1. INTRODUCTION

1.1 Immune system

The immune system of higher vertebrates consist of two components: innate and adaptive (Medzhitov and Janeway, 1998). In particular, host defense relies on a concerted action of both antigen-non specific innate immunity and antigen-specific adaptive immunity (Fearon and Locksley, 1996; Hoffmann et al., 1999; Medzhitov and Janeway, 1997b). Key features of the mammalian innate immune system include (*a*) the ability to rapidly recognize pathogen and/or tissue injury and (*b*) the ability to signal the presence of danger to cells of the adaptive immune system (Matzinger, 1994).

Evolutionary pressure has led to development of adaptive immunity, the key features of which are (*a*) the ability to rearrange genes of the immunoglobulin family, permitting creation of a large diversity of antigen-specific clones and (*b*) immunological memory. Yet this highly sophisticated and potent system needs to be instructed and regulated by antigen-presenting cells (APCs). Dendritic cells (DCs) are unique APCs because they are the only ones that are able to induce primary immune responses, thus permitting establishment of immunological memory (Banchereau et al., 2000; Banchereau and Steinman, 1998; Bell et al., 1999; Hart, 1997; Steinman, 1991).

1.1.1 Innate immune system

The innate immune system is a universal and ancient form of host defense against infection. Innate immune recognition relies on a limited number of germ line-encoded receptors defined pattern recognition receptors (PRR) (Hargreaves and Medzhitov, 2005). These receptors evolved to recognize conserved components or products of microbial metabolism produced by microbial pathogens. Together these microbial structures have been defined pathogen-associated molecular patterns (PAMPs). Relevant examples of PAMPs are: lipopolysaccharide (LPS), carbohydrates, double-stranded viral RNA, and non methylated DNA. Recognition of these molecular patterns allows the immune system to distinguish infectious non-self from non-infectious self (Fearon and Locksley, 1996).

Innate immune defenses are non-antigen specific and this system does not confer long-lasting immunity against a pathogen (Medzhitov and Janeway, 1997b).

The innate immune system includes two components: humoral and cellular.

The humoral component is mainly represented by: (1) complement system that is made up of many distinct plasma proteins that react with one another to opsonize pathogens and induce a series of inflammatory responses that help to fight infection; (2) type I interferons (IFN) α and β, important cytokines that contribute to defence against viral infections; (3) some inflammatory mediators such as cytokines that mediate local inflammation (interleukin (IL)-1, tumor necrosis factor (TNF) and chemokines) and cytokines that regulate the development of the inflammatory responses (IL-10 and transforming growth factor (TGF) -β) and some enzymes, such as lysozime, with an antimicrobic activity (Medzhitov, 2007).

The cellular component is represented by phagocytes such as macrophages, polymorphonuclear leukocytes (PMN: neutrophils, eosinophils and basophils) and DCs, natural killer (NK) cells, and mast cells.

If a microorganism crosses the epithelial barrier and begins to replicate in the tissues of the host, in most cases it is immediately recognized by the mononuclear phagocytes, or macrophages, that reside in these tissues. Macrophages mature continuously from monocytes that leave the circulation and migrate into tissues throughout the body. They are found in especially large numbers in connective tissue, in the submucosal layer of the gastrointestinal tract, in the lung (where they are also found in both the i*nterstitium* and the *alveoli*), in liver blood vessels (where they are known as Kupffer cells), and throughout the spleen, where they remove senescent blood cells. The second major family of phagocytes – the neutrophils – are short-lived cells that are abundant in the blood, but they are not present in normal, healthy tissues. Both of these phagocytic cells have a key role in innate immunity because they can recognize, ingest, and destroy many pathogens without the aid of an adaptive immune response (Borregaard and Cowland, 1997; Segal et al., 1981).

Macrophages, located in the submucosal tissues, are the first cells to encounter pathogens, but they are soon reinforced by the recruitment of large numbers of neutrophils to sites of infection. This occurs mainly following activation of the Complement System that releases chemotaxins such as C3a and C5a, which recruit neutrophils and, at the same time, opsonizes the pathogen and attempts to undermine its membrane integrity by the formation of the membrane attack complex (MAC) (Carroll and Fischer, 1997).

Macrophages and neutrophils recognize pathogens by means of their cell-surface receptors (mannose receptor, scavenger receptors, CD14, CR3) that can discriminate between the surface molecules displayed by pathogens and those of the host (Schlesinger, 1993).

Ligation of many of these cell-surface receptors by pathogens leads to phagocytosis of the pathogen, followed by its death inside the phagocyte.

A second important effect of the interaction between pathogens and tissue macrophages receptors is the activation of macrophages to release cytokines, chemokines, and other mediators that set up a state of inflammation in the tissue and bring neutrophils and plasma proteins to the site of an infection (Aderem and Underhill, 1999).

Many other cell type are enrolled in the innate immune responses, such as: NK cells, leukocytes that attack and destroy tumor cells, or cells that have been infected by viruses; mast cells that reside in connective tissues and mucous membranes, regulate the inflammatory response and are most often associated with allergy and anaphylaxis; basophils and eosinophils, that secrete chemical mediators involved in defending against parasites and play a role in allergic reactions, such as asthma (Galli et al., 2005; Kobayashi et al., 2005; Lanier, 2005; Ma et al., 2006).

Receptors that signal the presence of pathogens and induce cytokines also have the important role to induce the expression of co-stimulatory molecules on macrophages and on DCs, another type of phagocytic cell present in tissues that, as will be extensively discussed later, serve as a link between the innate and adaptive immune systems, as they present antigen to T lymphocytes, one of the key cell types of the adaptive immune system (Banchereau et al., 2000; Savina and Amigorena, 2007).

1.1.2 Adaptive immune system

The adaptive immune system may be described as composed by highly specialized systemic cells and processes that eliminate or prevent infections.

Adaptive immunity is triggered in vertebrates when a pathogen stimulates the innate immune system and generates a threshold level of antigen.

The major functions of the adaptive immune system include: (1) the recognition of specific "non-self" antigens in the presence of "self", during the process of antigen presentation; (2) the generation of responses that are tailored to maximally eliminate specific pathogens or pathogen infected cells; (3) the development of immunological memory, in which each pathogen is "remembered" by a signature antigen. These memory cells can be called upon to quickly eliminate a pathogen when subsequent infections occur (Janeway, 1992).

The cells of the adaptive immune system are B and T lymphocytes that derived from the same pluripotential hemopoietic stem cells, and are morphologically indistinguishable from one another until after they are activated.

Peripheral lymphoid organs contain a mixture of B- and T cells in at least three stages of differentiation: **"**naive cells" that have matured, left the bone marrow (BM) or thymus, have entered the lymphatic system, but that have yet to encounter their cognate antigen, "effector cells" that have been activated by their cognate antigen, and are actively involved in eliminating a pathogen and, "memory cells" – the long-lived survivors of past infections (Medzhitov and Janeway, 1998).

While B lymphocytes play a large role in the humoral immune response, T lymphocytes are intimately involved in cell-mediated immune responses.

1.2 T lymphocytes

The activation of T lymphocytes by APCs leads to their proliferation and the differentiation of their progeny into armed effector T cells (Liu and Janeway, 1991; Liu and Janeway, 1992). This depends on the production of cytokines, in particular the T-cell growth factor IL-2, which binds to high affinity receptor on the activated T cell. T cells whose antigen receptors are ligated in the absence of co-stimulatory signals fail to make IL-2 and instead become anergic or die (Bousso, 2008).

Proliferating T cells develop into armed effector T cells, the critical event in most adaptive immune responses. Once an expanded clone of T lymphocyte achieves effector function, its progeny can act on any target cell that displays antigen on its surface. There are three classes of effector T cell, specialized to deal with three classes of pathogen.

CD8 cytotoxic T lymphocyte (CTL) kill target cells that display peptide fragments of cytosolic pathogens, most notably viruses, bound to major histocompatibility complex class I (MHC I) molecules at the cell surface; they release perforin (which creates holes in the target cell membrane), granzymes (which are proteases that are activated intracellularly to trigger apoptosis), and often IFN-γ, which can block viral replication or even lead to the elimination of virus from infected cells without killing them or causes macrophage activation, which is a pre-requisite for the killing of intracellular bacteria such as *Mycobacterium tuberculosis* (Mtb). A membrane-bound effector molecule expressed on

CD8 T cells is the Fas ligand (CD178). When this binds to Fas (CD95) on a target cell it activates apoptosis in the Fas-bearing cell.

The development of CTL responses is necessary for the control of a variety of bacterial and viral infections. CTL traffic to peripheral sites of infection and specifically target infected cells. The CTL response to acute infection can generally be divided into four phases: (*a*) during the effector phase, naive CTL precursors are primed, undergo dramatic expansion, acquire effector function, travel to sites of infection, and mediate pathogen clearance by killing infected cells and secreting effector cytokines; (*b*) during the contraction phase, most effector CTL die, leaving behind 5%–10% of the original burst size as long-lived memory cells; (*c*) during the memory maintenance phase, memory CTL are maintained at stable levels throughout the life of the mouse and for many years in humans; and (*d*) the rapid recall response of memory CTL following re-exposure to the pathogen provides enhanced protection to the host (Butz and Bevan, 1998; Williams and Bevan, 2007).

