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Regulatory macrophages: setting the threshold for therapy

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Abstract

Macrophages exhibit remarkable plasticity and can change their phenotype in response to different environmental cues. They can become activated to kill intracellular microbes or they can assume regulatory properties to modulate immune responses. Regulatory macrophages are fundamentally different from classically, and we propose from non-classically activated macrophages; they arise in response to different stimuli and perform different physiological functions. They are likely to express unique biochemical markers that could be exploited to identify and potentially target these macrophage subsets in tissue. Furthermore, inducers of regulatory macrophages may have the potential to be used as anti-inflammatory therapeutics. Therefore, a better understanding of the various macrophage phenotypes may pave the way for new therapies that are directed at modulating macrophage functions or manipulating individual macrophage subsets.

Introduction

Over the past 50 years, a large body of work has convincingly demonstrated that classically activated macrophages and the mediators they produce are important components of host defense. However, as these cells have come under scrutiny, it has become apparent that the mediators produced by these activated macrophages could also contribute to autoimmune pathologies. Therefore, the activation status of these cells must be carefully regulated. Over the last two decades several groups have begun to identify and characterize macrophages with phenotypes that are distinct from classically activated macrophages. These alternatively activated cells exhibit a broad range of immunoregulatory activities and arise in response to a variety of different stimuli [1]. In many instances these cells not only fail to produce inflammatory mediators, but they also modulate immune responses through the secretion of immunosuppressive/anti-inflammatory cytokines. Given the possibility that these cells may play important roles in preventing autoimmune pathologies, we and several other groups have begun to ask questions about how macrophages make decisions that govern their phenotype and function. We considered these questions to be important, because we reasoned that studies pertaining to the regulation of macrophage activation may have the potential to lead to the development of macrophage-directed therapeutics that can ameliorate inflammatory pathologies.

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Our interest in regulatory macrophages emerged from an unexpected observation that we made several years ago i.e. that stimulating macrophages in the presence of high-density immune complexes resulted in a unique cytokine response that was markedly different from that of classically activated macrophages [2]. In essence, we observed that a combination of two stimuli, such as a TLR ligand plus immune complexes, resulted in macrophages that produce high levels of the anti-inflammatory cytokine IL-10 and little to no detectable IL-12 [1,3]. This was in stark contrast to classically activated macrophages i.e. macrophages stimulated with TLR ligand alone, that produce high levels of IL-12 but relatively low levels of IL-10.

Subsequent to our observations with immune complex-mediated alterations in macrophage physiology, we became aware of the work of several groups using a variety of different co-stimuli to modulate macrophage cytokine responses. One of the first macrophage modulators to be extensively studied was adenosine. This ubiquitous small molecule can cause profound alterations of macrophage physiology resulting in dramatic changes in cytokine production following stimulation [4]. Adenosine-treated macrophages exhibit a profound defect in the production of many inflammatory cytokines. Several other modulators of macrophage function have subsequently been identified, including apoptotic cells [5,6], cAMP analogues [7], prostaglandin E2 [7], TGF- β [8], and even IL-10 itself [9,10]. These “reprogramming” co-stimuli share the common characteristic of being relatively inefficient at inducing cytokine production from macrophages on their own; however, when combined with an inflammatory stimulus, such as a TLR agonist, they all modulate macrophage cytokine production, often decreasing inflammatory cytokine production and inducing the production of anti-inflammatory cytokines, such as IL-10, thereby generating what we will refer to in this Viewpoint as regulatory macrophages. These *in vitro* generated regulatory macrophages appear to share many of the characteristics of regulatory macrophages that have been identified *in vivo*, including embryonic macrophages and macrophages isolated from tumors or placentae [11].

Regulatory macrophages

We propose that one of the normal functions of regulatory macrophages is to modulate inflammatory immune responses, and thereby limit tissue damage. It should be emphasized, however, that while the role of regulatory macrophages appears to be to limit tissue damage, these cells do not appear to actively participate in wound healing. In this respect, they are frequently confused with the so-called “alternatively activated” macrophages that were originally identified by Siamon Gordon and his co-workers in the early 1990s [12]. These alternatively activated macrophages are induced by exposure to the T_H2 cytokine IL-4, and their physiology is described in detail in this volume and in numerous excellent reviews [13,14,15]. One of the characteristics of alternatively activated macrophages is the induction of arginase, which confers upon these cells the potential to convert arginine into polyamines and hydroxyproline. This property is not shared by regulatory macrophages, which do not contribute directly to the production of the extracellular matrix. Some consider all non-classically activated macrophages to fit into the broad category of so-called M2 macrophages, but there appear to be several characteristics that are unique to IL-4-treated alternatively-activated macrophages [16], that are not shared with many of the regulatory

