Review

Sphingosine 1-phosphate as a novel immune regulator of dendritic cells

Angelo Martino

Unit of Cellular Immunology, National Institute for Infectious Diseases, IRCCS 'Lazzaro Spallanzani', Via Portuense 292, 00149, Rome, Italy

(Fax, 39 065582825; Email, martino@inmi.it)

Although originally described as an intracellular second messenger, sphingosine 1-phosphate (S1P) has recently been shown to be involved in several physiological and pathological functions as an extracellular mediator. S1P receptors are widely expressed and thought to regulate important functions in cell signalling. Recently, the role of S1P on the immune system has evoked great interest. In particular, several aspects of the effects on antigen-presenting cells (APCs) as dendritic cells (DC) in mice and humans have been reported. In this review, we focus on the role played by S1P on the DC system and its effects in immune-related pathological states.

[Martino A 2007 Sphingosine 1-phosphate as a novel immune regulator of dendritic cells; *J.Biosci.* **32** 1207–1212]

1. Introduction

Lysophospholipids with biological mediator activity include lysoglycerophospholipids, epitomized by lysophosphatidic acid (LPA), and lysosphingolipids, such as sphingosine 1-phosphate (S1P). These lipids share three important characteristics. First, their structure contains a lipid domain and one or more polar charged substituents, and they are bound extensively *in vivo* by albumin and other plasma proteins. Second, they affect numerous functions of many cell types, from proliferation and survival to migration and secretion. There is a solid evidence for the involvement of these lipids in processes such as oxidative metabolism, angiogenesis, and carcinogenesis (Goetzl and Tigyi 2004; Goetzl and Lynch 2000). Third, they all signal cells predominantly through structurally related G protein-coupled receptors (GPCRs) (Hla *et al* 2001; Chalfant and Spieel 2005).

S1P is a polar sphingolipid metabolite that functions both as an extracellular and intracellular messenger to regulate diverse cell signalling pathways. S1P is generated by the conversion of sphingomyeline into ceramide by sphingomyelinase, ceramide into sphingosine by ceramidase, and sphingosine into S1P by sphingosine kinase (Hannun *et al* 2001). S1P is degraded by specific phosphatases regenerating sphingosine, or by a lyase cleaving it irreversibly into ethanolamine 1-phosphate and palmitaldheyde. Recently, there has been progress in cloning and characterizing the enzymes involved in sphingolipid metabolism. However, we still have a limited insight into the regulation of most of these functions. S1P has distinct roles in cell growth and survival, angiogenesis, vasculogenesis, neuritogenesis and immune functions, and the number of studies on S1P-mediated cell signalling has exploded in recent years. The extracellular actions of S1P are mediated by its interaction with a newly identified family of five specific GPCRs, S1P1–S1P5 (Lee *et al* 1998; Chun *et al* 2002). In addition, similar to other potent lipid mediators, S1P also has intracellular actions, implicated in inositol triphosphate-independent calcium mobilization, inhibition of activity of caspases, activation of non-receptor tyrosine kinases and the Raf/MKK/ERK signalling cascade, as well as other pathways involving cell proliferation and suppression of ceramide-induced apoptosis (Pyne and Pyne

Keywords. Cell differentiation; dendritic cells; immunity; lipids; sphingosine 1-phosphate

Abbreviations used: APC, antigen-presenting cells; DC, dendritic cells; GM-CSF, granulocyte–macrophage colony-stimulating factor; GPCR, G protein-coupled receptors; GTP, guanosine triphosphate; IFN, interferon; IL, interleukin; imDC, immature DC; LPA, lysophosphatidic acid; LPS, lipopolysaccharides; mDC, mature DC; MHC, major histocompatibility complex; NK, natural killer; PCR, polymerase chain reaction; RANTES, regulated on activation normal T cell expressed and secreted; S1P, sphingosine 1-phosphate; TNF, tumour necrosis factor

2000; Spiegel *et al* 1998; Cuvillier *et al* 1996). Endogenous S1P levels are modulated in a wide range of cell types in response to extracellular stimuli, such as growth factors, cytokines and GPCR agonists (Hannun *et al* 2001; Spiegel and Merrill 1996). However, the production of S1P is linked to the levels of its precursors.

