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The Translational Repressor T-cell Intracellular Antigen-1 (TIA-1) is a Key Modulator of Th2 and Th17 Responses Driving Pulmonary Inflammation Induced by Exposure to House Dust Mite

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Abstract

T-cell Intracellular Antigen-1 (TIA-1) is a translational repressor that dampens the production of proinflammatory cytokines and enzymes. In this study we investigated the role of TIA-1 in a mouse model of pulmonary inflammation induced by exposure to the allergenic extract (*Df*) of the house dust mite *Dermatophagoides farinae*. When intranasally challenged with a low dose of *Df*, mice lacking TIA-1 protein (*Tia-1*^{-/-}) showed more severe airway and tissue eosinophilia, infiltration of lung bronchovascular bundles, and goblet cell metaplasia than wild-type littermates. *Tia-1*^{-/-} mice also had higher levels of *Df*-specific IgE and IgG₁ in serum and *ex vivo* restimulated *Tia-1*^{-/-} lymph node cells and splenocytes transcribed and released more Th2/Th17 cytokines. To evaluate the site of action of TIA-1, we studied the response to *Df* in bone marrow chimeras. These experiments revealed that TIA-1 acts on both hematopoietic and non-hematopoietic cells to dampen pulmonary inflammation. Our results identify TIA-1 as a negative regulator of allergen-mediated pulmonary inflammation *in vivo*. Thus, TIA-1 might be an important player in the pathogenesis of bronchial asthma.

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Keywords

T-cell Intracellular Antigen-1; allergen-mediated pulmonary inflammation; cytokines; translation

1. Introduction

Asthma is a common syndrome characterized by reversible airflow obstruction, airway hyperresponsiveness, and chronic inflammation of the bronchial mucosa [1–2]. Asthma is commonly associated with atopy, the tendency to form IgE to innocuous environmental proteins (allergens) [3]. At least some of the lower airway pathology in asthma is thought to be driven by the adaptive immune response to allergens, with house dust mite allergens being the most prevalent environmental proteins to which asthmatic subjects are sensitized [4–5]. The presence of IgE specific for dust mite and other allergens permits immediate-type hypersensitivity responses in the respiratory mucosa in response to allergen exposure. Additionally, allergen-specific T cells generate cytokines that initiate and amplify the characteristic pathologic features [6–8]. These features (eosinophilia, neutrophilia, goblet cell metaplasia, and remodeling) are thought to reflect contributions from both T helper (Th)2 (IL-4, IL-5, IL-13) and Th17 (IL-17A, IL-17F)-type cytokines, all of which are readily demonstrable in biopsies from the airways of asthmatic individuals [9–12], and all of which are induced by mouse models of airway disease triggered by sensitization and challenge to antigen [13–17].

Cytokine generation is essential for the development of specific immune responses and clearance of pathogens, but has the potential to cause disease if dysregulated or prolonged [18–19]. Thus, the profile and magnitude of cytokines generated in response to a stimulus is controlled by several mechanisms. Transcription factors determine the development of specific Th1 (T bet), Th2 (GATA3) and Th17 (RoryT) subsets with specialized profiles of cytokine generation in response to cell activation [20–23]. Posttranscriptional control of nuclear export, cytoplasmic localization, translation initiation, and degradation of cytokine transcripts also plays a major role in setting the level of cytokine production [24–26]. Most posttranscriptional control mechanisms dampen cytokine expression as a check on the overproduction of potentially injurious inflammatory mediators [24–26]. A common type of posttranscriptional control is mediated by adenine/uridine-rich elements (AREs) found in the 3' untranslated regions (UTRs) of cytokine transcripts that recruit one or more ARE-binding proteins (ARE-BPs) to regulate the translation and stability of specific transcripts [25, 27–28]. These posttranscriptional control mechanisms are critical determinants of inflammatory mediator production in cells of both the innate and adaptive immune systems [24–28].

