

Current Understanding of the Role of Dendritic Cells and Their Co-Stimulatory Molecules in Generating Efficient T Cell Responses in Lepromatous Leprosy

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Abstract: Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* that needs continued vigilance, particularly for detection and treatment of hidden and undiagnosed cases. Cell-mediated immunity in leprosy has been identified as a key mechanism for the understanding of this disease. The dendritic cell (DC) is the most potent professional antigen-presenting cell and recently has been the focus of much interest as the main initiator of naive T-cell responses to several antigens. For rational use of DCs in adjuvant therapy of lepromatous leprosy, the patterns of synthesis and secretion of cytokines by DCs during some mycobacterial infections must be better understood. Therefore, the aim of this review is to illustrate some of the cellular events involved in the immune recognition of the antigens during leprosy and the role of antigen-presenting cell (DC) and their co-stimulatory molecules, such as DC-SIGN, CD-40, B7-1 and B7-2, in generating efficient T-cell responses.

Keywords: Leprosy, macrophages, dendritic cells, co-stimulatory molecules, antigen presentation.

1. INTRODUCTION

Leprosy is a chronic infection disease that primarily affects the skin, mucous membranes and nerves [1-3]. Currently, 2 to 3 million individuals are infected with *Mycobacterium leprae*, the etiologic agent of leprosy, and the detection of new cases continues to increase, reaching more than half million cases each year [4,5]. In addition, no useful vaccines have been developed, and no successful immunotherapeutic tools against leprosy are available. Leprosy presents a spectrum of clinical manifestations that are directly related to the different kinds of immune responses to the pathogen [6]. In the variations of the disease, patients with tuberculoid leprosy display pronounced immune response that restricts the growth of the pathogen, while, in contrast, patients with lepromatous leprosy present extreme susceptibility to *M. leprae* infection. Since the immune system in these two groups reacts completely differently on reinfection or *in vitro* antigen challenge, differentiation into the two major leprosy forms has been suggested. Currently, there is limited information on the primary immune response of the host during the distinct forms of leprosy and resistance or susceptibility to *M. leprae*. The presence of mRNA encoding for Interleukin-12 (IL-12) and gamma-interferon (IFN- γ) in lesions of tuberculoid patients has been described while IL-

4, IL-5 and IL-10 mRNA were found in lesions of LL patients [7]. These findings suggested that the differentiation of leprosy into a tuberculoid or lepromatous leprosy form is correlated with the presence of T helper-1 (Th-1) versus T helper-2 (Th-2) like cytokines. The determination of the T-cell response profile (whether Th-1 or Th-2) depends primarily on the nature of antigen presenting cells (APC). Therefore, in this paper, we review some of the antigen presentation issues including the role of DC-T cell interaction during immune response to leprosy infection.

2. FROM THE BEGINNING

The success of T cell mediated immune response against intracellular bacteria is determined by the efficiency of phagocytic cells in providing both MHC class I- and II-restricted antigen presentation functions. These APCs stimulate CD4+ T cells, specific for bacterial antigens. Phagocytic cells harboring replicative intracellular bacteria produce and release cytokines such as IFN- γ , which increases their bacteriostatic and bactericidal functions. In this context, macrophages [8] should represent a privileged APC partner for T cells. Moreover, activated macrophages produce IL-12 [9], which plays a pivotal role in host resistance to bacterial infection by stimulating NK cells, IFN- γ production, and Th1 responses [10]. However, DCs have been reported as the most potent APC for priming naive T cells [11], also producing IL-12 after contact with microbial stimuli [8]. Consequently, DCs are highly efficient in inducing antiviral and antitumor immune responses [12]. During the last decade, published data strongly suggest an effective role of DCs in

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controlling bacterial infection [8,13-17]. However the interaction of DCs with *mycobacteria* is still not completely understood.

3. THE ESSENTIAL NEED FOR ANTIGEN PRESENTATION

It is widely known that T cells require 2 types of signals for optimal activation [18]. The first signal is provided by the antigenic peptide binding to the T cell receptor (TCR) and simultaneously to major histocompatibility complex (MHC) on antigen-presenting cells (APCs). This feature provides antigen-specificity to the immune response. The second "costimulatory" signal is provided by binding of specific receptors on T cells to ligands on APCs. Literature widely describes the costimulatory pathway provided by binding of CD28 on T cell to B7-1 (CD80) and /or B7-2 (CD86), respectively on dendritic cells (DCs) and monocytes / macrophages-APCs [18].

DCs are important sentinels of the immune system and are, therefore, essential for the onset of a strong immune response against several incoming pathogens. Their function involves the capture and process of the antigens into peptide form in MHC class I and MHC class II molecules at peripheral tissues. On antigen capture and processing DCs mature and start migrating to the draining lymphonodes to present the antigenic peptides to T cells to elicit an immune response.

Studies of DC-T cells interactions have identified a novel receptor on DCs named DC-SIGN [19]. This receptor is a type-II mannose binding lectin that starts the initial contact formation between DCs and resting T cell by binding through ICAM-3 on a T cell. The identification of this receptor on DCs has shed new light on the mechanism of several pathogens that cause infection. DC-SIGN has been considered a monocyte-derived dendritic cell (MDDC) differentiation marker, especially on IL-4-treated monocytes [20]. Recent data show that DC-SIGN may function simultaneously as an adhesion receptor and as a phagocytic pathogen-recognition receptor, similar to the Toll-like receptors [21,22].

4. SOME FEATURES OF DCs

Dendritic cells (DCs) were first described by Steinman and Cohn (1973) in suspension cultures prepared from mouse spleens [23]. DCs are functionally divided into two groups: a) immature DCs localized in blood and peripheral organs and characterized by a distinct ability to internalize and process various antigens (Ags) through macropinocytosis and using the mannose receptor pathway [24,25], and b) mature DCs that presents a strong ability to prime and stimulate specific T cells to Ag and allogenic major histocompatibility complex (MHC) molecules. Several studies have described common precursors for DCs and phagocytic myeloid cells in mice and humans [26-28]. Such studies have proposed a dual role for DCs in the amplification of innate immune responses and in the activation of adaptative immune responses [29].

DCs maturation is induced by the exposure of immature DCs to inflammatory cytokines such as IL-1, IL-6 and Tumor Necrosis Factor-alpha (TNF). Such induction is followed by the increase of the expression of various co-

stimulatory molecules such as CD40, B7-1(CD80) and B7-2 (CD86) [30]. Expression of these co-stimulatory molecules by DCs plays a key role in generating efficient T-cell responses [18]. However, in the presence of some cytokines such as IL-10, DCs maturation is suppressed [31,32].

DCs are potent APC and they are now being focused as the main initiator of naïve T-cell responses to several antigens [33]. Since DCs are also efficient in capturing protein antigens *in situ* [21,22, 34-36] these features suggest that these DCs are biological adjuvants.

5. DENDRITIC CELLS AND SOME MYCOBACTERIAL AND VIRAL INFECTIONS

Sieling *et al.* (1999) reported the presence of CD1⁺ CD83⁺ monocyte-derived dendritic cells in tuberculoid lesions of leprosy patients, while Yamuach *et al.* (2000) showed T cell expression in tuberculoid leprosy lesions [37,38]. These reports strongly suggest the direct involvement of DCs in protective immunity against *M. leprae* infection. Our group observed a more efficient APC function for monocyte-derived DCs isolated from lepromatous leprosy patients when compared to autologous monocytes in T cell responses to *M. leprae in vitro*. This event was followed by B7 expression on different APC populations such as DCs and monocytes in different forms of leprosy, supporting a costimulatory role of B7 molecules in T cell activation in leprosy [39].

