Blood Cells, Molecules and Diseases 55 (2015) 293–303

Contents lists available at ScienceDirect

Blood Cells, Molecules and Diseases

journal homepage: www.elsevier.com/locate/bcmd

Characterization of hematopoietic GATA transcription factor expression in mouse and human dendritic cells☆

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ARTICLE INFO

Article history:
Submitted 31 March 2015
Revised 10 July 2015
Accepted 11 July 2015
Available online 14 July 2015

Editor: Mohandas Narla

Keywords:
Dendritic cells
Transcription factors
GATA1
GATA2
GATA3
GATA switch
PU.1

ABSTRACT

Dendritic cells (DCs) are key initiators and regulators of the immune response. The development of the DC lineage and their subsets requires an orchestrated regulation of their transcriptional program. Gata1, a transcription factor expressed in several hematopoietic cell lineages, has been recently reported to be required for mouse DC development and function. In humans, GATA1 is involved in the lineage separation between monocyte-derived DCs and Langerhans cells (LC) and loss of GATA1 results in differentiation arrest at the monocyte stage. The hematopoietic GATA factors (i.e. Gata1, Gata2, Gata3) are known to regulate each other's expression and to function consecutively throughout lineage commitment (so-called GATA switch). In humans, mutations in GATA2 are causative of MonoMAC disease, a human immunodeficiency syndrome characterized by loss of DCs, monocytes, B and NK cells. However, additional data on the expression of hematopoietic GATA factors in the DC lineage is missing.

In this study, we have characterized the expression of hematopoietic GATA factors in murine and human DCs and their expression dynamics upon TLR stimulation. We found that all hematopoietic GATA factors are expressed in DCs, but identified species-specific differences in the relative expression of each GATA factor, and how their expression fluctuates upon stimulation.

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1. Introduction

Dendritic cells (DCs) are key initiators and regulators of the immune system, both priming the immune response and inducing tolerance [1]. They are located both in lymphoid and non-lymphoid tissues, where they sample their environment continuously for self- and non-self-antigens [2, 3]. These "sampling" DCs are so-called immature DCs, and express a series of receptors that recognize pathogen-associated molecular patterns (PAMPs), including Toll-like receptors (TLRs). Upon PAMP recognition, DCs get activated, upregulate co-stimulatory molecule expression, migrate to the lymph nodes and present antigen-derived peptides to T-cells via MHC-I/II [4].

Two major types of DCs can be distinguished, namely conventional DC (cDC) and plasmacytoid DC (pDC) [5]. pDCs are mainly found in the blood stream. They are important mediators in the antiviral immunity by the large amounts of type-I interferon they produce upon TLR-7 (that recognizes ssRNA) or TLR-9 (that recognizes CpG-containing DNA) stimulation [6, 7]. cDCs are mainly resident tissue-dwelling or migratory and express TLR-2 (recognizing lipopeptides), TLR-4 (lipopolysaccharide (LPS)), TLR-5 (recognizing flagellin), TLR-6 (heterodimer with TLR-2), and TLR-9 (recognizing CpG-containing DNA), although they are recognized as potent bacterial responders [8, 9]. Under inflammatory conditions, yet another type of DC, so-called inflammatory DC, derives from circulating monocytes [5].

cDCs can be further subdivided into $CD8a^+$ cDCs and $CD4^+|CD11b^+$ cDCs in the mouse, which are comparable to human cDCs recently subdivided as $BDCA3^+$ and $BDCA1^+$ respectively [10, 11]. $CD8a^+$ cDCs highly express DEC205 and CD24, whereas $CD11b^+$ cDCs are $DEC205^{low}$ and express high levels of Sirp-α [12–16]. Migratory cDCs can be distinguished from resident DCs in the lymph nodes by a higher MHC-II expression [4, 5, 14].

