The chemokine system in diverse forms of macrophage activation and polarization

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Plasticity and functional polarization are hallmarks of the mononuclear phagocyte system. Here we review emerging key properties of different forms of macrophage activation and polarization (M1, M2a, M2b, M2c), which represent extremes of a continuum. In particular, recent evidence suggests that differential modulation of the chemokine system integrates polarized macrophages in pathways of resistance to, or promotion of, microbial pathogens and tumors, or immunoregulation, tissue repair and remodeling.

Chemokines are a superfamily of small proteins with a crucial role in immune and inflammatory reactions [1,2] (Box 1). Induction of leukocyte migration is the eponymous function of chemokines but they also affect angiogenesis, collagen production and the proliferation of haematopoietic precursors. There is compelling evidence that some chemokines are also involved in polarized immune responses [2] (Box 2). Chemokine receptors are differentially expressed on polarized Th cells. Typically, CXCR3 and CCR5 are preferentially expressed on polarized Th1 cells, whereas CCR3, CCR4 and CCR8 have been associated with the Th2 phenotype. CCR3 ligands also attract eosinophils and basophils, which are crucial for polarized type II responses (Box 2).

Cells belonging to the monocye–macrophage lineage have long been recognized to be heterogeneous. Because lineage-defined subsets have not been identified to date, macrophage heterogeneity is likely to reflect the plasticity and versatility of these cells in response to exposure to microenvironmental signals. Cytokines and microbial products profoundly and differentially affect the function of mononuclear phagocytes. It has now been recognized that cells belonging to the myelomonocytic differentiation pathway, including macrophages and dendritic cells (DCs), have a key role in polarized innate and adaptive responses. They act by promoting the orientation of adaptive responses in a type I or type II direction, as well as by expressing specialized and polarized effector functions [3–6].

Here, we will concisely review the key properties of the functional polarization of mononuclear phagocytes. In particular, we will focus on chemokines as a key component of the plasticity of the differentiation and activation of mononuclear phagocytes.

Confusing nomenclature of polarized mononuclear phagocytes: a proposal

Interferon-γ (IFN-γ), alone or in concert with microbial products [e.g. lipopolysaccharide (LPS)] or cytokines [e.g. tumor necrosis factor (TNF)], activates macrophages [7]. Classical macrophage activation is characterized by: high capacity to present antigen; high interleukin-12 (IL-12) and IL-23 production [8] and consequent activation of a polarized type I response; and high production of toxic intermediates [nitric oxide (NO), reactive oxygen intermediates (ROI)]. Many have referred to these cells as M1 macrophages, mirroring the Th1 nomenclature. IL-4 and IL-13 were subsequently found to be more than simple inhibitors of macrophage activation, in that they induce a distinct activation program, referred to as ‘alternative activation’ [3,9]. The term ‘alternatively activated macrophage’ has also been applied in a loose way to mononuclear phagocytes exposed to IL-10, glucocorticoid or secosteroid (vitamin D3) hormones [5]. These cells are in many respects ‘deactivated’, but in analogy to IL-4 and IL-13, these signals are more than simple deactivators (see later). Macrophages exposed to immune complexes (IC) and LPS are characterized by an IL-10high and IL-12low phenotype and promote type II responses; they have been called type II activated macrophages [4]. Finally, human monocytes differentiated with granulocyte–macrophage colony stimulating factor (GM-CSF) or M-CSF have M1 and M2 properties, respectively, and have been referred to as Mo1 and Mo2 [8].