Recently, Krutzik *et al.* [40], reported that Toll-like receptor (TLR) activation of human monocytes induces differentiation in two different types of cells: DC-SIGN + CD16+ macrophages and CD1+ DC-SIGN-dendritic cells. DC-SIGN + macrophages were detected in lesions and after TLR activation in all leprosy patients whereas CD1b+ dendritic cells were not detected in lesions or after TLR activation of peripheral monocytes in patients with the progressive lepromatous form, except during reversal reactions in which the bacilli were cleared by Th-1 responses. In tuberculoid lepromatous lesions, DC-SIGN +cells were positive for macrophage markers, but negative for DCs markers. Most recently, Soilleux, E *et al.* (2006) [41] demonstrated high DC-SIGN expression in lepromatous but not borderline tuberculoid leprosy in both HIV-positive and HIV-negative patients. They demonstrated that DC-SIGN may be induced on macrophages in lepromatous leprosy and may then contribute to *M. leprae* entry into these cells. Another, interesting finding was recently reported by Makino *et al.* (2005) [42], who demonstrated that (MMP)-II (major membrane protein) from *M. leprae* is able to stimulate DCs to activate memory T cells from paucibacillary leprosy patients. Interestingly, memory T cells from multibacillary leprosy patients, which are normally believed to be anergic, were activated by MMP-II-pulsed DCs similarly to those from healthy individuals.

Current literature suggests that DCs also strengthens the cellular immune response against other mycobacterium infections [43-48]. Macrophages and DCs seem to have different functions in the case of infection with *M. tuberculosis*, since macrophages secrete the proinflammatory cytokines TNF alpha, IL-1 and IL-6 and induce granulomatous inflammatory response, while DCs are primarily involved in inducing anti-mycobacterial T cell immune response [43] by

producing IL-12 and IFN- γ . Consistent with that purpose DCs strongly express: a) class II DR, DQ molecules and CD83, b) costimulatory molecules such as CD40, CD80 and CD86, and c) adhesion molecules such as CD58 and CD54. By contrast, interleukin-18 and IL-10 are secreted by macrophages at same time that a slight down-regulation of MHC class II DQ expression is observed.

Geijtenbeek *et al.* (2003) [49] demonstrated that DC-SIGN is an important receptor on DCs that captures and internalizes intact *Mycobacterium bovis bacillus* Calmette-Guerin (BCG) through the mycobacterial cell wall component ManLAM. It appears that *Mycobacterium tuberculosis* targets DC-SIGN both to infect DCs and to down-regulate DC-mediated immune responses. DC-SIGN was also implicated in the infection of human monocyte-derived DCs by *M. tuberculosis* [50]. However, DC-SIGN is also reported as a pattern recognition receptor that discriminates among *Mycobacterium* species through selective recognition of the mannose caps on LAM molecules [51].

The role of DCs has been investigated during the development of several diseases and in the host defense response against many pathological agents, especially human T-lymphotropic virus type 1 (HTLV-1) [52,53]. It has been suggested that monocytes infected by HTLV-1 cannot properly differentiate into DC leading to DC dysfunction in Adult T cell leukemia patients.

Interestingly, DC-SIGN not only helps capture of HIV-1 but also protects the virus in early endosomes, allowing HIV-1 transport within DCs to lymphoid tissues, where it enhances trans infection of T cells [54]. DC-SIGN works as a receptor to HIV-1 that binds to HIV gp120 and facilitates DC-induced HIV transmission of T cells [21].

6. DENTRITIC CELLS: ONE LINK BETWEEN ADAPTATIVE AND INNATE IMMUNE RESPONSES THROUGH IL-18 AND IL-12 PRODUCTION

Interleukin-18 is a pro-inflammatory cytokine that enhances innate and specific Th-1 immune response [55]. This cytokine is important for the generation of protective immunity to *Mycobacteria* [56,57]. It has been reported that the IL-18 precursor form is constitutively produced by DCs although the secretion of the biologically active form requires CD40 engagement on DCs [58]. Another cytokine, IL-10, has been shown to inhibit the activation of macrophages [59-61] and DC differentiation [62,63]. Interleukin-10 also inhibits IFN- γ production and Ag-specific proliferation of Th-1 [64]. It has been suggested that IL-18 is a weak inducer of IFN- γ synthesis from T cells without the cooperation of IL-12 or IFN- γ [65].

Interleukin-12 plays a pivotal role in the control of mycobacterial infection [66,67] since it is involved in both innate and acquired immunity level through IFN- γ production by natural killer (NK) and Th1 cells, respectively [9]. A new DC lineage has recently been identified, named interferon-producing killer DCs (IKDCs), distinct from conventional DCs and plasmacytoid DCs and with the molecular expression profile of both natural killer cells (NK) and DCs. They produce a high level of type I interferons (IFN) and interleukin-12 or IFN- γ depending on activation stimuli. On stimulation, ligands for Toll-like receptor (TLR)-9, IKDCs kill typical NK target cells using NK-activating receptors. Their

cytolytic capacity subsequently decreases, associated with the loss of NKG2D receptor (KlrK1) and its adaptors, Dap 10 and Dap 12. As cytotoxicity is lost, DC-like antigen-presenting activity is gained, associated with upregulation of MHC-II and costimulatory molecules, which generally distinguish them from classical NK cells. With their ability to kill target cells, followed by their ability to present antigen, IKDCs provide a link between innate and adaptative immunity [68].

The *in vitro* capacity of DCs to produce IFN- γ in response to IL-12 [69], suggests that DCs may participate directly in the control of bacterial infections. Literature reports macrophages as a source of IL-12 but some studies have demonstrated “ that DCs and not macrophages are the major source of IL-12 after infection with *Toxoplasma gondii* [8]. Several *M. tuberculosis*-derived lipoproteins induce IL-12 production in macrophages *via* Toll-like receptor 2 [70], an event that is also implicated in macrophage activation by lipoarabinomannans [71,72]. On exposure to BCG, IL-12 production by activated macrophages is apparently dependent on IFN- γ R expression [73]. This result suggests that production of IL-12 is not an early event of mycobacterial infection. After administration of BCG in mice, Jiao *et al.* (2002) [74] observed that DCs are more effective producers of IL-12 p40 in comparison to macrophages. Interestingly, IL-12 p70 production by murine and human DCs can be induced *in vitro* by mycobacteria [75-77].

Considering all these reports a hypothetical model for the interaction among DC, macrophages and T cells for tuberculoid leprosy can be proposed to explain the Th-1 response profile observed in this form of leprosy (Fig. 1). Macrophages infected by *M. leprae* express MHC-II, CD80 and CD86 on the cell surface simultaneously with the production of TNF alpha, IL-6, IL-1, IL-12 and IL-18. The cytokines IL-12 and IL-18 may synergistically act on T lymphocytes, leading to the IFN- γ secretion. In addition, *M. leprae* stimulated DCs secrete considerable levels of IL-12 and IFN- γ , in response to a less pronounced IL-12 production by *M. leprae* stimulated macrophage. Consequently, both IL-12 and IFN- γ (mainly produced by DC) act synergistically on T cell to induce IFN- γ secretion, leading to the activation of macrophages (without DC-SIGN expression) and production of Reactive Oxygen Intermediates (ROI) and Nitric Oxide (NO); these radicals and NO kill the *M. leprae* that is observed in the tuberculoid form leprosy. The high potency of DCs as APC would also be supported by the number of surface molecules displayed by these cells (MHC-II, CD 80, CD86, DC-SIGN, CD40, CD83, CD54 and CD58) that, as a whole, could amplify the immune response against *M. leprae* (Fig. 1).

