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Transduction of Phosphatase and Tensin Homolog Deleted on Chromosome 10 into Eosinophils Attenuates Survival, Chemotaxis, and Airway Inflammation

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Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is part of a complex signaling system that affects a variety of important cell functions. PTEN antagonizes the action of PI3K by dephosphorylating the signaling lipid phosphatidylinositol 3,4,5-triphosphate. In the present study, we used a TAT fusion protein transduction system to elucidate the role of PTEN in eosinophils and airway inflammation. A small region of the HIV TAT protein (YGRKKRRQRRR), a protein transduction domain known to enter mammalian cells efficiently, was fused to the N terminus of PTEN. Flow cytometric analysis of annexin V- and propidium iodide-stained cells was used to assess eosinophil survival. A chemotaxis assay was performed using a Boyden chamber. Cell analysis in bronchoalveolar lavage fluid and histological examinations were performed using OVA-challenged A/J mice. We found that TAT-PTEN was successfully internalized into eosinophils and functioned as a phosphatase in situ. TAT-PTEN, but not a TAT-GFP control protein, blocked the ability of IL-5 to prevent the apoptosis of eosinophils from allergic subjects. The eotaxin-induced eosinophil chemotaxis was inhibited by TAT-PTEN in a dose-dependent manner. Intranasal pretreatment with TAT-PTEN, but not TAT-GFP, significantly inhibited the OVA-induced eosinophil infiltration in bronchoalveolar lavage fluid. Histological examination of the lung, including H&E and Alcian blue/periodic acid-Schiff staining, revealed that TAT-PTEN, but not TAT-GFP, abrogated eosinophilic inflammation and mucus production. Our results suggest that PTEN negatively regulates eosinophil survival, chemotaxis, and allergic inflammation. The pharmacological targeting of PTEN may constitute a new strategy for the treatment of eosinophilic disorders. The Journal of Immunology, 2007, 179: 8105–8111.

The pathogenesis of asthma is characterized by the infiltration of tissues by inflammatory cells such as eosinophils, mast cells, and T cells. Several mediators released by these cells cause epithelial damage, leading to enhanced bronchial hyperresponsiveness and airway obstruction (1). Although eosinophils have been considered as the most important cells in this process, the results of a clinical trial using an anti-IL-5 Ab has raised questions about the role of eosinophils in bronchial hyperresponsiveness (2). A subsequent study demonstrated that treating asthmatics with anti-IL-5 Ab reduces airway eosinophil numbers and the deposition of extracellular matrix proteins in the bronchial subepithelial basement membrane, suggesting the involvement of eosinophils in airway remodeling (3). In support of this finding, eosinophil-deficient mice exhibit decreased subepithelial fibrosis and smooth muscle hyperplasia (4). Thus, the targeting of eosinophils is considered to be an attractive strategy for treating asthma.

Phosphatase and tensin homolog on chromosome 10 (PTEN) is a lipid and protein tyrosine phosphatase that dephosphorylates phosphotyrosine as well as the D3 position of phosphatidylinositol 3,4,5-triphosphate and phosphatidylinositol 3,4-diphosphate. The tumor suppressor gene PTEN is located at 10q23 and its mutation is detected in several neoplasms, including glioblastoma and breast and prostate cancers (5). The transduction of PTEN into tumor cell lines results in cell cycle arrest or apoptosis, suggesting that PTEN controls cell proliferation and survival (6–10). In Dictyostelium and mouse neutrophils, it has been demonstrated that the coordination of PI3K and PTEN is required for proper chemotaxis (11–13). The role of PTEN in immunity has also been investigated using PTEN-deficient mice. Suzuki et al. (14) have generated T cell-specific PTEN-deficient (Ptendo/−) mice in which the T cells exhibit autoreactivity, enhanced proliferation, and inhibition of apoptosis. Similar phenomena are observed in the B cells derived from B cell-specific PTEN-deficient (bPtendo/−) mice (15). These results indicate that PTEN negatively regulates most cellular functions in the immune system. However, little is known about the role of PTEN in eosinophil function.

