Chapter

Macrophage Polarization Is Decisive for Chronic Bacterial Infection-Induced Carcinogenesis

Mishi Wasson, Sonia Kapoor, Manoj Garg, Sandhya Singh and Hridayesh Prakash

Abstract

Macrophages are the special cells of the immune system and play both immunological and physiological role. One of the peculiar characteristics of macrophages is that they are double-edged and highly plastic component of immune system. Due to this characteristic, they are responsible for both progressions as well control of a variety of inflammatory, infectious and metabolic diseases and cancer. These are found in the body in three major phenotypes, which are known as M0 (also known as naïve); M1 (classically activated macrophages); and/or M2 (alternatively activated macrophages) at normal physiological conditions. We have been exploring macrophages in context of bacterial infection and previously demonstrated that M2 polarization of M1 effector alveolar macrophages during chronic/persistent *Chlamydia pneumonia*, *Mycobacterium tuberculosis* and *Helicobacter pylori* pathogens are decisive for the infection induced cancer development in host. Since chronic infection with these pathogens has been associated with adenocarcinoma, therefore, we feel that disruption of macrophage plasticity plays crucial role in the host for the development of cancer. On the basis of this, we propose that in such pathological conditions, management of M1/M2 imbalance is paramount for minimizing the risk of developing cancer by chronic and persistent infection.

Keywords: macrophages, immuno-epigenetics, metabolic programming, sterile inflammation, cancer

1. Introduction

Recent studies have demonstrated that macrophages display high grade of phenotypic plasticity due to which they can both enhance and inhibit immune response. This phenotypical plasticity of macrophages enables them to contribute to pathogenesis of large variety of diseases as well as homeostasis mechanisms. Due to this characteristic, these cells are now known as double-edge component of immunity as well. Many studies have demonstrated that these cells can enhance the progression as well as control many infectious and tumor [1] diseases. Both peripheral and tissue macrophages together constitute the reticuloendothelium system which plays a major role in both sensing microbial antigens and their subsequent eradication [2]. Macrophages are recruited to the inflamed/infected tissues, react to a variety of stimuli, and acquire either classical phenotype also known as M1 or alternative phenotype also known as (AAM, M2). Classically activated macrophages are immunostimulatory in nature and have Th1-orienting capacity while M2 are immunoregulatory in nature and have Th2 programming capacity [3]. The latter ones are anticipated to support the survival of various intracellular pathogens during persistency and believed to promote neoplastic transformation of infected tissue micromilieu (**Figure 1**). AAM accumulation in majority of adenocarcinoma (around 10% cases) confers poor prognosis during microbial persistency. Therefore in such abnormal pathological conditions, selective elimination of macrophages by ablating colony-stimulating factor 1(CSF-1) in LySMcre and op/op mouse model [4] or by the use of pharmacological drugs such as clodronate liposomes [5], which are among few possible modalities for mitigating macrophage-associated neoplasia. Within the frame of the above mentioned, this chapter will discuss various strategies to repolarize tumor-associated macrophages (TAM) during cancer development and uncover how selective activation of M1 macrophages could control infection-induced cancer but also existing anti-tumor immune therapies in both mouse and human model of tumors with special emphasis on gastric and lung tumors and inflammatory diseases like inflammatory bowel disease (IBD), which are responsible for global mortality. This may be achieved by targeting the major intracellular signaling component such as sphingolipids and Th2/Th17 responses, which promote M2 phenotype during persistent infection and potentially involve in the development of cancer.

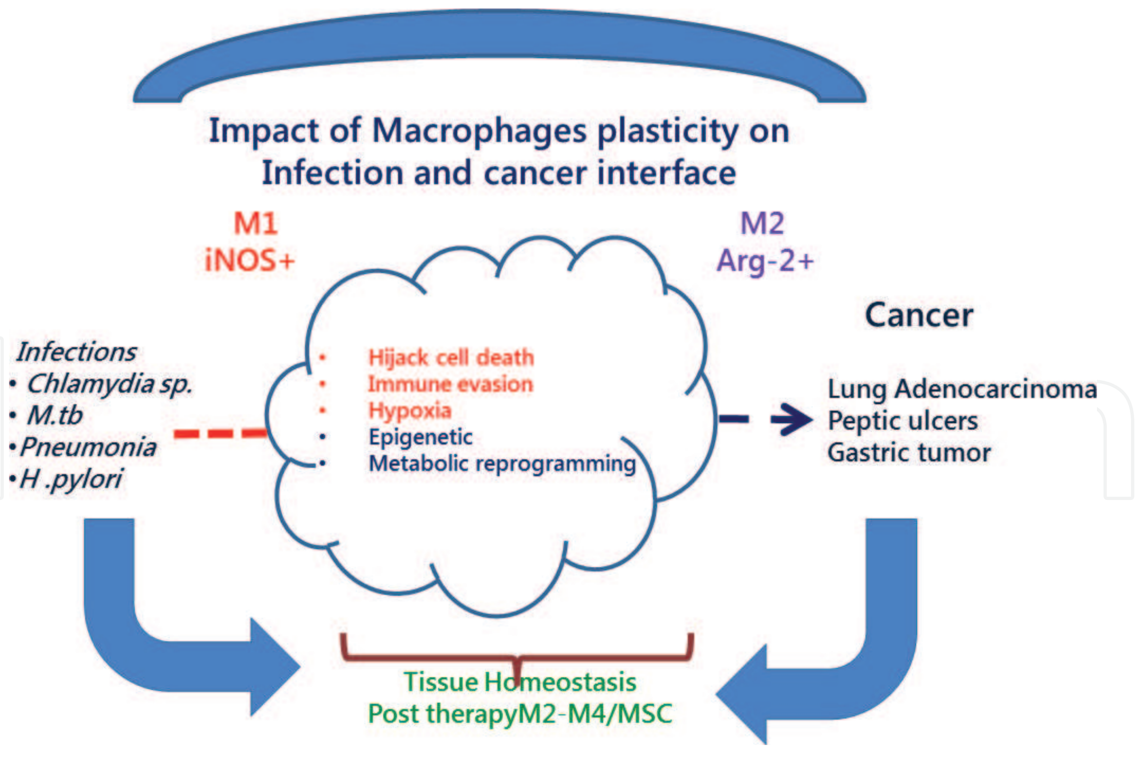

Figure 1.
Schematic representation of various approaches by which persistent infection with human pathogens disrupts functional plasticity of effector macrophages and promote cancer progression. The figure depicts how certain pathogens exploit various cellular and genetic mechanisms and promote M2 polarization of iNOS+ effector macrophages which are the special and double-edge component of the immune system. Phenotypic and functional polarization of effector macrophage is decisive event and anticipated to escort pathogens for neoplastic transformation of infected tissues during latent infections.