In contrast to the tuberculoid form, there is a low level or even lack of expression of some signal molecules of the immune system in lepromatous leprosy that may justify the defective T cell immune response observed. On the other hand, it would be possible that in lepromatous leprosy, IL-4 secreted by Th-2 cells may induce DC-SIGN expression on macrophages and, thus, enhance *M. leprae* entry into this cell through DC-SIGN pathway. Concurrently, IL-10 produced by macrophages would convert DCs to macrophages, which allows unlimited *M. leprae* entrance to this cell as shown in Fig. 2.

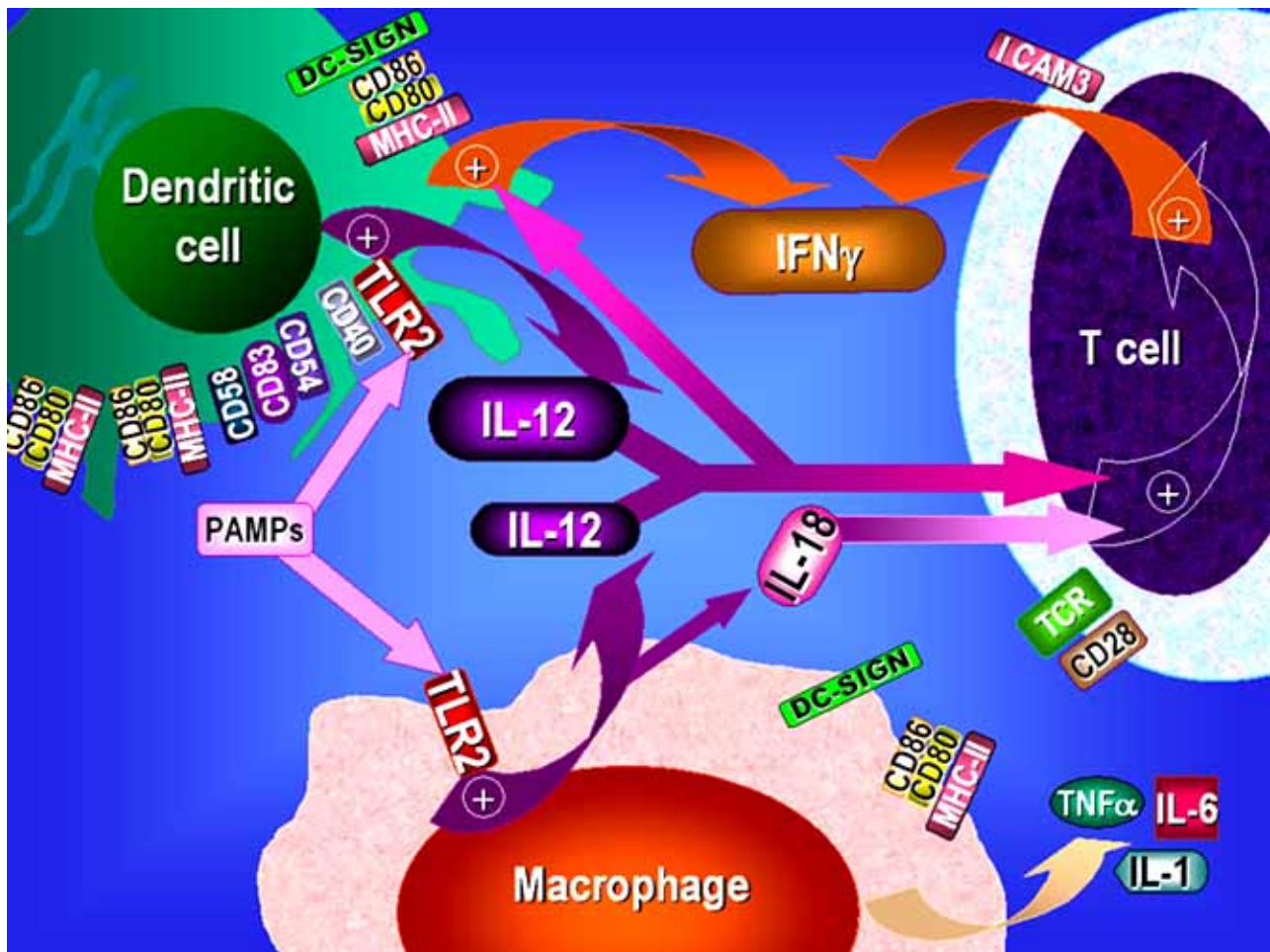


Fig. (1). DCs as determinant factors for the efficiency of T cell immune response. Initially antigen presenting cell-APC (DCs or macrophages) are activated by PAMPs (Pathogen Associated Molecular Patterns) through TLR2. Then, the expression of surface molecules as MHC-II, CD80 and CD86 on APCs increases in a higher level on DCs than macrophages. Simultaneously, several cytokines (TNF alpha, IL-6, IL-1, IL-12 and IL-18) are secreted. IL-12 and IL-18 act synergistically on T cell leading to the IFN γ secretion. In addition, IL-12 produced by macrophages also induces DCs to produce IFN γ . Different APCs can be activated to express costimulators for Th1 type cells and therefore this model could be applied to the efficient cell immune response against *M. leprae* observed in tuberculoid leprosy.

In addition, TLR-2 mutations may lead to LIR gene expression that would shift IL-12 production towards IL-10 production (Fig. 2). Thus IL-10 would downmodulate the innate immune response and consequently *M. leprae* active replication by decreasing ROI and NO production by macrophages. Taken together, these sequential events would lead to the severe susceptibility to *M. leprae* observed in patients with lepromatous leprosy.

7. DCs: BACTERICIDAL AND BACTERIOSTATIC ACTIVITY

Three major pathways of antigen uptake have been described in DC: (i) phagocytosis, (ii) receptor-mediated endocytosis and (iii) macropinocytosis [12]. Conventional phagocytosis is the main entry for microorganisms, including mycobacteria [29]. However, this activity can be associated with bactericidal and/or bacteriostatic mechanisms against invasion in phagocytic cells.

