Available online at www.sciencedirect.com
ScienceDirect
Immunobiology 211 (2006) 577–585
Immunobiology
www.elsevier.de/imbio

Differential regulation of C-type lectin expression on tolerogenic dendritic cell subsets

Sandra J. van Vliet[1], Ellis van Liempt[1], Teunis B.H. Geijtenbeek, Yvette van Kooyk*

Department of Molecular Cell Biology and Immunology, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, The Netherlands

Abstract

Antigen presenting cells (APC) express high levels of C-type lectins, which play a major role in cellular interactions as well as pathogen recognition and antigen presentation. The C-type lectin macrophage galactose-type lectin (MGL), expressed by dendritic cells (DC) and macrophages, mediates binding to glycoproteins and lipids that contain terminal GalNAc moieties. To investigate MGL expression patterns in more detail, we generated two new monoclonal antibodies and set up a quantitative real-time PCR analysis to determine MGL mRNA levels. MGL is not expressed by blood-resident plasmacytoid DC and thus represents an exclusive marker for myeloid-type APC. Dexamethasone treatment upregulated MGL expression on DC both at the protein and mRNA level in a time- and dose-dependent manner. In contrast, DC generated in the presence of IL-10 did not display enhanced MGL levels. Furthermore, dexamethasone and IL-10 also differentially regulated expression of other C-type lectins, such as DC-SIGN and Mannose Receptor. Our results demonstrate that depending on the local microenvironment, DC can adopt different C-type lectin profiles, which could have major influences on cell–cell interactions, antigen uptake and presentation.

Keywords: C-type lectins; Dendritic cells; Macrophages; Plasmacytoid DC

Introduction

Professional antigen presenting cells (APCs), such as dendritic cells (DCs) and macrophages (Mφ), are seeded throughout all peripheral tissues where they scan their surroundings for incoming pathogens or local environmental changes. Mφ mainly represent traditional tissue-resident scavenging cells important in the effector phase of the immune response. Similar to Mφ, DC also play an essential role in uptake of self- or pathogenic antigens. DC, once activated by proinflammatory stimuli or infectious pathogens, migrate towards the draining lymph node, where they initiate adaptive immunity (Taylor et al., 2005; Mellman and Steinman, 2001). Recently, Pozzi et al. (2005) demonstrated that also Mφ can migrate to draining lymph nodes and activate naïve $CD8^+$ T cells, although with lower efficiencies than DC. Next to immunity, DC contribute to tolerance via the induction of T cell unresponsiveness or apoptosis or via the induction of regulatory T cells. These processes can be mimicked in vitro by the addition of glucocorticoids (GC), such as dexametha-

Abbreviations: APC, antigen presenting cell; ASGP-R, asialoglycoprotein receptor; DC, dendritic cell; GILZ, glucocorticoid-induced leucine zipper; GC, glucocorticoids; mAbs, monoclonal antibodies; MGL, macrophage galactose-type lectin; MR, mannose receptor; Mφ, macrophage; pDC, plasmacytoid DC

*Corresponding author. Tel.: +31 204448080; fax: +31 204448081.
E-mail address: Y.vankooyk@vumc.nl (Y. van Kooyk).
[1]These authors contributed equally to this paper.

0171-2985/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.imbio.2006.05.022

sone, or by adding IL-10 to DC cultures (Xia et al., 2005; Steinman et al., 2003).

Different DC lineages can develop from separate precursors or represent various activation states of a single subtype. DC clearly possess a unique plasticity to adapt to environmental stimuli, leading to different functional phenotypes based on cell surface markers and production of cytokines and/or reactive metabolites (Janeway and Medzhitov, 2002). Furthermore, recent evidence indicates that mouse splenic DC and Mφ renew from a common bone marrow progenitor that is able to develop into both subtypes depending on differential cytokine signaling (Fogg et al., 2006). In humans, several pathways exist for the development of the different DC subtypes, such as Langerhans cells, plamacytoid DC (pDC) and interstitial DC, each requiring their own set of growth factors and/or cytokines (Shortman and Liu, 2002).

