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Minireview

Mycobacterium tuberculosis and Dendritic Cells: Recognition, Activation and Functional Implications

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The highly complex nature of interactions of *Mycobacterium tuberculosis* with cells of the immune system has puzzled researchers the world-over in understanding the pathogenesis and immunology associated with tuberculosis (TB). This has contributed to the delay in development of effective vaccine(s) for TB. Several excellent studies have provided only a glimpse of the kind and degree of immune responses elicited following infection by mycobacteria. Preferred entry via respiratory route results in the capture of mycobacteria by alveolar macrophages that eventually become their long-term hosts. Since the pathogen is rarely cleared this has resulted in the human population serving as a large reservoir for mycobacteria. Owing to their unique ability to prime naïve and memory T cells, dendritic cells (DCs) play important and indispensable roles in the initiation and maintenance of protective immune responses following infection. The kind of immune response initiated by DCs with respect to mycobacteria determines the character of immune responses mounted by the host against the pathogen. The profile of cytokines and chemokines secreted as a result of infection of DCs by mycobacteria further plays an important role in defining the course of infection. This minireview attempts to highlight key interactions of mycobacteria with dendritic cells. We discus the uptake of mycobacteria by DCs followed by DC activation and the spectrum of immune responses initiated by infected/activated DCs, followed by numerous ways the pathogen has devised to subvert protective responses.

Keywords: Dendritic cells, Mycobacteria, Immune response, Immune evasion, *Mycobacterium tuberculosis*

Introduction

Tuberculosis (TB) caused by the acid-fast bacterium *Mycobacterium tuberculosis* is a major health problem in Third World countries as well as a major health threat across USA and Europe. World Health Organization (WHO)'s statistics have indicated that currently one-third of the world's population is infected with *M. tuberculosis* and if control is not strengthened further, between 2002 and 2020, approximately one billion people are likely to be newly infected, over 150 million people would get sick and 36 million would die of TB[1]. Consequently, TB is now rated as one of the most common infectious diseases, and has the highest mortality rate in the world. The only vaccine available is the attenuated strain of *M. bovis*, namely *M. bovis* BCG (hereafter BCG)[2]. However, immunization with BCG shows varied protection across geographical distributions. While it confers protection to ~80% of the population in the UK, its efficacy in countries such as India is 0% in the adult population, although it has considerable success in controlling childhood TB[3-6].

Therefore, there is a need to develop a new or an improved BCG vaccine to control the spread of TB. To this end, one of the prerequisites is a thorough understanding of the interactions of *M. tuberculosis* with various cells of the immune system. This includes all aspects starting from its uptake, followed by activation or inactivation of the infected cell and the nature of innate and adaptive immune responses mounted against the pathogen resulting in possible elimination from the system.

M. tuberculosis infects primarily via respiratory route[7]. Although it can cause disease in most organs, pulmonary TB is the most common. The immune responses mounted against mycobacteria are successful in containing, but not eliminating the

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Abbreviations: TB, tuberculosis; BCG *M. bovis* BCG; DCs, dendritic cells; TLRs, toll-like receptors; DC-SIGN DC-Specific ICAM-3 Grabbing Non-integrin; manLAM, mannosylated lipoarabinomannan; NF-κB, nuclear factor-kappa B; Th1T helper 1; CFP-10, culture filtrate protein-10; ESAT-6, early secretory antigenic target of 6 kDa; GM-CSF, granulocyte macrophage colony stimulating factor; MHC, major histo-compatibility complex; IFN, interferon; LPS, lipopolysaccharide.

pathogen. Acute active TB results in a small percentage of infected individuals (e.g. in HIV^+ individuals), probably due to lack of initiation of an effective immune response. Capture of *M. tuberculosis* by alveolar macrophages results in the recruitment of various cell types to the site of infection leading to the formation of a granuloma. The granuloma comprising of giant cells, T and B lymphocytes and fibroblasts is an important structure that contains and thereby prevents the spread of infection to other organs and tissues[7]. In the granuloma, the organism may be dormant or non-replicating, actively replicating or metabolically altered with limited replicating cycles. Breakdown of immune response results in reactivation of the bacilli, leading to necrosis and damage to the lung tissue. Thus, a constant battle is waged between the bacterium and the host. The fact that the pathogen is very rarely cleared, indicates that it has devised various ways to evade the immune attack.

Although macrophages serve as the long-term hosts for mycobacteria, *M. tuberculosis* infects Dendritic cells (DCs) as well and its interactions with DCs are crucial for the development of protective immunity[8,9]. In this minireview, we have attempted to summarize our knowledge pertaining to interactions of *M. tuberculosis* with DCs and their implications on immune responses mounted by the host against the pathogen.

Dendritic Cells: Form and Function

Dendritic cells (DCs) are a family of cells that specialize in the uptake and presentation of antigen to T cells. They are the most potent antigen presenting cells of the immune system and are central to the initiation of immune responses[10,11]. They originate from stem cell precursors in the bone marrow and colonize peripheral tissues such as skin, trachea and intestine, where they scavenge pathogens/antigens. The uptake of antigens that range from soluble proteins, viruses, bacteria, parasites and even whole cells could be via endocytosis by clathrin-coated pits or caveolae, macropinocytosis or phagocytosis. Uptake of antigens is followed by migration of DCs to the nearest draining lymph node. During migration, in response to soluble and contact-dependent factors provided by the engulfed antigen and assisted by the local cytokine and cellular milieu, DCs undergo a process of 'maturation'. Maturation is characterized by the upregulation of a number of cell surface receptors and increase in the surface density of existing receptors. This includes high level expression of MHC class I and II molecules together with a range of costimulatory molecules such as CD80 (B7.1), CD86 (B7.2), CD40 and CD54 (ICAM-1) and CD83 (in the case of human DCs)[11].