CD4 helper T (Th) *cells* play a central role in the regulation of immune responses and in particular in determining the expansion and functional differentiation of antigen specific B and effector CD8 T lymphocytes . These cells have no cytotoxic or phagocytic activity but are able to manage the immune response by directing other cells to perform these tasks. In particular they have the capacity to help B cells to differentiate into plasma cells, that secrete antibodies, to induce macrophages to develop enhanced microbicidal activity, to recruit neutrophils, eosinophils, and basophils to sites of infection and inflammation, and, through their production of cytokines and chemokines, to orchestrate the immune responses.

The highly heterogeneous $CD4^+$ Th cell mediated immune response is represented by two main polarized forms: Th1 and Th2 cells (Romagnani, 1992; Zhu and Paul, 2008).

Clear evidence suggests that Th1 and Th2 cells develop from the same naive Th cell under the influence of both environmental and genetic factors acting at the level of antigen presentation. Genetic and environmental factors can have influence independently or in association with a series of modulatory factors that include the following: (1) the ligation of T-cell receptor (TCR); (2) the activation of co-stimulatory molecules; (3) the predominance of a given cytokine in the microenvironment of the responding Th cell; and (4) the number of post-activation cell divisions.

Th1 cells, characterized by the prevalent production of IL-2, IFN-γ, and TNF-β, without IL-4, IL-5, IL-9, and IL-13 production, drive the type-1 pathway (“cellular immunity”) to

fight viruses and other intracellular pathogens (with a particular important role in resistance to mycobacterial human infections), eliminate cancerous cells, and stimulate delayed-type hypersensitivity (DTH) skin reactions.

By contrast, Th2 cells, characterized by the prevalent production of IL-4, IL-5, IL-9, IL-10 and IL-13 in the absence of IFN-γ and TNF-β production, drive the type-2 pathway ("humoral immunity") and up-regulate antibody production to fight extracellular organisms (Mosmann and Coffman, 1989; Paul and Seder, 1994; Romagnani, 2004).

Furthermore a third type of Th lymphocyte that produce both Th1- and Th2-type cytokines (termed Th0) has been described in human and mouse systems (Abbas et al., 1996; Romagnani, 1994). Whether Th0 cells are precursors for Th1 and Th2 cells or represent a separate, stably differentiated population remains unclear. It is possible that Th0 cells are involved in eliminating many pathogens, where a balance of both regulated cell-mediated immunity and an appropriate humoral response will eradicate an invading pathogen with minimum immunopathology (Kamogawa et al., 1993).

The ability of cytokines to stimulate different effector mechanisms and thus differential immune responses is also reinforced by the production of cytokines by each subset, which cross-regulate each other's function as well as development. For example, IFN-γ produced by Th1 cells inhibits the development of Th2 cells as well as humoral responses, whereas the production of IL-4 and IL-10 by Th2 cells inhibits Th1 development and activation as well as macrophage activation and bactericidal activity (Fitch et al., 1993; Moore et al., 1993; Sher and Coffman, 1992).

Thus, Th1-dominated responses are potentially effective in eradicating infectious agents, including those with a intracellular life cycle within host cells. When the Th1 response is poorly effective or exhaustively prolonged, it may result in host damage. In contrast, Th2 responses are apparently insufficient to protect against the majority of infectious agents. In fact, the protective role of Th2 cells against infectious agent is indirect and mediated by their role in promoting B cell growth and causing their differentiation into antibody producing plasma cells. Th2 cells are also able to contrast the life of parasites in the host and tend to limit potentially harmful Th1-mediated responses. In this light Th2 cells may be regarded as a part of down regulatory mechanism for exaggerated and/ or inappropriate Th1 responses. The Th1/Th2 paradigm applied to the study of chronic inflammatory disorders or autoimmune diseases allowed to understand that a number of diseases are mediated by Th1 cells, the two clearest examples being multiple sclerosis and thyroid autoimmunity. In other disorders, Th1/Th2 polarization is less prominent, or rather Th2

responses tend to predominate, such as in systemic lupus erythematosus, progressive systemic sclerosis or allergic diseases. It is of note that in experimental models in animals, a number of diseases can be prevented by switching immune responses from Th1 to Th2 or from Th2 to Th1. Moreover, the Th1/Th2 concept suggests that modulation of the relative contribution of Th1- or Th2-type cytokines makes possible to regulate the balance between protection and immunopathology, as well as the development and/or the severity of some immunologic disorders (Del Prete, 1998; Romagnani, 2006).

Another type of T lymphocyte is the *regulatory T cells* (Treg) that play a critical role in maintaining self-tolerance as well as in regulating immune responses (Sakaguchi, 2004). Increasing Treg numbers and/or enhancing their suppressive function may be beneficial for treating autoimmune diseases and for preventing allograft rejection (Bluestone and Tang, 2005).

The molecular basis of suppression in some cases is through their production of cytokines, including TGF-β, IL-10, and IL-35. Although TGF-β is not absolutely required for suppression in some settings, particularly *in vitro*, it is very important in mediating suppression in several circumstances *in vivo* (Li et al., 2006).

IL-10 production is critical for Treg-mediated prevention and cure of inflammatory bowel disease (Asseman et al., 1999).

1.3 Dendritic cells (DCs)

1.3.1 Activation of DCs and launching of protective immunity

DCs play a key role in initiating and controlling the magnitude and the quality of adaptive immune responses. Immature DCs act as immunological sensors to alert for potentially dangerous microbes, either by directly recognizing microbial components or by receiving signals formulated by the innate immune system that is exposed to microbes. Immature DCs decode and integrate such signals and carry this information to adaptive immune cells (Pulendran et al., 2001). Thus, the type of adaptive immune response is highly dependent on the nature of the activating stimuli that DCs receive from the innate immune system.

In the steady state, DCs reside in both peripheral tissues and lymphoid organs. DC subsets also circulate in the blood. To elicit anti-microbial immunity, DCs undergo a complex process of maturation, a ‘metamorphosis’ from an antigen-capturing cell into an efficient APC. This process includes (1) changes in morphology such as the loss of adhesive

structures, cytoskeleton reorganization, and the acquisition of high cellular motility (Trombetta and Mellman, 2005); (2) loss of endocytic/phagocytic receptors; (3) secretion of chemokines in coordinated waves according to the type of immune cells that need to be attracted (Penna et al., 2002; Piqueras et al., 2006; Tang and Cyster, 1999); (4) up-regulation of co-stimulatory molecules, such as CD40, CD80 (B7.1), and CD86 (B7.2), a process that represent the crucial first step in adaptive immunity because naïve T cells will respond to antigen only when one cell presents both a specific antigen to T-cell receptor and a B7 molecule to CD28 on the T cell (Caux et al., 1994); (5) translocation of major MHC class II compartments to the cell surface (Cella et al., 1997a); and (6) secretion of cytokines that differentiate and polarize the attracted immune effectors (Heufler et al., 1996).

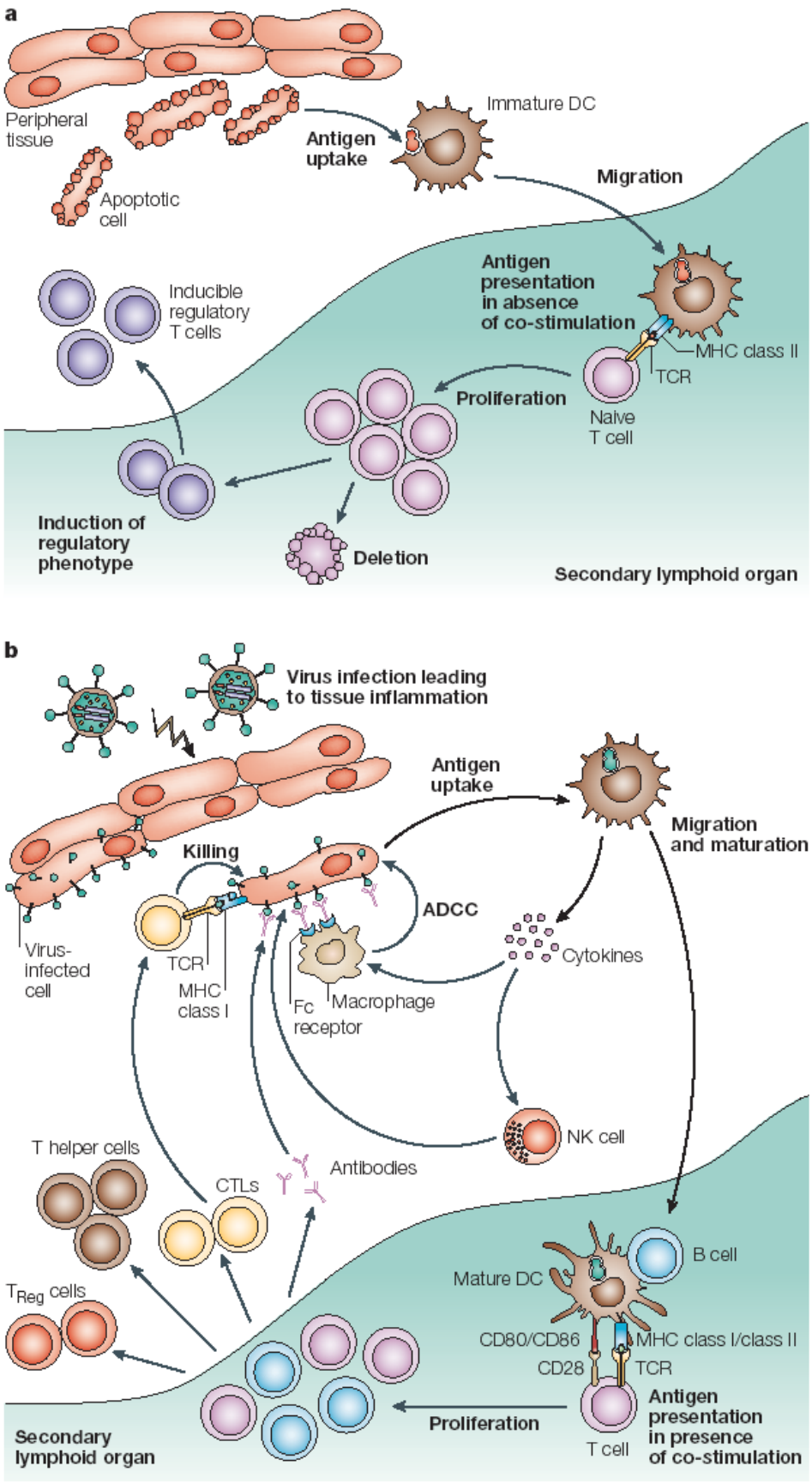
Immature and mature DCs have different functions **(Fig. 1)**; tissue DCs constantly sample their environment, capture antigens and migrate in small numbers to draining lymph node. In the absence of inflammation, the DCs remain in an immature state, and antigens are presented to T cells in the lymph node without co-stimulation, leading to either the deletion of T cells or the generation of inducible regulatory T cells. Thus immature DCs have the role to induce tolerance **(Fig. 1a)**.