The role of S1P and other sphingolipids in immune cells has been the subject of intense research (Lin and Boyce 2006). All immunoregulatory lipid mediators are produced by innate immune cells such as macrophages, dendritic cells (DC) and mast cells, and act on T and B lymphocytes as well as phagocytes and endothelial cells through their respective receptors. The influence of these mediators on growth-related actions and chemotaxis may be different for immune cells. For T cells, these functions comprise alteration of proliferation and suppression of apoptosis as well as effects on chemotaxis, cytokine generation, cytotoxic activity and regulatory functions (Goetzl and Graler 2004). Which effect will predominate is determined by lipid concentration, GPCR density and the array of coupled G proteins in the host cells. The particular capacity of S1P to affect T cell functions is also related to its ability to interfere with several functions of antigen-presenting cells (APCs) as DC. Data from our group and other groups showed the tremendous capacity of S1P to interfere with the immune functions of DC in the regulation of immunity. Thus, in this review, we summarize the recent understanding of the effect of S1P in immune cells and discuss in particular its involvement in the DC system.

2. S1P in the regulation of T cell functions

Circulation of mature lymphocytes between the blood and secondary lymphoid organs plays a pivotal role in the immune response. S1P is present in physiological fluids in high-nanomolar to low-micromolar concentrations and has multiple effects on many of these types of cells. Human and mouse blood CD4 T cells and B cells predominantly express S1P1 and S1P4 GPCRs, as assessed by real-time polymerase chain reaction (PCR) and western blotting (Dorsam *et al* 2003). In contrast, human T cell lines express similar levels of S1P2 and S1P3 GPCRs without alteration in these levels by numerous stimuli (Goetzl *et al* 1999). Mouse and human CD8 T cells express the same two major S1P receptors as CD4 T cells but also express S1P5. Activation of each of these normal lymphocytes, including stimulation with anti-CD28 plus anti-CD3 superantigens or phorbol esters trascriptionally downregulates S1P1 and S1P4. Some drugs strikingly reduce the lysophospholipids (LPL)-evoked biochemical and functional signals coupled to the respective receptors (Graeler *et al* 2003). The major effect of S1P on T cell functions is highly concentration dependent. At levels of 1–100 nM, which are found in tissues, S1P exerts principally supportive and stimulatory effects on the T cells (Graeler and Goetzl 2002). In this range, S1P evokes and directly enhances chemotaxis in response to chemokine stimulation. *In vitro*, at concentrations of 300–600 nM, S1P inhibits the chemotaxis induced by chemokines on T cells, suppressing T cell movement from the periphery to the lymph nodes. Although these events have not been correlated mechanistically, S1P causes systemic lymphopenia *in vivo* in rodents (Graeler and Goetzl 2002).

The distribution of B cells in the secondary lymph nodes and their recirculation into efferent lymph and blood are regulated by the interaction of S1P and S1P1 (Matloubian *et al* 2004). B cells from the marginal zone of the spleen, which are in closer contact with the blood, are presumably exposed to the plasma concentration of S1P and express S1P1 and S1P3 GPCRs (Cinamon *et al* 2004). The inhibition of B cell chemotaxis to germinal centre chemokines, such as CXCL13, by S1P and S1P1 maintains the B cells in the marginal zone. Furthermore, naïve and memory B and T cells express functional S1P1 GPCRs and exhibit a chemotactic response to S1P. Since S1P1 receptor expression is downregulated in B and T cells upon antigen activation, S1P has far less of an effect on the immune activities of effector lymphocytes than on those of naïve and memory cell subsets.

S1P has also been demonstrated to be able to suppress apoptosis and control effector cells by $CD4^+CD25^+$ T regulatory cells (Wang *et al* 2004). Certain specialized and very distinctive mechanisms controlling the downregulation and re-expression of S1P receptors on T cells have been shown *in vitro*, but most of the protein phosphorylation events remain to be delineated at the molecular level.