Here, and in previous publications [6], we have used M1 and M2 to refer to the two extremes of a spectrum of possible forms of macrophage activation. In particular, we
Box 1. Essentials of the chemokine system in leukocyte recruitment

Chemokines are small, secreted, chemoattractant proteins with a conserved secondary structure with a flexible N-terminal segment followed by three antiparallel β-sheets and a C-terminal α-helix. According to the relative position of cysteine residues, members of this large family have been classified into four subfamilies (CXCL, CC, C and CX3C). Chemokines recruit different leukocyte populations by acting at seven transmembrane domain G-protein coupled receptors (Figure I). At present, six receptors for CXC (CXCR1–6), ten receptors for CC (CCR1–10), one for C (CXCR1), and one for CX3C (CX3CR1) chemokines have been identified [69]. In addition, promiscuous non-signaling decoy receptors have been identified [47,49,63].

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**Figure 1.** Leukocyte expression and ligand specificity of chemokine receptors at a glance. Receptors, selected cognate ligands and predominant receptor repertoires in different leukocyte populations are listed. The selected ligands are identified with one old acronym and with the new nomenclature, in which the first part of the name identifies the family and L stands for ‘ligand’ followed by a progressive number. Red identifies predominantly ‘inflammatory’ or ‘inducible’ chemokines, green ‘homeostatic’ agonists, yellow molecules belonging to both realms. Chemokine acronyms are shown as: BCA, B cell activating chemokine; BRAK, breast and kidney chemokine; CTACK, cutaneous T-cell attracting chemokine; ELC, Epstein–Barr virus-induced receptor ligand chemokine; ENA-78, epithelial cell-derived neutrophil-activating factor (78 amino acids); GCP, granulocyte chemotactic protein; GRO, growth-related oncogene; HCC, homeostatic CC chemokine; IP, IFN-inducible protein; I-TAC, IFN-inducible T-cell chemoattractant; LXCCL4, lymphoid tissue chemokine; MIP, macrophage inflammatory protein; MPIF, myeloid progenitor inhibitory factor; TECK, thymus expressed chemokine. Abbreviations: PMN, neutrophils; Eo, eosinophils; Ba, basophils; MC, mast cells; Mo, monocytes; Ma, macrophages; iDCs, immature dendritic cells; mDCs, mature DCs; T naive, naïve T cells; T act, activated T cells; T skin, skin-homing T cells; T muc, mucosal-homing T cells; Treg, regulatory T cells.

Propose and use M2 as a generic name for the various forms (‘alternatively activated’ sensu stricto; type II; Mo2; M2) of macrophage activation other than the classic M1. The M1/M2 nomenclature has the obvious advantage of reflecting the Th1/Th2 dichotomy, already extended to other cell populations [e.g. Tc1, Tc2; natural killer (NK1), NK2]. The generic use of M2 to define macrophage activation other than the classic M1 is justified based on the sharing of selected functional properties (e.g. low IL-12) and their general involvement in type II responses, immunoregulation and tissue remodeling. We propose to refer to the three well defined forms of M2 as: M2a (where ‘a’ also stands for alternative), induced by IL-4 or IL-13; M2b, induced by exposure to IC and agonists of Toll-like receptors (TLRs) or IL-1R; and M2c, induced by IL-10 and glucocorticoid hormones (Figure 1).

Functional properties of polarized-mononuclear phagocytes

Figures 1 and 2 summarize selected properties of polarized macrophage populations. These properties are similar for mouse and human cells, unless specified. Polarized macrophages differ in terms of receptor expression, cytokine production, effector function and, as discussed later, chemokine repertoires. M1 macrophages exposed to the classic activation signals IFN-γ and LPS express opsonic receptors [e.g. FcγRIII (CD16)], whereas M2 macrophages are characterized by abundant levels of non-opsonic receptors (e.g. the mannose receptor). Arginine metabolism is characterized by high levels of inducible nitric oxide synthase (iNOS; NOS2) in M1 macrophages, whereas the arginine pathway predominates in M2a and M2c polarized macrophages, which results in generation of ornithine and polyamines. This is not the case for M2b macrophages exposed to IC and LPS [4]. IFN-γ increases the expression of TLR4 and of key components of the MyD88 signaling pathway in human cells, whereas IL-10 inhibits it [10]. However, there are important differences between mice and humans in terms of promoters and regulation of TLRs [11].