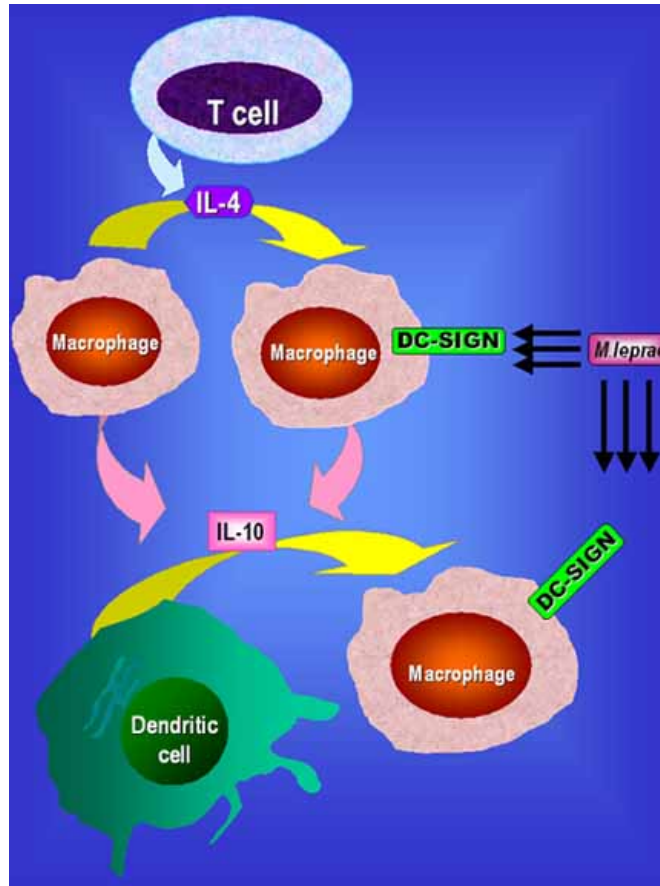
Recent studies indicate that DCs have only poor bactericidal activity, resulting in *M. tuberculosis* growth in human and murine DCs *in vitro* [46,76]. However, the antimicrobial

activity of DCs widely varies depending on their maturity and microenvironment. Interestingly, *mycobacterial* growth is inhibited in murine DCs treated with IFN- γ and LPS [76] while IL-10 may convert human DCs to macrophage presenting anti-mycobacterial activity [44]. Since Ag presentation by infected DCs is not a long term event, possibly due to some alternative mechanism, DCs could also represent a reservoir of mycobacteria therefore the lack of DCs mediated mycobactericidal activity could explain the persistence of the BCG bacilli within the DCs population *in vivo*. According to Jiao *et al.* [74] DCs are rapidly infected by BCG *in vivo* and mycobacteria are still present 2 weeks after infection. Although BCG survives in the cells, it seems that DCs control BCG growth *in vivo*, suggesting the presence of bacteriostatic but not a bacteriolytic activity.

8. DCs AND THE EXPRESSION OF TOLL-LIKE RECEPTORS (TLR): A KEY ROLE IN INNATE IMMUNE RESPONSE AGAINST MYCOBACTERIA

DCs are characterized by two essential roles: (i) amplification of innate immune responses and (ii) activation of the

A



B

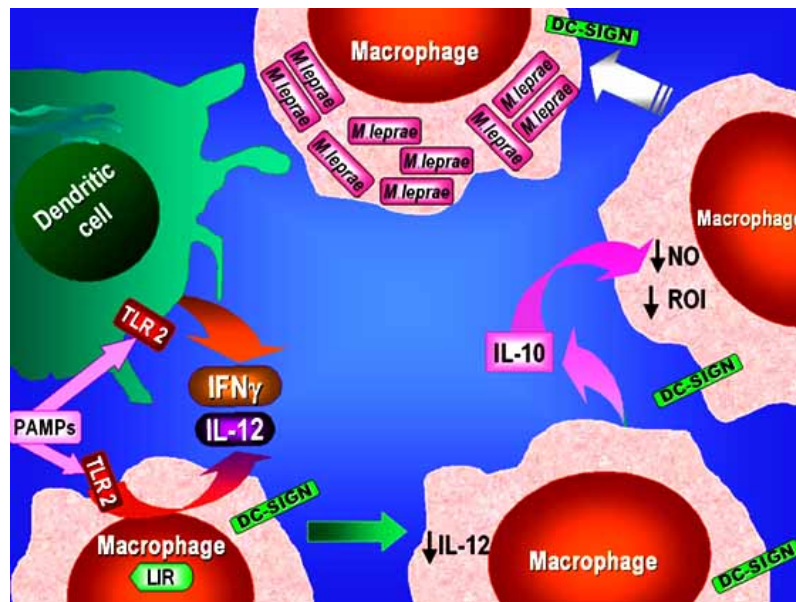


Fig. (2). A different pathway leading to the inefficient cell-immune response against *M. leprae* proposed for lepromatous leprosy. **(A)** IL-4 could induce DC-SIGN expression on macrophages. At the same time IL-10 could convert DCs in macrophages. Consequently *M. leprae* dramatically get into macrophages. **(B)** The LIR gene expression shifts the IL-12 production to IL-10 production by macrophages. This last cytokine inactivate macrophages by reducing Reactive Oxygen Intermediates (ROI) and Nitric Oxide (NO) production. The absence of these molecules inside macrophages allows the intense *M. leprae* proliferation observed in lepromatous leprosy.

adaptive immune responses. The early or innate response to infections by pathogens was considered as a non-specific mechanism of host defense until 1996, when a protein from *Drosophila* named Toll, was shown to be required not only during flies embryogenic development but also for an effective immune response against the fungus *Aspergillus fumigatus* [78]. Since then, significant advances in our understanding of the function of Toll proteins have been made including their important role in innate immunity. Currently, eleven human Toll-like receptors (TLR1-TLR11) have been identified in 12 mouse TLR. Toll-like receptors are type I transmembrane proteins expressed primarily in immune cells which are involved in the first line defense expressed by macrophage, DCs, mucosal epithelial cells, neutrophils and dermal endothelial cells [79]. They belong to the class of pattern recognition receptors (PRR), capable of triggering responses to different organisms such as viruses, bacteria, protozoa and fungi, through detection of the pathogen-associated molecular patterns (PAMPs). This mechanism uses a highly conserved signal transduction pathway that is similar (identical) in plants, drosophila, nematode, avian and mammals [80,81]. In vertebrates, the main function of the innate immune machinery is to detect the presence of PAMPs on invading microorganisms and to initiate production of reactive oxygen intermediates (ROI), inflammatory cytokines, interferon and chemokines as protective measures to defend the host. Up-regulation of these molecules is probably necessary for initiating and triggering a signaling cascade in cells for development of an antigen-specific adaptive immune response. During the last decade, after association of Toll-like receptors (TLRs) and class II MHC antigens expression by DCs and cytokine production, it was noticed that DCs might play a key role during initiation of innate immune responses [16,29,82,83]. These cells have been shown to respond to a wide variety of microorganisms through their TLRs [84].

The Toll-like receptor 2 (TLR2) is especially critical for development of the immune response against mycobacterial infections. Mutations in TLR2 lead to susceptibility to severe forms of mycobacterial infection. Interestingly, according to Kang and Chae (2001) [85] lepromatous leprosy patients display a conformational polymorphism in the intracellular domain of TLR2, which interacts with several bacterial structures such as lipoproteins, peptidoglycans, and lipoteichoic acid. Two polymorphisms of the TLR-2 gene, Arg 753, Gln and Arg 766 have been linked to a higher incidence of sepsis in a white population and of lepromatous leprosy in an Asian population, respectively [86]. Interestingly, these authors did not find any association with the tuberculoid form of leprosy. In fact it was the first evidence for correlation of a mutation in the intracellular domain of TLR-2 with susceptibility to lepromatous leprosy. More recently, the same authors [87] reported that monocytes obtained from leprosy patients with the TLR2 mutation are less responsive to cell lysate from *M. leprae* when compared to monocytes from patients without the mutation. In addition, the mutated samples showed significantly lower serum levels of IL-12, in comparison with TLR2 wild-type samples, associating the conformation of the intracellular domain of TLR2 with IL-12 production in leprosy. In mice, TNF alpha production in response to *M. leprae* was absent in TLR2-deficient macrophages while activation of NF-kappa beta by human Arg 677

Trp TLR2 was abolished in response to *M. leprae* and *M. tuberculosis* [88].