Finally, the mechanism of expression of S1P GPCRs by mononuclear phagocytes and natural killer (NK) cells has only recently been examined and needs further investigation. Although some aspects of T cell immunoregulatory signals from S1P have been elucidated, many questions remain unanswered or only partially answered. The role of S1P on DC and subsequent T cell activation particularly needs to be discussed.

3. Role of S1P in the DC system: chemotaxis and Th1/Th2 polarization by DC

DC are specialized APCs characterized by their ability to migrate to target sites and secondary lymphoid organs to process antigens and activate naïve T cells (Lanzavecchia and Sallusto 2001). They are considered promising tools for immunotherapy (Banchereau *et al* 2001). In peripheral tissues, immature DC (imDC) capture antigens and undergo a maturation process while migrating to the lymphoid organs. During this event, they upregulate major histocompatibility complex (MHC) and co-stimulatory molecules (Cella *et al* 1997). They also produce high levels of inflammatory

and immunoregulatory cytokines such as tumour necrosis factor (TNF)-α and interleukin (IL)-12. Mature DC (mDC) can activate naive antigen-specific T lymphocytes leading to effector T cell differentiation and memory T cell expansion, thus providing immediate and belated protection against pathogens or tumours. It is well established that the interaction between DC, T cells and innate cells is necessary for regulating the immune response in pathological states (Shreedhar *et al* 1999; Gerosa *et al* 2002). All these players of the immune system express receptors for S1P, which is present in the serum or local tissues at normal or high concentrations in several diseases. S1P and related lysophospholipids are released by platelets and constitute a major part of serum and plasma (Goetzl and Lynch 2000). S1P is also secreted by inflammatory cells such as mast cells upon ligation of their Fc receptor for immunoglobulin E and raised levels are found in asthmatic lungs after antigenic challenge (Jolly *et al* 2002). Thus, a role of S1P as a mediator in allergy and asthma has been proposed. Furthermore, high levels of S1P have been associated with other pathological conditions such as atherosclerosis and diabetes (Merill *et al* 1997; Xu *et al* 2004). In this context, DC play a crucial role in triggering the immune response and in maintaining the inflammatory status. The capacity of S1P to influence the immune response mediated by DC in these conditions results in a very important regulator key.

The first evidence of S1P's influence on DC biology was from Idzko *et al* (2002). The authors showed the existence of functional expression of S1P receptors on the surface of DC, and the treatment of imDC with S1P triggers intracellular Ca^{2+} transients, actin remodelling and chemotaxis. The increase of Ca^{2+} is due to the mobilization of intracellular stores, via activation of G-proteins and phospholipase C. The mechanism underlying the actin response is presumably regulated by interaction of phosphoinosites with actin-binding proteins and requires G-proteins and small guanosine triphosphate (GTP)-binding proteins of the rho family. In contrast, mDC lose their chemotactic sensitivity to S1P.

Recruitment of inflammatory cells and DC into peripheral tissues and their migration to lymphoid organs are controlled primarily by different chemokines such as monocyte chemotactic protein, regulated on activation normal T cell expressed and secreted (RANTES), and sequential expression of their receptors. The selective chemotactic activation towards imDC implies that S1P might play a role in the accumulation of imDC at peripheral target sites. In contrast, the effect of S1P towards mature DC might clear the way for chemokine-driven migration to secondary lymphoid organs, impairing the systemic triggering of the immune response and contributing to the local pro-inflammatory response. According to these data, mDC treated with S1P shift the immune response of naïve CD4 T cells towards a T-helper (Th)2 cytokine profile. Thus, S1P may not only regulate the trafficking of DC but also the quality of DC-mediated T cell response. Different disease states, such as atopic dermatitis, are associated with an increase in the local number of mast cells and DC. Since patients with atopic dermatitis have been shown to have high mast cell releaseability and a propensity to generate a Th2-dominant immune response, it is reasonable to speculate that S1P has role to play in DC biology in these patients (Tsai *et al* 2005).

