Available online at www.sciencedirect.com
ScienceDirect
Immunobiology 213 (2008) 715–728
Immunobiology
www.elsevier.de/imbio

The lymph vessel network in mouse skin visualised with antibodies against the hyaluronan receptor LYVE-1

Christoph H. Tripp[a], Bernhard Haid[a], Vincent Flacher[a], Michael Sixt[b], Hannes Peter[c], Julia Farkas[c], Robert Gschwentner[d], Lydia Sorokin[e], Nikolaus Romani[a], Patrizia Stoitzner[a,*]

[a]*Department of Dermatology and Venereology, Innsbruck Medical University, Anichstrasse 35, A-6020 Innsbruck, Austria*
[b]*Max-Planck-Institute for Biochemistry, Martinsried, Germany*
[c]*Department of Microbiology, University of Innsbruck, Innsbruck, Austria*
[d]*Department of Zoology, University of Innsbruck, Innsbruck, Austria*
[e]*Institute for Physiological Chemistry and Pathobiochemistry, Münster University, Germany*

Received 23 July 2008; accepted 23 July 2008

Abstract

Langerhans cells and dermal dendritic cells migrate to the draining lymph nodes through dermal lymphatic vessels. They do so in the steady-state and under inflammatory conditions. Peripheral T cell tolerance or T cell priming, respectively, are the consequences of migration. The nature of dendritic cell-containing vessels was mostly defined by electron microscopy or by their lack of blood endothelial markers. Selective markers for murine lymph endothelium were hitherto rare or not available. Here, we utilised recently developed antibodies against the murine hyaluronan receptor, LYVE-1, to study the lymph vessel network in mouse skin in more detail.

In hairless skin from the ears, lymph vessels were spread out in a horizontal plane. They formed anastomoses, and they possessed frequent blind endings that were occasionally open. Lymph vessels were wider than blood vessels, which were identified by their strong CD31 expression. In body wall skin LYVE-1 reactive vessels did not extend laterally but they dived straight down into the deeper dermis. There, they are connected to each other and formed a network similar to ear skin. The number and width of lymph vessels did not grossly change upon inflammatory stimuli such as skin explant culture or tape stripping. There were also no marked changes in caliber in response to the TLR 7/8 ligand Imiquimod.

Double-labelling experiments of cultured skin showed that most of the strongly cell surface MHC II-expressing (i.e. activated) dendritic cells were confined to the lymph vessels. Langerin/CD207$^+$ cells within this population appeared later than dermal dendritic cells, i.e. langerin-negative cells. Comparable results were obtained after stimulating the skin *in vivo* with the TLR 7/8 ligand Imiquimod or by tape stripping.

In untreated skin (i.e. steady state) a few MHC II$^+$ and Langerin/CD207$^+$ cells, presumably migrating skin dendritic cells including epidermal Langerhans cells, were consistently observed within the lymph vessels. The novel

Abbreviation: TLR, toll-like receptor.

*Corresponding author.
E-mail address: patrizia.stoitzner@i-med.ac.at (P. Stoitzner).

0171-2985/$ - see front matter
doi:10.1016/j.imbio.2008.07.025

antibody reagents may serve as important tools to further study the dendritic cell traffic in the skin under physiological conditions as well as in conditions of adoptive dendritic cell transfer in immunotherapy.

Keywords: Dendritic cell migration; Inflammation; Lymphatic vessels; Steady-state migration

