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Bacterial-induced cell reprogramming to stem cell-like cells: new premise in host-pathogen interactions

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Abstract

Bacterial pathogens employ a myriad of strategies to alter host tissue cell functions for bacterial advantage during infection. Recent advances revealed a fusion of infection biology with stem cell biology by demonstrating developmental reprogramming of lineage committed host glial cells to progenitor/stem cell-like cells by an intracellular bacterial pathogen *Mycobacterium leprae*. Acquisition of migratory and immunomodulatory properties of such reprogrammed cells provides an added advantage for promoting bacterial spread. This presents a previously unseen sophistication of cell manipulation by hijacking the genomic plasticity of host cells by a human bacterial pathogen. The rationale for such extreme fate conversion of host cells may be directly linked to the exceedingly passive obligate life style of *M. leprae* with a degraded genome and host cell dependence for both bacterial survival and dissemination, particularly the use of host-derived stem cell-like cells as a vehicle for spreading infection without being detected by immune cells. Thus, this unexpected link between cell reprogramming and infection opens up a new premise in host-pathogen interactions. Furthermore, such bacterial ingenuity could also be harnessed for developing natural ways of reprogramming host cells for repairing damaged tissues from infection, injury and diseases.

Background

The body's lineage-committed differentiated tissue cells are the residence of many bacterial pathogens that cause numerous human diseases. These pathogens often establish infection in their preferred niches by manipulating or subverting differentiated cell functions [1,2]. However, to accomplish these daunting tasks bacterial pathogens must fulfill several criteria

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[1,3]. For intracellular bacteria, many additional challenges and careful orchestrations are necessary to evade host immune attack, sustain bacterial survival and promote dissemination. Therefore, intracellular bacteria usually take precautions and reside within their favorable host niches for colonization and to gain full advantage of properties their preferred host cells offer. Although tissue niches with limited immune cell traffic are safe haven for propagation of intracellular bacteria, their dissemination, the next critical step of bacterial life cycle after colonization, particularly via systemic routes is challenging due to bacterial confinement to their specialized tissue niches. Better understanding of how intracellular bacteria overcome such challenges and pass infection to other tissues provide new tools for targeting the progression of bacterial infections.

New research continues to identify specific host cell functions and pathways that are required for many different bacterial pathogens during their infectious processes [4,5,6,7,8]. Developing strategies that target the critical host cell functions required for infection would have broad-spectrum efficacy and much less likelihood to permit pathogens to acquire resistant mutation and become drug resistant. Thus, usage of host-encoded functions essential for infection could be particularly timely, since the emergence of drug-resistant bacterial strains is a major concern for public health [9,10]. However, tackling such host-encoded functions as strategies for combating infection is challenging, since diverse pathogens use different tactics for their survival and propagation. Although tailor-made strategies for targeting individual pathogens with specific host requirements are possible, it is more beneficial and cost effective if we are able to identify common molecular host targets or pathways that can be applied to many bacterial pathogens simultaneously. Because pathogens are co-evolved alongside hosts with many common or evolutionary conserved strategies for cell manipulation, discovery of novel host cell modifying mechanisms from model organisms provide new insights into host-encoded functions that could be shared with many bacterial pathogens. It is likely that potentially effective common host-encoded functions can be identified from those bacterial pathogens, which are known to depend substantially or totally on host cell functions for every phase of their bacterial life cycle. *Mycobacterium leprae*, the causative organism for human leprosy, is one such intracellular pathogen that totally depends on host cells for maintaining bacterial survival and propagation [11], and thus could be a model organism for identifying both novel and common host-encoded functions.

One common property of host cells is the genomic plasticity, the extent to which host cells can alter their transcriptome in such a manner that allows these cells to adapt to changes in microenvironment [12]. Plasticity exists in adult tissue cells to varying degrees and this property is responsible for natural repair processes following tissue damage, often due to endogenous stem/progenitor cell populations [13,14]. It is now known that indeed adult tissue cell plasticity can be manipulated experimentally by changing expression of genes to reprogram somatic cells back to embryonic stage or change lineage commitment both in vitro and in vivo [15,16,17]. Plasticity of host cells can also be subjected to manipulation by intracellular bacterial pathogens. In this review, we describe how bacterial pathogens hijack plasticity of tissue cells to manipulate host cells during infection using ML and its preferred host niche, Schwann cells, as a model system. We also briefly discuss the implications of

these findings for bacterial infectious diseases in general, and how such bacterial ingenuity can be employed as a potential strategy for converting somatic cells to stem cell-like cells for tissue regeneration.

