Chapter 2

Dendritic Cells: Location, Function, and Clinical Implications

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Abstract

Dendritic cells (DCs) are antigen-presenting cells derived from bone marrow precursors and form a widely distributed cellular system throughout the body. DCs exert immune-surveillance for exogenous and endogenous antigens and the later activation of naive T lymphocytes giving rise to various immunological responses. Different growth factors and cytokines can modulate the differentiation and function of DCs, GM-CSF, M-CSF, Flt3, and TGF-β, resulting in a large variety of DCs with different functional abilities. Thus, DCs are classified as plasmacytoid DCs (pDCs), conventional DCs (cDCs), and DCs derived from monocytes (mDCs). Functionally, the cDCs may be divided into two states: immature and mature. Immature DCs are specialist in uptaking and processing antigens; in contrast, mature DCs are professional in antigen presentation. It has been observed that immature cDCs can induce immune tolerance while mature cDCs may induce Th2 or Th1 immune responses. It is worth noting that different subpopulations of DCs have the ability to secrete different cytokine patterns, resulting in the induction of different immunological responses. Furthermore DCs are involved in the pathophysiology of several diseases such as contact hypersensitivity, autoimmune diseases, or cancer, but they can also be used as therapeutic tools in these conditions.

Keywords: dendritic cells, immunotherapy, cancer, autoimmune diseases

1. Introduction

Dendritic cells (DCs) are antigen-presenting cells, characterized by a distinctive morphology and expression of markers such as CD11c and major histocompatibility complex class II

molecules (MHCII). In addition, DCs can recognize pathogens, tissue damage signals, and tumor antigens and then migrate to the secondary lymphoid organs where they present antigens and activate T lymphocytes. DCs may induce the development of diverse immunological responses, either Th1, Th2, Treg, or Th17. There is a great variety of DCs with different phenotypes and localizations that form a cellular system distributed throughout the body and that is responsible for immune-surveillance. DCs are classified into conventional DCs (cDCs), plasmacytoids (pDCs), and DCs derived from monocytes (mDCs) [1, 2].

DCs are involved in many diseases, for example, contact hypersensitivity and autoimmune diseases, so intensive research is underway with the purpose of finding alternatives to induce the secretion of tolerogenic cytokines to decrease the activity of DCs in this type of pathologies [3, 4]. On the other hand, DCs have an important role in cancer. It has been observed that tumor cells may inhibit the maturation of DCs and induce the modification of their phenotype to provoke a Th17 or Treg response, which favors the proliferation of tumor cells. In addition, DCs are used as a promising alternative in cancer immunotherapy. To date, only one DC-based vaccine is available clinically; therefore, the study of immunomodulatory molecules that increase the maturation of DCs for their subsequent use in antitumor immunotherapy is underway [4, 5].

In the present chapter, the origin, phenotype of DCs precursors, and the description of the subpopulations located in the organism will be reviewed. Also, we analyze the different functional states of DCs and their relationship to the secreted cytokine pattern. Finally, we consider the role of DCs in some pathologies.

2. Dendritic cells biology

2.1. Origin and differentiation

2.1.1. Cytokines in DCs differentiation

DCs originate from hematopoietic precursor cells located in the bone marrow. Years ago, it was thought that DCs could have a lymphoid or a myeloid origin; then, after some experiments realized in 2007, it was found that DCs may originate from the common myeloid progenitor (CMP) and from the common lymphoid progenitor (CLP), giving rise to classical or conventional CDs, as well as plasmacytoid CDs [6]. It is important to note that key growth factors are needed for DC differentiations, such as Flt3L, granulocyte macrophage colony-stimulating factor (GM-CSF), and M-CSF [7] (**Figure 1**).

Among the different growth factors involved in DCs differentiation, the most important is Flt3-L, where the receptor is Flt3 (Fms-like tyrosine kinase 3), a receptor of a protein tyrosine kinase located especially in DCs precursor in bone marrow, so it has been proposed that Flt3-L is involved in the differentiation of cDCs and pDCs (**Figure 1**). It has also been suggested that Flt3-L/Flt3 regulates the maintenance and development of DCs in lymphoid and mucosal organs [8], as Flt3-L is sufficient for the differentiation of pDCs and cDCs from precursor

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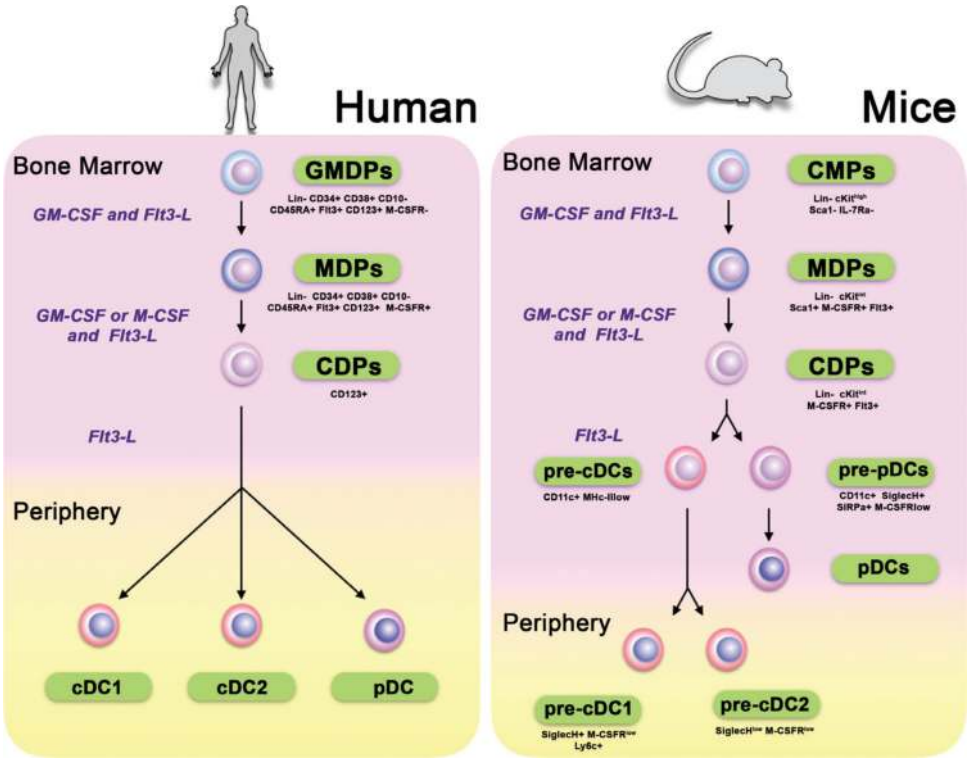

Figure 1. Differentiation of DCs in human and mouse. In human, three DCs precursors are recognized: GMDPs, MDPs, and CDPs. As the cells differentiate, they acquire different phenotype. It is accepted that under the influence of Flt3-L, cDC1, cDC2, and pDCs originate from CDPs. In mouse, it has been shown that there are several precursors: CMPs, MDPs, and CDPs. The latter differentiate into pre-cDCs and pre-pDCs. The pre-cDCs differentiate toward pre-cDC1 and pre-cDC2 giving rise to the cDC1 and cDC2, respectively. The pre-pDCs differentiate into pDCs. In the figure the phenotype of each cell and the cytokines involved in the differentiation process such as cell myeloid progenitors (CMPs), macrophages and dendritic cells progenitors (MDPs), common myeloid progenitors (CDPs), granulocytes, macrophages and DCs progenitors (GMDPs), pre-conventional dendritic cells (pre-cDCs), pre-plasmacytoid dendritic cells (pre-pDCs), pre-conventional dendritic cells 1 (pre-cDC1), pre-conventional dendritic cells 2 (pre-cDC2), conventional dendritic cells 1 (cDC1), conventional dendritic cells 2 (cDC2), plasmacytoid dendritic cells (pDCs) has been placed.

cells with various phenotypes *in vitro*, and when its expression is forced. In addition, Flt3-L deficient mice have shown only 10% of the pDCs and cDCs that are normally located in wild type, besides the administration of Flt3-L to those mice restored the levels of pDCs and cDCs, while the administration of Flt3-L to wild-type mice increased the levels of pDCs and cDCs in spleen [7, 9, 10].

