ERJ Express. Published on January 17, 2019 as doi: 10.1183/13993003.01510-2018

EUROPEAN RESPIRATORY *journal*

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Original article

IL-17F, rather than IL-17A, underlies airway inflammation in a steroid insensitive toluene diisocyanate-induced asthma model

Rongchang Chen, Qinglin Zhang, Shuyu Chen, Haixiong Tang, Peikai Huang, Shushan Wei, Lihong Yao

Please cite this article as: Chen R, Zhang Q, Chen S, *et al*. IL-17F, rather than IL-17A, underlies airway inflammation in a steroid insensitive toluene diisocyanate-induced asthma model. *Eur Respir J* 2019; in press (https://doi.org/10.1183/13993003.01510-2018).

IL-17F, rather than IL-17A, underlies airway inflammation in a steroid insensitive toluene diisocyanate-induced asthma model

Rongchang Chen[1#], Qinglin Zhang[1#], Shuyu Chen[2#], Haixiong Tang[3#], Peikai Huang[1], Shushan Wei[1], Lihong Yao[1*]

[1]State Key Laboratory of Respiratory Diseases, Guangzhou Institute of Respiratory Health, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University, Guangzhou, China

[2]The Second Affiliated Hospital of Guangzhou Medical University, Guangdong Provincial Key Laboratory of Allergy & Clinical Immunology, State Key Laboratory of Respiratory Disease; Guangzhou Medical University, Guangzhou, China

[3]Department of Respiratory Medicine, Minzu Hospital of Guangxi Zhuang Autonomous Region, Nanning, China

[#]These authors contributed equally to the article.

*Corresponding author: Lihong Yao, Ph.D., State Key Laboratory of Respiratory Diseases, Guangzhou Medical University, No.195, West Dongfeng Road, 510180, Guangzhou, China, E-mail: yaolh4321@126.com,

Key message

In a TDI-induced steroid insensitive murine asthma model, IL-17A restricts allergic responses through suppressing Th2 inflammation and eosinophil recruitment, while IL-17F modulates airway inflammation by driving Th17 response and neutrophil infiltrates.

Abstract

Steroid insensitivity constitutes the major problem for asthma management. Toluene diisocyanate (TDI) is one of the leading allergens of asthma that induces both Th2 and Th17 responses and is often associated with poor responsiveness to steroid treatment in the clinic. We sought to evaluate the effects of inhaled and systemic steroids on a TDI-induced asthma model and to find how IL-17A and IL-17F function in this model. BALB/c mice were exposed to TDI for generating an asthma model, and were treated with inhaled fluticasone propionate (FP), systemic prednisone (Pred), anti-IL-17A, anti-IL-17F, recombinant IL-17A or IL17F. Both FP and Pred showed no effects on TDI-induced airway hyperreactivity (AHR), bronchial neutrophilia and eosinophilia, epithelial goblet cell metaplasia. TDI-induced Th2 and Th17 signatures were not suppressed by FP or Pred. Treatment with anti-IL-17A after TDI exposure led to increased AHR, aggravated mucus production and airway eosinophil recruitment, accompanied by amplified Th2 responses. While anti-IL-17F ameliorated TDI-induced AHR and airway neutrophilia, with decreased Th17 responses. Recombinant IL-17A

and IL-17F showed opposite effects to the monoclonal antibodies. In conclusion, IL-17A and IL-17F exert distinct biological effects during airway inflammation of a TDI-induced asthma model, which is irresponsive to both inhaled and systemic steroids.

Keywords

asthma; toluene diisocyanate; steroid insensitive; IL-17A; IL-17F

Introduction

About 10~25% of adult-onset asthma is occupational associated, with diisocyanates (including toluene diisocyanate and methylene diphenyl diisocyanate) the most commonly reported causes [1], which often respond poorly to steroid treatment, leading to poor prognosis even after cessation of the exposure [2]. Deeper understanding of the disease is in urgent need to find more therapeutic targets. Toluene diisocyanate (TDI)-induced asthma is often characterized by accumulation of a large number of neutrophils and a smaller number of eosinophils into the airways [3]. Multiple mechanisms are thought to be involved in the induction of TDI-induced asthma, including immunologic, genetic, neurogenic and etc, leading to the definition of two subtypes of TDI-induced asthma: immunologic and nonimmunologic [4]. Evidence from human and animal models suggests that different subgroups of CD4+ T cells (mainly Th1, Th2 and Th17) together with their secreted cytokines contribute critically to TDI-induced asthma [4], therefore further studies are needed to uncover their complex functions.

During the past decade, the role of IL17 in immune and allergic disorders is attracting increasing attention. IL-17 is a family of cytokines mainly produced by Th17 cells and consists of six members, named IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (also known as IL-25) and IL-17F [5]. Among them, IL-17A and IL-17F share the closest amino acid sequence identity and function through IL-17 receptors. Recent clinical and animal studies supported that IL-17 is critically involved in asthma pathogenesis. Elevated IL-17 concentrations were found in the airways of allergic asthma patients [6] and positively correlate to asthma severity and steroid insensitivity [7]. Inhibition of IL-17 using gene knockout mice or blocking antibody reduces OVA-induced airway hyperresponsiveness, bronchial inflammatory cell infiltration as well as airway vascular remodeling [8-10]. However, Schnyder-Candrian S and coauthors discovered that exogenous IL-17 inhibited OVA-induced pulmonary eosinophil recruitment, Th2 inflammation, and bronchial hyperreactivity [11]. Those

opposite findings revealed that IL-17 conveys dual effects, which may be attributed to the conflicting biological functions of individual IL-17 family members, especially for IL-17A and IL-17F [12]. Researchers have already demonstrated an increased level of IL-17 in TDI asthma. Anti-IL17 neutralizing antibody could decrease TDI-induced airway inflammation and hyperresponsiveness [13, 14], suggesting important roles of IL-17 in TDI asthma. Yet, the separate functions of different subtypes of IL-17 have not been assessed.

We have previously established a TDI-induced asthma model with pronounced airway neutrophilia and eosinophilia. The two aims of this study were to evaluate the effects of inhaled and systemic steroids on TDI-induced asthmatic responses, and to examine the roles of IL-17A and IL-17F in TDI asthma.

Methods

For detailed methods including animal protocols and experimental procedures, please see the *Online supplementary material*.

Ethics statement

All animal experiments described here complied with the guidelines of the Committee of Guangzhou Medical University on the use and care of animals and were approved by the Animal Subjects Committee of Guangzhou Medical University.

Statistics

Data are expressed as mean±SD. Results were interpreted using one-way ANOVA and Bonferroni's difference post hoc test with SPSS 22.0. Differences were considered statistically significant when $p < 0.05$.

Results

TDI-induced airway hyperresponsiveness and pathological changes were not prevented by inhaled fluticasone propionate (FP) or systemic prednisone (Pred)

Allergic airway inflammation was induced by exposing BALB/c mice to TDI. As expected, this led to significant neutrophil and eosinophil accumulation with structural and functional abnormalities of the airways. Despite their known effects in mild and moderate asthma, corticosteroids do not seem to do any help in our TDI-induced asthma model. Intraperitoneal injection of 5 mg/kg prednisone (Pred) or intranasal instillation of 300 μg/kg fluticasone propionate (FP) once daily beginning from the first inhalation for a consecutive of one week did inhibit TDI-induced airway mucus production as

assessed by PAS staining and Muc5ac mRNA expression (Fig 1, *a, d and e*), yet TDI-induced airway inflammation, epithelial hyperplasia, airway hyperresponsiveness, smooth muscle thickening did not change after Pred or FP treatment (Fig 1, *a, b, c and f*). Similarly, neither inhaled FP nor systemic Pred affected the increased numbers of neutrophils and eosinophils in bronchoalveolar lavage fluid (BALF) of TDI sensitized and challenged mice (Fig1, *g*). In spite, greater numbers of neutrophils were seen in steroid-treated mice [25.55±1.97 (TDI+Pred group) *vs* 21.76±2.33 (TDI+FP group) *vs* 20.20±2.13 (TDI group) × 10^4/mL, though not significant]. Total serum IgE titers did not differ among all TDI-exposed groups (TABLE 2).

TABLE 1 Percentages (%) of T helper cell subsets in CD4+ cells in steroid-treated mice

	control	TDI	TDI+Pred	TDI+FP
BALF CD4 IFNγ$^+$	2.02±0.46	4.60±0.65**	4.32±0.65	4.34±1.19
BALF CD4 IL-4$^+$	0.09±0.08	2.61±0.67**	2.80±0.68	2.73±0.52
BALF CD4 IL17A$^+$	2.43±0.25	7.88±1.08**	8.24±0.92	7.93±1.46
lung CD4 IFNγ$^+$	0.20±0.07	0.97±0.21**	1.05±0.24	0.91±0.38
lung CD4 IL-4$^+$	0.11±0.02	0.61±0.10**	0.69±0.21	0.91±0.16
lung CD4 IL17A$^+$	0.73±0.10	1.99±0.39**	1.79±0.25	1.84±0.43

Data are presented as mean±SD. ** in TDI group indicates $p<0.01$ compared with control group. No significant differences were observed between TDI and TDI+Pred group, nor between TDI and TDI+FP group.

Both Th2 and Th17 responses are involved in TDI-induced asthma that can not be suppressed by inhaled FP or systemic Pred

TDI-induced asthma is thought to be mediated by a mixed Th1, Th2 and Th17 response [13, 15]. Here, we set to determine the percentages of those subtypes of T helper cells in this asthma model and to evaluate the effect of steroid treatment. BALF and lung single cell suspensions were harvested and stained for flow cytometry analysis. Results revealed that the percentages (%) of CD4 IFNγ+ (Th1), CD4 IL4+ (Th2), CD4 IL17A+ (Th17) cells in BALF CD4+ cells of TDI-exposed mice were 4.60±0.65, 2.61±0.67, 7.88±1.08, and the percentages (%) in lung CD4+ cells were 0.97±0.21 (CD4 IFNγ+), 0.61±0.10 (CD4 IL4+), 1.99±0.39 (CD4 IL17A+), compared with 2.02±0.46 (CD4 IFNγ+), 0.09±0.08 (CD4 IL4+), 2.43±0.25 (CD4 IL17A+) in BALF of control mice and 0.20±0.07 (CD4 IFNγ+), 0.11±0.02 (CD4 IL4+), 0.73±0.10 (CD4 IL17A+) in lung CD4+ cells of control group. While treatment with either inhaled FP or systemic Pred had no effects on those

TDI-induced T helper cell subsets (TABLE 1).