Differential cytokine production is a key feature of polarized macrophages. The M1 phenotype is typically IL-12high and IL-10low, whereas M2 macrophages are typically IL-10high and IL-12low. Human M1 macrophages also produce high levels of IL-23 [8]. Components of the IL-1 system are differentially regulated in polarized...
Box 2. A simplified view of chemokines in polarized T-cell responses

During type I and type II immune responses, master cytokines regulate chemokine production by stromal and inflammatory cells. Chemokines then support selective recruitment of polarized T cells and specific type I and type II effector cells expressing distinct panels of chemokines receptors (Figure I). Polarized type I and type II T cells express differential chemokine receptors. Typically, the CC chemokine receptors CCR3, CCR4 and CCR8 have been associated with a type II phenotype, whereas functional CXCR3 and CCR5 are preferentially expressed on polarized type I T cells. CCR3 ligands also attract eosinophils and basophils, crucial for polarized type II responses. Although differentially expressed, chemokine receptors are not markers for polarized T cells, in that there is no absolute association between chemokine receptor expression and cytokine repertoire of polarized T-cell populations. For instance, CCR4, expressed at much higher levels in polarized type II cells, is also expressed in non-polarized T-cell populations, and it is induced in type I cells following activation.

Figure I. Chemokines and chemokine receptors in polarizing circuitry. Abbreviations: DCs, dendritic cells; Ba, basophils; Eo, eosinophils; MC, mast cells; Treg, regulatory T cells; IC, immune complexes.

macrophage populations. IL-4, IL-13 and glucocorticoid hormones increase expression of the decoy receptor IL-1RII, whereas IFN-γ and LPS inhibit it and upregulate the signaling IL-1RI, and IL-1R accessory protein [6]. IL-4 and IL-13 induce IL-1 receptor antagonist (IL-1ra) production and inhibit IL-1 [12]. Therefore, pro- and anti-inflammatory components of the IL-1 system are coordinately regulated by signals that polarize macrophages in an M1 or M2 direction (Figure 2).

M2 cells are generally characterized by low production of proinflammatory cytokines (IL-1, TNF and IL-6). However, macrophages exposed to IC and LPS (M2b) (Figure 1) are an exception, in that they retain high levels of inflammatory cytokine production with concomitant high IL-10 and low IL-12 [4]. In spite of their high production of inflammatory cytokines and toxic molecules, M2b cells protect mice against LPS toxicity [4,13]. Moreover, they promote Th2 differentiation and humoral antibody production.

Transcriptional profiling has added a new dimension to the characterization of different forms of macrophage activation [14,15], although a systematic comparison of the various populations shown in Figure 1 is missing. In an ongoing, comprehensive effort conducted in our group on human monocytes and macrophages, whole genome transcriptional profiling yielded data compatible with the general scheme outlined in Figure 1[16–18]. In particular, it was found that IL-10 activates a transcriptional program much more restricted than LPS, IL-13 or IC [17]. In monocyte-derived DCs, when combined with LPS, the effect of IL-10 was not limited to inhibition of proinflammatory molecules, such as IL-12 and inflammatory cytokines [17]. IL-10 alone or in concert with LPS activates four distinct transcriptional programs: (i) control and deactivation of immunity and inflammation, including suppression of inflammatory cytokines and induction of signaling lymphocytic activation molecule [SLAM (CD150)]; (ii) tuning of cytokine or G protein-coupled receptor signaling, including superinduction of suppressor of cytokine signaling 3 (SOCS3), regulator of G protein signalling-16 (RGS-16) and phosphatases, and...
suppression of the γ isoform of phosphatidylinositol 3-kinase (PI3-Kγ); (iii) remodeling of the extracellular matrix, including induction of the long pentraxin PTX3 and versican; (iv) B-cell function and lymphoid tissue neogenesis, including induction of IL-7 and CXCL14. Thus, IL-10 is more than a mere deactivator of macrophage and DC function (Figure 1).

