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COMMENTARY

Leprosy Bacillus Triggers the Wrong Cells

In a recent issue of *Nature Medicine,* Krutzik, *et al*. report a novel finding on the role of macrophages and dendritic cells in leprosy. In lepromatous leprosy patients' blood and lesions, triggering of dendritic cells was impaired, suggesting a defect in the initiation of adaptive immunity.

INTRODUCTION

Dendritic cells (DC) and macrophages (Mf) play major roles in innate immunity and provide a first line of defense against invading pathogens like *Mycobacterium leprae*. Mf and particularly DC also play a key role in the onset of subsequent adaptive immunity by triggering pathogen specific T-cell and B-cell responses, and by the formation of immunological memory such that the immune system will be able to remember previous encounters with pathogens later in life. Besides representing key cells of the immune system in combatting bacterial infections, however, Mf and DC, paradoxically also provide a necessary safe-haven for so-called intracellular bacteria, of which mycobacteria in general and *M. leprae* in particular are prime examples: *M. leprae* is widely believed to be unable to dwell outside phagocytes, and exploits these otherwise hostile immune cells to survive and replicate in the human body. Thus, changes in the volatile equilibrium between host and pathogen will dictate whether the host ("immunity") or the pathogen ("immune escape") is favored.

Infection of Mf and DC by bacteria is mediated via a series of cell-surface receptors, including Toll-like Receptors (TLR), the Mannose Receptor and the Dendritic cell (DC)-specific surface receptor DC-SIGN (DC-specific ICAM-3–grabbing nonintegrin). These receptors interact with specific biochemical structures on the surface of bacteria and trigger phagocytosis, anti-microbial activity and release of cytokines by the infected cell[1–3]. DC-SIGN ordinarily interacts with cell surface molecules called ICAM-2 and ICAM-3 that are expressed on a variety of host cells. Interactions between DC-SIGN and ICAM-2 on endothelial cells induce tethering and rolling of immature DCs and thus promotes extravasation of these cells from the blood to inflammatory foci.

Mycobacterial products can trigger TLR-family members and induce immature DCs to differentiate into mature DCs, cells that are specialised in superior induction of T cell mediated immunity. Mature DCs release inflammatory cytokines, highly efficiently present captured antigens to naïve T cells and drive their differentiation and activation into effector and memory T cells. Presentation of antigens to T cells is achieved through surface molecules called MHC class-I and class-II molecules, as well as MHC class-I-like CD1 molecules. MHC class-I and class-II molecules present short protein fragments of pathogens, whereas CD1 molecules present complementary components such as bacterial lipids.

DC are present in very low numbers in the blood (<1%), and have therefore been difficult to study. Most studies on DC have therefore used human blood monocytes that were differentiated *in vitro* into DC-like cells using the cytokines IL-4 and GM-CSF. This technique allows acquisition of much higher cell numbers that are easier to work with. These cells have a DC-like phenotype, express *both* DC-SIGN and CD1b (DC-SIGN$^+$CD1b$^+$), and are able to activate T cells potently.

Summary of the Krutzik, *et al.*[4] study

To their surprise, however, Krutzik, *et al*. now report that this cell type is not observed *in vivo* in the lymphoid tissues they analyzed. Importantly, they find that stimulation of cells via their TLR—as a mimic of bacterial infection-induced differentiation of blood monocytes mostly into *either* DC-SIGN$^+$CD1b$^-$ *or* DC-SIGN$^-$CD1b$^+$ cells,

whereas only small percentages of double positive cells were seen, the predominant cell type induced by the widely used IL-4/GM-CSF combination. Thus, DC-SIGN and CD1b molecules were expressed mainly on different cell types.

Elegant *in vitro* studies further revealed that the DC-SIGN$^+$CD1b$^-$ cells carried typical Mf markers. The TLR induced expression of DC-SIGN was particularly prominent following exposure to the mycobacterial 19-kDa lipopeptide that binds to TLR2/1, and was mediated by the innate cytokine IL-15. These Mf were able to bind and phagocytose mycobacteria via DC-SIGN, and secreted high levels of inflammatory cytokines which are necessary to activate innate and adaptive immunity. In contrast, TLR induction of DC-SIGN$^-$CD1b$^+$ cells was dependent on GM-CSF. These cells resembled DCs and were substantially more capable of activating T cells than DC-SIGN$^+$CD1b$^-$ cells. DC-SIGN$^-$CD1b$^+$ cells lacked mature DC markers such as CD83, suggesting they had not fully matured yet. Finally, the latter cells were less able to bind BCG compared to the DC-SIGN$^+$CD1b$^-$ Mf like cells.

