Macrophage as a mediator of immune response: Sustenance of immune homeostasis

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Macrophages have evolved as a key player in our immune system. As macrophages remain groomed to combat invasions by pathogens they also exhibit distinct polarized (M1/M2) states while adapting to the local environmental milieu. Detailed molecular mechanisms governing the prevalence of the usual steady state as well as altered (M1/M2) homeostasis programs under normal and pathogenic conditions remain to be deciphered. In this review, we have discussed the significance of immune homeostasis in macrophages and the involvement of Wnt – Frizzled signalling therein. Overall this review throws light upon some important questions related to the immune homeostasis scheme, answers to which will offer new insight in the field of immunology.


Introduction

Among the myriad of specialized cells that orchestrate our immune system, macrophages are one of the crucial members responsible for fighting against infection by pathogens [1]. Monocytes, which originate from their bone marrow resident myeloid precursor, give rise to macrophages [2-3]. When circulating monocytes infiltrate tissues they get transformed into macrophages. The tissue macrophages not only eliminate encountered pathogens through phagocytosis but also process and present the foreign antigens via MHC molecules to lymphocytes causing lymphocyte activation [4]. Phagocytosis by macrophages is regulated by cytoskeletal modifications which are coordinated at least partly by Wnt – Frizzled signalling [5]. Not all tissue macrophages however, originate from monocytes [6-9], and although some origins have been specified the precise paths leading to the mature cell types remain largely uncharacterized.

The various kinds of macrophages differing in both phenotype as well as function may arise from different cell differentiation pathways and environmental inputs obtained from neighbouring cells [10]. While obliterating invading pathogens and other foreign materials from tissues, macrophages usher in several modes of signalling that are totally dependent on the existing framework. Cytokines in the milieu and the nature of pathogenic stimuli influence the mode of activation of tissue macrophages [11-13]. While pro-inflammatory cytokine secretion is associated with persistence of macrophages in the M1 mode (classical activation mode), anti-inflammatory cytokines are associated with an alternative mode of activation, namely the M2 mode [14-15]. The M1 macrophages promote antigen presentation associated with Th1 (T helper) cell activation, facilitating inflammation. M2 macrophages on the other hand support Th2 cell activation, which while being an inherent component of allergic responses, is also involved in wound healing and repair of tissue often associated with resolution of inflammation [12, 14-16]. Apart from M1 and M2, a third
category of macrophages that demand attention are the Tumor Associated macrophages (TAMs). Though TAMs resemble M2 macrophages to a certain extent, their transcriptional profile is quite different from that of M2 \cite{10}.

The exact molecular mechanisms directing the divergence and polarization of macrophages to the specific subtypes have not been clearly dissected. Whether the M1 subtype evolves into M2 with the nature and duration of the prevalent infections remains an open question. More so, details of the mechanism of immune homeostasis that keep macrophages groomed to react to local stimuli during the steady state (basal activation) as well as polarized state (differential modes of activation) also remain unsettled. This review focuses on the intrinsic functions of macrophages in the context of some recent developments as well as pending questions with respect to mechanisms of immune homeostasis.

**Macrophages exhibit diverse functions: the driving gear therein?**

Macrophages reside in almost all types of tissues present in the body. Their function is dictated by the tissue type they reside in. Macrophages present in the brain and spinal cord, known as microglia uphold an active immune defence scheme in the central nervous system. Alveolar macrophages likewise clear the air spaces from infectious, toxic or allergic particles that cross the protective barriers of our respiratory tract. Similarly, peritoneal macrophages present in the peritoneum and Kupffer cells of the liver act to confront infectious agents encountered effectively. Although a major portion of the tissue macrophages is derived from blood monocytes \cite{17}, tissue macrophages can also be generated independently. Macrophages of the central nervous system (CNS) for example, are derived from the yolk-sac, independent of monocytes \cite{18}. Recent studies on “fate-mapping” in mice also indicate lack of involvement of monocytes in the survival and maintenance of specific populations of tissue macrophages \cite{19}. It is only natural to assume that there will be a certain level of distinctness in the combat mechanism of each macrophage type as different combat mechanisms may arise from different modes of pathogenic recognition, endocytic entry and subsequent signal transduction. Such a scenario is in agreement with the diverse origin of macrophages and their diverse range of...
functions.

