INTRODUCTION

Dendritic cells (DCs) are a heterogeneous population of antigen-presenting cells (APCs) derived from hematopoietic progenitors that bridge the transition between the innate and adaptive immune responses, while maintaining self-tolerance and Th1/Th2 homeostasis, by priming other cells in either an immunogenic or tolerogenic direction ***(Finkelman et al., 1996).*** Through their role in both innate and adaptive immunity, DCs play a major part in transplant engraftment and rejection and in graft-versus-host disease (GvHD). Preferentially tolerogenic or immunogenic DC subtypes offer targets for immunotherapy, to optimize transplant success rates and prolong disease-free and overall survival ***(Pereira and Paiva, 2011).***

DCs are critical for initiating immune responses against both pathogenic and nonpathogenic bacteria. In an immature stage, DCs reside in peripheral tissues, continuously sampling the microenvironment, sensing the presence of pathogens, and releasing chemokines and cytokines to amplify the immune response ***(Banchereau and Steinman, 1998)***. It has been clearly evidenced that, depending on the nature of the stimuli received, myeloid DCs can develop into different subsets that possess unique biological functions, determined by the combination of surface molecule expression and cytokine secretion ***(Rizzello et al., 2011)***.

The pathogenic role of DC dysfunction (and its role as a potential therapeutic target) is the focus of continuing research, with descriptions of DC involvement in pathologic changes which have been associated with Th1/Th2 dysregulation. This includes autoimmune disease (rheumatic, neurologic and endocrine diseases ***(Lee et al., 2010),*** characterized by a Th1 response ***(Berger, 2000)*** and allergy (asthma, atopic dermatitis, and drug hypersensitivity ***(Kaiko and Foster, 2011)*** with a skew towards Th2. Plasmacytoid DC being implicated in diseases with a Type I IFN-signature ***(Sozzani et al., 2010).*** The rebalancing of Th1- and Th2-type responses, through Th2 stimulation in autoimmunity and Th1 shift in allergy, could be harnessed therapeutically ***(Yang and Gao, 2011).***

The induction of tolerance has been reported in immature, and partially mature DC phenotypes (similar to steady-state migratory veiled DC which tolerize lymph node T-cells towards self), whereas only the fully mature stage of DC differentiation would be immunogenic and able to release proinflammatory cytokines ,with inflammatory stimuli converting a tolerogenic signal to a stimulatory one .Current knowledge suggests that the induction of regulatory T cells (T_{reg}), and not just the lack of inflammatory signals, contributes to the maintenance of tolerance by immature DCs ***(Rutella et al., 2006).***

Various authors have shown that DC maturity/immaturity can be manipulated, further optimizing the immunotherapeutic

potential of these cell populations.DC maturation can be stimulated *in vitro* with increasing concentrations of growth hormone, resulting in increased IL-12 secretion and increased lymphocyte activation ***(Liu et al., 2010)*** with a Th1 shift, while the early addition of tumour-necrosis factor alpha (TNF-α) to mononuclear cell cultures enhances cell survival and increases DC maturation markers (CD80, CD83, CD86, and HLA-DR), also heightening IL-12 secretion by mature DC ***(Ebrahimi et al., 2009).***

The identification of population subtypes with a preference for tolerance or for immune stimulation will offer targets for immunotherapy and cellular manipulation, to optimize transplant success rates, decreasing early and late transplant-associated death, as well as primary disease relapse, prolonging disease-free survival and overall survival ***(Pereira and Paiva, 2011).***

There seem to be several pathways to generate DCs. Blood monocytes give rise to DCs when cultured with the appropriate cytokines. DCs progenitors are also present in bone marrow. $CD34^+$ subsets of haematopoietic progenitors give rise to all blood cells and DCs ***(Kaouther and Ridha, 2011).***

AIM OF THE WORK

To establish a protocol for in vitro differentiation of human peripheral blood monocytes into dendritic cells upon treatment with specific growth factors.

