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Uncoordinated 119 Preferentially Induces Th2 Differentiation and Promotes the Development of Asthma

Magdalena M. Gorska,*† Nicolas Goplen,* Qiaoling Liang,* and Rafeul Alam*†


Asthma is a chronic airway disease that is characterized by bronchial hyperresponsiveness and airflow obstruction. There are 300 million people worldwide with asthma, and the prevalence of the disease is increasing (1). Chronic airway inflammation is fundamental to asthma pathogenesis. The seminal event in the development of inflammation in allergic asthma is the generation of the Th2 cells as a result of exposure to allergens (2). Naive CD4 T cells differentiate into Th2 cells upon receiving coordinate signals from TCR and IL-4R (3). Although much is known about signals stemming from IL-4R, the Th2-promoting TCR pathways are not fully understood. TCR does not have intrinsic enzymatic activity and relies on nonreceptor tyrosine kinases for signal generation (4, 5). Tyrosine kinases of Src and Tec families are known to play an important role (6, 7). There are two TCR-linked Src family members Lck and Fyn. The Tec family includes Txk and Itk. Lck and Itk have been shown to favor Th2 development, whereas Fyn and Txk support the differentiation of Th1 cells (6–9). A recent study indicates that tyrosine kinases of the Abl family, Abl and Arg, are critical for IFN-γ production, but redundant for IL-4 synthesis in nonpolarized T cells (10).

We and others cloned the adaptor protein uncoordinated 119 (Unc119) (11–13). We subsequently showed that Unc119 controls the constitutive transport of Lck to the plasma membrane via endosomes. Unc119 is also critical for stimulation of the Lck enzymatic activity upon TCR triggering (14, 15). The effect of Unc119 on Fyn is short-lasting and of significantly lower magnitude (14, 16). Unc119 is required only for an initial short burst of Fyn kinase activity, but has no impact on Fyn activation during later stages of cell stimulation. Furthermore, Unc119 has no effect on the subcellular distribution of Fyn (15). In addition, we have recently reported that Unc119 is a potent inhibitor of Abl family kinases (17). Thus, Unc119 differentially affects three tyrosine kinases that are known to influence Th cell differentiation. In this study, we explore the role of Unc119 in Th1 and Th2 differentiation and in the pathogenesis of allergic asthma.

Materials and Methods

Abs

Rabbit polyclonal anti-Abl, anti-Arg, anti-CrkL, anti-JunD, goat polyclonal anti-Itk and anti-actin, mouse monoclonal anti-GATA3 (clone C17.8), rat monoclonal anti-mouse CD3 (clone 145-2C11), Syrian hamster monoclonal anti-mouse CD28 (clone 37.51), mouse monoclonal anti-human CD3 (clone OKT3), mouse monoclonal anti-human CD28 (clone CD28.2), rat monoclonal anti-mouse IL-12/IL-23 (p40 subunit; clone C17.8), rat monoclonal anti-mouse IFN-γ (clone XMG1.2; all from eBioscience, San Diego, CA), and rat monoclonal anti-mouse IL-4 (clone 1B.11; National Cancer Institute Preclinical Repository).

Subject population

Allergic asthmatics, subjects with rheumatoid arthritis (RA) or allergic rhinitis subjects without asthma and without RA and healthy controls were recruited in accordance to a protocol approved by an institutional review board. The asthmatic population (n = 11; 23–59 y old) was characterized by a positive skin test for common allergens and reversible bronchoconstriction as demonstrated by an increase in FEV1 of at least 12% after a short-acting bronchodilator and/or positive methacholine test (PC20 for methacholine ≥ 8 mg/ml). Severity of asthma ranged from mild intermittent (FEV1 > 80%) to severe persistent (FEV1 < 60%) per the National Asthma Education Expert Panel Guidelines. Patients with asthma maintained their routine medications, which included inhaled steroids with or without long-acting bronchodilators, rescue bronchodilators, and/or leukotriene receptor antagonists. None were given systemic steroids, theophylline, or omalizumab. Patients with allergic rhinitis without
asthma (n = 10; 18–59 y old) were recruited on the basis of a positive skin test and symptoms of allergic rhinitis with or without conjunctivitis, but not asthma. These patients continued their topical (nasal) medications, but withheld anti-histamines for 48 h. None were given allergen immunotherapy. Patients with RA (n = 7; 36–65 y old) visited our clinic because of accompanying rhinitis (allergic and/or nonallergic). RA was diagnosed previously according to guidelines of the American Rheumatism Association. Patients with RA maintained their routine medications, which included a nonsteroidal anti-inflammatory drug as needed, celecoxib, or hydroxychloroquine. None were given biologics, methotrexate, or systemic steroids. Healthy subjects (n = 11; 20–57 y old) had no major medical history, including history of asthma, allergic rhinitis, RA, and negative skin prick tests for common allergens. Exclusion criteria for all subjects were: respiratory and other infections within the last mo, immunodeficiency, and cigarette smoking.

