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Induction and maintenance of Th2 immune responses by T-lymphocyte-derived microparticle

Shosaku Nomura*, Michiomi Shimizu, Maiko Niki and Tomoki Ito

First Department of Internal Medicine, Kansai Medical University, 2-3-1 Shin-machi, Hirakata, Osaka 573-1191, Japan.

ABSTRACT

Thymic stromal lymphopoietin (TSLP), induced *via* OX40-ligand (OX40L) expressed on dendritic cells (DCs), is a key player in the induction of the T helper (Th)2 cell-mediated allergic cascade. Therefore, the TSLP-DC-OX40L axis might be the principal pathway in the inflammatory cascades in allergic diseases. Microparticles (MPs) are small membrane vesicles released from many different cell types by exocytotic budding of the plasma membrane in response to cellular activation or apoptosis. Elevated levels of platelet-derived MPs, endothelial cell-derived MPs, and monocyte-derived MPs in venous and arterial beds have been reported in almost all thrombotic diseases. However, the clinical role of T-lymphocyte-derived MPs (TLMPs) is poorly understood. In the present study we focused on the ability of TLMP to promote OX40L-mediated Th2 responses. Purified human naïve or memory $CD4^+$ T cells were stimulated with recombinant OX40L or TSLP-treated DCs (TSLP-DCs) in the presence of TLMP, and cytokine production by the primed T cells was examined. Although most TLMPs contain phosphatidylserine, only a few express lymphocyte functional antigen-1. TLMP remarkably enhanced TSLP-DC-driven or OX40L-driven Th2 responses from naïve T cells and the Th2 functional attributes of CRT^+ $CD4^+$ Th2 memory cells by the increased production of interleukin (IL)-5, IL-9 and IL-13. TLMP functions as a positive regulator of the TSLP-DC-OX40L axis that initiates and maintains Th2 cell-mediated inflammatory responses, and therefore, it might be a new therapeutic target for the treatment of allergic disorders.

*Corresponding author: shosaku-n@mbp.ocn.ne.jp; nomurash@hirakata.kmu.ac.jp

KEYWORDS: T-lymphocyte-derived microparticles, OX40L, Th2 responses, TSLP, dendritic cell

INTRODUCTION

Allergic disorders are characterized by inflammatory processes in which T helper type 2 (Th2) cells are crucial for the initiation and maintenance of allergic immune responses [1]. Th2-cell-derived cytokines such as interleukin (IL)-4, IL-5, and IL-13 induce the activation of an immunological effector system containing B cells, eosinophils, and mast cells, leading to increased immunoglobulin (Ig)E concentrations, mast cell degranulation, mucus hypersecretion, and eosinophil-mediated inflammation [2]. Previously, the integral role of dendritic cells (DCs) in the cellular cascade that initiates Th2 responses in allergy has been reported [3]. Furthermore, thymic stromal lymphopoietin (TSLP) [4], an epithelial cell-derived cytokine, equips DCs with a programming role in Th2 polarization in allergic inflammation [5]. TSLP is highly expressed by keratinocytes in the skin of patients with atopic dermatitis [5] and by airway epithelial lesion in patients with asthma [6]. In mice, the overexpression of TSLP causes atopic dermatitis-like skin lesions with the infiltration of Th2 cells and elevated serum IgE, which trigger bronchial allergic inflammation [7]. TSLP receptor-deficient mice failed to induce antigen-specific inflammatory Th2 cell responses and develop asthma in response to inhaled antigen [8]. Thus, TSLP appears to be the

first epithelial cell-derived cytokine that directly triggers DC-mediated Th2 allergic inflammation, which is a critical downstream cascade in allergic disease pathogenesis [9]. Human $CD11c^+$ myeloid DCs were shown to be activated by TSLP-primed naïve Th cells to differentiate into inflammatory Th2 cells that produced IL-4, IL-5, IL-13, and high levels of tumor necrosis factor (TNF)-α, with low levels of IL-10 and interferon (IFN)-γ [5]. In this process, OX40 ligand (OX40L), which is preferentially induced on human DCs by TSLP, was identified as a principal mediator to induce the generation of inflammatory Th2 cells [10]. Thus, the interacting axis of OX40L on DCs and OX40 on T cells is an essential element in inducing aberrant inflammatory Th2 cells that cause allergic disorders. In addition, memory Th2 cells are the principal cells responsible for the maintenance of chronic allergic inflammation and the relapse of allergic inflammation upon re-exposure to allergens [11, 12]. TSLP-activated DCs (TSLP-DCs) also play a role in the homeostatic expansion of allergen-specific Th2 memory cells and further polarization of the Th2 phenotypes through OX40L expression [13], contributing to the maintenance of chronic allergic inflammation.

Microparticles (MPs) are small membrane vesicles released from many different cell types by the exocytotic budding of plasma membrane in response to cellular activation or apoptosis [14-16]. MPs are commonly measured by flow cytometry and range in size from 0.1-1.0 μm [14, 15]. MPs disseminate various bioactive effectors originating from the parent cells. Therefore, MPs can alter vascular function and may induce biological responses involved in vascular homeostasis [15]. Although most MPs in human blood originate from platelets, they are also released from other cells such as leukocytes, erythrocytes, endothelial cells, smooth muscle cells, and cancer cells [14-17]. Elevated MP concentrations in venous and arterial beds have been reported in almost all thrombotic diseases [18-20]. Elevated levels of MPs are also associated with inflammation, cellular activation and dysfunction, angiogenesis, and transport [15, 21-25]. Interestingly, whereas MPs were initially considered to be a major source of proinflammatory effectors, it is now suggested that they might have anti-inflammatory and immunosuppressive properties, potentially helping in resolving local inflammation [26-29]. Indeed, besides their down-regulating effect on some DC functions [30], MPs can also block endothelial cell activation *via* phosphatidylserine (PS) or annexin-A1 binding [31] macrophage activation by means of several mechanisms [32-34]. These newly described regulatory roles of MPs during inflammation suggest an effect of MPs on DCs that might play a role in allergic diseases [35].

In the present study, we showed that T-lymphocyte-derived MP (TLMP) is a positive regulator of the TSLP-DC-OX40L axis that potentiates the differentiation of human naïve Th cells to Th2 cells and the expansion of memory Th2 cells, as well as markedly enhances the production of allergy-associated cytokines, IL-5, IL-9 and IL-13.

