Transcription Elongation Factor P-TEFb Is Involved in IL-17F Signaling in Airway Smooth Muscle Cells

Masayuki Nakajima^a Mio Kawaguchi^a Masashi Matsuyama^a Kyoko Ota^a
Junichi Fujita^a Satoshi Matsukura^b Shau-Ku Huang^{c, d} Yuko Morishima^a
Yukio Ishii^a Hiroaki Satoh^a Tohru Sakamoto^a Nobuyuki Hizawa^a

^aDepartment of Pulmonary Medicine, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan; ^bRespiratory Disease Center, Showa University Northern Yokohama Hospital, Kanagawa, Japan; ^cJohns Hopkins University, Asthma and Allergy Center, Baltimore, MD, USA; ^dNational Health Research Institutes, Taiwan, Taiwan

Keywords

Airway smooth muscle cells · Interleukin-8 · Interleukin-17F · Bromodomain-containing protein 4 · Positive transcription elongation factor b

Abstract

Background: IL-17F is involved in the pathogenesis of several inflammatory diseases, including asthma and COPD. However, the effects of steroids on the function of IL-17F signaling mechanisms are largely unknown. One of the transcription elongation factors, positive transcription elongation factor b (P-TEFb) composed of cyclin T1 and cyclin-dependent kinase 9 (CDK9), is known as a novel checkpoint regulator of gene expression via bromodomain-containing protein 4 (Brd4). **Methods:** Human airway smooth muscle cells were stimulated with IL-17F and the expression of IL-8 was evaluated by real-time PCR and ELISA. Next, the phosphorylation of CDK9 was determined by Western blotting. The CDK9 inhibitor and short interfering RNAs (siRNAs) targeting Brd4, cyclin T1, and CDK9 were used to identify the effect on IL-17F-induced IL-8 expression. Finally, the effect of steroids and its signaling were evaluated. **Results:** IL-17F markedly induced the transcription of the IL-8 gene and the

expression of the protein. Pretreatment of CDK9 inhibitor and transfection of siRNAs targeting CDK9 markedly abrogated IL-17F-induced IL-8 production. Transfection of siRNAs targeting Brd4 and cyclin T1 diminished IL-17F-induced phosphorylation of CDK9 and IL-8 production. Moreover, budesonide decreased CDK9 phosphorylation and markedly inhibited IL-17F-induced IL-8 production. **Conclusions:** This is the first report that P-TEFb is involved in IL-17F-induced IL-8 expression and that steroids diminish it via the inhibition of CDK9 phosphorylation. IL-17F and P-TEFb might be novel therapeutic targets for airway inflammatory diseases.

© 2018 S. Karger AG, Basel

Introduction

We and other groups have independently cloned the human IL-17F gene [1–3]. The expression of this gene is increased in the airways of patients with asthma and COPD, and its levels are correlated with disease severity [1, 4, 5]. In addition, we have demonstrated that a loss of single nucleotide polymorphism function in the IL-17F

Edited by: H.-U. Simon, Bern.

gene is inversely correlated with asthma risk and encodes an antagonist for the wild-type IL-17F [6, 7]. IL-17F is potentially involved in neutrophilic airway inflammation [8]. Overexpression of IL-17F in the mouse reveals an increased number of neutrophils in the airways [9, 10]. Neutrophilic airway inflammation is one of the key features of severe asthma and COPD. Levels of IL-8 are markedly higher in sputum, tracheal aspirates, and plasma from asthmatic patients compared with healthy subjects, and its levels are associated with an increased neutrophil number [11]. Similarly, its levels are clearly elevated in sputum from patients with stable COPD and are correlated with disease severity [12, 13]. Although the mechanisms of neutrophil infiltration into the airway have not been fully identified, emerging evidence has suggested a major involvement of airway smooth muscle cells (ASMCs) in the regulation of airway inflammation through the induction of IL-8 [14–16]. However, the role of IL-17F in ASMCs remains to be elucidated.