To elucidate the importance of intracellular signaling molecules in eosinophils, several approaches have been attempted. Although the application of pharmacological inhibitors is a common technique for targeting molecules of interest, its nonspecificity is frequently problematic. In contrast to proliferating cells, eosinophils have low turnover rates; hence, the validity of using antisense oligonucleotides or small interfering RNAs to assess signaling in eosinophils remains debatable. The overexpression of wild-type or dominant-negative protein by plasmid
transfection is not possible because eosinophils are short-lived and terminally differentiated cells. To overcome these problems, we used a TAT fusion protein system that facilitates cellular internalization of the protein (16). It has been shown that the dominant-negative forms of Ras or PI3K efficiently block IL-5 signaling and corresponding functions in eosinophils (17–19).

In the present study, we generated TAT-PTEN to investigate the role of PTEN in eosinophils and airway inflammation. TAT-PTEN, but not a TAT-GFP control protein, blocked the ability of IL-5 to prevent apoptosis in the eosinophils obtained from allergic subjects. The eotaxin-induced eosinophil chemotaxis was inhibited by TAT-PTEN in a dose-dependent manner. Intranasal pretreatment with TAT-PTEN, but not TAT-GFP, significantly inhibited the OVA-induced eosinophilic inflammation in a murine model of asthma.

Materials and Methods

Generation of the TAT-PTEN construct

The TAT-PTEN expression vector was provided by Dr. S. Dowdy (20). The expression cassette containing the wild-type PTEN (21) under the control of the CAG promoter (22) was supplied by the Riken Laboratory (Tsukuba, Japan). A cDNA fragment encoding TAT-PTEN was amplified by PCR (each cycle was conducted at 94°C for 40 s, 56°C for 30 s, and 72°C for 1 min, for a total of 35 cycles) using an AmpliTaq Gold DNA polymerase (Applied Biosystems) from the PTEN-containing cDNA with the forward primer that included the TAT sequence at the 5′ region (5′-TACGGTGTAAAGAGATCGT-3′, underlined) and the reverse primer (5′-TCAGACTTTTGAATTTGTGTATGCTG-3′, underlined) and the reverse primer (5′-TCAGACTTTTGAATTTGTGTATGCTG-3′). The PCR product was cloned into a pCR-TOPO cloning vector (Invitrogen Life Technologies). To extend the His tag sequences at the 5′ region, an additional PCR (each cycle was conducted at 94°C for 40 s, 56°C for 30 s, and 72°C for 1 min, for a total of 35 cycles) was performed with the pCRII-TAT-PTEN construct as a template and the following primers (forward, 5′-ATGCGGCAAGACAATCATCACATCACATCACACAGCGCCTACCTCAGTGAAGAACGTCCCATCGCGGTGGTT-3′, His sequence is underlined; and reverse, 5′-TCAGACTTTTGAATTTGTGTATGCTG-3′).

The final PCR product was cloned into the pCRII-TOPO vector and the sequence of the obtained construct (pCRII-His-TAT-PTEN) was confirmed by using an Applied Biosystems PRISM 3700 Genetic Analyzer.

Purification of the TAT-PTEN protein

The polystyline-tagged TAT-PTEN gene was expressed in TOP10–competent Escherichia coli cells. TAT-PTEN was purified by sonication (50 kHz, amplitude: 20) in buffer Z (8 M urea, 20 mM HEPES (pH 8.0), 100 mM NaCl, 1 mM PMSF, and 1 μM peptatin A) containing 20% glycerol. Cell lysates were resolved by centrifugation, absorbed onto a Ni Sepharose High Performance column (GE Healthcare Bio-Sciences), washed, and then sequentially eluted with 50, 100, and 250 mM imidazole in buffer Z (20 mM HEPES (pH 8.0), 150 mM NaCl, 1 mM PMSF, and 20% glycerol) using a low-volume 10,000 MWCO Slide-A-Lyzer dialysis cassette (Pierce). The protein concentration was measured using the DC protein assay (Bio-Rad). Expression of the TAT-PTEN protein was confirmed by SDS-PAGE and Western blotting. The phosphoinositide phosphatase activity of the protein was determined using a PTEN malachite green assay kit (Upstate Biotechnology) with prepared phospholipid vesicles (0.1 mM diCPCP, 0.5 mM DOPS, 20 mM HEPES (pH 7.4), 1 mM EDTA, and 4.2% ammonium molybdate in 4 N HCl). Each fusion protein was flash frozen at −80°C.