Tissue inflammation induces the maturation of DCs and the migration of large numbers of mature DCs to draining lymph nodes. The mature DCs express peptide–MHC complexes at the cell surface, as well as appropriate co-stimulatory molecules. This allows the priming of $CD4^+$ Th cells and $CD8^+$ CTLs, the activation of B cells and the initiation of an adaptive immune response. To control the immune response, $CD4^+CD25^+$ Treg cell populations are also expanded **(Fig. 1b)**.

DCs are activated by numerous agents derived from microbial components, microbes, dying cells, cells of the innate immune system, and cells of the adaptive immune system **(Fig. 2)**. In particular the immune system recognizes microbes through PRRs that are subdivided in at least three families of molecules: Toll-like receptors (TLRs), cell surface C-type lectin receptors (CLRs), and intracytoplasmic nucleotide oligomerization domain (NOD)-like receptors (NLRs). These receptors recognized the microbes through PAMPs, which are invariant across pathogens. Microbes directly activate DCs through their PRRs or indirectly, for instance, by capture of apoptotic/necrotic products of other cells dying in response to microbial exposure. Microbes also induce a wide repertoire of cells, such as epithelial cells, fibroblasts, and cells of the innate immune system, to secrete cytokines capable of activating DCs.

The innate immune system controls the immune system by modulating the type and function of myeloid (m)DCs; infact innate immune cells secrete different sets of soluble factors in response to various stimuli. Granulocytes and macrophages secrete IL-1, IL-6, and TNF upon microbial recognition. NK cells secrete IFN-γ. Mast cells secrete granulocyte macrophages-colony stimulation factor (GM-CSF), IL-4, and TNF. Keratinocytes secrete IL-15 and GM-CSF as well as thymic stromal lymphopoietin (TSLP) in allergic lesions (Medzhitov and Janeway, 1997a; Medzhitov and Janeway, 1998).
Plasmacytoid (p)DCs secrete large amounts of type I IFN upon viral encounter (Liu, 2005). Immature DCs and monocytes activated by GM-CSF and/or FMS-like tyrosine kinase 3 ligand (Flt3L) during extravasation are exposed to these factors, resulting in the differentiation into mature DCs with distinct phenotypes. These distinct DCs promote distinct types of T-cell immunity. Thus, DCs are the key player to convey information from the innate immune cells to the adaptive immune cells.

Fig. 1 Different functions of immature and mature DCs.
a) Immature DCs induce tolerance.
b) Mature DCs induce immunity.
ADCC, antibody dependent cell-mediated cytotoxicity; TCR, T-cell receptor.



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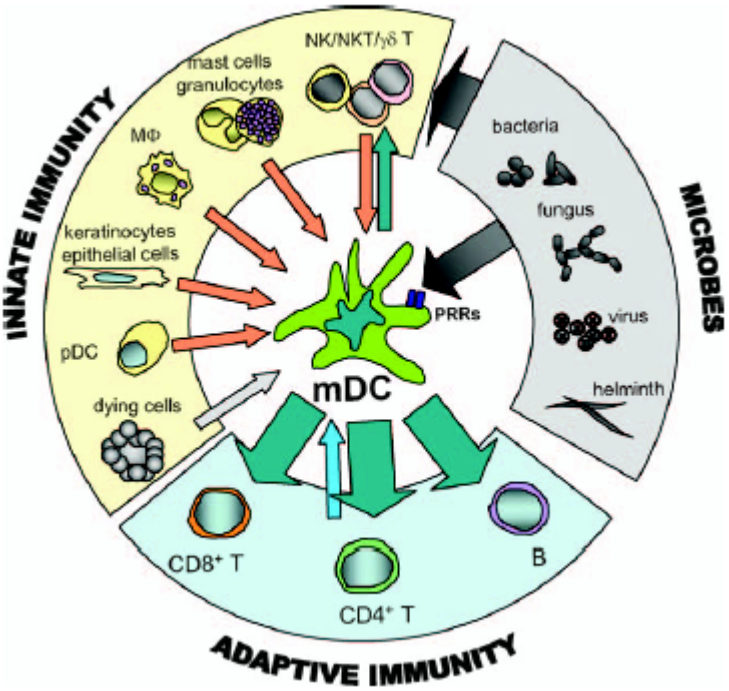

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Fig. 2 Regulation of mDC activation.

1.3.2 DC types and subtypes

DCs are heterogeneous (Shortman and Liu, 2002). Although all DCs are capable of antigen uptake, processing and presentation to naive T cells, the DC subtypes differ in location, migratory pathways, detailed immunological function and dependence on infections or inflammatory stimuli for their generation.

One division that has been recently proposed is into "*conventional DCs*" (cDCs), and "*precursors of DCs*" (pre-DCs) (Shortman and Naik, 2007).

cDCs already have a dendritic form and exhibit DCs functions in steady state. They can be divided into several categories: *(a) Migratory DCs* and (*b) Lymphoid-tissue-resident DCs*.

(a) Migratory DCs: are the classical text-book described DCs (Bell et al., 1999); they act as sentinels in peripheral tissue; migrate to the lymph nodes through the lymphatics, bearing antigens from the periphery; present these antigens to T cells in the lymph node.

Examples of this category include 2 types of skin DC subsets with myeloid origin: epidermal Langerhans cells (LCs) and interstitial dermal DCs (intDCs) (Valladeau and Saeland, 2005).

Skin DC subsets show different phenotypes and biological functions: they express different sets of molecules, for example epidermal LCs express CD1a, Langerin, and E-cadherin, while dermal intDCs express Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), CD11b, factor XIIIa, and CD14; then these two mDC subsets differentially respond to chemokines so that are characterized by differential migration kinetics to inflammatory site (Nestle et al., 1994).

Furthermore it has been demonstrated that, intDCs induce the differentiation of naive B cells into immunoglobulin M (IgM)-secreting plasma cells through the secretion of IL-6 and IL-12 but are not very efficient at priming naive $CD8^+$ T cells. In contrast, LCs are particularly efficient at inducing cytotoxic high-avidity $CD8^+$ T cells, while they are not able to promote the development of naive B cells into IgM secreting plasma cells (Dubois et al., 1998). LCs are also strong activators of naive $CD4^+$ T cells, inducing their polarization into T cells secreting IFN-γ (Th1) as well as cells secreting IL-4, IL-5, and IL-13 (Th2). In contrast, intDCs preferentially induce $CD4^+$ T cells, which help immunoglobulin production from B cells (Tfh). LCs and intDCs appear to be equally potent at activating the proliferation and differentiation of memory T and B lymphocytes.

These recent findings led to propose that intDCs preferentially induce humoral immunity, while LCs induce cellular immunity (**Fig. 3**) (Ueno et al., 2007)

(b) Lymphoid-tissue-resident DCs: are also involved in both immunity and tolerance, but they do not migrate through the lymph; their function and life-history are restricted to one lymphoid organ. lymph node-resident DCs capture microbial antigens rapidly delivered through lymphatics and conduits, and upon stimulation through PRRs, these DCs induce the proliferation and IL-2 secretion of antigen-specific T cells (Itano et al., 2003). In the steady state, lymph node-resident DCs captured self-antigens and induce tolerance. Examples include thymic cDCs and splenic cDCs.