2. Pathogens disrupt macrophage plasticity and effector response during persistency

Recent study has demonstrated that a bacterial product known as trabectedin is toxic to macrophages. This product inhibits NF-Y and KLF-2/4 which is important for the differentiation of macrophages in tumor micromilieu [6]. Similarly mitigating NF-κB, STAT3 and HIF-1 are involved in the activation of naïve macrophage to M1 effector phenotype and hold tremendous therapeutic option for modulating macrophages activation. Histopathological analysis of persistently infected lungs reveals the infiltration by specialized macrophage known as foamy macrophages. These are lipid-loaded macrophages and quite refractory in their nature. These macrophages behave more like AAM and are actively involved in the clearance of cellular debris and dead bacteria containing neutrophils and DC [7]. In some cases of coronary atheroma patients, these macrophages acquire phenotype similar to TAM (tumor-associated macrophages) and harbor dead bacteria in their endosome [8]. The presence of these macrophages thus promotes non-immunogenic inflammation which is similar to cancer-associated inflammation and supports opportunistic survival of deadly pathogens. Both phenotypic and functional polarizations of M1/M2 effector phenotype of macrophages are believed to be one of the prognostic factors contributing to the development of tumor during persistent/latent infections (**Figure 1**) in host. Once infiltrated in the infected lungs, these AAM/foamy macrophages potentially modify effector T cells and predispose them also as refractory which are otherwise proficient in the killing of infected cells. These macrophages secrete a plethora of cytokines/growth factors like VEGF-β, TGF-β, hypoxia-inducible factor, and sphingolipids which altogether contribute to neoplastic transformation of infected tissue. High gradient of VEGF and TGF-β promotes the differentiation of regulatory T cells [9] and inhibits the effector response of CD8+ T cells [10]. On the other hand, sphingolipids particularly S-1P/ceramide (either host or pathogen-derived) are known to promote mitophagy [11], M2 polarization of infiltrating M1, or naïve monocyte/macrophage populations [12]. In view of this, and to restore Th1 effector immune response during latent infection, reactivation of M1 effector phenotype of macrophage thus represents the most suitable therapeutic interventions. Apart from this, modulating the cytokine network also seems to be the most effective strategy for boosting immunity for the management of latent/persistent infections.

3. Bacterial persistency hijacks programmed cell death and autophagy and promotes immune metabolic reprogramming

Pathogenic bacteria have evolved several ways to survive efficiently in the phagocytes during their dissemination across the lymphatic system. Various pathogens adapt various strategies to this purpose which range from conferring resistance to the apoptosis [13], immune evasion [14], and metabolic programming of myeloid cells [15] as shown in **Figure 2**. Of these, conferring resistance and insensitivity for cell death in the infected cell seems to be one of the most fundamental processes. A range of bacterial pathogens like *Chlamydia trachomatis* (*C. tr*), *Chlamydia pneumonia* (*C. pn*), and *Helicobacter pylori* (*H. pylori*) which are associated with the pathogenesis of lung [16] and stomach cancer [17] respectively, exploit death and immune signaling for surviving in the hostile environment of antigen presenting (**Figure 2**) and effector cells. We [18, 19] and others [20] have demonstrated that *C. pn* and *C. tr* increase the stability of various endogenous regulators of apoptosis

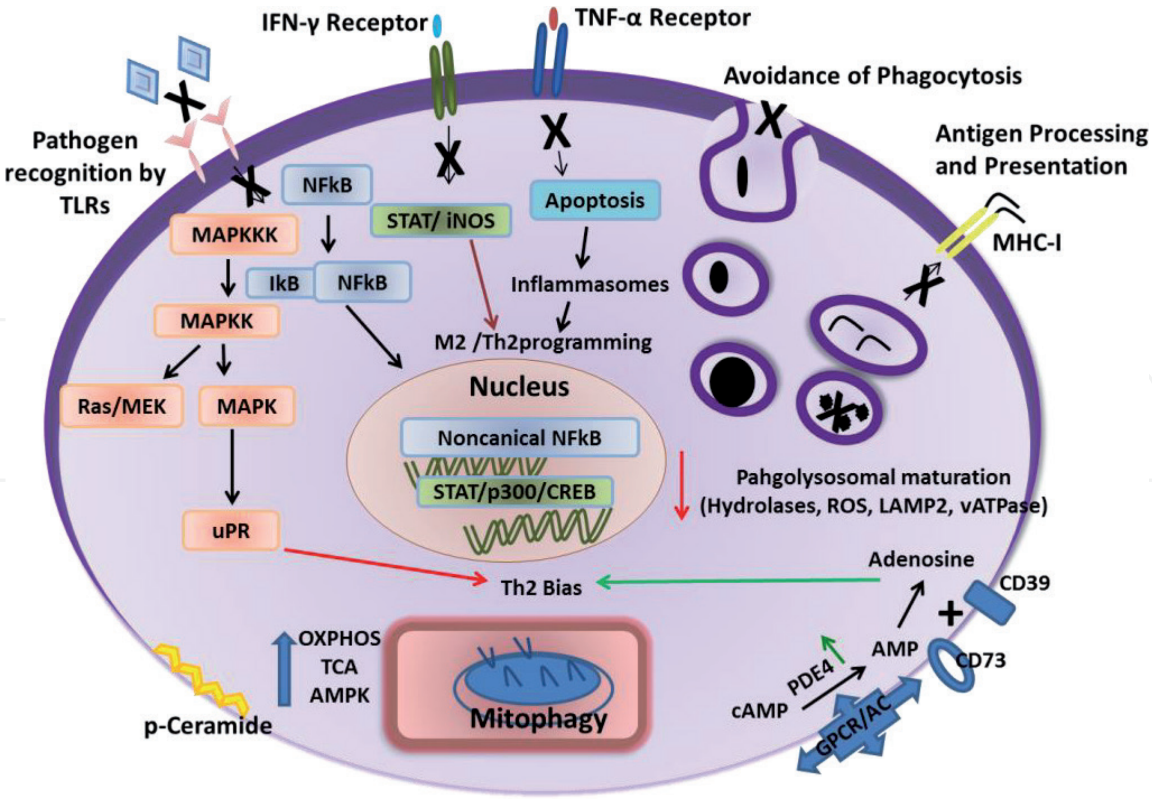

Figure 2.
Bacterial pathogens potentially exploit and interfere in various pathways in committed macrophage for subverting effector mechanisms during latency. Pathogenic bacteria interfere with various key signaling pathways which are important for the effector responses, e.g., recognition by receptors, uptake, and phagocytosis, lysosomal degradation, and alter signaling pathways and secretion of Th1 cytokines for establishing Th2 bias.