Eosinophil purification

Peripheral venous blood was obtained from subjects with and without atopic characteristics. The geometric means of serum IgE from normal and atopic subjects. The eosinophil purification was conducted in duplicate using 5-μM polystyline-polyethylene-free polycarbonate membranes (Nucleopore) in Boyden chambers (NeuroProbe). Human eosinotin was diluted in HBSS containing 0.02% BSA and placed in the lower wells (100 μl) at a concentration of 10 nM. After incubation of the eosinophils with and without the TAT fusion proteins for 3 min, 5 μl aliquots of the cell suspension were removed and fixed in 4% paraformaldehyde for 15 min. The cells were washed twice with cold PBS and stained with annexin V and propidium iodide. Purified eosinophils were placed on glass slides using Shandon Cytospin 3 (Thermo Scientific). The intracellular distribution of TAT fusion proteins was visualized under a laser confocal microscope (LSM510; Zeiss).

Cellular uptake of TAT fusion proteins

TAT-PTEN and TAT-GFP were labeled with FITC (Pierce) according to the manufacturer’s instructions, and excess FITC was removed by dialysis using the same cassette as that described above for protein purification. Eosinophils resuspended in HBSS with 1% FCS were incubated with 1 μM of the FITC-conjugated TAT-PTEN or TAT-GFP for 30 min at 37°C. The cells were washed twice in cold PBS and stained with annexin V and propidium iodide according to the manufacturer’s instructions. Eosinophil apoptosis was analyzed using a FACSscan cytometer (BD Biosciences), gating on the live cell population.

Chemotaxis assay

Chemotaxis of eosinophils was conducted in duplicate using 5-μM polystyline-polyethylene-free polycarbonate membranes (Nucleopore) in Boyden chambers (NeuroProbe). Human eosinotin was diluted in HBSS containing 0.02% BSA and placed in the lower wells (100 μl) at a concentration of 10 nM. After incubation of the eosinophils with and without the TAT fusion proteins for 3 min, 5 μl aliquots of the cell suspension were removed and fixed in 4% paraformaldehyde for 15 min. The cells were washed twice with cold PBS and stained with annexin V and propidium iodide according to the manufacturer’s instructions. Eosinophil apoptosis was analyzed using a FACSscan cytometer (BD Biosciences), gating on the live cell population.

Preparation of cytosolic cell extracts

Eosinophils were incubated with and without TAT-PTEN or TAT-GFP for the indicated times at 37°C. The cells were lysed in a lysis buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM Na2VO4, 1 mM NaF, 1 mM EDTA, 1 mM EGTA, 1 mM PMSF. 1% Triton X-100, 10% glycerol, and 1 μg/ml aprotinin, leupeptin, and pepstatin). After 20 min on ice, detergent-insoluble materials were removed by 12,000 × g centrifugation at 4°C. The whole cell lysates were boiled in 2× Laemmli reducing buffer for 4 min.

In some experiments, the cells were stimulated with 100 ng/ml IL-5 (R&D Systems) or 100 nM eotaxin (R&D Systems) for 3 min; the reaction was then terminated by adding nine volumes of ice-cold HBSS containing 1 mM Na2VO4. The cells were lysed using the Bio-Plex Cell Lysis Kit (Bio-Rad) for further quantification of phosphorylated and total Akt performed using Bio-Plex Phosphophoprotein Panel (Bio-Rad) and Luminex 200 (Luminex). Data are shown as a percentage of mean fluorescence intensity of unstimulated control.

Gel electrophoresis and Western blotting

SDS-PAGE was performed using Ready Gels J (Bio-Rad). The concentration of the polyacrylamide was 7.5–10%. The electrophoresed gel was blotted onto Hybond ECL membranes (GE Healthcare Bio-Sciences). Blots were incubated in a blocking buffer containing 10% BSA in TBST buffer (20 mM Tris–HCl, 137 mM NaCl (pH 7.6), and 0.05% Tween 20) for 1 h, followed by incubation in the primary Ab (0.1 μg/ml) for 1–2 h (mouse monoclonal anti-His Ab obtained from GE Healthcare Bio-Sciences and rabbit polyclonal anti-PTEN and anti-Akt Abs obtained from Santa Cruz Biotechnology). After washing three times in TBST buffer, the blots were incubated for 30 min with a HRP-conjugated secondary Ab (0.04 μg/ml) directed against the primary Ab. The blots were developed with an ECL substrate according to the manufacturer’s instructions (GE Healthcare Bio-Sciences). In some experiments, blots were reprobed with another Ab after stripping in a buffer containing 100 μl of 62.5 mM Tris-HCl (pH 6.7), 100 mM 2-ME, and 2% SDS at 50°C for 30 min.