Experimental manipulation of host cell plasticity

During mammalian development embryonic cells undergo a highly complex developmental program by acquisition, deletion or maintaining multiple transcriptional, epigenetic, and signaling programs to acquire various lineage-committed cell types of distinct functions. Although such terminally differentiated cells are stable in terms of operating their lineage committed programs in order to maintain the identity and specific cell functions in different tissues, recent advances have revealed that these committed programs of adult tissue cells are remarkably plastic and can easily be manipulated experimentally [12,16,18]. New areas of investigation for cell fate manipulation have rapidly evolved by the success of ectopic expression or deletion of critical genes required for either maintaining embryonic state or specific tissue lineage development, which are sufficient for reprogramming developmentally committed tissue cells all the way back to embryonic stem cells or converting into other cell phenotypes [19,20]. These revelations have attracted a wide range of interest as a strategy for cell manipulations which can eventually be harnessed for use in regenerative medicine, and also as research tools to gain new insights into basic developmental processes [21].

Hijacking notable plasticity of adult Schwann cells by M. leprae

Among differentiated tissue cells, Schwann cells, the glial cells of the adult peripheral nervous system (PNS), which derive from neural crest precursors and comprise myelin-forming and non-myelin-forming phenotypes [22,23], can be considered as an example of sophistication in cell differentiation. Yet they show remarkable plasticity illustrated by the ability of adult Schwann cells to switch between differentiated and de-differentiated states following nerve injury [24,25]. In response to injury-induced signalling, myelinated Schwann cells switch off the myelination program following loss of axonal contact and acquire a phenotype resembling immature Schwann cells, re-enter cell cycle and de-differentiate (Fig. 1). These de-differentiated Schwann cells in turn promote regeneration of axons and the myelin sheath, and in this manner adult Schwann cell plasticity contributes to the regeneration capacity of adult PNS even after severe injury [26].

Intriguingly, leprosy bacteria use adult Schwann cells with this notable plasticity as the natural primary target for the establishment of infection within the PNS. ML is a strictly obligate intracellular pathogen with a severely degraded bacterial genome, unable to generate its own energy and metabolic needs fully and thus depends totally on host cell functions for bacterial survival [11]. By selecting Schwann cells, ML have acquired several survival advantages [11,27]. Recent studies have suggested that ML use the regeneration properties of the PNS for the expansion of the bacterial niche within Schwann cells [27,28,29].

Setting the stage: from terminal differentiation to de-differentiation

In adult peripheral nerves, Schwann cells are developmentally matured and have acquired the stage of terminal differentiation necessary for fully functional nerves. ML 'simplifies' this sophisticated terminal differentiation by initiating myelin damage pathways and inducing cells to re-enter the cell cycle by activation of canonical and non-canonical Erk1/2 signalling pathways [29,30]. In fact, this was the first example to show that demyelination can be caused by activating Schwann cell Erk1/2 signalling without nerve lesions or inflammatory responses [28,29]. Subsequent studies using transgenic mouse models in which Erk1/2 signalling is sustained in adult PNS have further confirmed that, indeed, like in infection with ML, transgenic activation of Erk1/2 signalling in adult nerves could also elicit demyelination without lesions [31].

As in experimentally induced or natural nerve injury responses, ML-induced demyelination also generates de-differentiated cells [28; Fig. 1]. These Schwann cells maintain their lineage commitment in an immature state lacking the myelin sheath, and are usually equipped with properties for promoting remyelination of damaged nerves [32]. Interestingly, such de-differentiated Schwann cells are highly susceptible for ML invasion and are also likely to be a more favourable phenotype for intracellular bacterial growth [27]. Additionally, these Schwann cells also serve as safe haven for ML, since the PNS blood-nerve barrier protects the organism from host immune assault [33,34], and thus initial ML propagation in Schwann cells is likely to occur progressively due to lack of resistance from immune cells. Such favorable conditions, which are assisted with the nontoxic, non-cytopathic, non-apoptotic nature of ML, permit bacterial residence within Schwann cells for a long period with moderate cell proliferation, but without causing any cell transformation or apoptosis [27,30,35]. Thus, it is likely that once invaded, ML maintain infected de-differentiated Schwann cells in a viable and active state so that essential host factors necessary for bacterial survival can be properly secured. Recent studies have shown that the fate of Schwann cells following such uniquely compatible host-pathogen adaptation is the reversal of the developmental program of these lineage-committed Schwann cells [36].

Modeling the fate of infected Schwann cells

Considering the total host cell dependence for driving the bacterial life cycle to establish a productive infection, it is likely that ML has evolved to further sophisticatedly manipulate adult de-differentiated Schwann cells for bacterial advantage. However, in the case of human infection, it is unknown how long ML reside in de-differentiated Schwann cells, since these cells usually re-differentiate back to myelinated and non-myelinated Schwann cells in vivo, as in nerve injury [32; Fig. 1]. It is possible that ML occupy de-differentiated cells for a transient period until infection establishes within their preferred non-myelinated Schwann cells, as demonstrated in nerve biopsies from leprosy patients with high bacterial load [33].

