

Sphingosine 1-phosphate induces chemotaxis of immature and modulates cytokine-release in mature human dendritic cells for emergence of Th2 immune responses¹

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SPECIFIC AIMS

Sphingosine 1-phosphate (S1P) is a potent extracellular lysolipid acid mediator released after IgE stimulation of mast cells and presumably contributes to the promotion of inflammation in allergic diseases. We investigated the biological activity and intracellular signaling of S1P on immature and mature human dendritic cells (DC).

PRINCIPAL FINDINGS

1. Human DC express the mRNA for EDG-1, EDG-3, EDG-5, and EDG-6 receptors

Expression of mRNA for the different EDG receptor subtypes was analyzed in immature and mature DC by semiquantitative RT-PCR analysis. Similar amounts of mRNA for EDG-1, EDG-3, EDG-5, and EDG-6 receptors were revealed in both types of DC. No products were obtained after omitting reverse transcription in the reaction.

2. S1P has chemotactic activity for immature but not mature DC

Boyden chamber experiments revealed that S1P has chemotactic activity for immature DC, with a typical bell-shaped dose-dependent response curve (Fig. 1). Maximal and half-maximal reactions were observed at 10^{-6} M and 10^{-8} M S1P, respectively. The chemotactic index for the well-characterized chemotaxin C5a in immature DC was $\sim 2.08 \pm 0.07$. Preincubation of DC with pertussis toxin (PTX), which blocks activation of G_i-proteins, completely inhibited the chemotactic activity of S1P. In mature DC, S1P did not stimulate migration whereas the chemokine MIP-3 β provoked

this response, with a chemotactic index of $\sim 2.12 \pm 0.17$.

3. S1P induces Ca²⁺ mobilization and actin polymerization in immature, but not in LPS-differentiated DC

Reorganization of the actin cytoskeleton and Ca²⁺ transients are prerequisite for cell migration. The effect of S1P on the actin network in immature and mature DC was analyzed by flow cytometry. The phospholipid S1P triggered actin polymerization in immature DC. The response was rapid, with a 50% increase in the factin content within 25 s of stimulation. Maximal and half-maximal effects were observed at 10^{-6} M and 10^{-8} M S1P, respectively. Again, PTX totally abrogated this response. No actin reorganization was measured in LPS-treated DC upon stimulation with S1P, but there was in response to MIP-3 β .

Stimulation of immature DC with S1P induced a rapid and dose-dependent [Ca²⁺]_i increase, with maximal and half-maximal responses at 10^{-5} M and 10^{-7} M S1P concentrations, respectively. Experiments performed in the presence of 4 mmol/l EGTA indicated that mobilization of Ca²⁺ from intracellular stores was implicated. To investigate whether S1P induces Ca²⁺ transients via G_i-protein-coupled receptors, DC were preincubated with 4 μ M PTX for 2 h. Pertussis toxin pretreatment strongly inhibited S1P-induced Ca²⁺ increase in immature DC. In contrast to immature DC,

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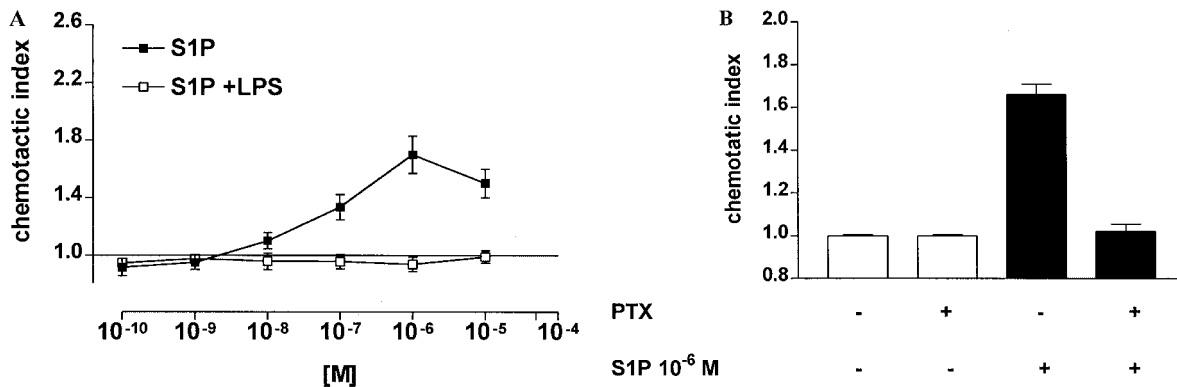