Mononuclear phagocytes are exposed to a multiplicity of signals in vivo with different temporal patterns. Therefore, polarization of macrophage function should be viewed as an operationally useful, simplified conceptual framework, describing a continuum of diverse functional states. With this general caveat, available information suggests that classically activated M1 macrophages are potent effector cells integrated in Th1 responses, which kill microorganisms and tumor cells and produce copious amounts of proinflammatory cytokines (Figure 1). By contrast, M2 macrophages tune inflammatory responses and adaptive type 1 immunity, scavenge debris and promote angiogenesis, tissue remodeling and repair. Different versions of the M2 phenotype, M2a, M2b and M2c, accentuate these general properties. Integration with, and promotion of, type II responses prevail for IL-4- or IL-13-stimulated M2a macrophages, whereas suppression and regulation of inflammation and immunity are predominant in IL-10-stimulated M2b cells.

Chemokine receptor repertoire during monocyte–macrophage differentiation

Circulating monocytes are heterogeneous in several respects and recent evidence indicates that the chemokine receptors CX3CR1 and CCR2 are differentially expressed in mouse mononuclear phagocytes [19]. A first subset, mostly overlapping with the CD16+ subpopulation, is characterized by high CX3CR1 expression and low levels of inflammatory chemokine receptors (CCR1 and CCR2), as well as L-selectin. These cells, indicated as ‘resident monocytes’, preferentially home to non-inflamed tissues, where they exhibit a long half-life and serve as precursors for resident tissue macrophages. A second subset, characterized by high CCR2 and low CX3CR1 levels, preferentially homed to inflamed tissues. These cells have been referred to as ‘inflammatory monocytes’ and are involved in immune response triggering [19].

CXCL14 is a highly selective murine monocyte attractant expressed in a variety of tissues, including gut and skin [20]. It might therefore act as a homeostatic attractant, however, its action is amplified by prostaglandin E2, which is produced at sites of inflammation [20].

Recruitment and activation of circulating monocytes is an important process in inflammatory and immune responses and inflammatory chemokines are major players in this process. Freshly isolated monocytes express a wide panel of inflammatory chemokine

Figure 1. Inducers and selected functional properties of different polarized macrophage populations. Macrophages polarize and acquire different functional properties in response to environment-derived signals. Macrophage exposure to IFN-γ and LPS drives M1 polarization, with potentiated cytotoxic and antitumoral properties, whereas M2 macrophages are in general more prone to immunoregulatory and protumoral activities. In particular, M2a (induced by exposure to IL-4 and IL-13) and M2b (induced by combined exposure to immune complexes and TLR or IL-1R agonists) exert immunoregulatory functions and drive type II responses, whereas M2c macrophages (induced by IL-10) are more related to suppression of immune responses and tissue remodeling. Abbreviations: DTH, delayed-type hypersensitivity; IC, immune complexes; IFN-γ, interferon-γ; RNI, reactive nitrogen intermediates; ROI, reactive oxygen intermediates; SLAM, signaling lymphocytic activation molecule; SRs, scavenger receptors; TLR, Toll-like receptor.
receptors: CCR1, CCR5, and CCR2, in particular. A non-redundant role of the CCR2–CCL2 axis has been demonstrated in gene-targeted mice [1]. Other receptors, such as CCR3, CCR4 and CCR8, have also been described on monocytes, although their expression levels are usually much lower and their ligands display a significantly lower efficacy in sustaining monocyte migration. Receptors for inflammatory CXC chemokines, such as CXCR1 and CXCR2, are also detectable, however, human monocytes are typically unresponsive to IL-8 (CXCL8) and related agonists. In resting human monocytes, these receptors are uncoupled from the signaling machinery, and become functional only after cell activation by specific agonists through a still undefined mechanism (see later).

