Journal of Dermatological Science xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Sphingosine 1-phospate differentially modulates maturation and function of human Langerhans-like cells

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ARTICLE INFO

Article history:
Received 27 August 2015
Received in revised form 19 December 2015
Accepted 6 January 2016

Keywords:
Langerhans cells
Sphingosine 1-phosphate
Maturation
Cytokine release
Endocytosis
Migration

ABSTRACT

Background: As mediators between innate and adaptive immune responses, Langerhans cells (LCs) are in the focus of recent investigations to determine their role in allergic inflammatory diseases like allergic contact dermatitis and atopic dermatitis. Sphingosine 1-phosphate (S1P) is a crucial lipid mediator in the skin and potentially interferes with LC homeostasis but also functional properties, such as cytokine release, migration and antigen-uptake which are considered to be key events in the initiation and maintenance of pathological disorders.

Objective: Here, we used human Langerhans-like cells to study the influence of S1P-mediated signalling on LC maturation, cytokine release, migration and endocytosis.

Methods: Immature Langerhans-like cells were generated from the human acute myeloid leukaemia cell line MUTZ-3 (MUTZ-LCs) and human primary monocytes (MoLCs). S1P receptor expression was determined by quantitative RT-PCR and western blotting. Expression of maturation markers were investigated by flow cytometry. The influence of S1P signalling on cytokine release was quantified by ELISA. Migration assays and FITC-dextran uptake in the presence of S1P, specific S1 P receptor agonists and antagonists as well as fingolimod (FTY720) were analysed through fluorescence microscopy and flow cytometry.

Results: S1P receptor protein expression was confirmed for $S1P_1$, $S1P_2$ and $S1P_4$ in MUTZ-LCs and $S1P_1$ and $S1P_2$ in MoLCs. In mature cells S1P receptors were downregulated. S1P did not induce maturation in MUTZ-LCs, whereas in MoLCs CD83 and CD86 were slightly upregulated. IL-8 release of MUTZ-LCs matured in the presence of S1P was not altered, however, reduced IL-6 and IL-12p70 levels were observed in mature MoLCs. Interestingly, immature MUTZ-LCs revealed a significantly increased S1P-dependent migratory capacity, whereas CCL20 induced migration was significantly decreased in the presence of S1P. Furthermore, migratory capacity towards CCL21 in mature MUTZ-LCs but not MoLCs was significantly lower when cells were stimulated with S1P. S1P, FTY720 and specific S1P receptor agonists did not modulate the endocytotic capacity of immature MUTZ-LCs and MoLCs. These findings were further supported by testing specific antagonists of $S1P_{1-4}$ in the absence or presence of S1P.

Conclusion: Our data demonstrate that S1P regulates key events of human LC maturation including cytokine release and migration. These findings are of particular importance when considering the potential use of S1P in inflammatory skin disorders.

1. Introduction

In their function as antigen presenting cells (APCs), dendritic cells (DCs) are recognized to play a critical role in balancing immunity and tolerance [1–3]. In human skin, multiple DC subsets are present of which Langerhans cells (LCs), localized in the epidermis, are unique in their first-line exposure to exogenous stimuli. LCs consistently capture material, including invading pathogens as well as skin allergens, and upon maturation migrate towards associated lymph nodes to present processed antigens to T

Abbreviations: AML, acute myeloid leukaemia; APC, antigen presenting cell; CMC, cytokine maturation cocktail; DC, dendritic cell; GPCR, G protein-coupled receptor; LC, Langerhans cell; MoDC, monocyte-derived DC; MoLC, monocyte-derived LC; PRR, pattern recognition receptor; S1P, sphingosine 1-phosphate; S1PR, S1P receptor; TLR, Toll-like receptor.

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http://dx.doi.org/10.1016/j.jdermsci.2016.01.002
0923-1811/

cells [4]. Antigen uptake hereby can be determined via specific pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) and C-type lectin receptors but also via receptor independent phagocytosis or macropinocytosis. LCs subsequently initiate an adaptive immune response and in particular a crucial differentiation of hapten-specific effector T cells. Recent investigations therefore intend to further determine the role of LCs in the context of an initiation as well as the maintenance of inflammatory skin disorders such as allergic contact dermatitis and atopic dermatitis.

