Endothelial cell PTP1B regulates leukocyte recruitment during allergic inflammation

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Berdnikovs S, Abdala-Valencia H, Cook-Mills JM. Endothelial cell PTP1B regulates leukocyte recruitment during allergic inflammation. Am J Physiol Lung Cell Mol Physiol 304: L240–L249, 2013. First published December 28, 2012; doi:10.1152/ajplung.00375.2012.—Pulmonary eosinophilia is a consistent hallmark of allergic lung inflammation. Infiltration of eosinophils into ovalbumin (OVA)-challenged lungs is dependent on the adhesion molecule vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells. Ligation of VCAM-1 activates endothelial cell protein tyrosine phosphatase 1B (PTP1B), which is required for VCAM-1-dependent leukocyte migration in vitro. To examine whether nonhematopoietic PTP1B modulates eosinophil recruitment in vivo, mice deficient in PTP1B were irradiated and received wild-type hematopoietic cells to generate chimeric PTP1B+/−/− mice. In response to OVA challenge, the chimeric PTP1B+/−/− mice had reduced eosinophilia in the lung tissue and bronchoalveolar lavage, indicating a role for PTP1B in nonhematopoietic cells during leukocyte recruitment. To determine whether endothelial cell PTP1B regulates eosinophil recruitment, mice with an inducible endothelial cell-specific PTP1B deletion (iePTP1B mice) were generated and the PTP1B deletion was induced after antigen sensitization before antigen challenge. In response to OVA challenge, the iePTP1B mice with the endothelial cell PTP1B deletion had an increased accumulation of eosinophils bound to the luminal surface of the endothelium in the lung vasculature and had a decrease in leukocyte recruitment into the lung tissue. In the iePTP1B mice, expression of adhesion molecules, cytokines, or chemokines that regulate leukocyte recruitment during inflammation was not altered, consistent with other studies that deletion of endothelial adhesion molecule signals does not alter lung cytokines and chemokines. In summary, these data suggest that VCAM-1 activation of PTP1B in the endothelium is necessary for eosinophil recruitment during allergic inflammation. Moreover, these studies provide a basis for targeting VCAM-1-dependent signaling pathways in allergy therapies.

PTP1B; allergic inflammation; endothelial; leukocyte extravasation; lung

LEUKOCYTE MIGRATION from peripheral blood into tissues is a crucial component of inflammation and immune surveillance (14, 39, 49). Pulmonary recruitment of eosinophils is a consistent feature of allergic lung inflammation. The mechanisms underlying eosinophil recruitment to the lung following allergen exposure are complex, involving the coordinate actions of adhesion molecules, the chemokine eotaxin, and T-cell-derived cytokines such as IL-5 (38, 54). In particular, it has been shown that eosinophil recruitment to the lung in murine models of allergic inflammation is dependent upon eosinophil binding to the adhesion molecule vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells (12, 26, 44). Leukocyte binding to endothelial cell adhesion molecules including VCAM-1 (CD106) triggers active endothelial cell “outside-in” signals that regulate leukocyte migration in vitro (1, 3, 34, 40, 48, 51). These signals have been demonstrated to result in localized alterations in the function of cell junction proteins and/or actin-dependent contractile forces in the endothelial cells, thereby transiently opening endothelial cell junction and permitting migration of leukocytes (14, 19). After binding to the endothelium, the majority of leukocytes migrate between endothelial cell junctions. Genetically modified mice with compromised opening of cell junctions exhibit a reduction in leukocyte extravasation (10, 46, 49). Thus endothelial cells are actively involved in leukocyte transendothelial migration (14, 19).

Leukocyte migration is regulated by endothelial cell tyrosine phosphatases, and it is reported that protein tyrosine phosphatases regulate endothelial cell junctions in vitro. We reported that ligation of VCAM-1 activates the protein tyrosine phosphatase 1B (PTP1B) (1, 16). In this pathway, VCAM-1-stimulated protein kinase C-α (PKCα) phosphorylates and activates PTP1B, which then induces signals for activation of ERK1/2 (1). Importantly, inhibition of PTP1B blocks VCAM-1-dependent lymphocyte transendothelial migration in vitro (16). However, it is not known whether endothelial PTP1B functions in vivo during leukocyte recruitment.