The intercellular signaling mechanism of IL-17F-induced gene expression has become clear [17, 18]. The extracellular signal-regulated kinase 1/2 (ERK1/2) signaling pathway is a major upstream signal transduction pathway for IL-17F [19]. In contrast, its downstream signaling mechanisms remain to be elucidated. Recently, RNA polymerase II (Pol II) pausing has been recognized as a novel and important checkpoint during the process of transcriptional initiation for gene expression [20]. Pol II initiates transcription and transitions to early elongation, but it pauses approximately 20–60 bp downstream of the transcription start site. This is called promoter-proximal pausing and is mediated by pausing factors, such as negative elongation factor (NELF) and 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole sensitivity-inducing factor (DSIF) [21]. In contrast, positive transcription elongation factors act to restart transcription from the promoter-proximal pausing. Among them, positive transcription elongation factor b (P-TEFb) has a pivotal role in pause release by phosphorylating the Pol II carboxy-terminal domain, NELF and DSIF. P-TEFb is composed of cyclin-dependent kinase 9 (CDK9) and cyclin T1, and is recruited via the coactivator bromodomain-containing protein 4 (Brd4) [22]. The activity of P-TEFb depends on CDK9 kinase activity [23]. However, it is currently unknown whether P-TEFb is involved in IL-17F-induced gene expression. Moreover, little is known about the effect of steroids on the function of IL-17F and P-TEFb activation. Corticosteroids are widely used in long-term management and acute exacerbations for asthma and COPD. Therefore, investigation of the regulatory mechanisms of

IL-17F-induced IL-8 expression in ASMCs may contribute to the pathogenesis of airway inflammatory diseases. In this study, we demonstrated, for the first time, activation of P-TEFb by IL-17F and the effect of steroids.

Methods

Cell Culture

ASMCs were purchased from Lonza (Walkersville, MD, USA) and cultured in SmBM medium with SmGM-2 SingleQuots (Lonza) containing insulin, fibroblast growth factor, gentamicin, 5% fetal bovine serum, and epidermal growth factor at 37°C with 5% CO₂ in humidified air. Confluent cells at passages 2–4 were used as described below.

Detection of IL-8 Gene Expression

Total RNA was extracted using an RNeasy Mini Kit (Qiagen, Chatsworth, CA, USA) from 1×10^6 ASMCs at 4, 12, and 24 h after stimulation with 10 and 100 ng/mL of IL-17F (R&D Systems, Tokyo, Japan). cDNAs were synthesized from 1 μ g of total RNA using the ReverTra Ace qPCR RT Kit (TOYOBO, Tokyo, Japan), followed by real-time PCR. The sequences of primers were as follows: IL-8, forward, 5'-TCTGCAGCTCTGTGTGAAGG-3', reverse, 5'-AAATTTGGGGTGGAAAGGTT-3'; G3PDH, forward, 5'-ACCACAGTCCATGCCATCAC-3', reverse, 5'-TCCACCACCCTGTTGCTGTA-3'. Real-time PCR was performed using a THUNDERBIRD SYBR qPCR Mix (TOYOBO), gene-specific primers, and an ABI 7500 real-time PCR system. The data are presented as the fold induction relative to the control group. Data are expressed as the mean $\pm$ SEM ($n = 6$ experiments).

Detection of IL-8 Protein Production

Culture supernatants were collected at 4, 12, 24, and 48 h after stimulation with IL-17F (10, 100 ng/mL). IL-8 protein levels in the supernatants and cell lysates of IL-17F-stimulated cells were determined with a commercially available ELISA kit (R&D Systems) according to the manufacturer's instruction, and expressed per 10^6 cells. Data are expressed as the mean $\pm$ SEM ($n = 6$ experiments).

Effect of Budesonide on IL-17F Induced IL-8 Expression

For analysis of the effect of steroids on IL-8 expression, ASMCs were treated in the presence or absence of budesonide (Calbiochem, Tokyo, Japan) at varying doses, and a vehicle control, 0.1% DMSO, for 1 h before treatment with IL-17F (100 ng/mL). The supernatants were collected at 24 h after stimulation. IL-8 protein levels in the supernatants were determined as described above and expressed as the mean $\pm$ SEM ($n = 6$ experiments).

Detection of CDK9 and p65 by Western Blotting

For analysis of activation of CDK9 and NF- κ B, ASMCs were treated with IL-17F (100 ng/mL) and in some cases with or without transfection with the short interfering RNAs (siRNAs; 10 nM) targeting Brd4 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and control (Ambion, Tokyo, Japan) using HiPerFect transfection reagent (Qiagen). The total cellular extracts (1×10^6 cell equivalents/lane) were subjected to 7.5–15% Tris-glycine gel electrophoresis

Fig. 1. IL-17F induced IL-8 gene expression and protein production in ASMCs. **a** IL-8 gene expression by IL-17F. Real-time PCR was performed as described in Materials and Methods. ASMCs were stimulated with IL-17F (10, 100 ng/mL) for 4, 12, and 24 h ($n = 6$). * $p < 0.05$ versus medium control; # $p < 0.05$ versus 10 ng/

mL of IL-17F-stimulated cells. **b** IL-8 protein expression by IL-17F. ELISA was performed as described in Materials and Methods. The cells were stimulated with IL-17F (10, 100 ng/mL) for 4–48 h ($n = 6$). * $p < 0.05$ versus medium control; # $p < 0.05$ versus 10 ng/mL of IL-17F-stimulated cells.