A crucial aspect in the immune response to infections is the adaptation of tissue macrophages to the infectious agents inhabiting the local milieu. Macrophages display the inherent ability to ingest and inactivate infectious agents through phagocytosis, which correlates with a trend towards the M1 subtype and the secretion of proinflammatory cytokines like TNF-α [10, 20-23]. Thus while effective phagocytosis during the innate immune response by macrophages facilitates clearance of infections, an excess of inflammatory cytokines generated during uncontrolled phagocytosis may lead to the perpetuation of inflammation and tissue injuries, as depicted in figure 1 [10, 21, 24-25]. Pathogenic conditions linked with chronic inflammation may lead to sepsis like conditions [26-29]. Interestingly, progression of sepsis apart from giving rise to M1 type macrophages also engages M2 polarized macrophages [30] with an alternative state of activation in anti-inflammatory cytokine secretion. These anti-inflammatory cytokines for example, TGF-β and IL10, while promoting resolution of inflammation and tissue repair, also endorse immunosuppression [31-35]. Both suppression and activation of immune responses furthermore, involve interplay of macrophages and T cells (both CD4 and CD8) in the context of differential modes of antigen presentation, a feature of the adaptive immune response program. Th1 and Th2 cells accordingly modulate cytokine secretion and signalling networks that fine-tune immune activation and/or immune suppression [16, 36-39]. Yet another feature embedded within the macrophage mediated immune response program is efferocytosis [40], the process by which macrophages engulf and eliminate apoptotic cells that may be infected from sites of inflammation. Failure to execute proper and effective efferocytosis may lead to the accumulation of unwanted necrotic material [40]. Efferocytosis is receptor mediated [41], Rho and Rac1 activity coordinating the engulfment of the apoptotic cells. This ensures the formation of a spacious phagosome, termed efferosome, which surrounds the newly engulfed apoptotic cells and eventually kills them.
Macrophages remain groomed to act according to the local environment. But what is the mechanism behind such a response by the macrophages? How is immune homeostasis sustained in its different essences for both the M1 and M2 macrophages? How in the first place does the innate immune system equip macrophages with the potential to circumvent infection? Could a steady state basal signalling serve to keep the macrophage guarded to confront infection? Macrophage identity is maintained at the transcriptional level by the transcription factor PU.1, which activates transcription of some crucial genes encoding essential macrophage regulators by influencing the functions of histone modifying enzymes \[6, 10\]. In a healthy living system macrophage defence programs are maintained by a steady state homeostasis that may involve specific signaling pathways. Upon pathogen encounter, these macrophages may differentiate into a particular polarized phenotype i.e. either M1 or M2 depending on the existing cytokine secretion profile. Transition from the M1 phenotype to the M2 phenotype may occur in cases of chronic infection and sepsis, but the molecular mechanism of such a transition remains unknown. Whether the reverse M2 – M1 transition is possible in vivo is also not clear. Although existing literature suggests the prevalence of M1/M2 macrophages with an altered state of homeostasis under pathogenic conditions, how such altered steady states are generated as well as maintained in vivo remains a major question. Does the switch to such transformed steady states lie in the transcription profile of the macrophages? Fig1 explains the different levels of immune homeostasis in macrophages and upholds the unanswered questions. Exploring the answers to such questions will be indeed challenging.

### Potential involvement of a steady state signalling in sustaining innate immune response in macrophages

Although macrophages have long been recognized for displaying innate immune response to bacteria and virus \[32, 41-43\], not much is known about the steady state signalling that sustains such in-built innate immune defence programs. We have recently demonstrated that NF-κB, a transcription factor functioning at the core of our immune system remains activated at a basal level in macrophages in the steady state. A low level of NF-κB (p65) is found to be present in the

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**Figure 3. Canonical and Noncanonical Wnt Signalling.** In the canonical pathway, Wnt interacts with its receptor, Frizzled which activates Dishevelled (Dvl). This inhibits GSK3β rendering β-catenin free to translocate to nucleus and interact with TCF/LEF thus leading to transcription of target genes. The non canonical pathway can work without β-catenin. It involves either Rac1 mediated or calcium response mediated signalling. Activated Dvl can activate Rac1 which further activates JNK leading to the translocation of phosphorylated Jun to the nucleus. Dvl mediated Rac1 activation can also activate IKKβ leading to p65 nuclear translocation where it causes transcription of target genes. On the other hand, Dvl can also activate PLC which via conversion of phospholipids to inositol trisphosphate generates elevated levels of Ca2+. This activates Calcineurin which then causes dephosphorylation of NFAT causing its nuclear translocation.
nucleus of macrophages in the absence of activating stimuli \[44\]. Several other findings support our data \[45\]. Interestingly, the low level of constitutive p65 activity in macrophages has been linked to steady state levels of both CD14 and IFN-β, which help sustain host protective immune response to bacteria/LPS and virus. We discovered that a steady state level of Wnt5a signalling is accountable for the observed constitutive NF-kb activity \[44\]. This basal NF-kB activity leads to the expression of CD14 / IFNβ, the promoter sequence of which at the genome level contains p65 binding elements \[44\] (Figure 2). Such steady state signaling can coordinate with other aspects of macrophage functions as explained in Figure 2 \[44, 46-49\].