proteins called IAP and inhibit the activity of both apoptotic and inflammatory Caspases 3, 8, and 11 during latent infection. Upregulation of CIAP2 and XIAP proteins [19] during acute and persistent/latent infection has shown the increase in noncanonical signaling of NF-κB which is a master transcription factor involved in both cell death inhibition and inflammatory programming and autophagy for Th1 effector response during infection. Our recent study has shown that *C. pneumonia* potentially interfere with M1 programming of infected macrophages [21] when stimulated with their cognate innate and inflammatory stimuli which is due to increased expression of HIF-1 and p38MAPK proteins [22] which are known to promote unfolded proteins response (UPR) in the infected macrophages [23] which in turn predispose macrophages refractory to immune stimulation. Within macrophages, mitochondria is potentially involved in the innate immune response of macrophages against a variety of successful intracellular pathogens mainly by flushing catatonic peptides like LL37, CAP 12, and CAP 18 to cytoplasmic compartment for efficient capture and killing of pathogen in mature phagolysosomes [24]. Recent studies have amply demonstrated that most of the opportunistic pathogens interfere in the mitochondrial physiology by promoting mitophagy which jeopardizes innate immune defense of macrophages against pathogens. In such cases, tweaking mitochondria by using Smac mimetic-based interventions holds promises in the management of persistent infection. Although we have recently demonstrated that Smac mimicry [21] is capable of mounting an efficient immune response against mild pathogens, it fails to do so against pathogenic microbes like *Leishmania donovani*. Since pathogenic microbes enhance the expression of p38MAPK/HIF-1 pathways [25] for sabotaging macrophages functions, therefore we feel that at the moment targeting p38MAPK/HIF-1 in conjunction to Smac mimetics would be paramount for controlling pathogenic microbes. This is a quite intriguing aspect of the field and needs further in-depth investigation. In the same line, many pathogens are known to interfere with autophagy process which is yet another potential mechanism which influences antigen presentation by APC to T cells for the clearance of pathogens. Many pathogens attack mTORc1 complex and disrupt the autophagosome apparatus [26, 27] and inhibit their presentation by APC to T cells for immune surveillance during latent phase. Therefore pharmacological tweaking of autophagy may offer one potential strategy for clearing dead pathogens effectively from the lung tissue. Irrespective of acute or persistent phase, all pathogens utilize host metabolism for their survival inside the host. During persistency, many pathogens consume carbohydrate and protein reservoir of infected macrophages and alter their metabolic rates. L-Tryptophan [28] and glucose are critical for macrophages, and their fluctuation largely dictates the effector response of infected macrophages as well as the fate of pathogen in APC/phagocytes. During persistent infection, *Chlamydia* sp. utilize cellular depot of L-tryptophan amino acid by activating IDO gene and metabolize it to L-kynurenine which impedes glycolysis [29] in the macrophages. M1 effector macrophages rely on cellular depot of glucose for both activation and differentiation into effector phenotype; therefore, during latent infection, pathogens disrupt in the glucose metabolism and promote hypoglycemia rendering them refractory for optimum defense against pathogens.