As in nerve injury-like responses, it is inevitable that initial human infection of adult peripheral nerves undergoes a similar de-differentiation program because ML overtime produce highly unfavorable conditions that deregulate the well-regulated Schwann cell-axon

communication system essential for maintaining functional PNS [27,28]. This signals terminally differentiated Schwann cells to generate de-differentiated cells as a part of natural property of plasticity, which is hijacked by ML. Although this early stage of ML infection cannot be studied in humans as there is no justifiable evidence for clinical manifestation of infection or nerve damage in affected individuals, these events can be recapitulated in model systems since plasticity of adult PNS and de-differentiation program following nerve injury or injury-like responses is highly conserved in human, rodents and other mammals including the natural animal host of ML, the nine-banded armadillo [37,38]. Indeed, new evidence suggests that nerve damage can be recapitulated in armadillo models by systemic infection with ML [38].

Infection reprograms committed Schwann cell fate to stem cell-like stage

Recent studies have recapitulated the early events of ML infection in a mouse model in order to understand the fate of these de-differentiated Schwann cells in response to long-term exposure to intracellular bacteria. Masaki et al. isolated de-differentiated Schwann cells from adult wild-type and Sox2-GFP transgenic mice after injury and separated Schwann cells from axons, which spontaneously generated de-differentiated Schwann cells. They then purified cells by FACS sorting using an antibody to Schwann cell surface marker $p75^{NTR}$ or GFP expression under the control of Sox2 promoter, respectively. The latter was important because it shows that isolated cells are GFP+/Sox2+, which is expressed in adult peripheral nerves only after Schwann cells undergo de-differentiation following injury [39]. Although Sox2, which is a stem cell marker (16), is expressed transiently in these adult de-differentiated cells in vivo they are lineage committed Schwann cells marked by master regulators of Schwann cell lineage such as Sox10 (22; Fig. 1). By using both purified pool and clonal de-differentiated Schwann cells infected with ML, Masaki et al showed that infected Schwann cells, as compared to uninfected/control cells, gradually ‘turn off’ Schwann cell differentiation/myelination- and lineage-associated genes and ‘turn on’ numerous developmental genes. The latter comprises mostly the mesoderm development including homeodomain/Hox, EMT (epithelial mesenchymal transition), as well as neural crest related genes, such as Hox-d, -a, -b gene clusters, Twist, Snai and Msx2 transcripts [36]. These findings suggested that ML gradually shut down the Schwann cell differentiation program and lead lineage-committed Schwann cells to a highly immature stage resembling progenitor/stem cell-like cells (pSLC). Indeed, properties of the stem cell-like phenotype and behavior of pSLC have been shown based on their reprogrammed stem cell-like transcriptome and the capacity to re-differentiate into multiple tissue types including bones, smooth muscles, skeletal muscle and adipocytes [36].

Conversion of infected Schwann cells to an early neural/mesoderm development program

A major tissue remodeling program that is central to early mesoderm development during embryogenesis is epithelial-mesenchymal transition (EMT), which is orchestrated by a number of developmentally regulated transcription factors (TFs) and generates cells that act as progenitors of different mesenchymal tissues [40]. Interestingly, master regulators of EMT, Twist-1, -2, Snail2 and Msx2, which are capable of inducing EMT in epithelial cells

and converting them to mesenchymal stem-like phenotypes [41] , were among the highly upregulated TF genes in ML infected Schwann cells (36). Interestingly, these key EMT genes such as Twist and Snail1/2 are also expressed in neural crest stem cells [42]. Demethylation of the promoter region of Twist1 in these reprogrammed cells further suggests the change in cell fate accompanies a change in epigenetic status. It is possible that ML hijack an EMT-like process in de-differentiated Schwann cells and change the cell fate to a neural crest/mesenchymal phenotype. However, these ML-driven cell fate changes in Schwann cells are highly complex as infection involves upregulation of multiple developmental genes of both mesenchymal and neural crest associated genes. Also of particular interest is the modulation of TFs of the homeodomain/Hox family and Sox family in Schwann cells in response to ML. It is known that the fate of somatic cells can be altered by forced expressions of Hox genes [43]. On the other hand, Sox2, as described above, is a critical TF involved in maintaining embryonic stem (ES) stem cells, neural stem cells and neural crest stem cells, as well as one of the factors required for reprogramming fibroblasts to induced pluripotent stem (iPS) cells with ES cell-like properties [16, 44,45,46,47], suggesting good reasons why ML target these embryonic TFs during Schwann cell reprogramming. An important finding is that these ML-induced events are not associated with the tumor suppressor genes like p53 or Schwann cell tumor associated gene NF1, which is inactivated in neurofibromatosis type 1, suggesting reprogramming events in Schwann cells are not associated with tumor formation [36]. Indeed, previous studies using human primary adult Schwann cells infected with ML have shown that infected human cells do not undergo any transformation even after long-term incubation despite moderate cell proliferation and lack of apoptosis induction [30, 35].

