Disponible en ligne sur
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

TRANSFUSION
CLINIQUE ET BIOLOGIQUE

Transfusion Clinique et Biologique xxx (2017) xxx–xxx

Research perspective

Differentiation of human dendritic cell subsets for immune tolerance induction

Différenciation de cellules dendritiques humaines pour l'induction de la tolérance immune

N. Deluce-Kakwata-nkor [a], L. Lamendour [a], V. Chabot [b], A. Héraud [a], Z. Ivanovic [c], F. Halary [d,e], F. Dehaut [b], F. Velge-Roussel [a,f,*]

[a] *EA 4245 cellules dendritiques, immuno-modulation et greffes, université François-Rabelais de Tours, UFR de médecine, 10, boulevard Tonnellé, 37032 Tours cedex, France*

[b] *Service recherche du laboratoire d'histocompatibilité et d'immunogénétique, établissement français du sang Centre-Atlantique, 2, boulevard Tonnellé, BP 40661, 37206 Tours cedex 3, France*

[c] *EFS Aquitaine-Limousin, place Amélie-Raba-Léon, CS 21010, 33075 Bordeaux, France*

[d] *UMR 1064, Inserm, centre de recherche en transplantation et immunologie (CRTI), université de Nantes, Nantes, France*

[e] *Institut de transplantation urologie néphrologie (ITUN), Hôtel-Dieu, CHU de Nantes, 30, boulevard Jean-Monnet, 44093 Nantes cedex 01, France*

[f] *UFR des sciences pharmaceutiques, avenue Monge, 37000 Tours, France*

Abstract

Objectives. – Since no further progress was achieved, in order to improve the long-term organ transplantation outcome, the immune tolerance appears as an interesting therapeutic goal. Dendritic cells (DCs) are specialized cells participating in the homeostasis of the immune response. Moreover, subsets of DCs, identified in humans, appear to have their respective competences in immune response modulation. Our objective is to purify from PBMC or to differentiate DC subsets from monocytes using several strategies and evaluate their IL10 secretion.

Methods. – $CD14^+$ cells were purified from peripheral blood mononuclear cell (PBMC) by affinity beads and cultured with cytokines up to 7 days. The pDCs were purified with anti-BDCA-2 beads from PBMC fraction enriched by Percoll® gradient. The moDCs, pDCs and moLCs subsets were analyzed by phenotype labelling and FACS analyses and IL10 secretion measured by ELISA.

Results. – The moDCs were characterized by the CD209 expression and a lower expression of CD1a markers. Expression of CD207 and CD1a markers characterized moLCs and $CD123^+$/BDCA-2^+ pDCs. Variable IL-10 secretions were shown between the three DC subsets, both at basal and activated levels.

Conclusions. – As the several DC populations studied have different capacities of IL-10 synthesis, they might play, among others, distinct roles in the induction of immune tolerance.

Keywords: Dendritic cells; DC subsets; Immuno-modulation; IL-10

Résumé

Objectifs. – L'induction de tolérance immune reste un challenge important dans le domaine de la transplantation d'organe. Les cellules dendritiques (DCs), piliers de la réponse immunitaire, jouent un rôle crucial aussi bien dans l'induction d'une immunité spécifique que dans celle d'une tolérance immune. Chez l'homme, il existe au moins quatre types de DCs effectrices majeures, les DCs conventionnelles (cDC), les DCs plasmacytoïdes (pDCs), les DCs inflammatoires (MoDCS) et les cellules de Langerhans (LCs). L'objectif du projet est de préparer différents sous-types de DCs (moDCs, pDCs, moLCs) afin d'analyser leurs capacités à synthétiser de l'IL-10.

* Corresponding author. EA 4245 cellules dendritiques, immuno-modulation et greffes, UFR de médecine, université François-Rabelais, 10, boulevard Tonnellé, 37032 Tours cedex, France.

E-mail address: velge@univ-tours.fr (F. Velge-Roussel).

http://dx.doi.org/10.1016/j.tracli.2017.08.002
1246-7820/

Please cite this article in press as: Deluce-Kakwata-nkor N, et al. Differentiation of human dendritic cell subsets for immune tolerance induction. Transfusion Clinique et Biologique (2017), http://dx.doi.org/10.1016/j.tracli.2017.08.002

Méthodes. – La différenciation des moDCs et moLCs est faite à partir de monocytes CD14$^+$ isolés des PBMCs, en présence de cytokines spécifiques. La purification des pDC est faite avec des billes anti-BDCA-2 à partir des PBMC.
Résultats. – Les moLCs différenciées sont caractérisées par l'expression de la Langerine (CD207) et de CD1a. Les moDCs se caractérisent par l'expression de CD209 et une plus faible expression de CD1a et les pDCs par l'expression conjointe de CD123 et BDCA-2. La synthèse d'IL10 est différente entre les trois sous-types de DCs étudiés au niveau basal comme activé.
Conclusions. – Les différentes populations de DC étudiées, parce qu'elles présentent des capacités variées, entre autres, pour la synthèse d'IL-10, pourraient jouer des rôles distincts dans l'induction de tolérance immune.

