REVIEW

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Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm

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Plasticity is a hallmark of cells of the myelomonocytic lineage. In response to innate recognition or signals from lymphocyte subsets, mononuclear phagocytes undergo adaptive responses. Shaping of monocyte-macrophage function is an essential component of resistance to pathogens, tissue damage and repair. The orchestration of myelomonocytic cell function is a key element that links inflammation and cancer and provides a paradigm for macrophage plasticity and function. A better understanding of the molecular basis of myelomonocytic cell plasticity will open new vistas in immunopathology and therapeutic intervention.

Myelomonocytic cells are an essential component of innate immunity[1]. They originate from bone marrow precursors, and new light has been shed on their differentiation[2,3]. Plasticity and diversity have long been known to be hallmarks of the monocyte-macrophage differentiation pathway[4]. Indeed, adaptive responses to environmental signals are now recognized for both mature and immature elements in the myelomonocytic differentiation pathway[5,6].

In addition to acting as a first line of resistance against pathogens (the unsung heroes of immunity) and activating adaptive responses, myelomonocytic cells undergo reprogramming of their functional properties in response to signals derived from microbes, damaged tissues, and resting or activated lymphocytes. Here we review this adaptive aspect of the functional plasticity of myelomonocytic cells with emphasis on their bidirectional interaction with lymphocyte subsets and their integration into adaptive (lymphocyte-mediated) immunity, using cancer as a paradigm.

Adaptive responses to innate recognition

One of the hallmarks of adaptive immunity is the ability to mount an enhanced and tailored immune response after secondary exposure to the same antigen. Likewise, sensing of microbial components by macrophages results not only in their functional activation but also in the reshaping of subsequent responses to microbial encounters. Thus, phagocyte-mediated innate immunity also has a built-in adaptive component, and the ability to mount a polarized response is a reflection of this[7,8]. Recognition of microbial moieties such as lipopolysaccharide (LPS) has long been known to be a potent activator of macrophages[3]. Recognition of microbial molecules can modify the pattern-recognition receptor repertoire of myelomonocytic cells, thus reshaping their subsequent responses. Regulation of the scavenger receptors MARCO and dectin-1 by microbial recognition is an example of this, and the change in receptor repertoire of cells carrying those receptors profoundly affects subsequent macrophage responses in terms of phagocytosis and cytokine production[7,9].

Under appropriate conditions, exposure to LPS results in hyporesponsiveness to subsequent challenge at the macrophage and organism level (referred to as 'endotoxin tolerance')[10]. Endotoxin tolerance mirrors the immunosuppressive phenotype observed in sepsis. Endotoxin tolerance might actually be a misnomer, because transcriptomal analysis of macrophages has indicated that endotoxin tolerance represents a case of gene reprogramming[11]. Endotoxin-tolerant macrophages have been found to express a set of molecules that overlap those expressed by alternatively activated (M2-polarized) macrophages[10,12]. This includes higher expression of interleukin 10 (IL-10), arginase 1 and the chemokines CCL17 and CCL22. Thus, endotoxin tolerance, far from being a form of unresponsiveness, represents an adaptive response with skewing of macrophage function to a phenotype of tissue repair and immunoregulatory.

In response to microbe recognition, macrophages produce copious amounts of fluid-phase pattern-recognition molecules. These molecules act as functional ancestors of antibodies (as so-called 'ante-antibodies')[13]. The repertoire of fluid-phase pattern-recognition molecules of myelomonocytic cells includes molecules that belong to the collectin family (for example, mannose-binding lectin), ficolin family (for example, L-, H- and M-ficolin) and pentraxin family (for example, pentraxin 3)[13]. Pentraxin 3 represents a paradigm of the interaction between the cellular and humoral arms of innate immunity[14]. This molecule, newly produced in mononuclear phagocytes and stored in a granular compartment in neutrophils, has a nonredundant role in resistance to such pathogens as *Aspergillus fumigatus*. The effector mechanisms involve the recognition of and binding to microbial moieties, activation of the complement cascade and opsonization-mediated destruction of pathogens[13,14]. Additionally, by binding to P-selectin, pentraxin 3 attenuates the recruitment of neutrophils to sites of inflammation and thereby

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dampens inflammation[15]. Therefore, pentraxin 3, as well as other soluble pattern-recognition molecules produced by phagocytes, has an amplification and regulatory role in innate immunity[13].