Given the significant function for PTP1B in promotion of VCAM-1-dependent leukocyte migration in vitro (16) and the central role for VCAM-1 in the recruitment of eosinophils in allergic inflammation (6), we determined whether nonhematopoietic PTP1B or endothelial PTP1B was required for eosinophil migration in vivo. PTP1B-deficient mice are useful for in vivo studies because they are physiologically normal under basal conditions. PTP1B-deficient mice have been used to study insulin and leptin receptors in vivo (18, 24, 53, 55). It is also reported that inhibition of PTP1B elevates cytokine receptor signaling in leukocytes in vitro (7, 41) and we have reported that antigen-challenged PTP1B knockout mice have elevated allergic inflammation (6). Therefore, to determine whether PTP1B in endothelial cells is necessary for eosinophil recruitment, we used two approaches: reconstitution of PTP1B+/−/− mice with wild-type (WT) hematopoietic cells and mice with an inducible endothelial-specific deletion of PTP1B (iePTP1B mice). Allergen challenge in PTP1B+/−/− mice reconstituted with WT bone marrow cells resulted in decreased leukocyte recruitment to the lung, indicating a role for PTP1B in nonhematopoietic cells. Transgenic mice with a deletion of PTP1B in the endothelium after antigen sensitization had increased accumulation of eosinophils bound to the luminal surface of the endothelium and decreased leukocyte recruitment into the lung without affecting levels of adhesion molecules, cytokines, or chemokines that regulate leukocyte recruitment during allergic inflammation. These findings suggest that endothelial PTP1B is necessary for leukocyte extravasation into the lung during antigen challenge.

METHODS

Mice. The studies are approved by the Northwestern University Institutional Review Committee for animals.
Chimeric mice with nonhematopoietic cell PTP1B+/−. Six- to eight-week-old thymocyte differentiation antigen Thy1.2+ PTP1B+/− mice (kind gift of Dr. Michel Tremblay, McGill Univ., Montreal, Canada) were lethally gamma irradiated with a split dose of 1,000 rad (650 rad followed by 350 rad 3 h later to limit toxicity) using a gamma cesium irradiator. Two hours following irradiation, PTP1B+/− recipient mice were reconstituted with 5 million WT Thy1.1+ bone marrow cells in 200 μl saline/mouse by retroorbital injection under sterile conditions. As a control, WT donor bone marrow was transferred to irradiated WT recipient mice. Mice were treated with Baytril (40–50 μg/mg oral dose per mouse per day) to avoid infection and morbidly. At 12 wk after the bone marrow transfer, success of bone marrow reconstitution was confirmed by flow cytometry. In the resulting chimeric mice, spleen leukocytes were >99% Thy1.1+ (data not shown).

Inducible endothelial-specific deletion of PTP1B. To generate mice with inducible endothelium-specific deletion of PTP1B (iePTP1B), three strains of mice were crossed: 1) vascular endothelial (VE)-Cadherin-tTA (mouse bearing tetracycline transactivator (TA) under VE-Cadherin promoter, kind gift of Dr. Laura Benjamin, Beth Israel), 2) TetO-Cre [mouse expressing Cre recombinase under doxycycline-suppressed tetracycline operator sequence (TetO) tetracycline-responsive promoter, Jackson Labs] and 3) PTP1BloxP/loxP (mouse with floxed PTP1B locus, kind gift of Dr. Benjamin Neel, Univ. of Toronto, Toronto, Canada). Each strain was backcrossed on the C57BL6 background at least five times prior to crossing of strains. PTP1BloxP/loxP mice were bred either to VE-Cadherin-tTA or TetO-Cre mice to generate fl/+tTA and fl/+Cre mice. These two strains were crossed for the Fl generation of PTP1BloxP/loxP(tTA + Cre+) (iePTP1B) mice for the experiments. Only Fl iePTP1B mice were used for the experiments because in our experience, continued breeding of the iePTP1B mice with iePTP1B mice resulted in some mice on doxycycline with Cre expression leak and deletion of PTP1B. All mice in the experiments were examined by Western blot for PTP1B expression in isolated endothelial cells and spleen leukocytes. iePTP1B mice were maintained on doxycycline (2 mg/ml) added to drinking water in water-deprivation cages throughout their pre- and postnatal development to suppress deletion of PTP1B (iePTP1B). Doxycycline water bottles were removed, taking advantage of the differential adherence of fibroblasts to select against them. The resulting cell preparations were >95% ATI lung endothelial cells as determined by expression of endothelial cell adhesion molecule (EpCAM; CD326) by flow cytometry (EpCAM antibody staining, BD Pharmingen) (data not shown).