Mots clés : Cellules dendritiques ; Sous-types de DCs ; Immunomodulation ; IL-10

1. Introduction

Dendritic cells (DCs) are professional antigen-presenting cells that induce immunity upon detection of pathogens, while maintaining tolerance in response to innocuous molecules due to their functional plasticity [1,2]. Two main DC subsets, at last, have been identified in the blood, spleen, tonsil and lymph nodes including conventional DCs (cDCs), consisting of either BDCA1/CD1c$^+$ DCs or BDCA3/CD141$^+$ DCs and plasmacytoid DCs (pDCs) consisting of CD123$^+$ cells. cDCs are effective at antigen-specific stimulation of CD4$^+$ and CD8$^+$ T cells whereas pDCs specialize in producing type I interferons in response of virus motifs [3]. In the skin, three DC subsets have been described: Langerhans cells characterized by the expression of Langerin (CD207$^+$) [4], CD1a$^+$ dermal DCs, and CD14$^+$ dermal DCs, which all migrate into skin-draining lymph nodes [5]. Additional revised taxonomy of human blood DC subsets have been described based on Single-cell RNA-seq including two subpopulations within BDCA1/CD1c$^+$ DCs (CD1 C$^+$-A and CD1 C$^+$-B) which are distinguished by their strong signature of inflammatory genes [6]. Moreover, a new DC subset "AS DCs" which share properties with pDCs have been identified in human blood, nevertheless, pDCs remains as "the natural interferon-producing cells" with weaker T cell proliferation induction ability [6]. Since their discovery, DCs have proved to play a central role in regulating immune responses. Tolerant DCs are often characterized by a low expression of surface MHC II molecules and co-stimulatory molecules (CD40, CD80 and CD86) and low T cell stimulatory ability [7]. Moreover, they usually show reduced IL-12 and increase IL-10. During the last decades, significant advances have been made in establishing methods to manipulate DCs in vitro to generate tolerant DCs [8] using cytokines such as: Il-10 alone [9] or in combination of transforming growth factor (TGF-ß1) [10]. Alternatively, pharmacological mediators including: 1,25-dihydroxyvitamin D3 [11], histone deacetylase inhibitors (HDAC) [12] or immunosuppressive drugs such as mycophenolate mofetil or rapamycin [13,14] which modulate DCs in that sense. Despite the importance of Tol-DCs, the capacities of each DC subset to induce and promote immune tolerance remain unknown. We explore DC subset capacities of secreting IL-10 versus pro-inflammatory cytokines using PRRs agonist.

2. Methods

2.1. Monocyte isolation and culture

Cytapheresis products were obtained from Centre Atlantic Transfusion Department (EFS-CA). They were issued from the healthy adult volunteers who had given their written informed consent and the university ethic committee approved the procedure. Mononuclear cells were obtained from peripheral blood of healthy donors using Ficoll (Dutscher) density gradient centrifugation. The monocytes were then purified by a positive selection using CD14 microbeads (Miltenyi Biotec) (> 90% of purity). For immature monocyte-derived DCs (moDCs), monocytes were differentiated for 6 days in RPMI 1640 (Dutscher) medium supplemented with 10% FCS (Dutsher), 66 ng/mL granulocyte macrophage colony stimulating factor (GM-CSF, Miltenyi Biotec) and 25 ng ng/mL IL-4 (Miltenyi Biotec). At day 6, cells were collected and flow cytometry analysis was performed. For human moLCs, monocytes were cultured for 7 days in RPMI 1640 medium supplemented with the 2% human albumin (HAB, Vialebex LFB), 50 ng/mL GM-CSF (Miltenyi Biotec), and 10 ng/mL TGF-ß1 (Miltenyi Biotec), renewed at day 3. At day 7, cells were collected and FACS analysis was performed. The pDCs were isolated by negative selection using plasmacytoid dendritic cells isolation kit II (Miltenyi Biotec), from an enriched DC cells fraction, which was obtained by gradient centrifugation of PBMCs, Percol® (Healthcare) gradient centrifugation of PBMCs. Cells were gated based on their characteristic pattern of SSC and FSC. Doublets were excluded based on forward scatter height (FSC-H) and forward scatter width (FSC-W) and analysed for the double expression of CD123+ (IL-3R$^+$) and CD303 (CLEC4 C, BDCA-2). The purity of population is up to 98,1%.

2.2. Flow cytometry analysis

Cells (1×10^5/100 µL) were stained for 30 min at 4 °C with the following anti-human antibodies at the appropriate concentration or with the relevant isotypes: CD83-FITC (BD Biosciences), CD14-PE (Beckman coulter), CD86-PE (BD Biosciences), HLA-DR–APC (BD Biosciences), CD207-APC (Biolegend), CD1A-AF488 (Biolegend), CD123-APC (Biolegend), BDCA-2-APC (Biolegend), CD209-PE (Beckman

Please cite this article in press as: Deluce-Kakwata-nkor N, et al. Differentiation of human dendritic cell subsets for immune tolerance induction. Transfusion Clinique et Biologique (2017), http://dx.doi.org/10.1016/j.tracli.2017.08.002