(DRC, Tokyo, Japan), followed by transfer onto polyvinylidene difluoride membranes (Bio-Rad, Tokyo, Japan). The Abs to CDK9, phospho-CDK9, and phospho-p65 were purchased from Cell Signaling Technology (Cell Signaling Technology, Danvers, MA, USA). The Abs to p65 (Rel A) and β -actin were purchased from Santa Cruz Biotechnology.

Effect of CDK9 and Cyclin T1 Inhibition on IL-17F-Induced IL-8 Expression

For analysis of the involvement of CDK9, ASMCs were treated in the presence or absence of a CDK9 inhibitor, CDK9 inhibitor II (Calbiochem), or a vehicle control, 0.1% DMSO, for 1 h before stimulation with IL-17F (100 ng/mL). The supernatants were collected at 24 h after stimulation for analyses with ELISA. IL-8 protein levels in the supernatants were determined as described above. To further confirm the involvement of CDK9, predesigned siRNAs (10 nM) for CDK9 and cyclin T1 (Santa Cruz Biotechnology) and the control (Ambion) were also used for transfection into ASMCs as described above. The supernatants were then collected at 24 h after stimulation with 100 ng/mL of IL-17F and subjected to analysis by ELISA. Data are expressed as the mean + SEM ($n = 6$ experiments).

Effect of Brd4 Inhibition on Activation of CDK9 and IL-17F-Induced IL-8 Expression

For analysis of the involvement of Brd4, ASMCs were transfected in the presence or absence of predesigned siRNAs (10 nM) for Brd4 (Santa Cruz Biotechnology) and negative control siRNAs were used. The siRNA transfection into ASMCs by HiPerFect transfection reagent was performed as described above. Following transfection, Western blotting analysis was performed as described

above. The Abs used were anti-CDK9, anti-phospho-CDK9, and anti- β -actin. The supernatants were collected at 24 h after stimulation with 100 ng/mL of IL-17F. IL-8 protein levels in the supernatants were evaluated by ELISA and expressed as the mean + SEM ($n = 6$ experiments).

Effect of NF- κ B Inhibition on IL-17F-Induced IL-8 Expression

For analysis of the involvement of NF- κ B, ASMCs were treated in the presence or absence of a NF- κ B inhibitor, BAY 11-7082 (Calbiochem) at varying doses, or a vehicle control, 0.1% DMSO, for 1 h before treatment with IL-17F (100 ng/mL). The supernatants were collected at 24 h after stimulation for analyses with ELISA. IL-8 protein levels in the supernatants were determined as described above. To further confirm the involvement of NF- κ B, predesigned siRNAs (10 and 100 nM) for NF- κ B (Santa Cruz Biotechnology) and control (Ambion) were also used for transfection into ASMCs as described above. The supernatants were then collected at 24 h after stimulation with 100 ng/mL of IL-17F and subjected to analysis by ELISA and expressed as the mean + SEM ($n = 6$ experiments).

Data Analysis

The statistical significance of differences was determined by analysis of variance (ANOVA). The values are expressed as the mean $\pm$ SEM from independent experiments. Any difference with p values < 0.05 was considered significant. When ANOVA indicated a significant difference, the Scheffe F-test was used to determine the difference between groups. The Student t test was performed for some assays. p values < 0.05 were considered significant.

Fig. 2. Effect of budesonide on IL-8 protein expression. ASMCs were pretreated with budesonide (0.1, 1, and 10 nM) for 1 h before the 24-h stimulation of IL-17F (100 ng/mL), and then IL-8 protein levels in supernatants were measured by ELISA. Data are expressed as means $\pm$ SEM ($n = 6$). * $p < 0.05$ versus IL-17F-stimulated cells with vehicle control (DMSO).