Human monocyte maturation into tissue macrophages is accompanied by a loss of CCR2 expression and increased expression of CCR1 and CCR5 [21,22]. This chemokine receptor switch might indicate a multistep navigation process, with the CCL2–CCR2 axis being involved in initial recruitment, followed by a subsequent CCR1–CCR5-dependent positioning in the tissue.

Areas in inflammatory and neoplastic tissues are characterized by low oxygen tension. Hypoxia strongly affects the chemokine system in macrophages. In particular, human macrophages exposed to low oxygen conditions do not migrate in response to CCL2 [23,24] and CCL2 production is also inhibited in hypoxic conditions [23–25]. By contrast, CXCR4 expression and function in different myeloid populations is strongly increased through the activation of hypoxia-inducible factor 1α [26]. Thus, reciprocal modulation of CCL2 and CXCR4 in hypoxic tissues, such as areas of necrotic tumors associated with hypoxia, is probably part of a multistep navigation program, in which a dynamic regulation of the chemokine

**Figure 2.** M1 and M2 macrophages: the extremes of a continuum. Macrophage activation is associated with profound changes in gene expression profiles. Exposure to different tissue-derived stimuli induces distinct polarization profiles, associated with the expression of selected molecules. Classical macrophage activation (M1 macrophage) is induced by exposure to IFN-γ and LPS, and it is associated with a distinct set of molecules (shown in red). Different forms of alternative activation (M2 macrophage) can be due to different stimuli, with distinct molecular profiles. IL-4 and IL-13 induce M2a (yellow), immune complexes + LPS induce M2b (magenta), and IL-10 induces M2c (green). Molecules in common for M2a and M2c (induced both by IL-4 + IL-13 and IL-10) are shown in blue. Abbreviations: IFN-γ, interferon-γ; IL-1 ra, IL-1 receptor antagonist; LPS, lipopolysaccharide; MR, mannose receptor; RNI, reactive nitrogen intermediates; ROI, reactive oxygen intermediates; TLR, Toll-like receptor.
Figure 3. The chemokine repertoires of polarized M1 and M2 macrophages. During polarization, macrophages profoundly modify the panel of chemokines produced. M1 polarization is accompanied by production of inflammatory CC chemokines and IFN-γ-responsive chemokines that recruit Th1, Tc1 and NK cells, and coordinate a type I immune response particularly suited for intracellular pathogen killing and tumor resistance. IL-4 and IL-13 exposure sustains M2a polarization, which is accompanied by production of chemokines agonist at CCR3, CCR4 and CCR8, consequent recruitment of eosinophils, basophils and Th2 cells, and organization of a type II immune response. M2b polarization is critically dependent on exposure to immune complexes and TLR or IL-1R agonists, and it is characterized by selective production of CCL1, with consequent recruitment of Tregs and immunoregulation. Exposure to IL-10 drives M2c polarization, which is characterized by CCL16 and CCL18 production and consequent recruitment of eosinophils and naïve T cells, respectively. Induction of CXCL13 requires co-stimulation by IL-10 and LPS. Abbreviations: Ba, basophils; DTH, delayed-type hypersensitivity; Eo, eosinophils; IC, immune complexes; IFN-γ, interferon-γ; IL-4, interleukin-4; IL-1R, IL-1 receptor; iNOS, inducible nitric oxide synthase; MR, mannose receptor; NK, natural killer; ROI, reactive oxygen intermediates; TAMs, tumor associated macrophages; Treg, regulatory T cells.
receptor profile supports initial recruitment of macrophages, controlled by the CCR2–CCL2 circuit, and their subsequent final positioning, under the control of CXCR4–CXCL12.