Th1-, Th2-, and Th17-related cytokines were also quantified. Levels of Th2-related IL-4, IL-5, IL-13 and Th17-related IL-17A, IL-17F in both BALF and lung homogenates were higher in TDI sensitized and challenged mice compared with vehicle-exposed (TABLE 2). It is the same for IL-6 and IL-1β, which are critical for the differentiation and maturation of Th17 cells, though IL-1β was not detected in BALF of this model. Other cytokines including IL-18, as well as the eosinophil chemoattractants CCL11 and CCL24 also increased significantly in TDI asthma. FP or Pred blunted the release of IL-5 but promoted IL-6 production in BALF, yet had no significant effects on the others (TABLE 2). IFNγ, IL-22, IL-23 were not detected.

Th2 and Th17 signatures would result in distinct gene expression patterns in lung epithelia [16]. So we assessed the relative expression of those genes in the whole lung. As shown in TABLE 3, the mRNA levels of Th2 markers Ccl11, Clca3 and Th17 markers Cxcl1, Cxcl3, Csf3 were extensively up-regulated by TDI. Still, inhaled FP or systemic Pred did not alter those gene expression patterns. It was the same for Il17a and Il17f mRNA expression.

TABLE 2 Levels of a list of cytokines in BALF in steroid-treated mice

	control	TDI	TDI+Pred	TDI+FP
BALF IL-4	0.38±0.42	19.43±4.15**	16.77±4.20	17.10±4.54
lung IL-4	5.15±1.10	27.88±7.66*	21.64±3.05	23.01±2.62
BALF IL-5	4.92±1.36	64.37±17.06**	15.40±2.92**	14.54±5.78**
lung IL-5	1.29±3.37	43.14±7.80**	39.55±8.82	43.88±6.46
BALF IL-13	2.39±0.48	18.68±5.46**	18.26±4.66	19.25±4.17
lung IL-13	1.89±4.05	74.68±15.80**	58.91±12.05	63.19±16.39
BALF IL17A	2.72±1.02	14.68±3.72**	20.99±6.05	17.39±4.35
lung IL17A	10.63±2.35	21.23±1.79**	26.32±3.88	24.07±4.20
BALF IL17F	0.00±0.00	34.90±4.09**	38.72±11.94	41.78±22.52
lung IL17F	6.70±.31	37.32±10.50*	31.50±2.66	30.48±2.83
BALF IL-6	8.76±2.20	98.57±29.93**	325.79±109.18**	347.92±112.60**
BALF IL-18	8.46±1.94	310.75±98.40**	325.75±40.97	276.21±65.13
BALF CCL11	8.31±2.02	140.16±21.06**	110.76±23.16	106.23±37.58
BALF CCL24	8.88±5.05	223.81±47.17*	183.97±24.44	201.24±14.32
lung IL1β	31.14±6.91	70.00±7.92*	73.28±10.97	70.68±30.47
serum IgE#	2.31±1.17	54.78±11.65**	41.57±6.93	44.29±10.00

Data are presented as mean±SD. * in TDI group indicates p<0.05 compared with control group, and ** indicates p<0.01. ** in TDI+Pred or TDI+FP group indicates p<0.01 compared with TDI group.
#The unit for IgE is ng/mL, and pg/mL for all the others.

TABLE 3 Fold changes of mRNA expression in lungs of steroid-treated mice

	control	TDI	TDI+Pred	TDI+FP
Ccl11	1.00±0.26	7.85±2.22**	7.21±2.27	6.28±2.52
Clca3	1.00±0.12	41.45±8.52**	47.30±11.95	35.32±19.47
Cxcl1	1.00±0.14	8.37±2.99**	7.57±2.54	8.97±3.36
Cxcl3	1.00±0.25	4.30±1.17**	7.06±3.64	7.50±3.65
Csf3	1.00±0.35	18.15±4.22**	32.99±12.71	32.91±11.33
Il17a	1.00±0.42	28.71±4.68**	33.59±5.78	35.18±5.65
Il17f	1.00±0.09	2.61±0.72*	2.68±0.76	2.12±0.46

mRNA expression was normalized to control values and expressed as n-fold. Data are presented as mean±SD. * in TDI group indicates p<0.05 compared with control group, and ** indicates p<0.01. No significant differences were observed between TDI and TDI+Pred group, nor between TDI and TDI+FP group.

Blockade of IL-17A exacerbates TDI-induced airway inflammation

As IL-17A and IL-17F were both increased in TDI asthma, we wondered whether blocking each of them would help to ameliorate the disease. Monoclonal antibodies (100 μg/mouse) against IL-17A and IL-17F were respectively given to the mice after each airway challenge. Surprisingly, we observed completely different outcomes after neutralizing IL-17A and IL-17F in TDI-exposed mice. Despite decreasing the level of secreted IL-17A in BALF, IL-17A mab exacerbated TDI-induced airway hyperresponsiveness and inflammation, led to more severe epithelial cell hyperplasia and remodeling, and drove greater numbers of eosinophils into the airway lumen (Fig 2), coupled with markedly enhanced expression of eosinophil chemokine CCL24 in the lung (Fig 3, *c*). Administration of IL-17A mab did not inhibit the percentages of CD4 IL17A+ cells in BALF and lung, but gave rise to the number of CD4 IL4+ cells (Fig 3, *a and b*). At the same time, we detected larger amounts of Th2 cytokines IL-4 and IL-5 in BALF after the mice were treated with IL-17A mab, whereas levels of IL-6, IL-18 and IL-1β in TDI asthmatic mice were not affected by IL-17A mab (Fig 3, *c*; Fig 4, *a*). Accordingly, TDI-induced increased mRNA expression of Th2 markers Ccl11, Clca3 was enhanced, in contrast with suppressed expression of Th17 markers Cxcl1, Cxcl3 and Csf3 (Fig 4, *b*). We got opposite results after treating the TDI- sensitized and challenged mice

with recombinant IL-17A (Online supplement, Fig S2 and Fig S3), except for the unaffected number of CD4 IL4+ cells in BALF and lung despite their suppressed functions of secreting Th2 cytokines (Online supplement, Fig S3, a-c). These results suggest that IL-17A is restraining TDI-induced airway hyperreactivity and allergic responses.

Anti-IL-17F atternuates AHR and airway neutrophil inflammation in TDI asthma

On the other hand, blocking IL-17F exerted a list of promising protective functions. Intraperitoneal injection of IL-17F mab at the dose of 100 μg per mouse per time after each TDI challenge for a total of 3 times resulted in dramatically decreased airway inflammation and hyperresponsiveness, extensively compromised epithelial hyperplasia, goblet cell metaplasia and mucus production, as well as a significantly small number of neutrophils in BALF, while airway eosinophil recruitment was not inhibited (Fig 2). Besides, percentages of CD4 IFNγ+, CD4 IL4+ and CD4 IL17A+ cells in BALF and lung were also lowered after IL-17F mab treatment (Fig 3, *a and b*). Interestingly, IL-4, IL-5, IL-13, CCL11, CCL24 in BALF or lung homogenates did not show obvious differences between mice treated with isotype IgG and mice treated with IL-17F mab (Fig 3, *c*; Fig 4, *a*), yet gene expression of Th17 markers Cxcl1, Cxcl3 and Csf3 was downregulated after neutralizing IL-17F with monoclonal antibody in TDI sensitized and challenged mice (Fig 4, *b*). At the same time, IL-17F mab treatment also inhibited the release of IL-6, IL-18 in BALF and IL-1β expression in lung (Fig 3, *c*; Fig 4, *a*). Conversely, treatment with recombinant IL-17F aggravated the TDI-induced airway neutrophilic inflammation and Th17 related responses (Online supplement, Fig S2 and Fig S3), though airway hyperreactivity to methacholine was not significantly increased by IL-17F (Online supplement, Fig S2, *f*).

Discussion

In the present study, we assessed the effects of inhaled and systemic corticosteroids on TDI-induced asthmatic responses. Unexpectedly, both administration routes displayed few effects, indicating that we've identified a new steroid insensitive asthma model that can be generated by a single allergen. In the mean time, we found for the first time that IL-17A is restraining while IL-17F is contributing to TDI-induced airway hyperreactivity and inflammation, demonstrating distinct roles of IL-17A and IL-17F.

The family of diisocyanates consists of a list of highly reactive chemicals that have been commonly used in industrial production, including TDI, hexamethylene diisocyanate (HDI), diphenylmethane

diisocyanate (MDI), etc. They are all well-established important respiratory allergens and are connected with the majority of occupational asthma cases [17]. Here we prepared TDI-induced asthma model through dermal sensitization followed by inhalation as previously reported. Without neglecting the importance of respiratory sensitization, the skin also acts as an important exposure route for initiating immune sensitization. Skin exposure is well recognized as a mechanism for inducing immune sensitization in TDI asthma, including production of allergen-specific IgE [4]. Though the reported incidence of dermal exposure to TDI varies substantially [18], it predisposes the body with very low concentration of TDI and is technically much easier.