In a study of caucasian leprosy patients, no individuals carried the Arg677TrpSNP, while 9.4% of the study population was found to be heterozygous for the Arg753Gln polymorphism. Detection of this polymorphism among patients with different ethnicities may yield important information regarding to differences of risk profiles of susceptibility to bacterial infections [89].

In addition, TLR2 expression also seems to be implicated in leprosy nerve lesions. Nerve damage is a clinical feature of leprosy and a major source of patient morbidity. According to Oliveira *et al.* [90] *M. leprae* ligands induce Schwann cells apoptosis through TLR2 binding and, consequently, triggers the innate immune response that contributes to the nerve injury.

Apparently the expression of some genes presents characteristic patterns in different clinical forms of leprosy. Genes of the leukocyte immunoglobulin-like receptor (LIR) family are more expressed in lesions of lepromatous patients presenting the disseminated form of the infection. In functional studies, LIR-7 suppressed innate host defense mechanisms by shifting production of IL-12 toward IL-10 in monocytes. This event blocks the antimicrobial activity triggered by TLR [91]. Therefore, these authors conclude that gene expression profiles may be useful in defining clinical forms of disease.

Altogether, these studies show the importance of DCs in the efficiency of T cell immune response (Fig. 1). This is reinforced by the effects of TLR-2 mutations in the severeness of the clinical form of leprosy, involving the shift of macrophage activity from IL-12 toward IL-10 production (Fig. 2).

9. DCs AND LANGERHANS CELLS

Langerhans cells (LCs), one of the cell populations within the epidermis, contribute to the initiation of primary T cell immune responses [92-94]. LCs take up and process antigens that enter through the skin and migrate from the epidermis into the dermis. After that, they go to the regional lymph nodes *via* lymphatic vessels. During this process, LCs undergo maturation and acquire the potential to activate antigen-specific naïve cells. Human LCs are of hemopoietic origin, but cytokine regulation of their development is not fully understood. Notch ligand delta-1 is expressed in a skin as well as Granulocyte-macrophage colony-stimulating factor (GM-CSF) and transforming growth factor- β 1 (TGF- β 1). Notch receptors are a family of highly conserved heterodimeric transmembrane proteins involved in several cell processes, such as proliferation, differentiation, and apoptosis (95, 96). Mammals have Notch receptors with different tissue and cellular distributions. Among cells of the hemopoietic lineage, Notch mRNA and protein expression can be detected in human immature CD 34+ hemopoietic progenitors, lymphoid, myeloid, and erythroid precursors; as well as B and T cells, monocytes, and neutrophils [97,98]. The activation of Notch-1 by Jagged-1 may induce maturation of human DC [99].

Immature DCs are mostly located in peripheral tissues and are highly efficient in Ag uptake by phagocytosis, recep-

tor-mediated endocytosis, and macropinocytosis. After that, DCs mature, become specialized in Ag presentation, and migrate to local lymph nodes to initiate immune responses. Maturation can be induced by different signals such as tissue damage or infection [30].

In human and mouse DCs, TLR-3 and TLR-4 act in synergy with TLR-7, TLR-8 and TLR-9 in the induction of a selected set of genes. Thus, synergic TLR stimulation increases production of IL-12 and 23, the Delta-4/ Jagged-1 ratio, and consequently, induces the stimulation of Th-1 immune response through DC. In this way, DCs are able to discriminate pathogens promoting Th-1 responses [100]. No information about Notch receptor is reported in leprosy. It is probable that the Th-1 immune response observed in the tuberculoid leprosy has the participation of DCs and Notch receptors.

10. DCs-AN ADJUVANT?

As mentioned before, DCs are the most potent antigen-presenting cells and, therefore, the potential for their use in vaccination has opened new perspectives for the development of immunotherapy strategies against cancer and infectious diseases. Interestingly, in renal cell carcinoma, DC maturation seems to be involved in the success of the cancer treatment [101,102]. In metastatic renal cancer, vaccine therapy based on DCs seems to be associated with better outcome and regression of disease [103-105].

An alternative form of immune therapy, using the cell wall skeleton of BCG (BCG-CWS), also seems to lead to a better prognosis for many cancer patients [106]. The BCG-CWS may represent the adjuvant feature of mycobacteria in CFA and in immune therapy for cancer. Recently, Tsuji *et al.* [107] suggested that BCG-CWS induces TNF alpha secretion from DCs, through TLR2 and TLR4, and consequently, the DCs maturation. Since the stimulation and the maturation of DCs induce the increase of antigen-presenting ability, these studies suggests the importance of using BCG-CWS for DCs stimulation, probably by completing the DCs maturation that endows them with the ability to prime T cell responses. The nature of the original stimulus that promotes DCs maturation and establishes the cytokine expression profile might determine different types of T cell responses.

Recent studies suggest that different types of Dcs, due to their cytokine-mediated plasticity, may be used not only as ideal cellular adjuvants for therapeutic vaccines against cancer and severe infections, but also under specific conditions such as in transplantation and autoimmune diseases [108]. Moreover, the knowledge of the key role of DCs in the priming and regulation of the immune response may lead to the identification of novel and powerful adjuvants capable of selectively orienting the immune response towards protection. The identification of safe and effective adjuvants inducing a correct DC activation and antigen presentation is an urgent need not only for enhancing the immunogenicity of vaccines against infections agents, but also to get through the tolerance against self-tumor associated-antigens (TAAs) in patients with malignancies, which is required for the development of effective cancer vaccines. It is reasonable to assume that novel and more selective and effective immune adjuvants will be identified in the near future as we understand more about the DCs role in the immune response ini-

tiation and regulation. Thus, this knowledge may lead to new perspectives for the development of prophylactic and therapeutic vaccines against infections diseases and cancer.

11. CONCLUDING REMARKS

This review concentrates on some points regarding DCs and their role in antigen presentation: The expression of surface molecules by DCs, their production of specific cytokines and interaction with T cells during mycobacterial infections and others pathologies as ATL and cancer point to an important role of these cells. The potent antigen-presenting activity of DCs suggests that they are "primers" of the immune system. The central problem in lepromatous leprosy is the weak or absence of cell-mediated immunity response to *M. leprae*, resulting from the inability of T cells to respond to the *M. leprae* antigens, although they are capable of responding to a wide array of other antigens. Therefore, the development of effective immunotherapy against leprosy may be through activation of DCs and, leading as a consequence to a better antigen presentation, production of IFN- γ and IL-12 and, consequently, inducing a much more efficient T cell response against the etiologic agent of leprosy.