The sphingolipid sphingosine 1-phosphate (S1P) appears to play a pivotal role in skin immunity modulating functions of keratinocytes [5,6], fibroblasts [7] and APCs such as macrophages but also DCs [8,9]. A complex signalling network is mediated via 5 G protein-coupled receptors (GPCRs), denoted as sphingosine-1-phosphate receptors $(S1P)_{1-5}$, in addition to intracellular pathways [10]. S1P and fingolimod (FTY720), an immunosuppressive drug with clinical application in multiple sclerosis, regulate essential DC functions such as cytokine production and chemotaxis both in human and murine model systems whereas for LCs exclusively a reduced migratory capacity in mice was reported [11–15]. Furthermore, in the context of DC antigen uptake, striking differences have been reported. S1P impaired endocytosis in immature mouse DCs from the XS52 cell line [16] whereas S1P induced endocytosis in mature but not immature bone marrow-derived mouse DCs (BMDCs) [17,18]. In immature human monocyte-derived DCs (MoDCs) endocytotic capacity remained unaltered in the presence of FTY720 [12].

In the present study, we aimed to clarify the role of S1P on key events of human LC maturation including cytokine release, migration and endocytotic capacity. Human Langerhans-like cells, obtained upon cytokine-dependent differentiation of the acute myeloid leukaemia (AML) cell line MUTZ-3 or primary monocytes, were used as the most common human in vitro LC model systems to investigate the effect on maturation by S1P.

2. Material and methods

2.1. Maintenance of MUTZ-3 cell line and differentiation of MUTZ-LCs

The AML cell line MUTZ-3 (ACC 295; DSMZ, Germany) was maintained in a 24-well tissue plate (BD Biosciences, Germany) at a density of $0.5–1.0 \times 10^6$ cells/ml per well. Growth medium consisted of alpha medium (w/o L-Glutamine, with nucleosides; Biochrom, Germany) supplemented with 20% fetal calf serum (FCS) (Biochrom), 2 mM L-glutamine and 10% conditioned medium obtained from the human bladder carcinoma cell line 5637 (ACC 35; DSMZ) [19]. Medium was exchanged every 3 days. MUTZ-LCs were obtained after 10 days of differentiation (2.0×10^5/ml) in a defined cytokine medium containing 10 ng/ml TGF-β, 100 ng/ml GM-CSF (all from MiltenyiBiotec, Germany), 2.5 ng/ml TNF (eBioscience, Germany) and 50 μM 2-mercaptoethanol (Sigma–Aldrich, Germany) and medium was exchanged completely at day 5. To ensure a consistent differentiation profile of $CD1a^+$ $CD207^+$ $CCR6^+$ MUTZ-LCs, only MUTZ-3 progenitors at passage 15–25 were transferred to cytokine medium. The identity of the cell line throughout the experiments was confirmed by STR analysis (ATCC, USA).

2.2. Generation of MoLCs from human monocytes

MoLCs were differentiated from human monocytes as described previously [20,21]. Briefly, buffy-coat samples from different healthy donors (DRK-Blutspendedienst Ost, Germany) were obtained after informed consent and approved by the ethics committee of the Charité – Universitätsmedizin, Berlin. Following density gradient centrifugation using NycoPrepTM 1.077 (Axis–Shield, Norway), peripheral blood mononuclear cells (PBMCs) resulted in the interphase. After manifold washing with phosphate buffered saline (PBS, PAA Laboratories, Austria), monocyte adherence was used for purification. Differentiated MoLCs were obtained after 7 days of monocyte cultivation in complete medium, consisting of RPMI 1640 (Sigma–Aldrich) containing 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (all from PAA Laboratories, Austria) and 10% heat inactivated FCS (Biochrom), supplemented with GM-CSF (100 ng/ml), IL-4 (20 ng/ml) and TGF-β1 (20 ng/ml; all from MiltenyiBiotec). Cell separation via CD1a MicroBeads (clone HI149; MiltenyiBiotec) was performed at day 5. Cytokine-dependent differentiation led to immature $CD1a^+$ $CD207^+$ $CD324^+$ TROP-2^+ Axl^+ MoLCs.