DENDRITIC CELLS

Dendritic cells (DCs) were first described in the mid 1970s by Ralph Steinman, who observed in the spleen a subpopulation of cells with a striking dendritic shape. These cells were non phagocytic, loosely adherent, and of low buoyant density. It was soon appreciated that these bone marrow (BM)-derived cells existed in all lymphoid and most non lymphoid tissues ***(Banchereau et al., 2002).***

DCs are highly potent APCs that are distinct from other immune cells by being equipped with molecular machinery that enables them to very efficiently take up, process, and present antigens on major histocompatibility (MHC) class I and II molecules to T cells. In addition, they are equipped with a range of pathogen sensing molecules such as toll-like receptors (TLRs), nucleotide- binding oligomerization domain proteins (NOD), and C-type lectins that allow them to detect pathogen products and sense inflammation. Signaling through these receptors triggers migration of DCs from peripheral tissues to secondary lymphoid organs (SLOs) bringing DCs carrying antigens into close association with naïve T cells ***(Randolph et al., 2008).*** And thus they induce a cellular immune response involving both $CD4^+$ T helper cells (Th) and cytotoxic $CD8^+$ T cells (CTL). Moreover, DCs are also important in inducing humoral immunity as explained by their capacity to activate naïve and memory B cells. Besides, natural killer cells (NK)

and natural killer T cells (NKT) may also be activated by DCs ***(Schott, 2006).***

Cardinal features of DCs are (i) their ability to efficiently take up and present self and pathogen-derived antigens to other cells of the immune system such as T cells and B cells, and (ii) their capacity to migrate from peripheral tissues such as skin and mucosa to secondary lymphoid tissues where they can activate lymphocytes and initiate the immune response. This migratory behavior is pivotal and provides a critical cellular link between the external environment where pathogens might enter the body and the secondary lymphoid tissues where immune responses are initiated ***(Chopin et al., 2012).***

DC progenitors leave the BM and give rise to circulating precursors. They differentiate into immature DCs (iDCs) that are distributed throughout the peripheral tissues and mucosa and act as sentinels of the immune system. They are characterized by a high capacity for antigen uptake and processing. In response to inflammatory stimuli, iDCs rapidly undergo a complete genetic reprogramming. During the first twenty-four hours, DCs progress from immature to mature cells that are characterized by a high capacity for antigen presentation and T-cell priming. The mature DCs then migrate to the SLOs where they interact with T cells. This complex "maturation" process involves not only the upregulation of costimulatory surface proteins and the optimization of antigen

presentation capacities, but also the production of cytokines and chemokines that profoundly influence the outcome of the T-cell response. The signals in DC involved in each of these phenomena are not completely understood. The signals delivered by DC are believed to direct the T-cell response into either a Th1 or Th2 response. Also, they can drive the differentiation of regulatory T cells (Treg) involved in self-tolerance ***(Kaouther and Ridha, 2011).***

Dendritic Cell Development

DCs are continuously produced from BM hemopoietic stem cells (HSC) in response to growth and differentiation factors such as *fms*-like tyrosine kinase-3 ligand (Flt3L) and granulocyte-macrophage colony-stimulating factor (GM-CSF). These Long-term repopulating HSCs constantly self renew but can also give rise to short-term repopulating cells that have lost much of their self-renewal capacity. These precursors differentiate further into the multi-lineage progenitor (MPP). MPP can give rise to both the common-lymphoid progenitor (CLP) and the common myeloid progenitor (CMP). Both CLP and CMP can differentiate into several DC subsets ***(Manz et al., 2001).***

HSCs commit into multipotent progenitors that give rise to either a CLP or a CMP. A population that lies downstream of CMP has been found to differentiate either into DC or macrophages, and was therefore named the macrophage-

dendritic cell progenitor (MDP).Full commitment to the DC lineage is acquired at the common DC progenitor (CDP) stage whereas CDP can either differentiate into plasmacytoid DCs (pDCs) or into a pre-DC. The latter will further differentiate into mature conventional DCs (cDCs) in the peripheral tissues, or secondary lymphoid organs (**Fig.1**) ***(Chopin et al., 2012).***