Other important cytokine is granulocyte and macrophage colony-stimulating factor (**Figure 1**). It has been observed that it can induce the differentiation of DCs *in vitro* and *in vivo* (**Figure 2**); however, GM-CSF-deficient mice show normal levels of resident DCs of lymphoid organs, although there were some alterations in the levels of resident mucous or migratory DCs. So, it is believed that GM-CSF is not related to the differentiation of DCs in the steady state, but

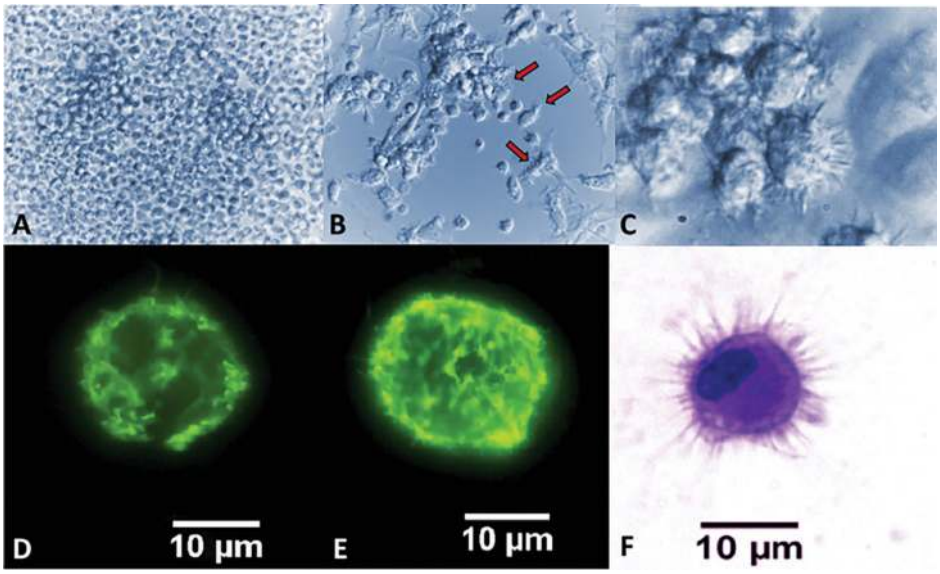

Figure 2. Differentiation of DCs from bone marrow precursors. The figure shows the differentiation of DCs from bone marrow precursor. (A) Culture of bone marrow cells at 24 h with GM-CSF. (B) and (C) The culture of bone marrow precursor cells after 6 days of culture with GM-CSF. The presence of poorly adherent cells with a great number of long and thin extensions is observed. (D) An immature DC MHCII positive is shown after 6 days of culture of bone marrow precursor cells with GM-CSF. (E) MHCII expression in DCs, after culturing for 2 h in the presence of LPS, a large increase in MHCII expression is observed. (F) A large number of extensions are observed in a DC stained with toluidine blue. The arrows point to DCs.

in the replenishment of DCs in non-lymphoid organs. In spite of the above, GM-CSF has been widely used in the differentiation of DCs *in vitro* that are subsequently used for therapeutic purposes [11, 12].

On the other hand, another cytokine involved in DCs differentiation is macrophage colony-stimulating factor (**Figure 1**). M-CSF is involved in DCs differentiation from monocytes and is very important for the differentiation of pDCs from certain MCSFR + cell populations in bone marrow. Nevertheless, knockout mice for M-CSF and its receptor did not show changes in DC levels in lymphoid organs, although there were decreased levels of monocytes and Langerhans cells (LCs) [13]. After all, now Flt3-L is considered as the most important cytokine in DCs development; nonetheless, M-CSF and GM-CSF are also relevant cytokines in DCs development and activation in non-lymphoid tissues [4, 13].

2.1.2. DCs precursors

The differentiation of DCs is carried out from various cell progenitor populations located in bone marrow (**Figure 1**). Multiple experiments have been conducted in order to characterize the different populations that can give rise to DCs, macrophages, or lymphocytes. These experiments include the isolation of cell populations by flow cytometry and the later treatment with Flt3-L, M-CSF, and GM-CSF or cultivated with stromal cells producing these cytokines

[8]. As a result, the first cell population with potential to differentiate into DCs, macrophages, monocytes, and polymorphonuclear cells was described and named cell myeloid progenitor (CMP), characterized by localizing in bone marrow and showing a Lin− c-Kit-high Sca1− IL-7Ralpha− phenotype. The CMP differentiation potential was evidenced when polymorphonuclear cells, macrophages, and DCs were obtained after CMPs were cultured with GM-CSF and Flt-3L-producing stromal cells [14]. Later, CMPs lose their potential to differentiate into granulocytes when initiated with the expression of M-CSFR. These progenitor cells are called macrophages and dendritic cell progenitors (MDPs), characterized by the phenotype Lin− Sca1− M-CSFR+ Flt3+ c Kit int CX3CR1+. The capacity of differentiation of the MDPs was evaluated by *in vitro* assays, where macrophages and DCs were obtained when MDPs were cultivated in the presence of M-CSF or GM-CSF. *In vivo* assays in irradiated mice showed that the inoculation of MDPs was directly involved in the differentiation of DCs and macrophages especially in lymphoid organs [13, 14].

Then, MDPs begin to decrease the c-Kit expression, which is indicative of differentiation into common dendritic cell progenitors (CDPs), characterized by the expression of the phenotype Lin− $cKit^{int}$, Flt3+ M-CSFR+. CDPs have the ability to differentiate into cDCs and pDCs with different phenotypes. So, when CDPs are cultivated in the presence of Flt3-L, a cell population CD11c+ MHCII+ is obtained; whereas, when CDPs are treated with GM-CSF a different cell population is obtained, since it shows the phenotype CD11c+ CD11b+ MHCII+. Also, when CDPs are treated with GM-CSF and Flt3-L cDCs and pDCs are acquired. *In vivo*, the potential of CDPs was evident when their inoculation in irradiated mice showed that CDPs were directly involved in the differentiation of CD11c+ CD8+ and CD11c+ CD8− cDCs and CD11c+B220+ pDCs. In conclusion, CDPs have the potential to differentiate just to pDCs and cDCs [13, 15].

CDPs have the potential to differentiate to Pre-pDCs and Pre-cDCs (pre-cDCs1 and pre-cDCs2). Pre-pDCs are characterized by the low expression of M-CSFR low [1], while it has been reported that Pre-cDCs is characterized by the expression of CD11c, Siglec-H, SIRPa low, and MHCII int. Pre-cDCs can be differentiated into Pre-cDC1s and Pre-cDC2s cells. The Pre-DC1s cells are known by decreasing the expression of M-CSFR, Siglec-H, and Ly6c. Ly6c is a monocyte marker. In the case of Pre-cDC2s, they maintain the expression of M-CSFR and Ly6c, but decrease the expression of Kit and Siglec-H. It is important to mention that Pre-DC1s, Pre-DC2s, and pDCs are located in the periphery, such as blood or lymphoid organs, whereas Pre-cDCs, Pre-pDCs, DCP, MDP, and CMP cells are located in the bone marrow [10, 16].

On the other hand, the DCs differentiation in human is a little different from its counterpart in mice (**Figure 1**). The cell with potential to differentiate into granulocyte, macrophage, and DCs is named granulocytes, macrophages, and DCs progenitor (GMDPs). This population is located in the bone marrow and may have the following phenotype: Lin− CD34+ CD38+ CD10− CD45RA+ Flt3+ CD123+ M-CSFR-. When these cells initiate the expression of M-CSFR, the phenotype changes and they are called macrophages and DCs progenitor, population with the capacity of differentiation to macrophages and DCs. Consequently, MDPs increase the expression of CD123, so they acquire the ability to differentiate just to DCs (pDCs and cDCs), so this cell population is called common DC progenitors (CDPs) [10] (**Figure 1**).