In mice, lymphoid-tissue-resident cDCs can be separated into $CD8^+$ cDCs that express high levels of CD8α on the cell surface, and $CD8^-$ cDCs that lack this marker (Vremec et al., 1992). The $CD8^+$ and $CD8^-$ cDCs differ in immune functions, including cytokine production and the presentation of antigens on MHC class I molecules (den Haan et al., 2000; Hochrein et al., 2001). The $CD8^-$ cDCs in turn are divisible into $CD4^+CD8^-$ and $CD4^-CD8^-$ cDC subsets (Vremec et al., 2000).

pre-DCs are cells without immediate dendritic form and DCs function, but with a capacity to develop into DCs with little or no division.

One DC-lineage cell that occurs as a pre-cDC in steady state is the pDC, also known as the natural IFN-producing cell. This is a round, non-dendritic, relatively long-lived and circulating cell, which, after stimulation by viral or other microbial infections, produces large quantities of type I IFN. The same inflammatory stimuli also initiate the conversion of pDCs into a dendritic form and the cells acquire some DCs antigen-processing and antigen-presentation properties (Grouard et al., 1997; Liu, 2005; Yoneyama et al., 2004).

Another example included in this category is represented by monocytes.

Finally, a novel DC populations that are not found in the steady state appear as a consequence of infection or inflammation. These were termed "*inflammatory DCs*" (Shortman and Naik, 2007). One example is the DCs produced *in vivo* when pDCs are stimulated by the influenza virus (O'Keeffe et al., 2002). Another example is the DCs that appear after the infection of mice with *Listeria monocytogenes* . These are termed TipDCs as they produce TNF and inducible nitric-oxide synthase (iNOS) (Serbina et al., 2003).

It has been also hypothesize that inflammatory monocytes can produce inflammatory DCs (Randolph et al., 1998; Randolph et al., 1999).

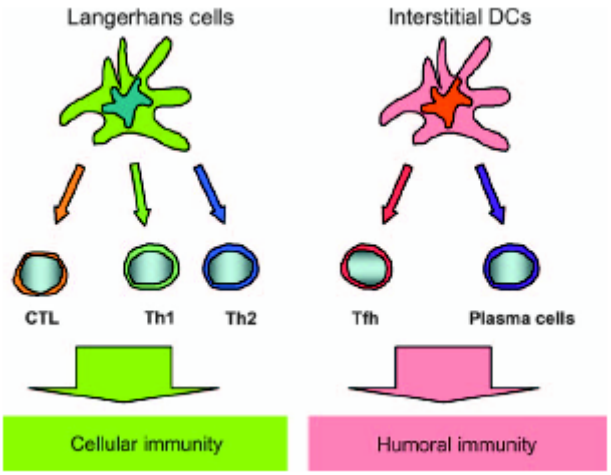

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Fig. 3 intDCs preferentially induce humoral immunity, while LCs induce cellular immunity.

1.3.3 DCs in the health/disease balance

DCs orchestrate a repertoire of immune responses that bring about resistance to infection and silencing or tolerance to self.

But, a system as complex as that of DC might suffer dysregulation leading to the development of distinct types of diseases.

For example, the critical role of DCs in the launching of protective anti-microbial immune responses make them primary targets for microbes who want to survive and eventually thrive.

It is known that DCs induce resistance to infection. In particular, when microbial antigens are injected in association with DCs into mice, the animals acquire adaptive immunity to some pathogens (i.e. *Borrelia burgdorferi*, chlamydiae, *Leishmania major*, fungi, *Toxoplasma gondii*, *plasmodium malariae* and human immunodeficiency virus - HIV). Conversely, DC depletion reduces defences to viruses like citomegalovirus (CMV), herpes virus (HSV)-2 and Lymphocytic Choriomeningitis Virus (LCMV) (Dalod et al., 2002; Probst and van den Broek, 2005). In humans, a lack of circulating DCs during bacterial sepsis and dengue virus infection is associated with a poor prognosis. A key concept is that

DCs mature in distinct ways in response to different microbial components, thereby launching alternative versions of host immunity.

In contrast, several microbes or viruses have the capacity to actively block DCs maturation (*Salmonella typhi*, anthrax lethal factor protein, *Plasmodia*, a *Mycobacterium ulcerans* mycolactone, herpes simplex, HIV, CMV, varicella zoster, hepatitis C virus - HCV); or DCs differentiation from monocytes (*Candida albicans* and Mtb) furthermore, some pathogens can alter other levels of DCs physiology to evade an immune response injecting toxins into phagocytes, including DCs, and destroying the cells required for innate and adaptive protection (*Yersinia pestis*, *Salmonella typhi*), inducing apoptotic cell death in DCs (Albert et al., 1998)(Influenza, measles and HSV-2), inhibiting the migration step of DC function by blocking expression of chemokine (C-C motif) receptor type 7 (CCR7), a chemokine receptor that guides DCs into lymphatic vessels and onwards to lymphoid tissues (CMV, herpes and Mtb) (Cyster, 1999; Khader et al., 2006; Randolph et al., 2005).

Microbes also can alter the function of DCs so that they switch T-cell responses from protective Th1 to non-protective Th2, as in infections with *Aspergillus fumigatus*, malaria and hepatitis C, or to IL-10 production in the case of *Bordetella pertussis*.

Thus, to counteract the variety of mechanisms for pathogenesis of infectious disease, DCs are now being considered in the design of vaccines to prevent and treat infection by enhancing immunogenesis.

It could be also possible that an inappropriate responses to self constituents, in select genetic backgrounds, can lead to chronic inflammatory conditions, termed autoimmune diseases. DCs bearing self antigens are able to induce autoreactive T cells in mouse models of multiple sclerosis, cardiomyopathy and systemic lupus erythematosus (Bondanza et al., 2003; Eriksson et al., 2003). It is increasingly appreciated that a pivotal step leading to human autoimmunity is an overproduction of a particular cytokines, and subsets of DCs can be a major source.

Despite their role in inducing autoimmunity, DCs are also relevant to the therapy of these diseases.

Not only during infectious diseases or autoimmunity, but also during cancer, allergy, and transplant rejection, DCs instigate unwanted responses that causes disease, but, again, DCs can be harnessed to silence these conditions with novel therapies (Gabrilovich, 2004; Lambrecht and Hammad, 2003; Merad et al., 2004; Schuler-Thurner et al., 2002; Soumelis et al., 2002; Traidl-Hoffmann et al., 2005).

Infact, given their capacity to modulate immune responses, DCs are an attractive target for the development of both preventative and therapeutic vaccines.

However, many elementary problems in the experimental research have yet to be solved, especially relating to the heterogeneity of DCs and their role in various diseases. Addressing these problems would greatly stimulate the progression of clinical application.

Furthermore, the knowledge of ontogeny and developmental pathways of DCs and the standardization of definitions of DC subsets, would also have significant therapeutic value and could be useful in clinical studies.

1.4 The origin of DCs

Since the initial description of DCs by Steinman and Cohn as "the population of cells enriched from mouse spleens that were responsible for the so called 'mixed-leukocyte reaction' (MLR) activity" (Steinman and Cohn, 1973), it has now become evident that there are many distinct DC subtypes, each with a particular location and specialized function in the immune system (Shortman and Liu, 2002). Most of these DC subtypes have a short lifespan once they enter lymphoid tissues to interact with T cells, so they must be continually renewed (Kamath et al., 2002; Kamath et al., 2000). The precursors of DCs and the developmental pathways that lead to the different DC subtypes are therefore important, not just in the initial establishment of the DCs network, but as dynamic components of the response to microbial invasion and in the active maintenance of self-tolerance.

Recent researches suggest that differentiation of cDCs, located in mucosal surfaces and lymphoid organs, does not occur in the BM, but locally, in these peripheral locations. This hypothesis relies on the fact that cDC subpopulations existing in lymphoid and nonlymphoid organs are found neither in the BM nor in the blood and that DCs number increases considerably in the lymphoid organs during infection; in addition, DCs precursor populations have been described in the circulation.

The concept of local DCs differentiation implies the existence of circulating DC precursors that would be recruited to peripheral antigen-capture locations and lymphoid organs, where they would differentiate into DCs. Peripheral DCs differentiation would therefore be responsible for the maintenance of the different DC subsets existing under steady state (Ardavin, 2003; Shortman and Naik, 2007).

1.4.1 The origin of steady-state DCs

DCs, together with all blood cells, have their ultimate origin in haematopoietic stem cells (HSCs). The earliest self-renewing HSC in the adult BM gives rise to a series of downstream precursor cells that are progressively committed to particular cell lineages. One of the earliest steps is the development of precursor cells that have a strong bias to either myeloid or lymphoid development, exemplified by the isolation from BM of the common lymphoid progenitor (CLP) and the common myeloid progenitor (CMP) populations (Akashi et al., 2000; Kondo et al., 1997).