Cellular uptake of TAT fusion proteins

TAT-PTEN and TAT-GFP were labeled with FITC (Pierce) according to the manufacturer’s instructions, and excess FITC was removed by dialysis using the same cassette as that described above for protein purification. Eosinophils resuspended in HBSS with 1% FCS were incubated with 1 μM of the FITC-conjugated TAT-PTEN or TAT-GFP for 30 min at 37°C. The cells were washed twice in cold PBS and stained with annexin V and exclude propidium iodide. Purified eosinophils were placed on glass slides using Shandon Cytospin 3 (Thermo Scientific). The intracellular distribution of TAT fusion proteins was visualized under a laser confocal microscope (LSM510; Zeiss).

Survival assay

An apoptosis detection kit (MBL) was used to quantitatively determine eosinophils undergoing apoptosis, by virtue of their ability to bind to annexin V and exclude propidium iodide. The chemoattractant response of the buffer was resuspended at 0.5 × 106 cells/ml in RPMI 1640 medium supplemented with 10% FCS. After incubation with TAT-PTEN or TAT-GFP, the eosinophils were further cultured with 1 ng/ml IL-5 (R&D Systems) in 24-well tissue culture plates for 24 h at 37°C. The cells were washed twice in cold PBS and stained with annexin V and propidium iodide according to the manufacturer’s instructions. Eosinophil apoptosis was analyzed using a FACSscan cytometer (BD Biosciences), gating on the live cell population.
Bronchoalveolar lavage fluid (BALF) cell analysis and histological examination in mice

Care and use of the animals followed the guidelines of the Principles of Laboratory Animal Care formulated by the National Society for Medical Research. Specific pathogen-free male A/J mice (10–12 wk old) were purchased from SLC. The mice were initially immunized four times with 10 μg of OVA plus 2 mg of alum on days 0, 28, 35, and 49. After the sensitization, the mice were intranasally challenged with 40 μl of 10 mg/ml OVA from days 50–53. The mice were divided into four groups for the intranasal administration of pretreatment and challenge: 1) PBS plus PBS, 2) PBS plus OVA, 3) TAT-PTEN (3 μmol/mouse) plus OVA, and 4) TAT-GFP (3 μmol/mouse) plus OVA. On day 54, BALF was obtained from the mice by intubating and washing the lungs with 1 ml of saline until 5 ml of fluid was recovered. The cells were pelleted from the lavage fluid, resuspended in 1 ml of saline, and placed on glass slides for counting and fixation using Shandon Cytospin 3 (Thermo Scientific). The slides were then stained with Diff-Quik (Sysmex) and cell differentiation was assessed microscopically. The supernatant of the BALF was concentrated 10-fold by freeze-drying to examine the cytokine/chemokine levels. The measurement of cytokine/chemokine was performed using Bio-Plex Cytokine Panel (Bio-Rad) and Luminex 200. The lungs were fully inflated using 10 cm of H₂O pressure and fixed with 20% formaldehyde for H&E and Alcian blue/periodic acid-Schiff (AB/PAS) staining.

Statistical analysis

Results are expressed as means ± SEM. The data were analyzed for statistical significance using the Mann-Whitney U test and ANOVA. Post hoc analysis was performed using the Bonferroni/Dunn test.