4. Human pathogens promote epigenetic changes in macrophages during persistency

At genome level, *C. tr* infection causes global hypoacetylation and hypermethylation of lysine residues on core histones which alter histone post-translational modifications which differ between acute and persistent infections. Upregulation of pH2AX (Ser139) and H3K9me3 which are hallmarks of DNA double-strand breaks (DSBs) and senescence-associated heterochromatin foci (SAHF), respectively, during *Chlamydophila trachomatis* [30] infection suggested teratogenic manifestation of *Chlamydia* persistency. This is largely due to increasing levels of reactive oxygen species (ROS) which is produced during latent infection. ROS contribute to DNA double-stranded breaks leading to persistent DNA damage, which in turn triggers SAHF formation in an ERK-dependent manner [30]. CPAF and CADD proteins from *Chlamydophila* pathogens are known to perturb host cell cycle machinery and inhibit recruitment of the DNA damage response proteins pATM and 53BP1 to damaged sites interfering with DNA repair mechanisms [30]. Despite impaired DNA repair, infected cells continue to proliferate which are in turn supported by enhanced oncogenic signals such as ERK, CyclinE, and SAHF. These changes altogether lead to the malignant transformation of infected tissue. Similarly, other pathogens like *Campylobacter rectus* [31], which is associated with oral cancer, downregulate Igf2 gene and enhance DNA methylation at its promoter which can be attributed to bacteria-mediated epigenetic modifications to the host genome. Other pathogens like *Salmonella enterica* serovar Typhi, which is one of the prognostic factors for the susceptibility for gallbladder carcinoma, exploit MAPK and AKT pathways [32] which initiate and sustain neoplastic transformation of infected host. Macrophages sense and trigger immune response against pathogens via TLR-linked signaling cascade. Under normal circumstance, almost all pathogens trigger TLR signaling pathways for activating macrophages; however, only few obligate intracellular pathogens, in hitherto, interfere with TLR signaling directly or indirectly and limit defense mechanisms [33] of effector macrophage like pattern of cytokines secretions, their uptake, and phagocytosis by macrophages. Although there are multiple ways how a pathogen can interfere with TLR signaling, so far TLR2/4 triggered hypoxia and associated sterile inflammatory response, and/or TLR masking/shedding mechanisms have been identified and proposed [34, 35]. *Yersinia enterocolitica* and *Candida albicans* are known to induce immunosuppression through TLR2-mediated IL-10 release and differentiation of T-helper cells to CD4+CD25+ regulatory T cells [36]. *Yersinia* species secrete a virulence (V) antigen, LcrV, which binds to CD14 and TLR2, trigger IL-10 secretion, and mediate immunosuppression. It has recently been shown that a particular residue in the N-terminal region of LcrV targets TLR2 and is required for altering IL-10 induction via TLR2 [37]. Likewise, *H. pylori* escapes from recognition by the TLRs due to the removal of phosphate groups from the 1′ to 4′ positions of lipid A in LPS, which confers low negative charge to this molecule and increases the chance of escaping TLR recognition. The recognition of non-LPS ligands by TLR2 leads to anti-inflammatory responses that are associated with IL-10 production [38]. Flagellin of *H. pylori* is one of the PAMP which potentially modifies the N-terminal recognition domain of TLR5 and helps in escaping the innate immune responses. Manipulation of amino acid 89–96 of the recognition domain of TLR5 results in low affinity to flagellin binding [39]. Under recurrent/latent infection state, TLR2-mediated signaling, hitherto, inhibits IFN-γ response and hijacks Th1 programming of macrophages. A pathogen like *M. avium* inhibits IFN-γ signaling in TLR2-dependent manner where it enhances the expression of dominant-negative STAT1b. Similarly, 19KD protein of *Mycobacterium tuberculosis* inhibits IFN-γ-induced expression of HLA-DR and FcγR1 expression on human macrophages [40]. In addition to the induction of anti-inflammatory signals by TLRs, certain pathogenic microbes have developed strategies to either block or avoid their recognition by TLRs and subsequent activation of the innate defense. According to one recent study, phospholipids and Ypk protein of *Treponema pallidum* interfere in TLRs (TLR3, TLR4, and TLR9) signaling [41] by blocking the function of LPS-binding protein and CD14. Several bacterial pathogens have altered specific PAMP structures to circumvent recognition by TLR4 or TLR5; pathogens, such as *Porphyromonas gingivalis* or *Leptospira*, which have specialized LPS structures that only interact with TLR2; likewise, in *Helicobacter pylori*, the flagellin [39, 42] is not appropriately recognized by TLR5, approving the survival of the bacteria without loss of virulence. Virulent strains of *Salmonella typhi* escape from their recognition by host PRR by various mechanisms, which predominately include modifying their lipid A by various mechanisms including deacylation, palmitoylation, and the addition of aminoarabinose [43]. Pathogens have evolved in several ways of avoiding NO-mediated killing that plays a central role in effector response in phagocytes. *Salmonella typhi* reside in a specialized membrane compartment called the *Salmonella*-containing vacuole (SCV), which is similar to inclusion in the case of *Chlamydia* sp. in macrophages, and use a T3SS called *Salmonella* pathogenicity island 2 (Spi2), which protects them from reactive nitrogen intermediates. Spi2-deficient strains of *S. typhi* get colonized in iNOS+ compartment efficiently [44] with the intracellular organisms in the SCV. Intracellular organisms have also developed mechanisms to detoxify NO-mediated effects. These include the ability to repair damage caused by reactive nitrogen intermediates and to detoxify these molecules. Pathogens have evolved the strategies of inhibiting iNOS activity which is the characteristic feature of M1 effector macrophages. Mucosal pathogen *Citrobacterro dentium* causes a marked reduction in the level of iNOS activity in macrophages [45]. There are many reported examples of bacterial pathogens altering inflammatory cytokines related to signaling. *Staphylococcus aureus* proteins A and M bind directly to the TNF-α receptor 1, on respiratory epithelium, which then potentiates a chemokine and cytokine cascade and subsequent disease [46]. Similarly, *Shigella flexneri*, through exploring type III effector, OspG, which is a protein kinase, activates ubiquitin-conjugated enzymes, thereby affecting phospho-ikBα degradation and subsequent NF-κB activation. Both *Chlamydia pneumonia* and *Chlamydia trachomatis* promote shedding of TNF receptor 1 by activating TACE activity and shunt TNF signaling though TNFR2 [47] and resist antibacterial and inflammatory action of TNF which is major component of effector macrophages.

5. Potential interventions for reactivating refractory macrophages for therapy outcome

While the application of antibiotics is sufficient to control acute infection however during persistent infection, the outcome of treatment mostly remains refractory. This is due to increased density of refractory macrophages in various affected tissues which resists many therapies as seen in many similar diseases like cancer and metabolic disease which is mediated with tissue accumulation of type 2 or tumor-associated macrophages. It is now well accepted by medical community that increased densities of these macrophages are associated with poor prognosis in many infectious, tumor, and metabolic disease. In such conditions antibiotics and/or chemotherapy would require an additional regimen for effective treatment. During past decades, the growing evidence suggested that TAMs clearly play an important role in tumor progression, metastasis, and resistance to available chemotherapies by modulating the microenvironment inside the tumor mass as well as in the stoma. Therefore, it is important to reeducate or target the TAMs (M2-like) to antitumor M1-like macrophage phenotype for successful treatment of several human malignancies. In the remaining sections of this chapter, we have discussed various macrophage-specific and nonspecific interventions for reactivating refractory population of macrophages for improving existing therapies.

5.1 Neoadjuvant for retuning refractory macrophages

Many interventions have been made to reactivate or retune the TAM, but most of them could not influence the disease outcome profoundly. In this context our recent studies have shown neoadjuvant impact of low-dose radiation for retuning TAM, T cell-aided therapy [48], and subsequent normalization of vasculature in solid tumor-bearing animals. Since infection induced adenocarcinoma is manifested with high grade infiltration of foamy macrophages, which are like M2 TAM, therefore, on the basis of our tumor studies, we propose low dose gamma irradiation as one of the non-specific therapeutic interventions for the management of persistent infection-induced tumor development.