As the mainstay therapy in asthma, corticosteroids display great capacity for inhibiting allergic Th2 response and inducing eosinophil apoptosis [19]. Yet there are approximately 10% of asthma patients that do not respond to steroid treatment, consuming a disproportionate amount of asthma-associated healthcare costs [20]. Studies have shown that the number of neutrophils correlates with poor responses to cortiscoteroids and decreasing lung function [21-23]. High number of neutrophils may reflect a non-Th2-dominated mechanism and, possibly, a steroid resistant asthma phenotype, predicting more severe disease. Here, we prepared the TDI-induced asthma model as previously described. Allergic airway inflammation is dominated by accumulation of eosinophils and neutrophils, paralleled by increased numbers of Th2 and Th17 cells in both BALF and lungs of TDI-exposed mice. Compared with eosinophils, more neutrophils were seen in BALF of this model, together with predominated Th17 cells. Yet to our surprise, treatement with inhaled fluticasone propionate (300 μg/kg) or systemic prednisone (5 mg/kg) failed to inhibit TDI-induced airway eosinophil infiltration and most of the Th2-related responses, though IL-5 and airway mucus production were actually blunted. At the same time, TDI-induced AHR, epithelial damage and hyperplasia and Th17 signatures also showed no responsiveness to inhaled or systemic steroids. We even detected greater numbers of neutrophils (though not significant) in steroid-treated mice, accompanied by markedly higher levels of IL-6, an upstream cytokine for Th17 maturation, indicating that steroids not only exhibit no effects on TDI-induced eosinophilic inflammation, but also tend to propogate neutrophil inflammation and Th17 responses. Similar results were obtained when the TDI-asthmatic mice were treated with systemic prednisone at a higher dose of 10mg/kg (data not show). The results indicate that our TDI-induced asthma model is actually a steroid insensitive asthma model, which agrees with findings in clinical patients [2, 24]. But this sounds

contradictory to an earlier published paper, showing potent anti-inflammatory effects of dexamethasone for TDI-induced eosinophilia [25]. Though it has been well recognized that eosinophils would undergo apoptosis after glucocoticoid treatment in asthma [19], steroid resistant eosinophilic airway inflammation has also been described in both patients and animal models [26, 27]. Further studies are needed to find out the reasons why eosinophils may have different responses to steroid treatment.

As one of the most important features of TDI-induced asthma, the large numbers of neutrophils accumulating in the airway have made huge contributions to steroid resistance and greatly hamper control of the disease. Depletion of neutrophils could prevent TDI-induced AHR, epithelial damage and significantly reduce airway inflammation [28]. Therefore, targeting neutrophils might prove potential therapeutical effects. Members of the IL-17 family, mainly IL-17A and IL-17F, are known to be potent neutrophil mobilizing chemokines [5]. They attract neutrophils into mucosal surfaces by increasing the release of neutrophil-modulating cytokines and chemoattractants (including IL1β, IL-6, IL-8, granulocyte colony-stimulating factor, etc.) from epithelial and endothelial cells [29]. Genetic deletion or neutralization of IL-17A diminishes neutrophil invasion and confers protection against organ injury and infection in mice [30-31]. IL-17A can also enhance neutrophil fungal killing functions and induce its production of reactive oxygen species (ROS) [32]. Studies have already demonstrated an important role of IL-17 in TDI-induced asthma [13, 14], and previously we detected higher levels of IL-17A in this TDI-induced asthma model [33]. So in the current study, we treated the TDI- sensitized and challenged mice with IL-17A monoclonal antibody, hoping to find some therapeutic effects. Unexpectedly, IL-17A antibody not only showed no effects for TDI-induced airway neutrophilc inflammation, but also augmented eosinophil aggregation, Th2-related responses, AHR, bronchial epithelial hyperplia and mucus production, indicating that IL-17A functions to counteract eosinphilia and Th2 inflammation, which is verified a second time after we treated the TDI-inhaling mice with exogenous IL-17A. These appear to be in striking contrasts to the aforementioned studies [13, 14] and different from observations in OVA- or house dust mite (HDM)-induced asthma models [34, 35], but might help to account for the controversial dual roles of IL-17 (which consists of not only IL-17A) in allergic airway inflammation [8-11]. Consistent with our study, Nakae S et al. and Hellings PW et al. discovered that IL-17 deficiency or neutralization significantly enhanced BALF IL-4, IL-5 and aggravated OVA-induced eosinophila [8, 9]. One latest study in a

mouse model of allergic broncho-pulmonary aspergillosis (ABPA) [12] also supports the notion that antagonizing IL-17A can boost allergen-induced airway eosinophilia and Th2 responses.

In contrast to the effects of IL-17A mab, IL-17F mab treatment had completely different outcomes. As mentioned before, IL-17F, another member of the IL-17 family, is also capable of driving neutrophilc inflammation [29]. With the greatest sequence homology (>50%) shared between IL-17A and IL-17F, few studies have disentangled the specific effects of these two IL-17 family members in pathologic inflammation. Compared with IL-17A, IL-17F is much less studied. It was originally discovered in bronchoalveolar lavage cells from asthma patients upon ragweed allergen challenge [36]. Later, in 2002, a Japanese team demonstrated that a coding region variant of IL-17F gene is linked to chronic inflammatory airway diseases [37]. Recent studies proved that airway IL-17F expression positively correlates the number of neutrophils and asthma severity [38, 39]. In this study, BALF IL-17F level in TDI-exposed mice was higher than in control ones. In line with the findings in HDM-induced model [35], neutralizing IL-17F with monoclonal antibody resulted in considerably subsided bronchial neutrophil infiltration, ameliorated airway epithelial injury and hyperplasia, decreased AHR and blunted Th17 related responses, together with less eosinophils (though not significant) in the airway. At the same time, IL-17F mab treatment also suppressed the excessive secretion of IL-6, IL-18 and the upregulated IL1β expression in TDI asthmatic mice, all of which are critical mediators for neutrophil migration and activation [40]. Opposite results were seen when the TDI sensitized and challenged mice were treated with recombinant IL-17F. These data suggest that IL-17F is the major culprit for TDI-induced neutrophilc inflammation and pathological changes.

In conclusion, we discovered a novel steroid insensitive asthma model that is induced by TDI, providing an alternative approach for investigating mechanisms involved in severe asthma. In addition, we also found that IL-17A restricts TDI-induced allergic airway inflammation through conteracting Th2 responses and preventing eosinophil influx, whilst IL-17F contributes to TDI-induced airway neutrophilic inflammation and bronchial hyperresponsivenes, which might be a effective therapeutical targets for severe asthma in the future.

Funding

This study was supported by National Postdoctoral Program for Innovative Talents (Grant No. BX201700060), National Key R&D Program of China (Grant No. 2017YFC1310601), National Natural Science Foundation of China (Grant No. 81800022), Project funded by China Postdoctoral

Science Foundation (Grant No. 2018M633034), Natural Science Foundation of Guangdong Province (Grant No. 2018A0303130244), Guangzhou Healthcare Collaborative Innovation Major Project (Grant No. 201604020012), Young scientist fundation of Guangxi Medical University (Grant No. GXMUYSF201636), and Scientific Research Starting Foundation for Postdoctoral Fellows of Guangzhou.

Author contributions

Y.L. and T.H. designed the experiment, analyzed the results and wrote the manuscript. Y.L., C.S. and T.H. performed the experiments. C.R., Z.Q., H.P. and W.S. helped with the experiment and data analysis. C.R. and Z.Q. helped to revise the manuscript. All the authors agreed that the final approval of the version to be published and ensured questions relating to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

1. Gautrin D, Newman-Taylor AJ, Nordman H, Malo JL. Controversies in epidemiology of occupational asthma. Eur Respir J 2003;22(3):551-9.

2. Paggiaro PL, Vagaggini B, Bacci E, Bancalari L, Carrara M, Di Franco A, et al. Prognosis of occupational asthma. Eur Respir J 1994;7(4):761-7.

3. Pollaris L, Devos F, De Vooght V, Seys S, Nemery B, Hoet PH. Toluene diisocyanate and methylene diphenyl diisocyanate: asthmatic response and cross-reactivity in a mouse model. Arch Toxicol 2016;90(7):1709-17.

4. Lummus ZL, Wisnewski AV, Bernstein DI. Pathogenesis and disease mechanisms of occupational asthma. Immunol Allergy Clin North Am 2011;31(4):699-716.

5. Isailovic N, Daigo K, Mantovani A, Selmi C. Interleukin-17 and innate immunity in infections and chronic inflammation. J Autoimmun 2015;60:1-11.

6. Molet S, Hamid Q, Davoine F, Nutku E, Taha R, Pagé N, et al. IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. J Allergy Clin Immunol 2001;108(3):430-8.

7. Chesné J1, Braza F, Mahay G, Brouard S, Aronica M, Magnan A. IL-17 in severe asthma. Where do we stand? Am J Respir Crit Care Med 2014;190(10):1094-101.

8. Nakae S, Komiyama Y, Nambu A, Sudo K, Iwase M, Homma I, et al. Antigen-specific T cell sensitization is

impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. Immunity 2002;17(3):375-87.

9. Hellings PW, Kasran A, Liu Z, Vandekerckhove P, Wuyts A, Overbergh L, et al. Interleukin-17 orchestrates the granulocyte influx into airways after allergen inhalation in a mouse model of allergic asthma. Am J Respir Cell Mol Biol 2003;28(1):42-50.

10. Lu S, Li H, Gao R, Gao X, Xu F, Wang Q, et al. IL-17A, but not IL-17F, is indispensable for airway vascular remodeling induced by exaggerated Th17 cell responses in prolonged ovalbumin-challenged mice. J Immunol 2015;194(8):3557-66.

11. Schnyder-Candrian S, Togbe D, Couillin I, Mercier I, Brombacher F, Quesniaux V, et al. Interleukin-17 is a negative regulator of established allergic asthma. J Exp Med 2006;203(12):2715-25.

12. De Luca A, Pariano M, Cellini B, Costantini C, Villella VR, Jose SS, et al. The IL-17F/IL-17RC Axis Promotes Respiratory Allergy in the Proximal Airways. Cell Rep 2017;20(7):1667-1680.

13. Kim SR, Lee KS, Park SJ, Min KH, Lee KY, Choe YH, et al. PTEN down-regulates IL-17 expression in a murine model of toluene diisocyanate-induced airway disease. J Immunol 2007;179(10):6820-9.

14. Liu SY, Wang WZ, Yen CL, Tsai MY, Yang PW, Wang JY, et al. Leukocyte nicotinamide adenine dinucleotide phosphate-reduced oxidase is required for isocyanate-induced lung inflammation. J Allergy Clin Immunol 2011;127(4):1014-23.