Macrophage polarization: a useful oversimplification

Mirroring T helper type 1–T helper type 2 (T_H1-T_H2) polarization, two distinct states of polarized activation for macrophages have been recognized: the classically activated (M1) macrophage phenotype and the alternatively activated (M2) macrophage phenotype[3,4] (**Fig. 1a,b**). Bacterial moieties such as LPS and the T_H1 cytokine interferon-γ (IFN-γ) polarize macrophages toward the M1 phenotype. In contrast, M2 polarization was originally discovered as a response to the T_H2 cytokine IL-4 (ref. 16). M2 macrophages show more phagocytic activity, high expression of scavenging, mannose and galactose receptors, production of ornithine and polyamines through the arginase pathway, and a phenotype of low expression of IL-12 and high expression of IL-10, the IL-1 decoy receptor and IL-1RA[3,4,8]. In general, these cells participate in polarized T_H2 responses, help with parasite clearance, dampen inflammation, promote tissue remodeling and tumor progression and have immunoregulatory functions. M1 and M2 macrophages have distinct chemokinome profiles, with M1 macrophages expressing T_H1 cell–attracting chemokines such as CXCL9 and CXCL10 and M2 macrophages expressing the chemokines CCL17, CCL22 and CCL24 (refs. 8,17). Chemokines can also affect macrophage polarization, with CCL2 and CXCL4 driving macrophages to an M2-like phenotype[18,19]. M1- and M2-polarized macrophages have distinct features in terms of the metabolism of iron, folate and glucose[20–22].

Macrophages can also be polarized into an 'M2-like' state, which shares some but not all the signature features of M2 cells (**Fig. 1c,d**). For example, various stimuli, such as antibody immune complexes together with LPS or IL-1, glucocorticoids, transforming growth factor-β (TGF-β) and IL-10, give rise to M2-like functional phenotypes that share properties with IL-4- or IL-13-activated macrophages (such as high expression of mannose receptor, IL-10 and angiogenic factors)[23]. Variations on the theme of M2 polarization are also found *in vivo* (for example, in the placenta and embryo, and during helminth infection, *Listeria* infection, obesity and cancer)[24–29]. As a result of *in vivo* pathophysiological conditions characterized by a diversity and temporal evolution of activating signals, macrophages with intermediate or overlapping phenotypes have been observed. For example, transcriptome analysis has shown that monocytes infected with human cytomegalovirus have an atypical M1-M2 polarization biased toward the M1 phenotype yet express M2 genes such as *IL1RA*, *IL10*, *CCL18* and *CCL22* (ref. 30). Similarly, $CD11c^+$ adipose tissue macrophages from obese mice have a mixed profile, with upregulation of several M1 and M2 gene transcripts[31]. A new macrophage phenotype has been identified in response to oxidized phospholipids that differs distinctly from that of conventional M1 and M2 macrophages[32]. Furthermore, a shift in monocyte-macrophage phenotypes during the course of several diseases such as sepsis, cancer and obesity has been reported[10,33,34]. In a *Listeria monocytogenes* infection model, patrolling monocytes with low expression of the marker Gr-1 initially have an inflammatory M1 phenotype that subsequently changes to an M2 phenotype associated with tissue remodeling[28]. These studies emphasize the heterogeneity and plasticity of macrophage functional states and indicate that typical M1 and M2 phenotypes are extremes of a spectrum in a galaxy of functional states[4,8,35].