Isolation and stimulation of human CD4 T cells

Mononuclear cells were isolated from the peripheral blood of healthy volunteers using Histopaque-1077 (Sigma-Aldrich, St. Louis, MO) as described by the reagent manufacturer. CD4 T cells were purified from mononuclear cells by negative selection using the human CD4+ T Cell Isolation Kit II (Miltenyi Biotech, Auburn, CA). CD4 T cells were stimulated with plastic-bound anti-CD3 and anti-CD28 Abs (2 µg/ml of each Ab in the plate-coating buffer-PBS) or left unstimulated in RPMI medium containing 10% FBS for 24 or 72h. Cells were lysed in the modified RIPA buffer (14).

Immunoprecipitation and Western blotting

Immunoprecipitation, PAGE, protein transfer to polyvinylidene difluoride membranes and Western blotting were performed as explained elsewhere (14).

Immunoelectron microscopy assay

Mouse CD4 T cells were stimulated with an anti-CD3 Ab and an anti-Armenian hamster IgG secondary Ab as described (18). Cells were lysed in the modified RIPA buffer (14). IκB was isolated from cell lysates by immunoprecipitation using anti-IκB Ab. The immunoprecipitated IκB was incubated in the kinase buffer (100 mM HEPES, pH 7.4, 50 mM NaCl, 10 mM MgCl2, 1 mM DTT, 500 µM DTT, 25 mM β-glycophosphate) containing an additional 5 µCi [32P]-ATP, and the tyrosine kinase substrate-enzyme (Sigma-Aldrich) at 5 µM at 30°C for 5–10 min. Samples were run on polyacrylamide gels, transferred, and autoradiographed (14).

Th cell differentiation and measurement of cytokine release by ELISA

CD4 T cells were isolated from C57BL/6 splenocyte suspensions by negative selection using the mouse CD4 T cell Isolation Kit (Miltenyi Biotech). CD4 T cell differentiation into Th1 and Th2 cells was performed according to the well-established protocol (19, 20). On day 7, cells were washed and stimulated with PMA (50 ng/ml) and ionomycin (1 µM) for 16 h. In some experiments, imatinib mesylate (LC Laboratories, Woburn, MA) at 10 µM was added to cultures. The concentration of cytokines in the culture supernatants was measured by ELISA according to the manufacturer (BD Biosciences) protocol. Procedures on mice were approved by the Institutional Animal Care and Use Committee at National Jewish Health.

RNAi, plasmids, and retroviral infection of T cells

shRNAs for mouse Unc119 and luciferase (control) were designed and cloned into the retroviral vector Banskeher-GFP as described (15). Mouse Unc119 cDNA was cloned into the bicistronic retroviral vector GFP-RV (21) as explained elsewhere (14, 17). CD4 T cells were infected with retroviruses at 36 and 60 h after initiation of culture, according to well-established protocols (14, 15).

Mouse model of acute asthma

OVA sensitization and challenge of mice was performed as described (22). At 48 h after the last challenge, lungs were removed and CD4 T cells were isolated as previously explained (23). Lung pieces were digested with collagenase. Recovered cells were washed, and CD4 T cells were isolated by positive selection using CD4 MicroBeads (Miltenyi Biotech). Lung CD4 T cells were examined for Unc119 expression by flow cytometry (see below). In a second set of experiments lungs, were lysed in the modified RIPA buffer for analysis of the Unc119 protein expression by Western blotting.

Flow cytometry

Purified human or mouse CD4 T cells were immunostained for Unc119 according to a previously described method (15). Stained cells were analyzed using the CyAn ADP cytometer (Beckman-Coulter, Fullerton, CA).

