Review Article

Colony stimulating factors and macrophage heterogeneity

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Macrophages or mononuclear phagocytes are heterogeneous populations present throughout the body which can adapt quite dramatically to the surrounding milieu, both in the steady state and during immune/inflammatory responses. To assist in our understanding of this diversity, they have been classified into polarization or "activation" states, termed M1 and M2, respectively. This in vitro classification commonly incorporates interferon γ (± lipopolysaccharide) as the stimulus for M1 macrophages and IL-4 or IL-13 for M2 macrophages. Attempts are underway to place tissue macrophages, isolated from ongoing immune/inflammatory reactions, into these categories. However, more flexible classifications are needed to take into account the diversity of macrophage functions. We have compared the in vitro properties of monocytes/macrophages treated with macrophage-colony stimulating factor (M-CSF or CSF-1) and granulocyte macrophage-CSF (GM-CSF), the former because of its role in macrophage lineage development in the steady state and the latter because of its proinflammatory and immune-potentiating properties. Data will be presented on "M2-like" properties of CSF-1-treated populations and "M1-like" features of the GM-CSF-treated counterparts with links to IL-12 family biology. It is proposed that such CSF-dependent changes should be considered in discussions of macrophage polarization. The concept of "CSF-1 resistance" in macrophages, whereby steady state CSF-1-dependent signaling has to be overcome by pro-inflammatory stimuli, such as GM-CSF, interferon γ etc, will be discussed.

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Introduction

The article is not intended to be a comprehensive review of the topic. It will highlight the contribution of our laboratory to the topic since it has emanated from a talk given at the 31st Annual Meeting of the Japanese Society of Inflammation and Regeneration in August 2010. It will therefore also contain some personal views on what are some of the outstanding questions around this topic and some recommendations on what might be done to address them.

Colony stimulating factors

Colony stimulating factors (CSFs) were originally defined by their ability in vitro to generate myeloid cell colonies from precursor cells following proliferation and differentiation[1]. It was later realized that CSFs could also affect mature myeloid cell function[2-4]. Two CSFs which can govern the functions of macrophage lineage populations are macrophage-CSF (M-CSF or CSF-1) and granulocyte macrophage-CSF (GM-CSF). Their receptors are quite distinct, that for CSF-1 being the c-FMS tyrosine kinase (CD115), while that for GM-CSF being a dodecamer built from α (binding) and β (signaling) subunits[5-8]. There is evidence that both CSFs can enhance monocyte/macrophage survival as well as "activate" and differentiate them[4,9-16]. The *Csf1*^{op}/*Csf1*^{op} mouse shows that in the steady state CSF-1 is important for maintaining macrophage populations in a number of tissues, for example, the bone-resorbing osteoclasts[17,18]; in contrast, the major phenotype of the GM-CSF-/- mouse is pulmonary alveolar proteinosis, with defective alveolar macrophage function but with a relatively normal myeloid system[19].

Based on the ability of purified CSF-1 and GM-CSF to activate macrophages we first suggested that they both may be viewed as proinflammatory cytokines[3]. Several studies using neutralizing antibodies or gene deficient mice have given some credence to this concept and clinical trials targeting their activities or those of their receptors are in progress, mainly for rheumatoid arthritis (RA)[20]. What is unknown is to what extent during inflammation these CSFs can control macrophage numbers, either locally or systemically by mobilization, and/or can "activate" them[21].

