

## A Dangerous Liaison between Two Major Killers: *Mycobacterium tuberculosis* and HIV Target Dendritic Cells through DC-SIGN

Stefan H.E. Kaufmann and Ulrich E. Schaible

Department of Immunology, Max Planck Institute for Infection Biology, D-10117 Berlin, Germany

With 2.9 million and 1.7 million deaths in 2000, the HIV and *Mycobacterium tuberculosis*, respectively, again top the infamous WHO list of excess deaths caused by infectious agents (1; Fig. 1). To further worsen the situation, these two pathogens do not operate independently. More than half a million of those who died last year were already coinfecting with both pathogens and more than 50 million individuals coinfecting with HIV and *M. tuberculosis* envisage a similar fate in the upcoming years. One third of the total world population (2 billion people) is infected with *M. tuberculosis* and coinfection with HIV exacerbates the risk of developing active tuberculosis from 1 in 10 during lifetime to 1 in 10 during the year after HIV infection (2).

As long as the immune system remains competent, *M. tuberculosis* is normally held at bay, though the immune response fails to achieve sterile eradication. Once, however, the strength of the immune response declines, active disease can develop normally through reactivation of quiescent organisms and in some cases through reinfection (2, 3). This is where HIV comes into play. With its notorious capacity to target CD4 T cells, HIV impairs the major host cell responsible for preventing conversion of mere infection to active tuberculosis (4). Now an additional link in the dangerous liaison between HIV and *M. tuberculosis* has been uncovered. Two papers in this issue reveal that the dendritic cell (DC)-specific surface receptor DC-SIGN (DC-specific ICAM-3-grabbing nonintegrin) is not only exploited by HIV as shown previously (5–7) but also by *M. tuberculosis*. The tubercle bacillus and its cell wall glycolipid lipoarabinomannan seem to bind to and to induce, via DC-SIGN, an intracellular signal leading to IL-10 production, which in turn could impair activation of protective T cell responses directed against *M. tuberculosis* and HIV (8, 9). The detrimental consequences of HIV on *M. tuberculosis* infection have been known for some time. Now it appears that, through DC-SIGN-mediated immunosuppression, *M. tuberculosis* also plays its part in this fatal liaison.

DC-SIGN is a C-type lectin, which is specifically (though not exclusively) expressed on DCs and serves as natural receptor for ICAM-2 and ICAM-3 (10, 11). Interactions between DC-SIGN and ICAM-2 on endothelial cells induce tethering and rolling of immature DCs (Fig. 1). This interaction promotes extravasation of immature DCs from blood vessels to inflammatory foci. Interactions between ICAM-3 on T cells and DC-SIGN on mature DCs initiate formation of the immunologic synapse, which promotes T cell activation (10; Fig. 1). Clearly, the function of DC-SIGN is not simply to recognize microbial determinants and hence it is not to be considered a pattern recognition receptor (PRR) like Toll-like receptors (TLRs). Rather, DC-SIGN serves as an adhesion molecule, but can be coopted by microorganisms to their own advantage. Other C-type lectins coexpressed on DCs include DEC205 and the mannose receptor (MR), which shares the mannose specificity with DC-SIGN (11). DEC205 and the MR contain several carbohydrate recognition determinants (CRDs), i.e., 10 and 8, respectively, whereas DC-SIGN has only a single CRD. Similar to the MR, the cytoplasmic domain of DC-SIGN carries a tyrosine based endosomal sorting sequence for recycling to the cell surface, but in addition also a triacidic cluster similar to DEC205, which allows ligand targeting to lysosomes (11).

HIV becomes firmly attached to immature DCs by binding of the HIV envelope glycoprotein gp120 to DC-SIGN (5–7, 12). However, this interaction does not result in productive infection of DCs by HIV. Rather it allows transport of HIV by immature DCs from peripheral sites to draining lymph nodes. This is where DCs interact with and activate CD4 T cells thereby facilitating trans-infection of the latter (6, 7). In the case of HIV, viral transmission normally starts on epithelial surfaces such as in the genital tract. DCs in the genital epithelium, however, do not express DC-SIGN, whereas subepithelial DCs express DC-SIGN implying that HIV sequestration takes place there.