The maturation status of DCs is dynamic and is characterized by distinct phenotypic and functional modalities[12]. For example, immature DCs are programmed for antigen capture and display low levels of the above molecules, but express a range of endocytic and phagocytic receptors including the Toll-like receptors (TLRs). Mature DCs as a result of upregulated MHC (class I and II) and costimulatory molecules are very efficient T cell stimulators[13], but poor in capturing antigen as most receptors associated with antigen uptake are downmodulated. Therefore, antigens that modulate maturation of DCs play a major role in defining the nature of immune responses elicited against the pathogen and thereby determine the course of an infection[14]. Once inside the lymph nodes, DCs present the processed antigen to circulating naïve and memory T cells and initiate immune responses.

Recognition of Mycobacteria by DCs

Considerable evidences show that DCs can phagocytose mycobacteria and mycobacterial antigens[15], resulting in DC function and phenotype being modified. Along with macrophages, DCs lining the trachea may be one of the first cells that encounter mycobacteria and are, therefore, likely to be responsible for the ensuing immune responses. A number of surface receptors have been implicated in the recognition of mycobacteria by DCs, e.g. the mannose receptor (MR), CD11b (Mac-1), CD11c and DEC-205[16-18]. Some of these receptors are employed by macrophages as well.

One of the C-type lectin receptors extensively characterized with respect to mycobacteria is DC-Specific ICAM-3 Grabbing Non-integrin (DC-SIGN) — a major receptor on human DCs for *M. tuberculosis*[19]. DC-SIGN was previously reported to be a major receptor for HIV gp120 surface protein and was in fact called the gp120 binding protein[20]. However, it is now demonstrated that DC-SIGN serves as a pattern recognition receptor for viruses (e.g. ebola, dengue, HIV), bacteria (e.g. *M. tuberculosis*, *Salmonella*) and parasite (*Leishmania*)[20]. Although immature DCs also express

high levels of MR, CD11b and CD11c, neutralizing DC-SIGN with specific antibodies inhibits the interaction of DCs with both BCG and mannosylated lipoarabinomannan (manLAM) — a major sugar molecule expressed on the cell surface by virulent *Mycobacterium* spp. by >80%. On the other hand, neutralization of MR has no significant effect. This indicates that DC-SIGN is a preferred receptor for infection of DCs by *M. tuberculosis*.

Mycobacteria-DC-SIGN interactions at the molecular level have revealed that DC-SIGN discriminates between *Mycobacterium* spp. through selective recognition of the mannose caps on LAM molecules, expressed by slow-growing virulent *M. tuberculosis* and *M. avium* but not by the fast-growing mycobacteria such as *M. smegmatis* that express arabinose on their LAM molecules[21]. Further, the length of the neck-region of DC-SIGN plays no significant role in determining the susceptibility to TB in South African individuals[22].

Furthermore, manLAM inhibits lipopolysaccharide (LPS)-induced DC maturation by interacting with DC-SIGN. Neutralization of DC-SIGN restores LPS-induced DC maturation in the presence of manLAM[23]. Functional characterization of DC-SIGN-*M. tuberculosis* interactions has shown that specific targeting of DC-SIGN by *M. tuberculosis* through manLAM is a mechanism to impair DC maturation and to induce production of the anti-inflammatory cytokine interleukin (IL)-10[24]. This suggests that DC-SIGN, following binding to manLAM delivers a signal that interferes with mycobacteria-induced DC maturation signals. These results indicate that *M. tuberculosis* targets DC-SIGN to suppress cellular immune responses since immature DCs that secrete IL-10 would not only inefficiently stimulate T cells, but also induce a state of antigen-specific tolerance. These observations support an important role for DCs as hosts for mycobacteria.

In addition to DC-SIGN, many *M. tuberculosis* components have been identified as stimulators of toll-Like receptors (TLRs). These include LAM[25], a 19 kDa lipoprotein[26], the CpG repeat of non-methylated DNA[27] and peptidoglycan. These components interact with different TLRs, e.g., LAM and the 19-kDa lipoprotein act on TLR2, CpG DNA acts on TLR9, and peptidoglycan acts on TLR2 and TLR4. Thus, it has been postulated that *M. tuberculosis*-mediated adjuvant activity might be expressed through TLRs on DCs. Human TLR2 and TLR4 have been shown to be involved in *M. tuberculosis*-mediated intracellular signaling *in vitro*[15,28]. Further, while viable *M. tuberculosis* expresses distinct ligands that recognize and activate cells via TLR2 and TLR4, heat-killed *M. tuberculosis* fails to activate cells via TLR4. This suggests that interactions of *M. tuberculosis* with TLRs, in addition to physical binding, might involve rearrangement of ligands in a spatio-temporal manner, achievable only with live bacilli. These results could have important bearings on the development of vaccines that might require live attenuated bacilli as opposed to killed bacilli. Such a concept has been developed using *Listeria monocytogens*[29] and recently with *Salmonella typhimurium*[30], where killed but metabolically active bacilli have been used as vaccine candidates for effective T cell responses and protective immunity.

Activation of DCs following *M. tuberculosis* Infection

Phagocytosis of *M. tuberculosis* by DCs usually results in the activation of nuclear factor-kappa B (NF-κB) family of transcription factors together with the secretion of inflammatory cytokines and chemokines and release of reactive nitrogen and oxygen intermediates[31,32]. NF-κB has been implicated in the upregulation of surface markers on DCs[33]. These responses have determinant bearings on the surface phenotype, cytokine and chemokine patterns and T cell responses from the DCs[34]. However, as an exception to this rule many of the *M. tuberculosis* components in fact cause inactivation of the above mediators as discussed later.