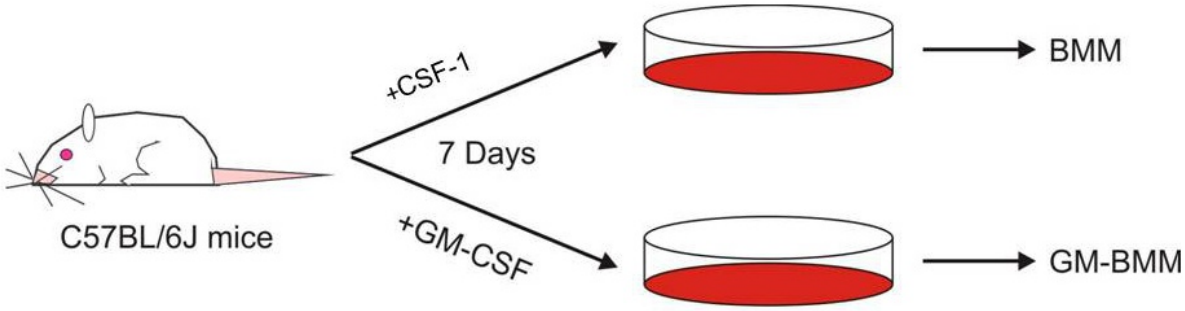

Figure 1. CSF-1- and GM-CSF-dependent macrophage populations
Briefly, murine bone marrow cells are cultured for 7 days in CSF-1 or GM-CSF to generate adherent bone marrow-derived macrophages (BMM) and GM-BMM, respectively[32].

Macrophage heterogeneity

It is apparent that macrophage populations are extremely diverse in properties with the capability to interconvert depending on their milieu[22-24]. This interconversion enables macrophages to carry out a wide array of functions to do with protecting the host from infection or other insults, on the one hand, while helping to restore homeostasis by clearing away debris and damaged tissue, and promoting tissue repair, on the other. It would be useful to know how to manipulate these various functions in vivo since host protection activities can lead to the problem of chronic inflammation while over-exuberant repair mechanisms might contribute to tumor growth and fibrosis. Unfortunately, it is proving difficult to target macrophages specifically at the gene level for a number of reasons[25]. In order to understand their

various roles, attempts have been made to categorize macrophages based on their in vitro activation into "classically activated" (M1) and "alternatively activated" (M2) states[22-24]; the most common stimuli invoked to induce these states in vitro are LPS ± interferon γ and IL-4 or IL-13, respectively. Attempts are being made to relate in vivo macrophage populations at sites of inflammation to these in vitro-defined states[26-28]. As a broad generalization, M1 macrophages are viewed as participating in the host response to infection or inflammation, while M2 macrophages are considered to participate in the immune response to helminths or in the resolution of an inflammatory reaction. However, it is likely that such a simplified delineation of macrophage phenotypes is not going to explain by any means the heterogeneity alluded to above[29].

