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Targeting Immune-Evasion Mechanisms as a Possible New Approach in the Fight against Tuberculosis

Roberto Nisini*

Department of Infectious, Parasitic and Immune-Mediated Diseases, National Institute of Health, Viale Regina Elena 299, 00161 Rome, Italy

Abstract

The emergence of multidrug resistant *Mycobacterium tuberculosis* (Mtb) strains, the difficulties in the development of new safe therapies or the production of a protective vaccine against tuberculosis make the study of the strategies developed by Mtb to escape from the host immune system an important source of potential targets of immuno-intervention in the fight against tuberculosis. A mechanism of immune-evasion specific for the post-primary tuberculosis based on the differentiation of disabled antigen presenting cells is described.

Keywords: *Mycobacterium tuberculosis*; Tuberculosis; Immune-evasion; Monocyte differentiation; Dendritic cell; Reactivation tuberculosis; Primary tuberculosis

Introduction

The World Health Organization has estimated that approximately one-third of the world's population is infected with *Mycobacterium tuberculosis* (Mtb) [1,2]. This enormous pool of infected individuals poses a major hurdle for global Tuberculosis (TB) control since between 8 and 9 million people develop active disease each year [3-5], even if the large majority of Mtb infected individuals remains asymptomatic, with a latent TB infection. These individuals are unable to clear the bacterium that remains dormant in some body compartments, within granulomas or cellular reservoirs [6]. However, in 5 to 10% of them Mtb may "resuscitate" causing reactivation TB [7-9]. Several lines of evidence support the notion that the immune system is primarily involved in the control of Mtb infection [10], as demonstrated by the severe forms of TB that occur in immuno-compromised individuals or in animal models of immuno-deficiency.

The immune system is activated during primary infections and effectively contributes to limit Mtb growth [11-13]. Cells of both the innate and acquired immune system contribute to control and maintain the infection latent, but are intriguingly unable to limit Mtb outgrowth during the Mtb reactivation that characterizes post-primary TB. The activation of the immune system following Mtb infection is documented in exposed individuals by anti Mtb specific antibodies and by the presence of Mtb-specific T lymphocytes. Antibodies may be detected by several techniques, including ELISA and the expansion of Mtb-specific T lymphocytes as the component of anti-Mtb memory immune response is demonstrated by the delayed type hypersensitivity to Mtb proteins, which characterizes subjects with a history of Mtb infection. Hypersensitivity has been extensively exploited as the read out in the tuberculin skin test for diagnostic purposes, since its introduction by Robert Koch (13). More recently, the presence of Mtb-specific T lymphocytes in infected individuals for diagnostic purposes can also be measured by Interferon-Gamma Releasing Assays (IGRA) [14]. As a consequence of the first infection, the majority of otherwise healthy patients experience a mild, self-limiting disease, primary TB, which clinically heals [4,15]. In particular, high amounts of antibodies are produced, T cells specific for Mtb antigens are primed and the majority of Mtb cells are killed by activated macrophages. However, Mtb may persist in some compartments, where it is forced to shift into a non-replicating, dormancy status [7,16,17]. If reactivation TB may be explained in patients with acquired immuno-deficiencies [8,18], it is much more complex to identify which perturbation of the immune system allows the Mtb shift from a non replicating to a metabolically active status in otherwise healthy patients with an expanded pool of efficient Mtb specific memory cells [19].

Immune-evasion as a Mtb tool to cause disease

Several hypotheses have been suggested to explain the extraordinary capacity of Mtb to cause disease in susceptible individuals and great emphasis has been given to its capacity to evade host immune responses [20,21].

There are several examples of pathogens escaping memory T and B cell responses. Some use mechanisms mainly relying on antigenic variation or mimicry. Viruses, such as HIV or HCV, undergo extensive mutation within a single infected individual with the consequence that memory cells primed at the beginning of the infection are unable to specifically recognize antigens coded by mutated genes with the progression of the disease. Bacteria, such as pneumococci, may exploit antigenic variation of their capsule so that IgG produced by memory B against a specific serotype are unable to opsonize bacteria of the same strain expressing different capsules. Other bacteria, such as group A *streptococcus* or group B *Neisseria meningitides*, synthesize components with similarity with human components and through this molecular mimicry, that causes autoimmunity in certain individuals, these bacteria take advantages of the immunological tolerance safeguarding self component to prevent the induction of a bacterial specific immune response.