macrophages described above. Regulatory macrophages do not express YM1 or RELM α , two markers that are reliably expressed on murine alternatively activated macrophages, and signaling in regulatory macrophages does not depend on STAT6, as it does in alternatively activated macrophages. Furthermore, the chemokine receptor repertoire differs between the two populations [17]. Thus, we consider regulatory macrophages to be a relatively broad category of macrophages whose main physiological role is to dampen inflammatory immune responses and prevent the immunopathology associated with prolonged classical macrophage activation. In this regard they are certainly distinct (if not opposite) from classically activated macrophages, and they are also quite different from macrophages treated with the T_H2 cytokines, IL-4 or IL-13 [18], the so-called alternatively-activated macrophages.

Most of the data pertaining to macrophage heterogeneity was initially obtained with mouse macrophages, but it is clear that human macrophages exhibit similar plasticity and can respond to IL-4 treatment by expressing higher levels of markers associated with alternatively-activated macrophages, such as CD206 and CCL18[19]. Human macrophages can also respond to most of the so-called “reprogramming” signals that give rise to regulatory macrophages, and dampen inflammatory responses.

IL-10 gene regulation

We consider the hallmark of regulatory macrophages to be the production of high levels of IL-10. Therefore, to better understand the physiology of these cells, we and others have begun to examine the regulation of IL-10 production. In T cells, Saraiva and O’Garra proposed a “strength of signal” hypothesis to explain IL-10 production [20]. We propose that a similar level of regulation may also explain IL-10 production in macrophages. In the simplest terms, activating macrophages with a (single) stimulus, such as a TLR agonist, results in the production of many inflammatory cytokines, but this stimulation does not appear to be sufficient to produce high levels of IL-10. The addition of the second “reprogramming” stimulus provides sufficient signaling strength to induce IL-10 production. Several of the reprogramming stimuli that we have examined activate ERK, a mitogen-activated protein kinase (MAPK) [21]. Such activation of ERK results in the phosphorylation of histones associated with the *il-10* promoter, and this makes the promoter more accessible to the transcription factors that induce *il-10* gene expression [22]. Thus, inflammatory signaling (alone) results in the activation of transcription factors, but these factors cannot efficiently access the *il-10* promoter because it is tightly packed in chromatin. The reprogramming signal activates ERK, which ultimately results in a more accessible promoter. These observations would predict that all stimuli that result in ERK activation would enable IL-10 production from macrophages, and this is certainly true of some growth factors. For example, M-CSF activates ERK and in our hands induces a macrophage population that produces high levels of IL-10 when stimulated. However, some transient ERK activators fail to induce IL-10, suggesting that there are aspects of this regulation that we do not, as yet, fully understand.

Immune cross-regulation

Macrophages are anatomically positioned to interact with lymphocytes, and therefore it is not surprising that these cells can exert dramatic influences on each other. Regulatory macrophages have been shown *in vitro* to be efficient antigen-presenting cells that induce highly polarized antigen-specific T-cell responses that are dominated by the production of T_H2 cytokines [3]. Conversely, the interaction of resident macrophages with regulatory T cells (Tregs) induces these macrophages to assume the characteristics of regulatory macrophages [19]. Similarly, the interaction of macrophages with B1 B cells results in the formation of a regulatory macrophage population that produces reduced levels of inflammatory cytokines and increased levels of IL-10 [10]. Thus, macrophages can influence the character of an adaptive immune response, and they can also change their physiology as a result of an on-going immune response.

Targeting therapies to macrophage subtypes

We propose that there are two general strategies to target therapies to macrophages. One is to *deplete* specific macrophage subtypes and the other is to *induce* the local development of a given macrophage subtype. The dramatic change in the physiology of macrophages when converting to regulatory macrophages would predict that therapeutics directed toward the induction of these cells would have the potential to affect immune responses. To test this, we and others have undertaken studies in animal models of inflammation to address the potential of regulatory macrophages in dampening inflammatory responses. One model of inflammation in which the activity of regulatory macrophages was examined is the lethal endotoxemia model. In this model, C57BL/6 mice were given a bolus of LPS intraperitoneally (i.p.). This induces a “cytokine storm” to which the mice typically succumb within 24–48 hours. It is quite difficult to reverse lethality in this model; however, if as little as one million regulatory macrophages are injected i.p. prior to the injection of endotoxin, then the outcome for these mice is dramatically changed. The immunomodulatory activity of these regulatory macrophages not only rescues the mice from lethal endotoxemia, but also alleviates nearly all the symptoms associated with endotoxin administration [23]. Thus, in this acute model of inflammation, a relatively small number of regulatory macrophages inhibit the pathology associated with LPS administration.

