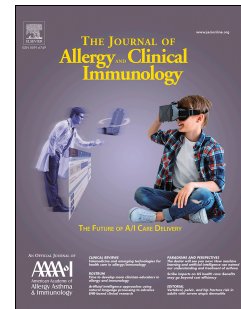


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Hideaki Morita, MD, PhD, Hirohisa Saito, MD, PhD, Kenji Matsumoto, MD, PhD



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Immune checkpoint molecules on ILC2s as potential therapeutic targets for allergic diseases

Hideaki Morita, MD, PhD,^{a,b,†} Hirohisa Saito, MD, PhD,^a Kenji Matsumoto MD, PhD^{a,†}

^aFrom the Department of Allergy and Clinical Immunology, National Research Institute for Child Health and Development, Tokyo, Japan

^bFrom the Allergy Center, National Center for Child Health and Development, Tokyo, Japan

†Corresponding authors:

Hideaki Morita, MD, PhD

Department of Allergy and Clinical Immunology, National Research Institute for Child Health and Development

2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan

Phone: +81-3-3416-0181

E-mail: morita-hi@ncchd.go.jp

and

Kenji Matsumoto, MD, PhD

Department of Allergy and Clinical Immunology, National Research Institute for Child Health and Development

2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan

Phone: +81-3-3416-0181

E-mail: matsumoto-k@ncchd.go.jp

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27 *1(LAIR-1), immune checkpoint molecule, allergic disease*

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For many years, the pathophysiology of allergic diseases was explained on the basis of allergen-specific immune responses (adaptive immunity), represented by Th2 cells and IgE antibodies. However, the discovery of group 2 innate lymphoid cells (ILC2s) that produce type-2 cytokines in a non-allergen-specific manner highlighted the importance of innate immunity in the pathophysiology of allergic diseases. Initial studies revealed that ILC2s are activated by such epithelial-derived cytokines as IL-33 and TSLP that are induced by allergens, and they may play a role in induction of allergic inflammation on body surfaces. ILC2s are also activated through interactions with other immune cells, including T cells, B cells and dendritic cells, suggesting that ILC2s may contribute to the amplification and chronicity of allergic inflammation through crosstalk between innate immunity and adaptive immunity.¹ Given that ILC2s are also reportedly involved in steroid resistance in bronchial asthma, development of a strategy to suppress ILC2s' activity is a growing field of research.

Immune checkpoint molecules are defined as ligand-receptor pairs that are expressed on immune cells and trigger immunosuppressive signaling. They play crucial roles in maintaining self-tolerance by suppressing autoreactive cells and in preventing collateral tissue damage by suppressing excessive inflammation. However, it has become clear that some pathogens and cancer cells use these molecules to induce immune exhaustion that allows them to evade immune responses against themselves. Therefore, immune checkpoint blockade therapy that activates anti-tumor or anti-pathogen immunity is attracting enormous attention as a novel treatment option for cancers and infections. Conversely, it might be possible to apply this concept in reverse to treat allergic diseases; that is, immune checkpoint molecules could be cross-linked in order to activate this pathway, which would then suppress allergic inflammation. Thus, immune

checkpoint molecules expressed on ILC2s may be promising therapeutic targets for allergic diseases (Table 1).²⁻¹⁰

In this issue of the *Journal*, Helou et al.² report that an immune checkpoint molecule, leukocyte-associated Ig-like receptor-1 (LAIR-1), that is expressed on ILC2s may be a potential therapeutic target for asthma. They found that (1) LAIR-1 was not expressed on lung ILC2s from naïve mice but was induced on lung ILC2s from inflamed tissues upon IL-33 administration, and (2) cross-linking of LAIR-1 by its known ligand, C1q, decreased type-2 cytokine production by ILC2s *in vitro*. They also demonstrated a suppressive role for LAIR-1 on ILC2s *in vivo*. In brief, following administration of IL-33 or *Alternaria*, ILC2s from LAIR-1-deficient mice produced more type-2 cytokines than ILC2s from WT mice, and those LAIR-1-deficient mice showed exacerbation of allergic airway inflammation and airway hyperreactivity. Finally, they investigated the roles of LAIR-1 on human ILC2s both *in vitro* and *in vivo*. *In vitro*, they found that knockdown of the *Lair 1* gene in human ILC2s resulted in significant upregulation of type-2 cytokine production and proliferation upon IL-33 stimulation. In addition, IL-33-induced allergic airway inflammation and airway hyperreactivity in humanized mice were significantly reduced by administration of C1q, a ligand of LAIR-1. Taken together, their findings suggest that the LAIR-1 pathway is crucial for regulating allergic airway inflammation by suppressing ILC2s' activity.