Noteworthy, Mtb capacity of immune-evasion does not fall within any of these categories of immune-escaping mechanisms being an extremely conservative bacterium with low rate of mutations and rare antigenic variations [22] and the majority of known immuno-dominant antigens are preserved among different Mtb strains and within the same

***Corresponding author:** Roberto Nisini, Department of Infectious, Parasitic and Immune-Mediated, National Institute of Health, Viale Regina Elena 29900161 Rome, Italy, Tel: +390649902659; E-mail: roberto.nisini@iss.it

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Mtb that infects a single individual [22]. Thus, a variety of alternative mechanisms have been proposed and *in vitro* proven to explain the survival of Mtb within the macrophage and its escape from immuno-recognition, such as the inhibition of phagosome–lysosome fusion, phagosome acidification and the resistance to killing by oxygenated metabolites [11,21]. Thus, by interfering with its intracellular degradation, Mtb would substantially block the processing of its antigens, the loading of immuno-dominant peptides onto MHC class II molecules, and/or the transport of MHC-peptide complexes to the cell surface [12] resulting hidden to T cell recognition.

The real impact of these *in vitro* studied mechanisms of immune-evasion in the pathogenesis of TB is difficult to be measured *in vivo*, but clashes with the observation of a robust T cell response in individuals who experienced primary TB. If immune-evasion mechanisms occur *in vivo*, we can speculate that immunologically naïve individuals with respect to mycobacterial infection are able to overcome these mechanisms, permitting the priming of Mtb specific immune response both at B and T lymphocyte levels, at odds with individuals who are experiencing reactivation TB, whose memory immune cells are eluded by immune-evasion.

An immune-evasion mechanism effective in post-primary TB only

As a contribution to explain why the immune system is able to control primary infections but not to prevent reactivation and limit Mtb outgrowth in post-primary TB occurring in immuno-competent individuals, we hypothesized that the function of Antigen Presenting Cells (APCs) may be a target of immune-evasion mechanisms evolved by Mtb. In particular, we hypothesized that, by infecting monocytes recruited to the inflammatory site together with specific T lymphocytes, Mtb could divert their differentiation into effective dendritic cells, thus limiting the possibility to maintain an adequate level of anti-Mtb cellular immune response. This mechanism of immune diversion, would preferentially take place in post-primary TB, when the pool of resident DC has to be replaced by monocyte derived DC.

In the first infection, which causes primary TB, Mtb is internalized, processed and eventually killed or induced to shift in dormancy by alveolar and lymph node macrophages cooperating with helper and/or cytotoxic Mtb specific T lymphocytes of various subsets. Circulating monocytes are recruited at the infection site where they are believed to differentiate into macrophages to replace those killed by Mtb, and tissue resident or circulating dendritic cells (DCs) are firstly recruited at the site of the infection where they mat phagocytize Mtb and then migrate to regional lymph nodes where they are responsible of the priming of Mtb specific T cells. In reactivation TB not only circulating monocytes and DCs are recruited at the site of reactivation, but also memory Mtb-specific T cells. Monocytes are precursors cells of both macrophages and DCs and we have shown that antigen loaded monocytes may differentiate into DCs if they present antigen to specific T helper cells [23], which secrete GM-CSF and other cytokines including IFN-γ or IL-4 upon antigen dependent activation [23]. We also showed that Mtb infected monocytes that are induced to differentiate into DCs by Mtb specific T cell clones or by added cytokines, such as GM-CSF and IL-4, IFN-α or IFN-γ, differently from uninfected monocytes or by monocytes infected with other pathogens such as *M. avium*, differentiate into an unusual type of DC with peculiar phenotypic and functional characteristics [24,25]. Briefly, DCs derived from Mtb-infected monocytes (Mt-MoDC) are characterized by a failure in the expression of CD1 molecules, a reduced capacity to up-regulate HLA class II DR and CD80 molecules, and by a severely