T-cell intracellular antigen-1 (TIA-1) is an ARE-BP that functions as a translational silencer [29]. TIA-1 binds to AREs in the 3' UTRs of mRNAs that encode inflammatory mediators such as tumor necrosis factor (TNF)- α [29]. TIA-1 also targets mRNAs that encode pro-inflammatory enzymes such as cyclooxygenase-2 (COX-2) [30–31]. Binding of TIA-1 inhibits translation initiation by a partially characterized mechanism [32]. Recent data implicate TIA-1 and TIAR proteins as key factors in the translation regulation of mRNAs bearing 5'-terminal oligopyrimidine tracts (5'TOPs) [33–34].

Because inhibition of translation initiation prevents the assembly of polysomes, TIA-1 displaces its associated transcripts into non-polysomal ribonucleoprotein (RNP) particles [32, 35]. By coordinately repressing the translation of multiple inflammatory mediators, TIA-1 applies a posttranscriptional “brake” that prevents pathological inflammation [25]. Mutant mice lacking TIA-1 (*Tia-1*^{-/-} mice) have a profound inflammatory diathesis. They are markedly more susceptible to lipopolysaccharide (LPS)-induced septic shock than WT

controls [29]. Moreover, *Tia-1*^{-/-} mice develop spontaneous arthritis, associated with pathological overexpression of TNF- α [36]. TIA-1 is widely expressed in hematopoietic and non-hematopoietic cells [37]. Whereas LPS-activated *Tia-1*^{-/-} macrophages overproduce TNF- α , T cell receptor-activated *Tia-1*^{-/-} T cells do not [38]. TIA-1, however, represses the translation of IL-4 and IL-13 in T cell receptor-activated T cells [39]. Thus, the effects of TIA-1 are cell-type and transcript specific.

In this study, we sought to determine the role of TIA-1 in the control of pulmonary inflammation induced by the allergenic extract (*Df*) derived from the house dust mite, *Dermatophagoides farinae*. The *Df* extract is biologically complex, can activate cells of the innate immune system to initiate the immune response [4, 40–41], and breaks tolerance through the respiratory mucosa, potentially more closely mimicking the pathophysiology of atopic sensitization in humans than traditional models using systemic immunization protocols with exogenous adjuvants [42]. Here we show that TIA-1 exerts major control over the expression of cytokines in parabronchial lymph nodes, thus dampening the Th2 and Th17, but not Th1, responses elicited by the allergen and leading to exaggeration of pulmonary pathology. We thus suggest that post-transcriptional control mechanisms operated by TIA-1 may contribute substantially to the pathogenesis of bronchial asthma.

2. Material and methods

2.1 *Df*-induced pulmonary inflammation

C57BL/6 wild type (WT) and *Tia-1*^{-/-} [29] littermate male mice were housed under specific pathogen-free conditions and maintained on a 12-hour light/dark cycle. On days 0, 4, 7, 11, 14, and 18, seven- to nine week-old WT and *Tia-1*^{-/-} mice were lightly anesthetized and treated intranasally with 1 μ g of protein extract from the dust mite *Dermatophagoides farinae* (*Df*; Greer Laboratories, Lenoir, NC) dissolved in 20 μ l of endotoxin-free NaCl 0.9% (Sigma, St. Louis, MO) or the saline alone [15].

Twenty-four hours after the last treatment, NaCl- or *Df*-treated mice were euthanized by i.p injection of an overdose of sodium pentobarbital (Sigma) and were exsanguinated. Mice were then cannulated with a polyethylene tube (22G; Terumo Medical Corporation, Elkton, MD), lungs were washed three times with 0.7 ml of ice-cold PBS containing 10% fetal bovine serum and 0.5 mM EDTA. In >95% of the mice, the volume recovered was consistently 1.9 – 2.0 ml. The recovered bronchoalveolar lavage (BAL) was centrifuged, the cells were separated from the fluid and cytocentrifuged onto slides and stained with Diff-quick (Fisher Diagnostic, Middletown, VA). At least 200 leukocytes on the cytospin preparations were counted from non-adjacent optical fields and classified as mononuclear cells (macrophages and lymphocytes), neutrophils, or eosinophils, according to the standard hemocytological criteria. All animal studies described in this paper were approved by the Animal Care and Use Committee of the Dana Farber Cancer Institute (Boston, MA).