Spleen cells were isolated as previously described (6) and red blood cells were lysed by hypotonic shock.

Immunoblotting. Cell lysates were resolved by 10% SDS-PAGE and transferred to nitrocellulose membranes by the semidy method according to manufacturer’s instructions (Bio-Rad). The membranes were blocked in 5% nonfat dried milk in Tris-buffered saline plus 0.1% Tween 20 (TBS-T) for 1 h at room temperature and washed three times for 5 min in TBS-T. The membranes were incubated with rabbit anti-mouse anti-PTP1B primary antibody (no. abs40, Millipore) in TBS-T plus 5% milk overnight, washed three times for 5 min in TBS-T, incubated with anti-rabbit IgG horseradish peroxidase (HRP)-linked secondary antibody (no. 7074; Cell Signaling Technology) in TBS-T plus 5% milk for 1 h, washed three times for 10 min in TBS-T, and examined for detection with the enhanced chemiluminescence kit (no. RP2132, Amersham) and autoradiography. Equal protein loading was determined by stripping the membrane with Restore Western Blot Stripping Buffer (Pierce, Rockford, IL) for 15 min and then labeling with mouse anti-β-actin antibody (no. 3700; Cell Signaling Technology). Densitometry was performed using ImageJ software (National Institutes of Health). The data were presented as the fold increase in the ratio of relative intensity of the band/relative intensity of band for the loading control.

Ovalbumin-induced experimental asthma in chimeric mice with nonhematopoietic PTP1B+/−. At 12 wk post-bone marrow reconstitution of irradiated mice, the mice were sensitized by intraperitoneal injection (200 μl) of OVA grade V 10 μg/album or saline/album on days 0 and 7 and then challenged on days 15, 17, and 20 with intranasal OVA fraction VI (50 μg in 50 μl saline) or 50 μl saline alone (4) (Fig. 1A). Tissues were collected and examined on day 21 (Fig. 1A).
eosinophil counts and serum preparations, and the lungs were lavaged with 0.5 ml ice-cold PBS to obtain bronchoalveolar lavage (BAL) cells. Middle lobe of the right lung was harvested for cytokine/chemokine analysis; left lung, superior and postcaval right lung lobes were used for endothelial cell isolation; and inferior lobe of the right lung was embedded in OCT freezing medium (Electron Microscopy Sciences) and used for cryosection and histological preparations. OVA-specific IgE was determined by ELISA, as previously described (9).

**Blood, BAL leukocytes, and lung eosinophils.** Mature blood eosinophils were prepared using Dscohm and counted using a hemocytometer. BAL cell cytospins were differentially stained using the Diff-Quick kit (Dade Behring) and counted according to standard hemocytometer methods. Blood, BAL leukocytes, and lung eosinophils were examined in the blood, BAL, and lung tissue. Total numbers of leukocytes in the BAL were significantly reduced in PTP1B-deficient chimeric mice (Fig. 2A). Differential counts showed that OVA-challenged PTP1B-deficient mice had reduced recruitment of eosinophils, monocytes/macrophages, and lymphocytes, but not neutrophils (Fig. 2B). There was also reduced inflammation in the lung tissue of OVA-challenged PTP1B-deficient chimeric mice compared with WT controls (Fig. 3A). There was no difference in blood eosinophils (Fig. 3B), serum OVA-specific IgE (Fig. 3C), or body weight between OVA-challenged chimeric PTP1B-deficient mice and OVA-challenged WT mice (Fig. 3D). In summary, PTP1B deletion in nonhematopoietic cells in vivo attenuates OVA-induced lung inflammation.