LAIR-1 is an immunoglobulin-like receptor containing two immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and has been reported to be expressed on such immune cells as T cells, B cells and NK cells. In addition, cross-linking of LAIR-1 on those cells directly inhibited their activation, indicating that LAIR-1 serves as an immune checkpoint molecule. However, LAIR-1 expressed on myeloid lineage cells has

different roles, including supporting the survival, differentiation and proliferation of those cells. Therefore, cross-linking of LAIR-1 may lead to both improvement and exacerbation of inflammatory diseases depending on the types of inflammation, and it may also weaken anti-tumor immunity. These findings suggest the importance of ensuring cell specificity when considering LAIR-1 as a therapeutic target.

The immune checkpoint molecules expressed on ILC2s can be categorized into two different groups based on the cell type(s) expressing their ligand. For one group, the ligand is expressed mainly on immune cells, including antigen-presenting cells. For the second group, the ligand is expressed mainly on non-immune cells, including epithelial cells and fibroblasts. The former group includes such checkpoint molecules as programmed cell death protein-1 (PD-1), CD200R, cytotoxic T-lymphocyte antigen 4 (CTLA-4) and T-cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif (TIGIT), and activation of ILC2s may be suppressed when these molecules interact with their ligands on antigen-presenting cells. The ligands of LAIR-1 are collagen expressed on non-immune cells and C1q, which is abundant in the serum; thus, LAIR-1 would be categorized into the second group. Given that ILC2s are tissue-resident cells, it would seem reasonable for the tissue-constituting cells to be responsible for keeping an eye on ILC2s so that they do not get out of control.

The same concept might also apply to killer cell lectin-like receptor G1 (KLRG1) expressed on ILC2s. In humans, KLRG1 was highly expressed on skin ILC2s from atopic dermatitis patients compared to from healthy controls. In addition, it was also upregulated on human ILC2s by *in vitro* stimulation with IL-33 or TSLP. The ligand of KLRG1 is a member of the cadherin family, which includes E-, N- and P-cadherin. Those cadherin ligands are expressed on tissue-constituting cells such as keratinocytes,

and E-cadherin ligation reportedly suppressed cytokine production by human ILC2s.¹⁰ However, in mice, KLRG1 is known to be a surface marker on ILC2s, and it is expressed on ILC2s in various organs at steady state. This suggests that KLRG1 may also contribute to setting a threshold for ILC2 activation in those organs. Of note, unlike KLRG1, LAIR-1 is expressed only on activated—not resting—lung ILC2s, suggesting that it may serve as a checkpoint molecule for preventing uncontrolled activation. Therefore, LAIR-1 may be a more suitable checkpoint molecule than KLRG1 to target in the treatment of allergic inflammation.

Another interesting issue may be involvement of immune checkpoint molecules in induction of IL-10–producing ILCs (IL-10⁺ILCs) that have regulatory functions. IL-10⁺ILCs were found in lung and nasal tissues with type-2 inflammation from both humans and mice^{5,7,8} and also in the peripheral blood of allergic rhinitis patients who underwent AIT.^{6,9} Because IL-10⁺ILCs were induced from ILC2s *in vitro* by IL-2, IL-33 and retinoic acid stimulation,^{5,7,9} they are thought to be derived from ILC2s *in vivo*. In addition, those induced IL-10⁺ILCs expressed such immune checkpoint molecules as CTLA-4 and TIGIT.⁵⁻⁹ However, it is not clear whether LAIR-1 will be induced on IL-10⁺ILCs like other molecules such as CTLA-4, and whether immune checkpoint molecules are involved in the induction of IL-10⁺ILCs from ILC2s.