2.2 Lung histology

Left lungs from NaCl- and *Df*-treated mice were fixed for at least 8 h in 4% paraformaldehyde in 0.1 M sodium phosphate (pH 7.6), washed twice with PBS containing 2% DMSO, suspended in 50 mM NH₄Cl overnight at 4°C and embedded in glycolmethacrylate as described previously [43]. Two-micrometer-thick glycolmethacrylate sections were stained by the chloroacetate esterase (CAE) reaction to assess inflammatory cell infiltrates, the periodic acid Schiff (PAS) reaction to depict mucus-secreting epithelial cells (goblet cells) or with Congo red dye to highlight eosinophil infiltrates.

The extent of cellular infiltration of the tissue was evaluated by a pathologist without knowledge of the particular mouse strain or procedure on fifteen bronchovascular bundles

(BVBs) of comparable large-caliber preterminal bronchi (diameter 200–220 μM) from the lung sections obtained for each mouse in the different experimental groups. The goblet cells positively stained for the presence of mucus were enumerated in at least four independent BVBs of each lung. Data were expressed as the average of goblet cell counts stained in each bronchus of each section per millimeter of bronchial basal lamina, as measured by Image J (National Institutes of Health image analysis software [<http://rsbweb.nih.gov/ij/>]) [15].

2.3 Measurement of total and Df-specific serum Igs

Mice were bled and sera were collected and saved at -80°C until assayed. To measure Df-specific IgG1 and IgE a 96 well plate was coated with Df (5 $\mu\text{g}/\text{ml}$) and incubated at 4°C overnight. The plates were blocked with a buffer containing serum (assay buffer; eBioscience, San Diego, CA) for 2 hours, then serial dilutions of sera were incubated for 2 hours at room temperature (for Df-specific IgG1), or overnight at 4°C (for Df-specific IgE). Immunoglobulin isotypes were measured by using either alkaline phosphatase-conjugated anti-mouse IgG1 (1:2500, Southern Biotech, Birmingham, AL) followed by substrate p-nitrophenyl phosphate (read at 405 nm), or biotinylated anti-mouse IgE (monoclonal clone R35–118 at 2 $\mu\text{g}/\text{ml}$, BD Biosciences, Franklin Lakes, NJ) and streptavidin-horseradish peroxidase, followed by substrate 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (read at 405 nm).

2.4 *In vitro* restimulation of splenocytes and lymph node cells with Df

Spleens and parabrachial lymph nodes (PLNs) were collected from each mouse and separately homogenized through a 70- μm nylon mesh (BD Biosciences) in complete medium [RPMI-1640 containing 10% FBS (Sigma), 1% non-essential amino acids (Sigma), 2 mM L-Glutamine (Sigma), 100 U/ml penicillin and 100 $\mu\text{g}/\text{ml}$ streptomycin (Sigma), 25 mM HEPES (Mediatech-Cellgro, Manassas, VA), 1 mM sodium pyruvate (Mediatech-Cellgro), 50 μM β -mercaptoethanol (Sigma)]. The red cells were lysed with RBC lysis buffer (154 mM NH_4Cl , 10 mM KHCO_3 , 125 μM EDTA) and the total number of cells was determined. Splenocytes and PLN cells were seeded at final concentration of $4 \times 10^6/\text{ml}$ and stimulated (72 h, 37°C) with Df 20 $\mu\text{g}/\text{ml}$. At the end of the incubation, supernatants were collected to evaluate cytokine (IL-4, IL-5, IL-13, IL-17A, and IFN- γ) release by specific ELISA (eBiosciences).

At the end of the culture, the rate of apoptosis in WT and *Tia-1*^{-/-} splenocytes, measured as binding of FITC-conjugated Annexin V (BD Biosciences), was assayed by flow cytometry on a FACSCanto™ flow cytometer (BD Biosciences) and data were analyzed with FlowJo software (Tree Star, Ashland, OR).