Initially, it was considered obvious that DCs would be myeloid-lineage cells. DCs have many similarities to macrophages, can be generated in culture from monocytes, and are clonally associated with granulocytes and macrophages in colonies of BM cells that are grown under the influence of the 'myeloid' hormone GM-CSF (Inaba et al., 1993).

It was therefore surprising when a lymphoid restricted precursor cell that was isolated from mouse thymus could generate DCs (Ardavin et al., 1993; Wu et al., 1991). It was first assumed that this was the basis of cDCs heterogeneity.

A series of careful studies, both on mouse precursors transferred into irradiated recipients and on human precursors in culture, showed that all DC subtypes tested could be generated from both CLPs and CMPs (Manz et al., 2001).

Using a limiting-dilution approach in a culture system that allowed DCs development from human precursor cells, it was shown that a single CLP or a single CMP could generate both cDCs and pDCs (Chicha et al., 2004).

These findings indicated that there is a remarkable developmental flexibility in DCs generation, compared with other haematopoietic cell lineages.

It was also showed that the crucial events in commitment to DCs formation, and in the development of the separate DC subtypes, are downstream of both CLPs and CMPs.

If the first step towards the development of the individual types of blood cell is considered to be the formation of precursor cells isolated as the CMP and CLP populations, further work has defined a series of precursors of each type, rather than just two committed precursor forms.

Of these, the myeloid precursors and the lymphoid precursors that express the FLT3 (FMS-related tyrosine kinase 3) receptor have the greatest capacity to form DCs both in mice and humans (Maraskovsky et al., 1996; Maraskovsky et al., 2000; McKenna, 2001). Both the

tissue-resident cDCs found in lymphoid organs and the pDCs can be generated from either $FLT3^+$ precursor type (Karsunky et al., 2003) (**Fig. 4**).

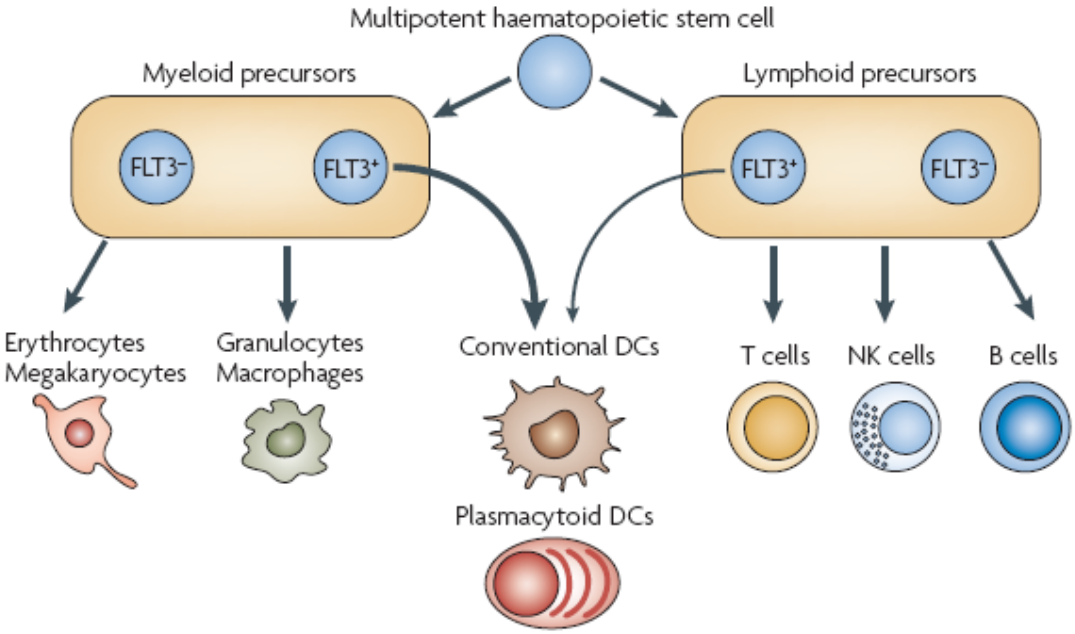
Which precursor type will actually generate DCs *in vivo* will depend on the availability of precursors, the local environment and the tissue involved. Myeloid precursors are the main source of DCs in most circumstances.

The view that haematopoiesis proceeds through a sequence of strictly ordered and irreversible differentiation steps has been replaced by the concept of a more gradual, multistep process with irreversibility as a late consequence of a series of changes.

The production of macrophages and DCs seems to be closely linked during haematopoiesis. Studies using mice have described a myeloid pathway of macrophages and DCs development, which proceeds through a macrophages–DCs precursor cell that is no longer able to produce many granulocytes, but can form DCs by two routes — the direct production of lymphoid-tissue-resident DCs, and the production of inflammatory DCs through a circulating monocyte intermediate (Fogg et al., 2006).

However, it has also been observed in several mouse studies that macrophages and DCs can be produced even by precursors that are otherwise committed to T-cell or B-cell production.

The retention of some potential to form macrophages and DCs by T-cell and B-cell precursors might explain why DCs, which are basically a myeloid cell type, can nevertheless be generated by lymphoid precursors (Shortman and Naik, 2007)(**Fig. 5**).



from Shortman et al. Nat. Rev. Immunol. 2007

Fig. 4 DCs development from haematopoietic precursors

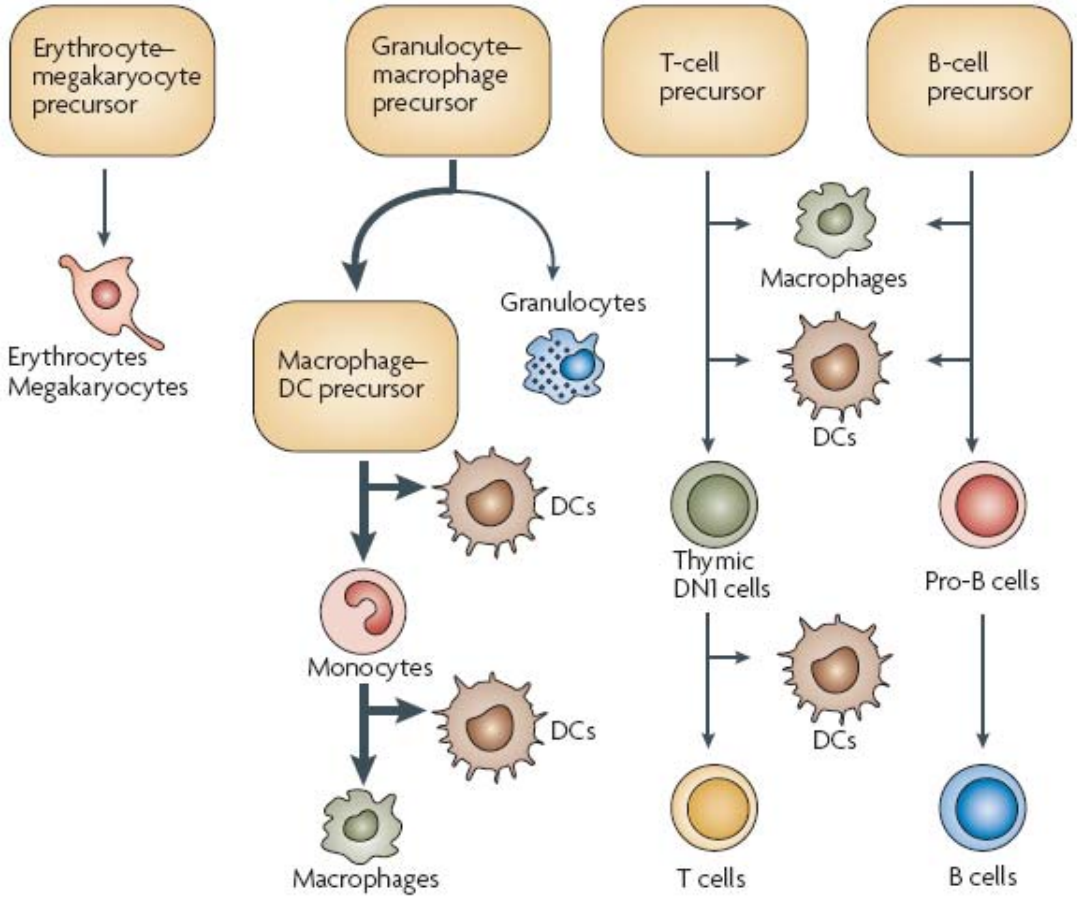


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Fig. 5 Model for the retention of macrophage and DCs potential during haematopoiesis

1.4.2 Differentiation of cDCs locally in the peripheral tissues

As previously described, some recent studies lead to the concept that different types of DCs develop through different branches of haematopoietic pathways that involve different immediate precursor cells.

Furthermore, these studies show that many individual tissues generate their own DCs locally, from a reservoir of immediate DC precursors, rather than depending on a continuous flux of DCs from the BM.

cDCs development in the spleen can probably be used as a model of lymphoid-tissue-resident DCs development in general (**Fig 6**).

The cDCs in the spleen of steady-state mice derive from an intrasplenic precursor, a pre-cDCs. This precursor population might be replenished from earlier precursor cells that are generated in the BM, which might occasionally seed the spleen from the bloodstream (Kamath et al., 2000).