Results

Cellular internalization and in situ activity of TAT-PTEN

Dowdy and colleagues (20) have described how TAT fusion proteins were internalized into mammalian cells within 30 min in a concentration-dependent manner. Thus, we constructed TAT-PTEN to examine the role of PTEN in eosinophil function and allergic inflammation (Fig. 1). Initially, we incubated eosinophils with 1 μM TAT-PTEN or TAT-GFP for 15 s, 5 min, and 30 min at 37°C to assess the cellular uptake of the proteins. After the incubation, the eosinophils were washed with cold HBSS and lysed. The lysates were subjected to electrophoresis and Western blotting with anti-PTEN or anti-GFP Ab and then reprobed with anti-His Ab. As shown in Fig. 2A, the blots with both anti-PTEN and anti-His Abs revealed that TAT-PTEN efficiently entered cells in a manner that depended on the length of incubation. In contrast, internalization of TAT-GFP reached a maximum at 15 s and plateaued for 30 min (Fig. 2B). To further confirm these results, we observed the intracellular distribution of the TAT fusion proteins in eosinophils. The confocal microscopic analysis of transduced TAT-PTEN (Fig. 3A) and TAT-GFP (Fig. 3B) demonstrated cytoplasmic localization accompanied with intense uptakes, possibly within endosomes. Our TAT-PTEN, unlike the dominant-negative form of TAT fusion proteins reported previously (17–19), is designed to function as an in situ phosphatase. PTEN is assumed to inhibit the PI3K pathway by dephosphorylating phosphatidylinositol 3,4,5-triphosphate, leading to the down-regulation of Akt, which lies downstream of PI3K. For this reason, we investigated the effect of TAT-PTEN on Akt activation in eosinophils to detect the in situ activity of the protein. After pretreatment of the eosinophils with 1 μM TAT-PTEN or TAT-GFP for 30 min, Akt phosphorylation induced by IL-5 or eotaxin was measured.

Effect of TAT-PTEN on eosinophil survival and chemotaxis

PTEN has been demonstrated to promote apoptosis in a number of tumor and immune cells (7–10, 24, 25). Thus, we investigated the role of PTEN in the maintenance of eosinophil survival. After incubating with TAT-PTEN or TAT-GFP for 30 min, eosinophils were incubated with IL-5 for 24 h and viability was assessed by annexin V and propidium iodide staining. After 24 h, in the absence of IL-5, almost one-half of the eosinophils had undergone apoptosis. In contrast, eosinophil viability was >80% after stimulation with IL-5. Incremental increases in the concentration of TAT-PTEN, but not TAT-GFP, significantly abrogated the survival of eosinophils from atopic donors (Fig. 5). Interestingly, however, the effect of TAT-PTEN was not observed in the eosinophils from normal subjects (Fig. 5). We next studied the effect of TAT-PTEN on eotaxin-induced eosinophil chemotaxis since PTEN has been observed to be involved in the migration of Dictyostelium and mammalian cells (11–13). Eosinophils were incubated with and without the TAT fusion proteins and then applied to the upper Boyden chambers. The lower chambers contained 10 nM eotaxin. The cells that migrated and adhered to the lower surface of the membrane were counted under the light microscope. TAT-PTEN, but not TAT-GFP, dose-dependently reduced the chemotaxis of eosinophils, regardless of their atopic state (Fig. 6).

Effect of TAT-PTEN on allergic inflammation

Kwak et al. (26) have reported that the intratracheal administration of adenovirus-carrying PTEN cDNA significantly inhibits eosinophil inflammation and bronchial hyperresponsiveness. We therefore investigated the in vivo effect of TAT-PTEN in a murine model of asthma. The OVA-sensitized mice were intranasally pretreated with and without TAT-PTEN or TAT-GFP, followed by intranasal OVA challenge. Airway inflammation was assessed by BALF cell analysis and histological examination. Following OVA challenge, the number of cells in the BALF increased due largely to an increase in eosinophils (Fig. 7). The increase in the numbers of BALF cells, particularly eosinophils, was significantly abrogated in mice pretreated with TAT-PTEN, but not in those treated with TAT-GFP (Fig. 7). A histological examination revealed that airway inflammation and the production of purple-stained mucus were augmented by OVA challenge (Fig. 8, C and D) compared with the PBS control (Fig. 8, A and B). The H&E staining revealed...
that airway inflammation was attenuated by the administration of TAT-PTEN (Fig. 8E). Pretreatment with TAT-PTEN markedly reduced the mucus-producing epithelial cells stained with AB/PAS (Fig. 8F). The effect elicited by TAT-PTEN was not observed in the case of TAT-GFP administration (Fig. 8G and H). To study the effect of TAT-PTEN on Th1/Th2 balance, we measured the levels of cytokine/chemokine in BALF. The increase in the IL-5 level caused by OVA challenge was significantly inhibited by TAT-PTEN, but not by TAT-GFP (Fig. 9A). In contrast, the administration of TAT-PTEN significantly increased the RANTES level in BALF (Fig. 9B). Since the down-regulation of vascular endothelial growth factor (VEGF) expression in allergen-induced asthmatic lung by PTEN has recently been demonstrated (27), we also measured the VEGF concentration in BALF. Although TAT-PTEN tended to decrease the VEGF level, the difference was not significant (data not shown).