15. De Vooght V, Vanoirbeek JA, Haenen S, Verbeken E, Nemery B, Hoet PH. Oropharyngeal aspiration: an alternative route for challenging in a mouse model of chemical-induced asthma. Toxicology 2009;259(1-2):84-9.

16. Choy DF, Hart KM, Borthwick LA, Shikotra A, Nagarkar DR, Siddiqui S, et al. TH2 and TH17 inflammatory pathways are reciprocally regulated in asthma. Sci Transl Med 2015;7(301):301ra129.

17. Kimber I, Dearman RJ, Basketter DA. Diisocyanates, occupational asthma and IgE antibody: implications for hazard characterization. J Appl Toxicol 2014;34(10):1073-7.

18. Lynch HN, Prueitt RL, Goodman JE. Critique of the ACGIH 2016 derivation of toluene diisocyanate Threshold Limit Values. Regul Toxicol Pharmacol 2018;97:189-196.

19. Woolley KL, Gibson PG, Carty K, Wilson AJ, Twaddell SH, Woolley MJ. Eosinophil apoptosis and the resolution of airway inflammation in asthma. Am J Respir Crit Care Med 1996;154(1):237-43.

20. Trevor JL, Deshane JS. Refractory asthma: mechanisms, targets, and therapy. Allergy 2014;69(7):817-27.

21. Saffar AS, Ashdown H, Gounni AS. The molecular mechanisms of glucocorticoids-mediated neutrophil survival. Curr Drug Targets 2011;12(4):556-62.

22. Uddin M, Nong G, Ward J, Seumois G, Prince LR, Wilson SJ, et al. Prosurvival activity for airway neutrophils in severe asthma. Thorax 2010;65(8):684-9.

23. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J 2014;43(2):343-73.

24. Malo JL, Cartier A, Côté J, Milot J, Leblanc C, Paquette L, et al. Influence of inhaled steroids on recovery from occupational asthma after cessation of exposure: an 18-month double-blind crossover study. Am J Respir Crit Care Med 1996;153(3):953-60.

25. Song J, Zhao H, Dong H, Zhang D, Zou M, Tang H, et al. Mechanism of E-cadherin redistribution in bronchial airway epithelial cells in a TDI-induced asthma model. Toxicol Lett 2013;220(1):8-14.

26. Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. American Thoracic Society. Am J Respir Crit Care Med 2000;162(6):2341-51.

27. Kim RY, Horvat JC, Pinkerton JW, Starkey MR, Essilfie AT, Mayall JR, et al. MicroRNA-21 drives severe, steroid-insensitive experimental asthma by amplifying phosphoinositide 3-kinase-mediated suppression of histone deacetylase 2. J Allergy Clin Immunol 2017;139(2):519-532.

28. De Vooght V, Smulders S, Haenen S, Belmans J, Opdenakker G, Verbeken E, et al. Neutrophil and eosinophil granulocytes as key players in a mouse model of chemical-induced asthma. Toxicol Sci 2013;131(2):406-18.

29. Nembrini C, Marsland BJ, Kopf M. IL-17-producing T cells in lung immunity and inflammation. J Allergy Clin Immunol 2009;123(5):986-94.

30. Kono H, Fujii H, Ogiku M, Hosomura N, Amemiya H, Tsuchiya M, et al. Role of IL-17A in neutrophil recruitment and hepatic injury after warm ischemia-reperfusion mice. J Immunol. 2011 Nov 1;187(9):4818-25.

31. Flannigan KL, Ngo VL, Geem D, Harusato A, Hirota SA, Parkos CA, et al. IL-17A-mediated neutrophil recruitment limits expansion of segmented filamentous bacteria. Mucosal Immunol 2017;10(3):673-684.

32. Taylor PR, Roy S, Leal SM Jr, Sun Y, Howell SJ, Cobb BA, et al. Activation of neutrophils by autocrine IL-17A-IL-17RC interactions during fungal infection is regulated by IL-6, IL-23, RORγt and dectin-2. Nat Immunol 2014;15(2):143-51.

33. Yao L, Zhao H, Tang H, Song J, Dong H, Zou F, et al. Chicken IgY facilitates allergic airway inflammation in a chemical-induced murine asthma model by potentiating IL-4 release. Toxicol Lett 2015;239(1):22-31.

34. Lu S, Li H, Gao R, Gao X, Xu F, Wang Q, et al. IL-17A, but not IL-17F, is indispensable for airway vascular remodeling induced by exaggerated Th17 cell responses in prolonged ovalbumin-challenged mice. J Immunol 2015;194(8):3557-66.

35. Chenuet P, Fauconnier L, Madouri F, Marchiol T, Rouxel N, Ledru A, et al. Neutralization of either IL-17A or IL-17F is sufficient to inhibit house dust mite induced allergic asthma in mice. Clin Sci 2017;131(20):2533-2548.

36. 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(8):4430-5.

37. Hizawa N, Kawaguchi M, Huang SK, Nishimura M. Role of interleukin-17F in chronic inflammatory and allergic lung disease. Clin Exp Allergy 2006;36(9):1109-14.

38. Sorbello V, Ciprandi G, Di Stefano A, Massaglia GM, Favatà G, Conticello S, et al. Nasal IL-17F is related to bronchial IL-17F/neutrophilia and exacerbations in stable atopic severe asthma. Allergy 2015;70(2):236-40.

39. Ricciardolo FLM, Sorbello V, Folino A, Gallo F, Massaglia GM, Favatà G, et al. Identification of IL-17F/frequent exacerbator endotype in asthma. J Allergy Clin Immunol 2017;140(2):395-406.

40. de Oliveira S, Rosowski EE, Huttenlocher A. Neutrophil migration in infection and wound repair: going forward in reverse. Nat Rev Immunol 2016;16(6):378-91.