2.5 Generation of bone marrow chimeras

Five-week old sex-matched WT and *Tia-1*^{-/-} mice were lethally irradiated with 1200 Rads (12 Gy) in 2 splitted doses, 4 hours apart. Within 24 hours from the irradiation, the bone marrow (BM) of WT and *Tia-1*^{-/-} donors was collected and 10^7 nucleated cells were infused via the tail vein into sex-matched irradiated mice in 200 μl of PBS. As a result of the bone marrow transfer, four groups of chimeric mice were generated: WT BM into WT mice (WT $\rightarrow$ WT), *Tia-1*^{-/-} $\rightarrow$ WT, WT $\rightarrow$ *Tia-1*^{-/-}, *Tia-1*^{-/-} $\rightarrow$ *Tia-1*^{-/-}. For the first eight weeks after the injection of BM cells, chimeric mice were fed with drinking water supplemented with enrofloxacin (Baytril®, Bayer HealthCare, Leverkusen, Germany). Ten weeks after the injection, mice were exposed to six doses of NaCl or Df according to the same protocol described above and euthanized 24 h after the last instillation. Before the beginning of treatment with NaCl or Df, peripheral blood was drawn from sample mice of each group for the evaluation of complete blood count.

2.6 Statistics

Data are expressed as mean $\pm$ SEM from the indicated number of experiments. Significance was determined with a two-tailed heteroscedastic Student's t-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3. Theory

TIA-1 represses the translation of IL-4 and IL-13 in T cell receptor-activated T cells. IL-4 and IL-13 are cytokines critically involved in asthma pathogenesis. In this study, we sought to determine the role of TIA-1 in the control of pulmonary inflammation induced by the allergenic extract (*Df*) derived from the house dust mite, *Dermatophagoides farinae*.

4. Results

4.1 *Df*-induced pulmonary inflammation is increased in *Tia-1*^{-/-} mice

To investigate the role of TIA-1 in the pulmonary inflammatory response induced by exposure to the allergenic extract (*Df*) of the house dust mite *Dermatophagoides farinae*, C57BL/6 mice lacking TIA-1 protein (*Tia-1*^{-/-}) and their wild-type (WT) littermates were administered a low dose of the allergen (1 μ g of protein in 20 μ l of NaCl 0.9 %) intranasally, twice weekly for three consecutive weeks. Because we anticipated that TIA-1 would act as a suppressor of inflammation in this model, we chose the 1 μ g dose of *Df* so as to elicit minimal inflammation in the WT C57BL/6 mice. Cohorts of WT mice and *Tia-1*^{-/-} mice were simultaneously treated with NaCl 0.9 % alone, as a control. Twenty-four hours after the last instillation, mice were euthanized and cannulated to collect the bronchoalveolar lavage (BAL) and the lungs were examined histologically.

Compared to WT mice that received saline, WT mice treated with the allergen showed an increased total number of cells in the airways (41.41 ± 2.21 vs. $20.09 \pm 1.00 \times 10^4$, $p < 0.001$; Fig. 1A), with a slight increase in the total number of mononuclear cells (monocytes/macrophages and lymphocytes), a mild neutrophilia and a moderate eosinophilia (Fig. 1B). The numbers of BAL fluid cells recovered from saline-treated *Tia-1*^{-/-} mice were similar to those recovered from the naïve WT controls. With *Df* treatment, the number of cells in BAL of *Tia-1*^{-/-} mice was significantly higher than in the WT (60.00 ± 2.78 vs. $41.41 \pm 2.21 \times 10^4$, $p < 0.001$; Fig. 1A). The difference in the number of cells retrieved from the BAL was associated with a 2-fold increase of eosinophils (22.90 ± 2.59 vs. $11.37 \pm 1.64 \times 10^4$, $p < 0.001$; Fig. 1B) and a modest but significant increase in the number of mononuclear cells (36.09 ± 1.54 vs. $28.80 \pm 1.17 \times 10^4$, $p < 0.001$) in *Tia-1*^{-/-} mice. The numbers of neutrophils were similar between the two strains (Fig. 1B).