Figure 1. S1P elicits chemotaxis of immature but not mature DC. A) DC were exposed to S1P for 90 min at 37°C in a Boyden chamber. C5a and MIP-3β were used as positive controls for immature and mature DC, respectively. The chemotactic index for C5a in immature DC was $\sim 2.08 \pm 0.07$ and the index for MIP-3β in LPS-differentiated cells was $\sim 2.12 \pm 0.17$. Data are expressed as mean \pm SE ($n=5$). B) After pretreatment with 10 μ g/ml PTX for 2 h, DC were exposed to 10⁻⁶ M S1P for 90 min at 37°C in a Boyden chamber. Data are means \pm SE ($n=4$).

S1P did not elicit Ca²⁺ transients in DC matured with LPS whereas MIP-3β was effective.

4. S1P reduces IL-12 and TNF-α production and augments IL-10 release in maturing DC

Recent evidence suggests that S1P also regulates the production of cytokines in different cell types. S1P did not effect basal cytokine production from immature DC. However, S1P together with LPS dose-dependently inhibited the secretion of IL-12 and TNF-α but up-regulated release of IL-10. Pertussis toxin did not influence the release of S1P-induced cytokine production.

5. S1P in maturing DC inhibits their capacity to induce Th1 immune response, promoting the outcome of Th2 cells

Naive CD4⁺CD45RA⁺ allogeneic T cells primed with immature DC differentiated to a similar extent into Th1, Th2, and Th0 cells, whereas those stimulated with mature DC differentiated mainly into IFN-γ-producing Th1 cells. S1P did not affect Th cell polarization induced by immature DC. In contrast, T cells primed with DC matured in the presence of S1P displayed an impaired Th1 and enhanced Th2 polarization (Fig. 2). Lower and higher production of IFN-γ and IL-4, respectively, was confirmed measuring the cytokines in the supernatants, and was already evident 5 days after initial T cell activation with mature DC. IL-5 followed behavior similar to IL-4.

CONCLUSIONS AND SIGNIFICANCE

The lysophospholipid S1P is a well-characterized extracellular mediator that is thought to be involved in angiogenesis as well as the pathogenesis of atherosclerosis and cancer. The release of high amounts of S1P

from mast cells after stimulation with IgE also suggests a role of this mediator in allergic reactions. Indeed, recent findings implicate a pathophysiological role of S1P in bronchial inflammation and remodeling in patients with asthma. The influence of S1P on inflammatory and immune responses is not well understood. The present study reveals functional expression of S1P receptors in DC.

Treatment of immature DC with S1P triggers intracellular Ca²⁺ transients, actin remodeling, and chemotaxis (Fig. 3). The increase in intracellular Ca²⁺ is due

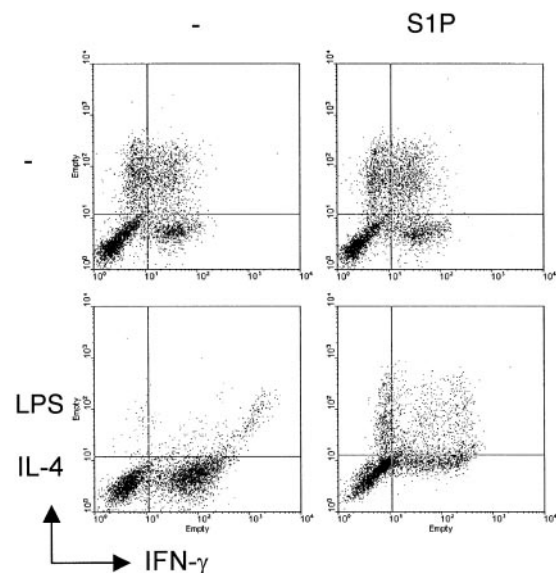


Figure 2. DC matured in the presence of S1P are impaired in their ability to initiate Th1 responses in vitro. Immature DC were left untreated, stimulated with 10⁻⁸ M S1P, or induced to undergo maturation with LPS in the presence or the absence of S1P (10⁻⁸ M or 10⁻⁵ M) for 24 h. DC then were used to prime purified allogeneic CD4⁺CD45RA⁺ naive T lymphocytes. After 10 days, T cells were restimulated with PMA and ionomycin and examined for intracellular IFN-γ and IL-4 by flow cytometry. Numbers indicate the percentage of positive cells in each quadrant.