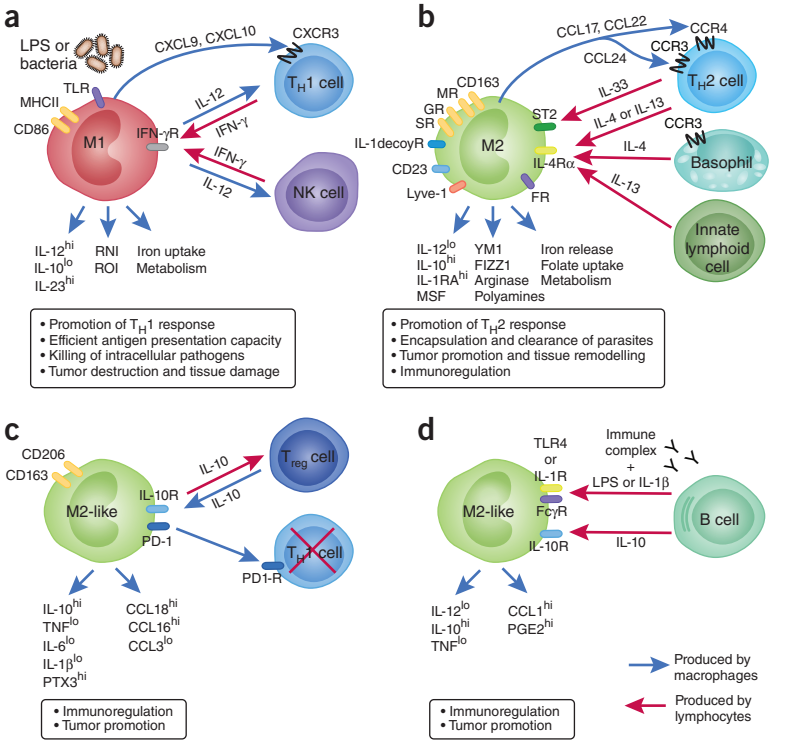

Figure 1 The orchestration of macrophage activation and polarization by lymphoid cells. (**a**) M1-polarized macrophages and their crosstalk with T_H1 and NK cells. (**b**) M2 polarization of macrophages driven by T_H2 cells, basophils and innate lymphoid cells through their secretion of IL-4, IL-13 or IL-33. (**c**) M2-like macrophages polarized by interaction with T_{reg} cells. (**d**) M2-like polarization of macrophages by interaction with B cells through antibody-mediated FcγR activation or cytokines. FR, folate receptor; GR, galactose receptor; IFN-γR, IFN-γ receptor; IL-1decoyR, IL-1 decoy receptor; MHCII, major histocompatibility complex class II; MP, macrophage; MR, mannose receptor; SR, scavenging receptor; ST2, receptor; PGE2, prostaglandin E_2; PTX3, pentraxin 3; RNI, reactive nitrogen intermediate; ROI, reactive oxygen intermediate.

Bidirectional macrophage-lymphocyte interactions

Myelomonocytic cells engage in a complex bidirectional interaction with adaptive and innate lymphoid cell subsets. We discuss examples of such two-way interactions below in the context of macrophage polarization.

By producing IFN-γ, T_H1 cells can drive classical M1 polarization of macrophages (**Fig. 1a**). These cells are characterized by their ability to release large amounts of proinflammatory cytokines (such as IL-12, IL-23 and tumor necrosis factor (TNF)), reactive nitrogen intermediates and reactive oxygen intermediates, higher expression of major histocompatibility complex class II and costimulatory molecules, efficient antigen presentation, and microbicidal or tumoricidal activity. M1 macrophages are part of a polarized T_H1 response and mediate resistance to intracellular pathogens and tumors and elicit tissue-disruptive reactions[3,8]. M1 macrophages, through their expression of cytokines and chemokines such as IL-12, CXCL9 and CXCL10, drive the polarization and recruitment of T_H1 cells, thereby amplifying a type 1 response[23]. M1 macrophages show a shift in iron homeostasis[21] by repressing ferroportin and inducing H ferritin, which favors iron sequestration and thereby contributes to bacteriostatic effects.