Results

Expression of IL-8 Gene and Protein

First, the levels of IL-8 gene expression were evaluated by real-time PCR. IL-8 gene expression was markedly induced by 100 ng/mL of IL-17F at the 4, 12, and 24 h time points when compared with the control (Fig. 1a). IL-17F markedly induced its expression in a dose-dependent manner at 4 and 12 h after stimulation. The levels of IL-8 protein production were evaluated by ELISA (Fig. 1b). IL-8 protein was detected in untreated cells, but its levels in supernatants were markedly increased in a dose-dependent manner, peaking at the 24-h time point, in ASMCs stimulated with IL-17F when compared with the control. Moreover, pretreatment of the cells with 0.1, 1, and 10 nM of budesonide markedly reduced IL-17F-induced IL-8 production in a dose-dependent manner, while 1 h pretreatment of the cells with vehicle alone did not affect its production (Fig. 2).

Activation of CDK9 by IL-17F

To examine the involvement of CDK9 in IL-17F-induced IL-8 expression, activation of CDK9 in IL-17F-stimulated cells was evaluated. CDK9 and β -actin were equally detected at all time points (Fig. 3a). In contrast, a transient phosphorylation of CDK9 was seen in ASMCs

upon stimulation with IL-17F, peaking between 10 and 30 min after stimulation and returning to baseline levels by 60 min.

Effect of CDK9 and Cyclin T1 Inhibition on IL-17F-Induced IL-8 Expression

Pretreatment of the cells with 10 μ M of the CDK9 inhibitor, CDK9 inhibitor II, markedly blocked IL-17F-induced IL-8 production, while 1 h pretreatment of the cells with vehicle alone did not affect its production (Fig. 3b). As shown in Figure 3c, IL-8 production induced by IL-17F was markedly inhibited in cells transfected with siRNAs targeting CDK9 or cyclin T1 when compared with cells transfected with control siRNAs.

Effect of Brd4 Inhibition on Activation of CDK9 and IL-17F-Induced IL-8 Expression

The cells transfected with siRNAs targeting Brd4 diminished IL-17F-induced CDK9 phosphorylation (Fig. 3d). In contrast, it was not inhibited by the transfection with control siRNAs. Similarly, IL-8 production induced by IL-17F was markedly inhibited in the cells transfected with siRNAs targeting Brd4 when compared with the cells transfected with control siRNAs (Fig. 3c).

NF- κ B Inhibition on IL-17F-Induced IL-8 Expression

Next, we sought to examine the involvement of NF- κ B signaling in IL-17F-induced IL-8 expression. Results showed that pretreatment of ASMCs with 0.5, 1, and 5 μ M of an NF- κ B inhibitor, BAY 11-7082, did not influence the levels of IL-17F-induced IL-8 protein production (Fig. 4a). Similarly, the siRNAs targeting p65 did not reduce IL-17F-induced IL-8 production (Fig. 4b).

Effect of Budesonide on the Activation of CDK9 and NF- κ B p65

Moreover, when the cells were pretreated with budesonide, it was noted that the level of IL-17F-induced CDK9 phosphorylation was diminished as compared to that seen in the vehicle control (Fig. 5a). In contrast, the pretreatment of the cells with budesonide or vehicle control did not affect IL-17F-induced NF- κ B p65 phosphorylation (Fig. 5b).

Discussion

The current study provided evidence demonstrating that the IL-17F signal mediates the Brd4-P-TEFb pathway to induce the expression of IL-8. Brd4 is located up-

Fig. 3. Kinetic activation of CDK9 by IL-17F in ASMCs. **a** The cells were incubated with IL-17F (100 ng/mL) for different time points as indicated. Western blotting analysis was performed with Abs against total (t)-CDK9, phosphorylated (p)-CDK9, and β -actin as indicated. The results shown are representative of 3 separate experiments. **b** Effect of the inhibition for CDK9 on IL-8 protein expression. ASMCs were pretreated with CDK9 inhibitor II (10 μ M) for 1 h before the 24-h stimulation of IL-17F (100 ng/mL), and then IL-8 protein levels in supernatants were measured by ELISA. Data are expressed as means $\pm$ SEM ($n = 6$). * $p < 0.05$ versus IL-17F-stimulated cells in the absence of the inhibitor. **c** The validation of blocking by siRNAs targeting Brd4 (10 nM), CDK9 (10

nM), and cyclin T1 (10 nM) was performed. After transfection of the siRNAs, ASMCs were stimulated with IL-17F (100 ng/mL) for 24 h. The levels of IL-8 protein production in the supernatants were measured by ELISA. Data are expressed as means $\pm$ SEM ($n = 6$). * $p < 0.05$ versus IL-17F-stimulated cells transfected with a control siRNA. **d** Effect of siRNAs targeting Brd4 on IL-17F induced phosphorylation of CDK9. The cells were transfected with siRNAs targeting Brd4 (10 nM) or control siRNAs (10 nM), and then ASMCs were stimulated with IL-17F (100 ng/mL) for 30 min. Western blotting analysis was performed with Abs against (t)-CDK9, (p)-CDK9, and β -actin. The results shown are representative of 3 separate experiments.