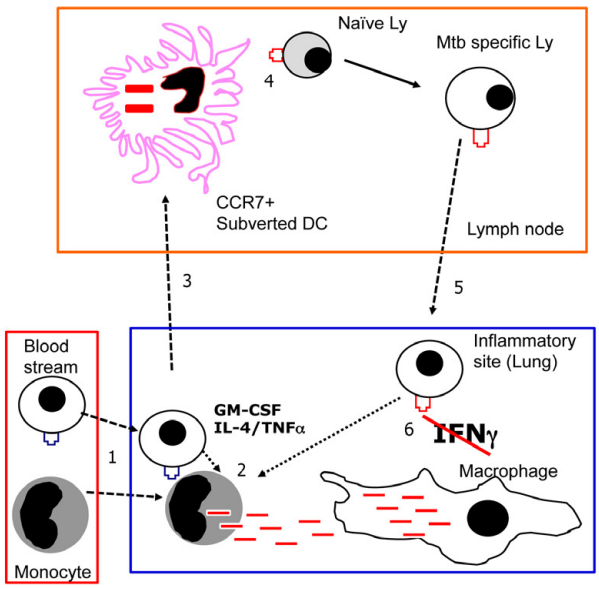

Figure 1: An escape mechanism based on the Mtb dependent differentiation of subverted DC from infected monocytes. Monocytes and memory T lymphocytes (Ly) are recruited into the inflammatory site in post-primary TB (1). Monocytes are infected by Mtb and present Mtb antigens to memory T Ly, which activate and secrete cytokines such as GM-CSF and IL-4, IFNγ or TNF-α (2). Sensing these cytokines, infected monocytes differentiate in subverted dendritic cells ($CD1a^{-}$, $HLA\text{-}DR^{low}$, $CD80^{low}$, $CCR7^{+}$) and migrate to lymph nodes (3). In lymph nodes, subverted DC can prime Mtb specific T lymphocytes, which are unable to secrete IFNγ (4). these lymphocytes migrate to the inflammatory site (5), but upon antigen presentation by infected APCs, they cannot help macrophages to kill intracellular Mtb, which consequently continues to grow, kill macrophages, infect other cells, including newly recruited monocytes and cause tissue damage (6).

impaired capacity to prime IFN-γ producing T lymphocytes. Thus, we proposed the Mtb interference with monocyte differentiation into fully competent DCs as an additional mechanism of immune evasion that may occur in reactivation TB only (Figure 1). In fact, the T cell dependent differentiation of Mtb infected monocyte into subverted DCs cannot take place in primary TB, since a memory T cell response is developing at this stage and several data suggest that the T cell response in primary TB requires an estimated mean time of 30-45 days [26]. Moreover, the number of circulating monocytes is higher than the number of circulating DCs, thus monocytes recruited and infected at the inflammatory site exceed DCs, making it possible that the pool of subverted DCs derived from infected monocytes functionally outnumbers DCs in post-primary TB.

The described interference with monocyte differentiation would finally result in the reduction of T lymphocytes capable of secreting IFN-γ, a cytokine that is considered of primary importance in Mtb control [27].

Notably, the described mechanism of immuno-evasion is shared by BCG [28], but not by non pathogenic mycobacteria such as *M. avium* [24] or several tested gram positive bacteria (personal communication). Since LPS was shown to negatively influence monocyte differentiation in to DC [29], it is reasonably that also gram negative bacteria such as *Salmonella* sp. could interfere with monocyte differentiation as a mechanism of immuno-evasion.

However, this phenomenon seems to be particularly relevant in the pathogenesis of chronic infections, where resident DC have to be replaced after their migration to lymph nodes and in TB in particular.

Interestingly, our *in vitro* obtained data are in line with the observation that patients with advanced stages of TB result negative at IGRA, currently used for TB diagnostics. Moreover, the lack of CD1 molecules observed in Mtb subverted DC is of particular relevance, since Mtb is the microorganisms with the highest content of antigenic lipids, which are presented by these antigen-presenting molecules to CD1-restricted lipid-specific T lymphocytes. It has been demonstrated that CD1-restricted T cells are expanded in humans as a result of a prior Mtb infection, contributing to the initiation of a cell-mediated immune response against the pathogen. In contrast CD1-restricted T cell responses are absent or drastically reduced in patients with active pulmonary tuberculosis [30], and this reduction could be a possible consequence of a reduced differentiation of CD1 expressing DCs because of the described Mtb interference with monocyte differentiation.

Possible innovative strategies of immuno-intervention for TB treatment

We have shown that a Mtb cell wall constituent, α-glucan, is directly involved in causing the subverted differentiation of monocyte through its binding to membrane receptors including the CR3 [28]. The binding of α-glucan to monocyte receptors triggers a p38 dependent intracellular signaling that results in the subverted differentiation and CD1a reduced gene transcription [31]. Interestingly, the involvement of p38 was demonstrated by the use of a p38 inhibitor, which was sufficient to block the Mtb dependent interference and resulted in the differentiation of infected monocyte into fully competent DCs [31].

The fight against TB is based on general hygiene measures, including the early diagnosis and isolation of infected individuals and on the treatment of cases and contacts. Treatment of latent infected individuals may be a possible, but difficult strategy that would be substituted by the development of an efficacious vaccine. Unfortunately, the only available vaccine, BCG is largely inefficacious and the research for a more efficacious vaccine has encountered unexpected difficulties and failures. The increase isolation of Mtb strains resistant to available antibiotics and chemotherapeutics and the dreadful association with HIV infection forces the search for novel antibiotics, but only few products have been licensed for use in multidrug resistant TB in the last years. In this scenario, a better understanding of the mechanisms used by this pathogen to cause disease is of extreme importance for the possible identification of novel targets for intervention. The mechanism of immune-evasion we proposed may represent a target for pharmacologic or immunologic intervention aimed at increasing the pool of effective T cell involved in the containment of Mtb outgrow. Among several other possible alternative measures aimed at fighting Mtb pathogenetic mechanism, the immuno-mediate block of α-glucan binding on monocyte, by specific antibodies, or the identification of safe drugs able to inhibit the signaling of the involved receptors could limit the differentiation of subverted monocyte-derived DCs and ultimately favor the expansion of effective T cells in post-primary TB.

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