Alternatively, as the spleen remains a haematopoietic organ in mice, the pre-cDCs might be generated endogenously. A late branch in the cDCs developmental pathway, detected by high or low expression of CD24 on the precursor cells, leads to pre-cDCs in the spleen that are pre-committed to form either $CD8^+$ or $CD8^-$ cDCs, respectively (Vremec et al., 2007). The cDCs so formed are in an immature state and are still capable of some homeostatic proliferation.

In contrast to the cDCs, the pDCs are generated in the BM by a pathway that branches off from that of cDCs. The pDCs found in the mouse spleen and other tissues probably arrive there from the bloodstream.

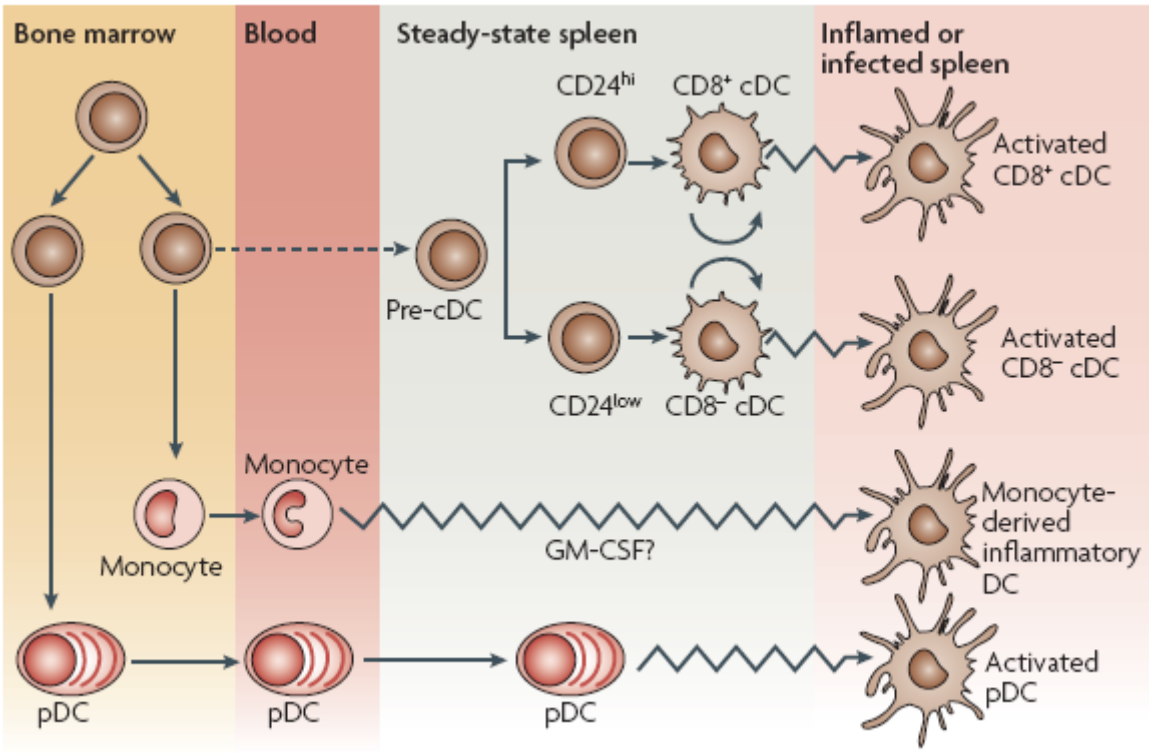
This steady-state situation changes after microbial stimulation or inflammation. In addition to full activation of the resident cDCs and the pDCs in the spleen, a new type of inflammatory DCs is then generated from monocytes, a DCs type that is not present in the steady state.

Careful studies of the kinetics of thymic cDCs development using parabiotic mice have also shown two patterns of thymic cDCs development, one in phase with the cyclical entry of T-cell precursors into the thymus and subsequent T-cell development, the other unrelated to T-cell developmental kinetics and indicative of a more continuous input from the bloodstream (Donskoy and Goldschneider, 2003).

Another example is represented by LCs, infact mouse skin seems to contain a significant reservoir of immediate LC precursors or immature LCs, most of which are normally

quiescent but are capable of local clonal expansion to produce LCs. These precursors can in turn be generated from blood monocytes after LCs depletion (Ginhoux et al., 2006).

These findings of a monocyte origin for the regenerating epidermal LCs population of mice *in vivo* fit well with findings from an *in vitro* human skin-reconstitution model, where $CCR2^{+}CD14^{hi}$ monocytes produced LCs (Schaerli et al., 2005). They are also in accordance with cell-culture systems, in which LCs can be generated from myeloid precursors, under the influence of cytokines that include GM-CSF and TGF-β.



from Shortman et al. Nat. Rev. Immunol. 2007

Fig. 6 Pathways to splenic DCs

1.5 Monocytes as DCs precursors

It has been described how experimental evidence obtained over the last decade has shown that the DCs system comprises a large collection of subpopulations, located in both lymphoid and non-lymphoid organs, and which are endowed with specific functions.

An essential problem in DCs immunobiology is the definition of the developmental pathways that lead to the generation of these distinct DC subpopulations

Data derived from several experimental approaches have established that DCs can be generated from myeloid or lymphoid hematopoietic progenitors through yet non-fully defined immediate DC precursor populations.

Except for pDCs, DCs differentiation does not occur in the BM, but in peripheral locations to which immediate DC precursors home and where they differentiate into specific DC subpopulations (Ardavin, 2003; Shortman and Naik, 2007).

Important issues that remain only partially solved, however, are the phenotypic and functional characterization of these immediate DC precursors, and how their differentiation is controlled under steady state and inflammatory and/or infectious conditions has not yet completely established.

Research in the last few years, using a variety of experimental systems, has contributed to the description of precursor populations for certain DC subsets, although the mechanisms controlling their recruitment and differentiation *in vivo* are not fully understood.

The best characterized of these potential immediate DC precursors, due essentially to hematological, rather than immunological, studies, are monocytes, although paradoxically, how their differentiation into DCs is controlled is largely unknown.

1.5.1 Monocyte subsets

Monocytes belong to the mononuclear phagocyte system, and were initially described as circulating precursors for tissue macrophages. Although the majority of murine monocytes are positive for the myeloid marker Gr-1, it was recently described a minor subset of monocytes that are Gr-1−, and represent around 20% of total monocytes. These two monocyte subsets also differ in the expression of molecules involved in leukocyte migration and homing: Gr-1^{+} monocytes are $CCR2^{+}$ $CX3CR1^{low}$ $CD62L^{+}$, whereas Gr-1^{-} monocytes are $CCR2^{-}$ $CX3CR1^{high}$ $CD62L^{-}$. Interestingly, Gr-1^{+} monocytes are recruited to inflammatory locations, whereas the Gr-1^{-} subset appears to be recruited to non-

inflammatory sites by a CX3CR1-dependent mechanism. Murine Gr-1^{+} and Gr-1^{-} monocyte subsets have been claimed to be functionally similar to the CD14^{+}CD16^{-} and CD14^{-}CD16^{+} human monocyte subpopulations, respectively (Geissmann et al., 2003).

1.5.2 Monocyte-derived inflammatory DCs in the mouse models

Although different research groups have addressed the identity of DC precursors in steady state, the characterization of DC precursors responsible for de novo DCs formation during infection has remained elusive.

A number of reports suggest that DC precursor recruitment and de novo DCs formation could also take place in inflammatory foci and lymphoid organs during infection (Martin et al., 2002; Serbina et al., 2003; Yoneyama et al., 2001).

In particular, studies describing the accumulation of DCs in lymphoid tissues during infection or showing in non-infectious inflammatory conditions that monocytes can differentiate into DCs suggest that monocytes can be identified as potential *in vivo* DC precursors during infection (Geissmann et al., 2003; Le Borgne et al., 2006; Naik et al., 2006; Randolph et al., 1999; Serbina et al., 2003).

Questions regarding the ontogenic relationship of DCs with monocytes and macrophages have been long-standing.

In the eighties it was known that monocytes continuously exit the bloodstream and enter body tissues, where many differentiate to macrophages, and that, since there was no net accumulation of macrophages in tissues during the steady state, monocytes entry into tissues might be just sufficient to replace dying macrophages, or monocytes might only transiently traverse tissues (van Furth and Cohn, 1968).

In the 1996 than it was discovered that, during resolution of inflammation, at least some monocytes leave tissues by migrating to draining lymph node (Bellingan et al., 1996).

However, the state of differentiation of monocyte-derived cells that arrive in lymph node remained a concept to characterized.

In 1998 for the first time it was observed that monocytes differentiated into DCs in an *in vitro* model of transendothelial trafficking without addition of exogenous cytokines, supporting the idea that monocytes could become DCs under physiologic conditions.

Monocytes that matured to DCs in this model did so most efficiently when they received a phagocytic stimulus during their transient residence in subendothelial matrix. These nascent DCs then trafficked across the endothelium in the ablumenal-to-lumenal direction

(Randolph et al., 1998), a movement that might mimic the migration of DCs from the periphery into the lumen of an afferent lymphatic vessel.