stream of P-TEFb, since the siRNAs targeting Brd4 blocked the phosphorylation of CDK9. Moreover, the activation of P-TEFb is important for IL-8 expression by IL-17F, since the CDK9 inhibitor and the specific siRNAs targeting CDK9 and cyclin T1 abrogated its expression. Furthermore, corticosteroids diminished IL-17F-induced IL-8 expression via the inhibition of CDK9 activation. Thus, the Brd4-P-TEFb pathway is pivotal for IL-17F-induced IL-8 expression in ASMCs.

Elongation is a critically controlled step in transcription regulation by several elongation factors [20]. Among these factors, P-TEFb has a pivotal role in the pause release of Pol II and expression of inflammation-mediated genes, such as IL-8 [24]. The treatment of a CDK inhibitor, 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole, or siRNAs for cyclin T1 decreased the expression of Dengue virus-induced IL-8 in Huh7 cells [25]. P-TEFb is also involved in TNF- α -induced IL-8 expression in the bronchial epithelial cell line, A549 [24, 26]. In contrast to IL-8,

Fig. 4. Effect of NF- κ B inhibition on IL-17F-induced IL-8 expression. **a** ASMCs were pretreated with the NF- κ B inhibitor, BAY 11-7082 (0.5, 1, and 5 μ M), for 1 h before the 24-h stimulation of IL-17F (100 ng/mL), and then IL-8 protein levels in supernatants were measured by ELISA. Data are expressed as means $\pm$ SEM ($n = 6$).

b After transfection of the siRNAs (10 and 100 nM) targeting p65, ASMCs were stimulated with IL-17F (100 ng/mL) for 24 h. The levels of IL-8 protein production in the supernatants were measured by ELISA. Data are expressed as means $\pm$ SEM ($n = 6$).

Fig. 5. Effect of budesonide on activation of CDK9 and p65. The cells were pretreated in the presence or absence of budesonide (1 nM) and a vehicle control (DMSO), before treatment with IL-17F (100 ng/mL). Western blotting analysis was performed with Abs against (t)-CDK9 and (p)-CDK9 (**a**) and total (t)-p65 and phosphorylated (p)-p65 and β -actin (**b**). The results shown are representative of 3 separate experiments.

treatment of the CDK9 inhibitor did not reduce virus-induced IFN- β production [27]. These findings suggest that P-TEFb is involved in the inflammatory cytokine gene expression. Additionally, little is unknown about the functional role of P-TEFb in ASMCs, and its association with IL-17F signaling. Here, we reported, for the first time, that P-TEFb is involved in regulating IL-17F-induced gene expression.

In this study, knocking down of Brd4 diminished not only CDK9 phosphorylation, but also inhibited the expression of IL-8. Recent reports demonstrated that Brd4 plays a major role in the pathogenesis of airway inflammation. Indeed, Brd4 activation in bronchial epithelial cells was induced by several inflammatory stimuli, such as RS virus, and diesel exhaust particles that are able to exacerbate airway inflammation [28, 29]. In addition, Brd4 regulates IL-1 β -induced IL-8 expression in airway epithelial cells [30]. Brd4 also contributes to ASM proliferation and cytokine release [31, 32]. Moreover, Brd4 is a member of the bromodomains and extraterminal domain (BET) family. BET controls human Th17 differentiation and is necessary for the transcription of Th17 cytokines, such as IL-17A and IL-21 [33, 34]. Inhibition of Brd4 suppresses Th17 differentiation, suggesting that Brd4 is an important target for Th17-mediated responses.

While IL-17F induced IL-8 expression via the Brd4-CDK9 pathway, targeted disruption of cyclin T1 or CDK9 did not completely diminish its expression. P-TEFb is

mostly composed of CDK9 and cyclin T1, but 20% of the cellular CDK9 binds to other C-type cyclins, such as cyclin T2 and cyclin K [35]. In addition, other CDK isoforms, such as CDK4 and CDK6, are also involved in IL-1-induced IL-8 expression [36]. For these reasons, inhibition of cyclin T1 and CDK9 might show a partial effect on its expression.