Modulation of Surface Phenotype

Interaction of human DCs with *M. tuberculosis* or BCG results in increased surface densities of a number of molecules such as MHC II, CD40, CD54, CD58, CD80, CD86 and CD83 and IL-12 production that are involved in interactions with T cells[35-38], suggesting that BCG treatment directly induces DCs to mature. In addition, the cell wall skeleton of BCG consisting of mycolic acids, arabinogalactan, and peptidoglycan (PGN) activate TLR2 and TLR4[15]. In humans, TLR2 and TLR4 were shown to be involved in DC maturation by BCG peptidoglycan. Tumor necrosis factor (TNF)-α, a major cytokine induced following NF-κB activation is triggered by TLR2 and TLR4 agonists and considered to be a key maturation

inducer of human DCs[15]. In fact, exogenous addition of TNF-α up-regulates CD80, CD86, CD83 and CD150 on DC surface.

Activation of DC-SIGN via manLAM results in incomplete maturation of human DCs[39] with low levels of MHC class I and II, CD86, CD83 and CCR7. Further, these DCs are susceptible to killing by NK cells, indicating their immature status since mature DCs are resistant to NK cell-mediated lysis[11]. These DCs, however, show reduced phagocytic ability and reduced ability to prime naïve T cells.

Infection of DCs with *M. tuberculosis* also results in the down-regulation of CD1 molecules[40]. Furthermore, *M. tuberculosis* inhibits the differentiation of DCs from human monocytes in the presence of interferon (IFN)-α, wherein the cells acquire a phenotype reminiscent of macrophages[41]. These cells retain CD14 without acquiring CD1 molecules and only weakly express B7 family of costimulatory molecules and MHC class I and II molecules on their cell surface. Further, murine DCs when infected by mycobacteria both *in vitro* and *in vivo* lose their antigen presentation ability[8,42-44]. The above studies suggest that depending upon the receptors involved, the interaction of DCs with mycobacteria could either result in activation of DCs characterized by upregulated levels of molecules implicated in productive T cell responses or the inactivation of DCs leading to impaired T cell responses. Similarly, it has recently been identified that components of the *Salmonella* pathogenicity island 2 prevent DC and effective T cell activation, thereby preventing generation of adaptive immunity[45]. Further, it was also demonstrated that live, but not heat-killed *Salmonella* during its transition from the extracellular stage to the intracellular stage within DCs prevent the processing and presentation of its highly immunogenic antigen FliC and thereby prevents activation of $CD4^+$ T cells[46]. These results indicate that strategies to evade host-mediated immune responses by modulating DC function are mimicked not only by *M. tuberculosis* but also by other pathogens.

In an interesting study, it was observed that in the granuloma of *M. tuberculosis* infected mice, highly vacuolated macrophages, often called 'foamy macrophages' express a phenotype characteristic of DCs[47]. These macrophages express high levels of DEC-205, CD11b, CD11c and high levels of MHC class II and CD40-markers that typify mature DCs. However, these cells express the above phenotype only during early chronic stages of the disease and quickly lose the phenotype once the disease progresses towards late stage chronic infection. It was postulated that the expression of DC-like markers by 'foamy macrophages' could potentiate immune responses at sites of infection that would aid in containment of the pathogen within the granuloma.

Cytokine and Chemokine Profiles during Mycobacteria-DC Interactions

Cytokine profile secreted by *M. tuberculosis*-activated DCs determines whether efficient and effective interactions with T cells would ensue, that would in turn determine the nature of immune responses to mycobacterial infections. Infection of DCs with either *M. tuberculosis* or BCG is associated with increased expression of IL-12, TNF-α, IL-1 and IL-6[35-38]. These cytokines play major roles in protective anti-mycobacterial immune responses. On the other hand, mycobacterial infection of DCs increases the secretion of IL-10, which might inhibit cellular responses to mycobacteria through downregulation of IL-12 production. In another study, lung DCs phagocytose *M. tuberculosis*, resulting in production of IL-12 and stimulation of naïve T cells for IFN-γ production[37].

Further, interactions of *M. tuberculosis* vis-à-vis TLR and DC-SIGN have shown interesting correlates. While interactions with TLR results in DC activation characterized by high IL-12 secretion during early infection, interactions with DC-SIGN prevent DC activation by blocking NF-κB activation that results in high secretion of IL-10[48]. This is possible during later stages of infection when soluble manLAM secreted by *M. tuberculosis* from infected macrophages would have access to DC-SIGN, resulting in the inactivation of DCs. In addition, triggering of DC-SIGN on human DCs modulates TLR-induced activation of NF-κB resulting in the acetylation of NF-κB p65 subunit. Acetylation of NF-κB has been shown to prolong and increase IL-10 transcription and thereby enhances anti-inflammatory responses[49]. This indicates that when the scales are tipped towards TLR, effective control of TB could be envisaged. In contrast, should the scales be tipped towards DC-SIGN impaired control of TB is a possible outcome.

While neutralization of TNF-α significantly blocks DC maturation induced by LPS, it inhibits induction

of DC maturation upon BCG infection[38]. This indicates that TNF-α plays a minor role in BCG-induced DC maturation. However, neutralization of TNF-α results in low IL-12 secretion by activated DCs. BCG cell wall skeleton induces DCs to secrete TNF-α, IL-1β, IL-6 and IL-12[15]. Further, it was examined whether the production of IL-10 by DCs affected their IL-12 responses to mycobacterial infection and the generation of protective immune responses *in vivo*. Compared to wild-type DCs, IL-$10^{-/-}$ DCs secrete high IL-12 upon BCG infection and CD40 stimulation *in vitro*. Further, they are more efficient than wild-type DCs at inducing IFN-γ production to mycobacterial antigens in the draining lymph nodes following adoptive transfer. This effect is associated with increased trafficking of DCs lacking IL-10 to the draining lymph nodes and enhances IL-12 production within the draining lymph nodes[50,51]. These data indicate that autocrine IL-10 exerts a dual inhibitory effect on the induction of primary immune responses by DCs at two levels. First, by down-regulating the migration of infected DCs to the draining lymph nodes and second by modulating the IL-12 production by DCs in the draining lymph nodes.