Discussion

In the present study, we developed a novel TAT fusion protein to investigate the role of PTEN in eosinophils and airway inflammation. TAT-PTEN, but not TAT-GFP, reduced the IL-5-induced survival of eosinophils obtained from allergic subjects. The eotaxin-induced eosinophil chemotaxis was inhibited by TAT-PTEN. The intranasal administration of TAT-PTEN, but not TAT-GFP, significantly inhibited the OVA-induced eosinophilic inflammation. This is the first report to clarify the role of PTEN in eosinophils. Moreover, we demonstrated the utility of TAT-PTEN as a therapeutic modality for the treatment of allergic diseases.

Eosinophils play a pivotal role in the pathogenesis of airway inflammation and remodeling in asthma. Therefore, it is of paramount importance to investigate eosinophil signaling and its functional relevance. Pharmacological inhibitors of intracellular signaling molecules have been widely used for this purpose. However, the data are often difficult to interpret because of the lack of inhibitor specificity. Although eosinophils are nonproliferating and terminally differentiated cells, transfection with plasmids or transduction with inhibitory nucleotides, such as antisense oligonucleotides and small interfering RNAs, may be unrealizable. To solve the above-mentioned problems, the TAT fusion protein system has become the focus of scientific attention. The first description of this system was made independently by Green and Loewenstein (28) and Frankel and Pabo (29), who demonstrated that the 86-aa HIV TAT protein could rapidly enter cells and subsequently transactivate the viral long terminal repeat promoter. Later, Fawell et al. (16) expanded on these findings by revealing that chemically cross-linking a 36-aa domain of TAT to heterologous proteins facilitated cell internalization. According to their results, the TAT fusion proteins were predominantly localized in the cell surface area following incubations of up to 20 min, with progressive accumulation in diffuse cytoplasmic, nuclear, and nucleolar regions with incubations of 30 min to 6 h. Several groups

![Image](https://example.com/image1)

**FIGURE 3.** Intracellular localization of (A) TAT-PTEN and (B) TAT-GFP in eosinophils. Eosinophils were incubated with 1 µM FITC-conjugated TAT-PTEN or TAT-GFP for 30 min at 37°C. After preparation of a cytospin specimen, the cells were visualized under a confocal microscope.

![Image](https://example.com/image2)

**FIGURE 4.** The in situ phosphatase activity of TAT-PTEN in eosinophils stimulated with IL-5 or eotaxin. Eosinophils were incubated with 1 µM TAT-PTEN or TAT-GFP for 30 min and then stimulated with 100 ng/ml IL-5 or 100 nM eotaxin for 3 min. Quantification of the phosphorlated and total Akt was performed using the Luminex System. The data are shown as a percentage of mean fluorescence intensity of unstimulated control and are expressed as means ± SEM (n = 4). *, p < 0.05 vs without the protein (ANOVA).

![Image](https://example.com/image3)

**FIGURE 5.** Effect of TAT-PTEN on eosinophil survival. Eosinophils were incubated with and without the TAT fusion proteins for 30 min. The cells were further cultured with IL-5 (1 ng/ml) for 24 h and then the viability of eosinophils was assessed by annexin V and propidium iodide staining. The data are expressed as means ± SEM (n = 3). *, p < 0.05 vs without the protein (ANOVA).