Adaptive transfer of shRNA-expressing CD4 T cells

CD4 T cells were isolated from splenocyte suspensions of OT-2 TCR transgenic mice (gift from Dr. Philippa Marrack, National Jewish Health, Denver, CO). T cells were activated with OVA peptide (aa323–339) and irradiated C57BL/6 splenic APCs in Th2-skewing conditions (day 0) as described (24). Cells were infected with retrovirus for Unc119 shRNA or luciferase shRNA (36 and 60h) and sorted by flow cytometry (selection marker-GFP). Sorted cells were expanded in cytokines for 2 d. On day 7, sorted cells or PBS were injected via a tail vein into C57BL/6 recipients (7 × 104 cells per mouse).Recipient mice were challenged intranasally with OVA (150 µg in 15 µl PBS) 24 h before and 24, 48, and 72 h after injection. Forty-eight hours after the last challenge, the airway reactivity to aerosolized methacholine was measured by the respiratory apparatus Flexivent (Scareq, Montreal, Quebec, Canada) (22), and bronchovascular lavage (BAL) was performed. Lungs were sectioned and stained with H&E, periodic acid and fluorescent Schiff’s reagent or anti-GFP Ab and DAPI as explained elsewhere (22, 25). The morphometric analysis of stained sections was performed as described (22).

Statistical analysis

Statistical analyses in human studies were performed using Mann-Whitney U test. Statistical analyses in mouse studies were done by Student t test. Results shown are as mean ± SD. p < 0.05 was considered statistically significant.

Results

The expression of Unc119 is upregulated in CD4 T cells from asthmatic subjects and in the asthmatic lung

T cells play a central role in allergic inflammation, and Unc119 is important for their activation. To address the role of Unc119 in asthma, we first attempted to determine whether the expression of Unc119 is changed in T cells from allergic asthmatic subjects. Flow cytometric analysis showed an increased Unc119 protein level in peripheral blood CD4 T cells from allergic asthmatic subjects (Fig. 1A, 1B). The Unc119 protein expression in CD4 T cells from allergic patients without asthma was similar to that in healthy control subjects (Fig. 1A, 1B). The result indicates that more widespread Th2 inflammation is required for induction of Unc119 expression in CD4 T cells. The Unc119 protein level was normal in subjects with rheumatoid arthritis, which is a Th1/Th17-type disease (Fig. 1A, 1B). We also examined the expression of Unc119 in CD4 T cells isolated from the lung tissue from a mouse model of asthma. C57BL/6 mice were immunized and challenged with OVA. The expression of Unc119 was higher in lung CD4 T cells from the OVA-challenged mice compared with those from the saline-treated animals (Fig. 1C). The Unc119 level was also higher in the lung lysate from OVA-challenged mice (Fig. 1D).

The expression of Unc119 in human CD4 T cells is induced by prolonged TCR stimulation

We investigated the mechanism responsible for induction of Unc119. Because Unc119 impacts on TCR signaling, we asked whether TCR signaling could affect the Unc119 protein level. We studied peripheral blood CD4 T cells from healthy donors. Stimulation with the combination of anti-CD3 and anti-CD28 Abs resulted in upregulation of the Unc119 protein (Fig. 1E). Importantly, the induction of Unc119 required a prolonged TCR stimulation (72 h). The late induction of Unc119 is intriguing as it has been previously demonstrated that only prolonged TCR signals favor Th2 commitment (26). We also observed that a shorter incubation (24 h) with or without anti-CD3/anti-CD28 Abs actually resulted in a drop of Unc119 protein level below that seen in freshly purified CD4 T cells (Fig. 1E).

The Unc119 protein is upregulated in Th2 cells

We examined the expression of Unc119 in the course of Th1 and Th2 differentiation (Fig. 1F). At 24 h, Unc119 expression declined below the level detected in freshly isolated CD4 T cells.
results are consistent with the data in Fig. 1E. At 48 h, Unc119 expression increased dramatically in Th2 cells and stayed at this highly elevated level throughout development. Unc119 was also re-induced in Th1 cells, but at a slower kinetics and to a significantly lower level than that observed in Th2 cells.