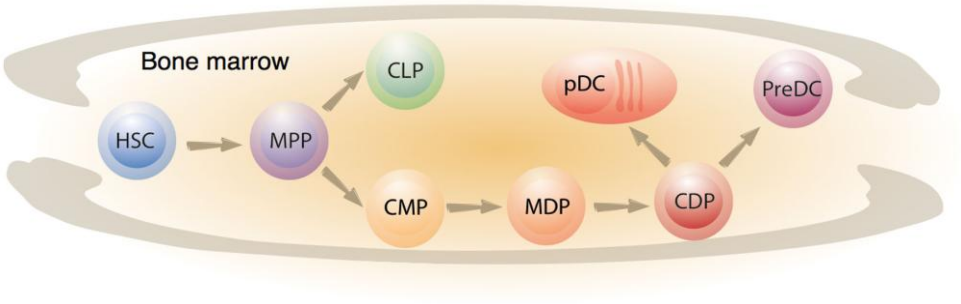

Figure (1): Ontogeny of DC precursors ***(Chopin et al., 2012).***

Dendritic cell subsets:

A signature of DCs is their heterogeneity. The DC network is composed of multiple subtypes of DCs that vary in their origin, anatomical localization, lifespan, and function which makes defining DC subsets a problem. Unraveling the developmental history of these subtypes has been complicated in part by the rarity of DCs in tissues (approximately 1% of cells) and their short life span (a few days). Simplistically, four major populations of DCs have been described namely pDCs, cDCs, Langerhans cells (LCs), and the monocyte-derived DCs (MoDCs) ***(Chopin et al., 2012).***

I- Plasmacytoid DCs:

On Giemsa staining under light microscopy human pDCs are round in shape and look like plasma cells. They are slightly smaller than CD 14^+ monocytes, but bigger than resting lymphocytes. Plasmacytoid DCs reside within lymph nodes, spleen, thymus, BM and in the cerebrospinal fluid. These cells were found to correspond to a subset of circulating blood DCs, an immature $CD11c^-$ population, referred to as pre-pDCs, and to be distinct from "conventional" myeloid $CD11c^+$ DCs (mDCs). Pre-pDCs express CD4 but lack T-cell receptor alpha (TCRα), TCRβ, TCRγ, TCRδ, or CD3 chains. Furthermore, they do not express B-cell lineage (CD19, CD21) or myeloid (CD13, CD14, CD33) markers. Pre-pDCs typically mature and produce large amounts of alpha/beta interferons (IFN-α/β) in response to viral and bacterial stimuli. This property further identified them as the enigmatic "natural type I IFN-producing cell" in blood ***(McKenna et al., 2005).***

Human pDCs were originally purified as $Lin^-CD4^+MHCII^+$. Besides the lack of common myeloid markers such as CD11b, CD11c, CD13 and CD33, a collection of surface molecules could be identified that set pDC apart from mDC subsets. Human pDC characteristically express high levels of the interleukin (IL)-3 receptor alpha chain (CD123). A further distinction is the expression of CD45RO by mDC, compared to CD45RA expressed by human pDC. Blood Dendritic Cell Antigen-2 (BDCA-2) is a member of

the C-type lectin family of transmembrane glycoproteins. The engagement of BDCA-2 may internalize antigen for the presentation to T-cells. The monoclonal antibody against BDCA-2 is highly specific for human pDC. However, its use for pDC enrichment is limited, as BDCA-2 engagement results in inhibition of the type I IFN production capability. Another pDC marker is BDCA-4/neuropilin-1, a receptor for members of the semaphorin family that also functions as co-receptor for vascular endothelial growth factor (VEGF) ***(Barchet et al., 2005).***

Flt-3L is the key differentiation factor for the development of pDC from hematopoietic stem cells in humans and mice. Its injection in vivo dramatically increases the numbers of both mDCs and pDCs in human blood ***(McKenna et al., 2005)***.