Introduction

Dendritic cells of the skin, i.e. epidermal Langerhans cells and dermal dendritic cells (Valladeau and Saeland, 2005; Romani et al., 2008), initiate immune responses against antigens/pathogens that enter the body through the skin. Thus, they serve as sentinels of the immune system. Only a few recent examples exist of innate immune responses carried out by skin dendritic cells, in particular Langerhans cells (De Witte et al., 2007). In contrast, ample evidence exists about the ability of skin dendritic cells to induce adaptive immune responses (Romani et al., 2006), contact hypersensitivity being the classical example for this (Streilein and Bergstresser, 1984). Several important questions are not yet answered, though. First of all, as opposed to the well-defined epidermal Langerhans cell, dermal dendritic cells are still not unequivocally characterised. Subsets may exist (Angel et al., 2006; Bursch et al., 2007; Ginhoux et al., 2007; Poulin et al., 2007), and a transformation from resident macrophages (Dupasquier et al., 2004; Zaba et al., 2007) into dermal dendritic cells upon organ culture or *in vivo* was recently observed (Dupasquier et al., 2008). The relative contributions of Langerhans cells versus dermal dendritic cells are just beginning to be understood, thanks to the advent of mouse models that selectively deplete Langerhans cells (Bennett et al., 2005; Kaplan et al., 2005; Kissenpfennig et al., 2005). Surprisingly, Langerhans cells may be less immungenic *in vivo*, perhaps even down-regulatory (Kaplan et al., 2005), as anticipated from some of the *in vitro* data. Furthermore, it is not entirely clear as to how skin dendritic cells contribute to the maintenance of peripheral tolerance (Mayerova et al., 2004; Shibaki et al., 2004). Langerhans cells and/or dermal dendritic cells can constitutively capture self-antigens from the skin and carry them to the peripheral lymph nodes (Hemmi et al., 2001). As long as this happens in the steady state, peripheral tolerance is sustained (Steinman and Nussenzweig, 2002). The relative contributions of Langerhans cells and dermal dendritic cells in this process have not yet been elucidated, though.

Irrespective of these uncertainties about the behaviour of skin dendritic cells, there is one precondition that needs to be fulfilled at any rate. Dendritic cells must migrate from the skin to the draining lymph nodes, i.e. those sites where primary immune reactions are initiated, be they immunogenic or tolerogenic in nature. Many conditions under which dendritic cell migration takes place have been described in the past. Migration is regulated at the levels of adhesion, enzymatic digestion of the extracellular matrix, chemotaxis to chemokines (Romani et al., 2001; Randolph et al., 2005) and by specialised conduit systems in the lymph nodes (Sixt et al., 2005). The necessary signals are induced by inflammatory cytokines and toll-like receptor (TLR) ligands as well as physical stimuli. Various TLR ligands have been described as potential modulators of the skin. Ligation of TLRs induces the production of inflammatory cytokines and chemokines as well as maturation and migration of DC. Recent data from mouse models strongly suggested that DC migration can be improved by treating skin with proinflammatory cytokines or TLR 7 ligand Imiquimod (Nair et al., 2003).

Comparably few data are available about the routes of dendritic cell migration, mostly the lymph vessels of the skin (reviewed in Angeli et al., 2006). Not least, this was due to the fact that antibodies recognising lymph vessels have not been available for long (Breiteneder-Geleff et al., 1999; Gale et al., 2007). This prompted us to study in more detail the lymph vessel network in mouse skin and to better define dendritic cells travelling inside these vessels. This was achieved by using an antibody against lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1), exclusively expressed on lymphatic endothelial cells in mouse dermis (Banerji et al., 1999; Prevo et al., 2001). LYVE-1 is a receptor for hyaluronan, a carbohydrate component of the cutaneous extracellular matrix, presumably involved in the regulation of interstitial-lymphatic flow (Huang et al., 2006), lymphangiogenesis (Chen et al., 2005), and possibly also in innate immune functions (Böckle et al., 2008).

We focused on the morphology and spatial orientation of lymphatic vessels in murine skin. Furthermore, we investigated the occurrence and proportions of skin dendritic cells within lymphatic vessels of inflamed and steady-state skin, with the aim to gain more insights into the regulation of dendritic cell migration.

Materials and methods

Mice and media

Mice of inbred strains C57BL/6 and BALB/c were purchased from Charles River Laboratories (Sulzfeld,