![Image](https://example.com/image4)

**FIGURE 6.** Effect of TAT-PTEN on eosinophil chemotaxis. Eosinophils were incubated with and without the TAT fusion proteins for 30 min. After incubation, the cells were subjected to the chemotaxis assay using Boyden microchambers. Eotaxin (10 nM) was used as the chemoattractant. The data are expressed as means ± SEM (n = 4), *, p < 0.05 vs without the protein (ANOVA).
have demonstrated that the TAT fusion proteins block specific signaling molecules such as Ras and PI3K in eosinophils (17–19). In the present study, we generated TAT-PTEN that was efficiently incorporated in eosinophils. It has been demonstrated that the expression of wild-type PTEN in PTEN-deficient tumor cell lines reduces phosphatidylinositol 3,4,5-triphosphate levels and inhibits Akt phosphorylation (30). We therefore examined the in situ phosphatase activity of TAT-PTEN in eosinophils and found that the Akt phosphorylation induced by IL-5 or eotaxin was inhibited by TAT-PTEN.

PTEN appears to play a particularly important role in regulating apoptosis in a variety of cell types. Somatic deletions or mutations of the PTEN gene are commonly detected in a large fraction of tumors (5) and overexpression of wild-type PTEN in PTEN−/− cell lines induces apoptosis (7–10). PTEN also inhibits Ag receptor signaling and cell survival in B cells and T cells (24, 25). Our results revealed that the transduction of PTEN inhibited the survival of eosinophils derived from atopic donors, but not those from normal subjects. Although mechanisms regarding the differential response of eosinophils based on atopic state are unclear, one possible explanation is as follows. We have previously found that Lyn, Jak2, and Raf-1, but not MAPK, are essential for the survival of eosinophils stimulated with IL-5 (31, 32). However, the role of PI3K in signaling between IL-5 and GM-CSF in eosinophil apoptosis remains controversial (33, 34). IL-5 and GM-CSF share a βc receptor that is critical for signal transduction. These results indicate that the Lyn (Jak2)-Ras-Raf-1 pathway may be common in survival signaling in the eosinophils from both atopic and normal subjects. In contrast, eosinophils from atopic donors, or primed in vivo in the allergic condition, possibly utilize the PI3K pathway as well as the Ras-Raf-1 pathway. In support of this finding, Pinho et al. (35) have established a model of allergic pleurisy in mice and found that treatment with the PI3K inhibitor abrogated the accumulation of eosinophils associated with an increased number of apoptotic events. Thus, it is reasonable that TAT-PTEN accelerates the apoptosis of atopic eosinophils by antagonizing the effect of PI3K.

The role of PTEN has recently been elucidated in Dictyostelium and mouse neutrophils (11–13). In the resting condition, PI3K is uniformly distributed in the cytoplasm, whereas PTEN is localized in the cell periphery. PI3K is translocated to the leading edge of the cell associated with delocalization of PTEN from the front in response to chemotaxtrants. Recently, Li et al. (36) have demonstrated that activated RhoA in the rear of leukocytes activates Rho-associated

FIGURE 7. Effect of TAT-PTEN on BALF cells in a murine model of asthma. TAT-PTEN or TAT-GFP was intranasally administrated in OVA-sensitized A/J mice. The mice were intubated for lavage with a total of 5 ml of saline. Cells were pelleted from the lavage fluid and the cell number was counted under the light microscope following Diff-Quik staining. The data are expressed as means ± SEM (n = 5). **, p < 0.05 vs PBS-treated/OVA-challenged mouse or TAT-GFP-treated/OVA-challenged mice (Mann-Whitney U test).

FIGURE 8. Effect of TAT-PTEN on the histological characteristics of lung tissue. Sections of lung were stained with H&E (A, C, E, and G) or AB/PAS (B, D, F, and H) stain. PBS-treated/PBS-challenged mouse (A and B), PBS-treated/OVA-challenged mouse (C and D), TAT-PTEN-treated/OVA-challenged mouse (E and F), and TAT-GFP-treated/OVA-challenged mouse (G and H). Original magnification, ×200.

FIGURE 9. Effect of TAT-PTEN on cytokine/chemokine production. The concentration of IL-5 (A) and RANTES (B) in the BALF supernatant was measured using Bio-Plex Cytokine Panel and Luminex 200. The data are expressed as means ± SEM (n = 5). *, p < 0.05 vs PBS-treated/OVA-challenged mice; **, p < 0.05 vs PBS-treated/OVA-challenged mice or TAT-GFP-treated/OVA-challenged mice (Mann-Whitney U test).
coiled-coil-forming protein kinase (ROCK), subsequently forming a complex with and phosphorylating PTEN. Activated PTEN located at the back and lateral sides restricts phosphatidylinositol 3,4,5-triphosphate accumulation in the front of the cells, which is required for proper chemotaxis. Our previous report demonstrated that the ROCK pathway is activated by eotaxin and critical for eosinophil chemotaxis (37). Thus, the activation of Rho and ROCK may be responsible for endogenous PTEN activation in eosinophils. In some cells, however, overexpression of PTEN inhibits cell migration (38).