2.2. Dendritic cells subsets

2.2.1. Location

There is a great variety of DCs with different phenotypes and location. In general, DCs have been divided into conventional, plasmacytoid, and monocyte-derived [1]. The cDCs can be divided into cDC1s and cDC2s. The cDC1s are characterized by being CD8+ CD103+ in mouse and BDCA3+ (CD141+) in human. CDC2s phenotype is CD11b+ CD4+ CD8− in mouse and are BDCA1+ (CD1c+) in human. The plasmacytoid pDCs are positive for B220, mPDCA1 and Siglec-h in mice, while in humans they are BDCA4+ and BDCA2+ [17]. Another group of DCs derived from monocytes (mDCs) appears only when there is an inflammation. Langerhans cells, normal residents of the epidermis and epithelia, are not considered on the same lineage of the DCs mentioned above, since they originate from precursor cells that migrated to the skin before birth and differentiated into LCs during the first week of life [18].

In relation to the origin of the DCs, they all differ from bone marrow progenitor cells that have, as their common denominator, the expression of Flt3 and sometimes M-CSFR [19]. DCs are more numerous in lymphoid organs and epithelia. In addition, DCs can express various molecular markers depending on their location. Thus, pDCs, CD1s, and CD2s can be observed in different tissues of the organism [20]. **Figure 3** shows to which cluster of cDCs each cell belongs. It is necessary to consider the phenotype and particular location of DCs in relation to their function on that tissue. For example, the degree of maturation of DCs in lymphoid organs is different from that of DCs in other tissues, since DCs are sentinel cells responsible for the recognition of pathogens and signals of tissue damage, which induces their migration to lymphoid organs to carry out the activation of different subsets of T, natural killer (NK), NKT, and B lymphocytes. It has also been studied and analyzed for a long time that the inflammatory or tolerogenic microenvironment induced by the cytokines present in tissues is essential in the determination of the functions that DCs can have [17].

It is important to know the types of DCs located in the organism, as well as the cytokines involved in its activation, so the following explains the different types of DCs located in lymphoid organs, skin, gut, and blood (**Figure 4**).

2.2.1.1. DCs in lymphoid organs

2.2.1.1.1. Lymph nodes

In the lymph nodes, there are several subsets of DCs, one of them are the CD103+ migratory cDCs from peripheral tissues and generally exhibit a mature phenotype characterized by an increase in MHCII, CD80, CD86, and CD40. There are also two classes of resident DCs: CD8+, CD4+, or CD11b+, which possess an immature phenotype, unless there is an inflammatory environment in the lymph node. Also, the presence of CD141+ and CD1a+ DCs, reminiscent of the population of cells with the same marker in dermis, has been observed; therefore, these cells are considered as migratory [1, 20].

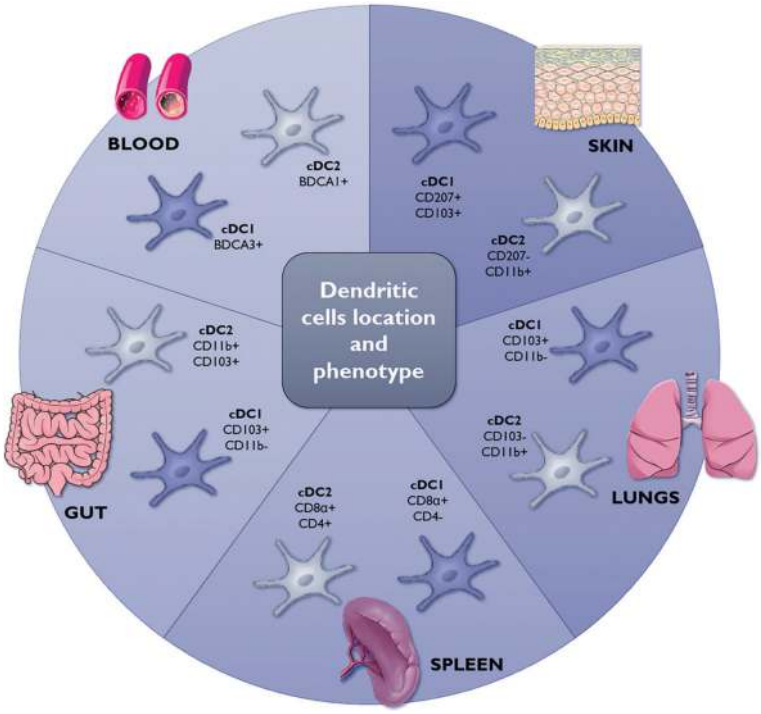

Figure 3. Dendritic cells location and phenotype. The cDCs (cDC1 and cDC2) may be located in lymphoid organs, blood, and mucous membranes. In lymphoid organs, the cDC1 (CD8+ CD4−) and cDC2 (CD8+ CD4+) are located. In mucoses such as respiratory tract and digestive tract, cDC1 (CD103+ CD11b−) and cDC2 (CD103+ CD11b+ or CD103− CD11b+) are also found. In blood, the cDC1 are BDCA3+ and the cDC2 express BDCA1+. In skin, the cDC1s are characterized by the expression of CD207+ CD103+, and the cDC2 for the expression of CD207− CD11b+.

2.2.1.1.2. Spleen

In the spleen, all DCs are CD8+ and are approximately 20% of the total spleen cells. DCs are classified into subsets according to CD11b marker expression. A subset is $CD11b^{low/-}$ DCs and shows an immature phenotype ($MHCII^{low}$ $CD80^{low/-}$ $CD86^{low/-}$ $CD40^{low/-}$), proliferates in the presence of Flt3L, and expresses molecules such as CD205, CD207, and Clec9a. CD11+ DCs subsets are divided into DCs expressing high or low levels of the endothelial cell-specific adhesion molecule (ESAM). These two classes of DCs also proliferate in the presence of Flt3L and are CD4+ (ESAM) [21].

2.2.1.1.3. Thymus

In the thymus, there are at least three subsets of DCs: CD8+ cDCs (50%), Sirpα+ cDCs (20%), and pDCs (30%) [20]. CD8+ cDcS is likely to be derived from specific precursor cells. In this regard, studies have been investigated using the reporter of IL-7 receptor,

