expressed relative to β-actin mRNA expression (mean ± s.d. from three different experiments).

Discussion

In this study, we have demonstrated that mucosal eotaxin-3 mRNA was elevated significantly in the active lesions of UC patients. This elevation was specific to the active UC patients, as the increase in the active lesions of CD patients was modest and significantly lower than that detected in the active lesions of UC patients. A recent study by Manousou *et al.* revealed a similar observation of significant elevation in mucosal eotaxin-3 mRNA expression in IBD patients, especially in UC patients [31], but they did not find any differences between active and inactive disease. The reason for this discrepancy concerning disease activity might be associated with the methods used. They performed eotaxin-3 analysis using a reverse transcription (RT)–PCR-based method, but we used a semi-quantitative real-time PCR method, which serves as a much more sensitive and quantitative analysis. In addition, they defined disease activity by the clinical activity index based on clinical symptoms, whereas we determined disease activity according to endoscopic and histological findings. Finally, the majority of patients enrolled in Manousou's study (22 of 32 IBD patients) were clinically active. Thus, to our knowledge, the current study is the first report showing an elevation of mucosal eotaxin-3 expression in the active lesions of UC patients, and to a lesser degree in the active lesions of CD patients, suggesting a role for eotaxin-3 in the pathophysiology of active IBD patients.

The molecular mechanisms responsible for eotaxin-3 expression in the colonic mucosa have not been identified fully. To investigate the regulatory mechanisms involved in eotaxin-3 expression in the UC mucosa, we used colonic myofibroblasts isolated from the normal human colonic mucosa [23]. Among the various cytokines, Th2 cytokines IL-4 and IL-13 exerted remarkable effects on eotaxin-3 induction. Manousou *et al.* evaluated eotaxin-3 mRNA expression recently in the colon cancer cell lines HT-29 and Caco-2 cells, and suggested that colonic epithelial cells might be a source of eotaxin-3 in the inflamed mucosa of IBD patients. However, as shown in Fig. 2b, eotaxin-3 mRNA expression in both HT-29 and Caco-2 cells was very weak, even under IL-4 and IL-13 stimulation, compared with the responses of human colonic myofibroblasts. This finding negatively supports the hypothesis that colonic epithelial cells are the source of eotaxin-3 in the mucosa. In contrast, the current observations suggest strongly that colonic myofibroblasts are one of the major sources of eotaxin-3 in the inflamed mucosa of IBD patients. A limitation of the present study is the use of colonic myofibroblasts derived from normal mucosa. To clarify the role of colonic

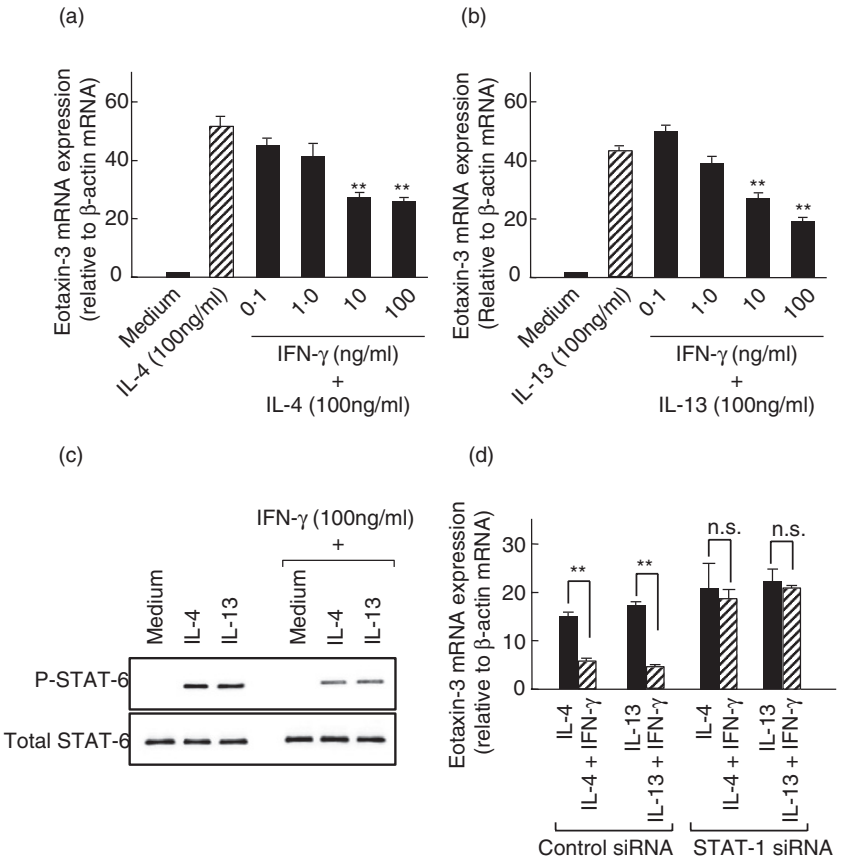

Fig. 6. Effects of interferon (IFN)-γ on interleukin (IL)-4 and IL-13-induced eotaxin-3 mRNA expression. (a,b) Colonic myofibroblasts, derived from normal mucosa, were stimulated for 24 h with IL-4 (100 ng/ml) or IL-13 (100 ng/ml) in the presence or absence of increasing concentrations of IFN-γ. Eotaxin-3 mRNA expression was expressed relative to β-actin mRNA expression [mean ± standard deviation (s.d.) from three different experiments]. $^{**}P < 0{\cdot}01$ *versus* IL-4 or IL-13 alone. (c) Effects of IFN-γ on IL-4- and IL13-induced signal transducer and activator of transcription-6 (STAT6) activation. The cells were preincubated with IFN-γ (100 ng/ml) for 3 h, and then stimulated with IL-4 (100 ng/ml) and IL-13 (100 ng/ml) for 15 min. The phosphorylated (p-) and total STAT-6 were detected by Western blotting. (d) Effects of STAT-1 silencing on eotaxin-3 expression. The cells were transfected with the siRNA specific for STAT-1 and incubated for 24 h with IL-4 (100 ng/ml) or IL-13 (100 ng/ml). Eotaxin-3 mRNA expression was expressed relative to β-actin mRNA expression (mean ± s.d. from three different experiments). $^{**}P < 0{\cdot}01$.

myofibroblasts in the pathophysiology of IBD, in future further studies should be performed using colonic myofibroblasts derived from IBD patients. In addition, eotaxin-3 expression in colonic epithelial cells should be confirmed using non-transformed cells.

