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Letter to the Editor

Semaphorin 4D is expressed constitutively by human eosinophils

Dear Editor

The semaphorin family comprises soluble and membrane-bound proteins originally identified as axonal guidance cues that function during neuronal development. Emerging evidence suggests that a subset of semaphorins (sema3A, sema4A, sema4D, sema6D, sema7A), called 'immune semaphorins', function in the immune system. Sema3A use neuropilin-1/Plexin-A1 receptor complex. Sema3A was required for DC transmigration and that sema3A produced by the lymphatics promoted actomyosin contraction at the trailing edge of migrating DCs.¹ Sema4A and Sema4D use Tim-2 and CD72, respectively, as receptors during immune responses. On the other hand, sema4A and sema4D use Plexin-B1 during cell migration and angiogenesis. Sema4A is preferentially expressed on B cells and dendritic cells, and is involved in the activation of T cells, whereas sema4D, which is expressed constitutively by T cells, is involved in the activation of B cells and dendritic cells.² Sema4A enhances the in vitro activation and differentiation of T cells and the in vivo generation of antigen-specific T cells. In addition, the Sema4A receptor is Tim-2, a member of the family of T-cell immunoglobulin domain and mucin domain (Tim) proteins that is expressed on activated T cells.³ Moreover sema4A plays not only in T cell priming, but also in the regulation of Th1/Th2 responses.⁴ And immune-cell-expressed ligand sema4A and the Treg-cell-expressed receptor neuropilin-1 (Nrp1) interact both in vitro, to potentiate Treg-cell function and survival, and in vivo, at inflammatory sites.⁵ Sema4D plays a critical role in immune responses by the novel mechanism of turning off negative signaling by CD72. The sema4D–CD72 interaction results in strong B cell costimulation by the mechanism of turning off negative signaling.⁶ Sema7A use β 1 integrin as receptor. Sema7A is highly expressed on activated T lymphocytes and its localization in the immunological synapse amplifies pro-inflammatory cytokine expression by antigen presenting cells.⁷ These immune semaphorins and their receptors have been reported to be expressed in various immune cells. However, there are not enough reports on these effect on eosinophils. So we have examined the effect of immune semaphorins on eosinophils.

Eosinophils were purified from heparinized peripheral blood of healthy volunteers as previously described.⁸ It has already been reported that sema7A receptor, β 1 integrin is expressed on eosinophils. We examined gene expression of the other immune semaphorin receptors (Neuropilin-1, Plexin-A1, Plexin-B1, CD-72) in untreated and cytokine-treated eosinophils by RT-PCR. All receptors' gene were expressed in untreated eosinophils and not

enhanced by the cytokine stimulation with IL-3, IL-5, TNF- α , β -NGF or VEGF (data not shown). We then investigated protein expression of the receptors by flow cytometry. These receptors were not expressed on the surface of untreated and cytokine-treated eosinophils (data not shown). Next, we tested the effect of sema4A and sema4D on O₂⁻ generation induced by IL-5 (10 ng/ml) or GM-CSF (1 ng/ml). Superoxide generation was measured by a cytochrome C reduction method as described elsewhere.⁹ Sema4A and sema4D (1 ng/ml) did not have any effect on the enhanced O₂⁻ generation (data not shown). Finally, we tested the effect of semaphorin 3A, 4A, 4D and 7A on cytokine and chemokine production induced by IL-5 (10 ng/ml) or GM-CSF (1 ng/ml). A panel of cytokines and chemokines (IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, TNF α , TNF β , IFN- γ , G-CSF, GM-CSF, MIP-1 α , MIP-1 β , MCP-1, Eotaxin, IP-10) in the supernatants was measured by using Luminex multiplex kits on a Luminex 100 multiplex bead array system (Luminex Corporation, Austin, TX, USA) to screen for cytokines and chemokines produced by eosinophils cultured with sema3A, sema4A, sema4D or sema7A (1 ng/ml) and/or IL-5 (10 ng/ml) or GM-CSF (1 ng/ml) for 48 h. These semaphorins have not evident effect for production cytokines and chemokines induced by IL-5 or GM-CSF (data not shown). From the above, it have been demonstrated that sema3A, sema4A, sema4D and sema7A do not have a direct effect on eosinophils.

Recently, it was reported that sema7A expressed on eosinophils. Sema7A protein on the surface of blood eosinophils was increased more by IL-3 than by GM-CSF or IL-5.¹⁰ Therefore, we examined for expression of semaphorin on eosinophils. First, we examined gene expression of sema4D in untreated and cytokine-treated eosinophils by RT-PCR. Sema4D gene was expressed in untreated eosinophils (Fig. 1A) and not enhanced by the cytokine stimulation with IL-1 β , IL-3, IL-5, IL-33, TNF- α or TSLP (Fig. 1B). We then investigated protein expression of the receptors by flow cytometry. Sema4D was expressed in untreated eosinophils and not enhanced by the cytokine stimulation with IL-5 or GM-CSF (Fig. 2).

Our study showed that immune semaphorin receptors' gene were expressed in eosinophils, but protein of these receptors were not expressed on the surface of untreated and cytokine-treated eosinophils from peripheral blood. Therefore, these semaphorins did not have direct effect on eosinophils. We used the several cytokines (IL-3, IL-5, GM-CSF, TNF- α , β -NGF, and VEGF) which were reported the activated effect for eosinophils or the enhanced expression for semaphorin receptors for priming.^{10,11} There is a possibility that up-regulation of semaphorin receptors and direct effect of semaphorin are led in eosinophils by other priming methods. Previous study reported that sema7A expressed on eosinophils and sema7A protein on the surface of blood eosinophils was increased more by IL-3 than by GM-CSF or IL-5.¹⁰

Peer review under responsibility of Japanese Society of Allergy.