Consistent with the data from BAL analysis, histological evaluation of lung tissue revealed bronchovascular inflammation in mice that received *Df*, but not in those treated with saline. The infiltrates consisted of Congo red staining eosinophils, neutrophils, lymphocytes and plasma cells surrounding BVVs (Fig. 2A and 2B). Moreover, the administration of *Df* induced the metaplasia of mucus-producing goblet cells (Fig. 2A-g,h; arrows). These features were even more evident in the lungs of mice treated with *Df*, while the histology of saline-treated *Tia-1*^{-/-} mice were similar to the WT controls (Fig. 2A and 2B). Morphometric analysis confirmed that *Df*-treated *Tia-1*^{-/-} mice have significantly higher numbers of inflamed BVVs than WT controls (11.52 ± 0.43 vs. $7.95 \pm 0.48/15$ BVVs, $p < 0.001$; Fig. 2C). Similarly, *Tia-1*^{-/-} mice have ~70% more mucus-producing goblet cells (measured as the number of cells staining with periodic acid Schiff per millimeter of bronchial basal lamina) than WT controls (34.68 ± 2.10 vs. $20.24 \pm 1.96/\text{mm}$, $p < 0.001$; Fig. 2C).

4.2 The increased *Df*-induced pulmonary inflammation in *Tia-1*^{-/-} mice is associated with the increase of Th2- and Th17-polarized immune responses

Next, we sought to determine whether increased *Df*-induced pulmonary inflammation in *Tia-1*^{-/-} mice correlated with an effect of TIA-1 protein on the production of the inflammatory cytokines that drive allergic inflammation in the lungs. Splenocytes and PLN cells from *Df* treated WT and *Tia-1*^{-/-} mice were restimulated *in vitro* with *Df* (at 20 µg/ml for 72 h), and cytokines in the supernatants were detected by ELISA.

As shown in Figure 3, splenocytes from *Tia-1*^{-/-} mice released significantly higher levels of IL-4, IL-13, and IL-17A, but not IFN-γ, than WT controls (Fig. 3A). Splenocytes from *Tia-1*^{-/-} mice also released higher levels of IL-5 but in this case the increase was not significant. PLN cells from *Tia-1*^{-/-} mice released higher levels of IL-5, IL-13 and IL-17A, but not IFN-γ, than WT controls (Fig. 3B). However IL-17A was the only cytokine that was significantly increased in the PLN *Tia-1*^{-/-} group. These results indicate that TIA-1 normally dampens the expression of some Th2 and Th17 cytokines in *Df*-challenged lung.

In order to confirm that the differences in cytokine release between *Df*-restimulated WT and *Tia-1*^{-/-} lymphoid cells were not reflecting a different viability *in vitro*, at the end of the incubation with *Df*, splenocytes from the two strains were separated from the supernatants, washed and stained with FITC-conjugated annexin V. The binding of FITC-annexin V to WT and *Tia-1*^{-/-} cells was evaluated by flow cytometry. The results demonstrated that WT and *Tia-1*^{-/-} splenocytes had a similar percentage of apoptotic cells after being cultured with *Df* for 72 h (data not shown).

We also evaluated whether the altered response of the adaptive immune system to *Df* in *Tia-1*^{-/-} mice also affected the humoral compartment and the synthesis of immunoglobulins (Igs). Serum was collected from WT and *Tia-1*^{-/-} mice exposed to NaCl or *Df* and *Df*-specific IgE and *Df*-specific IgG₁ were measured by ELISA. In both strains, treatment with *Df* led to the synthesis of antigen-specific IgE and IgG₁. In accordance with an increased Th2 response in *Tia-1*^{-/-} mice, this group of mice had significantly higher *Df*-IgE and *Df*-IgG₁ compared to WT (0.106 ± 0.015 vs. 0.066 ± 0.009 p < 0.05 and 0.793 ± 0.061 vs. 0.516 ± 0.058 p < 0.001 O.D. at 405 nm, respectively; Fig. 4B).

4.3 Both parenchymal and hematopoietic cells contribute to the increased inflammatory response to *Df* in *Tia-1*^{-/-} mice