2.3. S1P and S1PR agonists/antagonists

S1P (Cayman Chemical, USA) stock was dissolved in methanol, stored at −80 °C and S1P solution was freshly prepared for each experiment. Methanol was evaporated using N_2 and S1P was diluted in PBS containing 0.4% fatty acid free bovine serum albumin (BSA; Carl Roth, Germany). Fingolimod (FTY720; Cayman Chemical, USA), a clinically used agonist of $S1P_1$ and $S1P_{3-5}$, as well as its phosphorylated form fingolimod-phosphate (FTY720-P; Cayman Chemical) were used at concentrations of 0.1, 1 and 10 μM. Due to a higher mRNA expression of *S1PR2* and *S1PR4* for unstimulated MUTZ-LCs, the selective $S1P_2$ agonist CYM5520 (5 μM) and the selective $S1P_4$ agonist CYM50179 (1 μM) were used and purchased from Sigma–Aldrich. In addition, the $S1P_1$ antagonist W146 (2 μM; Cayman Chemical), $S1P_2$ antagonist JTE-013 (5 μM; Tocris Bioscience, UK), $S1P_3$ antagonist CAY10444 (5 μM Cayman Chemical) and $S1P_4$ antagonist CYM50358 (5 μM; Tocris Bioscience) were used.

2.4. Stimulation and maturation of MUTZ-LCs and MoLCs

MUTZ-LCs were seeded in alpha medium (2.5×10^5 cells/ml) without supplements and exposed to S1P (0.1; 1 and 10 μM), Pam_3CSK_4 (1 μg/ml; InvivoGen, USA) or a cytokine maturation cocktail (CMC) consisting of 50 ng/ml rh-TNF-α, 25 ng/ml rh-IL-1β (all from eBioscience), 100 ng/ml rh-IL-6 (MiltenyiBiotec) and 1 μg/ml PGE_2 (Tocris) for 24 or 48 h. MoLCs were stimulated with 1 μg/ml Pam_3CSK4, 1 μg/ml poly(I:C) (InvivoGen) or a CMC containing 20 ng/ml rh-TNF-α, 30 ng/ml rh-IL-1β and 1 μg/ml ultrapure lipopolysaccharide (LPS) from *Escherichia coli* serotype 0111:B4 (InvivoGen) or 1 μg/ml poly(I:C) for 24 h or 48 h, respectively. As control, MUTZ-LCs and MoLCs were maintained in alpha medium and complete medium, respectively. Stimulation with Pam_3CSK_4 and poly(I:C) was performed in the absence or presence of S1P with 1 h preincubation.

2.5. Flow cytometry

Phenotypical characterization of MUTZ-LCs and MoLCs was assessed by flow cytometry. Cells were labelled with the following monoclonal antibodies: FITC-conjugated mouse anti-CD1a (clone HI149), anti-CCR6 (clone R6H1, all from eBioscience), anti-CD86 (clone FM95, MiltenyiBiotec) and corresponding isotype control (eBioscience), PE-conjugated mouse anti-CD207 (clone 10E2), anti-CD83 (clone HB15e, all from Biolegend), anti-CD209 (clone DCN47.5, MiltenyiBiotec), anti-CCR7 (clone 150503, BD Biosciences) and corresponding isotype control (eBioscience), Alexa Fluor-conjugated mouse anti-Axl (clone 108724) and corresponding isotype control (all from R&D Systems, USA) and unconjugated mouse anti-TROP-2 (clone 162–46, BD Bioscience) and corresponding isotype control (eBioscience), followed by secondary

Please cite this article in press as: S. Bock, et al., Sphingosine 1-phospate differentially modulates maturation and function of human Langerhans-like cells, J Dermatol Sci (2016), http://dx.doi.org/10.1016/j.jdermsci.2016.01.002