Unc119 has opposing effects on IL-4 and IFN-γ production

We examined the role of Unc119 in T helper cytokine production. The biologic relevance of a protein is best studied in a loss of function model. We previously demonstrated a substantial knockdown of Unc119 in primary mouse CD4 T cells by infection with a retrovirus encoding a short hairpin (sh) RNA (15). We also showed that Unc119 knockdown did not affect the expression of Lck, CD3ε, and CD4 (15). Therefore, to study the role of Unc119 in T helper cytokine production, mouse CD4 T cells were stimulated via TCR in Th1- or Th2-skewing conditions and harvested at 0, 24, 48, 72, 96, 120, and 144 h. Cells were lysed and lysates were blotted with Unc119 and anti-actin Abs (n = 3).
Unc119 promotes the release of Th2 cytokines, especially the release of IL-4, whereas it inhibits IFN-γ production.

Unc119 differentially affects Itk and Abl kinases

We attempted to examine the molecular mechanism that would link Unc119 to Th1/2 cytokine synthesis. As already mentioned, Unc119 activates the Th2-promoting kinase Lck (14, 15). Lck phosphorylates and activates the tyrosine kinase Itk, which substantially contributes to IL-4 gene transcription (6). We tested the effect of Unc119 on Itk enzymatic activity in an immune complex kinase assay. The activation of Itk in Unc119 knockdown and Unc119-overexpressing CD4 T cells was significantly impaired and augmented, respectively (Fig. 3A, 3B).

Interestingly, Unc119 affected both basal and TCR-regulated Itk activity. Accordingly, we have previously observed that Unc119 influences the basal activity of Lck (14). Therefore, Unc119 is critical for activation of two Th2-promoting tyrosine kinases—Lck and Itk. We next investigated the molecular mechanism of increased IFN-γ secretion by Unc119-knockdown T cells. We previously showed that Unc119 bound and inhibited Abl family kinases in fibroblasts (17). Abl kinases have been reported to favor IFN-γ production (10). Unc119 interacted with Abl in resting T cells (Fig. 3C). The association remained the same in cells stimulated with an anti-CD3 Ab. Abl tyrosine kinases are activated by multiple extracellular and intracellular stimuli (e.g., cell-to-cell adhesion, growth factors, Ag, genotoxic stress) (27).

Accordingly, Abl kinases show some activity even in the absence of TCR stimulation (28). We investigated the activation of Abl kinases using an Ab against the phosphorylated tyrosine 221 of the Abl-specific substrate—Crk (28). The phosphorylation of Crk was greatly augmented in Unc119-knockdown T cells (Fig. 3D). Our results indicate that Unc119 exerts a differential effect on Th1- and Th2-promoting tyrosine kinases in T cells.

Inhibition of Abl family kinases prevents excessive production of IFN-γ by Unc119-knockdown Th1 cells

Next, we asked whether Abl kinases were responsible for augmented IFN-γ release by Unc119-knockdown cells. To answer this question, we used the Abl kinase inhibitor imatinib mesylate (STI571, Gleevec) (10, 27, 28). CD4 T cells were activated under Th1 and Th2-skewing conditions, infected with shRNA-encoding retroviruses, and sorted as described previously. Imatinib or vehicle was added to cultures 48 h after the last infection. The 48 h time point coincides with the highest GFP fluorescence and maximum Unc119 knockdown. Cells were cultured under the skewing conditions with or without imatinib for an additional 48 h. Next, cells were washed and stimulated with PMA and ionomycin in the presence or absence of imatinib for an additional 16 h. The concentration of cytokines in the culture supernatants was measured by ELISA. We observed 2.9- and 7.5-fold reductions in IFN-γ secretion by imatinib-treated control and imatinib-treated Unc119-knockdown Th1 cells, respectively (Fig. 3E). The amount of IFN-γ in supernatants from imatinib-treated Unc119-knockdown cells was comparable to that in supernatants from imatinib-treated control cells. Imatinib did not significantly affect the IL-4 production by Th2 cells. Imatinib-treated control and Unc119-knockdown Th2 cells tended to produce slightly less IL-4 than the vehicle-treated counterparts but the difference did not reach statistical significance (Fig. 3F).

