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Human mesenchymal stem cells: New sojourn of bacterial pathogens

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SUMMARY

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M. tuberculosis*), is the leading infectious disease which claims one human life every 15–20 s globally. The persistence of this deadly disease in human population can be attributed to the ability of the bacterium to stay in latent form. *M. tuberculosis* possesses a plethora of mechanisms not only to survive latently under harsh conditions inside the host but also modulate the host immune cells in its favour. Various *M. tuberculosis* gene families have also been described to play a role in this process. Recently, human bone marrow derived mesenchymal stem cells (MSCs) have been reported as a niche for dormant *M. tuberculosis*. MSCs possess abilities to alter the host immune response. The bacterium finds this self-renewal and immune privileged nature of MSCs very favourable not only to modulate the host immune system, with some help from its own genes, but also to avoid the external drug pressure. We suggest that the MSCs not only provide a resting place for *M. tuberculosis* but could also, by virtue of their intrinsic ability to disseminate in the body, explain the genesis of extra-pulmonary TB. A similar exploitation of stem cells by other bacterial pathogens is a distinct possibility. It may be likely that other intracellular bacterial pathogens adopt this strategy to 'piggy-back' on to ovarian stem cells to ensure vertical transmission and successful propagation to the next generation.

Introduction

Tuberculosis, caused by *Mycobacterium tuberculosis* (*M. tuberculosis*), is the world's deadliest bacterial infectious disease taking one human life every 20 s globally (WHO, 2012). One third of the world population is latently infected by *M. tuberculosis*. India alone accounts for about 25% of all clinical tuberculosis cases globally. HIV co-infection, emergence of multi-drug resistance (MDR), extensive drug resistance (XDR) and total drug resistance (TDR) and the diabetic 'epidemic' are rendering TB eradication far more difficult (Sharma and Mohan, 2013).

Most individuals are asymptomatic as the bacilli can stay in a latent form. However, the probability of these individuals contracting the disease during their lifetime is 10%. The bacterial infection cycle can be broadly categorised into initial active infection in immunocompetent individuals, granuloma formation and reactivation when the host becomes immunocompromised. *M. tuberculosis* gains entry into the host via the respiratory tract and is ingested by macrophages and tissue dendritic cells, which release pro-inflammatory cytokines that recruit more dendritic cells, monocytes and neutrophils to the site of infection (Korbel et al., 2008). Upon activation DCs migrate to lymph nodes where they process and present antigens to T cells and hence activate adaptive immune response (Dheda et al., 2010). The activated T cells release cytokines like TNF-α and INF-γ, which in most cases lead to a resolution of infection, but in about 10% of the cases an infection is established leading to TB (Russell, 2007). Granulomas consist of a central core of macrophages infected with *M. tuberculosis* which get surrounded by activated macrophages, giant multinucleated cells, lymphocytes and dendritic cells. A subset of granulomas, due to proteinaceous dead cell mass, undergoes central caseous necrosis (McElvania Tekippe et al., 2010). TNF-α not only helps the host to fight against the initial bacterial infection

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but is also necessary for containment of the infection in the form of granuloma (Flynn et al., 1995).

When the host immune response becomes feeble, which is sensed by *M. tuberculosis* through a possible immune quorum sensing mechanism (Tundup et al., 2014), the bacteria become active, thrive inside the hosts (Gupta et al., 2012) and modulate the host immune response for their survival. Recently, stem cells have been identified as a new niche for mycobacteria thereby adding a new dimension to the pathogenesis mechanism employed by mycobacteria. *Mycobacterium leprae* (*M. leprae)* has been reported to reprogram adult neural cells into stem cells for its dissemination in the host (Masaki et al., 2013). In another study, *M. tuberculosis* has been shown to reside in mesenchymal stem cells (MSCs) (Das et al., 2013).

MSCs are not only immune privileged but also have a role in modulating the host immune response by a plethora of mechanisms (Raghuvanshi et al., 2010; Selmani et al., 2008). Pathogens like *M. tuberculosis* might utilize these properties of MSC for self-propagation in the host. For example, upon reactivation of *M. tuberculosis*, the host cell-mediated immunity, characterized by pro-inflammatory cytokines like IL-12, shifts to a pro-pathogen T cell (Th2) response (Howard and Zwilling, 1999). In this review, we discuss the probable role of MSCs in pathogenesis, reactivation and dissemination of *M. tuberculosis*. Further, the possibility of vertical transmission as a means of propagation to the next generation is also discussed.

Stem cells as niche for mycobacteria

A recent report by Das et al. (2013) shows that MSCs provide a niche for dormant *M. tuberculosis*. In this report when bone marrow stem cells (CD133+) were infected *in vitro*, it was observed that most of the infected cells expressed the cell surface marker CD271, a marker for MSCs. Viable bacteria could also be recovered from these cells 6 months after infection in a mouse TB model. The CD271+ cells also expressed ABCG2, a drug efflux pump that may help *M. tuberculosis* in drug evasion (Zhou et al., 2001). The obvious conclusion was that *M. tuberculosis* uses this mechanism to evade the host immune response and also render the current drug therapies inadequate. The decreased viability of *M. tuberculosis* in adipocytes can be attributed to the hypoxic conditions created during adipocyte differentiation that push the bacilli into a dormant state, rather than the undifferentiated status of BM-MSCs (Neyrolles et al., 2006). It remains to be studied whether differentiation of BM-MSCs into cells other than adipocytes would result in a similar decrease in viability of the bacillus.