Chemokine repertoires during macrophage activation and polarization

Distinct chemokine repertoires associate with M1 and the various forms of M2 macrophage activation. LPS activation of monocytes or macrophages results in the NF-κB-dependent transcription of inflammatory chemokines, such as CXCL1, 2, 3, 5, 8, 9 and 10 and CCL2, 3, 4, 5, 11, 17 and 22 [27]. In addition, LPS and IFN-γ induce the expression of CXCL10, CXCL9 and CCL5 [28–30]. LPS mediates induction of the CXCL10, CXCL9 and CCL5 genes through the activation of the transcription factor IFN regulatory factor-3 (IRF-3), which results in IFN-β expression and subsequent STAT1 (signal transducer and activator of transcription 1) activation [29]. The spectrum of chemokines produced during classical activation (M1) amplifies delayed-type hypersensitivity (DTH) reactions and resistance to intracellular pathogens and tumors (Figure 3).

CXCL16 and CX3CL1 share the property of being transmembrane chemokines [31]. CXCL16 is expressed in macrophages and DCs and is induced by IFN-γ and TNF [32,33]. It acts on CXCR6-expressing cells (T cells, NKT cells) [31–34] in membrane-bound and shed forms. Hence, it provides a loop of amplification of cell–cell interaction and recruitment in polarized type I responses (Box 2 and Figure 3).

M2-inducing signals generally inhibit the expression of M1 chemokines. The TLR- and IFN-γ-dependent induction of CXCL10, CCL5 and CXCL9 is inhibited by IL-4 and IL-10 [6]. The inhibitory effects of IL-10 on LPS-activated macrophages rely on both STAT3-dependent mechanisms [14,35] and the inhibition of NF-κB [36]. Furthermore, IL-10 directly inhibits CXCL10 and CXCL9 gene expression through the inhibition of STAT1 phosphorylation [30]. Glucocorticoids also suppress CXCL10 production by LPS-treated macrophages through the inhibition of STAT1 [37].

IL-10 also alters KC mRNA stability by preventing the usually stabilizing effect of LPS [38]. The proto-oncogene c-Maf might also have a role in IL-10 activity. This transcription factor represents one of the physiological mediators of IL-10 activity, and when overexpressed it inhibits transcriptional activation of the IL-12p35 and p40 genes and it also upregulates IL-10 and IL-4 [39].

Suppression of the transcriptional activation of IFN-γ and LPS-responsive genes by IL-4 requires STAT6, which acts by sequestering coactivator molecules required for the action of STAT1 and NF-κB [40]. M2 macrophages obtained from mice implanted intraperitoneally with the filarial nematode Brugia malayi display an IL-4-dependent inhibition of the proinflammatory chemokines CCL3 and CCL4 [41]. Mouse M2 macrophages have a crucial role in the correct cytokine balance during parasite infection [42].

Overall, evidence suggests that signals inducing M2 polarization (e.g. IL-10, IL-4, IL-13) downregulate NF-κB and STAT1 activities, and thus act as a common mechanism to limit the induction of inflammatory chemokines associated with the development of type I immunity and inflammation.

M2-inducing signals are not simple inhibitors of proinflammatory chemokines, in that they induce expression of a specific subset of chemokines generally

Figure 4. Levels of regulation of CCL22 produced by M2a mononuclear phagocytes. In the context of a type II response, macrophage exposure to IL-4 or IL-13 induces M2a polarization and stimulates production of CCL22, which further polarizes the immune response by recruiting other CCR4+ cells. Type I response control this CCL22-driven circuitry by IFN-γ-dependent inhibition of CCL22 production and by CD26-mediated CCL22 inactivation, owing to N-terminal processing, which leads generation of inactive variants (3–69, 5–69 and 7–69). The chemokine decoy receptor D6 participates in this process by selectively recognizing and scavenging the unprocessed [1–69] biologically active version of CCL22. Abbreviations: CLA, cutaneous lymphocyte associated antigen; DCs, dendritic cells; EC, endothelial cells; IFN-γ, interferon-γ; IL-4, interleukin-4; T reg, regulatory T cells.
associated with a type II response (Figure 3). IL-4 and IL-13 selectively induce CCL24 [43], CCL17, and CCL22 in M2a macrophages, with inhibition by IFN-γ [44]. IL-4 also induces the production of CCL2, a chemokine associated with Th2 polarization by analysis of gene-targeted mice [45]. The CCR4 agonist CCL17, produced by M2a cells, along with IL-10, inhibits the CpG-mediated M1 activation [46].

