

## Critical Role for Inflammatory Macrophages in Driving Antigen-dependent Th17 Cell Responses?

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### Abstract

Macrophages are heterogeneous cells with diverse phenotypes and sometimes opposing functions. These activities are dictated by activating stimuli in their microenvironment. For example, it is well described how CD4<sup>+</sup> T helper (Th) cell-derived cytokines result in different macrophage-activation states. However, much less is known on how differentially-activated macrophages, presenting antigen, can drive the major types of CD4<sup>+</sup> Th subpopulations, especially in human systems. Many studies have focussed on dendritic cells as the major antigen-presenting cell shaping T cell responses or on murine macrophage-secreted cytokines in the presence of mitogenic-stimuli, such as CD3/CD28, to induce Th polarization. Recent literature is, however, providing evidence that activated antigen-presenting macrophages can be as efficient as dendritic cells in polarising Th cells, especially Th17, and whilst both these cell types co-exist within inflamed tissue, macrophages are more abundant. The bias towards polarization of particular T cell subsets is strongly dependent on the activation state of macrophages. The concept of targeting macrophages to downregulate inflammatory responses may therefore have further reaching consequences by also abrogating pathogenic Th cells in autoimmune or inflammatory diseases.

### Commentary

Macrophages have a key role in regulating immune responses such as inflammation, wound healing, and maintenance of tissue homeostasis [1]. Macrophages are characterized by remarkable plasticity and diversity [2]. They rapidly respond to microenvironmental signals for example cytokines and acquire distinct phenotypes and functions. Key phenotypes have been characterized *in vitro*, as M1-activated proinflammatory/microbicidal macrophages induced by microbial products and IFN $\gamma$ , and, M2 anti-inflammatory/tissue healing/immunomodulatory macrophages induced by IL-4/IL-13/IL-10 [1,2]. These are, however, extremes on a continuum of the phenotypes found *in vivo*. The nature and balance of macrophage activation-states is important and their dysregulation has been linked to inflammatory diseases, autoimmunity or, at the other extreme, cancer and chronic wounds. Macrophages are professional antigen-presenting cells (APCs) and recent evidence suggests the heterogeneous nature of macrophages impacts their role in CD4<sup>+</sup> T-cell polarization [3-6].

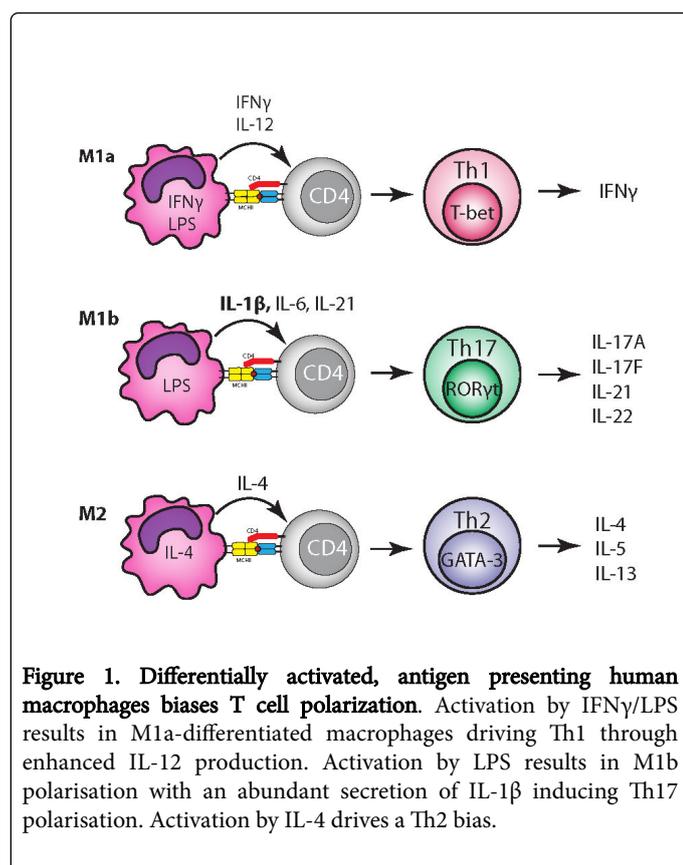
CD4<sup>+</sup> T helper (Th) cells orchestrate adaptive immune responses. Soluble and cell-contact dependent signals from APCs result in T cell differentiation into distinct functional phenotypes including Th1, Th2, Th17, and regulatory T (Treg) cells characterised by cytokine output and transcription factor expression [7]. Human M1 and M2 subsets differentially produce cytokines modulating T-cell polarization [8], however, their role in antigen presentation and downstream CD4<sup>+</sup> T cell polarization remains poorly studied. Dendritic cells are still commonly viewed as the most potent APC, especially driving naïve T cells to Th1 [9]. Macrophages, however, express MHC class-II and costimulatory molecules and efficiently present antigen to T cells *in vitro*. A greater abundance of macrophages is present within inflamed or immune-activated tissue such as the gut, where numbers of

macrophages but not dendritic cells correlate with Th17 activity [10]. New evidence suggests that they are at least as capable of presenting antigen and driving Th17 cell responses as dendritic cells and this is highly dependent on the macrophage activation status and the cytokines they produce [3]. A recent hypothesis supports this notion, suggesting that APCs in lymphoid tissue act in concert with tissue-resident APC to shape T cell responses [11]. Thus, in diseased tissue where macrophages outnumber dendritic cells by orders of magnitude, local antigen presentation and CD4<sup>+</sup> T cell polarization by macrophages may be significant.