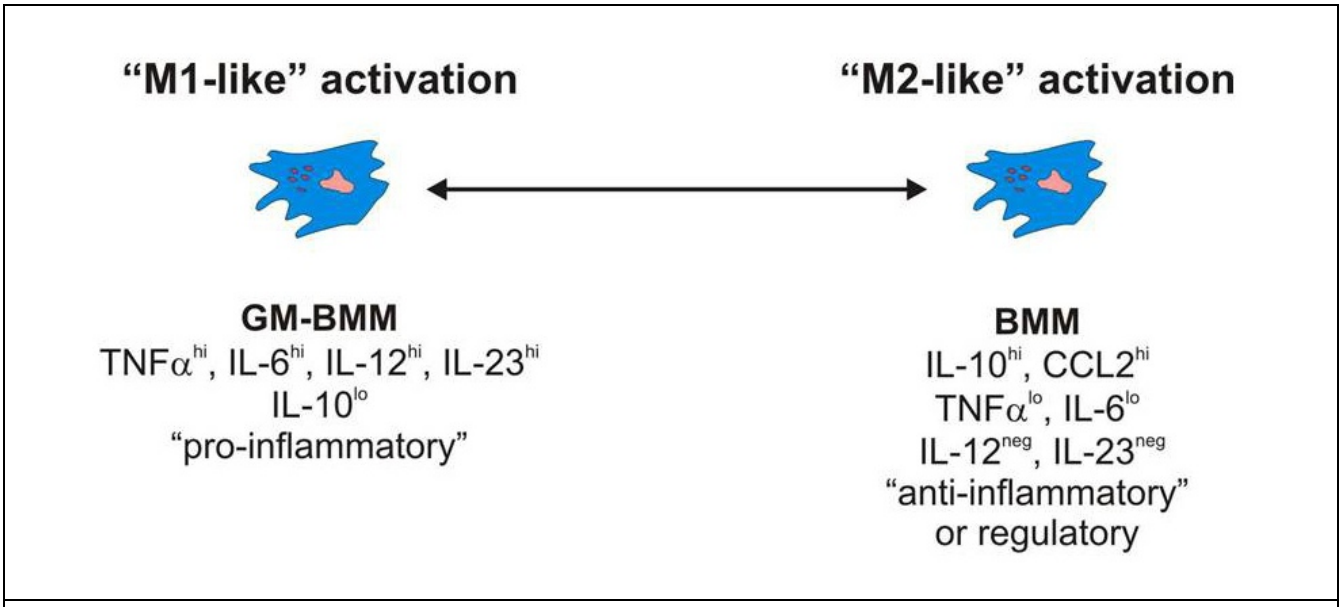

Figure 2. "M1-like" and "M2-like" macrophages
Based on gene expression of some cytokines at least (qPCR), GM-BMM and BMM have been proposed to have "M1-like" and "M2-like" properties, respectively[35].

CSFs and macrophage heterogeneity

As regards the contribution of GM-CSF and CSF-1 to macrophage lineage diversity, it is not unreasonable to propose that macrophages in many tissues in the steady state are exposed to locally generated CSF-1 while GM-CSF is more likely to be involved during inflammatory or immune reactions, except in tissues, such as lung, which are normally exposed to microorganisms[20]. In this context, CSF-1 circulates at readily detectable levels whereas GM-CSF is difficult to detect normally. GM-CSF is strongly implicated in the development of a particular type of dendritic cell (DC), namely a "monocyte DC" or an "inflammatory DC"[30]. Interestingly, when GM-CSF and CSF-1 are both present in cultures of human monocytes, the former directs cells towards a DC phenotype whereas CSF-1 opposes this and differentiates the population more towards a macrophage phenotype[31]. As a striking example of CSF-driven "plasticity", when murine bone marrow cells are cultured for a few days in GM-CSF the population arising has features of immature DCs yet, if the GM-CSF is then removed and the cells cultured in CSF-1+RANKL, osteoclasts are generated[32]. To highlight even further the additional permutations in macrophage lineage phenotypes arising out of CSF action, GM-CSF suppresses in vitro osteoclastogenesis driven from osteoclast precursors by CSF-1+RANKL[32].

Efforts have been made to relate the macrophage phenotypes following exposure to CSF-1 and GM-CSF to M1 and M2 polarization. Others have termed GM-CSF-treated human monocytes as M1 macrophages and CSF-1-treated counterparts as M2 macrophages[33,34] whereas we have used a more conservative "M1-like" and "M2-like" terminology to describe murine macrophages cultured in the respective CSFs[35]. This in vitro system is depicted in Figure 1. In Figure 2, the model arising out of our in

vitro studies is presented. The "plasticity" inherent within these cell types and exposed by the respective actions of these CSFs is indicated in Figure 3. NF-κB and AP-1 were implicated in this "plasticity"[35]. We also found in this mouse system that endogenous type I interferon, even in the absence of an additional stimulus such as LPS, can make a very significant contribution to the gene expression profiles resulting from CSF action[36]; this result highlights the potential contribution of endogenous cytokines to the macrophage heterogeneity observed, at least in vitro.