M. tuberculosis infected DCs also produce a range of chemokines and chemokine receptors that modulate T cell responses. It was observed that infected DCs express CCR7 (that allows DCs to home to draining lymph nodes) and become responsive to its ligand CCL21[52]. Concurrent with this CCR5 expression is lost. These DCs also express high levels of CCL3, CCL4, CXCL9 and CXCL10 within hours of infection. These chemokines also play a major role in the migration of NK and T cells.

Immune Responses by Mycobacteria Infected DCs

DCs elicit varied immune responses to mycobacteria and mycobacterial antigens. When compared with control DCs, BCG-infected DCs are much more potent in stimulating allogeneic immune responses as well as cellular responses *in vivo*[53]. DCs infected with BCG express more co-stimulatory molecules than TNF-α treated DCs and enhance mixed leukocyte reactions. When autologous T cells are co-cultured with BCG-exposed DCs, they become highly activated, as determined by the surface levels of CD25, CD54 and CD71 on both $CD4^+$ and $CD8^+$ T cells. Cytokine production from T cells cultured with BCG-exposed DCs is enhanced with elevated secretion of IL-2, IL-10 and IFN-γ by both $CD4^+$ and $CD8^+$ T cells[54].

The role of DCs in *M. tuberculosis* pathogenesis was exemplified recently by employing a novel approach that depleted DCs *in vivo* using diptheria-toxin/GFP transgenic mice[9]. Following depletion of DCs, mice were impaired in their ability to mount effective $CD4^+$ T cell responses. This resulted in a significant loss of control over bacterial replication, resulting in huge bacterial loads in the lungs and spleen.

In another study, DCs have been shown to play an important role in mounting protective immune responses against *M. tuberculosis* infection[55]. *M. tuberculosis* infected tsDCs (a conditionally immortalized dendritic cell line) are capable of eliciting antigen-specific T-cell responses and potent anti-mycobacterial protective immunity in a murine model of experimental TB infection. *M. tuberculosis* infected DCs induce protective responses comparable with BCG alone. Thus, these results demonstrate that *M. tuberculosis* activated DCs are able to induce protective immune responses that may be as effective as BCG vaccination in mice. Further, these results support the development of anti-mycobacterial vaccine strategies that are able to target DCs *in vivo*. In addition, they also suggest that vaccine vectors capable of activating DCs *in vivo* should increase the protective efficacy of the immunization protocol.

Similarly, DCs either infected with BCG or pulsed with *M. tuberculosis* antigen(s) have been found to impart significant protection against experimental mycobacterial infections. DCs infected with BCG when administered intra-tracheally in mice induce potent T cell responses and IFN-γ production to mycobacterial antigens resulting in a significant protection against aerosol *M. tuberculosis* infection[56]. Despite the fact that vaccination schedule for BCG-infected DCs is shorter than subcutaneous BCG vaccination (7 days *vs* 100 days), both types of vaccination show similar levels of protection. These data reaffirm that DCs can be potent inducers of cellular immune responses against mycobacteria and support the concept of combining DC strategies with mycobacterial vaccines for protective immunity against TB.

In a monkey model of TB, the importance of early sensing of *M. tuberculosis* infection by DCs, their migration to the lymph nodes and T cell trafficking was demonstrated[57]. It was shown that for effective

protection an early activation and migration of DCs to draining lymph nodes together with stimulation of antigen specific T cells are key factors for inducing protection. Delays in any of the above processes could significantly alter the outcome of mycobacterial infections. Further, it was emphasized that new and better vaccines should elicit fast DC turnover at sites of infection together with strong DC activation ensuring maximum antigen presentation to T cells. This could result in the production of key cytokines required for inducing protective immunity.

DCs pulsed with *M. tuberculosis* sonicate induce significant protection to a challenge with both moderate and high doses of virulent *M. tuberculosis* in a mouse model[58]. This protection is characterized by enhanced antigen specific T cell responses that result in high IFN-γ production in the lungs and lymphoid tissues together with significant reduction in bacterial loads. Likewise, DCs pulsed with a H2-M3 oligopolymorphic MHC class 1b binding *N*-formylated peptide-TB2 elicit antigen-specific $CD8^+$ T cell responses in a mouse model of experimental TB[59]. Thus, the above studies reiterate the notion that immunizations with antigen-pulsed DCs offer viable alternatives for vaccine strategies against mycobacterial infections.

Survival of Mycobacteria in DCs

The ability of mycobacteria to survive in DCs is controversial, with reports both in favor and against. Both a constrained survival of mycobacteria in DCs[60,61] as well as increased replication of mycobacteria in DCs when compared to tightly regulated replication in macrophages has been reported[62-65]. Like resting macrophages, DCs provide an environment within which mycobacteria can survive and replicate, albeit to a low extent[63,65]. It could be attributed to the fact that turnover of mycobacteria within DCs is not enough to kill the cell and the outcome of slow replication is reflected by constant availability of antigens for presentation to T cells that potentiate the immune response. Following activation with TNF-α and IFN-γ, macrophages kill mycobacteria resident in phagosomes, but this does not occur in activated DCs. Upon stimulation with IFN-γ, DCs are able to control the replication of mycobacteria, but they are not killed. Instead, they appear to reside in vacuoles separated from normal recycling pathway. DCs may, therefore, act as a reservoir for mycobacteria *in vivo*, particularly within lymph nodes to which they have migrated.