Bacterial strategy: keeping the wanted and removing the unwanted

In addition to the activation of developmental genes, silencing regulators of Schwann cell lineage/differentiation may also be critical for reprogramming Schwann cells out of the lineage and to the pSLC state. One key early event in reprogramming is the bacterial-induced removal of nuclear Sox10 [36], the master regulator of Schwann cell lineage, identity and differentiation/myelination [48]. Genes encoding myelin proteins that provide a unique status for differentiated Schwann cells are direct targets of Sox10, and the down regulation of myelin genes during ML infection may be associated at least partly with nuclear Sox10 removal [22, 48]. This bacterial strategy may be of significant advantage for ML propagation, since the myelin sheath occupation within almost the entire Schwann cell cytoplasm is unfavorable for ML, which reside strictly within the cytoplasm for bacterial multiplication. Nuclear Sox10 removal accompanies the silencing of Sox10, which is directly correlated with highly significant DNA methylation of Sox10 promoter region in reprogrammed cells, suggesting epigenetic regulation of Sox10 mediated by intracellular ML.

On the other hand, sustaining nuclear Sox2 expression is critical for maintaining infected Schwann cells in the de-differentiated state or perhaps promoting to the pSLC stage [36]. Because Sox2 is also a negative regulator of myelination [39,44,49], maintaining Sox2 expression, which is otherwise expressed only transiently in adult peripheral nerves after injury (39), in infected cells is critical not only for down-regulating differentiation but also

retaining Schwann cells at the de-differentiated stage. However, it is the removal of Sox10 and concomitant expression of Sox2 together with other developmental TFs that are likely to be the initial driving force for reprogramming of Schwann cells to pSLCs. Indeed, Sox factors and their partner proteins can play sequential roles in development [50], and similarly their interplay at target genes could also tip the balance of cellular maturity. Perhaps whilst the normal injury environment favors re-differentiation after injury, mediated with the help of Sox10 when conditions are appropriate, infection conditions that lose Sox10 may then tip the balance towards Sox2 transcriptional networks that favor the pSLC state. Underlying mechanisms by which ML perform these tasks should provide new insights into not only targeting ML infection before infection spreads within the PNS but also the regulation of Schwann cell de-differentiation and remyelination.

Bacterially reprogrammed host cells as a vehicle for dissemination of infection

A critical step of the bacterial life cycle after colonization in the primary host niche is dissemination via systemic routes. This is particular challenging for an extremely passive bacterium like ML with a strictly obligate intracellular life style, residing in a specialized and complex host niche such as the adult PNS. ML appear to overcome these challenges by conversion of parent Schwann cells to pSLC with acquired characteristics of differentiation to mesenchymal tissues as well as migratory and immunomodulatory properties [36,51]. Host cell dependence of ML for bacterial survival is well documented but host cell dependence for bacterial dissemination was unknown until recently. Converting parent host cells to stem-like cells with a migratory property provide ML with a vehicle to disseminate infection. Since pSLC are host derived, ML take the unique advantage of these migratory stem cell-like cells as a hideout during the hostile journey of transmission to other preferred niches without being detected by immune cells that normally traffic throughout the body. By converting Schwann cells to pSLC with neural crest/mesenchymal stem cell-like properties, ML take advantage not only to migrate but also transfer infection to skeletal and smooth muscles by re-differentiating stem cells into these tissues without systemic involvement (Fig. 2A). In leprosy patients, disseminated ML have been demonstrated in several mesenchymal tissues including skeletal muscles and smooth muscles [52,53,54]. These findings together provide a possible mechanism by which ML disseminate to smooth and skeletal muscle during human infection. This also provides an intriguing strategy for an extremely passive intracellular pathogen like ML to overcome challenges faced during dissemination.

Another striking property that ML take advantage of by reprogramming Schwann cells is the use of the efficient capacity of pSLC to transfer bacteria to other cell types. Before reprogramming, primary Schwann cells of both rodent and human origin usually retain intracellular ML after infection [28–30; 55]. Such initial bacterial retention capacity in adult Schwann cells may be of functional significance during human infection, since Schwann cells in leprosy patients are known to harbor ML for an extensive period, which may be critical for extremely slow bacterial expansion (ML doubling time is about 14 days), within this privileged niche [56,57]. However, once colonized, Schwann cells undergo a

reprogramming process, which acquires a property of effective ML transfer to skin fibroblasts and neural fibroblasts [55]. The latter is of particular significance, since they are abundant in the peripheral nerve microenvironment and thus could serve as an immediate target for ML during dissemination. Also, ubiquitous distribution of fibroblasts in almost all body tissues types suggests that pathogens are most likely to take advantage of these cells in order to reach or exit from their specific tissue niches.

Reprogrammed cells contribute to bacterial dissemination by granuloma formation

The changes in Schwann cells induced by ML appear to have unexpected bacterial advantages. The primary tissue niche of ML, adult Schwann cells, are non-immune neural tissue cells whose major functions are to produce the unique myelin sheath and support neurons for maintaining a proper functional nervous system [22]. Conversion of Schwann cells to pSLCs with immunomodulatory properties, releasing numerous chemokines and tissue remodeling factors, may set the stage for ML to disseminate infection via systemic routes.