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REFERENCES

- [1] Job CK. Nerve damage in leprosy. *Int J Lepr* 1989; 57: 532-9.
- [2] Johnson PC. Peripheral nerve pathology. In: *Neuropathology*. Willian and Wilkins: Baltimore, 1997; 1233-323.ed. Davis and DM Robertson.
- [3] Stoner GL. Importance of the neural predilection of *Mycobacterium leprae* in leprosy. *Lancet* 1979; 10: 994-6.
- [4] Smith WC. We need to know what is happening to the incidence of leprosy. *Lepr Ver* 1997; 68: 195-200.
- [5] World Health Organization. Leprosy-global situation. *Wkly Epidemiol Rec* 2000; 75: 225-32.
- [6] Ridley DS, Jopling WH. Classification of leprosy according to immunity: a five-group system. *Int J Lepr Other Mycobact Dis* 1966; 34: 255-73.
- [7] Yamamura MK, Uyemura RJ, Deans K, *et al.* Defining protective responses to pathogens: cytokine profiles in leprosy lesions. *Science* 1991; 254: 277-82.
- [8] Reis e Souza C, Hierry T, Sharton-Kersten D, *et al.* *In vivo* microbial stimulation induces rapid CD40 ligand-independent production of interleukin-12 by dendritic cells and their redistribution to T cell areas. *J Exp Med* 1997; 186: 1819-29.
- [9] Trinchieri G. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu Ver Immunol* 1995; 13: 251-76.
- [10] Hsieh CS, Mactonia SE, Tripp CS, *et al.* Development of Th1 CD4+ T cells through IL-12 produced by *Listeria*-induced macrophages. *Science* 1993; 260: 547-9.
- [11] Sallusto F, Lanzavecchia. Mobilizing dendritic cells for tolerance, priming and chronic inflammation. *J Exp Med* 1999; 189: 611-4.
- [12] Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature* 1998; 392: 245-52.
- [13] Inaba KM, Inaba M, Naito M, *et al.* Dendritic cell progenitors phagocytose particulates, including bacillus Calmette-Guerin organisms, and sensitize mice to *Mycobacterial* antigens *in vivo*. *J Exp Med* 1993; 178: 479-88.
- [14] Marriot I, Hammond TG, Thomas K, *et al.* *Salmonella* efficiently enter and survive within cultured CD11c+DCs initiating cytokine expression. *Eur J Immunol* 1999; 29: 1107-15.

- [15] Svensson M, Stockinger B, Wick MJ. Bone marrow-derived dendritic cells can process bacteria for MHC I and MHC II presentation to T cells. *J Immunol* 1997; 158: 4229-36.
- [16] Rescigno M, Citterio S, Thery C, *et al.* Bacteria-induced neobiosynthesis, stabilization, and surface expression of functional class I molecules in mouse dendritic cells. *Proc Natl Acad Sci* 1998; 95: 5229-34.
- [17] De Smedt T, Pajak B, Muraille E, *et al.* Regulation of dendritic cell numbers and maturation by lipopolysaccharide *in vivo*. *J Exp Med* 1996; 184: 1413-24.
- [18] Lenschow DJ, Walunas TL, Bluestone JA. CD28/B7 system of T cell costimulation. *Annu Rev Immunol* 1996; 14: 233-58.
- [19] Geijtenbeek TB, Kwon DS, Torensma R, *et al.* DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances transinfection of T cells. *Cell* 2000a; 100: 587-97.
- [20] Relloso M, Puig-Kroger A, Pello OM, *et al.* DC-SIGN (CD209) expresión is IL-4 dependent and is negatively regulated by IFN, TGF-beta and anti-inflammatory agents. *J Immunol* 2002; 168: 2634-43.
- [21] Engering A, Geijtenbeek TB, Van Vliet SJ, *et al.* The dendritic cell-specific adhesion receptor DC-SIGN internalizes antigen for presentation to T cells. *J Immunol* 2002; 168: 2118-26.
- [22] Cambi A, And Figdor CG. Dual function of C-type lectin-like receptors in the immune system. *Curr Opin Cell Biol* 2003; 15: 539-46.
- [23] Steinman RM and Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantification, tissue distribution. *J Exp Med* 1973; 137: 1142-62.
- [24] Reis e Souza C, Stahl PD, Austyn JM. Phagocytosis of antigens by Langerhans cells "*in vitro*". *J Exp Med* 1993; 178: 509-14.
- [25] Sallusto F, Lanzavecchia A. Dendritic cells use macropinocytosis and amnosome receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: Downregulation by cytokines and bacterial products. *J Exp Med* 1995; 182: 389-400.
- [26] Cauxc, Lebecque S, Liu Y-J, Banchereau B. Developmental pathways of human myeloid dendritic cells. In: Lotze T, Thomson AW (eds). *Dendritic cells: Biology and Clinical Applications*. Academic Press: San Diego, 1999; 63-92.
- [27] Young, JW. Cell fate development in the myeloid system. In: Lotze T, Thomson AW (eds). *Dendritic cells: Biology and Clinical applications*. Academic Press: San Diego, 1999; 29-49.
- [28] Vandenberghe S, Wu L. Dendritic cells origins: Puzzles and paradoxes. *Immunol Cell Biol* 1999; 77: 411-19.
- [29] Rescigno M, Granucci F, Ricciradi-Castagnoli. Dendritic cells at the end of the Millennium. *Immunol Cell Biol* 1999; 77: 404-10.
- [30] Banchereau J, Briere F, Caux C, *et al.* Immunobiology of dendritic cells. *Annu Rev Immunol* 2000; 18: 767-11.
- [31] Enk A, Angeloni V, Udey M, *et al.* Inhibition of Langerhans cell antigen presenting function by IL-10. *J Immunol* 1993; 151: 2390-7.
- [32] Steinbrink K, Wolf M, Jonuleit H, *et al.* Induction of tolerance by IL-10-treated dendritic cells. *J Immunol* 1997; 159: 4772-780.
- [33] Liu LM, MacPherson GG. Antigen acquisition by dendritic cells: intestinal dendritic cells acquire antigen administered orally and can prime naive T cells *in vivo*. *J Exp Med* 1993; 177: 1299-307.
- [34] Holt PG, Schon-Hegrad MA. Localization of T cells macrophages, and dendritic cells in rat respiratory tract tissue: implications for immune functions studies. *Immunol Today* 1987; 349-62.