Interestingly, IL-17F was shown to induce phosphorylation of NF- κ B p65, but blocking p65 did not reduce IL-17F-induced IL-8 expression. It is thought that NF- κ B is generally involved in IL-8 gene expression [37], but in our study the addition of an NF- κ B inhibitor did not reduce IL-17F-induced IL-8 expression. Consistent with this, previous reports have demonstrated that cadmium or rhinovirus induced IL-8 expression through an NF- κ B-independent pathway in airway epithelial cells [38, 39]. Thus, the exact role of NF- κ B signaling may vary depending on the cell types and stimulus used.

The effects of corticosteroids on IL-17F signaling are, at present, unclear. We reported that budesonide inhibited phosphorylation of CDK9, leading to decreased IL-8 expression. A previous study has demonstrated that corticosteroid receptors compete with P-TEFb recruitment to the IL-8 promoter in A549 [24]. We identified that corticosteroids inhibited phosphorylation of a subunit of P-TEFb, CDK9, leading to decreased IL-8 expression in ASMCs. Hence, corticosteroids may exert a postinitiation step in regulating IL-8 gene expression.

Airway neutrophilia is especially seen in the patients with severe asthma and COPD. However, the mechanism of neutrophil infiltration is not elucidated. Expression of IL-17F is increased in the airways of asthma and COPD patients [1, 7]. In a mouse model, overexpression of IL-17F induced airway neutrophilia [9, 10]. In particular, IL-17F is a key effector of neutrophilic reaction against aspergillus infection [40]. On the contrary, ASMCs are

known as a major IL-8-producing cell type, although its inducers have not been clarified. We showed that IL-8 expression is markedly increased in ASMCs in response to IL-17F, suggesting the potential importance of IL-17F in the regulation of neutrophilic inflammation.

In conclusion, this study provides a novel regulatory mechanism whereby IL-17F induced IL-8 expression in ASMCs through the involvement of Brd4-P-TEFb, which could be inhibited by steroids via their abilities to inhibit CDK9 activation. Thus, the IL-17F-P-TEFb axis might be an attractive pharmacotherapeutic target for controlling airway inflammatory diseases.

Acknowledgement

This work was supported by MEXT KAKENHI Grants-in-Aid for Scientific Research[©] 15K09209. S.K.H. was supported, in part, by National Health Research Institutes Taiwan.

Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Author Contributions

M.N. analyzed the data and wrote the manuscript. M.N., K.O., and J.F. performed the experiments. M.M., S.M., S.-K.H., Y.M., Y.I., H.S., T.S., and N.H. contributed to the interpretation of the results. M.K. supervised the entire research. All authors read and approved the final manuscript.

References

- 1 Kawaguchi M, Onuchic LF, Li XD, Essayan DM, Schroeder J, Xiao HQ, et al: Identification of a novel cytokine, ML-1, and its expression in subjects with asthma. *J Immunol* 2001; 167:4430–4435.
- 2 Hymowitz SG, Filvaroff EH, Yin JP, Lee J, Cai L, Risser P, et al: IL-17s adopt a cystine knot fold: structure and activity of a novel cytokine, IL-17F, and implications for receptor binding. *EMBO J* 2001;20:5332–5341.
- 3 Starnes T, Robertson MJ, Sledge G, Kelich S, Nakshatri H, Broxmeyer HE, et al: IL-17F, a novel cytokine selectively expressed in activated T cells and monocytes, regulates angiogenesis and endothelial cell cytokine production. *J Immunol* 2001;167:4137–4140.
- 4 Al-Ramli W, Préfontaine D, Chouiali F, Martin JG, Olivenstein R, Lemièrre C, et al: T_H17-associated cytokines (IL-17A and IL-17F) in severe asthma. *J Allergy Clin Immunol* 2009; 123:1185–1187.
- 5 Chang Y, Nadigel J, Boulais N, Bourbeau J, Maltais F, Eidelman DH, et al: CD8 positive T cells express IL-17 in patients with chronic obstructive pulmonary disease. *Respir Res* 2011;12:43.
- 6 Kawaguchi M, Takahashi D, Hizawa N, Suzuki S, Matsukura S, Kokubu F, et al: Interleukin-17F sequence variant (His161Arg) is associated with protection against asthma and antagonizes wild-type IL-17F activity. *J Allergy Clin Immunol* 2006;117:795–801.