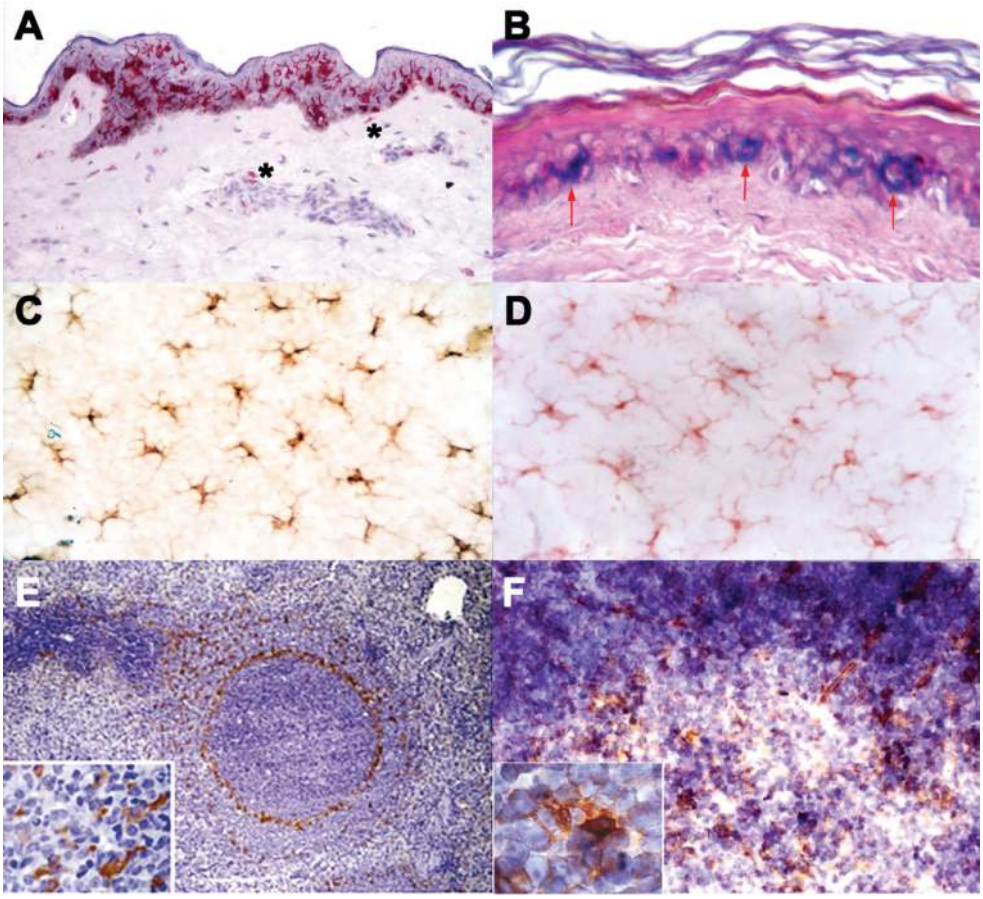

Figure 4. DCs at different locations. (A) and (B) human skin sections showing epidermal LCs positive for Langerin and CD1a, respectively. Arrows in (B) point to basal DCs. In (A), a few Langerin-positive cells (asterisks) are observed in the dermis. (C) and (D) Epidermal sheets of mouse skin with LCs positive for MHCII and CD205, respectively. In (E), a histological section of spleen showing Fascin-positive DCs is depicted. DCs are located in the T-dependent zone of the white pulp. In the thymus (F), a large amount of CD205-positive DCs is observed at the corticomedullary border.

which is a characteristic marker of lymphoid lineage, and of CD207, which is characteristic of CD8+ cDCs, and it has been found that only CD207 was expressed in thymic cDCs [22]. Using a new strategy named retroviral barcoding, it was determined that cDCs have a great similarity to spleen DCs and progenitors of bone marrow DCs [1]. Unlike CD8+ DCs, Sirpα+ DCs and pDCs develop extrathymically and are home to the thymus at steady state. Thymic homing of Sirpα+ DCs is dependent on a CCR2-mediated chemotaxis while pDC homing is dependent on CCR9 [23]. Both DC subsets are home to the thymus through blood vessels, but the specific tissues that originated from have not been comprehensively determined.

2.2.1.2. Blood

Multiple cell lines can be localized in the blood, such as granulocytes, monocytes, and lymphocytes, and to study blood DCs, several lineage markers (Lin), such as CD3, CD19, CD14, CD20, and CD56, are used to separate populations of DCs by means of flow cytometry assays [24]. Thus, populations of cDCs and pDCs can be identified in blood as they are Lin−. pDCs are characterized by expressing MHCII, BDCA2, and BDCA4, while the cDCs express MHCII and CD11c. Both types of DCs are negative for Lin markers. The cDCs divide into two subtypes, BDCA1 (CD1c) or CD141 (BDCA3) cells [25, 26].

2.2.1.3. Skin

In epidermis and dermis, different types of DCs can be found. In the epidermis, LCs constitute 2–4% and are characterized by expressing high concentrations of Langerin (CD207), CD45, and low concentrations of CD11c and MHCII. In humans, the expression of CD1a has also been observed, but not in mice. Unlike the other DCs, the differentiation of LCs is independent of Flt3; however, they are dependent for their development of Csf-1R, which also induces macrophage differentiation, M-CSF, in addition to chemokines CCL2 and CCL20 [18]. Langerhans cells were considered to be bone marrow dependent; however, it has been observed that LCs may have two distinct embryonic origins: the fetal liver and the yolk sac. In the mouse dermis, two populations of DCs, the CD103+ and CD8α+, have been observed, whose origin is based on the precursor of DCs positive for CLEC9A. It has been observed that when these cells are CD24^{low}, CD11b^{low}, and Sirpα+, they are involved in the development of Th2 and Th17 responses [27]. In humans, LCs have a phenotype very similar to that of mice, and they may respond to IL-15. On the other hand, in the dermis are several subsets of DCs: CD14+ CD1a− DCs, CD14− CD1a+ DCs, and 6-sulpho LacNAc+ DCs [28]. The CD14+ DCs are poor activators of CD8+ T cells in contrast to CD14− DCs. The subset CD141+ DCs is extremely successful in the activation of CD4+ T lymphocytes. The population of pDCs located in the dermis is very low; however, in inflammatory skin diseases such as psoriasis or lupus erythematosus, an increase of this type of skin cells has been observed [29].

2.2.1.4. Gut

DCs in the intestine are located in the lamina propria of the intestinal mucosa, especially in the Peyer's patches of the small intestine. These cells are usually CD103+, CD8+, and CD207+, express low concentrations of MHCII, and have been observed to proliferate when there are high concentrations of Flt3. There is a second type of DCs that are also located in the lamina propria, but which express the markers CD103 and CD11b, although CD103 is expressed in low levels. These types of DCs can also be localized in the muscular layer of the digestive tract, so they may be confused with CD11b+ macrophages [30].

2.2.2. Cytokine production

As previously mentioned, the DC differentiation is a process occurring in bone marrow. It depends on key cytokines (Flt3-L, GM-CSF, and M-CSF) and different cell precursors such

as the common myeloid progenitor and the common lymphoid progenitor resulting in the obtaining of classical (cDCs) and plasmacytoid DCs (pDCs [17, 18, 31, 32]. As a result of this process, different DC populations not only acquire different phenotypes but also colonize different tissues and perform different functions [31]. However, when precursors of dendritic cells are differentiated in the presence of different factors such as GM-CSF and tumor necrosis factor-α (TNF-α), at least two CDs populations give rise, one characterized by the expression of CD1a+/E-cadherin+ and another characterized by the expression of CD14+/CD68+. Both populations shared some characteristics, such as the secretion of some cytokines (IL-1, IL-1, IL-6, IL-7, IL-12, IL-15, IL-18, TNF-, TGF-, M-CSF, and GM-CSF). However, when these cells are treated with CD40 ligands, DCs acquire the ability to produce IL-10 and IL-13 [31].

The progression in the maturation/activation state of the different CDs populations involves not only changes in the expression of the receptors present in the cell membrane but also their ability to interact with the extra cellular environment and with other cells. In this sense, both immature CDs in lymphoid and mucosal tissue and differentiated CDs *in vitro* are characterized by (1) having a weak antigenic presentation capacity, since DCs express low levels of MHC-II molecules, and co-stimulatory molecules (CD40, CD80, and CD86), where expression increases when DCs are exposed to maturation stimulus such as CD40L or IFN-γ; (2) having a high expression of tissue damage receptors such as CD36 and Toll-like receptors [26]; (3) having a high expression of molecules involved in the capture of antigens of different chemical origin, such as FcγRI, CD1, CD205, CD207, CD209, and CLEC-9 [33–37], receptors involved in antigenic internalization and in the release of intracellular signals, promoting greater expression of adhesion molecules [38, 39]; (4) changes in the production of cytokines after undergoing the maturation/activation process. Mature CDs produce differentially cytokines related to promote the different immune responses (Th1, Th2, Th17, and Treg).

In this section, we describe the patterns of cytokine production, produced by the different subsets of DCs.

2.2.2.1. Conventional DCs

Human cDCs differentiated from bone marrow precursors in the presence of GM-CSF and IL-4 differentially show not only the expression of mRNA but also the production and release of different cytokines depending on their maturation stage.