The M2-associated agonist CCL22 is processed and inactivated by dipeptidylpeptidase IV (CD26), expressed on a variety of cell types and, among lymphocytes, preferentially on Th1 cells. CCL22 is also recognized and inactivated by the promiscuous receptor D6, a decoy and scavenger for inflammatory CC chemokines expressed on lymphatic endothelium [47–49]. In spite of its promiscuous recognition of CC chemokines, D6 binds and scavenges CCL22 but not the processed forms [(3–69) CCL22, (5–69) CCL22 and (7–69) CCL22]. Thus, CCL22, produced by polarized M2a cells, is tuned at the level of induction, processing and scavenging (Figure 4).

CCL18 expression is induced by Th2-associated cytokines, such as IL-4, IL-13 and IL-10, whereas it is inhibited by IFN-γ [50,51]. IL-10 induces production of both CCL18 and CCL16 in M2c human monocytes and macrophages [52,53] (Figure 3). Perhaps unexpectedly, CCL16 activates macrophages for tumor cytotoxicity [54]. However, consistent with a role in type II inflammation, CCL16 attracts eosinophils through the histamine receptor type 4 [55]. The receptor for CCL18 has not been identified. CCL18 attracts naïve T cells and is produced by tumor-associated macrophages [56]. Recruitment of naïve T cells in a microenvironment dominated by IL-10, which inhibits DC maturation, might result in tolerance and immunoregulation.

As discussed earlier, IL-10, together with LPS, stimulates CXCL13 production in human monocyte-derived DCs [17] and, less prominently, in monocytes. The CXCL13 receptor CXCR5 is expressed on B cells and also on a subset of CD4+ T cells, which home to lymphoid follicles and provide help for antibody production. Therefore, CXCL13 facilitates a three-party interaction among monocytes or DCs, CXCR5+ follicular type Th cells and B cells. In addition to CXCL13, IL-10 induces IL-7 under the same conditions [17]. CXCR5, and its cognate ligand CXCL13, and IL-7 are all part of a cytokine cascade that sustains the organization of secondary lymphoid tissues. Therefore, IL-10-induced CXCL13 in M2 cells and DCs might promote the organization of extranodal lymphoid follicles in chronic inflammatory conditions.

Modulation of the chemokine system associated with M2b activation (Figure 1) has not been investigated. In a whole genome transcriptional profiling effort, we recently found that M2b monocytes express high levels of CCL1 (A. Vecchi et al., unpublished) (Figure 3). Interestingly, regulation of CCL1 production, confirmed at the protein level, was unique, in that other signals did not induce it and a series of other chemokines were not induced by co-stimulation by IC and TLR or IL-1R engagement. CCL1 interacts with CCR8, which is expressed on eosinophils, Th2 cells, regulatory T cells and skin homing human T cells [57–61]. Thus, the unique association of CCL1 to M2b macrophages provides a mechanism of amplification of Th2 polarization and immunoregulation.

Thus, in contrast to the spectrum of M1-derived chemokines, M2-derived chemokines promote recruitment of leukocytes involved in tissue repair and remodeling, allergy, resistance to helminth infection and tumor progression (Figure 4).