- [35] Bujdosó R, Hopkins J, Dutia BM, Young P, McConnell I. Characterization of sheep afferent lymph dendritic cells and their role in antigen carriage. *J Exp Med* 1989; 170: 1285-301.
- [36] Crowley M, Inaba K, Steinman RM. Dendritic cells are the principal cells in mouse spleen bearing immunogenic fragments of foreign proteins. *J Exp Med* 1990; 172: 383-6.
- [37] Sieling PA, Jullien M, Dahlem M, *et al.* CD1 expression by dendritic cells in human leprosy lesions: correlation with effective host immunity. *J Immunol* 1999; 162: 1851-8.
- [38] Yamauuchi P, Bleharski JR, Uyemura K, *et al.* A role for CD40-CD40 ligand interactions in the generation of type 1 cytokine responses in human leprosy. *J Immunol* 2000; 165: 1506-12.
- [39] Santos DO, Santos SL, Esquenazi D, *et al.* Evaluation of B7-1 (CD80) and B7-2 (CD86) costimulatory molecules and dendritic cells on the immune response in Leprosy. *Jpn J Lepr* 2001; 70: 15-24.
- [40] Krutzik SR, Tan B, LiH, *et al.* TLR activation triggers the rapid differentiation of monocytes into macrophages and dendritic cell. *Nat Med* 2005; 11: 653-60.
- [41] Soilleux E, Sarno E, Hernandez M, *et al.* DC-SIGN association with the Th2 environment of lepromatous lesions: cause or effect? *J Pathol* 2006; 209: 182-189.
- [42] Makino M, Maeda Y and Ishii N. Immunostimulatory activity of major membrane protein-II from *Mycobacterium leprae*. *Cell Immunol* 2005; 233: 53-60.
- [43] Giacomini E, Iona E, Ferroni L, *et al.* Infection of human macrophages and dendritic cells with *Mycobacterium tuberculosis* induces a differential cytokine gene expression that modulates T cell response. *J Immunol* 2001; 166: 7033-41.
- [44] Först D, Rölinghoff M, Stenger S. IL-10 converts human dendritic cells into macrophage-like cells with increased antibacterial activity against virulent *Mycobacterium tuberculosis*. *J Immunol* 2000; 165: 978-87.
- [45] Tascon RE, Soares CS, Ragno S, *et al.* *Mycobacterium tuberculosis*-activated dendritic cells induce protective immunity in mice. *Immunology* 2000; 99: 473-80.
- [46] Henderson RA, Watkins SC, Flynn JL. Activation of human dendritic cells following infection with *Mycobacterium tuberculosis*. *J Immunol* 1997; 159: 635-43.
- [47] Demangel C, Britton WJ. Interaction of dendritic cells with *Mycobacteria*: where the action stars? *Immunol Cell Biol* 2000; 78: 318-24.
- [48] Mohagheghpour N, Van Vollenhoven A, Goodman J. Interaction of *Mycobacterium avium* with human monocyte-derived dendritic cells. *Infect Immun* 2000; 68: 5824-9.
- [49] Geijtenbeek TB, Van Vliet SJ, Koppel EA, *et al.* *Mycobacteria* target DC-SIGN to suppress dendritic cell function. *J Exp Med* 2003; 197: 7-17.
- [50] Tailleux L, Schwartz O, Herrmann J, *et al.* DC-SIGN is the major *Mycobacterium tuberculosis* receptor on human dendritic cells *J Exp Med* 2003; 197: 121-7.
- [51] Maeda V, Nigon J, Herrmann JL. The cell surface receptor DC-SIGN discriminates between *Mycobacterium* species through selective recognition of the mannose caps on lipoarabinomannan. *J Biol Chem* 2003; 278: 5513-6.
- [52] Makino MS, Shiomokubo S, Wakamatsu S, *et al.* The role of HTLV-I-infected dendritic cells in the development of HTLV-I-associated myelopathy/tropical spastic paraparesis. *J Virol* 1999; 73: 4575-81.
- [53] Makino MS, Wakamatsu S, Shimokubo S, *et al.* Production of functionally deficient dendritic cells from HTLV-I-infected monocytes: Implications for the dendritic cell defect in adult T cell leukemia. *Virology* 2000; 274: 140-8.
- [54] Geijtenbeek TB, Van Vliet, Van Duijnhoven GCF, *et al.* DC-SIGN, a dendritic cell-specific HIV-1 receptor present in placenta that infects T cells in Trans-A review. *Placenta* 2001; 15: S19-S23.
- [55] Mc Innes IB, Gracie AJ, Leung BP, *et al.* Interleukin-18: a pleiotropic participant in chronic inflammation. *Immunol Today* 2000; 21: 312-8.
- [56] Vankayalapati R, Wizel B, Weis SE, *et al.* Production of interleukin-18 in human tuberculosis. *J Infect Dis* 2000; 182: 234-9.
- [57] Sugawara I, Yamada H, Kaneko H, *et al.* Role of interleukin-18 (IL-18) in *Mycobacterial* infection in IL-18-gene-disrupted mice. *Infect Immun* 1999; 67: 2585-9.
- [58] Gardella S, Andrei C, Costigliolo S, *et al.* Interleukin-18 synthesis and secretion by dendritic cells are modulated by interaction with antigen-specific T cells. *J Leuk Biol* 1999; 66: 237-41.
- [59] Moore K W, O'Garra A, Malefyt RD, *et al.* Interleukin-10. *Annu Rev Immunol* 1993; 11: 165-90.
- [60] Bogdan C, Vodovotz Y, Nathan C. Macrophage deactivation by interleukin 10. *J Exp Med* 1991; 174: 1549-55.
- [61] Fiorentino DF, Zlotnik A, Mosmann TR, *et al.* IL-10 inhibits cytokine production by activated macrophages. *J Immunol* 1991; 147: 3815-22.
- [62] De Smedt T, Van Mechelen M, De Becker J, *et al.* Effect of interleukin 10 on dendritic cell maturation and function. *Eur J Immunol* 1997; 27: 1229-35.
- [63] Allavena P, Piemonti L, Longoni D, *et al.* IL-10 prevents the differentiation of monocytes to dendritic cells but promotes their maturation to macrophages. *Eur J Immunol* 1998; 28: 359-69.
- [64] Pretolani M, Storoer P, Goldman M. Interleukin-10. In "*The Cytokine Network and Immune Functions*" J. Thèze, ed. Oxford University Press. 1999; 45.