Plasmacytoid dendritic cells in the blood express L-selectin (CD62L), and similar to naïve lymphocytes are able to enter lymphatic tissues, including lymph nodes, spleen, thymus, and mucosal-associated lymphoid tissues, through the hematogenous route. In lymph nodes, they then reside in the in the T-cell zone in close association with the high endothelial venules (HEV). pDC are also recruited to sites of local inflammation, where they become activated. Accumulations of pDC in the skin are prevalent in psoriasis lesions, cutaneous systemic lupus erythematosus (SLE). Also they are found in mucosal tissues in allergic rhinitis ***(Barchet et al., 2005).***

The TLRs expressed by pDC are restricted to those that enable recognition of DNA and RNA viruses. Human pDC express only TLR7 and 9. TLR9 is engaged by unmethylated CpG -rich DNA that is common in bacteria, but also prevalent in the genomes of DNA viruses. Accordingly, pDC have been shown to recognize Herpes simplex virus (HSV-1, HSV-2) in a TLR9 dependent fashion. In contrast, TLR7 mediates recognition of ribonucleotide homologs, like loxoribine, synthetic single stranded (ss) RNA sequences and ssRNA viruses, such as Influenza virus. While TLRs with specificity for bacterial products are expressed on the cell surface, TLR7 and TLR9 are confined to an acidic endosomal compartment and signal exclusively through the adapter molecule MyD88. Thus, after endocytosis into pDC, viruses may reach lysosomes, where viral nucleic acids interact with TLR7-9, triggering a MyD88-dependent signaling pathway. Importantly, this pathway can be triggered also by non replicating and inactivated viruses, allowing pDC to initiate type I IFN responses even in the absence, or preceding the onset of viral replication ***(Iwasaki et al., 2004).***

When stimulated by viruses or TLR7/9 ligands, human and mouse pDC are capable of producing up to 10 pg/cell of type I IFN, making them 10–100-fold more efficient than other cell types including mDC. Transcription of IFN-α and IFN-β genes is primarily controlled by members of the interferon regulatory factor family (IRF), in particular IRF-3 and/or IRF-7 ***(Honda et al., 2005).***

Specifically, viral infection activates IRF-3, which is constitutively expressed, leading to an initial secretion of low levels of IFN-β and IFN-α. These interferon species initiate an autocrine loop by signaling through the IFN-α/β receptor, inducing expression of IRF-7. This transcription factor, upon activation, induces the expression of additional IFN-α genes, boosting IFN secretion. Most cell types, including classical DC, require upregulation of IRF-7 in response to type 1 IFN feedback signaling, in order to secrete IFN-α. In contrast, pDC are capable of secreting IFN-α even in the absence of positive feedback signaling. It was shown that this ability is due to constitutive expression of IRF-7 in pDC, and the ability of MyD88 to recruit IRF-7, but not IRF-3, through a molecular complex. Thus, engagement of TLRs in pDC triggers a signaling cascade that rapidly activates IRF-7 and transcription of IFN-α genes independent of IRF-3 activation. In contrast, engagement of TLR7 and TLR9 in classical DC triggers secretion of pro-inflammatory cytokines and chemokines, but not type I IFNs. Basic expression of IRF-7 in pDC but not in classical DC is commonly suggested to explain the difference in the capacity to produce type I IFN ***(Barchet et al., 2005).***

Type I IFNs are most widely studied for their antiviral properties. However, they are also potent regulators of the innate and adaptive immune response. Through secretion of type I IFNs, pDC enhance cytotoxicity of NK cells and $CD8^+$ T cells, and protect DC from the cytopathic effect of viruses, thus assisting their antigen presenting function. pDC also secrete

additional cytokines, IL-12 and IL-6, which cooperate with type I IFNs in regulating immune responses. Through secretion of IL-12 and type I IFNs, pDC induce IFN-γ secretion in NK cells, $CD8^+$ T cells, and $CD4^+$ T helper cells, promoting clearance of intracellular pathogens. Through secretion of IL-6 and type I IFNs, pDC promote differentiation of memory B cells into antibody secreting plasma cells, facilitating the production of anti-viral antibodies. pDC are also efficient producers of proinflammatory chemokines, particularly CCL3, which may enable them to recruit other leukocytes to inflamed sites. pDCs activated by pathogens, TLR ligands or after engagement of CD40L undergo maturation, which enhances their antigen presentation potential ***(Megjugorac et al., 2004).***