The chemotaxis induced by S1P in DC was recently investigated by Czeloth *et a*l (2005) in murine mDC but not imDC migrating to S1P. Conversely, its analogue FTY720 did not trigger the migration *per se*. The link between DC and S1P confirms that after inflammatory stimulation, their interaction stimulates cytokine secretion such as IL-6, TNF-α and IL-8, maintaining the local pro-inflammatory response. Regulation of the production of pro-inflammatory cytokines and the capacity to attract novel DC contributes to the persistence of inflammation (Oz-Alan *et al* 2006).

4. S1P interferes with the differentiation of competent DC from peripheral monocytes

DC originate from the haematopoietic stem cells in the bone marrow. Recently, there have been considerable insights into the origin of the DC subset and their modulation of cytokines in neighbouring cells. Progenitors of DC in the bone marrow migrate via the blood stream and home in to the peripheral tissues where they encounter several essential growth factors in the microenvironment such as granulocyte–macrophage colony-stimulating factor (GM-CSF), IL-4, IL-15 and IL-3 secreted by various cell types including endothelial cells, mast cells, keratinocytes and fibroblasts. Such growth factors determine the fate of the progenitors; whether they will differentiate into immature Langherans DC, interstitial DC or plasmacytoid DC. Migrating DC can also originate primarily from blood monocytes (Chapuis *et al* 1997). An estimated 25% of circulating inflammatory monocytes will differentiate into migrating DC, whereas the others will give rise to resident macrophages (Randolph *et al* 1999). This balance becomes complicated during pathological inflammatory states. At the sites of inflammation, cytokines and chemokines promote not only the activation of resident DC and macrophages but also the recruitment of DC precursors such as monocytes. What drives the differentiation of monocytes into DC or macrophages *in vivo* is still unknown, but it has been shown that several factors may influence this process *in vitro*. It is conceivable that the differentiation of monocytes into competent DC or macrophages in body tissues is shaped by the local environment present during their transit. Thus, several conditions favour the development of one or the other cell type. DC precursors

permeating peripheral tissues including the skin receive stimuli from local inflammation (Randolph *et al* 1999). In this context, microorganisms, inflammatory cytokines and other molecules such as gangliosides shed from tumour cells can interact with DC precursors, interfering with the early step of their differentiation. Such meddling can lead to the generation of 'semi-professional' APCs characterized by a less stimulatory mode, or induce the differentiation of macrophages (Navarro-Péguet *et al* 2003; Martino *et al* 2004; Chomorat *et al* 2000). Increasing evidence hence demonstrates that interfering with DC generation may negatively modulate the specific immune response. We recently showed that peripheral circulating monocytes in the presence of S1P, GM-CSF and IL-4 (widely used for the generation of immature DC *in vitro*), differentiate into a particular subset of DC characterized by the lack of CD1a on their surface and some aspects of mDC. Indeed, a $CD1a^-$ DC population derived from S1P-treated precursors presented a higher expression of such co-stimulatory molecules and subverted the response to bacterial products such as lipopolysaccharides (LPS) (Martino *et al* 2007). Nevertheless, the priming of monocytes with S1P induces a differentiation of DC that is unable to respond to further inflammatory stimuli but produces large amounts of TNF-α without stimulation. In contrast, these DC are not able to secrete immunomodulatory IL-12. This event contributes to the inflammatory response in local tissues and impairs the triggering of a systemic T cell response. In fact, S1P-treated monocyte-derived DC, in the presence or absence of LPS, do not induce the production of either interferon (IFN)-γ or IL-4 in naïve CD4 T cells upon stimulation with anti-CD3. This incapacity to involve the T cell response could be due to the inability of DC to produce immunoregulatory cytokines as IL-12.