To determine the relative contribution of TIA-1 in hematopoietic cells *versus* lung parenchymal cells in response to intranasally administered *Df*, we generated radiation-induced chimeric mice. Irradiated WT and *Tia-1*^{-/-} mice were reconstituted with bone marrow (BM) from WT and *Tia-1*^{-/-} mice in order to produce 4 groups of chimeric mice: WT BM into WT mice (WT → WT), WT → *Tia-1*^{-/-}, *Tia-1*^{-/-} → WT, and *Tia-1*^{-/-} → *Tia-1*^{-/-}. Ten weeks after the reconstitution a complete blood cell count was performed to verify the reconstitution of peripheral blood cells and mice were exposed to six doses of NaCl 0.9% or *Df* (1 µg/dose) over three weeks. Twenty four hours after the last instillation mice were euthanized and analyzed as described above. First, we evaluated the airway response to *Df* in terms of BAL cellularity. The intranasal instillation of *Df* induced an increase in total number of cells in mice of all groups compared to mice that received saline (data not shown). Among the two groups with WT parenchymal cells, *Tia-1*^{-/-} → WT mice showed a small increase in total number of BAL cells compared to WT → WT ($45.00 \pm 3.84 \times 10^4$ vs. $40.00 \pm 2.48 \times 10^4$ cells; Fig. 5A). The two groups of mice with host tissues lacking TIA-1 showed a significantly higher number of BAL cells compared to chimeras with WT parenchymal cells and *Tia-1*^{-/-} → *Tia-1*^{-/-} mice had a moderate but not significant increase compared to WT → *Tia-1*^{-/-}, mice ($48.19 \pm 2.19 \times 10^4$ vs. 60.29 ± 5.64

$\times 10^4$ cells; Fig. 5A). The analysis of cell subpopulations in BAL showed that the *Df*-induced influx of neutrophils and eosinophils was similar in WT $\rightarrow$ WT and *Tia-1*^{-/-} $\rightarrow$ WT groups (neutrophils: $2.86 \pm 0.49 \times 10^4$ and $2.33 \pm 0.51 \times 10^4$, respectively; eosinophils: $6.40 \pm 1.88 \times 10^4$ and $6.14 \pm 1.13 \times 10^4$, respectively; Fig. 5B). The absence of TIA-1 from parenchymal tissue resulted in a slight (~20% and ~30%, respectively) increase in BAL neutrophils and eosinophils (WT $\rightarrow$ *Tia-1*^{-/-}: $3.46 \pm 0.77 \times 10^4$ and $9.23 \pm 1.87 \times 10^4$ vs. WT $\rightarrow$ WT: $2.33 \pm 0.51 \times 10^4$ and $6.14 \pm 1.14 \times 10^4$, respectively; Fig. 5B), when the mice received WT bone marrow. However, the highest number of neutrophils and eosinophils was found in the BAL of mice with both parenchymal and hematopoietic cells of *Tia-1*^{-/-} origin ($4.70 \pm 0.76 \times 10^4$ and $15.39 \pm 3.69 \times 10^4$, respectively; Fig. 5B). Thus, neutrophils and eosinophils in BAL of *Tia-1*^{-/-} $\rightarrow$ *Tia-1*^{-/-} mice were increased by 2- and 2.5-fold versus *Tia-1*^{-/-} $\rightarrow$ WT and 1.4- and 1.7-fold versus WT $\rightarrow$ *Tia-1*^{-/-}, respectively.

These results using bone marrow chimeras indicated that TIA-1 has effects on both parenchymal and hematopoietic cells that conspire to dampen the allergic inflammatory response induced by exposure to *Df*.

5. Discussion

TIA-1 is a multifunctional RNA-binding protein that regulates the splicing and translation of multiple mRNAs [29, 44–45]. Mutant mice lacking TIA-1 have an inflammatory diathesis manifest by the development of spontaneous inflammatory arthritis [29]. The inflammatory phenotype is thought to result from increased translation of transcripts encoding TNF α , IL-1 β , IL-6, COX-2 and MMPs [30–31]. TIA-1 binds to adenine/uridine-rich elements found in the 3'-untranslated regions of these transcripts to inhibit translation initiation by a partially characterized mechanism [32–33]. By coordinately dampening the translation of several transcripts encoding inflammatory mediators, TIA-1 has profound effects on the inflammatory response [36].

Here we report that *Tia-1*^{-/-} mice are significantly more sensitive to dust mite antigen-induced pulmonary inflammation than WT controls. In the absence of TIA-1, inflammatory cell infiltrates in bronchial airways, as well as inflammatory cell infiltrates, bronchovascular bundles, and mucin production in pulmonary tissues are increased compared to WT controls (Fig. 1, 2). The absence of TIA-1 enhances the immune response to *Df* as evidenced by a significant increase in *Df*-specific IgE and IgG1 antibody production (Fig. 4). The systematic effects of a lack of TIA-1 are also evidenced by increased activation of restimulated splenocytes and parabronchial LN cells (Fig. 3). These findings are consistent with previous results showing that TIA-1 dampens synovial inflammation and indicate that TIA-1 can dampen inflammation in different tissues.