We have also begun to examine the role for regulatory macrophages in a more chronic model of inflammation, experimental autoimmune encephalomyelitis (EAE). EAE is considered to be a mouse model for human multiple sclerosis (MS). In human MS, immune cells attack the myelin surrounding nervous tissue, interfering with nerve conduction and frequently resulting in partial or complete paralysis. In mice a similar condition is induced by immunizing mice with peptides derived from myelin-associated proteins in the presence of a strong adjuvant. These mice progressively develop paralysis of their extremities. The administration to these mice of a reagent that induces the development of regulatory macrophages can partially reverse paralysis. Mice begin walking normally as little as 24 hours after the induction of regulatory macrophages (unpublished).

Thus, regulatory macrophages can diminish inflammation and reduce pathology in two distinct models of inflammation; the LPS model involves an acute innate response to bacterial endotoxin, whereas the EAE model involves a progressive antigen-specific T-cell mediated autoimmunity. In both cases, regulatory macrophages can prevent or even reverse autoimmune pathology. Therefore, we propose that reagents that induce regulatory macrophages may have the potential to be beneficial in treating inflammatory diseases. A note of caution should be struck, however, regarding the potential susceptibility of non-classically activated macrophages to infections by intracellular pathogens. The recent occurrences of opportunistic infections in patients treated with immunosuppressive therapeutics, such as anti-cytokine antibodies [24], reminds us that limiting immune responses can come at a price of susceptibility to infections. Importantly in this context, regulatory macrophages continue to produce NO when appropriately stimulated [18], suggesting that they may retain some capacity to limit intracellular infections, despite the fact that this is not their primary physiological role.

Another macrophage-based therapeutic approach would be the depletion of macrophage subsets. This approach depends on the identification of reliable and specific biomarkers for macrophage subpopulations, which would provide an opportunity to deplete these cells from tissues. This may enhance immunity in those tissue compartments where regulatory macrophages predominate. It has been proposed, for example, that immunosuppressive macrophages abound in advanced tumors [25], and this may be a setting where macrophage subset depletion could contribute to rejection following tumor ablation. Given the heterogeneity of macrophages and the likelihood that classical and regulatory macrophages may have opposing functions, strategies to deplete all macrophages may remove beneficial, as well as the detrimental, macrophages. For this reason, we consider subset depletion to be potentially more worthwhile.

The identification of different macrophage subsets may also provide clinicians with valuable tools to diagnose disease, based on the macrophage phenotypes that arise in response to the disease. For these reasons, several groups have performed microarray analysis on various monocyte and macrophage subsets to begin to identify transcripts that are expressed or silenced as macrophages undergo phenotypic changes in response to stimulation. Several candidate transcripts that are overexpressed by regulatory macrophages have been identified [18], but to date good stable convenient surface markers that are specific for regulatory macrophages have not been identified. CCL1 has been identified as a product that is specifically produced by monocytes stimulated in the presence of Fc γ R engagement [26]. This work therefore identifies a potential secreted biomarker of regulatory macrophages. It also illustrates the utility of examining changes in chemokines and chemokine receptor expression as a powerful way of identifying changes in immune cells as they differentiate. The induction of CCL1 in this subpopulation of regulatory macrophages would predict that they would specifically recruit T_H2 cells, which preferentially express CCR8, the receptor for CCL1. Thus, regulatory macrophages can potentially promote T_H2 responses in two ways: they can preferentially recruit T_H2 T cells and, as APCs, they can bias T cell responses toward IL-4 secretion [3].

Conclusions

The macrophage's ability to react to different stimuli with a wide range of diverse responses has made this cell an important regulator of immune responses. Classically activated macrophages have long been known to play an important role in host defense. Regulatory macrophages, which we propose are distinct from both classically and non-classically activated macrophages, can play an important role in dampening immune responses and preventing autoimmune pathology. Understanding the mechanisms whereby each of the macrophage subsets are induced to assume these different roles may provide new opportunities to therapeutically manipulate immune responses to the benefit of the host.

Acknowledgments

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Figure 1.
The three major macrophage subtypes and some of the biochemical and physiological properties of each.