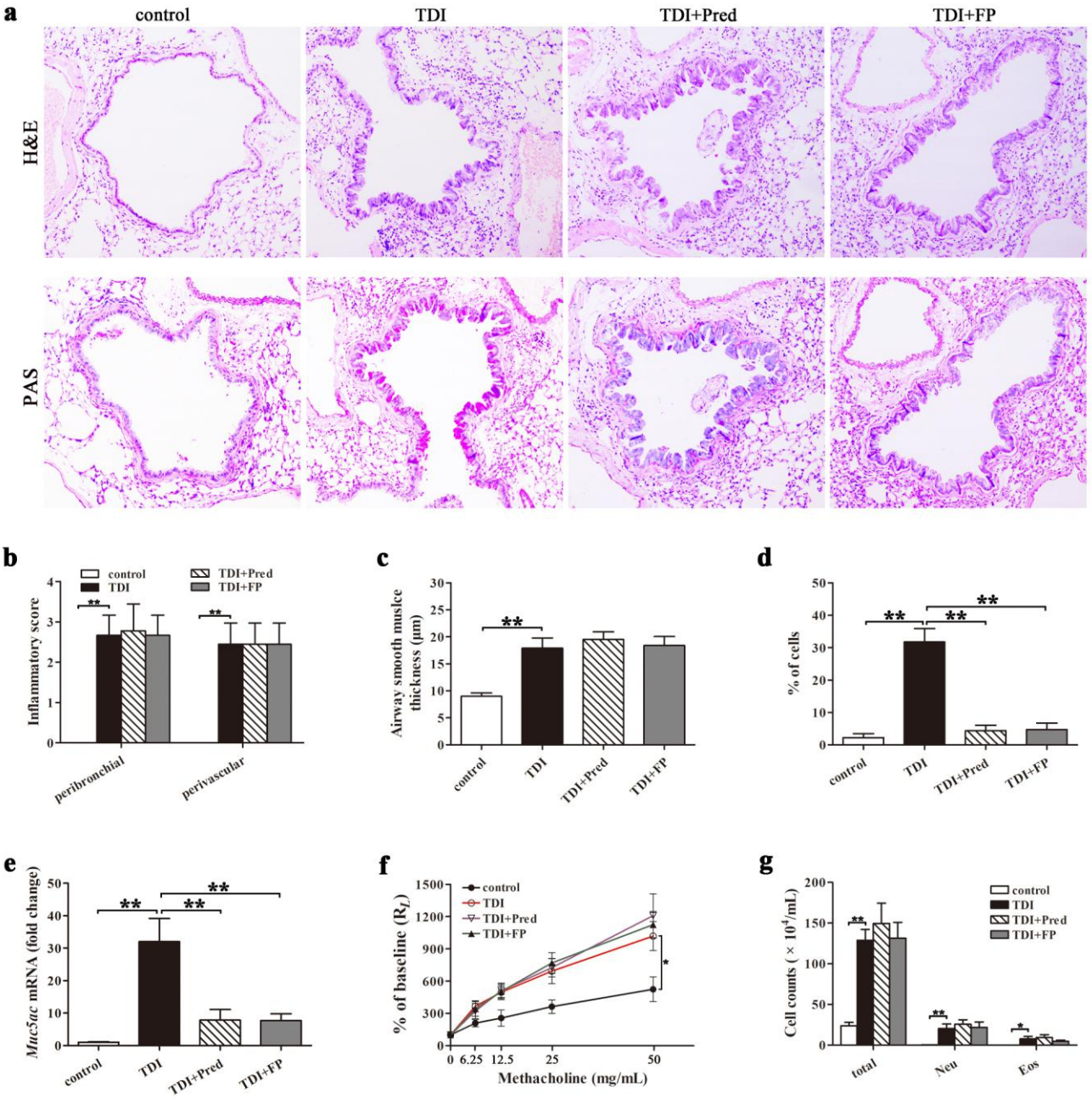
FIGURE LEGENDS

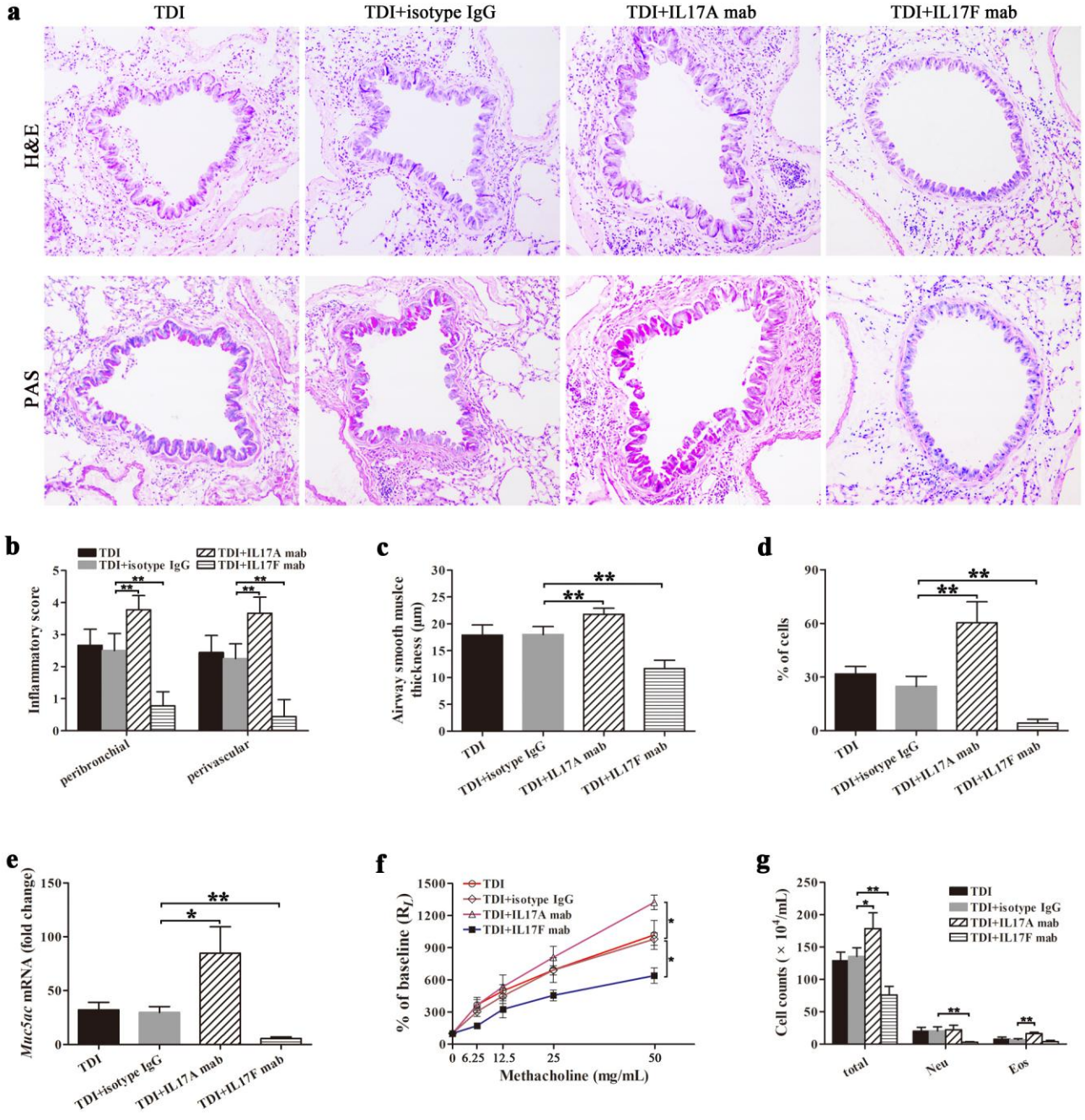
FIG 1. Inhaled fluticasone propionate (FP) or systemic prednisone (Pred) had no effects on TDI-induced airway inflammation and bronchial hyperreactivity. **a,** Representative H&E stained and PAS stained lung sections of different groups. Original magnification was 200×. **b-c,** Semiquantification of airway inflammation and and analysis of airway smooth muscle thickness were performed. n=8~10. **d,** Quantification of PAS-positive staining was determined by counting the number of PAS-positive epithelial cells. n=8~10. **e,** Expression of Muc5ac gene (qPCR) in the lung. n=4. **f,** Airway hyperresponsiveness was measured by lung resistance (R_L). Results were shown as percentage of baseline value. n=5. **g,** Numbers of total inflammatory cells and neutrophils, eosinophils. n=8~10. *p<0.05; **p<0.01.

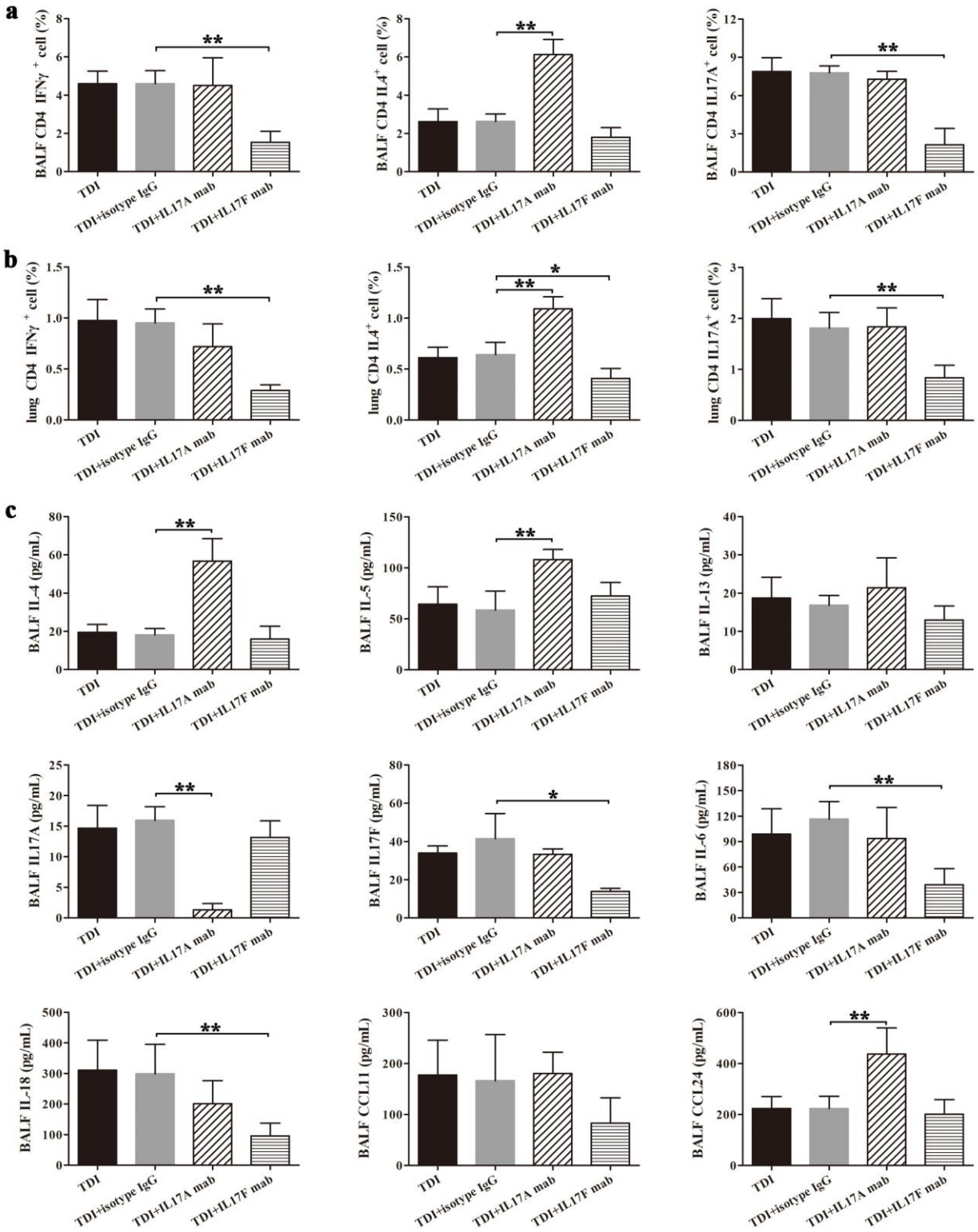
FIG 2. IL-17A monoclonal antibody (IL-17A mab) and IL-17F monoclonal antibody (IL-17F mab) had opposite effects on TDI-induced airway hyperreactivity and bronchial inflammation. **a,** Representative H&E- and PAS- stained lung sections of different groups. Original magnification was 200×. **b-c,** Semiquantification of airway inflammation as well as analysis of airway smooth muscle thickness was performed. n=8~10. **d,** Quantification of PAS-positive staining was determined by counting the number of PAS-positive epithelial cells. n=8~10. **e,** Expression of Muc5ac gene (qPCR) in the lung. n=4. **f,** Airway hyperresponsiveness was measured by lung resistance (R_L). Results were shown as percentage of baseline value. n=5. **g,** Numbers of total inflammatory cells, as well as neutrophils, eosinophils in BALF. n=8~10. *p<0.05; **p<0.01. No significant differences were observed between TDI group and TDI+isotype IgG group.