Evidence for the ability of macrophages to drive Th cell responses has recently been shown by several independent groups. CD4<sup>+</sup> T cells co-cultured *in vitro* with activated macrophages and specific antigen were skewed towards Th1 or Th2 depending on the macrophage stimuli [4]. Macrophages from spleen red pulp and from the gut lamina propria favored the generation of Foxp3<sup>+</sup> Treg cells [5,6]. Strikingly, dendritic cells in gut mucosa were dispensable for bacterial antigen-specific Th17 production and intestinal macrophages presenting antigen were essential for segmented filamentous bacteria-specific Th17 responses [12,13]. Similar trends have been shown in the human system, for example LPS or LPS/IFN- $\gamma$ -activated, antigen-loaded human macrophages skewed Th cells toward Th17 or Th1, respectively [3]. Taken together, these investigations highlight the essential role of macrophages in CD4<sup>+</sup> T cell polarization in which activation state and antigenic stimuli are critical.

The question arises as to how activated macrophage as APCs control T cell polarization. In a direct comparison between differentially activated human macrophages presenting antigen to autologous T cells, it was shown that the cytokine output profile is critical [3]. This study had the advantage in that the interactions between antigen-loaded activated macrophages and autologous T cells more closely recapitulate the physiological responses *in vivo*, in contrast to previous

studies that relied on adding cytokines together with mitogenic stimuli to induce T cell polarization. Antigen-loaded IFN $\gamma$ /LPS-activated macrophages were most efficient at driving Th1, associated with their upregulated production of IL-12, whilst LPS-activation induced the highest levels of IL-1 $\beta$ , that is known to be an important driver in human Th17 polarization [3]. By contrast, IL-4-activated macrophages did not result in significant polarization of either Th1 or Th17, or secretion of IL-12/IL-1 $\beta$ , in line with their recognised anti-inflammatory/tissue reparative nature (Figure 1).



In the same study, LPS-activated macrophages were at least as potent at inducing Th17 as dendritic cells from the same human donor, supporting the notion that macrophages make an important contribution to driving antigen-dependent Th17 responses within inflamed tissue. Th17 and Th1 polarization again correlated strongly with the output of the polarizing cytokines IL-1 $\beta$  and IL-12 by activated macrophages and dendritic cells, respectively. Likewise, human monocytes were reported to be more potent than dendritic cells at inducing Th17 expansion when activated by anti-CD3 and monocyte-derived factors, with IL-1 $\beta$  again identified as a key polarising cytokine [14]. A limitation of these studies was that macrophage polarising stimuli were restricted to LPS, IFN $\gamma$ /LPS, IL-10 and IL-4 that showed significant differences in their ability to secrete factors driving Th17. However, *in vivo*, the activating stimuli at a particular point in time are likely to be more complex with, a multitude of different ligands causing activation of macrophages in response to infection. It will therefore be important in future *in vitro* and *in vivo* studies, to assess how combinations of bacterial components, TLR ligands and other macrophage activating stimuli compare in their capacity to polarize Th cells. There are, however, precedents for macrophages activated *in vivo* driving Th17. One example is during

fungal infection where antigen presentation and macrophage activation through engagement of multiple receptors (e.g. TLR4, dectin1, mannose receptor) has been shown to result in efficient Th17 polarization to eliminate infection [15]. While much of the studies are limited to monocytes/macrophages derived from blood from healthy volunteers, tissue-derived macrophages from different sources may also have different biology. Further studies on macrophages from other tissues and from disease settings are necessary to consolidate results *in vitro*. Interestingly, human tumor-associated macrophages were the prominent antigen presenting cells in tumours and most efficient at inducing Th17 cells, potentially by high IL-1 $\beta$  secretion [16,17]. Moreover, a unique macrophage subset distinct from M1/M2 macrophages displaying a characteristic IL-1 $\beta$ <sup>high</sup> phenotype, supported the expansion of Th17. An additional study pointing towards the importance of IL-1 $\beta$  as a Th17 driver *in vivo* demonstrated that IL-1 $\beta$  but not IL-6 was required for the induction of Th17 cells by intestinal macrophages [18].

Thus, activated macrophages presenting antigen at sites of inflammation may play a greater role in driving T cell responses than initially anticipated. T cell polarization is dependent on their pre-activation status as well as the local antigenic stimuli and importantly the macrophage-derived cytokines. For human cells, enhanced IL-1 $\beta$  is strongly associated with Th17 differentiation while IL-12 is inhibitory, at least *in vitro*. It is notable that activated human macrophages show phenotypic and functional differences as compared to activated macrophages in the murine system e.g. iNOS expression [19]. While the focus here is on human studies, differences in murine macrophage activation and the resultant ability to drive Th polarization is also of interest, especially given that generation of murine Th17 relies more on IL-6 and TGF- $\beta$  than on IL-1. The predominance of Th17 at inflammatory sites in human studies might be explained by the presence of activated macrophages providing an essential Th17 polarizing environment. This highlights new potential avenues to target Th1 and Th17 cell driven inflammatory and autoimmune diseases through regulating macrophage phenotypes, or to boost activity in cancer where Th1 cell skewing would benefit. Knockdown of suppressor of cytokine signalling (SOCS3) expression in macrophages, for example, effectively decreases their IL-1 $\beta$  secretion and ability of antigen-loaded macrophages to drive Th1 and Th17 responses [20]. Further work is, however, needed in order to determine the efficacy of macrophage-targeting therapies to regulate aberrant T cell responses.

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