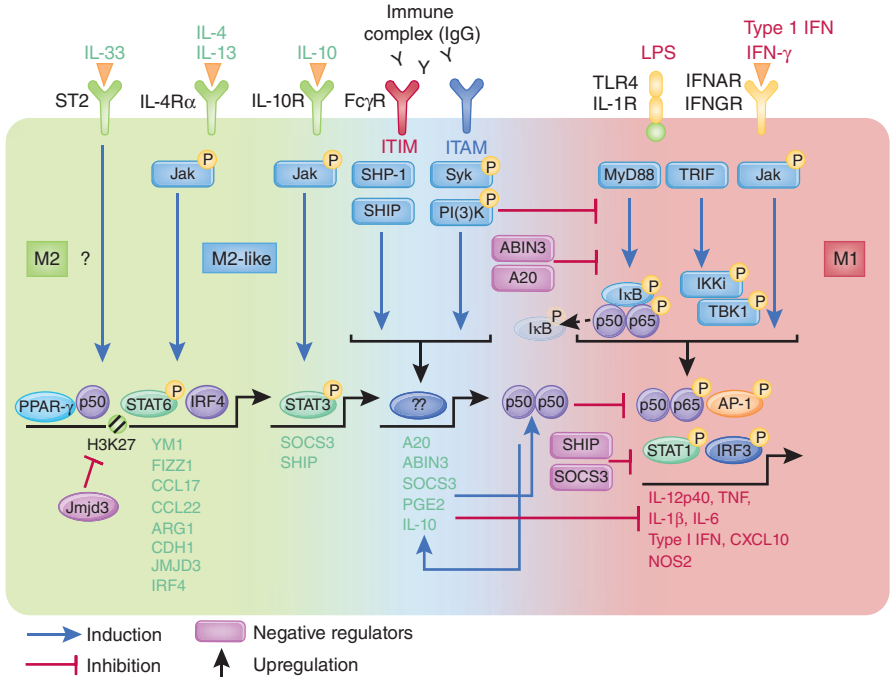

Figure 2 Molecular pathways of macrophage polarization. M1 stimuli such as LPS and IFN-γ signal through the TLR4, IFN-α, or IFN-β receptor (IFNAR) and IFN-γ receptor (IFNGR) pathways, inducing activation of the transcription factors NF-κB (p65 and p50), AP-1, IRF3 and STAT1, which leads to the transcription of M1 genes (red lettering indicates molecules encoded). In contrast, M2 stimuli such as IL-4 and IL-13 signal through IL-4Rα to activate STAT6, which regulates the expression of M2 genes (green lettering indicates molecules encoded). The regulation of these genes also involves JMJD3, IRF4, PPAR-γ and p50. IL-10 and immune complexes, plus LPS and IL-1, trigger M2-like macrophage polarization. IL-10 signals through its receptor (IL-10R), activating STAT3. Immune complexes trigger FcγR signaling, leading to the expression of molecules such as A20, ABIN3, SOCS3, prostaglandin E_2 and IL-10, which negatively regulate the TLR4 and IL-1R and interferon-signaling pathway. Activatory and inhibitory FcγR signaling is initiated by activation of Syk–phosphatidylinositol-3-OH kinase (PI(3)K) and tyrosine phosphatase SHP-1–inositol phosphatase SHIP, respectively. Methylation of histone H3K27 is a post-translational modification linked to gene silencing. A20, deubiquitinating enzyme; ABIN3, A20-binding NF-κB inhibitor; IgG, immunoglobulin G; IκB, NF-κB inhibitor; IKKi, inducible IκB kinase; ITAM, intracellular tyrosine-based activatory motif; ITIM, intracellular tyrosine-based inhibitory motif; Jak, Janus kinase; TBK1, NF-κB activator; TRIF, adaptor protein.

The interaction of natural killer (NK) cells with mononuclear phagocytes goes beyond IFN-γ production; indeed, NK cell cytolytic activity is exerted preferentially on M2-polarized macrophages (C. Bottino *et al.*, personal communication), which represents a potential mechanism for further skewing and amplification of the T_H1 response. Macrophages and NK cells are abundant in the placenta. Placental macrophages have an M2-like polarized phenotype[25], as is the case for embryonal macrophages[27]. The interaction of placental macrophages with NK cells results in the induction of proangiogenic cytokines (VEGF and IL-8)[36]. Furthermore, crosstalk between NK cells and placental $CD14^+$ myelomonocytic cells induces regulatory T cells (T_{reg} cells) in an indoleamine dioxygenase– and TGF-β-dependent manner[37]. Thus, the interaction between NK cells and macrophages is probably involved in shaping key aspects of the placenta, such as its unique vascularization and the maintenance of immunosuppression in the placental microenvironment.

T_H2 cell–derived IL-4 and IL-13 direct M2 polarization of macrophages during helminth infection and allergy[29,38]. Indeed, some prototypical mouse M2 markers (such as YM1, FIZZ1 and MGL proteins) were first identified in parasite infection and allergic inflammation[29,38,39]. IL-4-treated macrophages have a phenotype of low expression of IL-12 and high expression of IL-10, the IL-1 decoy receptor and IL-1RA and share many of the features characteristic of M2-polarized macrophages[1,8] (**Fig. 1b**). Importantly, IL-4-activated macrophages express a distinct set of chemokines, including CCL17, CCL22 and CCL24. The corresponding chemokine receptors CCR4 and CCR3 are present on T_{reg} cells, T_H2 cells, eosinophils and basophils[23]. Thus, the release of these chemokines results the recruitment of these cells and amplification of polarized T_H2 responses. M2 macrophages also have distinct metabolic properties. Through the upregulation of ferroportin and the downregulation of H ferritin and hemeoxygenase, M2 macrophages favor enhanced release of iron, which supports cell proliferation[21]. The expression of folate receptor-β and uptake of folate are other characteristic features of M2 macrophages[20]. Furthermore, E-cadherin is a selective marker of M2 macrophages and is linked to the mediation of homotypic cellular interactions such as macrophage fusion[40]. In general, M2 cells participate in polarized T_H2 responses, parasite clearance, the dampening of inflammation, the promotion of tissue remodeling, angiogenesis, tumor progression and immunoregulatory functions.

Many other cytokines can govern M2 polarization. IL-33 is a cytokine of the IL-1 family associated with T_H2 and M2 polarization[41,42]. IL-33 amplifies IL-13-induced polarization of alveolar macrophages to an M2 phenotype characterized by the upregulation of YM1, arginase 1, CCL24 and CCL17, which mediate lung eosinophilia and inflammation[42]. IL-21 is another T_H2-associated cytokine shown to drive M2 activation of macrophages[43].

Tissue remodeling has long been associated with M2 polarization[4,8]. IL-4-activated macrophages, as well as cells exposed to IL-10, TGF-β and tumor supernatants, selectively express the fibronectin isoform MSF (migration-stimulating factor)[44]. MSF lacks a typical RGD (Arg-Gly-Asp) motif and is a potent motogen for monocytes; however, its role in ontogeny and immunopathology remains to be defined. M2 macrophages support angiogenesis and lymphangiogenesis by releasing proangiogenic growth factors such as IL-8, VEGFA, VEGFC and EGF[4,45–47]. Macrophages act as 'bridge cells' or 'cellular chaperones' that guide the fusion of endothelial tip cells (vascular anastomosis) and facilitate vascular sprouting[45,48]. These tissue-resident macrophages express the receptor tyrosine kinase Tie-2, similar to the proangiogenic Tie-2-expressing monocytes (TEMs). Interestingly, transcriptome profiling has shown that TEMs share several characteristics with M2-polarized cells[49]. Further studies should determine the exact relationship between TEMs and Tie-2-expressing tissue macrophages. Macrophages expressing the hyaluronan receptor LYVE-1 have also been reported to promote angiogenic as well as lymphaniogenic functions and show M2-like characteristics[31].