- [65] Sareneva T A, Matikainen S, Kurimoto M, *et al.* Influenza A virus-induced IFN alpha /beta and IL-18 synergistically enhance IFN gamma gene expression in human T cells. *J Immunol* 1998; 160: 6032-8.
- [66] Cooper AM, Magram J, Ferrante J, *et al.* Interleukin-12 (IL-12) is crucial to the development of protective immunity in mice intravenously infected with *Mycobacterium tuberculosis*. *J Exp Med* 1997; 186: 39-45.
- [67] De Jon R, Altare F, Haagen A, *et al.* Severe *Mycobacterial* and *Salmonella* infections in interleukin-12 receptor-deficient patients. *Science* 1998; 280: 1435-8.
- [68] Chan CW, Crafton E, Fan HN. Interferon-producing killer dendritic cells provide a link between innate and adaptative immunity. *Nat Med* 2006; 12: 167-8.
- [69] Toshiaki O, Fukao T, Suzue K, *et al.* Interleukin 12-dependent interferon gamma production by CD8 alpha + lymphoid dendritic cells. *J Exp Med* 1999; 189-1981-6.
- [70] Brightbill HD, Libraty DH, Krutzik SR, *et al.* Host defense mechanisms triggered by microbial lipoproteins through Toll-like receptors. *Science* 1999; 285: 732-6.
- [71] Yoshida A, Koide Y. Arabinofuranosyl-terminated and mannosylated lipoarabinomannans from *Mycobacterium tuberculosis* induce different levels of interleukin-12 expression in murine macrophages. *Infect Immun* 1997; 65: 1953-5.
- [72] Underhill DM, Ozinsky A, Smith KD, *et al.* Toll-like receptor 2 mediates *Mycobacteria* induced pro-inflammatory signaling in macrophages. *Immunity* 1999; 96: 14459-63.
- [73] Flesh IE, Hess JH, Huang S, *et al.* Early interleukin-12 production by macrophages in response to *Mycobacterial* infection depends on interferon gamma and tumor necrosis factor alpha. *J Exp Med* 1995; 181: 1615-21.
- [74] Jiao X, Lo-Man R, Guernonprey P, *et al.* Dendritic cells are host cells for *Mycobacteria* *in vivo* that trigger innate and acquired immunity. *J Immunol* 2002; 168; 1294-1301.
- [75] Henderson RA, Watkins SC, Flynn JL. Activation of human dendritic cells following infection with *Mycobacterium tuberculosis*. *J Immunol* 1997; 159: 635-301.
- [76] Bodnar KA, Serbina NV, Flynn JL. Fate of *Mycobacterium tuberculosis* within murine dendritic cells. *Infect Immun* 2001; 69: 800-9.
- [77] Mohaghepour N, Van Vollenhoven A, Goodman J, *et al.* Interaction of *Mycobacterium avium* with human monocyte derived dendritic cells. *Infect Immun* 2000; 68: 5824-9.
- [78] Lemaitre B, Nicolas E, Michaut L, *et al.* The Dorsalventral Regulatory Gene Cassette *spätzle/Toll/cactus* Controls the Potent Antifungal Response in *Drosophila* Adults. *Cell* 1996; 86: 973-83.
- [79] Rock F, Hardiman G, Timans JC, *et al.* A family of human receptors structurally related to *Drosophila* Toll. *Proc Natl Acad Sci* 1998; 95: 588-93.
- [80] Hajjar A M, O'Mahony SD, Ozinsky A, *et al.* Cutting Edge: Functional Interactions Between Toll-Like Receptor (TLR) 2 and TLR1 or TLR6 in Response to Phenol-Soluble Modulin. *J Immunol* 2001; 166: 15-9.
- [81] Adrian Ozinsky A, David M. Underhill DM, *et al.* The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between Toll-like receptors. *Proc Natl Acad Sci* 2000; 97: 13766-771.
- [82] Mellman I, Turley SJ, Steinman RM. Antigen processing for amateurs and professionals. *Trends Cell Biol* 1998; 8: 231-7.
- [83] Tang HL, Cyster JG. Chemokine Up-regulation and activated T cell attraction by maturing dendritic cells. *Science* 1999; 30: 819-22.
- [84] O' Neill L Célula dendrítica e expressão de TLR. *Sci Am* 2005; 35: 68-75.
- [85] Kang TJ, Chao GT. Detection of Toll-like receptor 2 (TLR2) mutation in the lepromatous leprosy patients *FEMS Immunol Med Microbiol* 2001; 31: 53-8.
- [86] Kan TJ, Lee SB, Chae GT. A polymorphism in the Toll-like receptor 2 is associated with IL-12 production from monocyte in lepromatous leprosy. *Cytokine* 2002; 20: 56-62.
- [87] Bochud PY, Hawn TR, Aderem A. Cutting edge: a Toll-like receptor 2 polymorphism that is associated with lepromatous leprosy is unable to mediate *Mycobacterial* signaling. *J Immunol* 2003; 170: 3451-4.
- [88] Schroder NW, Hermann C, Hamann L, *et al.* High frequency of polymorphism Arg 753 Gln of the Toll-like receptor-2 gene detected by a novel allele-specific PCR. *J Mol Med* 2003; 81: 368-72.
- [89] Oliveira RB, Ochoa MT, Sieling PA, *et al.* Expression of Toll-like receptor 2 on human Schwann cells: a mechanism of nerve damage in leprosy. *Infect Immunity* 2003; 71: 1427-33.
- [90] Bleharski JR, Hi H, Meiken C, *et al.* Use of genetic profiling in leprosy to discriminate clinical forms of the disease. *Science* 2003; 301: 1527-30.
- [91] Hinkel A, Tso CL, Gitlitz B, *et al.* Immunomodulatory dendritic cells generated from nonfractionated bulk peripheral blood mononuclear cell cultures induce growth of cytotoxic T cells against renal cell carcinoma. *J Immunother* 2000; 23: 83-93.
- [92] Geissmann FX, Prost C. Monnet JP, *et al.* Transforming growth factor beta 1, in the presence of granulocyte/macrophage colony-stimulating factor and interleukin 4, induces differentiation of human peripheral blood monocytes into dendritic Langerhans cells. *J Exp Med* 1998; 187: 961-966.
- [93] Mohamadzadeh M, Berard F, Essert G, *et al.* Interleukin 15 skews monocyte differentiation into dendritic cells with features of Langerhans cells. *J Exp Med* 2001; 194: 1013-1019.
- [94] Larregina AT, Morelli AE, Spencer LA, *et al.* Dermal-resident CD14+ cells differentiate into Langerhans cells. *Nat Immunol* 2001; 2: 1151-1158.
- [95] Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: cell fate control and signal integration in development. *Science* 1999; 284: 770-776.
- [96] Osborne B, Miele L. Notch and the immune system. *Immunity* 1999; 11: 653-671.
- [97] Ohishi K, Varnum-Finney B, Flowers D, *et al.* Monocytes express high amounts of Notch and undergo cytokine specific apoptosis following interaction with the Notch ligand *delta-1*. *Blood* 2000; 95: 2847-2856.
- [98] Karanu FN, Murdoch B, Miyabayashi T, *et al.* Human homologues of *delta-1* and *delta-4* function as mitogenic regulators of primitive human hematopoietic cells. *Blood* 2001; 97: 1960-1972.
- [99] Weijzen A, Velders MP, El Mishad AG, *et al.* The notch ligand *jagged-1* is able to induce maturation of monocyte-derived human dendritic cells. *J Immunol* 2002; 169: 4273-4278.
- [100] Napolitani G, Rinaldi A, Bertoni F, *et al.* Selected toll like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. *Nat Immunol* 2005; 6: 769-776.
- [101] Merad M, Angevin E, Wolfers J, *et al.* Generation of monocyte-derived dendritic cells from patients with renal cell cancer: modulation of their functional properties after therapy with biological responses modifiers (IFN-alpha plus IL-2 and IL-12). *J Immunother* 2000; 23: 369-78.
- [102] Dall'Oglio M, Srougi M, Barbuto JÁ. Complete response of metastatic renal cancer with dendritic cell vaccine. *Int Braz J Urol* 2003; 29: 517-9.
- [103] Barbuto JÁ, Ensina LF, Neves AR, *et al.* Dendritic cell-tumor cell hybrid vaccination for metastatic cancer. *Cancer Immunol Immunother* 2004; 53: 1111-8.
- [104] Neves Ar, Ensina LF, Anselmo LB, *et al.* Dendritic cells derived from metastatic cancer patients vaccinated with allogeneic dendritic cell-autologous tumor cell hybrids express more CD86 and induce higher levels of Interferon-gamma in mixed lymphocyte reaction. *Cancer Immunol Immunother* 2005; 54: 61-6.
- [105] Hayashi A. Interferon gamma as a marker for the effective cancer immunotherapy with BCG-cell wall skeleton. *Proc Jpn Acad* 1994; 70: 205-9.
- [106] Hayashi A; Doi O, Azuma I, *et al.* Immuno-friendly use of BCG-cell wall skeleton remarkably improves the survival rate of various cancer patients. *Proc Jpn Acad* 1998; 74: 50-5.
- [107] Tsuji S, Matsumoto M, Takenchi O, *et al.* Maturation of human dendritic cells by cell wall skeleton of *Mycobacterium bovis* bacillus Clamette-Guérin: Involvement of Toll-like receptors. *Infect Immun* 2000; 68: 6883-90.
- [108] Santini S, Belardelli F. Advances in the use of Dendritic cells and new adjuvants for the development of therapeutic vaccines. *Stem cells* 2003; 21: 495-505.