**RESULTS**

PTP1B deficiency in nonhematopoietic cells decreases eosinophilia during allergic lung inflammation. Inhibition of endothelial cell PTP1B blocks VCAM-1-dependent leukocyte transendothelial migration in vitro (16), but whether PTP1B regulates leukocyte recruitment in vivo is not known. Therefore, because eosinophil recruitment in response to OVA is dependent on binding to VCAM-1, we determined whether PTP1B deficiency in nonhematopoietic cells in vivo reduces eosinophil recruitment during allergic inflammation. For this model, lethally irradiated PTP1B/−− Thy1.2+/+ recipient mice were reconstituted with WT bone marrow from Thy1.1+/+ mice, which express WT PTP1B. As a control, Thy1.1+/− WT PTP1B donor bone marrow was used to reconstitute WT recipient mice. The chimeric mice expressed ≥99% Thy1.1+ leukocytes (data not shown). Chimeric mice were sensitized with OVA/alum and then challenged with OVA in saline as in the timeline in Fig. 1A. Twenty four hours after the last OVA challenge, leukocytes were examined in the blood, BAL, and lung tissue. Total numbers of leukocytes in the BAL were significantly reduced in PTP1B-deficient chimeric mice (Fig. 2A). Differential counts showed that OVA-challenged PTP1B-deficient mice had reduced recruitment of eosinophils, monocytes/macrophages, and lymphocytes, but not neutrophils (Fig. 2B). There was also reduced inflammation in the lung tissue of OVA-challenged PTP1B-deficient chimeric mice compared with WT controls (Fig. 3A). There was no difference in blood eosinophils (Fig. 3B), serum OVA-specific IgE (Fig. 3C), or body weight between OVA-challenged chimeric PTP1B-deficient mice and OVA-challenged WT mice (Fig. 3D). In summary, PTP1B deletion in nonhematopoietic cells in vivo attenuates OVA-induced lung inflammation.

OVA-challenged PTP1B-deficient chimeric mice had reduced IL-5, IL-12, IL-13, and CCL24 but not IL-4, CCL-11, or IL-33. We determined whether cytokines and chemokines that regulate allergic inflammation were altered in OVA-challenged PTP1B-deficient chimeric mice. OVA-challenged mice with PTP1B deficiency in nonhematopoietic cells showed a significant decrease in lung tissue expression of IL-5, IL-13, and CCL24 compared with OVA-challenged WT controls, as measured by qPCR (Fig. 4, B, C, and E). Consistent with gene expression, there was a significant decrease in BAL protein levels of the cytokine IL-5 (Fig. 4F). There was also a decrease in protein expression of IL-12 (Fig. 4I). In contrast, there was no difference in mRNA and/or protein for IL-4, IL-33, or CCL11 between these two groups (Fig. 4, A, D, G, and F). In summary, deletion of PTP1B in nonhematopoietic cells results in decreased production of selected cytokines and chemokines.
As measured by flow cytometry for epithelial cell expression of EpCAM and endothelial cell expression of CD31 and CD105 (data not shown). As a control, lymphocytes (>95% purity) were isolated from spleens of the same animals and represented the hematopoietic compartment. Western blots for the relative ratio of PTP1B expression to the control, β-actin, showed no differences in hematopoietic or epithelial PTP1B protein levels between iePTP1B/dox mice compared with the control mice indicated in Fig. 5, B–D. There was a significant reduction in the ratio of PTP1B to β-actin expression in positively selected endothelial cells from iePTP1B/dox mice compared with iePTP1B/+ dox mice (Fig. 5, C and D).