5.2 Nanomedicine as immune adjuvant for refractory macrophages

Nanomedicine has emerged as one of the new modalities for reprogramming of both naïve as well as refractory macrophages toward their effector phenotype and thus represents one potential intervention for the management of latent infectious disease. We and others have recently demonstrated that due to their size and unspecific adjuvant properties, nanocarriers/nanocapsules can penetrate inflamed tissue microenvironment effectively and deliver drug in controlled and sustained rate for exerting adjuvant actions on macrophages in the inflamed and fibrotic lesions of infected tissue. Nanomedicine-based approaches may impact refractory macrophages at various levels, namely, (i) enhanced infiltration of fresh monocyte/macrophages, (ii) direct killing, and (iii) in situ polarization of AAM/foamy-like macrophages during chronic infection to assist clearing of infection. One of the interesting mechanisms by which nanoparticle may improve the therapy outcome is to control the differentiation of naive monocytes toward iNOS+ M1 effector macrophages and replace CD11b+/iNOS-/Arginase-1+ AAM during chronic infections. In this context, our recent work has shown that a certain biodegradable amino acid-based pNAPA nanocapsule can potentially stimulate naïve macrophage to the M1 effector phenotype. On the basis of these merits, the nanocapsules may be used as adjuvant for activating innate immune system for the management of infectious diseases and cancer. Another potential application of nanoparticles is to deliver drugs or biopharmaceuticals for preventing differentiation of effector phenotype of macrophage to refectory. In this context one study has shown that delivery of CCR2 and CCR5 siRNA-loaded nanoparticles was able to reduce the recruitment of monocytes to inflamed tissue [49]. Nanocarrier-based approaches can be used for the direct killing of the refractory macrophages as well. For instance, liposomal formulations have been developed for the delivery of bisphosphonates such as zoledronates and clodronates. Subcutaneous/orthotropic injections of these nanocarriers result in the depletion of AAM accompanied with impaired angiogenesis and reduction in metastasis. Nonspecific targeting is the major issue with nanocarriers which can be addressed by tagging these nanocapsules with specific ligands such as LyP1 and mannose receptors (e.g., CD206) which are highly expressed by TAM/AAM [50] for effective targeting of macrophages. PLA-PEG nanoparticles, cyclodextrin nanoparticles, and liposomal formulations have been developed for loading drug cargoes such as sunitinib, IL-12 plasmids, TGF-β inhibitors, and VEGF siRNA for reprogramming of refractory macrophages for skewing in situ Th1 effector immune response against latent infections [51–54].

5.3 Immunotherapeutics are the next-generation treatment modalities

One of the key characteristics of both AAM and TAM is to restrict Th1 immune response/T-cell programming by virtue of their releasing of Th2 cytokines and growth factors, which stimulate the neoplastic differentiation of inflamed fibroblast in tissue [55]. One of the major mechanisms by which these cells limit effector T-cell response is to engage programmed cell death ligands 1 and 2 (PD-L1, PD-L2) [56] which are expressed by the AAM/TAMs. Pulmonary infiltration of lipid rich foamy macrophages is a typical evidence of persistent infection-induced non-immunogenic/sterile inflammatory immune response during persistent/latent *C. pn* and *M. tb* infections. Foamy macrophages are special kinds of AAMs which have poor antibacterial defense mechanisms and serve as carriers of many pathogens during dissemination. These macrophages inhibit Th1 programming of CD4/8+ T cells and promote Th2 bias by secreting IL-4 and IL-13 in infected tissue micromilieu and help these bacteria in immune escape. These macrophages are known to express PD-1L which after binding to PD-1 T cells drives anergy in T cells. Binding of PD-1L to PD-1 receptor triggers functional anergy in cytotoxic T lymphocytes (CTL) which are otherwise effective in eradicating persistently infected dead cells. Many pathogens exploit these pathways as a major immune evasion mechanism for securing their opportunistic survival. For example, *Chlamydia* sp., *H. pylori*, and *Leishmania donovani* pathogens are known to modulate the expression of PD-L1 on macrophages [57–59] for dumping adaptive immune responses. In such conditions, blocking PD-L1 pathway by monoclonal antibody against PD-1/PD-L1 has been found to be effective in restoring phagocytic potential of macrophages for dead cell clearance and subsequent control of tumor in mice models. For this study, it is anticipated that co-administration of antibody against PD-1/checkpoint inhibitors (CTLA4) along with antibiotics would be beneficial for the management of latent infectious diseases. In the same line, vascular endothelial growth factor (VEGF), transforming growth factor (TGF-β), fibroblast growth factor (FGF), and platelet-derived growth factor

(PDGF), which are potentially secreted by AAM and promote sterile inflammation, also represent potential pharmacological targets for enhancing immunogenic death of cells during latent phase. Colony-stimulating factor 1 receptor (CSF1R) represents yet another promising target for therapeutic interventions because CSF1R signaling is crucial for differentiation, recruitment, and survival of TAMs [60].

5.4 Antibody/small molecule inhibitor targeting polarization of refractory macrophages

Intracellular pathogens, during both acute and latent infection, fiddle with various signaling pathways which range from receptor-associated cell death and innate immune signaling, antigen presentation, vesicular transport, and phagocytosis pathways. Although we have disused these in earlier section, here we will discuss the pharmacological and clinical significance of various approaches which may be decisive for mitigating cellular perturbations in the host for restoring immune defenses of macrophage during persistency. In this context, our recent study [21] has proposed that Smac mimetic (IAP-specific inhibitors)-based strategy has potential for enhancing immunogenic cell death of infected cells and reactivating refractory macrophages for improved clearance. Due to these virtues, several Smac mimetics have entered in the second-phase clinical trial against cancer, and we anticipate that the same is expected to help immune system for the management of persistent bacterial infection as well. Other than this, many pathogens exploit MAPK pathways [61] for their benefits and induce production of IL-10 cytokines in the macrophages which further inhibits T-cell programming mainly by promoting T-cell exhaustion [62]. Other than this, elevated levels of p38MAPK promote sterile/anti-inflammatory response, which supports opportunistic survival of pathogen inside macrophages. Likewise, many pathogens exploit cAMP/PKA pathways and acquire Th2 bias during their persistency [63] for securing their survival; TNF-α is a major and key cytokine responsible for receptor-mediated killing of infected cells. We (unpublished data) and others have shown that many intracellular pathogens, during persistency, potentially target this cytokine and inactivate cell death pathways in TACE- or ADAM-dependent manner. Pathogens like *Chlamydia* sp. secrete CPAF, CADD, and hsp60 exerting TACE activity and cause shedding of TNFR1 [21], which actually induces cell death. Interestingly these proteins which are secreted by chlamydial pathogens require MAPK activation for efficient shedding of TNFR1 [64]. Therefore on the basis of the above observations, designing a suitable MAPK/phosphodiesterase 4 (PDE4) inhibitors [65] thus represents a compelling approach for controlling bacterial persistency and associated immune evade mechanism. Sphingolipids are yet another dual-specific cellular targets [66] of many pathogens for deviating Th1 effector immune response [67]. We have recently demonstrated that S1P/ceramide rheostat is an important parameter which can largely dictate whether pathogen would undergo persistency or not [66]. In this direction, we have recently demonstrated that the gain of S-1P during acute *M. tb* infection affords protective immunity in host for controlling pathogen burden; however, the same is anticipated to promote mycobacterial persistency and thus in such conditions, employment of sphingolipid-based inhibitors, in hitherto, would favor host for breaking persistency and induction of protective immune responses.