Abbreviations: DC, dendritic cell; LC, Langerhans cell; PAMP, pathogen-associated molecular pattern; TLR, Toll-like receptor; cDC, conventional dendritic cell; pDC, plasmacytoid dendritic cell; ssRNA, single stranded RNA; LPS, lipopolysaccharide; BMDC, bone marrow derived dendritic cell; GM-CSF, granulocyte-macrophage colony-stimulating factor; Flt3L, FMS-like tyrosine kinase 3 ligand; PBMC, peripheral blood mononuclear cell; mo-DC, monocyte derived dendritic cell.

☆ Author's contributions: MS designed and performed experiments, analyzed the data, and wrote the manuscript. VS, IMDC, MH and EL performed experiments. TK and TB participated in discussions and revised the manuscript. LG designed experiments, contributed to data analysis and revised the manuscript. All authors reviewed and approved the final version.

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http://dx.doi.org/10.1016/j.bcmd.2015.07.006
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The development of DCs from hematopoietic progenitors is tightly regulated by transcriptional programs. Many transcription factors have been studied in depth by using genetically modified mice and reconstitution assays [17]. PU.1 [17–19], IRF-2 [20–23], IRF-4 [20–24], IRF-8 [20–22, 24], Batf3 [24], RelB [20, 21, 23], and E2-2 [25, 26] were all shown to be important in DC development, while some of them being required for a specific DC subset. Recently, Gata1 was described to be expressed in both cDCs and pDCs, and required for DC differentiation, survival and function [27]. Furthermore, previous studies showed that GATA1 is also essential for DC differentiation from human monocyte-derived DCs, since knockdown of GATA1 resulted in differentiation arrest at the monocyte stage, and also crucial for the LC–DC lineage bifurcation from monocytes [28].

Gata1 belongs to the GATA family of transcription factors, consisting of Gata1-6. Of these, Gata1, Gata2, and Gata3 are expressed in the hematopoietic system. All the GATA transcription factors are very similar to each other. They contain two conserved multifunctional zinc finger domains and recognize a common DNA binding motif WGATAR. The two zinc finger domains are involved in DNA-binding and protein–protein interactions. Gata1 is essential for the differentiation of erythrocytes, eosinophils and megakaryocytes [29], but seems to be dispensable for mast cell differentiation [30]. In humans, there are two isoforms of GATA1, i.e. full length GATA1 (GATA1-FL) and a short isoform (also known as GATA1s), both due to alternative splicing and translation [31]. Mutations in *GATA1* exon 2 leading to the expression of Gata1s only, result in Diamond–Blackfan anemia [31–33]. Gata1 regulates genes related to cell differentiation, cell cycle and survival, such as the anti-apoptotic protein Bcl-XL [28, 34–37]. Importantly, it has been shown that Gata1 and PU.1 antagonize each other during erythroid (Gata1 dependent) or macrophage (PU.1 dependent) differentiation, while in other lineages they can co-exist [28]. Gata2 is important throughout the hematopoietic system. It regulates the self-renewal of multipotent progenitor cells and is involved in erythro- and megakaryopoiesis [29, 38]. Gata2 binds and regulates the PU.1 promoter directly [39]. Conditional expression of Gata2 in mouse embryonic stem cells resulted in a decrease of PU.1 and an increase of Gata1, which blocked macrophage differentiation [39]. In humans, heterozygous mutations in GATA2 are linked to the MonoMAC syndrome, which is characterized by the loss of DCs, monocytes, B and NK cells [40]. Gata3 is a critical regulator of cells that orchestrate both the innate and adaptive immunity. It regulates hematopoietic stem cell maintenance [41] and plays an essential role in the development and function of T cells [42] and NK cells [43].

GATA transcription factors are often expressed in an overlapping manner or replace each other during differentiation, a mechanism which is so-called the GATA switch [44, 45]. This is important for example in the erythrocyte/megakaryocyte lineage bifurcation point. Gata2 is expressed in common megakaryocyte/erythroid progenitors. Repression of Gata2 by Gata1 facilitates terminal differentiation of erythrocytes. However, if both Gata1 and Gata2 remain expressed, it leads to the differentiation of megakaryocytes [44, 46–48].