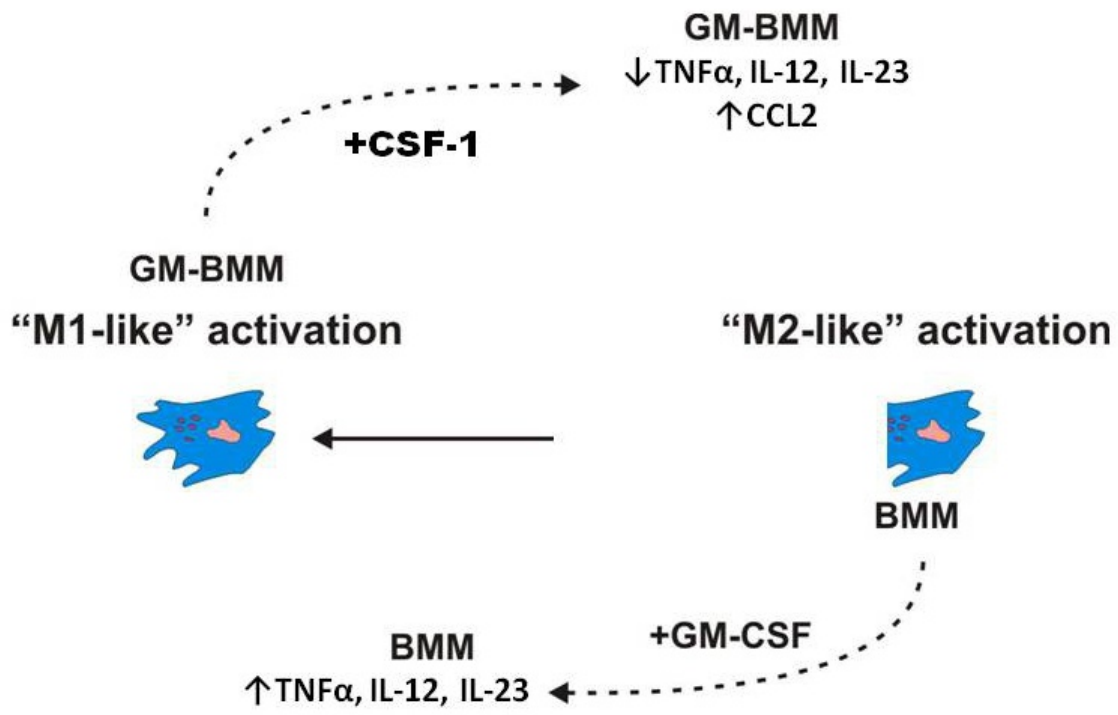

Figure 3. Macrophage "plasticity" in response to GM-CSF and CSF-1
BMM in the presence of GM-CSF can adopt features of GM-BMM ("M1-like") while, conversely, GM-BMM exposed to CSF-1 can resemble BMM ("M2-like"), as judged by the gene expression of certain cytokines[35].

The approach in my laboratory has been to view CSF-1-exposed monocytes/macrophages as being representative of a "default state" from which additional responses have to be referenced and to which the host tries to revert[20,35,37]. As a result, we suggested that consideration be given to including CSF-1 in macrophage cultures to provide a baseline. Based in part on the likelihood that tissue macrophages will be exposed normally to CSF-1 and on the observations that in some situations GM-CSF and CSF-1 can have competing effects, I proposed at the level of the macrophage that inflammation is a state of "CSF-1 resistance", i.e. the homeostatic functions of CSF-1 at the cellular level need to be overcome before proinflammatory changes can occur by analogy with insulin resistance in diabetes/obesity wherein insulin signaling can be compromised[20]. This concept is illustrated in Figure 4.

Any discussion on CSF-1 biology must now take into account the discovery of an additional ligand, IL-34, for the CSF-1 receptor[38]. In other words, we cannot, as in the past, assume that blockade of CSF-1 receptor activity will be equivalent to neutralizing only CSF-1 activity, i.e. informing us necessarily about CSF-1 biology.