5.5 Future perspectives: macrophage-based palliative strategies for tissue homeostasis post-antibiotic purging

Successful therapy post-antibiotic treatment infection should normalize the tissue microenvironment and restore homeostasis. This could be achieved by chelating oxidative stress and remnants of inflammatory response for the replenishment of tissue mass, which normally gets lost during various therapeutic procedures. Management of M1/M2 imbalance is believed to be the key for minimizing the risk of having cancer by chronic and persistent infection with intracellular pathogens. In the clinics, this can be achieved by exchanging refractory populations of macrophage with effector ones which can control the sterile reactions and tumorigenesis. However, due to the pro-inflammatory nature of iNOS+ effector macrophages, this may elicit another sequence of destruction, which alone may not be beneficial. Therefore in such delicate conditions, co-administration of M1 macrophage with mesenchymal stem cell regenerative approach seems to be optimum for reconstituting the affected tissues and organs. The potential inclusion of macrophage-mesenchymal cell-based therapeutic intervention could be categorized under prospective palliative therapies for restoration of physiological function post-treatment.

6. Conclusion

Since chronic infection with bacterial pathogens has been associated with adenocarcinoma, therefore, we believe that the management of M1/M2 imbalance is paramount for minimizing the risk of developing cancer by chronic and persistent infection of the lung, stomach, and cervix. This may be achieved by targeting major signaling pathways such as sphingolipids and Th2/Th17 responses which drive M2 phenotype and which are potentially involved in the development of cancer. In the light of the above, we propose that selective activation of M1 macrophages could improve existing antitumor immune therapies in both mouse and human models of tumors with special emphasis on gastric and lung tumors and inflammatory diseases like inflammatory bowel disease (IBD) which are responsible for global mortality.

Acknowledgements

S.K. acknowledges the grants from SERB-Department of Science and Technology (Project ECR/001173/2016) and Department of Biotechnology (Project BT/PR18562/BIC/101/424/2016), Government of India. This work was supported by Department of Biotechnology (DBT), under its Ramalingaswami Fellowship (BT/RLF/Re-entry/24/2014) and SERB-ECRA scheme (SERB File No. ECR/2016/001519) award to Dr. Manoj Garg.

Conflict of interest

The authors have no competitive/financial interest.

Acronyms and abbreviations

MAPK	mitogen-activated protein kinase
MAPKK	MAPK kinase
MAPKKK	MAPKK kinase
NF-κB	nuclear factor κB
STAT	signal transducer and activator of transcription
TLR	toll-like receptor
TNF-α	tumor necrosis factor-α

Author details

Mishi Wasson[1], Sonia Kapoor[2], Manoj Garg[2], Sandhya Singh[3] and Hridayesh Prakash[1]*

1 Amity Institute of Virology and Immunology, Amity University, Noida, Uttar Pradesh, India

2 Amity Institute of Molecular Medicine and Stem Cell Research, Amity University, Noida, Uttar Pradesh, India

3 Amity Institute of Physiology and Allied Sciences, Amity University, Noida, Uttar Pradesh, India

*Address all correspondence to: hprakash@amity.edu

References

[1] Nadella V, Singh S, Prakash H. Macrophages directed approaches are paramount for effective cancer immunotherapies. Integrative Cancer Science and Therapeutics. 2016;**3**:465-472

[2] Gordon S, Plüddemann A. Tissue macrophages: Heterogeneity and functions. BMC Biology. 29 Jun 2017;**15**(1):1-18

[3] Atri C. Role of human macrophage polarization in inflammation during infectious diseases. International Journal of Molecular Sciences. 2018;**19**:1801

[4] Hua L, Shi J, Shultz LD, Ren G, Harbor B. Genetic models of macrophage depletion. Methods in Molecular Biology. 2019;**1784**:1-15

[5] Weisser SB, Van Rooijen N, Sly LM. Depletion and reconstitution of macrophages in mice. 2. Derivation of polarized macrophages from bone marrow aspirates. Journal of Visualized Experiments. 2012;**1**(66):5-11

[6] Sessa C, De Braud F, Perotti A, Bauer J, Curigliano G. Trabectedin for women with ovarian carcinoma after treatment with platinum and taxanes fails. Journal of Clinical Oncology. 2019;**23**:1867-1874

[7] Teng O, Ke C, Ang E, Guan XL. Macrophage-bacteria interactions—A lipid-centric relationship. Frontiers in Immunology. 2017;**8**:1836

[8] Kozarov E. Bacterial invasion of vascular cell types. Future Cardiology. 2012;**8**:123-138

[9] Terme M, Pernot S, Marcheteau E, Sandoval F, Benhamouda N, Colussi O, et al. VEGFA-VEGFR pathway blockade inhibits tumor-induced regulatory T-cell proliferation in colorectal cancer. Cancer Research: 2013;**73**:539-549

[10] Gavalas NG, Tsiatas M, Tsitsilonis O, Politi E, Ioannou K, Ziogas AC, et al. VEGF directly suppresses activation of T cells from ascites secondary to ovarian cancer via VEGF receptor type 2. British Journal of Cancer. 2012;**107**:1869-1875

[11] Dany M, Ogretmen B. Ceramide induced mitophagy and tumor suppression. Biochimica et Biophysica Acta. 2016;**1853**:2834-2845

[12] Rodriguez YI, Campos LE, Castro MG, Aladhami A, Alvarez SE. Sphingosine-1 phosphate: A new modulator of immune plasticity in the tumor microenvironment. Frontiers in Oncology. 2016;**6**:1-16

[13] Kobayashi SD, Braughton KR, Whitney AR, Voyich JM, Schwan TG, Musser JM, et al. Bacterial pathogens modulate an apoptosis differentiation program in human neutrophils. PNAS. 2003;**100**:10948-10953

[14] Finlay BB, Mcfadden G. Review anti-immunology: Evasion of the host immune system by bacterial and viral pathogens. Cell. 2006;**124**:767-782