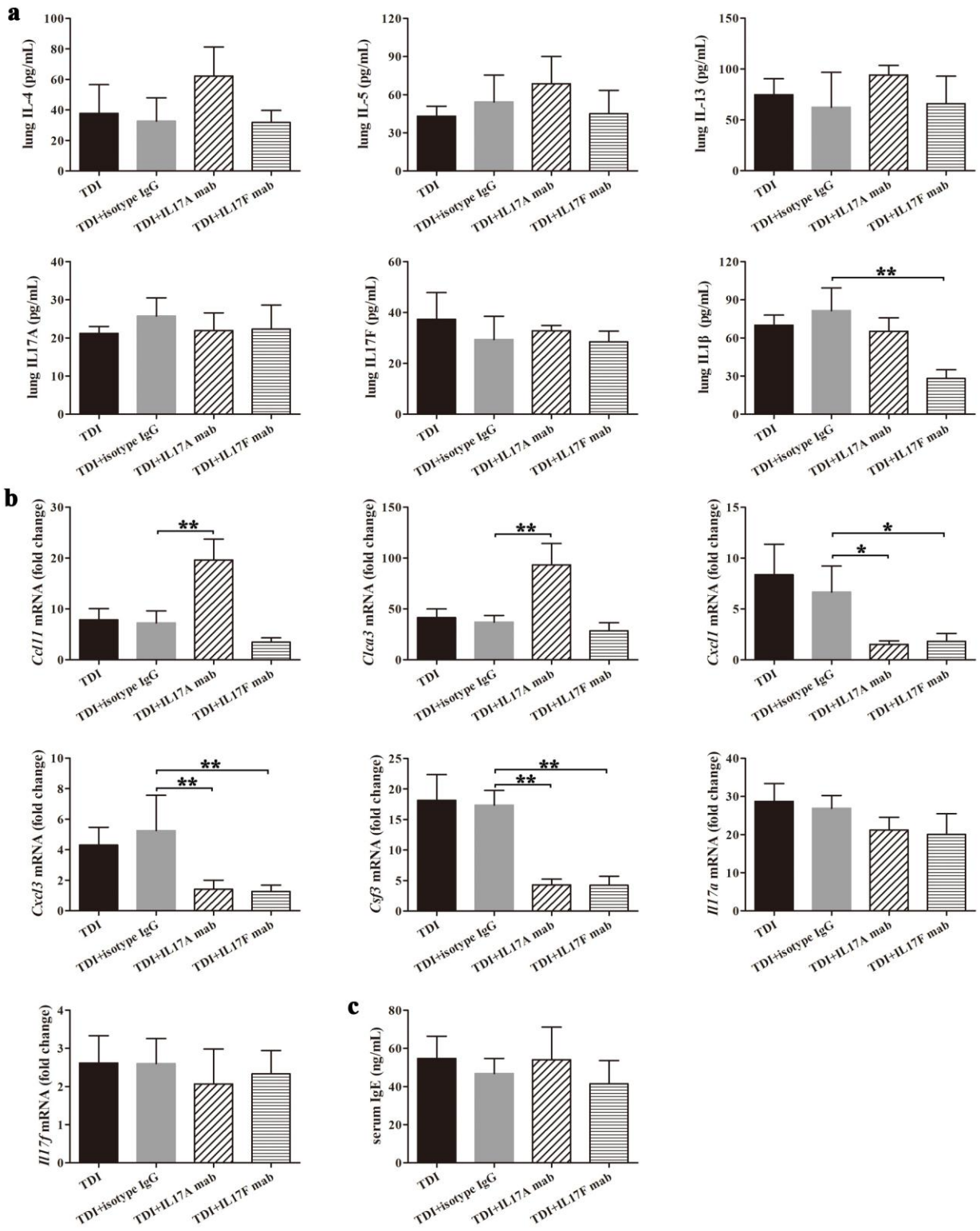
FIG 3. Blocking IL-17A and IL-17F displayed different capacity for orchestrating Th2/Th17 responses. **a,** Percentages of CD4 IFNγ+ (Th1), CD4 IL4+ (Th2), CD4 IL17A+ (Th17) cells in BALF CD4+ cells. n=5. **b,** Percentages of CD4 IFNγ+, CD4 IL4+, CD4 IL17A+ cells in CD4+ cells of lung single cell suspensions. n=5. **c,** Levels of IL-4, IL-5, IL-13, IL-17A, IL-17F, IL-6, IL-18, CCL11 and CCL24 in BALF were quantified by multiplex immunoassays or ELISA. n=8~10. *p<0.05; **p<0.01. No significant differences were observed between TDI group and TDI+isotype IgG group.

FIG 4. Neutralizing IL-17A and IL-17F had different effects TDI-induced pulmonary expression of Th2 and Th17 markers. **a,** IL-4, IL-5, IL-13, IL-17A, IL-17F and IL1β in whole lung homogenates were quantified by multiplex immunoassays or ElISA. n=4. **b,** Whole lung tissue expression of Th2 markers Clca3 and Ccl11, and Th17 markers Cxcl3, Cxcl1, and Csf3, as well as Il17a and Il17f were assessed by qPCR. n=4. **c,** Total serum IgE concentrations were determined. n=8~10. *$p<0.05$; **$p<0.01$. No significant differences were observed between TDI group and TDI+isotype IgG group.


a
control
TDI
TDI+Pred
TDI+FP
H&E
PAS
b
control
TDI
TDI+Pred
TDI+FP
Inflammatory score
peribronchial
perivascular
c
Airway smooth muslce thickness (μm)
control
TDI
TDI+Pred
TDI+FP
d
% of cells
control
TDI
TDI+Pred
TDI+FP
e
Muc5ac mRNA (fold change)
control
TDI
TDI+Pred
TDI+FP
f
control
TDI
TDI+Pred
TDI+FP
% of baseline (RL)
Methacholine (mg/mL)
g
control
TDI
TDI+Pred
TDI+FP
Cell counts (× 10⁴/mL)
total
Neu
Eos


a
TDI
TDI+isotype IgG
TDI+IL17A mab
TDI+IL17F mab
H&E
PAS
b
Inflammatory score
peribronchial
perivascular
c
Airway smooth muslce thickness (μm)
d
% of cells
e
Muc5ac mRNA (fold change)
f
% of baseline (RL)
Methacholine (mg/mL)
g
Cell counts (× 10⁴/mL)
total
Neu
Eos


a
BALF CD4 IFNγ+ cell (%)
BALF CD4 IL4+ cell (%)
BALF CD4 IL17A+ cell (%)
b
lung CD4 IFNγ+ cell (%)
lung CD4 IL4+ cell (%)
lung CD4 IL17A+ cell (%)
c
BALF IL-4 (pg/mL)
BALF IL-5 (pg/mL)
BALF IL-13 (pg/mL)
BALF IL17A (pg/mL)
BALF IL17F (pg/mL)
BALF IL-6 (pg/mL)
BALF IL-18 (pg/mL)
BALF CCL11 (pg/mL)
BALF CCL24 (pg/mL)
TDI
TDI+isotype IgG
TDI+IL17A mab
TDI+IL17F mab


a
lung IL-4 (pg/mL)
lung IL-5 (pg/mL)
lung IL-13 (pg/mL)
lung IL17A (pg/mL)
lung IL17F (pg/mL)
lung IL1β (pg/mL)
b
Cd11 mRNA (fold change)
Clca3 mRNA (fold change)
Cxcl1 mRNA (fold change)
Cxcl3 mRNA (fold change)
Csf3 mRNA (fold change)
Il17a mRNA (fold change)
Il17f mRNA (fold change)
c
serum IgE (ng/mL)
TDI
TDI+isotype IgG
TDI+IL17A mab
TDI+IL17F mab

Online supplementary material

Methods

Mice, allergen and treatments

6~8-week-old male BALB/c mice were purchased from Guangdong Medical Laboratory Animal Center. They were housed under specific pathogen-free conditions and maintained on a 12-hour light-dark cycle with free access to food and water. A TDI-induced asthma model was prepared based on our previous work [1]. In short, BALB/c mice were dermally sensitized with 0.3% TDI on the dorsum of both ears (20 μL per ear) on days 1 and 8. After that, on days 15, 18 and 21, the mice were placed in a horizontal rectangle chamber and challenged for 3 h each time via the airways with 3% TDI through compressed air nebulization (NE-C28, Omron). TDI was diluted in a mixture of 3 volumes of olive oil and 2 volumes of acetone for the sensitization and 4 volumes of olive oil and 1 volume of acetone for the challenge. Control mice were sensitized and challenged with the same amount of vehicle.

Fluticasone propionate (FP, SigmaAldrich, St. Louis, Missouri, US.) was dissolved in sterile 0.05% Tween-20 in PBS and administered i.n. at a dose of 300 μg/kg/mouse in a volume of 20 μL in mice lightly anesthetized with isofluorane. Prednisone (Pred, SigmaAldrich, 5 mg/kg), anti-IL-17A monoclonal antibody (IL-17A mab, eBioscience, 100 μg/mouse), anti-IL-17F monoclonal antibody (IL-17A mab, eBioscience, 100 μg/mouse), or the isotype control antibody (100 μg/mouse) was administered separately via the intraperitoneal (i.p.) route. Recombinant mouse IL-17A or IL-17F (PeproTech, Rocky Hill, NJ) was dissolved in sterile PBS and administered intranasally at the dose of 1 μg/mouse per time. FP and Pred were administrated once daily, beginning immediately after the first challenge to the last day of challenge for a consecutive of seven days; while the monoclonal antibodies and recombinant cytokines were given immediately after each inhalation. FP, Pred, monoclonal antibodies and recombinant cytokines were given at different time points and with different routes of administration because of their intrinsic pharmacodynamics and pharmacokinetic characteristics. These treatments are all aimed to be therapeutic and not prophylactic.

Airway responsiveness measurements

As previously described [2], twenty-four hours after the third inhalation, airway responsiveness was assessed by lung resistance (R_L) measurement (Buxco Electronics, Troy, NY, USA) mice in response to grading doses of aerosolized methacholine (6.25, 12.5, 25 and 50 mg/mL). R_L was recorded every

five minutes following each nebulisation step until a plateau phase was reached. Results were expressed as percentage of baseline value (value at 0 mg/mL methacholine) for each increased concentration of methacholine.

Specimens collection and cell isolation

For bronchoalveolar lavage fluid (BALF), mice were cannulated through the trachea to the left main bronchus and lavaged *in situ* by instilling and retrieving 0.5 mL of sterile saline into the left lung. BALF recovered exclusively from the left lobe was centrifuged at 500 rpm for 10 min at room temperature, and supernatants were stored at -80°C until further use. Whereas the pellet was immediately resuspended in 0.2 ml of sterile saline. Cell counts were then determined for each BALF sample, and differential cell counts were performed on cytospin preparations stained with haematoxylin and eosin (H&E).

To remove the intravascular pool of cells, lungs were perfused with 5 ml of sterile saline via the pulmonary circulation, through catheterization of the right heart. Then, the right lungs (not undergone BAL) were removed.

To prepare single cell suspensions, right lungs were diced using iridectomy scissors. Lungs were enzymatically digested with collagenase (200U/mL, Sigma) in RMPI 1640 medium for 30 minutes at 37°C. Pulmonary cell suspensions were obtained by grinding the tissue through 100 μm nylon sieves, and red blood cells were lysed using ammonium chloride buffer. Cell counts were then performed before staining for flow cytometry analysis.