Questions and Future Directions

The attempts to characterize macrophage "activation" states as M1 and M2 are well worthwhile provided the limitations are realized. Obviously, if confined to responses to agents, such as interferon γ ± LPS, IL-4 or IL-13, this categorization cannot possibly describe adequately the properties of macrophage populations in response to infection or every

other stimulus that these cells are likely to be exposed to in vivo. What really matters is what the macrophages are doing or producing at a particular point in time in a particular lesion or during a particular response. Therefore what is needed is more information from macrophages isolated locally and over time from affected tissues. With this information at hand specific methods need to be developed and employed to manipulate their properties to suppress unwanted actions and/or promote beneficial ones. However, it was mentioned above that it is proving difficult to deplete specifically macrophage populations and their gene products by transgenic technology[25]. In this context, our current approach is to endeavour to determine what effects neutralization of GM-CSF and CSF-1 by monoclonal antibody have on pathology and the macrophages associated with such pathology, as a way of defining the contributions of macrophage populations. Whether the macrophages under the influence of these CSFs are deemed to be "M1-" or "M2-like" is being assessed without too much concern as to whether this is the case or not.

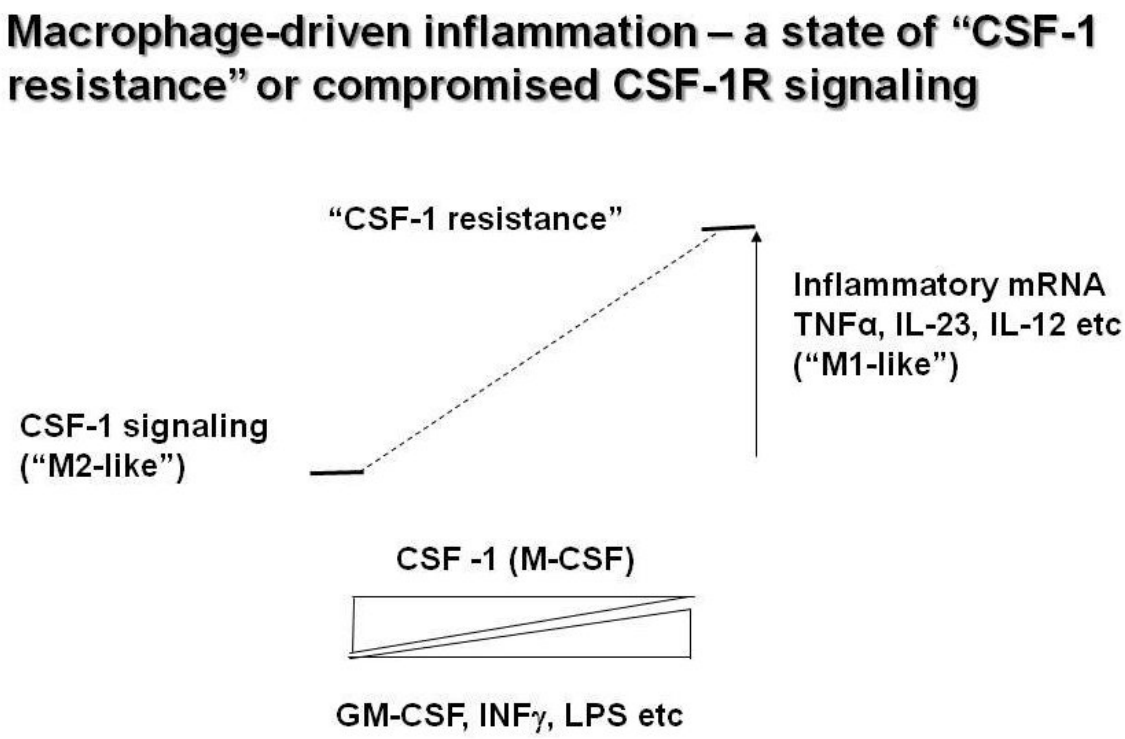

Figure 4. Macrophage-driven inflammation - a state of "CSF-1 resistance" or compromised CSF-1 receptor signaling