Studies have identified a new class of innate effector cells as a source of IL-13. Three newly defined cell types—natural helper cells, nuocytes and multipotent progenitor type 2 cells—were identified as the main source of IL-13 production in gut-associated lymphoid tissue during helminth infection[50–52]. We are tempted to speculate that these 'natural' sources of IL-13 contribute to the unusual properties of macrophages in the gastrointestinal tract, but this remains to be determined[53].

Progress has been made in defining the molecular pathways that underlie M2 versus M1 polarization (**Fig. 2**). IL-4 signals through either type I IL-4 receptors (IL-4Rα or IL-4Rγc) or type II IL-4 receptors (IL-4Rα or IL-13Rα1), whereas IL-13 signals only through type II IL-4 receptors[54]. Differences in the expression of type I or type II receptors on different cell types dictate their sensitivity to IL-4 and IL-13. Monocytes and macrophages have type I receptors as well as type II receptors and respond to both cytokines[1,54]. However, IL-13Rα2, a component of the type II receptor, can act as a decoy for IL-13 and dampens monocyte alternative activation[55]. Signaling downstream of the IL-4 receptors involves the activation of various Janus kinases, which culminates in the activation of STAT6, a master regulator of M2 genes[39,40,56]. STAT6 also induces expression of the transcription factor PPAR-γ, which acts in synergy with STAT6 to regulate the expression of M2-specific genes and macrophage polarization in obese mice[26]. At an epigenetic level, the histone demethylase JMJD3 regulates transcription of the M2-associated genes *Arg1, Chi3l3* (called '*Ym1*' here) and *Retnla* (called '*Fizz1*' here) by reciprocal changes in the methylation of histone H3 Lys4 (H3K4) and histone H3 Lys27 (H3K27)[57]. IL-4 induces upregulation of JMJD3, which then decreases H3K27 methylation at the promoters of those M2-associated genes to activate transcription. In contrast, JMJD3 inhibits the transcription of typical M1-associated genes. These data point toward an important role for chromatin remodeling in the regulation of macrophage activation[58]. It has been reported that JMJD3 regulates M2 macrophage polarization by inducing expression of the transcription factor IRF4 (ref. 59). Although early studies showed IRF4 to negatively regulate Toll-like receptor 4 (TLR4) signaling by binding to the adaptor MyD88, subsequent data have shown IRF4 to be essential for M2 macrophage polarization and the expression of M2 signature genes such as *Arg1*, *Ym1* and *Fizz1*.

T_{reg} cells can profoundly affect macrophage function (**Fig. 1c**). Human monocytes cultured in the presence of $CD4^+CD25^+Foxp3^+$ T_{reg} cells differentiate into M2-like macrophages[60]. In humans, these macrophages are characterized by higher expression of M2 markers such as CD163, CD206 and CCL18 and enhanced phagocytic capacity but lower expression of HLA-DR and LPS-induced inflammatory cytokines (such as TNF, IL-1β, IL-6 and CCL3; **Fig. 1c**). T_{reg} cell–derived IL-10 is involved in the suppression of inflammatory cytokines and the expression of CD163 and CCL18. Many of the immunosuppressive effects of IL-10 are mediated through activation of the transcription factor STAT3. IL-10-induced activation of STAT3 results in upregulation of SOCS3, which is an inhibitor of cytokine signaling pathways. In mice of the severe combined immunodeficiency strain, adoptive transfer of syngeneic T_{reg} cells into the peritoneal cavity polarizes the resident macrophages into an M2 phenotype similar to that described above[61]. Conversely, M2-polarized macrophages not only drive the differentiation of $CD25^+GITR^+Foxp3^+$ T_{reg} cells[62] but also regulate their recruitment by releasing CCL22 (ref. 63). In support of those observations, IL-4 gene therapy in an experimental autoimmune encephalomyelitis mouse model has been shown to upregulate CCL22 production by microglial cells, resulting in more recruitment of T_{reg} cells and disease protection[64]. Upregulation and activation of the receptor PD-1 on monocytes occurs during infection with human immunodeficiency virus[65]. In fact, PD-1 ligation induces IL-10 production by monocytes, which together with PD-1 inhibits $CD4^+$ T cell responses during such infection (**Fig. 1d**). Thus, the available evidence is consistent with a view of reciprocal regulation between macrophages and T_{reg} cells. However, the *in vivo* importance of this interaction remains to be fully ascertained.