Our results reveal that TIA-1 directly or indirectly represses the production of Th2 (i.e., IL-4, IL-5 and IL-13) and Th17 (i.e., IL-17) cytokines, but does not significantly affect the production of Th1 (i.e., IFN- γ) cytokines in *Df*-challenged mice. This may be a consequence of the binding of TIA-1 to sequences in the 3'-UTRs of these transcripts. Alternatively, TIA-1 may inhibit the production of another protein to indirectly affect the production of Th2 and Th17 cytokines. This possibility is supported by studies of bone marrow chimeras that implicate both hematopoietic and non-hematopoietic cells in the inflammatory phenotype observed in the absence of TIA-1. In these experiments, pulmonary inflammation is increased when TIA-1 is absent from either hematopoietic cells or non-hematopoietic parenchymal cells (Fig. 5). As the absence of TIA-1 in parenchymal cells increases the pulmonary infiltration of neutrophils and eosinophils, it is possible that TIA-1 represses the translation of transcripts encoding factors that recruit these cells to the inflamed lung.

We recently demonstrated that mutant mice lacking FAST, a protein that antagonizes the function of TIA-1 [46] are resistant to *Df*-induced pulmonary inflammation [47]. This suggests that TIA-1 and FAST may cooperatively modulate levels of cytokine and chemokine expression at the level of translation. Radiation chimeras revealed that the anti-inflammatory effects of FAST were due to reduced neutrophil infiltration and caused by the absence of FAST in lung parenchymal cells. We hypothesized that FAST prevents the TIA-1-induced translational repression of neutrophil chemoattractants in this model. These results are consistent with our findings that TIA-1 represses the recruitment of neutrophils to the inflamed lung (Fig. 5). Our findings also reveal that TIA-1 represses the recruitment of eosinophils to the inflamed lung. This differs from the effects of mice lacking FAST in which the recruitment of eosinophils is unaffected. This may be due to differential expression of TIA-1 and FAST in pulmonary cells that produce neutrophil and eosinophil chemoattractants. Although we have not yet identified these cells, a comparison of TIA-1 and FAST expression in inflamed lung may help identify the responsible cell types.

Taken together, our results reveal a post-transcriptional program that uses TIA-1 and FAST to modulate the translation of cytokines and chemokines that promote allergen-induced pulmonary inflammation. The finding that FAST is overexpressed in peripheral blood mononuclear cells in patients with asthma and atopy [48] strongly suggests that this post-transcriptional control pathway plays an important role in human disease. As such, these regulatory proteins may be good targets for the development of a new class of anti-inflammatory drugs for use in patients with allergic asthma.

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Abbreviations used in this paper

ARE	adenosine/uridine rich element
ARE-BP	ARE-binding protein
BAL	bronchoalveolar lavage
BM	bone marrow
BV	bronchovascular
BVB	BV bundle
CAE	chloroacetate esterase
CR	Congo red
<i>Df</i>	protein extract from <i>Dermatophagoides farinae</i>
PAS	period acid Schiff
PLN	parabronchial lymph nodes
Tia-1	gene encoding for T-cell intracellular antigen-1 protein
TIA-1	T-cell intracellular antigen-1 protein

3' UTR 3' untranslated region
WT wild-type

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RESEARCH HIGHLIGHTS

1. Dust mite challenged TIA-1^{-/-} mice have more severe pulmonary inflammation than wild type controls
2. Absence of TIA-1 enhances dust mite-induced Th2/Th17 cytokine production
3. Absence of TIA-1 in both hematopoietic and non-hematopoietic cells contributes to inflammation