MATERIALS AND METHODS

Cell culture and reagents

RPMI-1640 supplemented with 2 nM L-glutamine, 100 U/ml penicillin, 100 ng/ml streptomycin, and heat-inactivated 10% fetal bovine serum (Biosourse International) was used for cell cultures throughout the experiments. We used 15 ng/ml TSLP (R&D Systems), 1 μg/ml R848 (Invivogen) and recombinant IL-4 (R&D Systems). Recombinant OX40L/CD32-transfected L fibroblasts for OX40L stimulation and CD32-transfected L fibroblasts as controls were used for T cell stimulation experiments, as described previously [36].

Isolation and culture of blood DCs

Human peripheral blood DC subsets (myeloid DCs and pDCs) were isolated from peripheral blood mononuclear cells from healthy adult donors, as described previously [37]. $CD11c^+$ DCs were cultured in flat-bottom 96-well plates in the presence of TSLP or R848 at 5×10^4 cells in 200 μl of medium per well for 24 h.

Purification of a naïve and $CRTH2^+$ $CD4^+$ memory T cell subset

$CD4^+$ T cells were enriched using a $CD4^+$ T cell isolation kit II (Miltenyi Biotec) according to the manufacturer's instructions. Enriched $CD4^+$ T cells were stained with FITC-labeled lineage cocktail (CD8, CD14, CD16, CD19, CD56, BDCA2, TCR-α/β, and glycophorin A) or PE-Cys5.5-labeled anti-CRTH2 (Miltenyi Biotec) followed by allophycocyanin-labeled anti-streptavidin (BD Biosciences). Stained cells were sorted by fractions of $CD4^+$ $CD45RA^+$ naïve T cells and $CD4^+$ $CRTH^+$ Th2 memory cells (purity > 99%).

T cell stimulation

Two × 10^4 freshly purified naïve $CD4^+$ T cells (DC-to-T cell ratio, 1:4) were cocultured with allogeneic $CD11c^+$ DCs precultured with different conditions for 24 h, and 1 × 10^4 freshly purified $CRTH2^+$ $CD4^+$ Th2 memory cells (DC-to-T cell ratio, 1:2) were cocultured with autologous $CD11c^+$ DCs in 96-well round-bottom culture plates for 7 days. In some experiments, T cells were cultured for 5 days with irradiated OX40L/CD32-transfected L fibroblast (2.5 × 10^4/well) as a platform to immobilize the stimulating anti-CD3 (OKT3, 0.2 μg/ml) and anti-CD28 (0.2 μg/ml) monoclonal antibodies (mAbs). CD32-transfected L fibroblasts were used as control L fibroblasts (parental L fibroblasts).

TLMP purification and cell activation

Isolation of TLMPs was performed according to previous reports by Shefler *et al*. [38] and Wheway *et al*. [39]. In brief, the supernatants from stimulated or unstimulated T cells were centrifuged for 5 min at 1500 × g, large cellular debris was discarded and supernatants were spun for 45 min at 15,000 × g. TLMP pellets were resuspended in cell culture-grade phosphate-buffered saline (PBS), and samples were spun once more for 45 min at 15,000 × g in PBS to ensure the removal of any remaining soluble factors. Next, TLMP pellets were resuspended in RPMI 1640/10% fetal calf serum for quantification and use in assays. To analyze the expression of surface molecules on TLMP, cell pellets were stained with CF405M-conjugated Annexin V (AV, BD, Bioscience), PE-labeled CD4 mAb, and FITC-labeled anti-CD45, or anti-$CD11a^+$, and then analyzed by a FACSCalibur™ (BD, Bioscience). Isotope-matched mAbs (R&D Systems) were used as negative controls. Finally, various T cells were activated by incubation with purified TLMPs.

Surface marker analysis of T cells

Cells were stained with PE-labeled anti-CD124 (IL-4 receptor α) mAb to determine their expressions. Isotype-matched PE-labeled unrelated mAbs (R&D Systems and Beckman Coulter) were used as negative controls.

Analysis of T cell cytokine production

Cultured $CD4^+$ T cells were collected and washed. For the detection of cytokine production in culture supernatants, T cells at a concentration of 10^6 cells/ml were restimulated with immobilized anti-CD3 (OKT3, 5 μg/ml) and soluble anti-CD28 (1 μg/ml) for 24 h. The levels of IL-4, IL-5, IL-9, IL-10, IL-13, TNFα, and IFNγ were measured by enzyme-linked immunosorbent assay (ELISA) (kits from R&D Systems or eBioscience).

T cell expansion assay

Freshly isolated 2 × 10^4 $CRTH2^+$ $CD4^+$ Th2 memory cells were stimulated for 5 days with CD32-transfected or OX40L/CD32-transfected L fibroblasts precoated with anti-CD3 (OKT3, 0.2 μg/ml) and anti-CD28 (0.2 μg/ml) mAbs, as described above. The cultured T cells were collected and resuspended in an EDTA-containing medium to dissociate the clusters. Viable cells were counted by trypan blue exclusion of dead cells.

Statistical analysis

Results were represented as the means ± standard error of the mean (s.e.m.). Statistical analysis was performed using the paired Student t-test. A p value < 0.05 was considered statistically significant.

RESULTS

Surface molecules of TLMP

Numbers of annexin V^+ and $CD4^+$ TLMP were significantly increased following T cell activation. A significantly greater number of intracellular adhesion molecule (ICAM)-1^+ TLMP were detected following stimulation. The numbers of lymphocyte function-associated antigen-1 $(LFA\text{-}1)^+$ TLMP were variated (Table 1 and Fig. 1).

Table 1. Quantification of TLMP subpopulations.

AV-positive TLMPs*		
Specific antigen	Mean	S.D.
$CD4^+$	1367	814
$CD45^+$	1216	837
$CD4^+/CD45^+$	1119	724
$CD4^+/CD11a^+$	984	602
$CD4^+/CD11a^+/CD45^+$	897	462

*: TLMP were measured by flow cytometry (Fig. 1).
AV: annexin V; CD11a: LFA-1.