IL-17 can mediate the recruitment and activation of mononuclear phagocytes in diverse pathologies[66–68]. In addition, macrophages themselves can be an important source of IL-17 (P. Ward, personal communication). Neutrophils have been generally considered to be major effector cells in IL-17-producing helper T cell (T_H17 cell)–driven responses. The finding that IL-17 affects macrophage function calls for reappraisal of the role of mononuclear phagocytes in T_H17-oriented responses.

B cells also can directly regulate macrophage effector functions through the interaction of immunoglobulins with macrophage FcγR (the Fc receptor for immunoglobulin G) or via cytokine production. Macrophages stimulated by immune complexes in the presence of MyD88-dependent inflammatory stimuli (IL-1 or LPS) polarize to an $IL\text{-}12^{lo}IL\text{-}10^{hi}$ M2-like phenotype[69] (**Fig. 1d**). These cells have a unique chemokine profile in that they have high CCL1 expression[70]. CCR8, the cognate receptor of CCL1, is expressed on eosinophils, polarized T_H2 cells and T_{reg} cells and may actually have a role in the function of the last[23,70]. The binding of immune complexes to activatory FcγR on macrophages triggers a pathway dependent on the tyrosine kinase Syk, which inhibits not only TLR4 signaling but also type I interferon signaling through the upregulation of IL-10 and the negative regulators A20, ABIN3 and SOCS3 (ref. 71). Similarly, ligation of the inhibitory receptor FcγRIIb on macrophages by immune complexes induces the production of prostaglandin E_2, which inhibits the expression of TLR4-triggered inflammatory cytokines such as IL-6 and TNF[72].

The B-1 subset of B cells resides mainly in the peritoneum, and B-1 cells are constitutive producers of IL-10 (ref. 73). B-1 cell–derived IL-10 downregulates the expression of TNF, IL-1β and CCL3 but upregulates IL-10 expression in LPS-treated macrophages[74]. Conversely, macrophages from B cell–deficient μMT mice have high expression of these proinflammatory genes. Together these observations suggest that B cells can participate in the orchestration of macrophage function via antibodies and immune complexes as well as by the production cytokines.

Macrophage plasticity: cancer as a paradigm

Mononuclear phagocytes are a key element of cancer-related inflammation[75,76]. Cancer serves as a useful paradigm of macrophage diversity and plasticity[4,33,77]. Here we review how the regulatory pathways described above orchestrate the beneficial or pathological yin-yang interaction between tumor-associated macrophages (TAMs) and tumor cells (**Fig. 3**). Our emphasis will be on genetic evidence and on the general implications of studies on the tumor microenvironment.

Macrophages and related cell types (such as TEMs, the monocyte component of myeloid-derived suppressor cells) isolated from established metastatic mouse and human tumors generally have an M2-like phenotype, consistent with the smoldering nature of cancer-related inflammation[4,33,49,78]. Such macrophages generally have an $IL\text{-}12^{lo}IL\text{-}10^{hi}$ phenotype, show impaired expression of reactive nitrogen intermediates, less antigen presentation and tumoricidal capacity, and high expression of angiogenic factors (VEGF, EGF and semaphorin 4D), metalloproteases, cathepsins and the growth arrest–specific protein GAS6 (refs. 24,79–82). However, variations on this theme have also been noted depending on the tumor type. For example, macrophages in a mammary tumor model have a less polarized population with neither M1 nor M2 characteristics, although they have a lower abun-

dance of proinflammatory cytokines and less signaling[80]. Moreover, macrophage phenotype can vary in different areas of a tumor. In a mammary adenocarcinoma model, TAMs with high expression of major histocompatibility complex class II can localize to normoxic tumor tissues and express M1 markers as well as antiangiogenic chemokines, whereas TAMs with low expression of major histocompatibility complex class II were found in hypoxic tumor tissues, preferentially expressed M2 markers and had greater proangiogenic functions[83]. These results caution against the overinterpretation of studies on the basis of whole TAM populations.

Myelomonocytic cells influence nearly all steps of carcinogenesis and tumor progression[75,76,81,84]. These include the following: contribution to genetic alterations and instability; regulation of senescence[85]; promotion of angiogenesis and lymphangiogenesis[46,47,86]; suppression of adaptive immunity[87]; interaction with and remodeling of the extracellular matrix; and promotion of invasion and metastasis[47,88]. In turn, tumor cells shape their interaction with macrophages by escaping phagocytosis[89] and by promoting an M2-like polarization via chemokines and polarizing cytokines (such as CCL2 (ref. 19), CSF1, MSF, TNF, IL-10 and TGF-β[44,75,90]). Consistent with those mechanistic studies, in most but not all human tumors, a greater frequency of TAMs is associated with poor prognosis[77], as shown by Hodgkin's disease[91].