- 7 Hizawa N, Kawaguchi M, Huang SK, Nishimura M: Role of interleukin-17F in chronic inflammatory and allergic lung disease. *Clin Exp Allergy* 2006;36:1109–1114.
- 8 Nakajima M, Kawaguchi M, Ota K, Fujita J, Matsukura S, Huang SK, et al: IL-17F induces IL-6 via TAK1- NF- κ B pathway in airway smooth muscle cells. *Immun Inflamm Dis* 2017;2:124–131.
- 9 Hurst SD, Muchamuel T, Gorman DM, Gilbert JM, Clifford T, Kwan S, et al: New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. *J Immunol* 2002;169:443–453.
- 10 Oda N, Canelos PB, Essayan DM, Plunkett BA, Myers AC, Huang SK: Interleukin-17F induces pulmonary neutrophilia and amplifies antigen-induced allergic response. *Am J Respir Crit Care Med* 2005;171:12–18.
- 11 Ordóñez CL, Shaughnessy TE, Matthay MA, Fahy JV: Increased neutrophil numbers and IL-8 levels in airway secretions in acute severe asthma: clinical and biologic significance. *Am J Respir Crit Care Med* 2000;161:1185–1190.
- 12 Stănescu D, Sanna A, Veriter C, Kostianev S, Calcagni PG, Fabbri LM, et al: Airway obstruction, chronic expectoration, and rapid decline of FEV₁ in smokers are associated with increased levels of sputum neutrophils. *Thorax* 1996;51:267–271.
- 13 Saetta M, Turato G, Facchini FM, Corbino L, Lucchini RE, Casani G, et al: Inflammatory cells in the bronchial glands of smokers with chronic bronchitis. *Am J Respir Crit Care Med* 1997;156:1633–1639.
- 14 Watson ML, Grix SP, Jordan NJ, Place GA, Dodd S, Leithead J et al: Interleukin 8 and monocyte chemoattractant protein 1 production by cultured human airway smooth muscle cells. *Cytokine* 1998;10:346–352.
- 15 Pera T, Atmaj C, van der Vegt M, Halayko AJ, Zaagsma J, Meurs H: Role for TAK1 in cigarette smoke-induced proinflammatory signaling and IL-8 release by human airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2012;303:L272–278.
- 16 Hennessy S, van Thoor E, Ge Q, Armour CL, Hughes JM, Ammit AJ: IL-17A acts via p38 MAPK to increase stability of TNF- α -induced IL-8 mRNA in human ASM. *Am J Physiol Lung Cell Mol Physiol* 2006;290:L1283–1290.
- 17 Ota K, Kawaguchi M, Matsukura S, Kurokawa M, Kokubu F, Fujita J, et al: Potential involvement of IL-17F in asthma. *J Immunol Res* 2014;2014:602846.
- 18 Nakajima M, Kawaguchi M, Watanabe H, Morishima Y, Huang SK, Satoh H, et al: Role of IL-17F in the pathogenesis of psoriasis. *Curr Immunol Rev* 2016;12:112–117.
- 19 Kawaguchi M, Onuchic LF, Huang SK: Activation of extracellular signal-regulated kinase (ERK)1/2, but not p38 and c-Jun N-terminal kinase, is involved in signaling of a novel cytokine, ML-1. *J Biol Chem* 2002;277:15229–15232.
- 20 Shao W, Zeitlinger J: Paused RNA polymerase II inhibits new transcriptional initiation. *Nat Genet* 2017;49:1045–1051.
- 21 Yamaguchi Y, Takagi T, Wada T, Yano K, Furuya A, Sugimoto S, et al: NELF, a multisubunit complex containing RD, cooperates with DSIF to repress RNA polymerase II elongation. *Cell* 1999;97:41–51.
- 22 Jang MK, Mochizuki K, Zhou M, Jeong HS, Brady JN, Ozato K: The bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription. *Mol Cell* 2005;19:523–534.
- 23 Zhou M, Halanski MA, Radonovich MF, Kashanchi F, Peng J, Price DH, et al: Tat modifies the activity of CDK9 to phosphorylate serine 5 of the RNA polymerase II carboxyl-terminal domain during human immunodeficiency virus type 1 transcription. *Mol Cell Biol* 2000;20:5077–5086.