Wnt5a belongs to a large family of Wnt glycoproteins comprising about 18 members. Wnts (ligands) transmit signal upon binding to seven transmembrane span receptors termed frizzled that may operate in complex with LRP5/6 and/or ROR at the cell membrane during signal transduction \[5, 44, 50-53\]. Frizzled-5 happens to be a putative receptor for Wnt5a. It should, however, be noted that although modified versions of selective Wnt-Frizzled complex structures have been solved \[52, 54\], none of the ligand-receptor complexes have been truly biochemically characterized in their physiological contexts. Wnt signalling classically is divided into two different modes in the context of the transcriptional co activator β-catenin – the canonical mode, which is usually dependent on β-catenin and the non-canonical mode, which may operate without β-catenin \[51, 53\]. In the canonical mode of the Wnt pathway, the Wnt-LRP5/6-Frizzled complex in combination with the cytoplasmic adaptor protein Dishevelled (Dvl) inactivates the constitutive kinase activity of GSK3-β, which in the absence of the Wnt stimulus would phosphorylate β-catenin driving it towards proteosome mediated degradation. Lack of phosphorylation results in the accumulation of the stable β-catenin in the cytoplasm causing it to translocate to the nucleus thus activating transcription factors of the Tcf/Lef family to initiate target gene expression \[24, 52-53, 55-56\] (Figure 3). In the non-canonical mode of Wnt signalling of which Wnt5a is a representative, Wnt5a-Frizzled-ROR or Wnt5a-Frizzled initiated signalling alters the activity of Rho/Rac family GTPases through differential activation of Dvl \[53, 57\]. The subsequent activation of transcription factors such as JNK (AP1), NFAT and NF-κB through complex signalling networks is usually independent of nuclear translocation of β-catenin \[44, 47, 53, 58-60\]. Another wing of non canonical Wnt signalling is represented by the Wnt-Calcium (Ca\(^{2+}\)) pathway which often modulates β-catenin mediated signalling in order to sustain its own effects \[58, 61\]. This pathway may be exhibited by the Wnt5a-Frizzled2 complex, which activates Phospholipase C (PLC) causing cleavage of membrane phospholipids to inositol trisphosphate (InsP3) and DAG. DAG activates several isoforms of Protein kinase C (PKC) \[62\]. InsP3 upon binding to its cognate receptor in the endoplasmic reticulum leads to a surge in cytoplasmic Ca\(^{2+}\) levels. High calcium levels activate the calmodulin binding phosphatase calcineurin, which dephosphorylates NFAT thus priming it for transcriptional activation \[65\]. DAG primed PKC on the other hand promotes activation of AP1. Such Wnt5a – Fz2 – phospholipid mediated activation of an API – NFAT combination can lead to expression of several target genes \[64\] (Figure 3). It will be interesting to see if Wnt5a-Fz2 mediated signalling sustains a steady state activation of NFAT in macrophages thus contributing to the prevalence of innate immune response programs. Wnt5a-Frizzled2 signalling furthermore causes β-catenin dependent transcription, this being in compliance with the concept that actually it is the Wnt5a ligand-receptor combination in the cell type concerned that dictates the specificity of the Wnt5a mediated signal transduction scheme \[53\].

In our study on immune homeostasis in macrophages, the inherent Wnt5a-Frizzled5 signalling sustains a steady state activation of NF-κB (p65) through Rac1 GTPase activity \[44\]. It will be interesting to find out how Dvl, which also controls canonical Wnt signalling, functions with respect to Wnt5a – Frizzled5 signal transduction pathways to maintain the steady state activation of NF-κB (p65) in macrophages. The Wnt5a-Rac1-p65 homeostatic circuitry as documented by us has the potential to restrain infection at least partially through CD14 – TLR and IFNβ mediated bacterial and viral clearance \[44, 65-66\]. CD14 – TLR and IFNβ responsive pathways of pathogen recognition and clearance have been discussed in several excellent review articles \[67-70\]. The Wnt5a- NF-κB homeostatic circuit not only contributes to building the initial platform of macrophage defence against both bacteria and virus in the steady state, but it is also necessary for macrophage survival \[32, 41, 44, 46\] (Figure 2). Moreover, the Wnt5a-Fz5 signalling scheme allows phagocytosis by lipid raft clustering concomitant with Rac1-P13 kinase and IκB kinase activation which may or may not be CD14 dependent \[5, 44,46\] (Figure 2). It needs to be investigated whether other modes of Wnt signalling also participate in the sustenance of such steady state of immune response in macrophages.