Strong genetic evidence suggests that T_H2 cell–derived IL-4 and IL-13 can have a key role in orchestrating M2 activation of macrophages and their protumoral function. In a model of spontaneous mammary carcinoma driven by the polyoma virus oncoprotein PyMT[92], the T_H2-derived cytokines IL-4 and IL-13 induce M2 polarization of TAMs, thereby promoting tumor progression. Indeed, blockade of IL-4 or IL-4Rα signaling diminishes lung metastasis, which correlates with TAMs' lower expression of M2 genes (such as *Arg1* and *Tgfb1*) but higher expression of M1 genes (such as *Il6*, *Nos2* and *Il12a* (encoding IL-12p35)). Similarly, in a pancreatic cancer model, IL-4 induces large amounts of cathepsin activity in TAMs that then mediates tumor growth, angiogenesis and invasion *in vivo*[93]. Finally, in the 4T1 mammary carcinoma, NKT cells have been shown to polarize TAMs via IL-13 to an M2 phenotype, which supports tumor metastasis[56]. In fact, TAMs from mice deficient in CD1d (which lack NKT cells) or components of the IL-13 signaling pathway such as STAT6 and IL-4Rα have an M1-polarized tumoricidal phenotype that correlates with resistance to metastasis. T_{reg} cells are also frequently found in tumors and are associated with poor prognosis. IL-10 derived from tumor-associated T_{reg} cells triggers activation of the T cell–inhibitory receptor PD-L1 on TAMs, which favors the inhibition of tumor-specific T cell immunity[87]. TAMs themselves produce CCL22, which is a potent chemoattractant for T_{reg} cells in cancer[63].

The presence of T_H17 cells has been reported in several tumors[66,94,95]. The IL-17 pathway can have pro- or anti-tumor effects in different settings. In ovarian carcinoma, $CD4^+$ T cell–derived IL-17 can mediate the recruitment of myeloid cells into tumors and enhance tumor growth[66]. However, other studies have indicated that IL-17 not only mediates the recruitment of TAMs but also enhances their pro-tumoral properties through an IL-6–STAT3 circuit[95]. Thus, myelomonocytic cells are probably a key component of the yin-yang role of T_H17 cells in cancer.

There is little information on the interaction of NK cells with myelomonocytic cells in the tumor microenvironment. Results suggest that activated NK cells preferentially kill polarized M2 cells (C. Bottino *et al.*, personal communication). Similarly, NKT cells exert anti-tumor activity in a neuroblastoma model by killing cancer-promoting TAMs[96]. The elimination of cancer-promoting TAMs by T cells also underlies the activity of vaccination against the M2-associated molecule legumain[97]. It will be important to ascertain whether targeting TAMs has a role in ongoing NK cell–based therapeutic efforts[98].

B cells have emerged as additional participants in the regulation of macrophage function and cancer-related inflammation. A seminal study of the K14-HPV16 mouse model of multistage skin carcinogenesis has identified a B cell–mediated pathway of tumor-promoting inflammation and skewing of macrophage function. The pathway involves T cell–dependent autoantibody production by B cells directed against an extracellular matrix component, leading to the recruitment of mononuclear phagocytes and skewing of TAMs by immune complexes in an M2-like direction[99,100]. The regulation of macrophage function in this case was completely dependent on FcγR and did not involve complement. In a different setting, complement components have been linked to the recruitment of cancer-promoting myelomonocytic cells in transplanted[101] as well as primary mouse tumors (J. Lambris, personal communication). Cancer-associated fibroblasts isolated from neoplastic skin in a K14-HPV16 carcinogenesis model have an inflammatory phenotype that drives macrophage infiltration, angiogenesis and the development of transplanted squamous carcinoma[102]. B cells instruct innate immune cells to express IL-1β (via FcγR activation), which drives cancer-associated fibroblasts to a tumor-promoting inflammatory phenotype. In a transplanted tumor model setting (B16 melanoma), transfer of B-1 cells results in substantial induction of a pro-tumoral