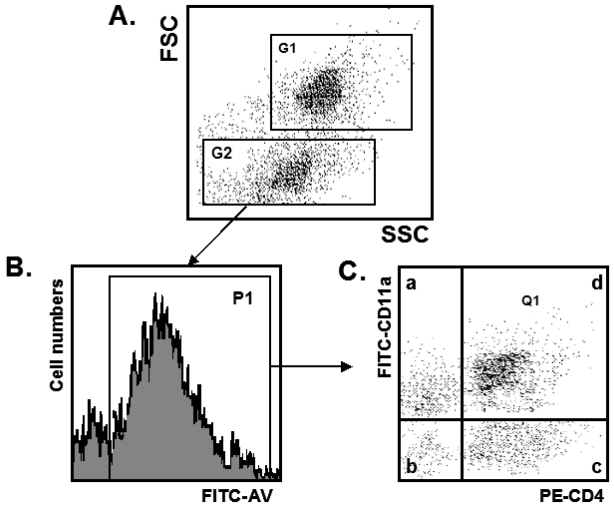

Fig. 1. Gating and staining strategy for the detection of TLMPs by FACS analysis. A: Gate limits in the Forward Scatter (FSC)/Side Scatter (SSC) plot for MP quantification (defined as >0.1 μm to <1 μm) were established before analyses using standard beads. Gate limits were set according to bead signal and according to MP size and granularity. G1 shows T-lymphocytes and G2 shows TLMPs. B: Annexin V-CF405M$^+$ TLMP (P1) were selected from G2. C: TLMPs binding to FITC- or PE-labeled antibodies (such as CD11a and CD4) were also selected from P1. Double staining with FITC- and PE-labeled antibodies from P1 was quantified from the Q1 region. FITC: fluorescein isothiocyanate. PE: phycoerythrin.

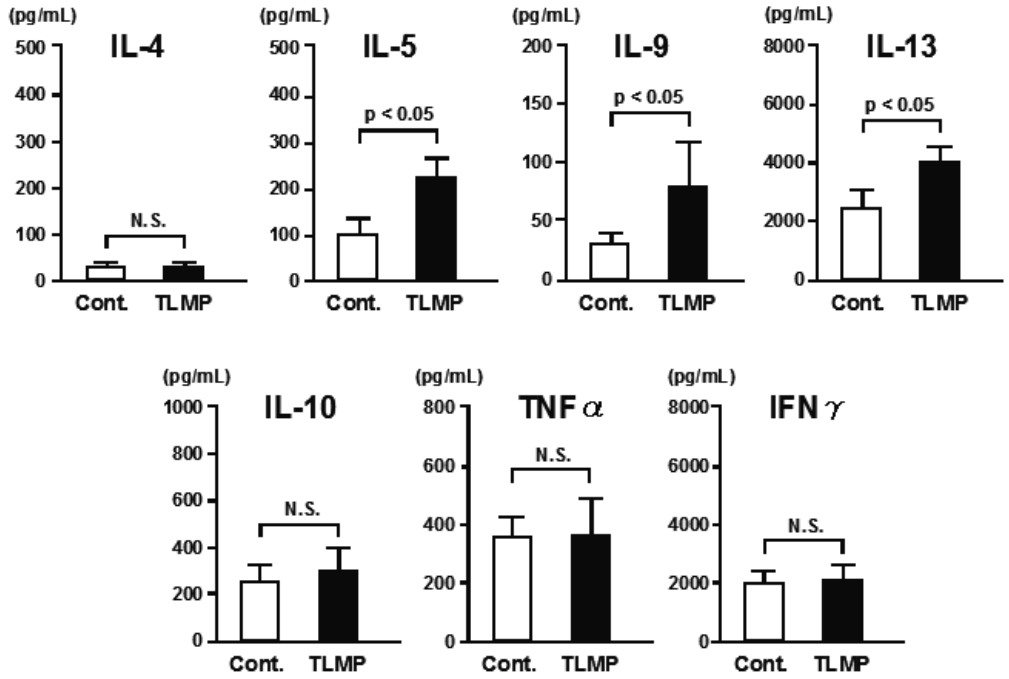

Fig. 2. Effects of TLMP on naïve Th cell differentiation. Freshly purified naïve CD4$^+$ T cells were stimulated with immobilized anti-CD3/CD28 mAbs on CD32 transfected-L fibroblasts in the presence or absence of TLMPs (50 μl). Five days after initiation of the culture, supernatants were collected and the amounts of cytokines indicated in the figures were determined by ELISA. Data are the means ± s.e.m. of five independent experiments. Statistical significance was determined using paired Student's t-test. N.S.: not significant.

TLMP enhances TSLP-DC-mediated Th2 cell responses *via* the OX40L axis

We next assessed the direct effect of TLMP on $CD4^+$ T cells as a Th2-polarizing axis. TLMP alone induced Th2 cells that produced IL-5, IL9, and IL-13 (Fig. 2A) but had no effect on IL-4, IL-10, TNFα, or IFNγ production when naïve T cells were stimulated with anti-CD3 and CD28 mAbs, which are dose-dependent for TLMP (Fig. 2B). We next examined whether TLMP and DC-derived OX40L could work synergistically in driving Th2 cell responses. Naïve $CD4^+$ T cells were cocultured for 7 days with allogeneic DCs pretreated with TSLP (TSLP-DCs), and cytokine production from the primed $CD4^+$ T cells was then examined. The addition of TLMP into the coculture of TSLP-DCs and naïve T cells induced a marked increase in the productions of IL-5, IL-9, and IL-13 (Fig. 3). This was also observed when naïve T cells were stimulated with OX40L-transfected L fibroblasts and anti-CD3/CD28 mAb (instead of TSLP-DCs). Increased productions of IL-4, IL-5, IL-9, IL-13, and TNFα with decreased productions of IL-10 and IFNγ were observed when T cells were stimulated with OX40L-transfected L cells (Fig. 4). In contrast, Th2 polarization was further promoted by the addition of TLMP, as indicated by the markedly elevated production of IL-5, IL-9, and IL-13 without affecting the production of IL-4, IL-10, TNFα, or IFNγ (Fig. 4). Therefore, TLMP and OX40L collaborate to promote Th2 differentiation.