Flow cytometry analysis

Bronchoalveolar lavage fluid and lung single-cell suspensions were fixed, permeabilized (Cytofix/Cytoperm buffer; BD Biosciences) and stained for 30 min with antibodies for CD4, IFNγ, IL-4, IL-17A (BD Biosciences). All appropriate isotype controls were used. Data were collected on a BD Biosciences Fortessa flow cytometer and analyzed using FlowJo software.

Histopathological analysis

The left lungs were harvested, fixed overnight in 4% neutral formalin and embedded in paraffin. Sections (4 μm) were stained with H&E to show morphological changes and inflammation. As previously described [1], two criteria were scored to quantify airway inflammation: peribronchial inflammation and perivascular inflammation. A value of 0 was allocated when no inflammation was detectable, a value of 1 for occasional cuffing with inflammatory cells, a value of 2 for most bronchi

or vessels surrounded by one layer (1 to 10 cells) of inflammatory cells, a value of 3 for most bronchi or vessels were surrounded by 2 layers (10 to 20 cells) of inflammatory cells and a value of 4 for most bronchi or vessels were surrounded by more than two layers (more than 20 cells) of inflammatory cells. Thickness of airway smooth muscle was measured by a modification of Cho JY's method [5]. Briefly, the thickness of the peribronchial smooth muscle layer (the transverse diameter) in large airways was measured from the innermost aspect to the outermost aspect of the circumferential smooth muscle layer. 20~24 image fields of eight sections from 8~10 mice per group were analyzed.

To visualize airway mucus production, sections were stained with periodic acid-Schiff base (PAS) and semi-quantified. PAS-positive epithelial cells of the total epithelial cells were counted to obtain a percentage and compare groups. 16~20 image fields of eight sections from 8~10 mice per group were analyzed.

Cytokine and chemokine analysis

Levels of cytokines and chemokines in BALF and lung homogenates, including Th2-related IL-4, IL-5, IL-13, Th17-related IL-17A, IL-17F, Th17 cell maturation associated IL1β, IL-6 [3, 4], neutrophil chemokine IL-18 [6], as well as eosinophil attractants CCL11 and CCL24, were measured using multiplex immunoassay or ELISA kits (eBioscience) according to Manufacturer's specifications.

Gene expression analysis

Lung tissue was homogenized in TRIzol Reagent (Takara, Guangzhou, China). Total RNA were extracted with an RNAiso Plus kit (Takara) and reverse-transcribed to complementary DNA using PrimeScript^{TM} RT reagent kit (Takara). Gene expression was quantified using SYBR Green Premix Ex Taq (Takara) by LightCycler 480 Fast Real-Time PCR System. The primers used were listed in Table 1.

TABLE 1. Murine qPCR primer sequences

Gene	Forward sequence (5'→3')	Reverse sequence (5'→3')
Gapdh	AAGAGGGATGCTGCCCTTAC	CCAATACGGCCAAATCCGTTC
Muc5ac	CAGGACTCTCTGAAATCGTACCA	AAGGCTCGTACCACAGGGA
Il17a	GAGAGCTTCATCTGTGTCTCTG	GCGCCAAGGGAGTTAAAGAC
Il17f	CGTGAAACAGCCATGGTCAAG	GGGACAGAAATGCCCTGGTT
Ccl11	TGCTCACGGTCACTTCCTTC	CTTGAAGACTATGGCTTTCAGGGTG
Clca3	AGGAAAACCCCAAGCAGTG	GCACCGACGAACTTGATTTT
Cxc11	AACCGAAGTCATAGCCACACT	CCGTTACTTGGGGACACCTT
Cxcl3	CACCCAGACAGAAGTCATAGCC	CCGTTGGGATGGATCGCTTT
Csf3	GTGCTGCTGGAGCAGTTGT	TCGGGATCCCCAGAGAGT

References

1. Yao L, Zhao H, Tang H, Liang J, Liu L, Dong H, et al. The receptor for advanced glycation end products is required for β-catenin stabilization in a chemical-induced asthma model. Br J Pharmacol 2016;173(17):2600-13.

2. Yao L, Zhao H, Tang H, Xiong J, Zhao W1, Liu L, et al. Blockade of β-catenin signaling attenuates toluene diisocyanate-induced experimental asthma. Allergy 2017;72(4):579-589.

3. Revu S, Wu J, Henkel M, Rittenhouse N, Menk A, Delgoffe GM, et al. IL-23 and IL-1β Drive Human Th17 Cell Differentiation and Metabolic Reprogramming in Absence of CD28 Costimulation. Cell Rep 2018;22(10):2642-2653.

4. Heink S, Yogev N, Garbers C, Herwerth M, Aly L, Gasperi C, et al. Trans-presentation of IL-6 by dendritic cells is required for the priming of pathogenic TH17 cells. Nat Immunol 2017;18(1):74-85.

5. Cho J1, Miller M, McElwain K, McElwain S, Broide DH. Combination of corticosteroid therapy and allergen avoidance reverses allergen-induced airway remodeling in mice. J Allergy Clin Immunol 2005;116(5):1116-22.

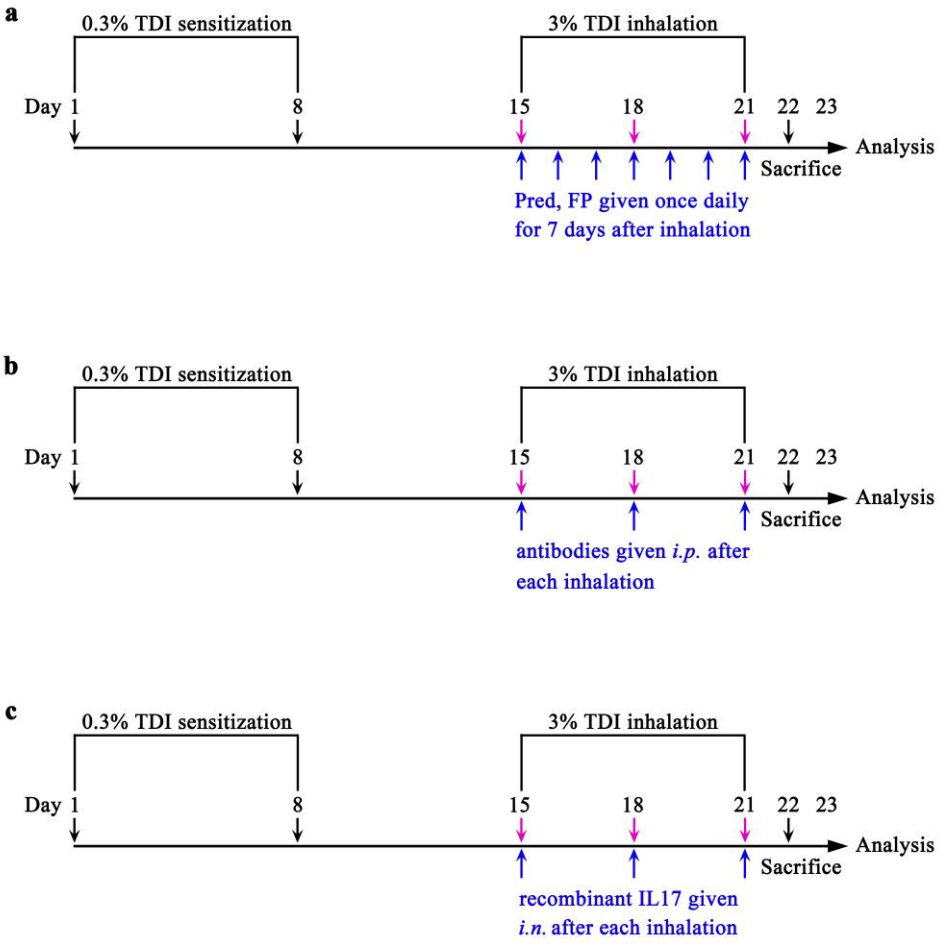
6. Leung BP, Culshaw S, Gracie JA, Hunter D, Canetti CA, Campbell C, et al. A role for IL-18 in neutrophil activation. J Immunol 2001;167(5):2879-86.