Interestingly, such a DC population has already been demonstrated in different experimental models and found to be associated with the suppression of immune response. Previous investigations revealed that *in vitro* induction of the CD1 molecule could be negatively regulated in DC derived from monocytes exposed to mycobacteria or such toxins as *Pertussis* toxin (Martino *et al* 2004; Martino *et al* 2006). Furthermore, it has been reported that S1P could be involved in tumour progression and fully competent $CD1a^+$ DC are the major players involved in the first steps of antitumour primary immune response (La Rocca *et al* 2004). Finally, in atherosclerotic plaques, it has been shown that an important trafficking takes place of monocyte-derived DC-like cells from atherosclerotic plaques during regression, but little emigration was detected from progressive lesions (Liodra *et al* 2004). S1P and other lipid mediators have been demonstrated to accumulate during atherosclerosis. So, we may hypothesize that the progression of atherosclerosis may be linked to the retention of DC in the subendothelium and the recruitment of monocytes unable to differentiate into competent migrating APCs.

5. Pathological implications of S1P in the DC system

Recent derivation of knock-out animal models for S1P receptors and metabolic enzymes facilitated studies on the pathological role of S1P in various processes. There is great interest in sphingolipid signalling in cancer biology, inflammatory diseases such as atherosclerosis and infectious diseases such as sepsis (Goetzl and Graler 2004; Goetzl and Lynch 2000).

Tumour cell apoptosis is induced by ceramide and sphingosine, which may be intermediates in the cell stress response, for example, when induced by a chemotherapeutic regimen. On the other hand, S1P is a pro-survival factor (Spiegel and Milstien 2002). Thus, overexpression of sphingosine kinase is associated with the transformation of fibroblasts (Xia *et al* 2000). Overexpression of S1P could be associated with the loss of balance of Th polarization capacity of resident DC. Indeed, an optimal immune response to tumour cells requires cytotoxic and Th1 cell response induced by APCs. In this context, the ability of S1P to facilitate the migration of mDC to the lymphoid organs and induce a shift towards a Th2 immune response could impair the efficiency of anti-tumoral immunity, facilitating the progress of cancer.

In inflammatory diseases such as atherosclerosis, S1P has been shown to be involved in several pathological processes. The S1P1 receptor is overexpressed in proliferating vascular smooth muscle cells and in atherosclerotic plaques (Kluk and Hla 2001). In particular, recruitment of monocytes at inflammation sites is required for the onset and progression of the disease. In atherosclerosis, monocyte-derived cells produce secretory factors that attract and activate smooth muscle cells, other immune cells and more monocytes. Mechanisms attracting monocytes to the lesions are partially defined but relatively little is known about the fate of monocytes once they migrate to the inflammatory lesions. The conversion of monocytes into DC emigrating out of tissues contributes to a continuous source of APCs and participates in the regulation of monocyte/macrophage homeostasis. Monocytes exiting the blood in the steady state and appearing at inflammation sites during atherosclerosis are blocked in macrophage homeostasis and accumulate abundantly, forming atherosclerotic plaques. In this context, the capacity of S1P to impair DC differentiation from peripheral monocytes may contribute to the progression of the disease. Furthermore, the capacity of DC to differentiate with inflammatory capacities, such as the high production of TNF-α, contributes to the chronicity of the inflammation. Moreover, this stimulates the production of S1P by activation of sphingosine kinase (Xia *et al* 1998). In other inflammatory diseases such

as atopic dermatitis, the lesions harbour an increased number of mast cells and DC that potentially secrete S1P. Patients with atopic dermatitis have an increased release of S1P from the mast cells and a propensity to generate a Th2 immune response. The level of S1P produced by the mast cells may act as a critical regulator of inflammation in these patients.

The capacity of antagonists or agonists of the S1P receptor to modify the tissue distribution of effector chemotactic responses of T cells represents a novel target for immunotherapy in transplantation and autoimmune diseases (Gardell *et al* 2006). FTY720, an agonist of the S1P receptor, has been evaluated in several studies *in vivo*. This modulates lymphocyte trafficking in a dual manner: by accelerating migration into the secondary lymphoid organs and blocking the egress in the medullary sinus of lymph nodes and afferent lymphatics. This leads to an overall decrease in circulating T and B cells, and inhibition of lymphocyte influx at sites of inflammation (Wei *et al* 2005). Little is known about the effect of this compound on the DC system, but recently, Idzko *et al* (2006) showed that the local administration of FTY720 inhibits the Th2-mediated cardinal feature of asthma by altering the function of lung DC without causing any systemic lymphopenia. These findings could pave the way for a feasibility study in which this compound or a more selective S1Pr agonist is administered via aerosol to patients with asthma.