Unc119 is important for the development of allergic asthma

Transfer of in vitro differentiated OVA-specific Th2 cells to nonimmunized recipients followed by airway challenge with OVA results in substantial airway inflammation and increased airway reactivity to methacholine (30, 31). We asked whether decreased expression of Unc119 (by shRNA) in transferred T cells would impact on their ability to induce the aforementioned features of allergic asthma. We have used CD4 T cells from OT-2 TCR transgenic mice as donor cells. These T cells recognize OVA peptide (aa 323–339) in the context of I-Aβ. CD4 OT-2 T cells were subjected to Th2-skewing conditions and infected twice at 36 and 60 h with the aforementioned retrovirus for shRNA for Unc119 or sh-luciferase. Cells were sorted by GFP expression and infected i.v. into C57BL/6 mice. Control mice received PBS (negative control). OVA was administered intranasally to recipient mice 24 h before injection, and the procedure was repeated 24, 48, and 72 h after the transfer. Mice were examined at 48 h after the last OVA challenge. Mice transferred with control (sh-luciferase) T cells had elevated amounts of Th2 cytokines in the BAL fluid (Fig. 5D–F) and developed eosinophilic inflammation of airways (Figs. 4A, 5A–C). In mice injected with Unc119-knockdown T cells, the concentration of Th2 cytokines and the numbers of infiltrating inflammatory cells in the lung were substantially reduced. In control
experiments, we examined the lungs for the presence of transferred T cells by staining with an anti-GFP Ab. We detected a considerable number of GFP+ sh-luciferase expressing cells in control mouse lungs (Figs. 4B, 5G). The number of lung-infiltrating GFP+ Unc119-knockdown T cells was reduced. This result is not surprising. First, owing to decreased Lck and Itk activities, Unc119-knockdown Th2 cells are likely to have some defects in migration. Second, the continuous influx of Unc119-knockdown T cells into airways might be
prematurely terminated because of diminished recruitment or activation of other inflammatory cells (reduced strength of a positive feedback).

We next examined the mice for functional outcomes of airway inflammation. Control mice developed excessive airway mucus production (Figs. 4C, 5H, I) and increased airway reactivity to methacholine (Fig. 5J). In mice receiving Unc119-knockdown T cells, the aforementioned features of asthma were significantly diminished. Thus, we have demonstrated a critical role for Unc119 in the development of an experimental Th2 disease. A schematic diagram of the opposing effect of Unc119 on Th1 and Th2 differentiation is presented in Fig. 6.

Discussion

In this study, we uncover the role of the signaling molecule Unc119 in Th1/2 cytokine production in vitro and its relevance for allergic asthma in vivo. The expression of Unc119 is augmented in mouse Th2 cells and CD4 T cells from asthmatic subjects. Unc119 is required for the production of Th2 cytokines, especially for the production of IL-4. In contrast, it inhibits the release of IFN-γ. The molecular mechanism involves opposing effects of Unc119 on Th2-promoting tyrosine kinases Lck and Itk and Th1-promoting tyrosine kinases of the Abl family (Fig. 6). The T cell-expressed Unc119 is critical for development of allergic asthma as demonstrated in adoptive transfer experiments.

As discussed earlier, Unc119 is critical for the plasma membrane anchoring and the activation of the TCR-associated Src family tyrosine kinase Lck (14, 15). In transgenic mice, which express dominant negative Lck, the development of Th2 cells is impaired whereas the maturation of Th1 cells is largely intact (7). Upon immunization, these mice are unable to produce IgE and IgG1 Abs, the two Ig isotypes that are driven by IL-4. In contrast, they retain full ability to produce IgG2a, an Ig that is stimulated by the Th1 cytokine IFN-γ. The Th2-promoting capacity of Lck can be attributed, at least in part, to the activation of Itk. Itk is an important substrate for Lck (32). Itk-deficient T cells have impaired ability to differentiate into IL-4-producing cells (6, 33). The mechanism involves diminished nuclear translocation of NFATc, reduced ERK activation and augmented T-bet expression. Interestingly, the expression of GATA3 is only slightly impaired (33). Itk-deficient mice do not mount Th2 responses in vivo as demonstrated in a parasite-infection model and in an allergic asthma model (6, 34). Thus, the impairment of Th2 cytokine production by Unc119-knockdown T cells, and the inability of these cells to induce experimental asthma in the adoptive transfer model, is likely caused by the diminished Lck and Itk activities.