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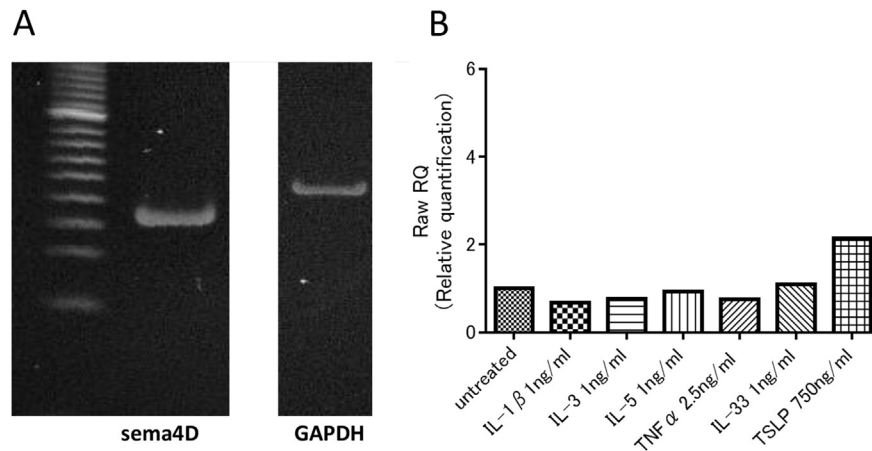


Fig. 1. A, Detection of sema4D in untreated eosinophils by real-time RT-PCR. The average Ct for sema4D was 27.608, and for GAPDH was 27.310. Amplified PCR products were detected by gel electrophoresis. B, Detection of sema4D in cytokine-treated eosinophils by real-time RT-PCR. RQ is expressed as fold-change using the $2^{-\Delta\Delta Ct}$ method in real-time PCR analysis.

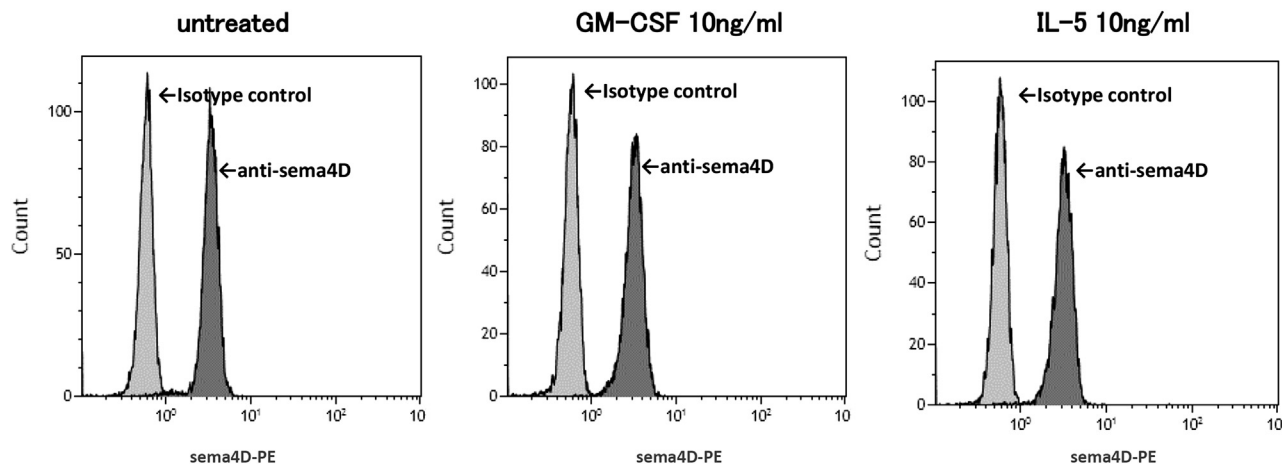


Fig. 2. Detection of sema4D in untreated and cytokine-treated eosinophils by flow cytometry. The cells were stained with isotype control antibody (isotype control), anti-sema4D Ab and analyzed by flow cytometry.

On the other hand, sema4D was expressed strongly and constitutively on peripheral blood eosinophils. Thus, sema4D was not upregulated any more in this study. Eosinophils are major effector cells in asthma. Airway epithelial cells expressed CD72 or plexin-B1. It was reported that sema4D acted as a critical positive regulator of the severity of inflammation observed in experimental allergic asthma. Its absence in vivo in Sema4D^{-/-} mice led to a downregulation of many features of allergic response such as predominantly eosinophilic BAL and lung tissue infiltration, mucous cell hyperplasia, BAL Th2 cytokine (IL-5 and IL-13) levels. It affected T cell activation and function suggesting its positive costimulatory role.¹² Further studies are required to define the function of eosinophil-expressed sema4D in lung allergic inflammation.

Acknowledgments

We thank Ms. Manami Negoro for her excellent technical assistance.

Conflict of interest

The authors have no conflict of interest to declare.

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Received 24 January 2015

Received in revised form 22 April 2015

Accepted 30 April 2015

Available online xxx