Immature DCs are characterized by having a phenotype CD11c+, CD86−, MHCI^{low}, MHCII^{low}, CD40−, CD80^{low}, CD54^{low}, OX40−, and CD8a−, whereas mature DCs are characterized by a phenotype CD11c+, CD83+, CD86+, MHCI^{low}, MHCII^{high}, CD40+, CD80+, CD54+, OX40+, and CD8a−. Both immature and mature DCs have different cytokine pattern secretion (**Table 1**) [17, 32].

It has been observed that the differential expression of cytokines is regulated not only according to the type of DCs but also by the activation pathway. lipopolysaccharide (LPS)-stimulated DCs have been shown to exhibit a positive regulation of IL-1α, IL-1β, and IL-6 and to a lesser degree of IL-15, TNF-α, and MIF. On the other hand, when DCs are stimulated with

Cytokines profile produced by cDCs					
Cytokine	**Immature DCS**	**Mature DCs**	**Activation path of mature DCs**		
			LPS	**Anti CD40**	**TNFα**
IL1a	High	Low	High	Low	Low
IL-1β	High	Low	High	Low	Low
IL-2	Negative	Negative	Negative	–	Médium
IL-4	Low	Medium	Medium	–	High
IL-6	Low	Medium	Low	Medium	Low
IL_d10	Low	Medium	Medium	Medium	Medium
IL-12	Low	High	High	High	High
IL-12 p35	Negative	High	High	Medium	Medium
IL-12 p40	Low	High	Medium	High	–
IL-12p70	Low	High	High	High	High
IL-13	Low	High	Low	Low	High
IL-15	Low	High	Medium	High	–
IL-18	Low	Medium	Low	Medium	–
IL-23	Low	High	High	Negative	–
MIF	High	High	High	High	–
IFN-γ	Low	Medium	Low	–	High
TGF-β whole	Low	Low	Negative	–	Low
TGF-β1	Low	Low	–	–	Medium
TGF-β2	Low	Low	–	–	–
TGF-β3	Low	Low	–	–	–
TNF-α	Low	High	High	Medium	Medium

Table 1. Cytokines secreted by immature DCs, mature and matured by different pathways.

anti-CD40 antibodies only discrete changes are observed in the levels of some cytokines, such as IL-6, IL-12p40, IL-15, and TNF-α, whereas IL-1α, IL-1β, IL-18, IFN-γ, TGFβ1-3, and MIF are not altered. This fact acquires relevance since those DCs that are stimulated via anti-CD40 may exhibit a phenotype of cDC1s or cDC2s being able to guide the immunological response to both TH1 and Th2. By contrast, DCs that are stimulated with LPS show a phenotype of cDC1s. This fact is due to a differential signal on the production of IL-23 which strongly interacts with IL-12 [24, 40].

This same pattern of differential activation has been observed in mature murine and canine DCs, stimulated with different types of signals, either with endogenous or with exogenous stimuli, where regardless of the type of stimulus, mature DCs show a phenotype with an

increased expression of CD11c, IL-10β, IL-12p40, IL-12β, IL-12β, IL-12β, IL-12β, IL-12β, IL-13, and TNF-α, thus contributing to awakening a Th1-type response. In contrast, TNF-α-stimulated DCs show increased expression of IL-2, IL-4, IL-12p40, IL-13, TNF-α, TGF-β, IFN-γ, and MCP-2 promoting a Th2 response. This differential production of cytokines appears to be due to the involvement of IL-13 that acts similarly to IL-4, thus promoting this response Th2 [41, 42] (**Table 1**).

2.2.2.2. Tolerogenic DCs

Characteristically, some subsets of DCs of myeloid origin that modulate the antigen-specific adaptive immune response by presenting auto-peptides to CD4+ T cells in the presence of inhibitory signals, anti-inflammatory cytokines, or other molecules that promote regulatory T cell populations are also capable of inducing the deletion or clonal anergy of autoreactive T cells.

Some mechanisms of the generation of tolerogenic DCs involve stimulation with IL-10, TGF-β, IL-6, TNF-α or its combination [43–46], as well as weak stimulation with bacterial products like LPS [47], pharmaceutical drugs like dexamethasone [48], and inhibitors of cell signaling such as PKCi or CTLA4 [49, 50]. These cells acquire a tolerogenic activity characterized by an immature phenotype with weak expression of co-stimulatory molecules, but with a differential production of anti- and proinflammatory cytokines.

This production of cytokines by tolerogenic DCs is dependent on the microenvironment in which they are found. Thus, the presence of IL-10 promotes a decrease in IL-6, IL-12, and IL-23, as well as an increase in the release of TGF-β, PGE_2, and IL-10, leading to an increase in Treg cell populations. In contrast, in the presence of high concentrations of TGF-β, tolerogenic DCs show a high expression of the co-inhibitory molecules ILT4, PDL-1, and PDL-2 [51, 52]. The synergism of IL-10 and TGF-β promotes similar cytokine production in DCs, but DCs also show a high CCR7-dependent migratory capacity with low antigenic activity.

When there is an early exposure to IFN-γ, DCs are guided to a tolerogenic phenotype with a reduced endocytic capacity as well as weak expression of IL-12, IL-23, and TNF-α, an effect that is maintained even after receiving a second proinflammatory stimulation [53] (**Table 2**).

2.2.2.3. Plasmacytoid cells

Since their description, pDCs have produced a great controversy about their origin and function. Unlike other DCs, pDCs generation is controlled by the expression of the transcription factor E2-2 [54]. pDCs express CCR9, CD9, CD19, CD123, CD303, and CD304 molecules. They also express the BDCA2 receptor, and the histidine transporter Slc15a4, which facilitate the signaling of TLRs and the production of IFN and other cytokines [55]. In mice, the pDCs express PDCA1 and Siglec-H [56]. In their immature or inactive state, they have a similar appearance to plasmatic cells, lacking dendritic cytoplasmic projections, do not show an ability for uptake and present antigens and produce large amounts of IFN types I and III. However, when these cells are activated, they rapidly undergo a morphological and functional conversion similar to that of cDCs with a capacity to stimulate T cells [2].

Phenotype and cytokine production by tolerogenic DCs			
Mechanism	**Phenotype**	**Cytokine**	**Tolerance mechanism**
IL-10	Low expression of costimulatory molecules CD11c+, MHCII^{low}, CD80^{low}, and CD86^{low} Weak migratory capacity CCR7^{low}	Low secretion of IL-6, IL-12, and IL-23 High secretion of IL-10, PGE2, and TGF-β	Anergy of CD4+ T cells Induction of Treg cells
TGF-β	Low expression of costimulatory molecules CD11c+ MHCII^{low}, CD80^{low}, and CD86^{low} High expression of inhibitory molecules ILT4^{high} and PD-LI/2^{high}	Low secretion of ll-β, IL-6, and IL-12 e IL-23 High secretion of IL-10	Anergy of CD4+ T cells Induction of Treg cells Inhibition of the secretion of INFγ by CD4+ T cells
IL10 + TGF-β	Low expression of costimulatory molecules CD11c+ MHCHII^{low}, CD80^{low}, and CD86^{low} High migratory capacity CCR7^{high} Weak antigenic presentation activity	Low secretion of IL-6, IL-12, IL-18, and IL-23 High expression of IL-4, IL-5, PGE2, and TGF-β	Anergy of CD4+ T cells Promotion of stimulated T-cells-producing IL2^{low}, IFN-γ^{low} IL-10^{high}
Dex	Low expression of costimulatory molecules CD11c+, MHCII^{low}, CD80^{low}, and CD86^{low} High migratory capacity CCR7^{high} and CXCR4^{high}	Low secretion of IL-12, IL-23, and TNF-α High secretion of IL-10	Anergy of CD4+T cells Induction of Tr1 cells-producing IL-10
IFN-γ early exposition	Low expression of costimulatory molecules CDIIc+ MHCII^{low}, CD80^{low}, and CD86^{low} Weak endocitic capacity	Low secretion of IL-12	Induction of Treg cells CD127$^{-/low}$, CD25^{high}, and FoxP3+ Induction of Treg cells IL10+, TGF-β+, and FoxP3+

Table 2. Tolerogenic DCs activation pathways with their cytokine secretion profile and phenotype.