**Immune homeostasis vs. macrophage polarization: Involvement of Wnt Signalling?**

Macrophage polarization is perhaps a continuous mode of activation based on the local milieu \[71-73\]. Just as a steady state (basal) signalling keeps macrophages groomed to circumvent infections as part of the innate immune response program, other homeostatic circuits may be able to sustain the differentially polarized states of macrophages under
various pathogenic conditions as in sepsis\textsuperscript{[26-27]}, rheumatoid arthritis\textsuperscript{[74]}, HIV infection\textsuperscript{[75]}, asthma\textsuperscript{[76]} and chronic obstructive pulmonary disease (COPD)\textsuperscript{[77-80]}. While it is true that transcriptional profiles of M1 vs. M2 macrophages have been studied and cytokines such as TNF-\(\alpha\) / IFN-\(\gamma\) and IL4 help propagate the M1 and M2 type of macrophages respectively, in vitro, it is not clear at the molecular level how these macrophages evolve and sustain themselves in our system in vivo \textsuperscript{[81-85]}. In COPD patients and also in experimentally induced COPD in mice, presence of both M1 and M2 polarized macrophages are observed\textsuperscript{[76, 79, 81]}. During the onset of experimental disease in mice, components of smoke may induce polarization towards the M1 phenotype, enhancing the release of proinflammatory cytokines like IL-6, IL-8, IL-1\(\beta\) and TNF-\(\alpha\). With time, the rates of phagocytosis/ efferocytosis by the prevailing M1 / M2 macrophages may change. In HIV infection, the mature macrophage can be polarized to both M1 and M2 and this polarization may be a crucial factor deciding susceptibility to HIV infection\textsuperscript{[75]}. In Asthma patients, although Th2 activation plays a dominant role in disease progression, there are some reports indicating a comparable role of both M1 and M2 macrophages in the development and sustenance of the disease\textsuperscript{[76]}.

In light of the fact that Wnt signalling is a primordial event in tissue patterning/organization during development and partakes of cell migration, wound healing and repair, it may be worthwhile to test the influence of appropriate Wnt family members in inflammation and its aftermath as contributed by the M1 and M2 subtypes of macrophages. The Wnt family comprises several members and it is quite possible that their effects in terms of sustenance of immune responses will be varied. Since Wnt5a mediated signals promote TNF-\(\alpha\) production\textsuperscript{[5, 50, 85]}, it is quite possible that Wnt5a maintains sustenance of the M1 subtype through distinctive homeostatic circuitries depending on the prevailing stoichiometry of ligand-receptor pairs in the different cells in context. At the same time it cannot be ruled out that distinct doses of Wnt5a in combination with LPS promote a tolerogenic or M2 like effect in macrophages\textsuperscript{[86-88]}. Thus different proportions of the same signal may allow transition from the M1 phenotype to M2 and vice versa depending on other factors present in the milieu. Perhaps appropriate harvesting of inflammatory (M1) and wound healing / immunosuppressive (M2) macrophages and careful analysis of the Wnt signals that sustain their phenotypes would throw light on the involvement of Wnt mediated homeostatic circuits in the maintenance of macrophage polarization. But, how useful is the prevalence of polarized macrophages through homeostatic circuits? Since M1 and M2 subtypes of macrophages are radically separated with respect to their status of activation, namely inflammation / immune activation and immune suppression\textsuperscript{[65, 89]}, would maintenance of a balance between the M1 and M2 states through appropriately targeted interventions of Wnt signalling intermediates under pathogenic conditions be beneficial? This remains an open question.

**Concluding remarks**

From the perspective of immune deficiency / immune suppression and autoimmunity it is important to understand steady state signalling or the status of immune homeostasis, especially in macrophages. Defects or modulations in the basal levels of immune response in macrophages under different situations may lead to either immune deficient conditions from lack of pathogen clearance or autoimmunity from altered macrophage mediated lymphocyte activation. Autoimmunity could ensue due to transformed regulations at different stages or levels of macrophage function ranging from annihilation of infection to wound healing and tissue repair\textsuperscript{[1, 3, 28, 74, 90-91]}. A detailed knowledge of signalling pathways that sustain immune homeostasis in macrophages under normal as well as pathogenic conditions is thus much needed.

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**Disclosures**

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