We observed reduced nuclear expression of JunB in Unc119-knockdown Th2 cells. JunB is critical for Th2 differentiation (35–37). JunB expression is positively regulated by the ERK pathway (38). ERK activation is stimulated by Lck and Itk (33, 39). Previously, we reported severe impairment of ERK activity in T cells with reduced Unc119 expression (14). Thus, we believe that the diminished JunB expression in Unc119-knockdown cells is a result of decreased Lck and Itk activities. We did not detect any significant drop in the GATA3 level. This result is consistent with Itk knockout data (33).
Unc119 regulates the Th1-promoting tyrosine kinases Abl, Arg and Fyn (this manuscript and Ref. 14, 16, 17). Unc119 constitutively associates with Abl and inhibits its enzymatic activity. Abl/Arg-double deficient T cells and Fyn-deficient T cells demonstrate selective impairment of IFN-γ production (9, 10). Abl kinases are critical for the activation of JNK in T cells (10). Abl kinases play much lesser role in the activation of ERK1/2 (10). Several reports indicate that JNK and ERK1/2 favor Th1 and Th2 development, respectively (40–42). Thus, the selective reduction in IFN-γ synthesis in Abl/Arg-double deficient T cells could be explained by the inhibition of the JNK pathway. Unc119 strongly inhibits Abl and Arg and moderately activates Fyn. Unc119 is important only for the early phase of Fyn activation. Unc119 knockdown T cells demonstrate augmentation of IFN-γ production. We believe that, in terms of IFN-γ synthesis, the effect of Unc119 on Abl kinases prevail over that on Fyn. We speculated that the IFN-γ production by Unc119 knockdown T cells is the result of hyperactivation of Abl family kinases and, possibly, an Unc119-independent late-phase Fyn activation (17). In addition, the diminished Itk activity may synergize in the stimulation of IFN-γ production through the induction of T-bet expression (33). However, the effect on Itk is not dominant, because the inhibition of Abl kinases by imatinib efficiently abrogates IFN-γ production in Unc119 knockdown T cells.

We observed a moderate elevation of T-bet in Unc119-knockdown Th1 cells. The effect of Itk on T-bet is well established (33). The role of Abl in the regulation of T-bet expression remains to be investigated. Abl may affect T-bet expression indirectly via hyperactivation of Abl family kinases.
activation of JNKs and their substrate c-Jun. c-Jun/ATF2 binding sites have been identified in the IFN-γ promoter (43). In addition, JNK2 regulates the expression of IL-12Rβ2 (41).

The expression of Unc119 is upregulated during Th2 development. Unc119 expression is also augmented by prolonged TCR stimulation. Other reports show that prolonged TCR stimulation is required for Th2 cytokine production (26). Our results suggest that increased expression of Unc119 might facilitate the Th2 differentiation and block the Th1 development. Overexpression of Unc119 augments the amount of Lck at the plasma membrane and increases Lck and Itk enzymatic activities (this study, 14, 15). Unc119 overexpression inhibits Abl activation (17). Thus, Unc119 overexpression leads to the enhancement and inhibition of the Th2- and Th1-promoting signaling pathways, respectively. Interestingly, Th2 cells have been shown to have increased Lck activity (44).

The heightened expression of Unc119 protein in CD4+ T cells from asthmatic subjects could result from a genetic predisposition (e.g., single nucleotide polymorphism) and/or exposure to the inflammatory milieu. Allergen may not be the only stimulus. The observed 66.3% increase in Unc119 expression in CD4+ T cell population is unlikely to have its roots solely in allergen-specific T cells, which are rare and account for 0.0001–0.1% of the total T cell population (45). Thus, other stimuli (e.g., cytokines) are likely to affect the Unc119 level. We believe that heightened expression of Unc119 in T cells from asthmatic subjects results in skewed tyrosine kinase signaling. Increased concentration of Unc119 results in augmented Lck and Itk activities and in diminished Abl activity. These signaling events promote Th2 differentiation and function as well as airway inflammation. Interestingly, Unc119 expression is normal in allergic subjects without asthma. We speculate that the stimulation threshold for Unc119 induction is not reached in atopic subjects because of limited inflammation. The difference in Unc119 level between allergic and allergic asthmatic subjects could also be due to a genetic predisposition.

We have demonstrated an increased expression of Unc119 in the lung tissue from a mouse model of asthma. T cells are probably not the only cells that upregulate Unc119 expression under inflammatory conditions. Unc119 regulates other inflammatory cells: eosinophils and myofibroblasts (11, 16). We have previously reported that an elevation of the Unc119 protein level via protein overexpression in the Caenorhabditis elegans nematode system. Genetics 141: 977–988.