Insights from our Studies

In an effort to decipher the physiological roles of antigens secreted by *M. tuberculosis* we have been working on the interactions of *M. tuberculosis* secretory antigens with DCs and their precursors and the outcome of such interactions in determining the immune responses to mycobacteria (reviewed in ref. 66). We have shown that many *M. tuberculosis* secretory antigens including culture filtrate protein (CFP)-10, early secreted antigenic target (ESAT)-6, MPT64, antigen 85B (Ag85B), whole cell lysate and total CFPs (when used collectively) induce the differentiation of mouse bone marrow leukocyte precursors into DCs[67]. These secretory antigen differentiated DCs show morphological and phenotypic similarities to DCs differentiated conventionally with granulocyte macrophage-colony stimulating factor (GM-CSF) and express high levels of costimulatory molecules (CD80, CD86, CD40, CD54) and MHC class I and II molecules. Further, these DCs are immature as stimulation with terminal maturation inducing agents further up-regulate the levels of costimulatory molecules as well as allogeneic responses. In parallel, we have also demonstrated that most of these antigens induce the maturation of both bone marrow derived and splenic $CD8^-$ and $CD8^+$ DCs[68].

However, functional characterization of these DCs shows that a challenge with mycobacterial components induce suppressor responses in an IL-10 and TGF-β dependent mechanism[69]. These results indicate that secretion of some of these antigens at sites of infection is a strategy employed by mycobacteria to subvert protective pro-inflammatory responses and induce suppressor responses at sites of infection.

We have recently demonstrated that not only do these DCs induce suppressor responses to mycobacteria, but also serve as depots for their survival. This is primarily achieved by down-modulating the oxidative burst and interfering with the activation status of key signaling molecules protein kinase C and calcium that contribute towards increased survival of mycobacteria within antigen activated DCs[70].

Dendritic Cells vs Macrophages in *M. tuberculosis* Infection

While macrophages and DCs can both process and present antigen to T cells, DCs are unique in their ability to initiate a primary immune response. Despite

many similarities, the two cell types respond differently to *M. tuberculosis* infection. While infection of DCs with *M. tuberculosis* induces their activation by upregulating costimulatory and MHC molecules[37] and secretion of IL-12 and IFN-γ, infection of macrophages with *M. tuberculosis* results in downregulation of MHC class I and II[71], IFN-γ responsiveness and IL-12 production, resulting in reduced inducible nitric oxide synthase activity and phox expression. Similarly, macrophage infection with BCG also results in down-regulation of peptide-loaded MHC class II molecules to the cell surface[72]. In addition surface levels of IFN-γ receptor are down-regulated in peripheral blood mononuclear cells of patients with active TB and the same are restored following anti-TB chemotherapy[73]. These results suggest that the down-regulation to IFN-γ responses during active TB occurs both at the level of ligand and the receptor.

Further, *M. tuberculosis* infected DCs and macrophages differ in their ability to influence the polarization of naïve T cells from spleens of ovalbumin peptide T cell receptor-transgenic mice. While a T helper 1 (Th1) response is obtained with DCs, addition of macrophages into the DC:T cell co-cultures hamper Th1 development by secretion of IL-10 and impairment of IL-12[34].

A critical step in the clearance of a pulmonary infection is the ability of DCs in the lung to migrate to the regional lymph nodes, where they initiate T cell responses. RNase protection assay reveals that CCR7 is greatly enhanced following infection with the virulent Erdman strain of *M. tuberculosis*[74]. On the other hand, mRNA levels for CCR7 in infected macrophages are extremely low. Further, it has been shown that DCs, but not macrophages, exposed to irradiated *M. tuberculosis* migrate to the draining lymph nodes. Once again while infected DCs primed Th1 responses with high IFN- γ levels and low IL-4 levels, infected macrophages failed to induce Th1 responses.

Summary

In conclusion, the interactions of *M. tuberculosis* with dendritic cells show opposite and often contrasting phenotypes and immune responses. These effects initiate at the time of recognition of the pathogen by distinct receptors. While recognition by TLRs result in activation of DCs and induce a response that benefits the host, recognition by DC-SIGN results in inactivation of DCs and elicit responses that favor the pathogen. Thus, both the time and form of recognition govern whether protective immunity would be achieved or not. In addition, the type of DCs involved during the interaction also determines the fate of infection. While DCs activated by some secretory antigens (e.g. CFP-10 and manLAM) tailor responses that favor the pathogen, DCs activated via TLRs by the bacilli favor the host. These results indicate that the nature of the antigenic moiety able to interact first with DCs determine the fate of the pathogen as also the host. Nevertheless, increasing evidences suggest that DCs offer immense potential in vaccine design and could constitute a major component of improved vaccine regimens.