TLMP enhances IL-4-mediated Th2 cell responses

We next examined the synergistic function of TLMP and IL-4 for Th2 polarization. Naïve T cells were stimulated with IL-4 and anti-CD3/CD28 mAb for 7 days. Unlike OX40L, IL-4 contributes to the generation of conventional Th2 cells that produce IL-5, IL-9, IL-13, and IL-10 but not TNFα (Fig. 5). When TLMP was added to the culture, the productions of IL-5, IL-9, and IL-13 were remarkably elevated without affecting IL-10, IFNγ, or TNFα (Fig. 5),

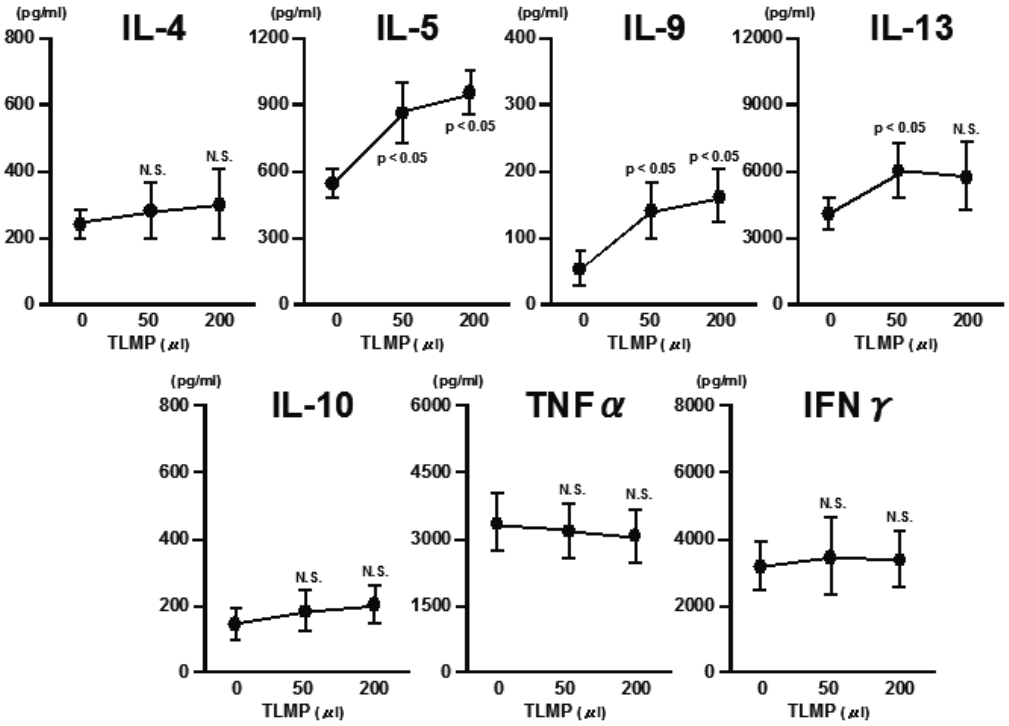

Fig. 3. Effects of TLMP on TSLP-DC-mediated Th2 cell responses. Blood myeloid $CD11c^+$ DCs were cultured with 15 ng/ml of TSLP (TSLP-DCs). Freshly isolated naïve $CD4^+$ T cells were cocultured with allogeneic TSLP-DCs (DC-to-T cell ratio, 1:4) in the presence of graded doses of TSLP (50 or 200 μl) for 7 days. Cytokine production by $CD4^+$ T cells was measured in supernatants by ELISA. Data are the means ± s.e.m. of five independent experiments. Statistical significance was determined using paired Student's t-test. N.S.: not significant.

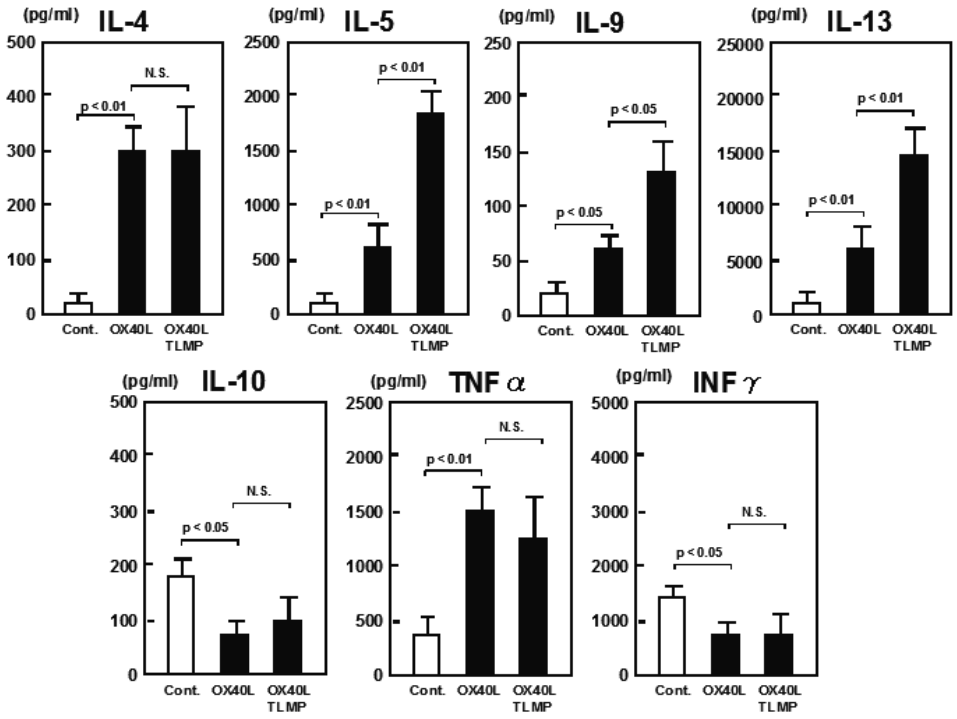

Fig. 4. Effects of TLMP on OX40L-mediated Th2 cell responses. Naïve Th cells were cultured on OX40L-transfected L fibroblasts with immobilized anti-CD3/CD28 mAbs in the presence or absence of TLMPs (50 µl). Naïve Th cells cocultued with CD32-transfected L fibroblasts (parental L cells) plus anti-CD3/CD28 mAbs were used as controls. Five days after the initiation of the culture, supernatants were collected and amounts of cytokines indicated in the figures were determined by ELISA. Data are the means ± s.e.m. of five independent experiments. Statistical significance was determined using paired Student's t-test. N.S.: not significant.

as tested in cultures containing TSLP-DCs (Fig. 3) and with OX40L-transfected L fibroblasts (Fig. 4). Thus, TLMP also enhances Th2 responses elicited by IL-4.