- 24 Luecke HF, Yamamoto KR: The glucocorticoid receptor blocks P-TEFb recruitment by NF- κ B to effect promoter-specific transcriptional repression. *Genes Dev* 2005;19:1116–1127.
- 25 Li LL, Hu ST, Wang SH, Lee HH, Wang YT, Ping YH: Positive transcription elongation factor b (P-TEFb) contributes to dengue virus-stimulated induction of interleukin-8 (IL-8). *Cell Microbiol* 2010;12:1589–1603.
- 26 Barboric M, Nissen RM, Kanazawa S, Jabraneferat N, Peterlin BM: NF- κ B binds P-TEFb to stimulate transcriptional elongation by RNA polymerase II. *Mol Cell* 2001;8:327–337.
- 27 Tian B, Zhao Y, Kalita M, Edeh CB, Paessler S, Casola A, et al: CDK9-dependent transcriptional elongation in the innate interferon-stimulated gene response to respiratory syncytial virus infection in airway epithelial cells. *J Virol* 2013;87:7075–7092.
- 28 Tian B, Yang J, Zhao Y, Ivanciuc T, Sun H, Garofalo RP, et al: BRD4 couples NF- κ B/RelA with airway inflammation and the IRF-RIG-I amplification loop in respiratory syncytial virus infection. *J Virol* 2017;91:e00007-17.
- 29 Yun YP, Joo JD, Lee JY, Nam HY, Kim YH, Lee KH, et al: Induction of nuclear factor- κ B activation through TAK1 and NIK by diesel exhaust particles in L2 cell lines. *Toxicol Lett* 2005;155:337–342.
- 30 Khan YM, Kirkham P, Barnes PJ, Adcock IM: Brd4 is essential for IL-1 β -induced inflammation in human airway epithelial cells. *PLoS One* 2014;9:e95051.
- 31 Clifford RL, Patel JK, John AE, Tatler AL, Mazengarb L, Brightling CE, et al: CXCL8 histone H3 acetylation is dysfunctional in airway smooth muscle in asthma: regulation by BET. *Am J Physiol Lung Cell Mol Physiol* 2015;308:L962–L972.
- 32 Perry MM, Durham AL, Austin PJ, Adcock IM, Chung KF: BET bromodomains regulate transforming growth factor- β -induced proliferation and cytokine release in asthmatic airway smooth muscle. *J Biol Chem* 2015;290:9111–9121.
- 33 Mele DA, Salmeron A, Ghosh S, Huang HR, Bryant BM, Lora JM: BET bromodomain inhibition suppresses T_H17-mediated pathology. *J Exp Med* 2013;210:2181–2190.
- 34 Cheung K, Lu G, Sharma R, Vincek A, Zhang R, Plotnikov AN, et al: BET N-terminal bromodomain inhibition selectively blocks Th17 cell differentiation and ameliorates colitis in mice. *Proc Natl Acad Sci USA* 2017;114:2952–2957.
- 35 Cho S, Schroeder S, Kaehlcke K, Kwon HS, Pedal A, Herker E, et al: Acetylation of cyclin T1 regulates the equilibrium between active and inactive P-TEFb in cells. *EMBO J* 2009;28:1407–1417.
- 36 Handschick K, Beuerlein K, Jurida L, Bartkuhn M, Müller H, Soelch J, et al: Cyclin-dependent kinase 6 is a chromatin-bound cofactor for NF- κ B-dependent gene expression. *Mol Cell* 2014;53:193–208.
- 37 Panday A, Inda ME, Bagam P, Sahoo MK, Osorio D, Batra S: Transcription Factor NF- κ B: an update on intervention strategies. *Arch Immunol Ther Exp* 2016;64:463–483.
- 38 Cormet-Boyaka E, Jolivet K, Bonnegarde-Bernard A, Rennolds J, Hassan F, Mehta P, et al: An NF- κ B-independent and Erk1/2-dependent mechanism controls CXCL8/IL-8 responses of airway epithelial cells to cadmium. *Toxicol Sci* 2012;125:418–429.
- 39 Kim J, Sanders SP, Siekierski ES, Casolaro V, Proud D: Role of NF- κ B in cytokine production induced from human airway epithelial cells by rhinovirus infection. *J Immunol* 2000;165:3384–3392.
- 40 Yang XO, Chang SH, Park H, Nurieva R, Shah B, Acero L, et al: Regulation of inflammatory responses by IL-17F. *J Exp Med* 2008;205:1063–1075.