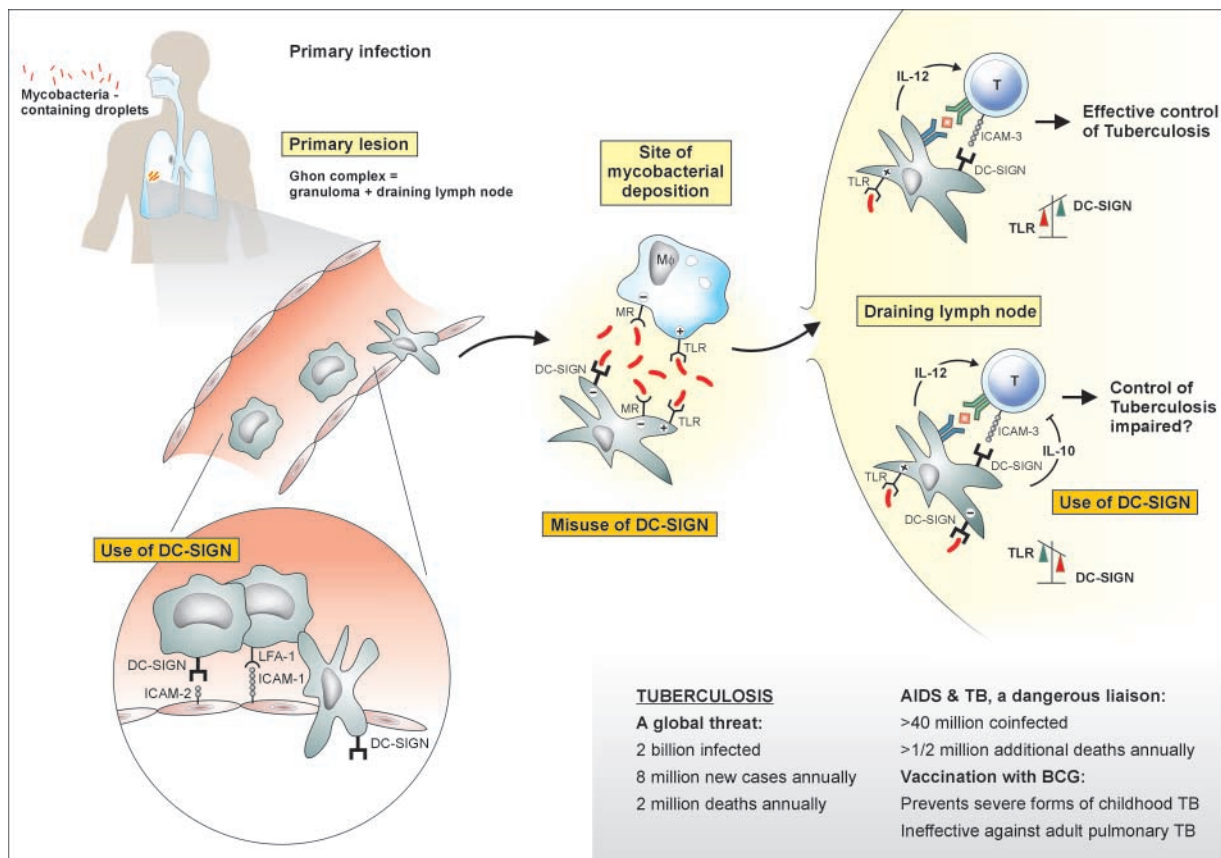
Two groups, in this issue, now show that mycobacteria exploit DC-SIGN as well (8, 9). Moreover, mannose-capped lipoarabinomannan (Man-LAM), a major component of the mycobacterial cell wall, was identified as the specific ligand. In contrast to Man-LAM, arabinose capped LAM (Ara-LAM) failed to bind. This is intriguing because

Address correspondence to Stefan H.E. Kaufmann, Department of Immunology, Max Planck Institute for Infection Biology, Schumannstr. 21/22, D-10117 Berlin, Germany. Phone: 49-30-28460-500; Fax: 49-30-28460-501; E-mail: kaufmann@mpiib-berlin.mpg.de

Man-LAM is abundant in slow growing mycobacteria comprising virulent mycobacteria, whereas Ara-LAM is abundant in fast growing atypical mycobacteria, which are avirulent. The studies by Geijtenbeek et al. employed slow growing but attenuated mycobacteria, namely the vaccine strain *M. bovis* bacillus Calmette–Guérin (BCG) and the avirulent laboratory *M. tuberculosis* strain H37Ra (8). The accompanying paper of Tailleux et al. (9), however, reveals that also virulent *M. tuberculosis* use DC-SIGN for cell entry. The mono-, di-, or trimeric mannose residues of LAM must comprise the binding structure for DC-SIGN. LAM is not only recognized by DC-SIGN but by a variety of other receptors expressed by DCs including the MR, CD11b, and CD11c. CD11 is part of the complement receptor 3 (CR3; reference 13). This receptor can bind mycobacteria through either a mannan binding site or C3b and C3bi fixed to the bacterial surface upon complement activation via the classical and the alternative pathway or via mycobacteria-mediated cleavage of C2 into a C3-convertase (14). The MR, as a C-type lectin, recognizes mannose residues through its CRD (15, 16). Yet, as determined by antibody blocking experiments in the present studies,