Expression of the PTEN mutant G129E, which is deficient in lipid phosphatase activity but retains protein phosphatase activity, inhibits the migration of U87MG glioblastoma cells (39). This effect is likely to be mediated through the direct interaction of PTEN with FAK, followed by its dephosphorylation. In chemotactic PTEN-deficient Jurkat T cells, ectopically expressed PTEN was distributed homogeneously in the cytoplasm (40). Nonetheless, in contrast to the results in Dictyostelium and murine neutrophils (11–13), PTEN attenuated actin polymerization and cell motility in Jurkat cells stimulated with stromal-derived factor 1 (40). In the present study, we observed that TAT-PTEN is largely distributed in the cytosol of eosinophils. Thus, the findings of Lacalle et al. (40) support our data demonstrating that TAT-PTEN blocks eotaxin-induced eosinophil chemotaxis. Taken together, it is possible that PTEN localization in migrating cells depends on cell type-specific factors.

Homozygosity for the null mutation of PTEN (PTEN−/− mice) results in early embryonic lethality (7, 40–42). PTEN−/− mice frequently develop a variety of cancers and autoimmune diseases (42–44). To overcome the lethality of PTEN−/−, various cell-specific PTEN mutations have been generated using the Cre-loxP system. The T cell-specific PTEN-deficient (Ptenlox/lox) T cells exhibit hyperproliferation, auto-reactivity, secretion of increased amounts of Th1/Th2 cytokines, and resistance to apoptosis (14). Similar phenomena, including hyperproliferation, resistance to apoptosis, and enhanced migration, are observed in PTEN-deficient (bPtenlox/lox) B cells (15). These results suggest inhibitory roles for PTEN in neoplasia formation and immune regulation. In a murine model of allergy, Kwak et al. (26) have established an elegant system for examining the role of PTEN. The intratracheal administration of adenosovirus carrying PTEN cDNA significantly reduced airway eosinophil infiltration and bronchial hyperresponsiveness, indicating that the effect of PTEN is suppressive. The application of TAT fusion proteins, such as the dominant-negative forms of Ras and PI3K, has been reported in a murine model of allergy (45, 46). In the present study, we generated TAT-PTEN that efficiently blocks eosinophilic inflammation, mucus production, and IL-5 production in vivo. These results are consistent with those of Kwak et al. (26). We also observed less IL-5 production in the TAT-GFP-treated mice than in the mice not treated with TAT proteins. It has been shown that TAT transduction causes a phalloidylsereine flip from the inner to the outer cell membrane (47), which is commonly observed during cell apoptosis. Although the mechanism underlying this is unclear, the electrostatic interaction between TAT-GFP and the cell membrane may account for the reduced IL-5 production in BALF. Lung expression of RANTES mRNA is up-regulated in the Th1-skewed condition of a murine asthma model following treatment with CpG oligodeoxynucleotides or the adoptive transfer of Th1 clones (48, 49). Our results demonstrated the increase in RANTES level in BALF induced by TAT-PTEN, suggesting that TAT-PTEN modulates allergic inflammation by inducing the Th1 condition. Lee et al. (27) have recently demonstrated the down-regulation of VEGF expression in allergen-induced asthmatic lung by PTEN, which may indicate a further mechanism of TAT-PTEN action.

In conclusion, we generated TAT-PTEN that efficiently blocks eosinophil survival, chemotaxis, and airway inflammation. Targeting PTEN can be a therapeutic modality in the treatment of several cancers and autoimmune diseases. However, the molecular regulation of PTEN in eosinophilic inflammation remains to be clarified. Further studies are necessary to elucidate the detailed signaling complex around PTEN in allergy, the outcome of which may lead to the development of new molecular targeting therapy.

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Disclosures

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References


