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Thymic stromal lymphopoietin expression in allergic pulmonary inflammation is Pin1-dependent

Stephane Esnault, PhD^a, Louis A. Rosenthal, PhD^{b,c}, Zhong-Jian Shen, PhD^a, Cara J. Westmark, PhD^a, Ronald L. Sorkness, PhD^{b,c,d}, and James S. Malter, MD^a

^aThe Waisman Center for Developmental Disabilities, Department of Pathology and Laboratory Medicine, University of Wisconsin School of Medicine and Public Health, Madison

^bThe Morris Institute for Respiratory Research, University of Wisconsin School of Medicine and Public Health, Madison

^cThe Department of Medicine, Clinical Science Center, University of Wisconsin School of Medicine and Public Health, Madison

^dThe School of Pharmacy, University of Wisconsin, Madison, Wis

To the Editor

Thymic stromal lymphopoietin (TSLP) may be a novel link between the activation of fibroblasts, epithelial cells, and keratinocytes after exposure to environmental stimuli and the subsequent development of allergic inflammation. In humans, TSLP potentiates the ability of dendritic and Langerhans cells to trigger a T_H2 immune response.^{1,2} TSLP-transgenic mice exhibited marked pulmonary, perivascular inflammation (lymphocytes and eosinophils), goblet cell hyperplasia, and subepithelial fibrosis,³ whereas TSLP receptor knockout mice showed an attenuated allergic reaction after ovalbumin sensitization and challenge.³ Keratinocytes treated with a combination of proinflammatory and T_H2 cytokines⁴ or epithelial cells activated through Toll-like receptor ligands⁵ upregulated TSLP expression and secretion. How TSLP expression is controlled at a molecular level remains largely unknown.

The peptidyl-prolyl isomerase, Pin1, has emerged as an important regulator of prosurvival and proinflammatory cytokine expression by both eosinophils and T cells.^{6,7} Active Pin1 binds to and likely isomerizes adenosine-uridine binding factor 1 (AUF1), reducing its affinity for GM-CSF and TGF- β mRNAs.^{6–8} Under such conditions, the mRNA is stabilized and cytokine expression enhanced. Conversely, *in vivo* Pin1 blockade reduced GM-CSF, IL-5, and TGF- β 1 expression, eosinophilia, and remodeling^{8,9} in both mouse and rat models of allergic pulmonary inflammation. The National Center for Biotechnology Information database searches revealed that the 3' untranslated region of rat, human, and

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mouse TSLP mRNA all contained an adenosine-uridine-rich element, suggesting Pin1 might regulate TSLP at the post-transcriptional level through AUF1.^{6–8}

Therefore, Brown Norway rats were sensitized with ragweed pollen extract and challenged with either aerosolized ragweed (RAG) or PBS. In addition to RAG sensitization and challenge, some rats also received an intraperitoneal injection of juglone (1 mg/kg body weight/d), a Pin1 inhibitor, the day before, the day of, and the day after the challenge (RAG + J) as recently described.⁹ Rats were analyzed 2 days after the allergen challenge as described previously.^{8,9} TSLP mRNA expression, as measured by quantitative reverse-transcription–real-time PCR analysis,^{8,9} was significantly increased in the lung after allergen challenge, which was completely suppressed by Pin1 blockade (Fig 1, *A*; ANOVA followed by planned pairwise comparisons with Fisher least significant difference test; *n* = 4 rats per group). As discussed, TSLP was upregulated by the combination of proinflammatory and type 2 cytokines such as TNF- α and IL-4.⁴ The level of TNF- α mRNA expression in bronchoalveolar lavage cells, as measured by quantitative PCR,^{8,9} was significantly enhanced by allergen challenge and also attenuated by Pin1 blockade (Fig 1, *B*; ANOVA followed by planned pairwise comparisons with Fisher least significant difference test; *n* = 4–6 rats per group). IL-4 mRNA, whose expression is Pin1-independent,⁹ was unaffected by juglone. To determine the role of Pin1 on TNF- α expression, splenocytes from Pin1 knockout mice and wild-type (WT) litter-mates were activated with antibodies to CD3 and CD28 as described.⁹ TNF- α mRNA was significantly lower in knockout than WT splenocytes (Fig 1, *C*; Student *t* test; *n* = 3 mice per group). Therefore, Pin1 activity was required for maximal TNF- α expression. Taken together, these results suggest that Pin1 controls TSLP expression *in vivo* in an allergen-driven model of pulmonary inflammation and may do so in part by inhibiting the expression of the proinflammatory cytokine TNF- α .