[15] Kelly B, Neill LAJO. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. Cell Research. 2015;**25**:771-784

[16] Chanudet E, Adam P, Nicholson AG, Wotherspoon AC, Ranaldi R, Goteri G, et al. *Chlamydiae* and mycoplasma infections in pulmonary MALT lymphoma. British Journal of Cancer. 2007;**97**:949-951

[17] Applications S. The infection connection. *Helicobacter pylori* is more than just the cause of gastric ulcers. Science & Society. 2006;**7**:5-8

[18] Prakash H, Becker D, Böhme L, Albert L, Witzenrath M, Rosseau S, et al. cIAP-1 controls innate immunity

to *C. pneumoniae* pulmonary infection. PLoS One. 2009;**4**:e6519

[19] Prakash H, Albrecht M, Becker D, Kuhlmann T, Rudel T. Deficiency of XIAP leads to sensitization for *Chlamydophila pneumoniae* pulmonary infection and dysregulation of innate immune response in mice. The Journal of Biological Chemistry. 2010;**285**:20291-20302

[20] Rajalingam K, Sharma M, Paland N, Hurwitz R, Thieck O, Oswald M. IAP-IAP complexes required for apoptosis resistance of *C. trachomatis*-infected cells. PLoS Pathogens. 2006;**2**:e114

[21] Nadella V, Mohanty A, Lalita Sharma SY, Mollenkopf H-J, Mazumdar VB, Palaparthi R, et al. Inhibitors of apoptosis protein antagonists (Smac mimetic compounds) control polarization of macrophages during microbial challenge and sterile inflammatory responses. 2018;**8**:1-21

[22] Lin N, Simon MC, Lin N, Simon MC. Hypoxia-inducible factors: Key regulators of myeloid cells during inflammation find the latest version: Hypoxia-inducible factors: Key regulators of myeloid cells during inflammation. The Journal of Clinical Investigation. 2016;**126**:3661-3671

[23] Grootjans J, Kaser A, Kaufman RJ, Blumberg RS, Program D, Jolla L. The unfolded protein response in immunity and inflammation Joep. Nature Reviews. Immunology. 2017;**16**:469-484

[24] Van Harten RM, Van Woudenbergh E, Van Dijk A, Haagsman HP. Cathelicidins: Immunomodulatory antimicrobials. Vaccine. 2018;**14**:63

[25] Yamala AK, Nadella V, Mastai Y, Prakash H, Paik P. Poly N-acryloyl-(L-phenylalanine methyl ester) hollow core nanocapsules facilitate sustained delivery of immunomodulatory drugs and exhibit adjuvant properties. Nanoscale. 2017;**28**:14006-14014

[26] Steele S, Brunton J, Kawula T. The role of autophagy in intracellular pathogen nutrient acquisition. Frontiers in Cellular and Infection Microbiology. 2015;**5**:1-11

[27] Marcelo C, Milton F, Aguilera O. Autophagy response: Manipulating the mTOR-controlled machinery by amino acids and pathogens. Amino Acids. 2014;**47**:2101-2112

[28] Diskin C, Pålsson-mcdermott EM. Metabolic modulation in macrophage effector function. Frontiers in Immunology. 2018;**9**:1-17

[29] Badawy AA. Kynurenine pathway of tryptophan metabolism: Regulatory and functional aspects. International Journal of Tryptophan Research. 2017;**10**:1178646917691938

[30] Chumduri C, Gurumurthy RK, Zadora PK, Mi Y, Meyer TF. Chlamydia infection promotes host DNA damage and proliferation but impairs the DNA damage response. Cell Host & Microbe. 2013;**13**:746-758

[31] Bobetsis YA, Barros S. Bacterial infection promotes DNA hypermethylation. Journal of Dental Research. 2007;**86**:169-174

[32] Scanu T, Spaapen RM, Bakker JM, Holden DW, Nath G, Neefjes J. *Salmonella* manipulation of host signaling pathways provokes cellular transformation associated with article *Salmonella* manipulation of host signaling pathways provokes cellular transformation. Cell Host & Microbe. 2015;**17**:763-774

[33] Van Avondt K, Van Sorge NM, Meyaard L. Bacterial immune evasion through manipulation of host inhibitory immune signaling. PLoS Pathogens. 2015;**2**:1-8

[34] Tan Y, Zanoni I, Cullen TW, Goodman AL, Jonathan C. Mechanisms

of toll-like receptor 4 endocytosis reveal a common immune-evasion strategy used by pathogenic and commensal bacteria. Immunity. 2016;**43**:909-922

[35] Gopalakrishnan A, Salgame P. Toll-like receptor 2 in host defense against *Mycobacterium tuberculosis*: To be or not to be-that is the question. Current Opinion in Immunology. 2017;**42**:76-82

[36] Netea MG, Sutmuller R, Hermann C, Van Der Graaf CAA, Van Der Meer JWM, Van Krieken JH, et al. Toll-like receptor 2 suppresses immunity against *Candida albicans* through induction of IL-10 and regulatory T cells. Journal of Immunology. 2019;**2004**:3712-3718

[37] Sing A, Rost D, Tvardovskaia N, Roggenkamp A, Wiedemann A, Kirschning CJ, et al. Yersinia V—Antigen exploits toll-like receptor 2 and CD14 for interleukin 10—Mediated immunosuppression. The Journal of Experimental Medicine. 2002;**196**:1017-1024

[38] Jr RMP, Fiske C, Wilson KT. Role of innate immunity in *Helicobacter pylori*-induced gastric malignancy. Physiological Reviews. 2010;**90**:831-858

[39] Gewirtz AT, Yu Y, Krishna US, Israel DA, Lyons SL, Peek RM. *Helicobacter pylori* flagellin evades toll-like receptor 5—Mediated innate immunity. The Journal of Infectious Diseases. 2004;**2279**:1914-1920

[40] Gehring AJ, Rojas RE, Canaday DH, Lakey DL, Harding CV, Boom WH, et al. The *Mycobacterium tuberculosis* 19-kilodalton lipoprotein inhibits gamma interferon-regulated HLA-DR and Fc gamma R1 on human macrophages through toll-like receptor 2. Infection and Immunity. 2003;**71**:4487-4497

[41] Hashimoto M, Asai Y, Ogawa T. Treponemal phospholipids inhibit innate immune responses induced by pathogen-associated molecular patterns. Journal of Biological Chemistry. 2003;**278**:44205-44213