By using the capacity to secrete multiple chemoattractants and survival factors, ML may use reprogrammed cells to create a secondary niche, recruit macrophages, transfer infection and establish a new habitat for further bacterial expansion and dissemination [36]. An important finding is the contribution of innate immune factors during Schwann cell reprogramming and their expression to the highest levels at pSLC stage [36,51]. Although early innate immune factors upregulated in the early stage of infection are likely to promote a reprogramming process, release of these immune factors particularly chemokines/cytokines are more prominent at the pSLC stage [51]. This was demonstrated by the capacity of pSLCs to recruit macrophages and form granuloma-like structures in both in vitro and in vivo models, resembling typical granulomas seen in tissue lesions from leprosy and tuberculosis patients [58,59,60]. Interestingly, some of the immune factors/chemokines released from pSLC are already known to foster granuloma formation [61,62].

Although mycobacterial granulomas are considered to be essential for containment of infection, recent studies on Zebrafish models have suggested that macrophage granulomas may also promote mycobacterial spread during early infection [58–60; 63]. However, unlike other pathogenic mycobacteria, ML use adult Schwann cells as primary non-immune tissue cells for initial colonization [34]. These findings suggest that once colonized, ML maintain Schwann cells in an active and viable state and take full advantage of Schwann cell plasticity to convert parent cells to pSLCs with the capacity to produce chemoattractants and trophic factors, which in turn promote the recruitment and survival of macrophages [36]. In vivo and in vitro analyses also provide further evidence that ML-laden macrophages in the granulomas contribute to the spread of the infection. In this manner it is possible that ML take a long route via remodeling events of host cells using multiple complex mechanisms to get access to final systemic dissemination of infection once established in a privileged niche like Schwann cells. This shows a striking example of how a human bacterial pathogen potentially makes use of its own remodeled host niche for dissemination of infection to other tissues. Ironically, the above-described bacterial strategies are a lesson from a neglected

pathogen causing a neglected human disease, leprosy, which still remains a major public health issue worldwide [64]. However, the sophistication the leprosy bacterium displays to manipulate its host cell niche is unprecedented and cannot be ignored.

Conclusions

Recent advances on bacterial-host cell interactions described above open up a new theme of fusion of infection biology and stem cell biology fields. These advances, by connecting tissue cell reprogramming to bacterial infections, may have implications on how we approach fundamental research into bacterial infectious diseases, which are becoming an ever more challenging global task than before due to emerging drug resistant bacteria. Undoubtedly, future studies on this direction with many bacterial pathogens will enhance our understanding of biology of both host-pathogen interaction and their symbiosis and co-evolution with much more sophistication. The striking example of adult Schwann cell reprogramming by leprosy bacilli provides a new level of sophistication in bacterial-host interaction that can be harnessed for studying many aspects of both pathogen and host biology. Remodeling normal host cells to stem-like cells that provide advantage for safe bacterial dissemination reveals an unexpected adaptation of host cell dependence from a bacterial pathogen. Also, mechanisms by which ML reprogram host cells will allow us to identify common mechanisms that we can adapt to dissect other bacterial-host interactions. Such directions may provide clues for identifying common host-encoded functions required for many bacterial infections and target them for developing strategies as alternative to or use in combination with antibiotics for halting the progression of bacterial infections. In the case of leprosy bacteria, reprogramming adult Schwann cells is likely to be an early critical event during infection of adult peripheral nerves way before complex inflammation-mediated neurological damage begins. Molecular basis of how ML perform these tasks should provide new insights into targeting ML infection before infection spread within the PNS, which would enormously benefit leprosy sufferers and prevent devastating nerve damage that disables these patients. The extent of host cell reprogramming capabilities of ML may also add new insights into current methods of artificially changing cell fate reversal used by overexpressing TFs using transgenic methods [18,19]. iPS cells generated by this technology are now widely used for understanding many aspects of biology, drug discovery and regenerative medicine [21,65]. However, the efficiency of reprogramming adult tissue cells to ES-like iPS cells remains extremely low, and the full potential of this technology for disease modeling and cell therapy has yet to be realized [18,66]. Although how ML reprograms host cells is a vastly complex process and unknown, certainly the long incubation time within cells gives greater opportunity to alter cellular dynamics without causing oncogenic transformation. Many studies have documented that various bacterial pathogens use a range of strategies to modulate host cell behavior, including secretion of nucleomodulins to alter host chromatin state [67], epigenetic modifications [68,69], hijacking host gene transcription [70], influencing metabolism [71,72], immune responses [5] and signaling pathways [4,73,74]. In addition, existing tissue progenitor/stem cells can also be modified by commensal bacteria in the gut [75] and other invading pathogens like *E.coli*, which mobilize hematopoietic stem cells [76]. Together, some commonality can be extracted from these examples and reprogramming induced by ML. Therefore, elucidating

mechanisms by which bacterial pathogens modulate the host cell machinery is not only valuable for developing strategies targeting host-encoded functions for prevention of infectious process, but also manipulate host cells for generating new approaches of medical importance for health benefit. As with the discovery of how bacterial pathogens protect themselves against infectious phages and plasmids using CRISPR/cas9 system as a mechanism for bacterial immunity [77,78], which later developed into a powerful method for gene editing and bioengineering [79], it is possible that unraveling the molecular detail of the mechanisms of bacterial-induced host cell reprogramming may provide us with new tools for tissue cell manipulations in medicine and tissue repair in regenerative medicine.