They are known to share with cDCs not only the same progenitor but also the dependence of some factors for their differentiation as the cytokine FMS-related tyrosine kinase 3 ligand (Flt3L) and interferon-regulating factor 8 (IRF8) [56]. Like other DCs, pDCs are deficient of lymphocytic lineage markers. When pDCs are activated by ligands of TLR7 and TLR9, they produce high amounts of IFNs types I and III, other cytokines such as IL-6 and IL-12 as well as chemokine ligands CCL3, CCL4, CCL5, CXCL9, CXCL10, and CXCL11 [57, 58]. The regulatory capacity of pDCs involves the expression of the indoleamine 2,3-dioxygenase (IDO) enzyme [52], a suboptimal presentation of antigens, and the induction of Treg cells [57, 58].

2.3. Processing and antigen presentation

DCs are capable of capturing and processing antigens very efficiently and for this they possess several molecules that have been identified and are discussed below. In general,

two antigen-processing pathways have been defined, the endocytic pathway and the cytosolic pathway, through which exogenous and endogenous antigens are presented, respectively [59].

2.3.1. Exogenous antigens: endocytic way

Exogenous antigens, such as those derived from bacteria, are captured by immature DCs by means of endocytosis, phagocytosis, or both, through different molecules such as Fc receptors of IgG or lectins such as CD205, which guides their internalization into endocytic compartments with increasing acidity: slightly acidic early endosomes, moderately acidic endolysosomes, and very acidic late endolysosomes [60]. The late endolysosomes are very rich in MHCII and it is in these compartments where the antigens are degraded in polypeptides of 13–18 residues by some acidic proteases such as thiol and aspartyl cathepsins specific for the substrate [61, 62]. In these compartments, cathepsin degrades the invariant chain (li) bound to MHCII to a 24 amino acid peptide called the class II invariant chain peptide (CLIP), which occupies the cleavage of chains α1 and β1 of MHCII. This degradation is regulated by the concentrations of cathepsin S and its inhibitor endogenous cystatin C. After the DCs have matured, cystatin C decreases and cathepsin S activity is increased, promoting the degradation of Ii toward CLIP [62, 63]. The cleavage of MHCII where CLIP is placed is the site that can occupy the processed antigens and accommodates peptides up to 30 amino acids in size. As the CLIP is occupying this cleft, it needs to be removed so that it can be occupied by degraded exogenous antigens [61]. This process is induced by human leukocyte antigen (HLA)-DM molecules, which structurally resemble MHCII and are not expressed on the cell surface of DCs, but act as a peptide exchanger, facilitating the capture of CLIP, leaving the cleft of MHC II free to be occupied by the degraded antigenic peptide, resulting in the stabilization of MHCII [64]. Finally, the MHCII/processed peptide complex is carried through transport vesicles to the plasma membrane where the peptide can be recognized by CD4+ T lymphocytes specific for the peptide antigen presented [65]. The overall process is shown in **Figure 5**.

2.3.2. Endogenous antigens: cytoplasmic way

Presentation of endogenous antigens by MHCI molecules is performed on all nucleated cells and involves the degradation of cytosolic proteins and the loading of the resulting peptides into newly synthesized MHCI within the rough endoplasmic reticulum [66]. It is worth noting that this antigen-processing route also processes viral proteins from infected cells and proteins from bacteria that were initially phagocytosed and processed into endosomes but escaped from them into the cytoplasm. All these proteins, located in the cytoplasm, are susceptible to be degraded by proteasomes [67]. The processing of cytoplasmic proteins begins when they are conjugated with several copies of ubiquitin which is recognized by proteasomes. The proteasomes are formed by four protein cylinders, two peripheral α rings, and two β central rings. The β rings have catalytic activity located in the β1, β2, and β3 subunits. Proteasomes have a broad specificity of protein substrates and can generate a large variety of peptides capable of being presented by MHC class I molecules [66, 67]. DCs are highly efficient in the processing and presentation of cytosolic proteins because they possess constitutively di-ubiquitin, which has a tandem of key functional protein motifs, and proteasomes with a substrate specificity that allows peptides resulting from degradation of proteins have a large amount of hydrophobic

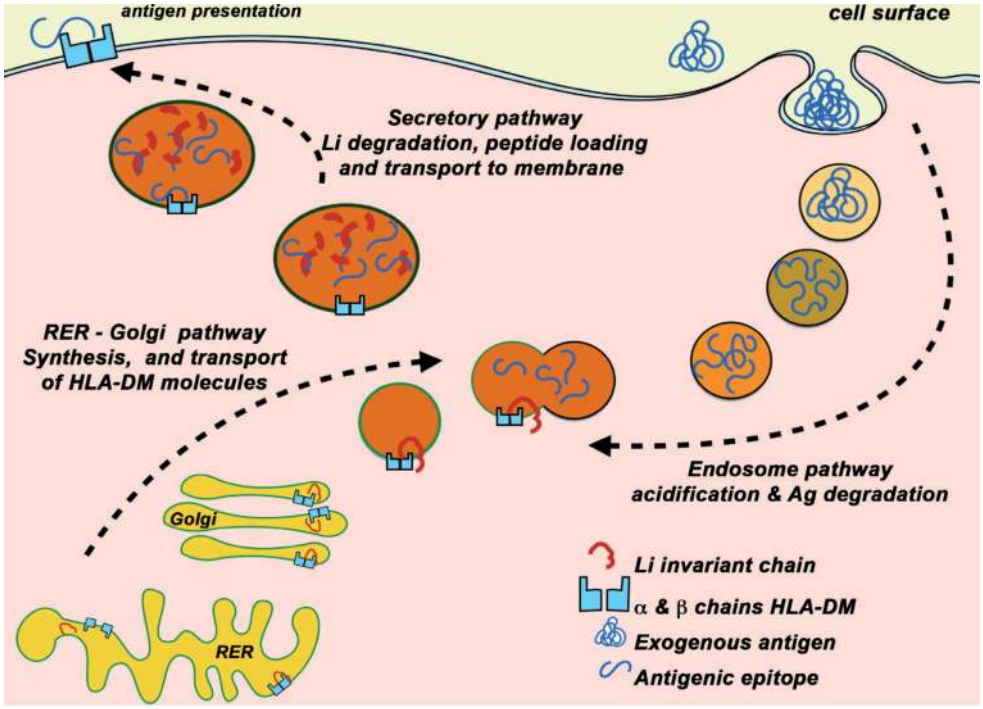

Figure 5. Exogenous antigens processing: endocytic way. Exogenous antigens are captured by immature DCs by endocytosis, phagocytosis, or both. Antigens are internalized into endocytic compartments where cathepsin degrades the invariant chain (li) bound to MHCII to a class II-invariant chain peptide (CLIP), which occupies the cleavage of MHCII. Then, CLIP is removed by HLA-DM molecules and the processed antigens occupy the space. Finally, the MHCII/processed peptide complex is carried through transport vesicles to the plasma membrane where the peptide can be recognized by CD4+ T lymphocytes.

or basic amino acids, which gives them a high affinity for MHC class I molecules [59, 68]. In particular, INF-γ and TNF-α, two proinflammatory cytokines, can induce a rapid increase of proteasomes in DCs, along with an increased expression of MHCI [69].

Peptides resulting from protein degradation are transported by an ATP-dependent process into the endoplasmic reticulum by a specific antigen-processing (TAP) transporter, which consists of two transmembrane proteins called TAP1 and TAP2 [67]. The transported peptides may have different lengths, but the transport is optimum for peptides of 8–16 amino acids [61]. Note that MHCIs are associated with TAP by means of a small protein called Tapasin, which retains MHCI in the endoplasmic reticulum until the peptide has conjugated to them. Inside the reticulum, the transported peptide is cut to a length of nine amino acids by a specific aminopeptidase in order to fit into the cleavage of MHCI [66]. When this happens, Tapasin releases the class I molecules coupled to the peptide antigen that can then be transported to the Golgi apparatus and from there to vesicles of transport to the plasma membrane of the cells where the antigenic peptides can be presented specifically to CD8+ T lymphocytes [59].