It is proposed that many macrophage populations in various tissues can be exposed even normally to sufficient tissue-derived CSF-1 to maintain them in a "M2-like" polarized state[26,35], with a relatively compromised ability to produce pro-inflammatory mediators. During inflammation, autoimmunity and/or infection, exposure of macrophages to increasing concentrations of stimuli, such as GM-CSF, interferon-γ and LPS, overcomes this CSF-1-driven polarization by interfering with CSF-1 signaling such that expression of certain pro-inflammatory molecules (illustrated here as TNF, IL-23 and IL-12 mRNA expression) is enhanced leading to a "pro-inflammatory" ("M1-like") state[26,35] — this "pro-inflammatory" polarization would be enhanced if a macrophage is exposed to less CSF-1 but, conversely, be reduced if it sees more. In other words, at least at the level of the macrophage lineage, inflammation can be viewed as a state of "CSF-1 resistance" or compromised CSF-1 receptor signaling. This hypothesis also proposes that when the levels of proinflammatory stimuli wane CSF-1 at the reaction site will assist in the resolution of the lesion by favouring the "M2-like" phenotype. Administered CSF-1 would favour this phenotype.

One problem within the literature for the M1 and M2 classification is that both murine and human

monocyte/ macrophage culture systems are used with little thought as to whether there is overlap is not. One of the difficulties in making such an essential comparison is that cells from different sites are cultured. For example, for convenience in the mouse, bone marrow-derived or peritoneal macrophages are often studied while in the human, blood monocytes are usually the cell type of choice. Even given these difficulties more comparative studies across species in which the same stimuli are assessed would be useful to enable some assessment of any future relevance of the murine data to human pathology.

Another confounding issue in the literature covering macrophage heterogeneity in vitro is the influence of potent endogenous mediators which are often present at rather low levels in macrophage cultures. Our findings referred to above on the influence of endogenous type I interferon on the gene expression profiles of CSF-treated macrophage populations is a salient example[36]. Other examples of such mediators capable of paracrine/autocrine feedback are IL-10 and TNF[39,40]. Whether such feedback has in vivo relevance is uncertain but their influence can complicate seriously the interpretation of the in vitro responses of macrophage populations to stimuli.

With respect to CSF-1- and GM-CSF-dependent biology, a particular issue is to what extent their control of macrophage numbers and/or "activation" is contributing to their role in inflammation/autoimmunity[21]. As mentioned, both can promote macrophage survival – this could help to explain in part the increased numbers of macrophages present at sites of inflammation, such as the rheumatoid synovium[21]; however, it is also possible that they can promote monopoiesis and monocyte mobilization from bone marrow or spleen, either directly or indirectly via downstream mediators, such as chemokines. We and others have recently found evidence that endogenous GM-CSF can enhance blood monocyte numbers during inflammation/autoimmunity[41,42]. CSF-1 receptor neutralization can also control the levels of a mature monocyte subpopulation in the steady state[43].

For CSF-1 biology there is a paradox which requires explanation. CSF-1 neutralization or that of its receptor can reduce the inflammatory response[20,44-50] and its administration can enhance such a response[51]. However, in some cases, CSF-1 administration can potentiate the repair of an injured tissue[52] and, as discussed above, it can reduce proinflammatory cytokine production in macrophage populations in vitro[31,35]. In order to help rationalize such observations it should be borne in mind that administration of excess of a ligand systemically may not give the (perhaps expected) opposite result to neutralizing the ligand locally in an inflamed tissue. Perhaps blocking CSF-1 action can reduce macrophage numbers at a site of inflammation which paradoxically has the same end result as its attempts to resolve the lesion.

In summary, it is advocated from the discussion above that more consideration be given to the contributions of CSF-1 and GM-CSF to the diverse features of tissue macrophages present in the steady state and during host responses to an altered milieu.

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