Prompted by these *in vitro* studies, one year later, was reported an indirect evidence of *in vivo* murine monocyte differentiation into DCs by using an experimental system based on the subcutaneous injection of fluorescent latex microspheres allowing to track the cells that had internalize them. These experiments showed that latex-containing $CD11b^+$ $F4/80^-$ cells, considered by the authors as inflammatory monocytes, were first detected at the injection site, to be found later in the draining lymph node, where they were described as $CD11c^+$ MHC II^+ DCs. Interestingly, these authors also addressed the differential potential of monocytes to differentiate into macrophages and DCs under the inflammatory conditions induced by the subcutaneous injection of latex microspheres. They concluded that around 25% of phagocytic monocytes differentiated into DCs and migrated to the draining lymph node, while the rest remained at the injection site and differentiated into macrophages (Randolph et al., 1999).

Using the same experimental model, in a later report was described that latex-containing lymph node DCs, claimed to derive from cutaneous inflammatory monocytes, express the lectin receptor DEC-205, a molecule expressed by dermal DCs and epidermal LC, both in the skin and after migration to the lymph node (Rotta et al., 2003).

However, in these experiments, was demonstrated that monocyte differentiation into DCs and subsequent migration to the draining lymph node was blocked if bacteria, or bacterial LPS were present in the injection area.

Thus, globally, the data derived from these reports suggest that *in vivo*, the differentiation of murine monocytes into macrophages or DCs must be subjected to a complex regulatory balance controlled by growth factors, inflammatory stimuli and pathogen compounds.

Nonetheless, in neither of these studies the population of inflammatory monocytes recruited to the skin, and considered as the putative precursors of the latex-containing lymph node DCs, was identified.

However, this issue has been addressed in a recent paper in which the experiments demonstrated that latex-containing monocytes that had differentiated into DCs, and migrated to the lymph node, expressed Gr-1, and therefore corresponded to the main circulating monocyte subpopulation (Qu et al., 2004).

In conclusion, it could be hypothesized that under physiological conditions similar to those created experimentally by the subcutaneous injection of latex microspheres, $Gr\text{-}1^+$ monocytes are recruited to inflammatory and/or infectious locations, where they

differentiate into DCs that migrate to the draining lymph node. The functional correlation between these monocyte-derived DCs migrating to the lymph node, and the lymph node DC subset derived from dermal DCs (Kamath et al., 2000), remains to be established. Consequently, their potential role in antigen processing, and subsequent induction of antigen-specific T cell responses, has to be also addressed.

The experimental models addressing the *in vivo* DC differentiation potential of monocytes discussed above, involved the migration of DCs derived from skin-recruited monocytes to the draining lymph node, through the afferent lymph vessels.

Data published on monocytes recruitment from the blood to the draining lymph node through high endothelial venules, during a skin inflammatory response, suggested the existence of an alternative physiological situation in which monocytes could differentiate into DCs. This process would occur after monocyte migration directly from the blood to the lymphoid organs, i.e. to the lymph nodes and mucosal lymphoid tissues through high endothelial venules, or to the spleen through the marginal sinus. In particular, it was showed that after adoptive transfer of CX3CR1$^{gfp/+}$ leukocytes by intravenous injection, Gr-1^{+} monocytes migrated into the lymph nodes draining the inflammed skin, through the high endothelial venules. This process was shown to be mediated by the MCP-1 chemokine, that was produced in the inflammed skin, transported to the lymph nodes through the afferent lymphatics and translocated to the luminal surface of the high endothelial venules.

Although the study described did not address the fate of the monocytes recruited to the lymph node, the hypothesis that under the experimental conditions considered in this report monocytes were induced to differentiate into DCs, was supported by data from two research groups (Palframan et al., 2001).

The first reported, by using an experimental system based on the intravenous injection of *Listeria monocytogenes*, that after recruitment to the spleen by a CCR2-dependent mechanism, monocytes differentiated into a specific DC subpopulation. Interestingly, these authors proposed that, rather than participating in the induction of pathogen specific T cell responses, this putative monocytes-deriveds DC subset could be involved in the innate immune response against *Listeria monocytogenes*, due to their TNFα- and nitric oxid-mediated microbicidal capacity. In this sense, the absence of this DC subset was shown to result in uncontrolled bacterial replication and host death, showing the importance of these monocytes-derived DCs for bacterial killing (Serbina et al., 2003).

In order to provide new insights on de novo DC differentiation during *in vivo* infection, it was also used a murine experimental model of cutaneous leishmaniasis induced by *Leishmania major* (*L. major*) injection.

Based on phenotypic studies, analysis of the kinetics of monocyte recruitment and differentiation into DCs, and monocytes transfer assays, these data demonstrated that during *L. major* infection, two de novo formed DC subsets were found in the popliteal lymph nodes (PO-LN). These included DCs derived from monocytes recruited to the dermis that differentiated locally and migrated to the PO-LNs (dermal mo-DCs) and from monocytes recruited to the PO-LN where they differentiated into DCs (LN mo-DCs). Dermal mo-DCs and LN mo-DCs were the DC subsets predominantly infected by *L. major*. Among de novo formed monocytes-derived DCs, dermal mo-DCs displayed a higher capacity to produce IL-12 and to stimulate *L. major*-specific T cells, suggesting their essential role in the induction of protective Th1 responses against Leishmania.

Thus, the demonstration of monocytes differentiation potential into DCs during *in vivo* infection and of local DCs differentiation in inflammatory foci suggested that the novo formed monocytes-derived DCs are essential in T cell immunity against pathogens (Leon et al., 2007).

So, a large number of experimental evidences, derived from the mouse models, demonstrated that monocytes are capable to differentiate into DCs, even if some recent papers reported that although monocytes appeared to be dedicated to DCs replenishment of nonlymphoid organs, such as the intestinal lamina propria and the lung, splenic DCs seem to arise from local precursors without a monocytic intermediate (Varol et al., 2007).

1.5.3 Differentiation of human monocytes into DCs

As above mentioned, in literature are reported a lot of mouse models that demonstrate how monocytes can differentiate into DCs also during infection or noninfectious inflammatory conditions, on the other hand, in humans it has not been defined which DC subset originates from monocytes.

Experiments of transendothelial migration of DCs generated from monocytes (Randolph et al., 1998) demonstrated that human monocytes have two potential fates associated with distinct patterns of migration, infact in the presence of endothelial cells grown on an extracellular matrix, the differentiation of monocytes diverged along two distinct pathways toward DCs or macrophages. DCs arisen from monocytes that migrate across endothelium

in an ablumental-to-lumenal direction, whereas macrophages developed from monocytes that remain in the subendothelial matrix. Despite that, it has not been conclusively established which are the processes that promote monocytes differentiation into DCs or into macrophages (Krutzik et al., 2005).

In culture, human monocytes acquire a macrophage phenotype both in the presence and in the absence of added cytokines, such macrophage colony-stimulating factor (M-CSF) (Gangenahalli et al., 2005), thus differentiation of monocyte into macrophage seems to represent a default differentiation program of monocytes upon extravasation (Lewis et al., 1999).

On the contrary it is well known that a defined cytokine cocktails are required for monocytes to differentiate into DCs starting from the 1994, when it was demonstrated that human monocytes could be directed to develop into potent immunostimulatory DCs when cultured in the presence of GM-CSF and IL-4 (Bender et al., 1996; Comes et al., 2002; Mohamadzadeh et al., 2001; Romani et al., 1996; Sallusto and Lanzavecchia, 1994; Santini et al., 2000; Zou and Tam, 2002).

1.6 Cytokines in the generation and maturation of monocytes derived DCs

With half of the circulating monocytes leaving the bloodstream each day, monocytes could constitute a considerable systemic reservoir of myeloid precursors. This notion is supported by studies with cytokine-driven culture systems that allow *in vitro* differentiation of monocytes into DCs (Chapuis et al., 1997; Geissmann et al., 1998; Kiertscher and Roth, 1996; Randolph et al., 1998; Sallusto and Lanzavecchia, 1994).

The combination of GM-CSF with IL-4 or TNF-α represents, by far, the most extensively characterized and utilized cytokine combinations that have been identified to support DCs differentiation *in vitro*. GM-CSF (as well as stem cell factor - SCF, and Flt3L), together with TNF-α, have been well described as being active in the differentiation of $CD34^+$ to cells with DCs phenotype (Caux et al., 1992; Santiago-Schwarz et al., 1992), whereas GM-CSF and IL-4 seem to be sufficient for culture of monocytes to DCs (Bender et al., 1996; Romani et al., 1996).

GM-CSF has been shown to function by inducing DCs progenitor expansion as well as promoting differentiation and survival of DCs (Markowicz and Engleman, 1990). In addition to inducing differentiation, TNF-α has been shown to strongly potentiate the

proliferation of $CD34^+$ HPCs induced by either IL-3 or GM-CSF (Caux et al., 1992; Caux et al., 1991) and to be required for the clonogenic growth of pure human DC colonies in the presence of GM-CSF (Young et al., 1995). Conversely, IL-4 functions to inhibit macrophage colony formation in addition to its role in inducing DCs growth and maturation from monocytes (Romani et al., 1994).