In an earlier report, Raghuvanshi et al. (2010) showed that MSCs infiltrate the granulomas that are formed post TB infection. MSCs achieve an immunosuppressive effect by releasing NO and TGF-β, which inhibit T-cell activation. This immunosuppression promotes susceptibility to *M. tuberculosis* infection. Moreover, MSCs exhibit a capability to convert CD4+ T cells into Tregs. These findings cumulatively suggest that MSCs may have a role in persistence of *M. tuberculosis* infection.

However, these are not the only associations reported between mycobacterial species and MSCs. Masaki et al. (2013) demonstrated the capability of *M. leprae* to reprogram adult Schwann cells to stem cell-like cells with mesenchymal characteristics to promote dissemination of infection. They showed that presence of intracellular leprosy bacilli leads to a decrease in the expression of Sox10, a master regulator of Schwann cell homeostasis, while the expression of the pluripotency marker Sox2 is maintained. These reprogrammed stem cells (named primordial Stem-Like Cells, pSLCs), express many hematopoetic markers, have a potential to differentiate into mesenchymal tissues, and produce a number of chemokines and cytokines, demonstrating their immune modulatory properties. Furthermore, pSLCs have the ability to migrate resulting in the spread of bacilli inside the host. Masaki et al. suggested two different ways by which *M. leprae* can achieve dissemination of infection. First, pSLCs harbouring *M. leprae* can spread the infection by directly differentiating into myoblast muscle cells. This way the leprosy bacillus spreads passively. Second, pSLCs migrate to the skeletal muscle-dermal interphase (SkMDIP), where they release cytokines to attract macrophages and form aggregates that resemble granulomas typically seen in mycobacterial infections. The bacillus can disseminate from stem cells to both M1 and M2 type of macrophages and these *M. leprae* infected macrophages can then move out of granuloma to different sites.

MSCs nitric oxide and reactivation of mycobacteria

Nitric oxide (NO), a known mycobactericidal agent produced by the innate immune response of the host, inhibits IL12 p40 through p38 MAP kinase-mediated regulation of calmodulin and c-rel (Boddupalli et al., 2007). Thus, NO also confers resistance to *M. tuberculosis* infection in addition to being an immunosuppressant (Mukhopadhyay et al., 2007). It is established that NO production by macrophages needs both IFN-γ and TLR-4 mediated signals (Shi et al., 2006). IFN-γ is a pro-inflammatory cytokine produced by activated T cells and is required for the maintenance of granulomas formed post infection (Russell, 2007). A disintegration of granulomas is observed in immunocompromised hosts that is often a vital cause of *M. tuberculosis* reactivation. Raghuvanshi et al. (2010) demonstrated that MSCs that infiltrate *M. tuberculosis*-infected organs produce NO and help in maintaining a dynamic equilibrium between *M. tuberculosis* and the host. They further showed that the level of IFN-γ decreases in immunocompromised individuals and thus the balance shifts in favour of *M. tuberculosis* as MSCs get impaired for NO production. This might be a biochemical mechanism employed by *M. tuberculosis* to sense when the immune response of the host becomes feeble. *M. tuberculosis* proliferation would increase when level of NO goes down, which might be an indirect signal for the bacillus to indicate impaired IFN-γ levels, a mark of suppressed immune response. This viewpoint is further supported by the fact that *M. tuberculosis* uses its cytochrome *c* oxidase to detect the levels of NO/O_2 (Mukhopadhyay et al., 2007). *M. tuberculosis* also employs members of the large PE/PPE protein family, present exclusively in the genus *Mycobacterium*, to stimulate macrophages to favour a Th2 response (Akhter et al., 2012). We earlier showed that PPE18 mounts a strong anti-inflammatory response by docking to the TLR2 to secrete IL10 (Nair et al., 2009). Hence, like macrophages MSCs have a dual role in *M. tuberculosis* pathogenesis, which is dependent on the immune status of the infected individual.

MSCs and the host-pathogen response

MSCs modulate the immune system by a number of ways. One mechanism involves induction of macrophages to a different phenotype. Co-culturing of macrophages with MSCs elicits the production of IL-10 and downregulates TNF-α and IL-12 (Eggenhofer and Hoogduijn, 2012). TNF-α produced by macrophages binds to the surface receptors on MSCs which initiates the downstream signalling events resulting in the production of prostaglandins. Prostaglandins serve as a negative feedback for TNF-α and promote IL-10 production. Thus, MSCs reprogram the local and infiltrating macrophages to an anti-inflammatory pathway (Fig. 1A). The members of the PE/PPE family may further contribute to this process. Furthermore, MSCs secrete HLA-G5 in an IL-10 dependent manner that suppresses allogeneic T-cell proliferation and contributes to

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