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References

1 World Health Organization (2005) *Fact sheet*. Geneva, Switzerland
2 Kaufmann S H E (2000) Is the development of a new tuberculosis vaccine possible? *Nat Med* 6, 955-960
3 Fine P E M (1995) Variation in protection by BCG – implications and -for heterologous immunity. *Lancet* 346, 1339-1345
4 Hess J H, Schaible U E, Raupach B & Kaufmann S H E (2000) Exploiting the immune system: towards new vaccines against intracellular bacteria. *Adv Immunol* 75, 1-8
5 Manabe Y C & Bishai W R (2000) Latent *Mycobacterium tuberculosis* - persistence, patience, and winning by waiting. *Nat Med* 6, 1327-1329
6 Foote S (1999) Mediating immunity to mycobacteria. *Nat Genet* 21, 345-346.
7 Flynn J A & Chan J (2001) Immunology of tuberculosis. *Ann Rev Immunol* 19, 93-129
8 Jiao X, Lo-Man R, Guermonprez P, Fiette L, Deriaud E, Burgaud S, Gicquel B, Winter N & Lecler C (2002) Dendritic cells are hosts for mycobacteria *in vivo* that trigger innate and acquired immunity. *J Immunol* 168, 1294-1301
9 Tian T, Woodworth J, Skold M & Behar S M (2005) *In vivo* depletion of Cd11c+ cells delays the CD4+ T cell response of *Mycobacterium tuberculosis* and exacerbates the outcome of infection. *J Immunol* 175, 3268-3272
10 Steinmann R M (1991) The dendritic cell system and its role in immunogenicity. *Ann Rev Immunol* 9, 271-296
11 Banchereau J & Steinman R M (1998) Dendritic cells and the control of immunity. *Nature* 392, 245-252
12 Steinman R M (1999) Dendritic cells. In: *Fundamental Immunology* (William E. Paul, ed) pp 547-573 Lippincott-Raven Publishers, PA

13 Reid S D, Penna G, & Adorini, L (2000) The control of T cell responses by dendritic cells subsets. *Curr Opin Immunol* 12, 114-121
14 Sousa C R (2001) Dendritic cells as sensors of infection. *Immunity* 14, 495-498
15 Tsuji R, Matsumoto M, Tekeuchi O, Akira S, Azuma I, Hayashi A, Toyoshima K & Seya T (2000) Maturation of human dendritic cells by cell wall skeleton of *Mycobacterium bovis bacillus* Calmette-Guerin: involvement of Toll-like receptors. *Infect Immun* 68, 6883-6890
16 Schlesinger L S (1993) Macrophage phagocytosis of virulent but not attenuated strains of *Mycobacterium tuberculosis* is mediated by mannose receptors in addition to complement receptors. *J Immunol* 150, 2920-2930
17 Prigozy T I, Sieling P A, Clemens D, Stweart P L, Behar S M, Porcelli S A, Brenner M B, Modlin R L, & Kronenberg M (1997) The mannose receptor delivers lipoglycan antigensto endosomes for presentation to T cells by CD1b molecules. *Immunity* 6, 187-197
18 Nigou J, Zelle-Rieser C, Gilleron M, Thurnher M & Puzo G (2001) Mannosylated lippoarabinomannans inhibit IL-12 production by human dendritic cells: evidence for a negative signal delivered through the mannose receptor. *J Immunol* 166, 7477-7485
19 Tailleux L, Schwartz O, Herrmann J L, Pivert E, Jackson M, Amara A, Legres L, Dreher D, Nicod L P, Gluckman, J C, Lagrange P H, Gicquel B & Neyrolles O (2003) DC-SIGN is the major *Mycobacterium tuberculosis* receptor on human dendritic cells. *J Exp Med* 197,121-127
20 Kooyk Y V & Geijenbeek T B H (2003) DC-SIGN: escape mechanism for pathogens. *Nat Rev Immunol* 3, 697-709
21 Maeda N, Nigou J, Hermann J L, Jackson M, Amara A, Lagrange P H, Puzo G, Giquel B, & Neyrolles O (2003) The cell surface receptor DC-SIGN discriminates between *Mycobacterium* species through selective recognition of the mannose caps on lipoarabinomannan. *J Biol Chem* 278, 5513-5516
22 Barreiro L B, Neyrolles O, Babb C L, van Helden P D, Giquel B, Hoal E G & Quintana-Murci L (2007) Length variation of DC-SIGN and L-SIGN neck-region has no impact on tuberculosis susceptibility. *Hum Immunol* 68, 106-112
23 Turville S G, Arthos J, Donald K M, Lynch G, Naif H, Clark G, Hart D & Cunnungham AL (2001) HIV gp120 receptors on human dendritic cells. *Blood* 98, 2482-2488
24 Geijtenbeek T B H, van Vliet S J, Koppel E A, Sanchez-Hernandez M, Vandenbroucke-Grauls C M J E, Apelmelk B & van Kyook Y (2003) Mycobacteria target DC-SIGN to suppress dendritic cell function. *J Exp Med* 197, 7-17
25 Means T K, Lien E, Yoshimura A, Wang S, Golenbock D T & Fenton M J (1999) The CD14 ligands lipoarabinomannan and lipopolysascharide differ in their requirement for Toll-like receptors. *J Immunol* 163, 6748-6755
26 Noss E H, Pai R K, Sellati T J, Radolf J D, Belisle J, Golenbock D T, Boom W H & Harding C V (2001) Toll-like receptor 2-dependent inhibition of macrophage class II MHC expression and antigen processing by 19 kDa lipoprotein of *Mycobacterium tuberculosis*. *J Immunol* 167, 910-918
27 Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sto S, Sanjo H, Matsumotao M, Hoshino K, Wagner H, Takeda K & Akira S (2000) A Toll-like receptor recognizes bacterial DNA. *Nature* 408, 740-745
28 Means T K, Wang S, Lien E, Yoshimura A, Golenbock D T & Fenton M J (1999) Human Toll-like receptors mediate cellular activation by Mycobacterium tuberculosis. *J Immunol* 163, 3920-3927
29 Brockstedt D G, Bahjat K S, Giedlin M A, Liu W, Leong M, Luckett W, Gao Y, Schnupf P, Kapadia D, Castro G, Lim J Y H, Sampson-Johannes A, Herskovits A A, Stassinopopoulos B, Bouwer H G A, Hearst J E, Portnoy D A, Cook D N & Dubensky Jr T W (2005) Killed but metabolically active microbes: a new vaccine paradigm for eliciting effector T cell-responses and protective immunity. *Nat Med* 11, 853-860
30 Lankowski A J & Hohmann E L (2007) Killed but metabolically active *Salmonella typhimurium*: appliction of a new technology to an old vector. *J Infect Dis* 195, 1203-1211
31 Fenton M J (1998) Macrophages and tuberculosis. *Curr Opin. Hematol* 1, 72-78.
32 Kaufmann S H E (2001) How can immunology contribute to the control of tuberculosis? *Nat Rev Immunol* 1, 20-30
33 Rescigno M M, Martino C L, Sutherland M R, Gold P & Ricciardi-Castagnoli P (1998) Dendritic cell survival and maturation are regulated by different signaling pathways. *J Exp Med* 188, 2175-2180
34 Rescigno M (2002) Dendritic cells and the complexity of microbial infection. *Trends Microbiol* 10, 425-431
35 Hickman S P, Chan J & Salgame P (2002) *Mycobacterium tuberculosis* induces differential cytokine production from dendritic cells and macrophages with divergent effects on naive T cell polarization. *J Immunol* 168, 4636-4642
36 Giacomini E, Iona E, Ferroni L, Miettinen M, Fattorini L, Orefici G, Julkunen I & Coccia E M (2001) Infection of human macrophages and dendritic cells with *Mycobacterium tuberculosis* induces a differential cytokine gene expression that modulates T cell response. *J Immunol* 166, 7033-7041
37 Henderson R A, Watkins S C & Flynn J L (1997) Activation of human dendritic cells following infection with *Mycobacterium tuberculosis*. *J Immunol* 159, 635–643
38 Kim K D, Lee H G, Kim J K, Park S N, Choe I S, Choe Y K, Kim S J, Lee E & Lim J S (1999) Enhanced antigen presenting activity and tumour necrosis factor alpha independent activation of Dendritic cells following treatment with *M. bovis Bacilus* Calmette-Guerin. *Immunology* 97, 626-633
39 Dulphy N, Hermann J L, Nigou J, Rea D, Boissel N, Puzo N, Charron D, Lagrange P H & Toubert A (2007) Intermediate maturation of *Mycobacterium tuberculosis* LAM-activated human dendritic cells. *Cell Microbiol* (in press)
40 Stenger S, Niazi K R & Modlin R L (1998) Down-regulation of CD1 on antigen-presenting cells by infection with *M. tuberculosis*. *J Immunol* 161, 3582-3588
41 Mariotti S, Teloni R, Lon E, Fattorini L, Giannoni F, Romahgnoli G, Orefici G & Nisini R (2002) *Mycobacterium tuberculosis* subverts the differentiation of human monocytes into dendritic cells. *Eur J Immunol* 32, 3050-3058
42 Gonzalez-Juarrero M, Turner O C, Turner J, Marietta P, Brooks J V & Orme I M (2001) Temporal and spatial arrangement of lymphocytes within lung granulomas induced by aerosol infection with *Mycobacterium tuberculosis*. *Infect Immun* 69, 1722-1728
43 Jonuliet H, Schmitt E, Schuler G, Knop J & Enk A H (2000) Induction of interleukin-10 producing nonproliferating CD4+