Inflammation in UC has been difficult to classify using the Th1/Th2 paradigm [32,33]. In patients with UC, the secretion of IL-4 as well as IFN-γ or IL-12 was not increased, and thus the inflammation is clearly not a Th1 or Th2 response [33]. The Th2 characteristics of UC inflammation have been explained recently by the increased IL-13 production by natural killer (NK) T cells, which manifest reactivity to antigens presented by epithelial cells [1,34]. Heller *et al*. demonstrated that lamina propria mononuclear cells (LPMCs) isolated from UC patients exhibited a strong capacity to secrete IL-13, and this was much more prominent compared to LPMCs isolated from CD patients [34]. However, we could not detect a significant IL-13 mRNA expression in IBD mucosa (data not shown), and there was no correlation between eotaxin-3 mRNA expression and IL-13 mRNA expression. The reason for this discrepancy is unclear, but these findings might be associated with different distributions of IL-4-secreting Th2 cells and IL-13-secreting NK T cells in the inflamed mucosa. In contrast, we found a significant positive correlation between mucosal eotaxin-3 mRNA expression and IL-4 mRNA expression. This was supported by the recent study that circulating Th2 phenotype lymphocytes were increased in UC patients [31]. Thus, these findings suggest that, in the inflamed mucosa of UC patients, Th2 cells may drive mucosal eotaxin-3 expression in colonic myofibroblasts via IL-4 secretion. Conversely, in future the role of IL-13 should be investigated further.

The IL-4-/IL-13-induced activation of the eotaxin-3 gene has been reported to be a STAT-6-dependent process mediated by a single STAT-6 binding motif located upstream of the transcription initiation site [20]. In human colonic myofibroblasts, IL-4 and IL-13 actually induced rapid phosphorylation of STAT-6, and the eotaxin-3 mRNA expression induced by IL-4 and IL-13 was blocked significantly and almost completely in the STAT-6 siRNA-transfected cells. These findings indicate that the IL-4- and IL-13-induced eotaxin-3 mRNA expression was actually mediated by STAT-6 activation.

Cytokine signalling is regulated strictly by the SOCS family of proteins [33,35]. Among them, SOCS1 is relatively specific to IFN-γ/STAT-1 and IL-4/STAT-6, whereas SOCS3 is specific to STAT-3. SOCS1 inhibits cytokine signalling by suppressing Janus kinase (JAK) activity and promoting the degradation of the activated cytokine–receptor complex. SOCS3 has been shown to have strong expression in the colon of CD and UC patients and in most mouse colitis

models [33]. These facts led us to explore the role of STAT proteins in IL-4- and IL-13-induced eotaxin-3 expression in colonic myofibroblasts. As shown in Fig. 4c, IL-4-induced eotaxin-3 mRNA expression was enhanced by silencing SOCS1, SOCS3 and SOCS5 expression. In particular, a marked enhancement was observed in the SOCS1-specific siRNA transfected cells. Similar responses were also observed for IL-13-induced eotaxin-3 expression. These findings suggest that SOCS1 plays a central role in suppressing IL-4 and IL-13 signalling to induce eotaxin-3 expression in colonic myofibroblasts. In contrast, an important role for SOCS3 in IL-4- and IL-13-induced eotaxin-3 expression has been reported in other cell types [20], but its role in colonic myofibroblasts was considered to be minimal.

IFN-γ is known to antagonize the actions of IL-4 by repressing the expression of a number of IL-4-induced genes, whereas IL-4 has been shown to antagonize IFN-γ [33]. In colonic myofibroblasts, IFN-γ suppressed IL-4- and IL-13-induced eotaxin-3 expression dose-dependently, and this was accompanied by the suppression of STAT-6 activation. Moreover, this inhibitory effect on eotaxin-3 induction was blocked completely by silencing STAT-1 expression. Thus, eotaxin-3 expression in colonic myofibroblasts is controlled positively by Th2 cytokines (IL-4 and IL-13), and regulated negatively by Th1 cytokine (IFN-γ). It can be speculated that eosinophil recruitment via eotaxin-3 expression might be determined by a balance between Th1 and Th2 cytokine expression in the mucosa.

In conclusion, eotaxin-3 was up-regulated in active UC patients. In addition, colonic myofibroblasts have been identified as a local source for eotaxin-3 in the colonic mucosa. IL-4- and IL-13 are potent inducers of eotaxin-3, whereas IFN-γ acts as a negative regulator of eotaxin-3 expression. These findings may provide important insights into the molecular mechanisms responsible for eosinophil accumulation and activation in the inflamed mucosa of UC patients. However, a clear role for eosinophils in the pathogenesis of IBD and the precise regulatory mechanism of eotaxin-3 expression remain unclear. In future, these questions could be explored further at the experimental level using cytokine- or eosinophil-deficient mice.

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Disclosures

The authors disclose no conflicts of interest.

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