TLMP enhances memory Th2 cell responses

We next examined the effect of TLMP on the activation of $CRTH2^+$ memory Th2 cells. Isolated $CRTH2^+$ memory T cells were stimulated with anti-CD3/CD28 mAb and OX40L-transfected L fibroblasts or the parental control L cells in the presence or absence of TLMP. OX40L stimulation enhanced the expansion of $CRTH2^+$ memory T cells, and this effect was further amplified when TLMP was added (Fig. 6).

Inhibition of TLMP function

We next examined the inhibition of TLMP function. As shown in table 2, anti-LFA antibody markedly inhibited the Th2-polarization function of TLMP.

DISCUSSION

MPs can be generated by nearly every cell type during activation, injury, or apoptosis [14-17, 40, 41]. In the circulation, MPs are derived from various vascular cell types, including platelets, erythrocytes, leukocytes, and endothelial cells [14, 15, 40-43]. All MPs, regardless of their cell of origin, contain negatively charged phospholipids, such as phosphatidylserine, in their outer membrane leaflet, accounting for their procoagulant properties [14, 15, 41]. MPs also participate in homeostasis under physiological conditions *via* their rapid-release mechanism [44]. The known emissions of membrane-anchored receptors, adhesion molecules, enzymes, and signaling proteins *via* MPs were complemented by the identification of functional mRNA and microRNA species [14, 15, 45]. In addition, it was reported that MPs participate in T cell proliferation or Th1/Th2 differentiation [35, 38, 39, 46-49].

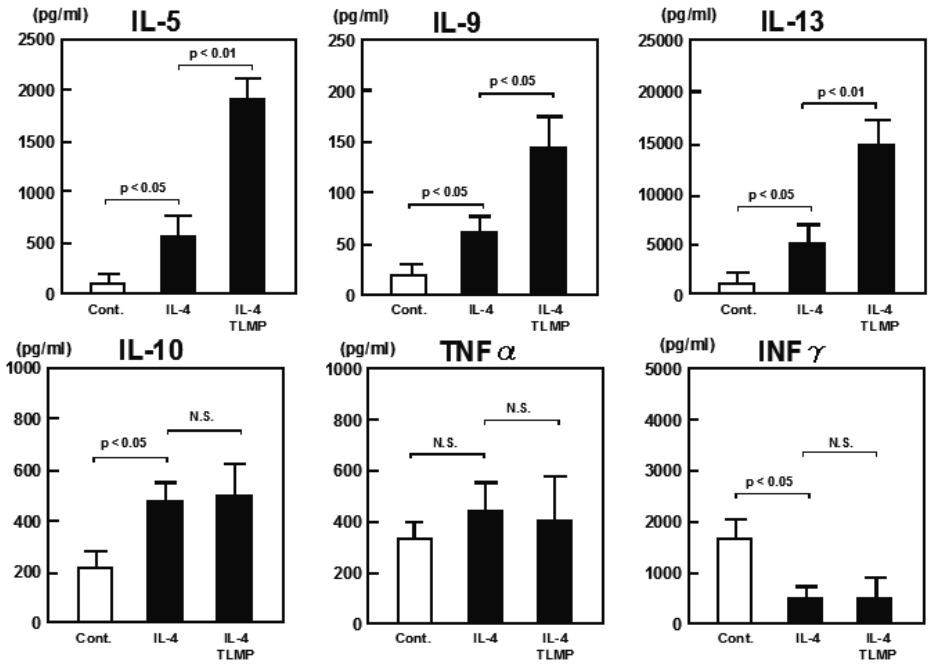

Fig. 5. Effects of TLMP on IL-4-mediated Th2 cell responses. Naïve Th cells were stimulated with immobilized anti-CD3/CD28 mAbs (CD32-transfected L fibroblasts (parental L cells) were used as a platform) and IL-4 (25 ng/ml) in the presence or absence of TLMPs (50 µl). Five days after the initiation of the culture, supernatants were collected and amounts of cytokines indicated in the figures were determined by ELISA. Data are the means ± s.e.m. of five independent experiments. Statistical significance was determined using paired Student's t-test. N.S.: not significant.

We here found that TLMP, an MP, participates in the Th2-polarizing DC-mediated OX40L effect as an important pathway to induce allergic responses. TLMP enhances IL-5 and IL-13 production in developing Th2 cells mediated by OX40L, and is involved in Th2 differentiation from naïve T cells as well as the maintenance of memory Th2 cells. Although OX40L causes allergic inflammation by the concomitant promotion of inflammatory cytokine TNFα production with the development of Th2 cells [10, 36], our results suggest that TLMP might contribute to Th2 immune responses by promoting the production of IL-5 and IL-13 from OX40L-driven Th2 cell differentiation and maintenance without affecting TNFα production.

IL-5 has been reported to support the terminal differentiation and proliferation of eosinophilic precursors and to recruit eosinophils to inflammatory sites [50]. Then, the recruited eosinophils produce cytotoxic substances such as major basic protein, eosinophilic cationic protein, and eosinophilic peroxidase, leading to tissue damage. In addition, IL-13, another cytokine upregulated by TLMP, might play a critical role in the development of bronchial asthma induced by airway hypersensitivity [51] and contribute to asthmatic airway obstruction by increasing mucin secretion in epithelial cells and mucus metaplasia [52, 53]. This was confirmed by a study showing that IL-13 affects both ciliated and secretory cell differentiation in animal models of asthma [54] and that it induces mucin secretion through the up-regulation of transforming growth factor (TGF) β [55]. Thus, in the context of Th2 cytokine-associated allergic responses, TLMP might be indirectly involved in the enhancement of inflammatory responses and aggravation of pathogenic conditions through the induction of increased IL-15 and IL-13 production.

Of note, TLMP enhanced the production of IL-9 in developing Th2 cells induced by either TSLP-DCs or OX40L. Th9 cells are closely associated with Th2 cells, as Th2 cells co-express both IL-4 and IL-9 in the early phase of Th2 differentiation, and IL-4, a Th2 cytokine, provides a key signal for Th9 induction [56].