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transferring to other tissues. This not only shows a complex host-pathogen partnership, but also hints at a target pathway for preventing infection spread.

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Highlights

- Induction of stem cells by *M. leprae* shows a fusion of infection biology with stem cell biology
- Stem-like cells acquire migratory and immunomodulatory properties and promote dissemination
- Reprogramming Schwann cells may be an early critical event during *M. leprae* infection
- Bacterial-induced host cell reprogramming may have applications in regenerative medicine

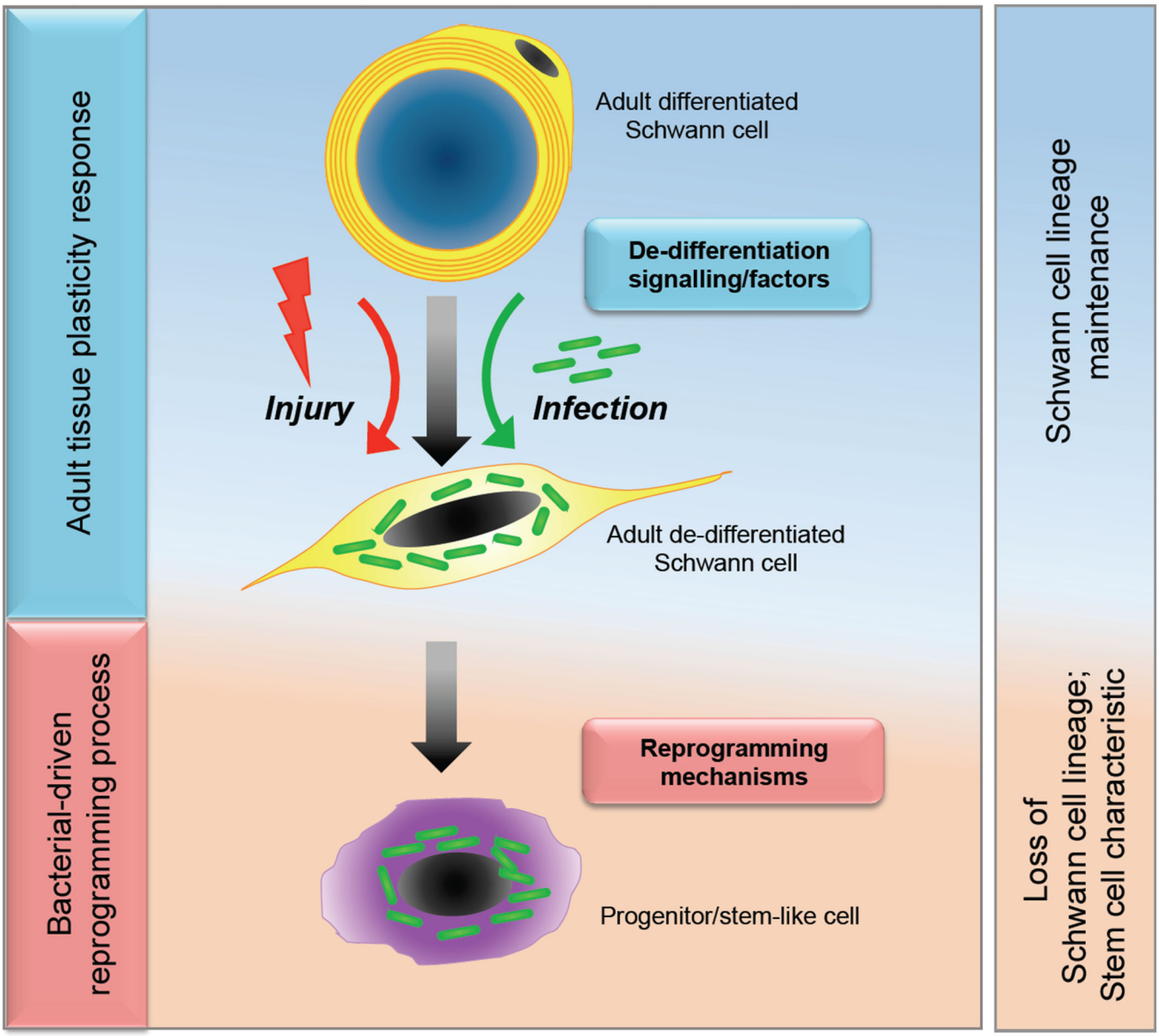

Figure 1. Setting the stage for host cell reprogramming: from terminal differentiation to de-differentiation and to stem-like cells

M. leprae hijack the innate ability of adult Schwann cell plasticity - switching off the myelin program and adopt a de-differentiated program- for de-differentiation by activating receptor-mediated signalling pathways (top). Unlike in the injury process that re-differentiates de-differentiated Schwann cells back to the terminally differentiated state for completion of the nerve repair process, M. leprae infection (green rods) drives de-differentiated Schwann cells further out of lineage and to an immature state generating stem cell-like cells for bacterial advantage (bottom).