Fig. 1. *Df*-induced airway inflammation in WT and *Tia-1*^{-/-} mice

WT and *Tia-1*^{-/-} mice were exposed to six doses of NaCl or *Df* 1 μ g intranasally over three weeks. Twenty-four hours after the last treatment mice were euthanized and bronchoalveolar lavage (BAL) was performed. Cells from the BAL were separated from the fluid, counted, cytocentrifuged onto slides and stained with Diff-Quick. (A) Total and (B) subpopulation differential cell counts from BAL of WT (■; $n = 22$ for NaCl-treated group and $n = 54$ for *Df*-treated group) and *Tia-1*^{-/-} (□; $n = 22$ for NaCl-treated group and $n = 65$ for *Df*-treated group) mice. Data are combined from seven independent experiments. Values are mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Fig. 2. *Df*-induced lung inflammation in WT and *Tia-1*^{-/-} mice

(A) Lung sections showing bronchovascular bundles (BVBs) from mice treated with NaCl (WT: a, e; *Tia-1*^{-/-}: b, f) or *Df* (WT: c, g; *Tia-1*^{-/-}: d, h) 24 h after the last intranasal challenge were stained by the chloroacetate esterase reaction (CAE, a–d) to assess inflammatory cell infiltrates or by the periodic acid Schiff reaction (PAS, e–h) to depict mucus-secreting cells (arrows). (B) Lung sections of *Df*-treated WT (a) and *Tia-1*^{-/-} (b) mice stained by Congo red dye to demonstrate accumulation of eosinophils (arrows) in BVBs. Pictures are from one representative mouse per strain and treatment from five separate experiments. Original magnifications, x20 (A) and x40 (B). (C) Quantitative analysis of pulmonary inflammation. The extent of cellular infiltration, on a total of 15 BVBs, and of goblet cell metaplasia, measured as the numbers of PAS- positive goblet cells per mm of bronchial basal lamina, was determined on glycolmethacrylate-embedded lung sections from WT (■; n = 39) and *Tia-1*^{-/-} (□; n = 46) mice exposed to *Df*. Data are combined from five independent experiments. Values are mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.

Fig. 3. Parabronchial lymph node cellularity and cytokine generation from *in vitro* restimulated WT and *Tia-1*^{-/-} parabronchial lymph node cells and splenocytes

Parabronchial lymph node cells and splenocytes were isolated from NaCl- and *Df*-treated WT and *Tia-1*^{-/-} mice and incubated *in vitro* with *Df*, as described in Methods. At the end of the incubation the indicated cytokines were measured in the supernatants by ELISA. Cytokine release from *Df*-restimulated (A) splenocytes and (B) parabronchial lymph node cells of WT (■) and *Tia-1*^{-/-} (□) mice exposed to *Df*. Data are combined from five (splenocytes) or three (lymph node cells) independent experiments. Values are mean ± SEM. **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

Fig. 4. Levels of *Df*-specific IgE and *Df*-specific IgG₁ in serum of WT and Tia-1^{-/-} mice. *Df*-specific IgE (A) and *Df*-specific IgG₁ (B) in serum of wild-type mice (■) and Tia-1^{-/-} (□) mice 24 h after the last intranasal instillation were measured by ELISA. Data are combined from six (*Df*-specific IgE) and seven (*Df*-specific IgG₁) independent experiments. Values represent the mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.

Fig. 5. *Df*-induced pulmonary inflammation and immunologic response in WT/*Tia-I*^{-/-} bone marrow chimeras

WT and *Tia-I*^{-/-} mice were irradiated and reconstituted with bone marrow from sex-matched WT and *Tia-I*^{-/-} mice in order to generate four groups of chimeras: WT + WT BM, WT + *Tia-I*^{-/-} BM, *Tia-I*^{-/-} + WT BM, and *Tia-I*^{-/-} + *Tia-I*^{-/-} BM. Mice were then exposed to six doses of NaCl 0.9% or *Df* 1 μg intranasally and euthanized 24 h after the last administration. (A) Total and (B) subpopulation differential cell counts from BAL of chimeras reconstituted with WT ($n = 14$ for WT recipients and $n = 11$ for *Tia-I*^{-/-} recipients) and *Tia-I*^{-/-} ($n = 16$ for WT recipients and $n = 14$ *Tia-I*^{-/-} recipients) bone marrow. Data are combined from three independent experiments. Values are mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.