**Inducible endothelial cell-specific deletion of PTP1B.** To determine whether PTP1B in endothelial cells regulates leukocyte recruitment during allergen challenge, we created an inducible endothelial-specific knockout of PTP1B under a VE-Cadherin promoter controlling doxycyclin-suppressed expression of Cre recombinase for induction of the deletion of the floxed PTP1B locus (iePTP1B mice). Thus the deletion of PTP1B could be induced after antigen sensitization, before the leukocyte recruitment to the lung that occurs during the antigen challenge phase (Fig. 1B). In this model, mice were maintained on doxycycline water throughout their pre- and postnatal development. Tail clips at weaning were used to select F1 mice based on concurrent gene expression of VE-Cadherin tTA, TetO Cre, or both were used as experimental controls (Fig. 5A). At 6–8 wk of age, mice were sensitized with OVA/alum on days 0 and 7 (Fig. 1B). One week following sensitization (day 15), doxycycline was removed from the drinking water for a group of mice to induce PTP1B deletion in endothelial cells (Fig. 1B). Mice were challenged with OVA in saline on days 22, 24, and 27 (Fig. 1B). Twenty four hours after the last OVA challenge, leukocytes were examined in the blood, BAL, and lung tissue.

To determine the specificity of PTP1B deletion in endothelial cells, lung endothelial cells, lung epithelial cells, and spleen lymphocytes were isolated from the iePTP1B mice without doxycycline (iePTP1B/dox) and the indicated control mice. The purity of isolated epithelial and endothelial cells was at least 95% and 85%, respectively, as measured by flow cytometry for epithelial cell expression of EpCAM and endothelial cell expression of CD31 and CD105 (data not shown). As a control, lymphocytes (>95% purity) were isolated from spleens of the same animals and represented the hematopoietic compartment. Western blots for the relative ratio of PTP1B expression to the control, β-actin, showed no differences in hematopoietic or epithelial PTP1B protein levels between iePTP1B/dox mice compared with the control mice indicated in Fig. 5, B–D. There was a significant reduction in the ratio of PTP1B to β-actin expression in positively selected endothelial cells from iePTP1B/dox mice compared with iePTP1B/+ dox mice (Fig. 5, C and D).

**Endothelial cell deletion of PTP1B decreases eosinophil recruitment during allergic inflammation.** OVA-challenged iePTP1B/dox mice had a significant decrease in total BAL leukocytes (Fig. 6A) compared with OVA-challenged control mice. There was a significant decrease in eosinophils and monocytes/macrophages and lymphocytes, but not neutrophils in BALs of the iePTP1B/dox mice (Fig. 6B). There were also fewer eosinophils in lung tissue of the OVA-challenged iePTP1B/dox mice (Fig. 7J) compared with OVA-challenged control mice (Fig. 7, B, D, F, and H). Because eosinophil recruitment in response to OVA is dependent on binding to VCAM-1 (12) and because VCAM-1 activates PTP1B in in vitro studies (16), we hypothesized that if PTP1B were required for eosinophil transendothelial migration on VCAM-1, there would be an accumulation of eosinophils on the luminal surface of the endothelium. Histological examination of the
lung tissue of OVA-challenged mice revealed that iePTP1B/−/− mice had increased numbers of eosinophils bound to luminal surface of endothelium in the lung compared with control groups (Fig. 7, J and K). There was no inflammation in the lungs of the saline-treated mice (Fig. 7, A, C, E, G, and I). There was no difference in blood eosinophils (Fig. 8 A), production of OVA-specific IgE antibodies (Fig. 8 B), or mouse body weight (Fig. 8 C) among the groups. These data with reduced lung eosinophil recruitment and increased eosinophil binding to the luminal surface of the endothelium in the iePTP1B/−/− dox are consistent with in vitro studies demonstrating that endothelial cell PTP1B functions in leukocyte transendothelial migration (16).