Until now, the expression of GATA factors other than Gata1 in DCs has not been explored. Considering the expression and need of Gata1 for DC differentiation, survival and activation, and taking into account the existence of GATA switches in other hematopoietic lineages, and the role of GATA2 in the development of MonoMAC disease, we decided to characterize in depth the expression of hematopoietic GATA factors in mouse and human DCs, either prospectively isolated or in vitro generated, and upon stimulation.

2. Materials and methods

2.1. Mice

All mice were kept under pathogen-free conditions with free access to food and water, under the guidelines for animal experimentation approved by the Animal Ethical Committee of the Netherlands Cancer Institute (NKI, Amsterdam, The Netherlands).

2.2. Human samples

Blood was taken from healthy donors after informed consent was obtained as approved by our institution medical ethics committee and in accordance with the 1964 Declaration of Helsinki.

2.3. Mouse dendritic cell culture

Bone marrow derived dendritic cells (BMDCs) were generated by culturing femoral and tibial bone marrow cells of male wild-type C57BL/6 mice (Jackson Laboratory, Bar Harbor, Main, USA).

2.3.1. GM-CSF cultures

BMDCs were cultured at a density of $0.5 * 10^6$/ml in Gibco® RPMI 1640 (Life Technologies, Carlsbad, CA, USA) supplemented with 5% heat inactivated fetal calf serum, 1% penicillin/streptomycin, 5 μM 2-mercaptoethanol and 20 ng/ml GM-CSF (Peprotech, Rocky Hill, NJ, USA); when indicated, 25 ng/ml IL4 (eBioscience, San Diego, CA, USA) was added. 0.5 μg/ml LPS (Santa Cruz Biotechnology, Dallas, Texas, USA) was added to the cells at day 7, and harvested for analysis at day 10.

2.3.2. Flt3L cultures

Red blood cells of the bone marrow were lysed with an isotonic ammoniumchloride buffer before culture. All cells of one femur and tibia were resuspended in 12 ml Gibco® RPMI 1640 supplemented with 10% heat inactivated fetal calf serum, 1% penicillin/streptomycin, 5 μM 2-mercaptoethanol and 200 ng/ml Flt3L (kind gift of B.N. Lambrecht, Ghent University) and cultured in a 6-well plate with 4 ml per well. 0.5 μg/ml LPS (Santa Cruz Biotechnology) or 2 μg/ml CpG-B ODN 1668 (Invivogen, Toulouse, France) was added at day 8 and the cells were harvested 24 h after stimulation for further analysis.

2.4. Human dendritic cell culture

After isolating the peripheral blood mononuclear cell fraction by percoll gradient by centrifugation at 2070 RPM during 20 min, monocytes were positively selected with $CD14^+$ MACS beads (Miltenyi Biotech, Auburn, CA, USA), following the manufacturer's guidelines. $CD14^+$ cells were cultured in Gibco® RPMI 1640 supplemented with 10% heat inactivated fetal calf serum, 1% penicillin/streptomycin, 10 ng/ml GM-CSF (Peprotech) and 10 ng/ml IL4 (Peprotech). Cells were stimulated with 0.5 μg/ml LPS (Santa Cruz Biotechnology) at day 7 and collected at day 8.

2.5. Flow cytometry

After harvesting cultured DCs either from mouse or human origin, cells were stained with antibody-cocktails, measured on a BD FACSCanto II flow cytometer (BD Bioscience, San Jose, CA, USA) and analyzed with FlowJo software (Ashland, OR, USA). For intracellular FACS, cells were fixed and permeabilized with the BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution Kit (BD-Bioscience) and stained according to manufacturer's guidelines (antibodies used are indicated in Table 1).

2.5.1. Prospective isolation of mouse splenic dendritic cell subtypes

Spleen tissue was digested in Gibco® RPMI 1640 (Life Technologies), supplemented with 1.5 WU/ml Liberase TL (Roche diagnostics, Lewes, United Kingdom), 200 U/ml DNAse (Sigma Aldrich, Dorset United Kingdom), and 0.5 μg/ml Agrastat (Merck Sharp and Dohme, Haarlem, The Netherlands) for 45 min after which single cell suspensions were obtained by passing suspensions through a 40 μm filter (BD bioscience).

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