As only a few surface proteins are expressed exclusively by DC; new potential markers are required that can distinguish between the different phenotypic DC subtypes. One family of proteins, known to be differentially expressed by the various DC subsets, are the C-type lectins (Figdor et al., 2002). C-type lectins recognize specific carbohydrate moieties in a Ca^{2+}-dependent manner. They function as cell–cell adhesion molecules (Geijtenbeek et al., 2000) and as pattern recognition receptors for pathogens (Gordon, 2002). Moreover, C-type lectins can internalize ligands, such as pathogens, but also self-glycoproteins for processing and presentation to T cells (Engering et al., 2002). The C-type lectin macrophage galactose-type lectin (MGL) is expressed on in vitro cultured monocyte-derived DC and Mφ (Suzuki et al., 1996). The carbohydrate recognition domain of MGL facilitates binding of terminal GalNAc-residues on glycoproteins, glyolipids or pathogens, in contrast to the well-known mannose/fucose-specific lectins DC-SIGN and mannose receptor (MR) (van Vliet et al., 2005). Although MGL was originally described to be a specific marker for cells at an intermediate stage of differentiation from monocytes to Mφ (Higashi et al., 2002b), other reports demonstrate MGL to be expressed by dendritic cells and alternatively activated macrophages (Higashi et al., 2002a; Raes et al., 2005).

To further analyze the expression pattern of MGL on human DC subtypes, two new monoclonal antibodies (mAbs) directed against the C-type lectin MGL were generated. Our findings extend the knowledge on MGL expression patterns both at RNA and protein level on functionally different immature DC subsets. We demonstrate that MGL is exclusively expressed by myeloid DC and not by blood-resident pDC. Only dexamethasone treatment, and not IL-10, can enhance MGL expression on tolerogenic DC at both the protein and mRNA level. Furthermore, we show that expression of the C-type lectins MGL, DC-SIGN and MR is differentially regulated, suggesting that depending on the cellular environment, DC can adopt various phenotypes with variable C-type lectin expression profiles.

Materials and methods

Cells and reagents

The cell lines CHO and CHO-MGL were maintained in RPMI containing 10% fetal calf's serum (Invitrogen, Carlsbad, CA). Immature DC were cultured for 3–7 days from monocytes obtained from buffy coats of healthy donors (Sanquin, Amsterdam, The Netherlands) in the presence of IL-4 (500 U/ml) and GM-CSF (800 U/ml, both from Biosource, Camarillo, CA). pDC were isolated from buffy coats using the BDCA-4 cell isolation kit according to the manufacturer's protocol (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). To some DC cultures, dexamethasone (at concentrations indicated in the figures, Sigma-Aldrich), IL-10 (10 ng/ml, Biosource) or LPS (100 ng/ml, Sigma-Aldrich, St. Louis, MO) was added. Monocytes were stimulated with IL-2 (200 U/ml, Biosource), IL-4 (500 U/ml), IL-10 (10 ng/ml), dexamethasone (10^{-6} M) or GM-CSF (1000 U/ml).

Generation of anti-MGL mAbs

Balb/c mice were immunized three times with recombinant MGL-Fc (van Vliet et al., 2005). After the final boost, spleen cells were fused with SP2/0 cells at a 1:1 ratio using PEG. Hybridoma supernatants were screened for the presence of anti-MGL mAbs on CHO-MGL transfectants. After two rounds of cloning, two hybridomas (1G6.6 and 18E4) were obtained that specifically recognize MGL.

Flow cytometry

Cells were incubated with primary antibody (5 μg/ml), followed by staining with a secondary FITC-labeled goat anti-mouse antibody (Zymed, San Francisco, CA) and analyzed on FACScalibur (BD Pharmingen, San Diego, CA). The following mAbs were used: isotype control 28-14-8 (mouse anti-mouse H2 D^b), AZN-D1 (DC-SIGN) and 3.29.B1 (MR) (Engering et al., 2002). The anti-MGL antibody MLD-1 was kindly provided by Dr. T. Irimura.

Immunohistochemistry

Cryosections of healthy humans tissues (7 μm) were fixed with 100% acetone and stained with primary