uptake of mycobacteria by DCs is only induced by interactions with DC-SIGN but not with other receptors (8, 9). Once engulfed, mycobacteria end up in a phagosomal compartment, where they are rapidly dissociated from DC-SIGN. However, in considering DCs as host cells for *M. tuberculosis* it is appropriate to ask whether these cells support mycobacterial growth in a similar way as macrophages do. Recent studies have shown that in contrast to macrophages mycobacteria do not grow readily inside DCs due to IL-10–induced reversion of DC maturation (17, 18). The data by Geijtenbeek et al. suggest that LAM and mycobacteria are targeted by DC-SIGN into LAMP-1<sup>+</sup> compartments of DCs (8). It is possible that mycobacterial phagosomes mature to late endosomal/lysosomal stages in DCs (19), whereas in macrophages mycobacteria arrest phagosome maturation at an early endosomal stage thereby promoting mycobacterial growth.

*M. tuberculosis* enters the host typically via aerosols and alveolar macrophages are considered the first cells to engulf *M. tuberculosis* and become infected. However, DCs have been identified in the airway mucosa in particular at sub-mucosal and interstitial sites of the respiratory tract (20).



**Figure 1.** Tuberculosis is still a global threat to mankind with no effective vaccine available as yet. Primary infection by *M. tuberculosis* is initiated by aerosol inhalation leading to infection of macrophages and DCs in the lung from where infected cells carry bacteria to the draining lymph nodes. Upon encounter of mycobacteria, DC-SIGN on DCs and alveolar macrophages engage with mycobacterial glycolipids, e.g., LAM, which triggers IL-10 secretion leading to an impaired T cell response. In contrast, engagement of TLRs will promote a protective inflammatory response characterized by IL-12 and IFN- $\gamma$  secretion. We consider a balanced stimulation of both arms important for protective responses accompanied by minimal immunopathology. Dysbalance could favor *M. tuberculosis* and hence disease development.

Hence, DCs could directly capture *M. tuberculosis* and then transport the pathogen from the primary site of bacterial implantation to the draining lymph node (the primary site of replication of *M. tuberculosis* in the lung and the draining lymph nodes are termed Ghon complex; Fig. 1). There, DCs can present mycobacterial antigens to T lymphocytes and in this way induce the protective T cell response. Similar to other C-type lectins, DC-SIGN can also function as a receptor of mannosylated antigens for presentation to T cells (21), the crucial mediators of protection against tuberculosis. Although IFN- $\gamma$ -producing CD4 T cells of the Th1 type are of major importance, other T cells, notably CD8 T cells and perhaps  $\gamma/\delta$  T cells and CD1-restricted  $\alpha/\beta$  T cells, participate as well (22). The major protective function is macrophage activation by IFN- $\gamma$  and hence protection is a typical Th1 phenomenon. In addition, mycobacterial killing by cytotoxic T cells, which release a lethal combination of perforin and granulysin, could contribute to protection (23).

Generally, blood-derived DCs are used for in vitro studies, which are obtained by culturing blood monocytes with GM-CSF and IL-4. The finding by Tailleux et al. that DCs in both lung and draining lymph nodes (the Ghon complex) express DC-SIGN and that DC-SIGN-positive lymph node DCs from tuberculosis patients carry *M. tuberculosis* antigens provide compelling evidence that, what has been observed in vitro, indeed corresponds to the in vivo situation (9).

Although Kwon et al. showed previously that DC-SIGN not only binds but also mediates internalization of HIV, which is required for trans-infection of T cells (6), Geijtenbeek et al. are the first to demonstrate an involvement of DC-SIGN in intracellular signaling by a pathogen (8). The authors found that LAM stimulated production of the anti-inflammatory and immunosuppressive cytokine IL-10 and, at the same time, impaired LPS-mediated DC maturation. Mycobacteria are potent inducers of the Th1 cell pathway and mycobacterial components have been shown to stimulate expression of costimulatory molecules and IL-12 production in DCs via TLR2 and TLR4 (24). Lipoproteins and insufficiently characterized cell wall components stimulate via TLR2 or TLR4, respectively. Hence, it is tempting to speculate that this proinflammatory pathway requires counterregulatory mechanisms to limit pathological sequelae. In this sense, DC-SIGN and probably other LAM receptors such as the MR could counteract TLR-mediated activation. Mycobacteria, however, may exploit this suppressive pathway thereby tipping the labile balance between proinflammatory/protective responses and anti-inflammatory/suppressive responses in favor of the latter situation and to their own benefit (Fig. 1). Furthermore, it has been proposed that during latent tuberculosis, where mycobacteria are contained within granulomas, protective IFN- $\gamma$  production has to be counterregulated by IL-10 to minimize immunopathogenicity (25). DC-SIGN could as well play a regulatory role here. How DC-SIGN signals into the DCs is not clear yet. However, the presence of immunoreceptor tyrosine-based activation motifs (ITAMs)

in its cytoplasmic tail suggest that DC-SIGN is capable of direct signaling (11).