In conclusion, in light of Helou et al.'s findings of expression and suppressive functions for an immune checkpoint molecule, LAIR-1, on ILC2s, targeting of LAIR-1 on those cells may have great potential for treatment of asthma and other allergic diseases.

However, there is as yet no direct evidence of the biological significance of crosslinking of LAIR-1 on ILC2s by antibodies. Various other issues also need to be resolved, including how to cross-link LAIR-1 only on ILC2s and how to identify patients who

may benefit from that cross-linking. We anticipate that these points will be clarified by future translational research on immune checkpoint molecules on ILC2s.

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1 **TABLE 1. Immune checkpoint molecules on ILC2s**

Molecule	Ligand	Organ (species)	Expression		Reference
			At steady state	In inflamed tissues or upon <i>in vitro</i> stimulation	
PD-1	PD-L1, PD-	Lung ILC2s (mouse)	-	++	Helou DG et al. Nat Commun. 2020. ³
	L2	Peripheral blood ILC2s (human)	-	++ (<i>in vitro</i> with IL-2, IL-7 and IL-33 stimulation)	Helou DG et al. Nat Commun. 2020. ³
CD200R	CD200	Lung ILC2s (mouse)	+	++	Shafiei-Jahani P et al. Nat Commun. 2021. ⁴
		Peripheral blood ILC2s (human)	+	++ (<i>in vitro</i> with IL-2, IL-7 and IL-33 stimulation)	Shafiei-Jahani P et al. Nat Commun. 2021. ⁴
LAIR-1	C1q, collagen	Lung ILC2s (mouse)	-	+	Helou DG et al. J Allergy Clin Immunol. 2021. ²
		Peripheral blood ILC2s (human)	+	++ (<i>in vitro</i> with IL-2, IL-7 and IL-33 stimulation)	Helou DG et al. J Allergy Clin Immunol. 2021. ²

KLRG1	E-, N-, and	ILC2s in lung, skin, intestine	+	+	Meininger I et al. Trends Immunol. 2020.
	P-cadherin	and mesenteric lymph nodes (mouse)			
		Skin ILC2 (human)	±	+ (atopic dermatitis patients) + (<i>in vitro</i> with IL-33 or TSLP stimulation)	Salimi M et al. J Exp Med. 2013. ¹⁰
CTLA-4	CD80,	Lung ILC2s (mouse)	-	+	Miyamoto C et al. Nat Commun. 2019. ⁸
	CD86				Seehus CR et al. Nat Commun. 2017. ⁷
		Nasal ILC2s (human)	-	+ (CRSwNP patients)	Morita H et al. J Allergy Clin Immunol. 2019. ⁵
		Peripheral blood ILC2s (human)	-	+ (allergic rhinitis patients who undergo AIT) + (<i>in vitro</i> with IL-33, retinoic acid plus IL-2 or IL-7 stimulation)	Boonpiyathad T et al. J Allergy Clin Immunol. 2021. ⁶ Golebski K et al. Immunity. 2021. ⁹ Morita H et al. J Allergy Clin Immunol. 2019. ⁵ Golebski K et al. Immunity 2021. ⁹

TIGIT	CD155,	Lung ILC2s (mouse)	-	+	Miyamoto C et al. Nat Commun. 2019. ⁸
	CD112				Seehus CR et al. Nat Commun. 2017. ⁷

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- 2 ILC2: group 2 innate lymphoid cells; PD-1: programmed cell death protein-1; PD-L1: programmed cell death ligand-1; LAIR-1:
- 3 leukocyte-associated immunoglobulin-like receptor 1; CTLA-4: cytotoxic T-lymphocyte antigen 4; CRSwNP: chronic rhinosinusitis
- 4 with nasal polyps; AIT: allergen-specific immunotherapy; TIGIT: T-cell immunoreceptor with Ig and immunoreceptor tyrosine-based
- 5 inhibitory motif (ITIM) domains.