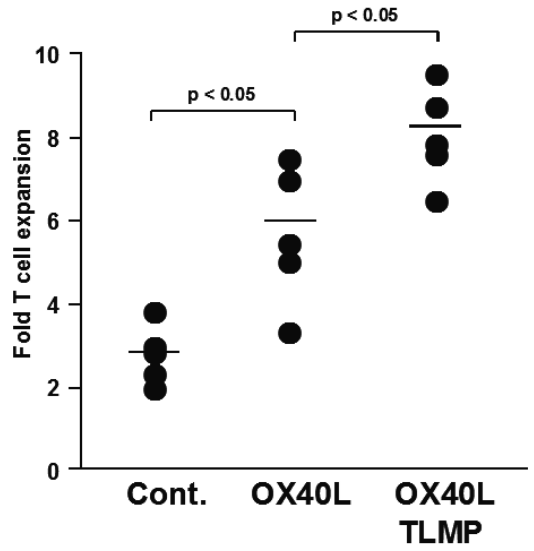

Fig. 6. Effects of TLMP on memory Th2 cell responses. Memory Th cells, isolated as $CD4^+CRTH2^+$ T cells, were stimulated with autologous TSLP-DCs (DC-to-T cell ratio, 1:2) for 7 days or with OX40L-transfected L fibroblasts plus immobilized anti-CD3/CD28 mAbs for 5 days in the presence or absence of TLMPs. After culture with OX40L-transfected L fibroblasts (OX40L) or parental L cells (control), viable T cells were counted by trypan blue exclusion test. Horizontal bars indicate the median of five independent experiments. Statistical significance was determined using paired Student's t-test (*, $p < 0.05$).

In addition, OX40L leads to a marked increase in Th9 differentiation from naïve $CD4^+$ T cells [57]. Although it has not been reported that TLMP induces IL-9 production in Th2 cells and basophils, IL-9 has been previously implicated in the pathogenesis of plasma and other allergic diseases in human studies [58-60]. Thus, TLMP is also involved in Th2-mediated allergic inflammation in accord with the production of IL-9, which mediates various effects on different cell types such as mast cell growth and the production of proinflammatory cytokines, IL-1β, IL-6, IL-5, and IL-13 [61]. Furthermore, IL-9 is suggested to be a key molecule for the differentiation of Th17 cells in conjunction with TGF-β_1 through the activation of STAT3 [62]. Therefore, the upregulation of IL-9 production induced by TLMP leads to an increase in inflammatory cells and worsening of regional allergic states. Our study now identifies the nature of the responses elicited by TLMP, which might pathophysiologically amplify both OX40L-driven inflammatory Th2 immune responses and IL-4-medicated classical Th2 cell differentiation. Although IL-4 is the critical Th2-polarizing factor [63], human DCs activated by TSLP or other stimuli do not have the capacity to produce IL-4 [5, 10]. Therefore, IL-4 is not the DC-derived original trigger of Th2 responses. Previous studies have suggested that basophils are

Table 2. Suppressive effects of anti-LFA-1 antibody for TLMP on TSLP-DC-, OX40L-, or IL-4-mediated Th2 cell responses.

Suppressive effects of anti-LFA-1 antibody			
TSLP-DC-mediated Th2 cell responses	TLMP	TLMP + anti FLA-1 mAb	p value
Induction of IL-5 (pg/ml)	896 ± 231	603 ± 62	< 0.01
Induction of IL-9 (pg/ml)	154 ± 40	72 ± 18	< 0.001
Induction of IL-13 (pg/ml)	6,040 ± 985	4,125 ± 711	< 0.05
OX40L-DC-mediated Th2 cell responses	TLMP	TLMP + anti FLA-1 mAb	p value
Induction of IL-5 (pg/ml)	1,721 ± 70	655 ± 94	< 0.01
Induction of IL-9 (pg/ml)	128 ± 33	69 ± 25	< 0.05
Induction of IL-13 (pg/ml)	14,150 ± 3,247	6,800 ± 1,526	< 0.05
IL-4-DC-mediated Th2 cell responses	TLMP	TLMP + anti FLA-1 mAb	p value
Induction of IL-5 (pg/ml)	1,955 ± 249	542 ± 134	< 0.01
Induction of IL-9 (pg/ml)	148 ± 51	68 ± 19	< 0.05
Induction of IL-13 (pg/ml)	15,345 ± 2,780	5,005 ± 920	< 0.05

The same experiments as in figs. 3, 4 and 5 were performed in the presence of anti-LFA antibody. Data are the means ± s.e.m. of five independent experiments. Statistical significance was determined using paired Student's t-test.

the most likely key source of IL-4 for the induction of Th2 immunity [64].

The precise mechanism of how TLMP functions as a positive regulator of the TSLP-DC-OX40L axis is unknown. However, lymphocyte-derived MP affects the function of the respiratory system [46-48]. In particular, Qiu *et al*. [46, 48] reported that MPs contribute to increased respiratory inflammatory responses and innate immune response maintenance in airway epithelium after MP engulfment by endothelial cells. These reports partially support our results. In addition, we assumed that the role of CD11a on TLMP was as a positive regulator of the TSLP-DC-OX40L axis, because anti-LFA antibody inhibited the Th2-polarization of TLMP. LFA-1 undergoes rapid conversion from an inactive to an active state as a result of an incremental increase in its intrinsic affinity for ligands, cellular signaling events, and avidity effects, such as lateral mobility or its clustering at the cell surface [65-68]. Activated LFA-1 recognizes and binds to ICAMs, which are inducibly expressed on antigen-presenting cells and on endothelium during inflammation [69]. These previous reports suggest that LFA-1-associated signaling might play an important role in immune responses induced by the TSLP-DC-OX40L axis. However, a greater understanding of how LFA-1-related functions affect TLMP is required.

CONCLUSION

We identified a specialized role of TLMP, which functions as a positive regulator of the DC-OX40L axis that promotes the differentiation of naïve Th cells into Th2 cells and maintains memory Th2 cells. Therefore, the regulation of TLMP production is a potential therapeutic target for the amelioration of allergic states. Thus, our results provide a new insight into the biological action of TLMP that underlies Th2-mediated allergic responses and might aid in the development of therapeutic strategies to ameliorate allergic diseases such as atopic dermatitis or asthma.

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CONFLICT OF INTEREST STATEMENT

The authors do not have any conflicts of interest to report concerning this work.