T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J Exp Med* 192, 1213-1222.

44 Steinbrink K, Jonuliet H, Muller G, Schuler G, Knop J & Enk A H (1999) Interleukin-10 treated human dendritic cells induce a melanoma-antigen-specific anergy in CD8+ T cells resulting in a failure to lyse tumor cells. *Blood* 93, 1634-1642

45 Tobar J A, Carreno L J, Bueno S M, Gonzalez P A, Mora J E, Quezada S A & Kalergis A M (2006) Virulent *Salmonella enterica serovar typhimurium* evades adaptive immunity by preventing dendritic cells from activating T cells. *Infect Immun* 74, 6438-6448

46 Alaniz R C, Cummings L A, Bergman M A, Rassoulian-Barrett S L & Cookson BT (2006) *Salmonella typhimurium* infection triggers dendritic cells and macrophages to adopt distinct migration patterns in vivo. *Eur J Immunol* 36, 2939-2950

47 Ordway D, Henao-Tamayo M, Orme I M & Gonzalz-Juarrero M (2005) Foamy macrophages within lungs granulomas of mice infected with *Mycobacterium tuberculosis* express molecules characteristic of dendritic cells and anti-apoptotic markers of TNF receptor-associated factor family. *J Immunol* 175, 3873-3881

48 Kaufmann S H E & Schaible U E (2003) A dangerous liason between two major killers: *Mycobacterium tuberculosis* and HIV Target dendritic cells through DC-SIGN. *J Exp Med* 197, 1-5

49 Gringhuis S I, den Dunn J, Litjens M, van Het Hof B, van Kooyk Y & Geijtenbeek T B (2007) C-type lectin DC-SIGN modulates Toll-like receptor signaling via Raf-1 kinase dependent acetylation of transcription factor NF-kappaB. Immunity (in Press)

50 Demangel C, Palendira U, Feng C G, Heath A W, Bean A G & Britton W J (2001) Stimulation of dendritic cells via CD40 enhances immune responses to *Mycobacterium tuberculosis* infection. *Infect Immun* 4, 2456-2461

51 Demangel C, Bertolino P & Britton W J (2002) Autocrine IL-10 impairs dendritic cells (DC)-derived immune responses to mycobacterial infection by suppressing DC trafficking to draining lymph nodes and local IL-12 production. *Eur J Immunol* 32, 994-1002

52 Lande R, Giacomini E, Grassi T, Remoli M E, Iona E, Mittinen M, Julkunen I, & Coccia E M (2003) IFN-α/β released by *Mycobacterium tuberculosis*-infected human dendritic cells induces the expression of CXCL10: selective recruitment of NK and activated T cells. *J Immunol* 170, 1174-1182