The findings were further extended by examining the expression of these new cell types in leprosy. Much like healthy donors, tuberculoid leprosy patients' monocytes yielded both DC-SIGN$^+$CD1b$^-$ Mf-like and DC-SIGN$^-$CD1b$^+$ DC-like cells following TLR activation. A striking finding was that lepromatous patients only yielded Mf but not DC like cells. Such a defect, however, was not observed when the above mentioned IL-4/GM-CSF combination was used to generate monocyte derived "classical" DCs *in vitro,* ruling out a general defect in their capacity to generate DC-SIGN$^-$CD1b$^+$ DC-like cells at all. Also, normal levels of DC-SIGN$^-$CD1b$^+$ DC-like cells were seen in lepromatous patients undergoing reversal reactions. More interestingly, the same cell type distribution was seen in tuberculoid, lepromatous, and reactional lesions. Also *in situ*, tuberculoid lesions contained both DC-SIGN$^-$CD1b$^+$ DC-like cells and DC-SIGN$^+$CD1b$^-$ Mf-like cells, whereas lepromatous lesions lacked the latter and mostly contained the former subset. In addition, the Mf cells could be demonstrated to contain *M. leprae* material in lepromatous but not tuberculoid lesions. Thus, since lepromatous patients lack (local) DCs the implication of these findings may be that they are unable to induce and activate proper T cell reponses to eradicate *M. leprae*.

Questions and discussion

The surprising findings by Krutzik, *et al*. obviously need confirmation and extension in other systems, but are certainly new and provocative. The finding that DC-SIGN$^+$ cells belong mostly to the Mf- but not DC-class is even highly provocative. Nevertheless, some caution may be warranted in overinterpreting this data to indicate that many DC-studies in the past have been performed on cells that hardly or not at all exist *in vivo*. Before such conclusions can be drawn more work is clearly needed. A small subset of cells in the Krutzik, *et al*. study actually is double-positive (DC-SIGN$^+$CD1b$^+$), both *in vitro* and *in vivo,* but may simply be a minor population that could be selectively expanded by IL-4/GM-CSF. It should be pointed out also that various DC and Mf subsets exist (5,6), and that there may even be a continuum of phagocyte types, each with its own level of plasticity. This would even further allow these cells to adapt to various conditions and acquire different phenotypes depending on the precise (cytokine-) environment ([5]). The micro environment in leprosy skin lesions or tonsils may not be ideal to favor "double positive DCs," but this does not exclude their existence or relevance in the human immune system. Of interest, also other Mf like subsets (CD16$^+$DC-SIGN$^-$; unfortunately, it is not indicated whether these cells were CD1b$^+$) were found in lepromatous lesions, pointing to the existence of a more complex local repertoire of phagocytic cells in leprosy lesions.

The sample size of patients and lesions studied by Krutzik, *et al*. seems rather small (the numbers of samples studied are not always clearly indicated in the manuscript) so that it is as yet uncertain to what extent the findings in the individuals analysed can be generalised to human leprosy *per se*.

The mechanisms behind the impairment of lepromatous patients' cells in inducing DC like cells remains unexplained. It is important to resolve this, as this may provide

novel therapeutic angles. The question is what phenotype would result when cells would have been stimulated more physiologically with *M. leprae* instead of unrelated TLR stimuli, but no data are reported on this issue. Furthermore, a general impairment in DC function in lepromatous leprosy is not easily reconciled with the characteristic and rather specific defect in T-cell responsiveness to antigens of *M. leprae* in lepromatous leprosy: general DC defects would be expected to lead to more general defect in T-cell responses, but this is not typically the case in lepromatous leprosy.

It also remains unknown if the defect in local DC like cells in lepromatous leprosy lesions is permanent or not: is this defect disease-activity dependent, or is it rather a permanent characteristic of lepromatous leprosy susceptible individuals? And if so, what are the host (genetic) factors that drive this defect?

Whatever the issues to be resolved, the study by Krutzik, *et al*. sheds new light on Mf and DC in innate and adaptive immune responses in general and in leprosy in particular. Therapies to activate and expand DCs in lepromatous patients may help to control disseminating infection.

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