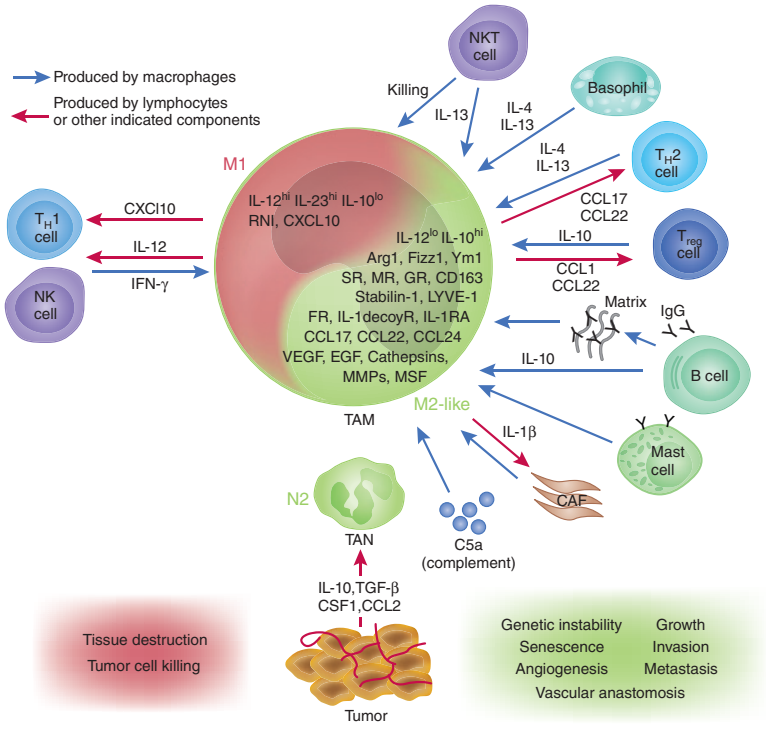

Figure 3 The yin-yang of myelomonocytic cells in tumor progression and their regulation by lymphoid cells. Myelomonocytic cells can have either beneficial or pathological roles in cancer depending on the cellular and tissue environment. Red, M1 polarization; green, M2 or M2-like polarization; red and green shading, functional outputs for M1 and M2 macrophages, respectively; black lettering in cells, salient features of M1 and M2 macrophages; arrows, crosstalk between macrophage and lymphoid cells. TAN, tumor-associated neutrophil.

M2 polarization of TAMs, whereas TAMs from B cell–deficient μMT mice produce a mainly M1 polarization[74]. M2 polarization of TAMs in this tumor model may be driven by the constitutive production of IL-10 by B-1 cells[74]. In apparent contrast to those findings, in a B cell lymphoma model, B cells targeted by rituximab (antibody to CD20) drive macrophages to an M2-like phenotype characterized by more phagocytosis of the malignant B cells, which is correlated with better tumor clearance[103]. Infiltration of plasma cell and macrophages is associated with poor prognosis in some human tumors[104]. B cells are now a confirmed target in the therapy of autoimmune diseases; therefore, the emerging role of B cells in shaping the pro-tumoral function of phagocytes might have important therapeutic implications.

The M2-like pro-tumoral phenotype of TAMs observed in most cancers is reversible[105,106]. Classically activated macrophages kill cancer cells and elicit tumor-destructive reactions centered on blood vessels[45]. IFN-γ can reeducate TAMs *in vitro*[107], thereby providing proof of principle of its anti-tumor activity in humans[107]. Along similar lines, activation of TLR9 by its ligand CpG, along with antibody to IL-10, switches TAMs from an M2 phenotype to an M1 phenotype [108]. Notch signaling in macrophages promote M1 polarization and enhance their anti-tumor activity[109]. Genetically blocking molecular determinants of macrophage polarization (such as STAT3, STAT6 or homodimers of the NK-κB subunit p50) results in reorientation of macrophage polarization, activation of specific immunity and anti-tumor activity[105,110,111]. It is therefore not unexpected that in a minority of human tumor types (such as colon cancer), TAM infiltration is a favorable prognostic indicator; in colon cancer, T cell infiltration has a considerable effect on prognosis[112], which suggests that early in progression, effective T cell responses orchestrate classic macrophage activation. There is also emerging evidence suggesting that neutrophils also have functional plasticity[5], and their N1-N2 polarization is reminiscent of macrophages in cancer[113]. The pathophysiological significance of neutrophil polarization is still being determined.

The orchestration of myelomonocytic cell function is an important element in pathways connecting inflammation and cancer[75]. In different tumor and tissue contexts, the cells involved (mature macrophages; Gr-1$^+$ monocytic cells; neutrophils; immature elements with myeloid-derived suppressor cell activity) and the pathways of regulation (such as IL-4 versus immune complexes[92,99]) differ. The identification of the molecular pathways responsible for the recruitment and skewing of macrophages in tumors provides a basis for ongoing therapeutic trials in patients[75]. However, fuller delineation of this diversity in human tumors will be needed for the clinical exploitation of myelomonocytic cell plasticity in cancer.

Concluding remarks

Plasticity and diversity are long-recognized hallmarks of mononuclear phagocytes. By responding to classic innate immunity signals and to lymphocyte mediators, mononuclear phagocytes act as integral components of adaptive responses to microbes, tissue injury and cell transformation. The ability of macrophages to profoundly reprogram their function, in a way, blurs the distinction between innate and adaptive responses. Interestingly, and perhaps unexpectedly, neutrophils show considerable plasticity reminiscent of that of their macrophage 'cousins'. Progress has been made in defining the surface phenotype, activating signals and molecular pathways associated with different forms of macrophage activation. Moreover, evidence has now accumulated showing that that the orchestration of macrophage function has a key role in different pathological conditions. Better understanding of phagocyte diversity and activation provides a basis for the development of still-unmet phagocyte-targeted therapeutic strategies.

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