**Endothelial cell deletion of PTP1B does not alter production of cytokines or chemokines that mediate allergic inflammation.** We determined whether cytokines and chemokines that regulate leukocyte infiltration in allergic inflammation were altered in OVA-challenged iePTP1B/−/− mice. OVA challenge to iePTP1B/−/− mice, iePTP1B/−/− dox mice, and the indicated control mouse strains increased expression of several mediators of allergic inflammation including IL-4, IL-13, CCL11, and CCL24 compared with the corresponding saline treatment (Fig. 9). However, OVA-challenged iePTP1B/−/− dox mice had no change in the cytokines IL-4, IL-5, IL-10, IL-13, IL-33, and IFNγ or the chemokines CCL11 or CCL24 compared with OVA-challenged control mice (Fig. 9). Consistent with no change in the cytokines, expression of the adhesion molecule

**Fig. 4.** Chimeric PTP1B−/− mice had decreased OVA-stimulated expression of several cytokines and chemokines that regulate allergic inflammation. Tissues were from mice in Fig. 2. A–F: 24 h after the last OVA challenge, lung tissue was preserved in RNA later solution (Qiagen) and then examined for cytokine and chemokine expression by qPCR. In these assays, β-actin was used as housekeeping gene (primers/probes from Applied Biosystems). G–I: BAL supernatants were examined for cytokine protein levels using the Th1/Th2 mouse cytokine multiplexing kit (Invitrogen). n = 6–8 mice per group. *P < 0.05 compared with saline controls, **P < 0.05 compared with saline and with PTP1B−/−, OVA group.

**Fig. 5.** Expression of PTP1B in inducible endothelial-specific deletion of PTP1B (iePTP1B) mice and control mice. A: representative gel electrophoresis showing genotyping of iePTP1B mice and control mice. All mice were genotyped at weaning to be selected for breeding or experiments. B: Western blot for PTP1B in alveolar epithelial type II (ATII) cells isolated from lungs of iePTP1B/−/− dox and iePTP1B/−/+ dox mice. Shown in the blots are 2 lanes for isolations from 2 representative mice from each group. C: endothelial cells were isolated from all mice in the experiments and examined by Western blot for PTP1B expression. Shown are representative Western blots for PTP1B expression in lysates from positive-selected endothelial cell isolation, column flow-through from the endothelial cell isolation, and lymphocytes isolated from spleens of the indicated mice. D: Western blots for PTP1B expression in C were quantified by densitometry (ImageJ, NIH). Shown is the relative intensity of PTP1B per intensity of the β-actin loading control. n = 2 mice per group. *P < 0.05 compared with the other groups.
VCAM-1, which is induced by cytokines, was not altered by the PTP1B deletion (data not shown). In summary, without altering cytokines and chemokines, deletion of PTP1B in the endothelium resulted in an increase in leukocytes bound to the luminal surface of the endothelium and decreased leukocyte recruitment into the lung during allergic inflammation.

DISCUSSION

In this study, we demonstrate that PTP1B deficiency in the nonhematopoietic compartment or PTP1B deletion in endothelial cells blocks allergic lung inflammation. PTP1B deficiency in the nonhematopoietic compartment of chimeric PTP1B−/− mice blocks recruitment of eosinophils, lymphocytes, and monocytes to the OVA-challenged lung. Without antigen challenge, PTP1B deletion did not affect basal numbers of circu-

Fig. 6. The iePTP1B−/− dox mice had decreased OVA-stimulated recruitment of cells to the BAL. The mice were sensitized and challenged with OVA as in the timeline in Fig. 1B. The iePTP1B mice express VE-Cadherin-tTA, TetO-Cre, and PTP1BloxP/loxP. PTP1Bfl/fl mice express PTP1BloxP/loxP. The tTA mice express VE-Cadherin-tTA. The Cre mice express TetO-Cre. A: total leukocyte counts in BAL. B: number of mononuclear cells, neutrophils, eosinophils, and lymphocytes in the BAL. In the top vs. bottom panel, there was a significant increase (P < 0.05) in all cell types in the OVA groups compared with corresponding saline controls. n = 6–8 mice per group. *P < 0.05 compared with the corresponding saline group and the other OVA-treated groups. **P < 0.05 compared with the corresponding saline groups.