2.3.3. Cross-priming

DCs have the ability to capture and process proteins from virally infected cells or tumor cells and that, when processed through the endocytic pathway, can be presented not only to CD4+ T lymphocytes but also to CD8+ T virgin lymphocytes [60]. This type of presentation is called cross-presentation or cross-priming. This process is determined by the nature of the antigens, the way they have been captured, and the subsets of DCs performing the presentation [65]. Thus, two approaches have been proposed to carry out this type of antigenic presentation. The cytosolic pathway involves the transport of antigens from the lumen of endosomes to the cytosol where they can be processed by proteasomes until they are coupled to MHCI in the endoplasmic reticulum [69]. The vacuolar pathway involves the coupling in endosomes of class I molecules to antigenic peptides derived from the degradation of endocytic compartments by lysosomal proteases [70].

3. Dendritic cells: clinical implications

3.1. Delayed contact hypersensitivity

The pathophysiology of contact hypersensitivity consists of two phases: the induction phase and the challenge phase. The induction phase begins when the haptens penetrate the stratum corneum of the epidermis and are endocytosed by the LCs. LCs are then activated and migrated through the lymphatic vessels to the paracortex of regional lymph nodes, where they present the haptens in the context of MHCII to CD4+ T lymphocytes [71]. These activated T lymphocytes specific for these haptens are expanded clonally and finally reach the circulatory torrent. During this process, they express the cutaneous leukocyte antigen (CLA), with which they have the possibility to return preferentially to the skin through the high endothelium postcapillary veins [72]. The lymphocytes generated in this process are antigen-specific memory lymphocytes [73]. This phase lasts in the human between 10 and 15 days and has no clinical repercussions.

The challenge phase begins when haptens, which have already stimulated the induction phase, are brought into second contact with the skin. These haptens are endocytosed and presented by LCs, keratinocytes, or dermal DCs [74], which can present them to antigen-specific memory T lymphocytes located on the skin. Memory T lymphocytes activate CD8+ cytotoxic T lymphocytes which are the main effector cells in contact hypersensitivity. These lymphocytes secrete inflammatory cytokines and chemokines and induce apoptosis in keratinocytes [75]. Then, intense chemotaxis of different leukocytes is produced toward the skin resulting in a large inflammatory skin reaction [76]. Among the leukocytes that are also attracted are CD4+ Treg lymphocytes that modulate the inflammatory response. In humans, this phase occurs at 72 h and persists for a few days, after which it rapidly decreases by mechanisms mediated by CD4+Treg lymphocytes [72].

3.2. Autoimmune diseases

Throughout the development, the maturation of the adaptive immune system and during the induction of immune responses, B and T lymphocytes show a high rate of genetic

recombination in the variable and hypervariable regions of the antigen receptors, so that the adaptive immune system has a wide variety of antigen receptors, being able to respond to virtually any type of molecule. Because of this phenomenon, there is a high probability that different self-antigens are recognized by the immune system, in addition to nonself antigens [1, 77]. To prevent the system from reacting against a self-antigen, autoreactive lymphocytes must be removed or trained as tolerant cells. Resident reticuloepithelial cells from cortex and marrow of the thymus perform a negative selection process to eliminate such autoreactive clones. In this sense, it has been demonstrated that thymic DCs are also actively involved in such negative selection processes, and more recently it has been shown that even peripheral DCs may migrate to the thymus and participate in this selection [1, 23]. This is highly significant as some populations of DCs are directly related to the development of autoimmune diseases, which has recently been shown in a study using transgenic mice transfected with diphtheria toxin A (DTA) coupled to a resistance cassette to neomycin which were crossed with CD11c-Cre mice.

In the progeny, the Cre complex removes the resistance cassette generating toxicity in CD11c+ cells, resulting in depletion of DCs including conventional, plasmacytoid, and LCs, which led to an increase in the frequency of CD4+ thymocytes and CD4+ lymphocytes in tissues together with the spontaneous development of multiorgan autoimmunity [78, 79]. On the other hand, the participation of immature DCs in the induction of Treg cells has been documented, where CD205 receptor stimulation led to a tolerant antigenic presentation, resulting in an increase of CD25+ CTLA-4 T cells, via ligands of co-stimulatory molecules such as CD28 and CD154, as well as a decrease in IL-2 production and CD4 [79, 80] T proliferation. Thus, immature DCs play a crucial role in the activation of Treg, not only to autoantigens but also against alloantigens, since it has been observed that the repeated stimulation of CD4+ T cells with immature allogeneic DCs in the absence of antigens leads to a differentiation toward Tregs cells [81].

On the other hand, it has been evidenced that in a state of non-inflammation (steady state), the tolerant response by the immature DCs depends strongly on the control of TGF-β1 [107]. LCs and bmDCs upregulate the expression of Axl, which belongs to the family of tyrosine kinase receptors, Tyro3, Axl Mer (TAM), which has the function of inhibiting the inflammatory response in DCs, in addition to participating in the elimination of apoptotic cells and the blockade of proinflammatory cytokine production, which together is essential for the maintenance of self-tolerance [27].

The participation of DCs in autoimmune processes has been controversial, since in different studies the ability of DCs to break tolerance and to induce autoimmune responses has been reported, and others have described the ability of DCs to preserve tolerance and avoid an autoimmune response. Thus, much of the controversy is due to the functional diversity of the different DCs populations, namely that while mature DCs can induce strong self-reactive responses, for example, in the central nervous system an amplification of experimental autoimmune encephalitis has been observed; it has also been observed that the use of immature DCs has the capacity to offer protection against the development of autoimmune reactions [81, 82].

Immature and tolerogenic DCs produce high levels of IL10, IL-2, and TGF-β, which effectively promotes the proliferation and activation of inducible regulatory T cells (iTreg), decreasing the autoimmune reaction; however, if the tolerance in different models cannot be restored, possibly it is due to the activity of CD220+ B cells that potently modulate Th17 responses, maintaining the proinflammatory state [79].

The use of DCs as a therapeutic tool in autoimmune reactions has to do not only with their ability to produce tolerance-inducing cytokines such as IL-10 and TGF but also with their biological capabilities such as the expression of molecules involved in the antigen presentation. For example, two populations of IL-10 modulated DCs (IL10DC) having a $CD83^{High}$, CCR7+, or $CD83^{low}$ CCR7-phenotype were recently compared. Assays suppression effector T cells showed that iTreg from the $CD83^{High}$ IL-10DCs induced greater suppression than the population of iTreg from the $CD83^{low}$ IL-10DCs, and had a higher migratory capacity to lymph nodes, suggesting that they are a good therapeutic candidate [52].

In addition to tolerogenic DCs, other DCs are involved in the modulation of autoimmune responses. Thus, other populations of DCs such as plasmacytoids have been evaluated. In a model of arthritis induced by methylated bovine serum albumin (mBSA), it is known that IFN-α prevents the inflammatory process, pDCs function was assessed in relation to TGF-β and IDO. IFN-α was found to increase the expression of IDO1 and the corresponding TGF-β signaling in the pDCs. Likewise, it was also observed that the depletion of the pDCs, either during the sensitization phase or already initiated the arthritic response, eliminates the protective effect of IFN-α. In addition, this same abrogating effect of IFN-α activity was observed when TGF-β signaling was blocked, but exclusively in the signaling phase, implying that this IDO1/TGF-β protection pathway is dependent on anti-inflammatory programs, while responses to restimulation are dependent on the participation of the pDCs [83].