REFERENCES

1. Georas, S. N., Guo, J., De Fanis, U. and Casolaro, V. 2005, Eur. Respir. J., 26, 1119.
2. Abbas, A. K., Murphy, K. M. and Sher, A. 1996, Nature, 383, 787.
3. Pulendran, B., Tang, H. and Manicassamy, S. 2010, Nat. Immunol., 11, 647.
4. Sims, J. E., Williams, D. E., Morrissey, P. J., Garka, K., Foxworthe, D., Price, V., Friend, S. L., Farr, A., Bedell, M. A., Jenkins, N. A., Copeland, N. G., Grabstein, K. and Paxton, R. J. 2000, J. Exp. Med., 192, 671.
5. Soumelis, V., Reche, P. A., Kanzler, H., Yuan, W., Edward, G., Homey, B., Gilliet, M., Ho, S., Antonenko, S., Lauerma, A., Smith, K., Gorman, D., Zurawski, S., Abrams, J., Menon, S., McClanahan, T., de Waal-Malefyt Rd, R., Bazan, F., Kastelein, R. A. and Liu, Y. J. 2002, Nat. Immunol., 3, 673.
6. Ying, S., O'Connor, B., Ratoff, J., Meng, Q., Mallett, K., Cousins, D., Robinson, D., Zhang, G., Zhao, J., Lee, T. H. and Corrigan, C. 2005, J. Immunol., 174, 8183.
7. Yoo, J., Omori, M., Gyarmati, D., Zhou, B., Aye, T., Brewer, A., Comeau, M. R., Campbell, D. J. and Ziegler, S. F. 2005, J. Exp. Med., 202, 541.
8. Al-Shami, A., Spolski, R., Kelly, J., Keane-Myers, A. and Leonard, W. J. 2005, J. Exp. Med., 202, 829.
9. Ziegler, S. F. and Liu, Y. J. 2006, Nat. Immunol., 7, 709.
10 Ito, T., Wang, Y. H., Duramad, O., Hori, T., Delespesse, G. J., Watanabe, N., Qin, F. X., Yao, Z., Cao, W. and Liu, Y. J. 2005, J. Exp. Med., 202, 1213.
11. Mojtabavi, N., Dekan, G., Stingl, G. and Epstein, M. M. 2002, J. Immunol., 169, 4788.
12. Prescott, S. L., Macaubas, C., Smallacombe, T., Holt, B. J., Sly, P. D. and Holt, P. G. 1999, Lancet, 353, 196.
13. Wang, Y. H., Ito, T., Homey, B., Watanabe, N., Martinn, R., Barnes, C. J., Mclntyre, B. W., Gilliet, M., Kumar, R., Yao, Z. and Liu, Y. J. 2006, Immunity, 24, 827.

14. Nomura, S. and Shimizu, M. 2015, J. Intens. Care, 3, 2.
15. Nomura, S. 2016, J. Atheroscler. Thromb., 23, 1.
16. Vajen, T., Mause, S. F. and Koenen, R. R. 2015, Thromb. Haemost., 114, 228.
17. Angelillo-Scherrer, A. 2012, Cir. Res., 110, 356.
18. Matsumoto, N., Nomura, S., Kamihata, H., Kimura, Y. and Iwasaka, T. 2004, Thromb. Haemost., 91, 146.
19. Simak, J., Gelderman, M. P., Yu, H., Wright, V. and Baird, A. E. 2006, J. Thromb. Haemost., 4, 1296.
20. Ederhy, S., Di Angelantonio, E., Mallat, Z., Hugel, B., Janower, S., Meuleman, C., Boccara, F., Freyssinet, J. M., Tedgui, A. and Cohen, A. 2007, Am. J. Cardiol., 100, 989.
21. Kim, H. K., Song, K. S., Chung, J. H., Lee, K. R. and Lee, S. N. 2004, Br. J. Haematol., 124, 376.
22. Sinning, J. M., Losch, J., Walenta, K., Böhm, M., Nickenig, G. and Werner, N. 2010, Eur. Heart J., 32, 2034.
23. Wang, J. G., Geddings, J. E., Aleman, M. M., Cardenas, J. C., Chantrathammachart, P., Williams, J. C., Kirchhofer, D., Bogdanov, V. Y., Bach, R. R., Rak, J., Church, F. C., Wolberg, A. S., Pawlinski, R., Key, N. S., Yeh, J. J. and Mackman, N. 2012, Blood, 119, 5543.
24. Suades, R., Padró, T., Alonso, R., Mata, P. and Badimon, L. 2013, Thromb. Haemost., 110, 366.
25. Suades, R., Padró, T., Alonso, R., López-Miranda, J., Mata, P. and Badimon, L. 2014, Thromb. Haemost., 111, 111.
26. Morel, O., Morel, N., Jesel, L., Freyssinet, J. M. and Toti, F. 2011, Semin. Immunopathol., 33, 469.
27. Sadallah, S., Eken, C. and Schifferli, J. A. 2011, Semin. Immunopathol., 33, 487.
28. Sadallah, S., Eken, C. and Schifferli, J. A. 2011, Clin. Exp. Immunol., 163, 26.
29. Pliyev, B. K., Kalintseva, M. V., Abdulaeva, S. V., Yarygin, K. N. and Savchenko, V. G. 2014, Cytokine, 65, 126.
30. Eken, C., Gasser, O., Zenhaeusern, G., Oehri, I., Hess, C. and Schifferli, J. A. 2008, J. Immunol., 180, 817.
31. Dalli, J., Norling, L. V., Renshaw, D., Cooper, D., Leung, K. Y. and Perretti, M. 2008, Blood, 112, 2512.
32. Eken, C., Martin, P. J., Sadallah, S., Treves, S., Schaller, M. and Schifferli, J. A. 2010, J. Biol. Chem., 285, 39914.
33. Eken, C., Sadallah, S., Martin, P. J., Treves, S. and Schifferli, J. A. 2013, Immunobiology, 218, 382.
34. Duarte, T. A., Noronha-Dutra, A. A., Nery, J. S., Ribeiro, S. B., Pitanga, T. N., Lapa, E. Silva, J. R., Arruda, S. and Boechat, N. 2012, Tuberculosis (Edinb.), 92, 218.
35. Turbica, I., Gallais, Y., Gueguen, C., Tharinger, H., Al Sabbagh, C., Gorges, R., Gary-Gouy, H., Kerdine-Romer, S., Pallardy, M., Mascarell, L., Gleizes, A. and Chollet-Martin, S. 2015, J. Leuko. Biol., 97, 737.
36. Ito, T., Wang, Y. H., Duramad, O., Hanabuchi, S., Perng, O. A., Gilliet, M., Qin, F. X. and Liu, Y. J. 2006, Proc. Natl. Acad. Sci. USA, 103, 13138.
37. Torii, Y., Ito, T., Amakawa, R., Sugimoto, H., Amuro, H., Tanijiri, T., Katashiba, Y., Ogata, M., Yokoi, T. and Fukuhara, S. 2008, J. Immunol., 181, 5340.
38. Shefler, I., Salamon, P., Reshef, T., Mor, A. and Mekori, Y. A. 2010, J. Immunol., 185, 4206.
39. Wheway, J., Latham, S. L., Combes, V. and Grau, G. E. R. 2014, J. Immunol., 193, 3378.
40. Piecin, A., Murphy, W. G. and Smith, O. P. 2007, Blood Rev., 21, 157.
41. Nomura, S., Ozaki, Y. and Ikeda, Y. 2008, Thromb. Res., 123, 8.
42. Combes, V., Simon, A. C., Grau, G. E., Arnoux, D., Camoin, L., Sabatier, S., Mutin, M., Sanmarco, M., Sampol, J. and Digna-George, F. 1999, J. Clin. Invest., 104, 93.
43. Latham, S., Chaponnier, L. C., Dugina, V., Couraud, P. O., Grau, G. E. and Combes, V. 2013, FASEB J., 27, 672.
44. Al-Nedawi, K., Meehan, B. and Rak, J. 2009, Cell Cycle, 9, 581.
45. Robbins, P. D. and Morelli, A. E. 2014, Nat. Rev. Immunol., 14, 195.
46. Qiu, Q., Xiong, W., Yang, C., Gagnon, C. and Hardy, P. 2013, Mol. Immunol., 55, 220.
47. Yongguang, Lu., Lang, Li., Hua, Yan., Qiang, Su., Junzhang, H. and Chunhui, Fu. 2013, Int. J. Cardiol., 168, 5396.
48. Qiu, Q., Xiong, W., Yang, C., Dai, X., Dan, X., Yang, Z., Jiao, Y., Xiang, Y., Liu, G. and Hardy, P. 2014, Apoptosis, 19, 1113.