6. Conclusion

Recent progress has increased our understanding of the function of S1P in the immune system. To date, it is well established that sphingolipids could be considered regulators of several immune functions. Among these, DC represent a key element, triggering the immune response in several diseases where an imbalance of lipid metabolism is involved. Thus, therapeutic strategies based on local administration or systemic delivery of sphingolipids or sphingomimetic drugs able to interfere with the homeostasis of sphingolipids may have promise in ablating unwanted immune responses.

Acknowledgements

Thanks to E Volpe for critical comments on the manuscript. This work was supported by the National Institute for Infectious Diseases ‘Lazzaro Spallanzani’.

References

Banchereau J, Schuler-Thurner B, Palucka A K and Schuler G 2001 Dendritic cells as vectors for therapy; *Cell* **392** 271–274

Cella M, Sallusto F and Lanzavecchia A 1997 Origin, maturation and antigen presenting function of dendritic cells; *Curr. Opin. Immunol.* **9** 10–16

Chalfant C E and Spieel S 2005 Sphingosine 1-phosphate and ceramide 1-phosphate: expanding roles in cell signalling; *J. Cell Sci.* **118** 4605–4612

Chapuis F, Rosenzwajg M, Yagello M, Ekman M, Biberfeld P and Gluckman J C 1997 Differentiation of human dendritic cells from monocytes in vitro; *Eur. J. Immunol.* **27** 431–441

Chomorat P, Banchereau J, Davoust J and Palucka A K 2000 IL-6 switches the differentiation of monocytes from dendritic cells to macrophages; *Nat. Immunol*. **1** 510–514

Chun J, Goetzl E J, Hla T, Igarashi Y, Lynch K R, Moolenaar W, Pyne S and Tigyi G 2002 International Union of Pharmacology XXXIV Lysophospholipid receptor nomenclature; *Pharmacol. Rev*. **54** 265–269

Cinamon G, Matloubian M, Lesneski M J, Xu Y, Low C, Lu T, Proia R L and Cyster J G 2004 Sphingosine 1-phosphate receptor 1 promotes B cell localization in the splenic marginal zone; *Nat. Immunol*. **5** 713–720

Cuvillier O, Pirianov G, Kleuser B, Vanek P G, Coso O A, Gutkind S and Spiegel S 1996 Suppression of ceramide-mediated programmed cell death by sphingosine 1-phosphate; *Nature* (*London*) **381** 800–803

Czeloth N, Bernhardt G, Hofmann F, Genth H and Forster R 2005 Sphingosine 1-phosphate mediates migration of mature dendritic cells; *J. Immunol.* **175** 2960–2967

Dorsam G, Graeler M, Seroogy C, Kong Y, Voice J K and Goetzl E J 2003 Transduction of multiple effects of sphingosine 1-phosphate (S1P) on T cell functions by the S1P1 G protein coupled receptor; *J. Immunol*. **171** 3500–3507

Gardell S E, Dubin A E and Chun J 2006 Emerging medicinal roles for lysophospholipid signalling; *Trends Mol. Med.* **12** 65–75

Goetzl E J and Tigyi G 2004 Lysophospholipids and their G protein-coupled receptors in biology and diseases; *J. Cell Biochem*. **92** 867–868

Gerosa F, Baldani-Guerra B, Nisii C, Marchesini V, Carra G and Trinchieri G 2002 Reciprocal activating interaction between natural killer cells and dendritic cells; *J. Exp. Med.* **195** 327–333

Goetzl E J and Lynch K R 2000 Lysophospholipids and eicosanoids in biology and pathophisiology; *Ann. NY Acad. Sci*. **905** XI–XIV

Goetzl E J and Graler M H 2004 Sphingosine 1-phosphate and its type 1 G protein-coupled receptor: trophic support and functional regulation of T lymphocytes; *J. Leukoc. Biol*. **76** 355–360