Activation induced upregulation of MHC class II and costimulatory molecules including CD80, CD86 and CD40 upon activaton, justifies the classification of pDC as members of the DC family of antigen presenting cells. However, pDC present antigens less efficiently, because they do not phagocytose, process and load antigens onto MHC molecules as effectively as mDC. In addition, even in fully matured pDC, levels of MHCII and costimulatory molecules remain significantly lower than in mDC ***(Barchet et al., 2005).***

II-Conventional DCs:

Conventional DCs, also called ‘classical’ or ‘myeloid’ DCs, were first identified by their ability to stimulate strong T cell responses**.** They express myeloid cell markers CD13 and CD33, and require exogenous GM-CSF for their survival. They

produce high levels of IL-12 when stimulated with tumor necrosis factor-α (TNF-α) or CD40 ligand and drive a potent Th1-polarized immune response ***(Mohamadzadeh et al., 2001).***

Conventional DCs can be divided into two main groups of cells. They are (i) the *migratory* DCs and (ii) the *tissue-resident* DCs. Migratory DCs reside in peripheral tissues such as the skin and mucosa where they efficiently sample environmental antigens and then migrate to the regional lymph node in afferent lymphatics to present antigens to T cells. They are composed of the dermal or interstitial DCs and can be divided into the $CD11b^+$ and CD11b− DCs. These DCs may also express the integrin αE, also known as CD103.CD103 is expressed on CD11b− DCs and can be found in a variety of other tissues. Despite a similarity in expression of surface molecules by these two DC subsets, the transcriptional machinery regulating these two populations is distinctly different **.**The second category of cDCs is composed of several subsets of DCs that are known as *tissue-resident DCs*. In contrast to their migratory counterparts, they do not circulate through peripheral tissues and thus can only process antigens found within the tissue in which they are localized. To overcome this potentially limited access to antigen, migratory DCs can transfer antigens to lymphoid resident DCs who via the process of *cross- presentation*, provide an alternate strategy for the amplification of $CD8^+$ T cell responses ***(Allan et al., 2006).***

Tissue-resident DCs are delineated by the expression of the surface molecules CD4 and CD8α and are found in secondary lymphoid organs such as the thymus, spleen, and lymph nodes. Three subsets have been defined which are (i) the $CD4^+$ DCs, (ii) the$CD8\alpha^+$ DCs, and (iii) the CD4−CD8α− (double negative, DN) DCs ***(Naik et al., 2006).***

Although there are a number of shared functions between these subsets, an interesting division has emerged: $CD8\alpha^+$ DCs are highly efficient indirect and cross-presentation of soluble, cell-associated, and pathogen-derived antigens to $CD8^+$ T cells. $CD4^+$ DCs and CD4−CD8α− DCs can also present MHC class I-restricted antigens in some settings ***(Chopin et al., 2012).***

CD8α^+ and CD103$^+$ DCs drivers of cross-presentation: The $CD8\alpha^+$ and $CD103^+$ DCs are cDCs that are of special interest due to their shared functional attributes in driving immune responses to pathogen infections and their capacity to cross-present antigens ***(Steinman, 2010).***

The CD8α^+ DC subset: $CD8\alpha^+$ DCs are distinct from other conventional murine DC subsets by being the key drivers of cross-presentation to a range of experimental pathogen antigens both *in vitro* and *in vivo*. $CD8\alpha^+$ DCs have been found to be critical for cross- presentation of self-antigens resulting in the induction of immune tolerance .This subset was also identified as the main subset involved in presenting pathogen-derived antigens. A number of mechanisms have been proposed