In the lung, the vast majority of mycobacteria seem to be engulfed by alveolar macrophages. It is therefore interesting to note that DC-SIGN has been found on alveolar macrophages, as well (11), although its function here remains unclear. Yet, macrophages seem to use several receptors for the crosstalk of *M. tuberculosis* including CR3 and MR, CR1, CR4, and CD14 as well as surfactant protein (SP)-A receptors and scavenger receptors (13). Hence, several pathways into macrophages seem to exist for mycobacteria. It remains to be established whether alveolar macrophages express on their surface a sufficient density of DC-SIGN. It is, however, possible that other C-type lectins, which also bind mannose such as the MR, fulfil similar functions on macrophages as DC-SIGN on DCs. Evidence for inhibition by MR signaling of TLR mediated IL-12 production has been provided (16). Hence, it is possible that both macrophages and DCs express inhibitory receptors in the form of different C-type lectins with specificity for mannose as an abundant carbohydrate residue of mycobacteria.

With such a labile balance, stochastic effects may come into play. Only minute numbers of mycobacteria arrive in the lung and establish infection. Thus, it is possible that the decision whether infection ultimately progresses into disease or remains contained is determined by the type of encounter between mycobacteria and host cell receptors: C-type lectins versus TLR. The final outcome of these interactions could well be further influenced by the type of host cells, macrophage versus DCs, that encounter mycobacteria. Accordingly, a dominance of TLR signaling would favor inflammatory and protective pathways, while a dominance of LAM signaling via DC-SIGN and MR could favor antiinflammatory and suppressive mechanisms (see Fig. 1).

Tuberculosis remains a major health threat and general agreement exists that an efficacious vaccine is needed for satisfactory control of this disease (26). The currently available vaccine, *M. bovis* BCG, can prevent miliary childhood tuberculosis, but fails to protect against the most prevalent form of disease, pulmonary tuberculosis in adults (26). Hence, this vaccine induces insufficient protection. Similarly, in the small proportion of infected individuals, who will develop active disease at a later stage of infection, protection is insufficient. Consistent with this notion, reinfection with *M. tuberculosis* can cause tuberculosis after chemotherapeutic eradication of primary infection (3). In the vast majority of infected individuals who do not develop tuberculosis but fail to eradicate the pathogen, protection can be considered sufficient as long as exogenous insult does not occur. However, these individuals remain vulnerable to immuno-compromising offense from outside, e.g., by HIV. Any efficacious vaccine needs to induce an immune response in susceptible individuals that is at least as good as the protection afforded by natural infection in the resistant population. It is tantalizing to speculate that exploitation of DC-SIGN (and probably MR) via LAM allows *M. tuberculosis* to undermine induction of an efficacious immune re-

sponse at the very early switchboard of its induction, the DCs. In the past, mycobacterial lipids, which manipulate host defense in favor of the pathogen, have already been discussed as virulence factors: LAM was found to inhibit macrophage activation and other mycobacterial phospholipids were shown to interfere with T cell activation (27). Although the molecular basis for these effects remains unclear, they could further contribute to immune suppression that occurs in late stage tuberculosis (28). Significant amounts of lipids including LAM are shed from mycobacteria during infection of macrophages (19, 29, 30). Therefore, it can easily be envisaged that in active tuberculosis abundant amounts of lipids are released into the circulation from caseous lesions containing high numbers of tubercle bacilli. Interaction with DC-SIGN and probably the MR of LAM and other mannosylated phospholipids such as the phosphatidyl inositol mannosides could trigger systemic IL-10 secretion and silencing of T cell responses (anergy) as described in patients with late stage tuberculosis (28). It remains to be seen whether similar rules also hold true for alveolar macrophages expressing receptors for LAM. It is possible that the plethora of different PRR on macrophages out-compete LAM-mediated inhibitory signaling, thus favoring protective effector functions.

Provided that misuse of DC-SIGN by mycobacteria and HIV favors the pathogens over the host, the findings that uptake of both predators can be blocked by antibodies against DC-SIGN in vitro provides some signs of hope (8, 9). This observation could form the basis for novel therapeutic strategies, e.g., using soluble receptors aimed at blocking transmission of HIV and immuno-suppression by *M. tuberculosis*. Therefore, these reports represent instructive examples in experimental medicine, showing how basic research may directly translate into medical control measures for the two major microbial killers of humankind.

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