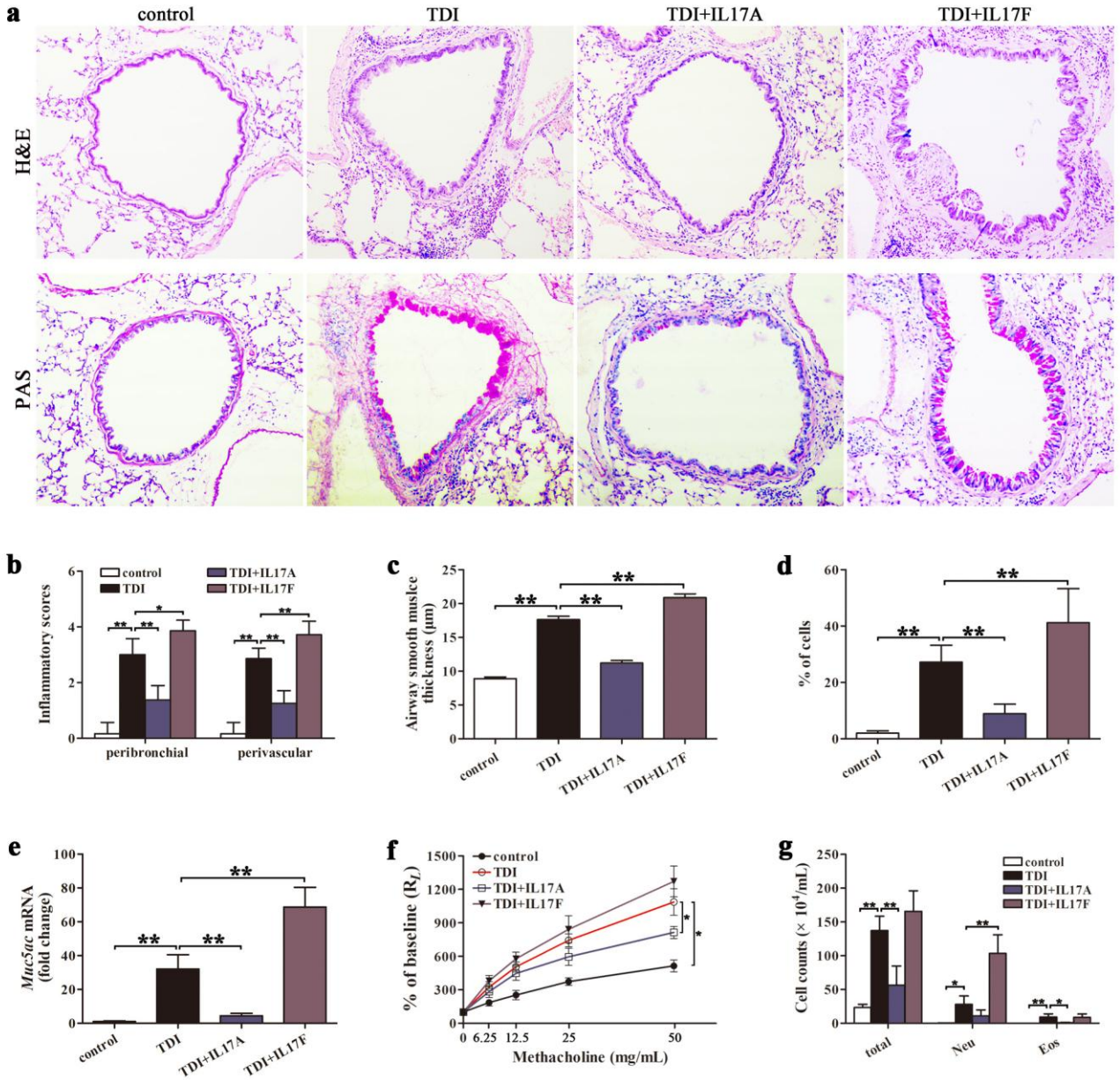
FIGURE LEGENDS

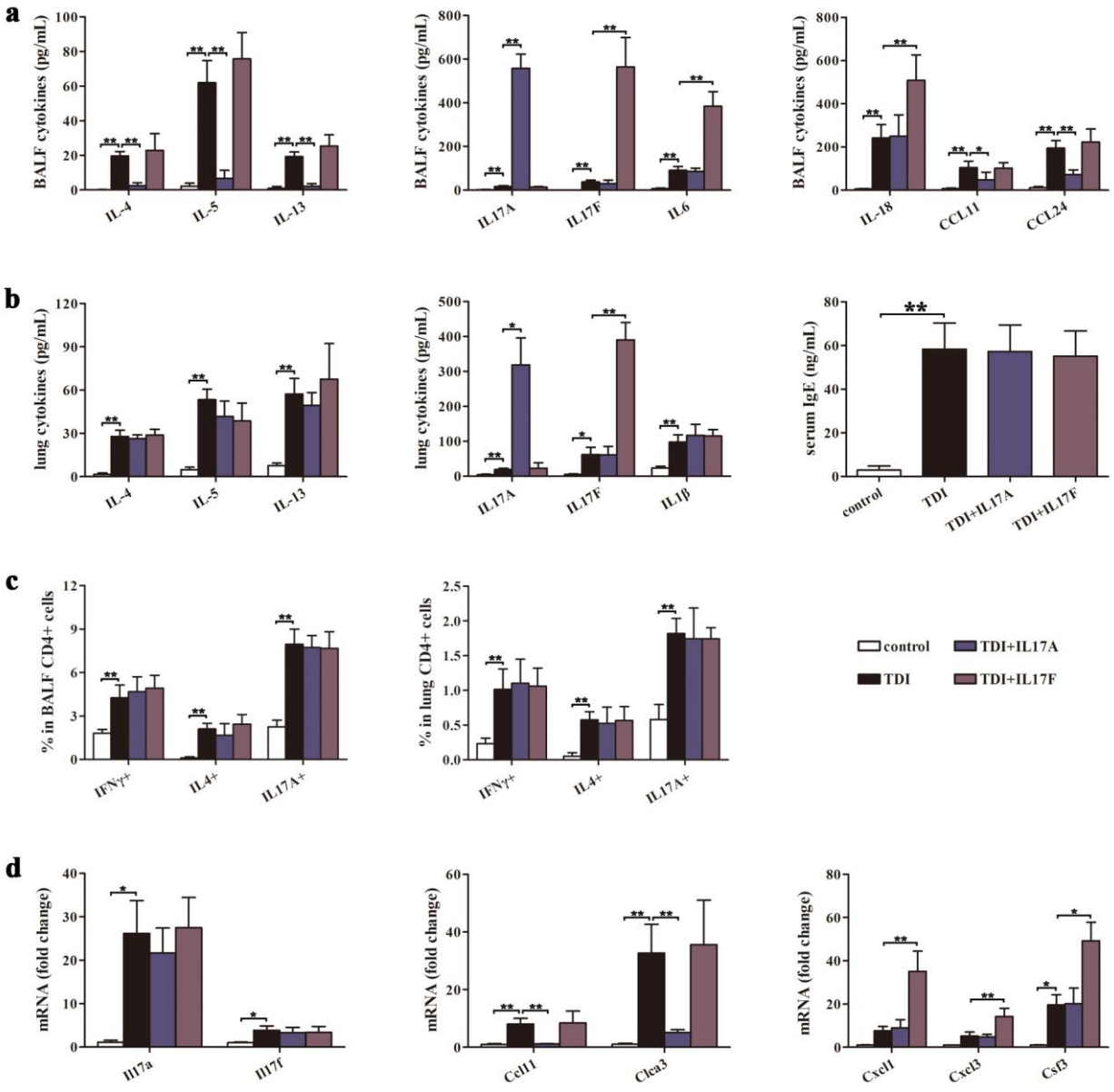
FIG S1. Experimental treatment schedules. **a,** Mice were dermally sensitized with 0.3% TDI on days 1 and 8. On days 15, 18 and 21, the mice were challenged with 3% TDI through compressed air nebulization. Prednisone (Pred, 5 mg/kg) and Fluticasone propionate (FP, 300 μg/kg) were respectively given to the mice through intraperitoneal injection or intranasal instillation once daily, beginning immediately after the first challenge to the last day of challenge for a consecutive of seven days. **b,** Mice were dermally sensitized and challenged with TDI. Anti-IL-17A monoclonal antibody, anti-IL-17F monoclonal antibody, or the isotype control antibody was administered separately via the intraperitoneal route at the dose of 100 μg/mouse per time immediately after each airway challenge. **c,** Mice were dermally sensitized and challenged with TDI. Recombinant IL-17A or IL-17F was administered intranasally at the dose of 1 μg/mouse per time immediately after each airway challenge.

FIG S2. Recombinant IL-17A and IL-17F had different effects on TDI-induced pathological changes as well as airway neutrophilic and eosinophilic inflammation. **a,** Representative H&E- and PAS-stained lung sections of different groups. Original magnification was 200×. **b-c,** Semiquantification of airway inflammation and analysis of airway smooth musle thickness were performed. n=8~10. **d,** Quantification of PAS-positive staining was determined by counting the number of PAS-positive epithelial cells. n=8~10. **e,** Expression of Muc5ac gene (qPCR) in the lung. n=4. **f,** Airway hyperresponsiveness was measured by lung resistance (R_L). Results were shown as percentage of baseline value. n=5. **g,** Numbers of total inflammatory cells and neutrophils, eosinophils in BALF. n=8~10. *p<0.05; **p<0.01.

FIG S3. Exogenous IL-17A and IL-17F showed distinct roles for TDI-induced Th2 and Th17 responses. **a-b,** Levels of different cytokines in BALF or lung homogenates were quantified by multiple immnoassays or ELISA. n=6~8. **c,** Percentages of CD4 IFNγ+ (Th1), CD4 IL4+ (Th2), CD4 IL17A+ (Th17) cells in BALF or lung CD4+ cells. n=5. **d,** Whole lung tissue expression of Th2 markers Clca3 and Ccl11, Th17 markers Cxcl3, Cxcl1, and Csf3, as well as Il17a, Il17f were assessed by qPCR. n=4. *p<0.05; **p<0.01.


a
0.3% TDI sensitization
3% TDI inhalation
Day 1
8
15
18
21
22
23
Analysis
Sacrifice
Pred, FP given once daily
for 7 days after inhalation
b
0.3% TDI sensitization
3% TDI inhalation
Day 1
8
15
18
21
22
23
Analysis
Sacrifice
antibodies given i.p. after
each inhalation
c
0.3% TDI sensitization
3% TDI inhalation
Day 1
8
15
18
21
22
23
Analysis
Sacrifice
recombinant IL17 given
i.n. after each inhalation


a
control
TDI
TDI+IL17A
TDI+IL17F
H&E
PAS
b
control
TDI
TDI+IL17A
TDI+IL17F
Inflammatory scores
peribronchial
perivascular
c
Airway smooth muslce thickness (μm)
control
TDI
TDI+IL17A
TDI+IL17F
d
% of cells
control
TDI
TDI+IL17A
TDI+IL17F
e
Muc5ac mRNA (fold change)
control
TDI
TDI+IL17A
TDI+IL17F
f
control
TDI
TDI+IL17A
TDI+IL17F
% of baseline (RL)
Methacholine (mg/mL)
g
control
TDI
TDI+IL17A
TDI+IL17F
Cell counts (× 10⁴/mL)
total
Neu
Eos


a
BALF cytokines (pg/mL)
IL-4
IL-5
IL-13
IL17A
IL17F
IL6
IL-18
CCL11
CCL24
b
lung cytokines (pg/mL)
IL1β
serum IgE (ng/mL)
control
TDI
TDI+IL17A
TDI+IL17F
c
% in BALF CD4+ cells
% in lung CD4+ cells
IFNγ+
IL4+
IL17A+
control
TDI
TDI+IL17A
TDI+IL17F
d
mRNA (fold change)
Il17a
Il17f
Ccl11
Clca3
Cxcl1
Cxcl3
Csf3