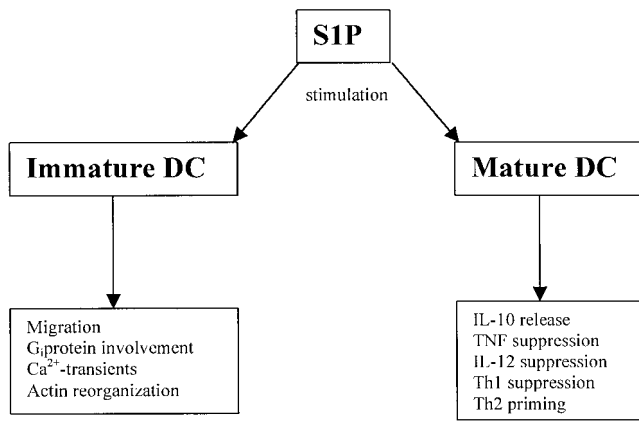


Figure 3. Biological function and signal transduction mechanisms of S1P in DC.

to mobilization from intracellular stores via activation of G_i -proteins and PLC. The mechanism underlying the actin response is presumably regulated by interaction of phosphoinositides with actin binding proteins and requires G_i -proteins and small GTP binding proteins of the rho family. Mature DC lose chemotactic sensitivity to S1P. Induction of Ca^{2+} transients and actin reorganization fully abrogated 24–48 h after treatment with LPS. DC originate from hemopoietic stem cells and migrate to peripheral target sites to uptake antigens. Recruitment of DC into peripheral tissues and the migration of maturing DC to regional lymph nodes are controlled primarily by different chemokines/chemoattractants such as monocyte chemoattractant protein 1–4, RANTES, macrophage inflammatory protein 3, and sequential expression of their receptors during maturation in a weight anchor/hoist sail model. The selective chemotactic activation toward immature DC implies that S1P might play a role in the accumulation of immature DC at peripheral target sites. Loss of the chemotactic activity of S1P toward DC during maturation might clear the way for MIP-3 β -driven migration to secondary lymphoid organs. Mature DC become insensitive to the chemotactic activity of monocyte chemoattractant proteins, platelet-activating factor, and adenosine because of transcriptional down-regulation of their

respective receptors. In contrast, mRNA levels of the S1P receptors were comparable in immature and LPS-differentiated DC. Due to the unavailability of selective investigational tools such as selective agonists, antagonists, or antibodies, we cannot exclude altered post-transcriptionally regulated expression of EDG receptors during DC maturation or identify which receptor subtype mediates the chemotactic response. On the other hand, one can speculate that the EDG receptors in immature and mature DC are differentially coupled to intracellular signaling pathways due to different expression pattern of G-protein subunits and G-protein subunit splice variants or post-translational modification of G-protein such as palmitoylation. DC exposed to LPS together with S1P showed independent of G_i -protein involvement and reduced secretion of IL-12 and TNF- α , but more abundant production of IL-10 (Fig. 3).

The differentiation of lymphocytes into distinct subsets is regulated during the priming process in secondary lymphoid organs by the local microenvironment generated by DC-secreted cytokines. As shown here, altered cytokine release by S1P-treated DC was associated with a Th1 to Th2 switch of in vitro primed T cell response. Thus, S1P may not only regulate the trafficking of DC, but also control the quality of DC-mediated T cell response. Atopic dermatitis lesions harbor an increased number of mast cells and DC, with part of DC closely resembling monocyte-derived DC. Atopic dermatitis patients have increased mast cell releasability and a propensity to generate Th2-dominated immune responses to environmental allergens. Therefore, it is reasonable to speculate about a possible pathophysiological role for S1P in these patients. It is known that allergen-IgE complexes can easily provoke mast cell release of S1P. In turn, S1P could contribute to the increased accumulation of DC in lesional atopic dermatitis skin and inhibit IL-12 production from maturing DC, thus favoring the emergence of Th2 immune responses.

In summary, our study implies that S1P might regulate the trafficking of DC and ultimately favor Th2 lymphocyte-dominated immunity. **FJ**