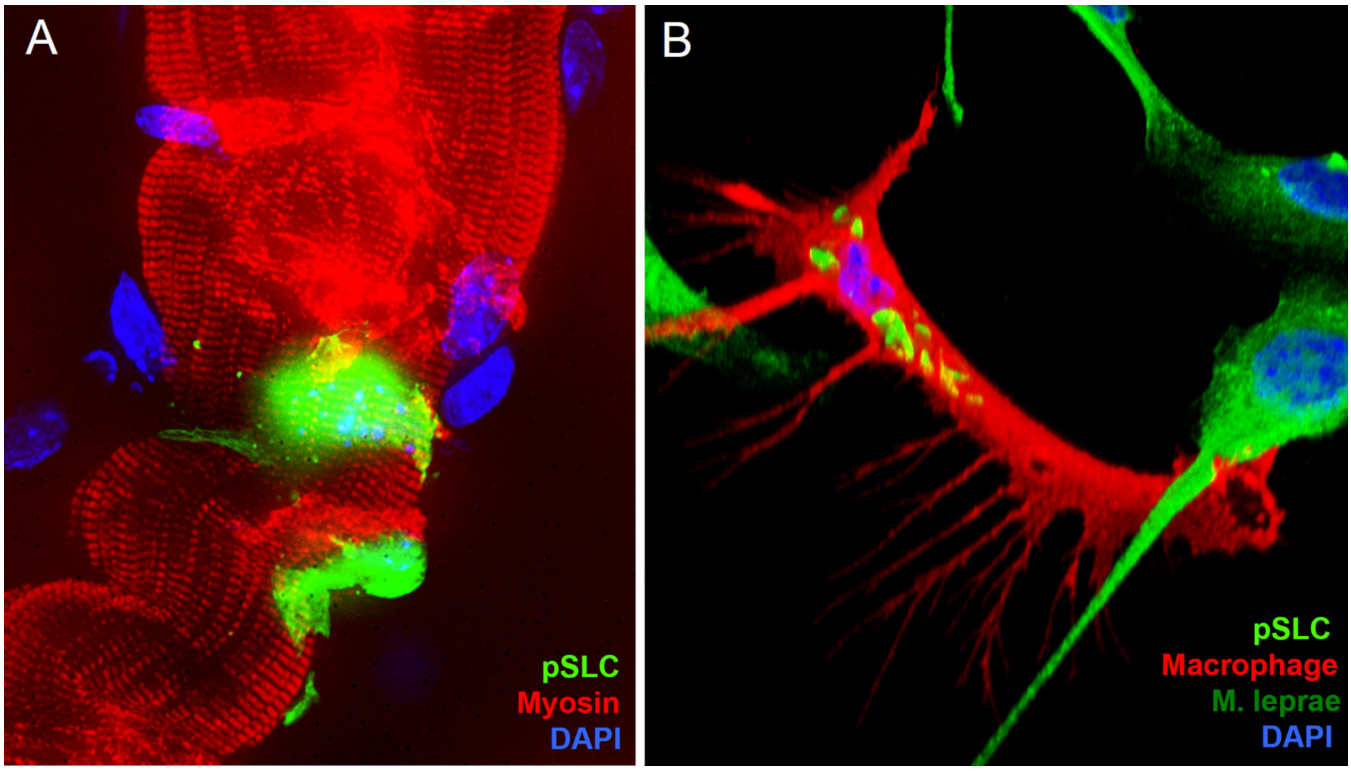

Figure 2. Stem cells generated by host cell reprogramming contribute to bacterial dissemination

(A) Leprosy bacteria reprogram primary adult Schwann cells to progenitor/stem-like cells (pSLC) (GFP/green) and use stem cell-like properties to fuse and differentiate directly to adult skeletal muscles in vivo (red; myosin labeling; DAPI in blue shows muscle nuclei), and thus spread the infection passively to skeletal muscles. **(B)** pSLC acquire immunomodulatory and efficient bacterial transfer capabilities during reprogramming and use these characteristics, particularly the release of chemokines and cytokines, to attract macrophages and transfer infection to them. Shown is bacterial transfer to a macrophage (F4/80 marker in red; ML is in green rods) by pSLC (green) in an in vitro model (Adapted from Masaki et al; 36)