Our *in vivo* data describe TSLP expression in total lung. Because TSLP is mainly produced by epithelial cells and fibroblasts,¹ we evaluated the contribution of Pin1 to TSLP production by lung fibroblasts *in vitro*. Fibroblasts were prepared from mechanically disaggregated lungs of Pin1 knockout or WT littermates in Dulbecco modified Eagle medium with glucose, supplemented with L-glutamine, sodium pyruvate, and 10% FBS. The adherent cells showed fibroblast morphology and stained strongly with antivimentin but very weakly with anti- α -smooth muscle actin (not shown). After 3 to 5 passages, the cells were serum starved for 3 days and activated for 6 to 7 hours (Fig 2, *B and A*, respectively) with murine IL-4 (20 ng/mL) $\pm$ TNF- α (20 ng/mL; R&D Systems, Minneapolis, Minn). TSLP mRNA levels were determined by quantitative PCR, normalized to 18S RNA, and expressed as fold change from resting cells. As shown (Fig 2, *A*), Pin1 knockout fibroblasts produced substantially less TSLP mRNA than WT when activated with IL-4 + TNF- α for 7 hours (Fig 2, *A*). WT lung fibroblasts treated with IL-4 plus TNF- α showed significant increases in TSLP, which was completely antagonized by juglone (Fig 2, *B*; mRNA fold change compared with resting cells (R); 1-sample and 2-sample *t* tests, respectively; *n* = 3 experiments per group). TSLP protein production by WT lung fibroblasts at 6 hours as determined by ELISA (R&D Systems) was consistent with the mRNA levels (15, 7, and 5 pg/mL for IL-4 + TNF- α , resting [R], and IL-4 + TNF- α + juglone, respectively). Therefore, Pin1 is required for basal as well as cytokine-induced TSLP mRNA and protein expression.

Whether these effects are at transcriptional and/or posttranscriptional levels as demonstrated for other cytokines remains to be established.^{6–9}

In summary, Pin1 modulates key features of the late phase of the allergic reaction including prosurvival and profibrotic cytokine expression, eosinophilia, and airway remodeling. These results further implicate Pin1 in T_H2 immune responses through the regulation of TSLP production by fibroblasts and strengthen the rationale for targeting this isomerase as a therapeutic strategy to reduce allergic inflammation. Because of its effects on the expression of multiple cytokines, Pin1 may be a ideal therapeutic target to control allergic disease and be more effective than anti-IL-5.

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References

1. Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol.* 2002; 3:673–80. [PubMed: 12055625]
2. Ebner S, Nguyen VA, Forstner M, Wang YH, Wolfram D, Liu YJ, et al. Thymic stromal lymphopoietin converts human epidermal Langerhans cells into antigen-presenting cells that induce proallergic T cells. *J Allergy Clin Immunol.* 2007; 119:982–90. [PubMed: 17320941]
3. Zhou B, Comeau MR, De Smedt T, Liggitt HD, Dahl ME, Lewis DB, et al. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat Immunol.* 2005; 6:1047–53. [PubMed: 16142237]
4. Bogiatzi SI, Fernandez I, Bichet JC, Marloie-Provost MA, Volpe E, Sastre X, et al. Cutting edge: proinflammatory and Th2 cytokines synergize to induce thymic stromal lymphopoietin production by human skin keratinocytes. *J Immunol.* 2007; 178:3373–7. [PubMed: 17339431]
5. Lee HC, Ziegler SF. Inducible expression of the proallergic cytokine thymic stromal lymphopoietin in airway epithelial cells is controlled by NFκappaB. *Proc Natl Acad Sci U S A.* 2007; 104:914–9. [PubMed: 17213320]
6. Shen ZJ, Esnault S, Malter JS. The peptidyl-prolyl isomerase Pin1 regulates the stability of granulocyte-macrophage colony-stimulating factor mRNA in activated eosinophils. *Nat Immunol.* 2005; 6:1280–7. [PubMed: 16273101]
7. Esnault S, Shen ZJ, Whitesel E, Malter JS. The peptidyl-prolyl isomerase Pin1 regulates granulocyte-macrophage colony-stimulating factor mRNA stability in T lymphocytes. *J Immunol.* 2006; 177:6999–7006. [PubMed: 17082615]
8. Shen ZJ, Esnault S, Rosenthal LA, Szakaly RJ, Westmark PR, Sorkness RL, et al. Pin1 regulates eosinophil TGF-β1 production and allergic lung fibrosis. *J Clin Invest.* 2008; 118:479–90. [PubMed: 18188456]
9. Esnault S, Rosenthal LA, Shen ZJ, Sedgwick JB, Szakaly RJ, Sorkness RL, et al. A critical role for Pin1 in allergic pulmonary eosinophilia in rats. *J Allergy Clin Immunol.* 2007; 120:1082–8. [PubMed: 17720236]

FIG 1.

Pin1 blockade reduces TSLP and TNF- α expression. **A** and **B**, Lung TSLP and bronchoalveolar lavage cell TNF- α mRNA levels in ragweed-sensitized rats that were challenged with PBS, ragweed (*RAG*), or RAG after juglone treatment (*RAG+J*). **C**, Fold change in TNF- α mRNA levels in CD3-activated plus CD28-activated WT and Pin1 knockout (*KO*) splenocytes compared to resting cells. Data presented as described.⁹

FIG 2.

Pin1 controls TSLP expression in murine lung fibroblasts. **A**, TSLP mRNA levels in lung fibroblasts from 2 Pin1 knockout (*KO*) and 2 WT mice after activation with IL-4 plus TNF- α for 7 hours. **B**, TSLP mRNA levels in WT lung fibroblasts that were untreated (*R*) or treated for 6 hours with IL-4 or IL-4 + TNF- α with (*J*) or without (-) juglone.