53 Demangel C & Britton W J (2000) Interaction of dendritic cells with mycobacteria: where the action starts. *Immunol Cell Biol* 78, 318-342

54 Cheadle E J, Selby P J & Jackson A M (2003) *Mycobacterium bovis bacillus* Calmette-Guerin-infected dendritic cells potently activate autologous T cells via B7 and nterleukin-12-dpendent mechanism. *Immunology* 108, 79-88

55 Tascon R E, Soares C S, Ragno S, Stavropoulos E, Hirst E M & Colston M J (2000) *Mycobacterium tuberculosis*-activated dendritic cells induce protective immunity in mice. *Immunology* 99, 473-480

56 Demangel C, Bean A G D, Martin E, Feng C G, Kamath A T & Britton W J (1999) Protection against aerosol *Mycobacterium tuberculosis* infection using *Mycobacterium bovis bacillus* Calmette Guérin-infected dendritic cells. *Eur J Immunol* 29, 1972-1979

57 Marino S, Pawar S, Fuller C, Reinhart T A, Flynn J & Kirschner L (2004) Dendritic cell trafficking and antigen presentation in the human immune response to *Mycobacterium tuberculosis*. *J Immunol* 173, 494-506

58 Rubakova E, Petrovskaya S, Pichugin A, Khlebnikov V, McMurray D, Kondratieva E, Baturina I, Kondatieva T & Apt A (2007) Specificity and efficacy of dendritic cell-based vaccination against tuberculosis with complex mycobacterial antigens in a mouse model. *Tuberculosis* 87, 134-144

59 Doi T, Yamada H, Yajima T, Wajjwalku W, Hara T & Yoshikai Y (2007) H2-M3-restricted CD8+ T cells induced by peptide-pulsed dendritic cells confer protection against *Mycobacterium tuberculosis*. *J Immunol* 178, 3806-3813

60 Laochumroonvorapong, P, Paul S, Manca C, Freedman V H & Kaplan G (1997) Mycobacterial growth and sensitivity to H2O2 killing in human monocytes *in vitro*. *Infect Immun* 65, 4850-4857

61 Tailleux L, Neyrolles O, Honore-Bouakline S, Perret E, Sanchez F, Abastado J P, Lagrange P H, Gluckman J C, Rosenzwajg M & Herrmann J L (2003) Constrained intracellular survival of *M. tuberculosis* in human dendritic cells. *J Immunol* 170, 1939-1948

62 Bodnar K A, Serbina N V & Flynn J L (2001) Fate of *Mycobacterium tuberculosis* within murine dendritic cells. *Infect Immun* 69, 800-809

63 Fortsch D, Rollinghoff M & Stenger S (2000) IL-10 converts Dendritic cells into macrophage-like cells with increased antibacterial activity against virulent *Mycobacterium tuberculosis*. *J Immunol* 165, 978-987

64 Buettner M, Meinken C, Bastian M, Bhat R, Stossel E, Faller G, Cianciolo G, Ficker J, Wagner M, Rollinghoff M & Stenger S (2005) Inverse correlation of maturity and antibacterial activity in human Dendritic cells. *J Immunol* 174, 4203-4209

65 Mohagheghpour N, van Vollenhoven A, Goodman J & Bermudez L E (2000) Interaction of *Mycobacterium avium* with human monocyte-derived dendritic cells. *Infect Immun* 68, 5824-5829

66 Latchumanan V K, Balkhi M Y, Sinha A, Singh B, Sharma P & Natarajan K (2005) Regulation of immune responses to *Mycobacterium tuberculosis* secretory antigens by Dendritic cells. *Tuberculosis* 85, 377-383

67 Latchumanan V K, Singh B, Sharma P & Natarajan K (2002) *Mycobacterium tuberculosis* antigens induce the differentiation of dendritic cells from bone marrow. *J Immunol* 169, 6856-6964

68 Natarajan K, Latchumanan V K, Singh B, Singh S & Sharma P (2003) Down-regulation of T helper 1 responses to mycobacterial antigens due to maturation of Dendritic cells by 10 kDa *Mycobacterium tuberculosis* secretory antigen. *J Infect Dis* 187, 914-928

69 Balkhi M Y, Sinha A & Natarajan K (2004) Dominance of CD86, TGF-β and Interleukin-10 from *Mycobacterium tuberculosis* secretory antigen activated dendritic cells regulate T helper 1 responses to mycobacterial antigens. *J Infect Dis* 189, 1598-1609

70 Sinha A, Singh A, Satchidanandam V & Natarajan K (2006) Impaired generation of reactive oxygen species during

differentiation of Dendritic cells (DCs) by *Mycobacterium tuberculosis* secretory antigen (MTSA) and subsequent activation of MTSA-DCs by mycobacteria results in increased intracellular survival. *J Immunol* 177, 468-478

71 Wang Y, Curry H M, Zwilling B S & Lafuse W P (2005) Mycobacteria inhibit IFN-γ induced HLA-DR expression by up-regulating histone deacetylaion at the promoter-region in human THP-1 monocytic cells. *J Immunol* 174, 5687-5694

72 Sendide K, Deghmane A-E, Av-Gay Y, Talal A & Hmama Z (2005) *Mycobacterium bovis* BCG attenuates surface expression of mature class II molecules through IL-10-dependent inhibition of cathepsin S. *J Immunol* 175, 5234-5332.

73 Singhal A, Jaiswal A, Arora V K & Prasad H K (2007) Modulation of gamma interferon receptor 1 by *Mycobacterium tuberculosis*: a potential immune response evasive mechanism. *Infect Immun* 75, 2500-2510

74 Bhatt K, Hickman S P & Salgame P (2004) A new approach to modeling early lung immunity in murine tuberculosis. *J Immunol* 172, 2748-2751