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Fig. 7. The iePTP1B−/− dox mice had decreased OVA-stimulated recruitment of cells to the lung tissue and increased number of eosinophils on luminal surface of the endothelium. Tissues were from mice in Fig. 6. A–J, eosin- methyl green staining of frozen lung sections. The representative images were obtained with a 20× objective. There was reduced eosinophil infiltration in lung tissue of OVA-challenged iePTP1B−/− dox mice compared with the other OVA-challenged control groups of mice. There was increased eosinophil accumulation on the endothelial luminal surface of OVA-challenged iePTP1B−/− dox mice compared with the other OVA-challenged control groups of mice. The enlargement in J is magnified 3-fold to illustrate increased numbers of eosinophils on luminal walls of the blood vessels (black arrows); this increased association of eosinophils was consistent in this group. Shown are representative images. K: number of eosinophils per 100 microns of luminal surface of the lung endothelium in OVA-treated experimental groups. Saline controls did not have eosinophils on the luminal surface of the lung endothelium (data are not shown). The iePTP1B mice express VE-Cadherin-tTA, TetO-Cre, and PTP1BloxP/loxP. PTP1Bfl/fl mice express PTP1BloxP/loxP. The tTA mice express VE-Cadherin-tTA. The Cre mice express TetO-Cre. n = 6–8 animals per group. *P < 0.05 compared with all other groups.
lating or tissue leukocytes, which is consistent with reports that PTP1B−/− mice do not differ in basal vascular function or inflammation (5, 6). The OVA-challenged chimeric PTP1B−/− mice also have reduced mRNA or protein for IL-5, IL-12, IL-13, and CCL24 but not IL-4 or IL-33. Several nonhematopoietic cell types including epithelium (32, 50) can produce IL-5, IL-12, IL-13, and CCL24 (17, 27, 45, 52), which suggests that nonhematopoietic PTP1B may be necessary for production of these cytokines in nonhematopoietic lung cells during allergic inflammation. This reduction in cytokines in allergen-

Fig. 8. OVA-challenged iePTP1B−/− dox mice do not have altered body weight, numbers of blood eosinophils, or serum OVA-specific IgE. Tissues were from mice in Fig. 6. A: eosinophils from peripheral blood were stained with Discomb stain and counted using hemocytometer. B: serum OVA-specific IgE was measured by ELISA. C: mouse body weight. *P < 0.05 compared with corresponding saline controls.

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challenged mice with nonhematopoietic PTP1B deficiency is in contrast to our report on allergen-challenged mice with complete PTP1B deficiency. Allergen-challenged PTP1B knockout mice have increased production of IL-4, IL-5, IL-12, and IL-33, and increased allergic inflammation, suggesting that PTP1B limits hematopoietic cell production of cytokines (6), perhaps through regulation of cytokine receptor signaling in hematopoietic cells as reported for cytokine receptors in vitro (7, 41). Nevertheless, nonhematopoietic PTP1B deficiency reduces allergic inflammation and production of some cytokines (IL-5, IL-12, and IL-13) but not other cytokines (IL-4 or IL-33).

Importantly, we also demonstrated that PTP1B in endothelial cells is required for leukocyte recruitment because iePTP1B/−dox mice, which displayed a deletion of PTP1B specifically in endothelial cells, had reduced eosinophils in the lung and increased accumulation of eosinophils on the luminal surface of the endothelium. This inhibition of inflammation in iePTP1B/−dox mice occurred without inhibition of allergen-induced lung cytokines or chemokines. Thus there was binding of eosinophils to the endothelium but reduced transendothelial recruitment of the eosinophils into the tissue. These data are consistent with our previous reports that in vitro, endothelial cell PTP1B is a mediator of VCAM-1 signaling (16) and reports that VCAM-1 is required for infiltration of eosinophils in response to OVA challenge to the lung (12). This is also consistent with our previous report in which chimeric mice with a deficiency in nonhematopoietic NADPH oxidase 2 (NOX2), a VCAM-1 signal upstream of PTP1B, had accumulation of eosinophils on the endothelium and reduced eosinophils in the lung without altering lung adhesion molecules, cytokines, or chemokines (4).