[42] Nigou J, Zelle-rieser C, Gilleron M, Thurnher M, Puzo G. Mannosylated lipoarabinomannans inhibit IL-12 production by human dendritic cells: Evidence for a negative signal delivered through the mannose receptor. Journal of Immunology. 2001;**166**:7477-7485

[43] Ernst RK, Guina T, Miller SI. *Salmonella typhimurium* outer membrane remodeling: Role in resistance to host innate immunity. Microbes and Infection. 2001;**3**:1327-1334

[44] Vazquez-Torres A, Xu Y, Jones-Carson J, Holden DW, Lucia SM, Dinaer MC, et al. *Salmonella* pathogenicity island 2-dependent evasion of the phagocyte NADPH oxidase. Science. 2000;**287**:7-10

[45] Vallance BA, Deng W, De Grado M, Chan C, Jacobson K, Finlay BB. Modulation of inducible nitric oxide synthase expression by the attaching and effacing bacterial pathogen *Citrobacter rodentium* in infected mice. Infection and Immunity. 2002;**70**:6424-6435

[46] Gómez MI, Lee A, Reddy B, Muir A, Soong G, Pitt A, et al. *Staphylococcus aureus* protein a induces airway epithelial inflammatory responses by activating TNFR1. Nature Medicine. 2004;**10**:842-848

[47] Kim DW, Lenzen G, Page A, Legrain P, Sansonetti PJ, Parsot C. The *Shigella flexneri* effector OspG interferes with innate immune responses by targeting ubiquitin-conjugating enzymes. PNAS. 2005;**102**:14046-14051

[48] Nadella V, Singh S, Jain Aklank, Jain M, Vasquez KM, Sharma A, et al. Low dose radiation primed

iNOS + M1macrophages modulate angiogenic programming of tumor derived endothelium. 2018;**57**:1-8

[49] Leuschner F, Dutta P, Gorbatov R, Novobrantseva TI, Donahoe JS, Courties G, et al. Articles therapeutic siRNA silencing in inflammatory monocytes in mice. Nature Biotechnology. 2011;**29**:1005-1010

[50] Yan Z, Wang F, Wen Z, Zhan C, Feng L, Liu Y, et al. LyP-1-conjugated PEGylated liposomes: A carrier system for targeted therapy of lymphatic metastatic tumor. Journal of Controlled Release. 2012;**157**:118-125

[51] Liao D, Liu Z, Wrasidlo WJ, Luo Y, Nguyen G, Chen T. Targeted therapeutic remodeling of the tumor microenvironment improves an HER-2 DNA vaccine and prevents recurrence in a murine breast cancer model. Cancer Research. 2011;**71**:5688-5697

[52] Liu X, Gao X, Zheng S. Modified nanoparticle mediated IL-12 immunogene therapy for colon cancer. Nanomedicine Nanotechnology, Biology, and Medicine. 2017;**13**:1993-2004

[53] Park J, Wrzesinski SH, Stern E, Look M, Criscione J, Ragheb R, et al. Combination delivery of TGF-β inhibitor and IL-2 by nanoscale liposomal polymeric gels enhances tumour immunotherapy. Nature Materials. 2013;**11**:895-905

[54] Xu Z, Wang Y, Zhang L, Huang L. Nanoparticle-delivered transforming growth factor-β siRNA enhances vaccination against advanced melanoma by modifying tumor. ACS Nano. 2014;**8**:3636-3645

[55] Burkholder B, Huang R, Burgess R, Luo S, Sloane V, Zhang W, et al. Tumor-induced perturbations of cytokines and immune cell networks. Biochimica et Biophysica Acta. 2014;**1845**:182-201

[56] Mino-kenudson M. Programmed cell death ligand-1 (PD-L1) expression by immunohistochemistry: Could it be predictive and/or prognostic in non-small cell lung cancer? Mechanisms of PD-L1 expression. Cancer Biology & Medicine. 2016;**1**:157-170

[57] Starkey MR, Nguyen DH, Brown AC, Essil A, Kim RY, Yagita H, et al. Programmed death ligand 1 promotes early-life chlamydia respiratory infection—Induced severe allergic airway disease. American Journal of Respiratory Cell and Molecular Biology. 2016;**54**:493-503

[58] Roy S, Gupta P, Palit S, Basu M, Ukil A, Das PK. The role of PD-1 in regulation of macrophage apoptosis and its subversion by *Leishmania donovani*. Clinical & Translational Immunology. 2017;**6**:e137-e110

[59] Silva R, Gullo I, Carneiro F. The PD-1:PD-L1 immune inhibitory checkpoint in *Helicobacter pylori* infection and gastric cancer: A comprehensive review and future perspectives. Porto Biomedical Journal. 2016;**1**:4-11

[60] Cannarile MA, Weisser M, Jacob W, Jegg A, Ries CH, Rüttinger D. Colony-stimulating factor 1 receptor (CSF1R) inhibitors in cancer therapy. Journal for Immunotherapy of Cancer. 2017;**5**:1-13

[61] Krachler AM, Woolery AR, Orth K. Manipulation of kinase signaling by bacterial pathogens. The Journal of Cell Biology. 2011;**195**:1083-1092

[62] Garra AO, Murphy KM. From IL-10 to IL-12: How pathogens and their products stimulate APCs to induce T(H)1 development. Nature Immunology. 2009;**10**:929-932

[63] Rodriguez KAM. The myriad roles of cyclic AMP in microbial pathogens, from signal to sword. Nature Reviews. Microbiology. 2013;**10**:27-38

[64] Paland N, Böhme L, Gurumurthy RK, Maurer A, Szczepek AJ, Rudel T. Reduced display of tumor necrosis factor receptor I at the host cell surface supports infection with *Chlamydia trachomatis*. The Journal of Biological Chemistry. 2008;**283**:6438-6448

[65] Li H, Zuo J, Tang W. Phosphodiesterase-4 inhibitors for the treatment of inflammatory diseases. Frontiers in Pharmacology. 2018;**9**:1-21

[66] Sharma L, Prakash H. Sphingolipids are dual specific drug targets for the management of pulmonary infections: Perspective. Frontiers in Immunology. 2017;**8**:1-6

[67] Baumruker T, Prieschl EE. Sphingolipids and the regulation of the immune response. Seminars in Immunology. 2002;**14**:57-63