In particular treatment with GM-CSF and IL-4 (or TNF-α) produce DCs with an immature phenotype that maintain the antigen capturing and processing capacity characteristic of immature DCs *in vivo* and display inefficient antigen presentation. Upon treatment with a number of maturative factors DCs acquire the characteristics of mature DCs, including morphology, loss of monocyte markers, loss of antigen uptake, upregulation of accessory molecules, translocation of MHC II to cell surface and finally a capacity to efficiently prime naive T cells (Shortman and Caux, 1997).

Although the majority of research and clinical protocols used GM-CSF in combination with either TNF-α or IL-4 to differentiate DCs from precursor, a wide variety of conditions have been reported to be able to support DCs generation (Zou and Tam, 2002).

About that, it was found that IFN-α induces DC differentiation as well as IL-4.

Although IFN-DCs had a more mature immunophenotype than IL-4-DCs, showing higher expression of CD80, CD86, and CD83, they still preserved comparable endocytic and phagocytic capacities and responsiveness to maturation stimuli (Santini et al., 2000).

IFN-DCs had strong antigen-presenting capacity, inducing intense proliferation of T cells to alloantigens or influenza virus. Moreover, IFN-DCs produced lower levels of IL-12p70 and higher levels of IFN-α, IL-4, and IL-10 than IL-4-DCs. As a consequence of this different pattern of cytokine secretion, IFN-DCs induced T cells to produce type 1 (IFN-γ) and type 2 (IL-4 and IL-10) cytokines, and as expected, IL-4-DCs induced only Th1 differentiation (Della Bella et al., 2004).

In particular, recently, it was described the differentiation of monocytes into a new of type of DC using IFN-β and IL-3 (IFN-β/IL-3 DCs). These cells express higher membrane levels of CD14 and lower levels of CD1a than other types of DCs and secrete lower levels of IL-12 in response to LPS. Interestingly IFN-β/IL-3 DCs induce strong proliferative response in mixed leukocyte reactions and are particularly efficient at eliciting IFN-γ and IL-5 production by allogeneic helper T cells. Furthermore their results demostrated that IFN-β/IL-3 DCs were very efficient at presenting exogenous peptides and were able to induce activation of antigen-specific CTL (Buelens et al., 2002).

According to some different reports also other cytokines, such as IL-13 and IL-15 contribute to the generation of DCs from monocytes.

In particular, IL-13 shares properties with IL-4 even if its receptor does not involve the common gamma chain present in the receptor complex of IL-4 and other cytokines.

Thus it was demonstrated that DCs cultivated in IL-13 and GM-CSF had phenotypic and functional characteristics comparable to IL-4 DCs. IL-13 DCs, showing a morphology and a membrane phenotype typical of DC (MHC II^+; $CD1a^+$; $CD14^-$; $CD3^-$; $CD20^-$), were also similar to IL-4 DCs in terms of macropinocytosis, stimulatory capacity of cord blood lymphocytes in MLR, and responsiveness to chemotactic signals (Piemonti et al., 1995).

Also IL-15 had been indicated as an important cytokine in the pathway of monocyte differentiation into DCs infact monocytes cultured with GM-CSF and IL-15 differentiate into $CD1a^+HLA\text{-}DR^+CD14^-$ DCs (IL15-DCs). Agents such as LPS, TNF-α, and CD40L induce maturation of IL15-DCs to $CD83^+$,$DC\text{-}LAMP^+$ cells. IL-15 DCs are potent antigenAPCs able to induce the primary (MLR) and secondary (recall responses to flu-matrix peptide) immune responses. As opposed to cultures made with IL-4DCs, a proportion of IL-15 DCs expresses LC markers: E-Cadherin, Langerin, and CCR 6. Accordingly, IL-15 DCs, but not IL-4 DCs, migrate in response to macrophage inflammatory protein (MIP)-3α/CCL20. However, IL-15 DCs cannot be qualified as "genuine" LC because, despite the presence of the 43-kD Langerin, they do not express bona fide Birbeck granules (Mohamadzadeh et al., 2001).

Even if a lot of *in vitro* methods to generate human DCs from monocytes are available, however, experimental data on monocyte differentiation and function *in vivo* are scarce.

Several cell types have been indicated as a possible source of cytokines capable of inducing monocyte differentiation into DC *in vitro*, including ephitelial cells, mastocytes and NKT (Brossart et al., 1998; Hegde et al., 2007; Wirths et al., 2002; Zhang et al., 2007).

For example it has been demonstrated that keratinocytes (KCs), that form the epidermal sheet, are able to produce GM-CSF and IL-15 and, so, to control the differentiation of a subpopulation of DCs with the typical phenotype and function of LCs (Mohamadzadeh et al., 2001). Also the airway epithelial cells, in response to inflammatory stimuli, have been demonstrated to have the capacity to differentiate monocytes into functional DCs with a process substantially mediated by epithelial-derived IL-15.

Moreover, recently, NKT cells have been indicated as one of the sources that produces, *in vivo*, cytokines capable to induce DCs differentiation (Hegde et al., 2007).

In particular, because it is well known that stimulation of human NKT cells directly *ex vivo* led the production of a wide variety of cytokines, including GM-CSF, IL-4, and IL-13 it has been hypothesized that interactions with monocytes might activate NKT cells to produce factors that influence monocyte differentiation.

Monocytes and NKT cells share expression of the chemokine receptors CCR5, CCR2, and CX3CR1, and both are recruited to sites of tissue inflammation (Kim et al., 2002; Yamazaki et al., 2001).

In addition, monocytes and NKT cells are present in blood, lymphoid tissues, and BM under non inflamed conditions (Matsuda et al., 2000). Thus, NKT cells and monocytes are likely to co-localize *in vivo* under normal and inflammatory conditions and may undergo self- or foreign antigen-driven interactions in either of these contexts.

Although several cell types have been indicated as a possible source of cytokines necessary to induce monocyte differentiation into DCs *in vitro*, the stimulus and context in which all the described cells would promote human monocytes differentiation into DCs instead of macrophages could not still be definitely defined.

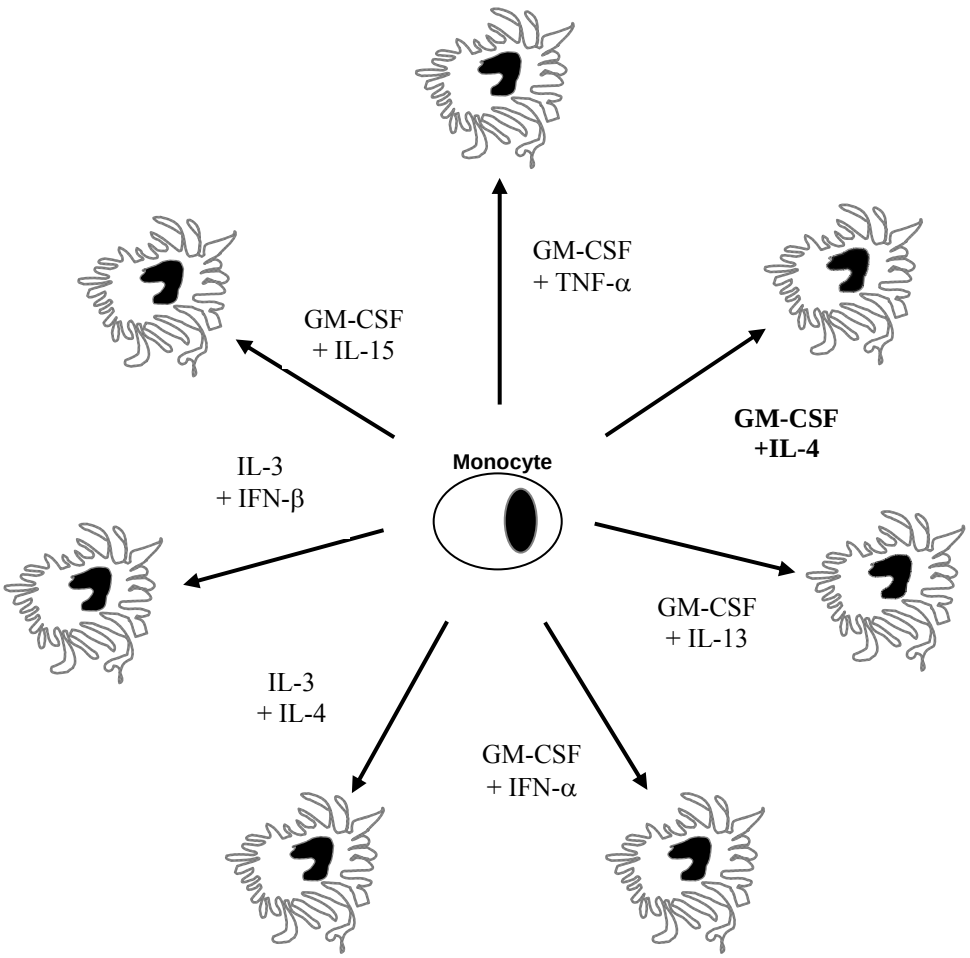


Fig. 7: Schematic representation of the different ways for the *in vitro* differentiation of human monocyte into DCs.