We have previously demonstrated in vitro that PTP1B plays an active role in promotion of VCAM-1-dependent lymphocyte transendothelial migration through an endothelial signal transduction cascade sequentially involving activation of endothelial cell NOX2 (36), PkCα (16), and PTP1B (16). This VCAM-1-activated PTP1B induces signals that lead to activation of ERK1/2 in endothelial cells (1). VCAM-1 activation of PTP1B and downstream activation of ERK1/2 are required for VCAM-1-dependent leukocyte migration in vitro (1, 16). In the present study with PTP1B deficiency in endothelial cells in vivo, there was a decrease in eosinophils, lymphocytes, and monocytes/macrophages, but not neutrophils, in OVA-challenged lungs of iePTP1B/−dox mice. In the OVA model of allergic inflammation, eosinophils migrate on VCAM-1 (12), lymphocytes migrate on intercellular adhesion molecule 1 (ICAM-1), and partially migrate on VCAM-1 (12, 30, 33), and monocytes can migrate on VCAM-1 and ICAM-1 (35). In contrast, neutrophils primarily migrate on platelet endothelial cell adhesion molecule (PECAM-1) (13). It is reported that VCAM-1 and ICAM-1 but not PECAM-1 activate PkCα (2, 3), and it is reported that VCAM-1 activation of PkCα then activates PTP1B (16). Thus leukocytes, which are reported to migrate on VCAM-1 and ICAM-1 in the OVA model, had reduced recruitment to the lung in the OVA-challenged iePTP1B/−dox mice. The loss of leukocyte recruitment in the iePTP1B/−dox mice is the first report that deleting the VCAM-1 signal PTP1B specifically in endothelial cells in vivo blocks eosinophil recruitment.

PTP1B regulates tyrosine phosphorylation, and it is reported that tyrosine phosphorylation can regulate cell junction proteins. During VCAM-1 signaling, the tyrosine phosphatase PTP1B activates signals that induce Thr202/Tyr204 phosphorylation and activation of ERK1/2 (1). Activation of ERK1/2 is also reported to be required for E-selectin-mediated opening of endothelial barriers and cancer cell transendothelial migration (47). ERK1/2 is reported to regulate endothelial cell junctions in endothelial cells stimulated with VEGF, cannabinoids, or peroxide (8, 31, 42). Furthermore, binding of monocytes or invasive cancer cells to endothelial cells activates tyrosine phosphorylation of vascular endothelial cadherin via an ERK signaling cascade (22, 23). It is also reported that PTP1B on the endoplasmic reticulum interacts with the plasma membrane preferentially at points of cell-cell contact in fibroblasts and epithelial ovarian cancer cells and that PTP1B interacts with cadherin complexes during the formation of these cell-cell junctions (25, 28, 29). In contrast, during leukocyte transendothelial migration endothelial cell-cell junctions dissociate. We have reported that VCAM-1-dependent transendothelial migration requires activation of PTP1B (16), that VCAM-1-stimulated PTP1B functions upstream of ERK1/2 (1), that PTP1B indirectly induces the activation of ERK1/2 (1), and that this PTP1B activation of ERK1/2 is required for transendothelial migration in vitro (1). The direct target of PTP1B in VCAM-1 signaling has not yet been identified. Studies to identify PTP1B targets during VCAM-1-dependent leukocyte migration through endothelial cell junctions are under investigation in our research group. In the present report, we demonstrate that in vivo, endothelial cell PTP1B is required for leukocyte transendothelial migration.

In conclusion, endothelial cell PTP1B participates in VCAM-1-dependent eosinophilia in OVA-challenged iePTP1B/−dox mice. Thus vascular PTP1B may be a novel endothelial target for drug intervention in the limitation of the VCAM-1-dependent component of inflammations that have been implicated in diseases such as asthma (11, 12), atherosclerosis (20, 43), and multiple sclerosis (21). Moreover, limiting VCAM-1-dependent inflammation in chronic disease may be an advantageous target, since it would spare PECAM-1-independent inflammation such as neutrophil clearance of infections.

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DISCLOSURES

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ENDOTHELIAL PTP1B REGULATES ALLERGIC INFLAMMATION

AUTHOR CONTRIBUTIONS


REFERENCES