Goetzl E J, Kong Y and Mei B 1999 Lysophosphatidic acid and sphingosine 1-phosphate protection of T cells from apoptosis in association with suppression of Bax; *J. Immunol*. **162** 2049–2056

Graeler M H, Kong Y, Karliner J S and Goetzl E J 2003 Protein kinase C epsilon dependence of the recovery from down-regulation of S1P1 G protein coupled receptors of T lymphocytes; *J. Biol. Chem*. **278** 27737–27741

Graeler M and Goetzl E J 2002 Activation-regulated expression and chemotactic function of sphingosine 1-phosphate receptors in mouse splenic T cells; *FASEB J*. **16** 1874–1878

Graeler M, Shankar G and Goetzl E J 2002 Suppression of T cell chemotaxis by sphingosine 1-phosphate; *J. Immunol*. **169** 4084–4087

Hannun Y A, Liberto C, Argraves K M 2001 Enzymes of sphingolipid metabolism: from modular to integrative signalling; *Biochemistry* **40** 4893–4903

Hla T, Lee M J, Ancellin N, Paik J H and Kluk M J 2001 Lysophospholipids-receptor revelations; *Science* **294** 1875–1878

Idzko M, Panther E, Corinti S, Morelli A, Ferrari D, Herouy Y, Dichman S, Mockenhaupt M *et al* 2002 Sphingosine 1-phosphate induces chemotaxis of immature and modulates cytokine-release in mature human dendritic cells for emergence of Th2 immune responses; *FASEB J*. **16** 625–627

Idzko M, Hammand H, van Nimwegen M, Kool M, Muller T, Soullié T, Willart M A, Hijdra D, Hoogsteden H C and Lambrecht B N 2006 Local application of FTY720 to the lung abrogates experimental asthma by altering dendritic cell function; *J. Clin. Invest*. **116** 2935–2944

Jolly P S, Rosenfeldt H M, Milstien S and Spiegel S 2002 The roles of sphingosine 1-phosphate in asthma; *Mol. Immunol*. **38** 1239–1245

Kluk M J and Hla T 2001 Role of sphingosine 1-phosphate receptor EDG-1 in vascular smooth muscle cell proliferation and migration; *Circ. Res*. **89** 496–402

Lanzavecchia A and Sallusto F 2001 The instructive role of dendritic cells on T cell responses: lineages, plasticity and kinetics; *Curr. Opin. Immunol*. **13** 291–298

La Rocca G, Anzalone R, Bucchieri F, Farina F, Cappello F and Zummo G 2004 CD1a and antitumour immune response; *Immunol. Lett*. **95** 1–4

Lee M J, van Brocklyn J R, Thangada S, Liu C H, Hand A R, Menzeleev R, Spiegel S and Hla T 1998 Sphingosine 1-phosphate as a ligand for the G-protein coupled receptor EDG-1; *Science* **279** 1552–1555

Lin D A and Boyce J A 2006 Lysophospholipids as mediators of immunity; *Adv. Immunol*. **89** 141–167

Liodra J, Angeli V, Liu J, Trogan E, Fisher E A and Randolph G J 2004 Emigration of monocyte-derived cells from atherosclerotic lesions characterizes regressive, but not progressive, plaques; *Proc Natl. Acad. Sci. USA* **101** 11779–11784

Matloubian M, Lo CG, Cinamon G, Lesneski M J, Xu Y, Brinkmann V, Allende ML Proia R L and Cyster J G 2004 Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1; *Nature* (*London*) **427** 355–360

Martino A, Sacchi A, Sanarico N, Spadaro F, Ramoni C, Ciaramella A, Pucillo L P, Colizzi V and Vendetti S 2004 Dendritic cells derived from BCG-infected precursors induce Th2-like immune response; *J. Leukoc. Biol*. **76** 827–834

Martino A, Volpe E, Auricchio G, Izzi V, Poccia F, Mariani F, Colizzi V and Baldini P M 2007 Sphingosine 1-phosphate interferes on the differentiation of human monocytes into competent dendritic cells; *Scand. J. Immunol*. **25** 84–91