Chemokine receptor repertoires during macrophage activation and polarization

The chemokine system is regulated at the level of agonist production and receptor expression [2]. Human monocytes exposed to bacterial LPS show a dramatic downregulation of the CCL2 receptor, CCR2 [62]. This effect is associated with destabilization of the transcript and is not dependent on induction of the agonist. Subsequent work has extended this observation to other proinflammatory signals, including TNF, IL-1 and IFN-γ, as well as to other cell types, such as DCs and activated T and NK cells. These results suggest that downregulation of certain inflammatory chemokine receptors (CCR2 most dramatically; also CCR5, CCR1) might deliver a stop signal to recruited mononuclear phagocytes to focus their action at sites of infection and inflammation [2,63]. When these proinflammatory signals leak into the systemic circulation, they might provide a negative signal to inhibit excessive mononuclear phagocyte recruitment at sites of inflammation and tissue damage by downregulating CCR2 expression. Reciprocally, anti-inflammatory signals, such as glucocorticoid hormones and IL-10, increase expression of certain chemokine receptors, including CCR2 and CCR5 [64–66]. Hence, certain M1 and M2 signals have reciprocal and divergent effects on the expression of certain chemokine receptors in human mononuclear phagocytes and this might serve as a strategy to finely tune the action of chemokines.

Mononuclear phagocytes express CXCR1 and CXCR2 but show little functional response to appropriate agonists [e.g. IL-8 (CXCL8)]. When cells are exposed to IL-4 and IL-13, increased receptor expression and coupling render these cells extremely sensitive to IL-8 (CXCL8) and related CXC chemokines [48]. Therefore, microenvironmental signals tune and shape the action of chemokines by regulating both receptor expression and coupling.

In M2c cells, IL-10 tends to increase expression of certain inflammatory chemokine receptors, such as CCR2 and CCR5 [66]. As discussed earlier, the significance of this observation might in fact relate to the effect of this cytokine when combined with primary proinflammatory signals [67]. IL-10 blocks downregulation of inflammatory chemokine receptors induced by LPS, alone or in combination with IFN-γ in monocytes and DCs. However, monocytes and DCs exposed to a combination of IL-10 and LPS, although retaining high levels of CCR2 and CCR5, do not migrate in response to appropriate agonists and show defective activation of signal transducing events. Inflammatory chemokine receptors in cells exposed to a combination of LPS and IFN-γ retain the ability of binding and sequestering agonists. Evidence was obtained that chemokine scavenging might also occur in vivo. It was therefore suggested that these chemokine receptors in M2c cells act
as functional decoy receptors for chemokines. The discovery that IL-10, in concert with LPS, superinduces SOCS3, RGS-16 and phosphatases, and inhibits PI3-Ky has provided a molecular basis for uncoupling of chemokine receptors in M2c cells in an IL-10-dominated inflammatory environment [17]. Hence, in an inflammatory environment dominated by IL-10, M2 cells expressing inflammatory chemokine receptors are set in a chemokine scavenging, anti-inflammatory mode.

Concluding remarks
Plasticity is a hallmark of the mononuclear phagocyte system. Fully polarized M1 and M2 cells in their various versions are extremes of a continuum. M1 cells might indeed be more diverse than so far realized. Moreover, differentiation might combine with polarizing signals to yield more diverse phenotypes, as illustrated by immature myeloid suppressor cells [68]. With this caveat, polarization of macrophage function is an operationally useful conceptual framework, which crystallizes a continuum of diverse functional states. M1 mononuclear phagocytes and the various M2 macrophages, referred to as M2a, M2b and M2c in this Review, express distinct repertoires of chemokines and chemokine receptors. Differential expression of components of the chemokine system in polarized mononuclear phagocytes integrates these cells into polarized circuits of resistance to, or promotion of, microbial pathogens and tumors and immunoregulation, tissue repair and remodeling. Transcriptional profiling and proteomic analysis are likely to add a new dimension to the analysis of macrophage differentiation and polarization, with the identification of new fingerprints useful to relate in vitro defined states to the complexity of pathology.

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References
5 Goerd, S. et al. (1999) Other functions, other genes: alternative activation of antigen-presenting cells. Immunity 10, 137–142
30 Ito, S. et al. (1999) Interleukin-10 inhibits expression of both interferon α- and interferon γ-induced genes by suppressing tyrosine phosphorylation of STAT1. Blood 93, 1456–1463
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