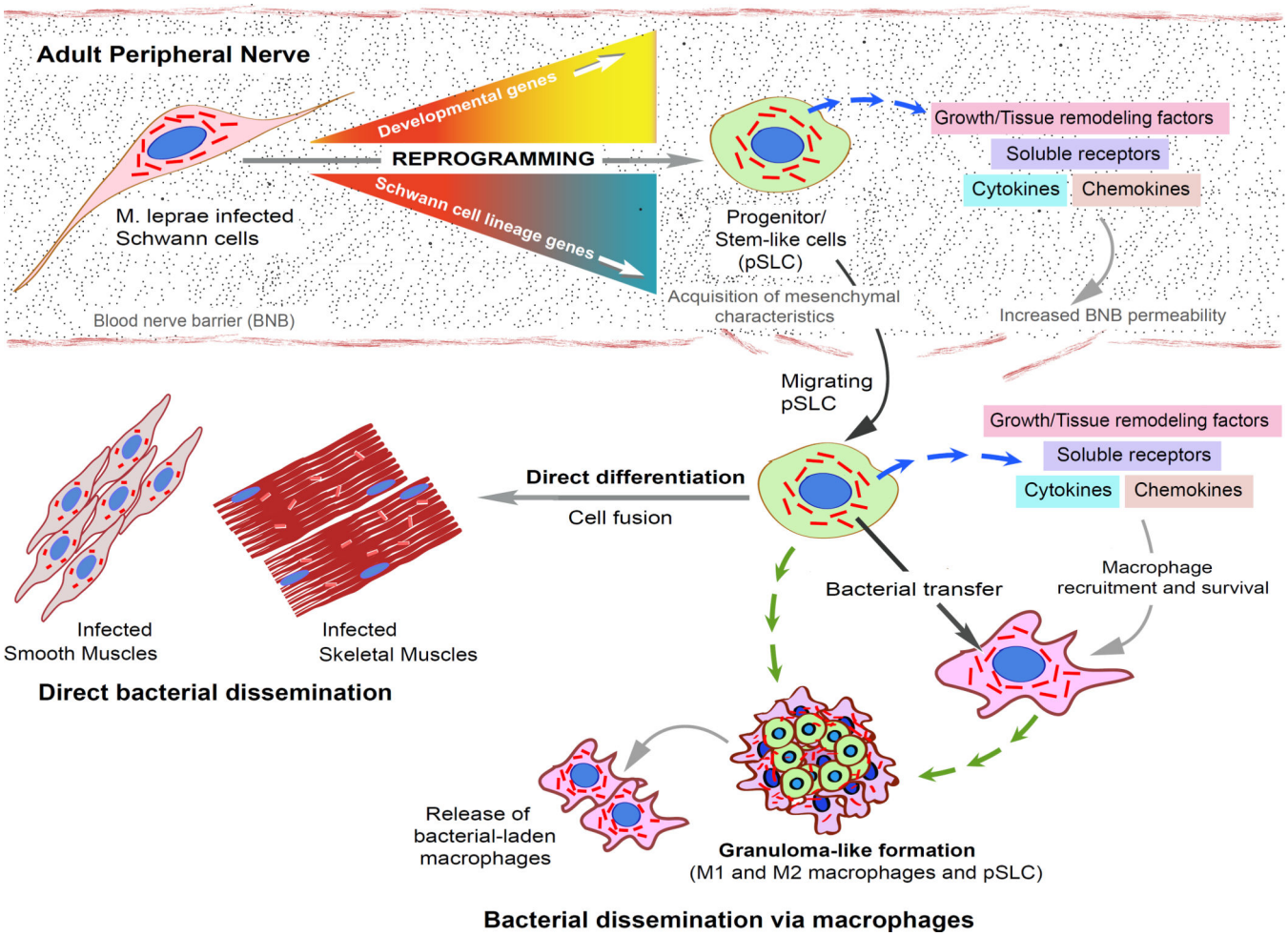

Figure 3. The proposed model for reprogramming adult Schwann cells to stem cell-like cells by intracellular M. leprae and subsequent events leading to dissemination of infection

Schwann cells in the adult peripheral nerves infected with ML undergo a reprogramming process that convert Schwann cells to pSLC by turning off Schwann cell differentiation/ myelination program-associated genes and upregulating embryonic genes of mesenchymal and neural crest development. Reprogramming renders pSLC to acquire migratory properties and immunomodulatory characteristics - releasing numerous chemokines, cytokines and growth/remodeling factors, which not only increased permeability of blood nerve barrier (BNB) but also attract macrophages. Acquired migratory properties promote ML-laden pSLC to exit breached BNB and disseminate to other preferred tissue niches such as smooth muscles and skeletal muscles where they can undergo direct differentiation, and thus transfer bacteria passively to these tissues. Chemoattractants released from pSLC recruit macrophages, transfer ML and form typical granuloma-like structures, which then release bacterial-laden macrophages, a mechanism by which reprogrammed cells may channel bacterial dissemination via systemic routes.