Martino A, Volpe E, Auricchio G, Colizzi V and Baldini P M 2006 Influence of *Pertussis* toxin on CD1a isoform expression on human dendritic cells; *J. Clin. Immunol*. **26** 153–159

Merrill A H Jr, Schmelz E M, Dillehay D L, Spiegel S, Shayman J A, Riley R T, Voss K A and Wang E 1997 Sphingolipids--the enigmatic lipid class: biochemistry, physiology, and pathophysiology; *Toxicol. Appl. Pharmacol*. **142** 208–225

Navarro Péguet J, Sportouch M, Popa I, Berthier O, Schmitt D and Portoukalian J 2003 Gangliosides from human melanoma tumors impair dendritic cell differentiation from monocytes and induce their apoptosis; *J. Immunol*. **170** 3488–3494

Oz-Arslan D, Ruscher W, Myrtek D, Ziemer M, Jin Y, Damay B B, Sorichter S, Idzko M, Norguaer J and Maghazachi A A 2006 IL-6 and IL-8 release is mediated via multiple signaling pathways after stimulating dendritic cells with lysophospholipids; *J. Leukoc. Biol*. **80** 287–297

Pyne S and Pyne N J 2000 Sphingosine 1-phosphate signalling in mammalian cells; *Biochem. J*. **349** 385–302

Randolph G J, Inaba K, Robbiani D F, Steinman R M and Muller W A 1999 Differentiation of phagocytic monocytes into lymph node dendritic cells *in vivo*; *Immunity* **11** 753–761

Shreedhar V, Moodycliffe A M, Ullrich S E, Bucana C, Kripke M L and Flores-Romo L 1999 Dendritic cells require T-cells for functional maturation *in vivo*; *Immunity* **11** 625–636

Spiegel S, Cuvillier O, Edsall L C, Kohama T, Menzeleev R, Olivera A, Pirianov G, Thomas D M, Tu Z, Van Broclyn J R and Wang F 1998 Sphingosine 1-phosphate in cell growth and cell death; *Ann. N. Y. Acad. Sci*. **845** 11–18

Spiegel S and Merrill A H Jr 1996 Sphingolipid metabolism and cell growth regulation; *FASEB J.* **10** 1388–1397

Spiegel S and Milstien S 2002 Sphingosine 1-phosphate, a key cell signalling molecule; *J. Biol. Chem.* **277** 25851–25854

Tsai M, Grimbaldeston M A, Yu M, Tam S Y and Galli S J 2005 Using mast cell knock-in mice to analyze the roles of mast cells in allergic responses *in vivo*; *Chem. Immunol. Allergy* **87** 179–197

Wang W, Graeler M H and Goetzl E J 2004 Physiological sphingosine 1-phosphate requirement for optimal activity of mouse CD4+ regulatory T cells; *FASEB J.* **18** 1043–1045

Wei S H, Rosen H, Metheu M P, Sanna M G, Wang S K, Jo E, Wong C H, Parker I and Cahalan M D 2005 Sphingosine 1-phosphate type 1 receptor agonism inhibits transendothelial migration of medullary T cells to lymphatic sinuses; *Nat. Immunol*. **6** 1228–1235

Xia P, Gamble J R, Wang L, Pitson S M, Moretti P A, Wattemberg B W, D'Andrea R J, Gamble J R and Vadas M A 2000 An oncogenic role of sphingosine kinase; *Curr. Biol*. **10** 1527–1530

Xia P, Gamble J R, Rye K A, Wang L, Hii C S, Cockerill P, Khew-Goodal Y, Bert A J, Barter P J and Vadas M A 1998 Tumor necrosis factor-alpha induces adhesion molecule expression through the sphingosine kinase pathway; *Proc. Natl. Acad. Sci. USA* **95** 14196–14201

Xu C, Schwartz-Hansen J and Edvinsson L 2004 Sphingosine signalling and atherogenesis; *Acta Pharmacol. Sin*. **25** 849–854

MS received 12 February 2006; accepted 17 April 2007

*e*Publication: 1 July 2007

Corresponding editor: Rajiv K Saxena