As previously mentioned, TLR7 and TLR9 play an important role in the biology of pDCs, which also involves them in autoimmune responses. In a murine model deficient mice of the Gfi1-transcriptional repressor, which modulates myeloid and lymphoid differentiation, show spontaneous autoimmunity like lupus, including high levels of IgM and IgG2a, autoantibodies against RNA and DNA, as well as an increased frequency of plasmoblasts and germinal centers. In contrast, Gif1 mice do not show this phenotype, but interestingly they show an increase in TLR7-dependent DCs activation, where stimulated DCs produce increased amounts of TNF-α, IL-6, and IFN-β as well as an increase in phosphorylation of the transcription factors NF-κB and IRF7, suggesting the control of the IFN-I-signaling pathway, so that apparently the negative regulation of TLR7 in DCs prevents the spontaneous development of lupus [81].

3.3. Cancer

Tumor cells have mechanisms to evade the immune system such as the decreased expression of class I molecules, the release of tolerogenic cytokines such as TGF-β and IL-10, as well as the induction of lymphocyte death. Generally, in the tumor stroma Treg cells, macrophages type 2, mast cells, inhibitory myeloid cells, and neutrophils may secrete cytokines that help

the growth of the tumor; also, the formation of free radicals may contribute to increase the mutation rate in tumor cells [84]. In the stroma, there are also immature DCs that can induce the activation of Tregs tumor antigen-specific antigen lymphocytes, which may be involved with increased tumor growth [85]. In addition, DCs have been isolated from patients with metastases or with advanced stages of the disease and it has been shown that DCs express co-inhibitory molecules such as PD-L1, arginase, and IDO, and produce TGF-β, IL-10, and prostaglandins E2, resulting in an inhibition of T lymphocytes. It is worth noting that the use of immunostimulators can inhibit this suppression effect induced by DCs [86, 87]. In some patients, small foci of extratumoral lymphoid tissue known as tertiary lymphoid tissue may be localized and in them it is common to find memory T lymphocytes and naive T lymphocytes, in addition to mature DCs. When DCs and T lymphocytes have been isolated from this tissue, the development of a Th1 response has been observed in addition to the increased survival of patients with cancer. It is important to emphasize that this lymphoid tissue is usually observed in the early stages of the disease.

3.3.1. Antitumoral immunotherapy

DCs can be used in antitumor immunotherapy to induce the development of effective immunological responses that decrease the size of the tumor mass and increase survival. CD8+ T lymphocytes and NK cells, through the release of perforins and granzymes or through the binding of FAS/FASL molecules, can induce the death of tumor cells. These lymphocytes may be activated by DCs that have presented antigens from a tumor in the lymph nodes closest to the tumor mass. Thus, DCs have been differentiated *in vitro* to be used as a type of adoptive immunotherapy in clinical protocols. There are multiple strategies that have been used with DCs as described below.

DCs derived from bone marrow precursor cells and DCs derived from monocytes have been used in clinical protocols, with the latter being the most used in immunotherapy against melanoma [4, 88]. Cytokines (IL-4, IL-15, and IFN-γ), TLR agonists such as nucleic acids (CpG), Imiquimod, LPS, monophosphoryl lipid A, BCG, as well as transfection of DCs with RNA, encoding cytokines, growth factors, and co-stimulatory molecules, have been used to mature DCs [89].

In relation to the antigens used, DCs treated with nucleic acids, whole tumor antigens, tumor lysates, and peptides have been used to carry out a specific antitumor response [8]. The clinical results have been variable, so DCs-based immunotherapy has been used in combination with the administration of antibodies, cytokines, and even radiotherapy and chemotherapy [90].

Regarding melanoma tumor antigens, several have been used to stimulate DCs to elicit a specific antitumor immunological response dependent on T CD8 lymphocytes, capable on inducing cell tumor death. In this sense, some of the antigens most used for antitumor immunotherapy against melanoma are MAGE proteins [91, 92], gp-100 [93], NY-ESO, and tyrosinase, among others. Also, in different clinical assays, DCs have been loaded with one or more tumor antigens, for example, DCs loaded with various melanoma-specific antigens (gp-100 and tyrosinase) were administered in patients with melanoma and had a regression success rate of 11% [94], whereas in another clinical study, in which the same antigens were used in conjunction with keyhole hemocyanin, 57% of those receiving the therapy showed no tumor growth and only 4% showed complete tumor regression [29].

The success of DCs loaded with proteins or with tumor peptides has been moderate and is generally because antigen-specific T lymphocyte proliferation is not high; however, DC-based immunotherapy has been more successful than other strategies (e.g., the use of peptide-based vaccines) [95]. It should be noted that the low success observed with DCs-based immunotherapy is due to the fact that most DCs are applied to patients in stages III and IV of the disease by a late detection of cancerous lesions, which are difficult to produce a successful immunological response with immunocompetent T lymphocytes [96, 97].

In relation to the delivery of tumor antigens for activating DCs, dead, apoptotic, or necrotic tumor cells have been used [5, 98]. DCs can phagocyte tumor cells by means of specific receptors. Thus, apoptotic cells are recognized by the integrin $\alpha V\beta 5$, by the CD36 molecule, or by means of the phosphatidylserine receptor [99] and necrotic cells are recognized by CD91, TLR-2, and TLR4 [99, 100]. One of the advantages of using dead tumor cells is that DCs can present antigens by means of MHCI activating CD8+ T lymphocytes by cross-presentation. In addition, the haplotype by which the antigens are presented is independent of the response, so this type of vaccine can be applied to any patient [3]. These types of vaccines have been shown to induce the activation of tumor-specific CD4+ and CD8+ T cells, as demonstrated by a study with 13 melanoma patients, where 3 showed tumor regression and 1 showed increased IFN levels [101].

Another strategy is the transfection of DCs with tumor RNA or encoding co-stimulatory molecules, cytokines, or growth factors [102]. DCs that have been transfected with RNA encoding tumor antigens can present tumor proteins by means of different HLA molecules, so that a large number of CD8+ T lymphocyte clones may be activated [103]. Some of the melanoma antigens used in this therapy are MAGE, gp-100, MART-1, and p53. The results have been moderately satisfactory since the development of an antitumor immune response characterized by the presence of CD8+ T lymphocytes and IFN secretion was observed; nonetheless, tumor regression was observed several times. Despite the moderate response, this type of vaccine has been shown to not only stimulate the proliferation and activation of NK cells but also induce a decrease in Treg cell levels in melanoma patients [104, 105].

Regarding the cytokines or growth factors used to activate or differentiate DCs in antitumor immunotherapy treatments, GM-CSF is one of the most widely used, since it can induce differentiation of DCs and monocytes *in vivo* [104]. On the other hand, RNA encoding for IFN, IL-7, and TNF has been transfected into DCs, resulting in cytokine secretion and DCs maturation. In some assays, it has also been attempted to transfect cells with RNA encoding cytokines, tumor antigens, and co-stimulatory molecules with the aim that with a single transfection and without the need for incubations of 24–48 h with cytokines or tumor antigens, the cells can present antigens and secrete cytokines [106].

4. Conclusions

Several aspects of the biology of DCs have been clarified, however are still missing some issues to be resolved.

1. Nonetheless in mice some precursors have been established for different subpopulations of DCs, in humans it is still necessary to clarify precisely which are the immediate precursors of the different subpopulations, in particular of the cDCs.
2. It is necessary to complete the phenotype of subpopulations of DCs in human. It is clear that as more molecules are described, in the future this will be a fulfilled task, but at the same time, it will make it more difficult to classify the DCs.
3. Protocols of *in vitro* differentiation of DCs from their precursors must be better defined. According to the latest studies, most of the results attributed to DCs are due to cellular heterogeneity, mixtures of DCs, and macrophages. In addition, it is also necessary to consider that there may be a mixture of DCs subpopulations. This issue is very important, especially when clinical trials are conducted against tumors or autoimmune diseases.
4. According to their location, DCs have several functions and this is always necessary to keep in mind, and more when it comes to understanding the particular immune responses of an organ or a tissue. Not all DCs have the same function; they can vary from organ to organ.

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