49. Song, J., Chen X., Wang, M., Xing, Y., Zheng, Z. and Hu, S. 2014, Scient. Rep., 4, 7583.
50. Yamaguchi, Y., Suda, T., Suda, J., Eguchi, M., Miura, Y., Harada, N., Tominaga, A. and Takatsu, K. 1988, J. Exp. Med., 167, 43.
51. Izuhara, K., Arima, K., Kanaji, S., Ohta, S. and Kanaji, T. 2006, Curr. Med. Chem., 13, 2291.
52. Shim, J. J., Dabbagh, K., Ueki, I. F., Dao-Pick, T., Burgel, P. R., Takeyama, K., Tam, D. C. and Nadel, J. A. 2001, Am. J. Physiol. Lung Cell Moll. Physiol., 280, L134.
53. Fujisawa, T., Ide, K., Holtzman, M. J., Suda, T., Suzuki, K., Kuroishi, S., Chida, K. and Nakamura, H. 2008, Respirology, 13, 191.
54. Laoukili, J., Perret, E., Willems, T., Minity, A., Parthoens, E., Houcine, O., Coste, A., Jorissen, M., Marano, F., Caput, D. and Tournier, F. 2001, J. Clin. Invest., 108, 1817.
55. Chu, H. W., Balzar, S., Seedorf, G. J., Westcott, J. Y., Trudeau, J. B., Silkoff, P. and Wenzel, S. E. 2004, Am. J. Pathol., 165, 1097.
56. Veldhoen, M., Uyttenhove, C., van Snick, J., Helmby, H., Westendorf, A., Buer, J., Martin, B., Wilhelm, C. and Stockinger, B. 2008, Nat. Immunol., 9, 1341.
57. Xiao, X., Balasubramaniam, S., Liu, W., Chu, X., Wang, H., Taparowsky, E. J., Fu, Y. X., Choi, Y., Walsh, M. C. and Li, X. C. 2012, Nat. Immunol., 13, 981.
58. Erpenbeck, V. J., Hohlfeld, J. M., Volkmann, B., Hagenberg, A., Geldmacher, H., Braun, A. and Krug, N. 2003, J. Allergy Clin. Immunol., 111, 1319.
59. Tsicopoulos, A., Shimbara, A., de Nadai, P., Aldewachi, O., Lamblin, C., Lassalle, P., Walls, A. F., Sénéchal, S., Levitt, R. C., Darras, J., Hamid, Q. and Wallaert, B. 2004, J. Allergy Clin. Immunol., 113, 462.
60. Soroosh, P. and Doherty, T. A. 2009, Immunology, 127, 450.
61. Noelle, R. J. and Nowak, E. C. 2010, Nat. Rev. Immunol., 10, 683.
62. Elyaman, W., Bradshaw, E. M., Uyttenhove, C., Dardalhon, V., Awasthi, A., Imitola, J., Bettelli, E., Oukka, M., van Snick, J., Renauld, J. C., Kuchroo, V. K. and Khoury, S. J. 2009, Proc. Natl. Acad. Sci. USA, 106, 12885.
63. O'Garra, A. 1998, Immunitu, 8, 275.
64. Nakanishi, K. 2010, Eur. J. Immunol., 40, 1836.
65. Evans, R., Patzak, I., Svensson, L., De Filippo, K., Jones, K., McDowall, A. and Hogg, N. 2009, J. Cell. Sci., 122, 215.
66. Hogg, N., Patzak, I. and Willenbrock, F. 2011, Nat. Rev. Immunol., 11, 416.
67. Abram, C. and Lowell, C. A. 2009, Annu. Rev. Immunol., 27, 339.
68. Kinashi, T. 2007, Adv. Immunol., 93, 185.
69. Verma, N. K. and Kelleher, D. 2014, Eur. J. Immunol., 44, 3484.