Review in Advance first posted online on December 23, 2008. (Minor changes may still occur before final publication online and in print.)

Alternative Activation of Macrophages: An Immunologic Functional Perspective

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Annu. Rev. Immunol. 2009. 27:451–83

The *Annual Review of Immunology* is online at immunol.annualreviews.org

This article's doi: 10.1146/annurev.immunol.021908.132532

0732-0582/09/0423-0451$20.00

Key Words

IL-4, IL-13, M2, macrophage polarization, parasite infection, allergy

Abstract

Macrophages are innate immune cells with well-established roles in the primary response to pathogens, but also in tissue homeostasis, coordination of the adaptive immune response, inflammation, resolution, and repair. These cells recognize danger signals through receptors capable of inducing specialized activation programs. The classically known macrophage activation is induced by IFN-γ, which triggers a harsh proinflammatory response that is required to kill intracellular pathogens. Macrophages also undergo alternative activation by IL-4 and IL-13, which trigger a different phenotype that is important for the immune response to parasites. Here we review the cellular sources of these cytokines, receptor signaling pathways, and induced markers and gene signatures. We draw attention to discrepancies found between mouse and human models of alternative activation. The evidence for in vivo alternative activation of macrophages is also analyzed, with nematode infection as prototypic disease. Finally, we revisit the concept of macrophage activation in the context of the immune response.

INTRODUCTION

MΦ: macrophage

Th: T helper cell (Th1, Th2, Th17)

Macrophages (MΦs) were initially recognized by Elie Metchnikoff as phagocytic cells responsible for pathogen elimination and housekeeping functions in a wide range of organisms, from invertebrates to vertebrates. In 1905, he summarized the evidence that phagocytic mononuclear cells from animals resistant to certain bacterial infections were more adept at killing those and unrelated bacteria, setting the basis for the concept of "macrophage activation" (1). However, 60 more years of combined work were required to define how MΦs become more efficient bacterial killers. In their work regarding MΦ activity in acquired resistance to intracellular bacteria, Mackaness, North, and their colleagues realized that protection against infection was mediated not only by humoral, but also by independent cellular factors (2, 3). In this response they defined a form of acquired resistance to intracellular bacteria that could not be passively transferred with serum, yet induced a spectrum of consistent morphologic and functional changes in the antibacterial activity of MΦs. Importantly, they noted that the antibacterial mechanism was not directed exclusively against the organism that provoked it.

Bloom, Bennett, and David were among those who helped to identify lymphocytes as the major antigen-specific cells responsible for MΦ microbicidal activation and consequent transfer of resistance against families of different intracellular pathogens (4, 5). Soon thereafter, the key factor for collaboration between these cells was discovered: interferon (IFN)-γ (6). IFN-γ is produced by activated $CD4^+$ T helper 1 cells (Th1), $CD8^+$ T cytotoxic 1 (Tc1) cells, and natural killer (NK) cells. This cytokine converts resting MΦs into potent cells with increased antigen presenting capacity, increased synthesis of proinflammatory cytokines and toxic mediators, and augmented complement-mediated phagocytosis. Thus, MΦs acquire the capacity for killing of bacteria, especially intracellular pathogens, and perhaps tumors. As the first type of antimicrobial MΦ activation to be recognized, it became known as "classical activation of MΦs."

In 1982, the immunological counterpart of IFN-γ, interleukin (IL)-4, was identified by Howard, Vitetta, Paul, and colleagues (7). Coffman, Mosmann, and collaborators provided evidence that IFN-γ and IL-4 are produced by mutually inhibitory $CD4^+$ T helper cells: Th1 and Th2 (8). In naive T helper cells, IL-4 and IFN-γ genes are silent; upon TCR engagement and costimulation Th cells begin to choose between Th1 and Th2 cell fates. Knowledge of mutual exclusion led to the discovery that Th2 cells also produce IL-10 to suppress Th1 cells (8). Th2 cells also secrete IL-13, a cytokine that partially shares ligand binding receptor complexes with IL-4 (9). In contrast to IFN-γ, IL-4 and IL-13 mediate immune responses typically characterized by eosinophilia, basophilia, mastocytosis, enhanced B cell class switching, and antibody production, with consequent plasma accumulation of IgE and IgG1. Th2 responses are essential for the control of extracellular parasites, including helminths, protozoa, and fungi, but also contribute to allergy, increased susceptibility to other pathogens, and complications of infection such as fibrosis (**Table 1**).

Initial observations regarding the role of IL-4 in MΦ activation showed that this cytokine was able to inhibit the respiratory burst and the production of IL-1β and IL-8 (10). It was also shown that IL-4 induced MHC class II expression, and MΦ-MΦ fusion and that IL-4 came to play an essential role in MΦ-mediated control of the protozoon *Trypanosoma cruzi* (11–13). Importantly, it was found that IL-13 induces both redundant and nonredundant effects to those of IL-4 in MΦs (9). With the finding in 1992 of upregulation of mannose receptor (MRC1) as a distinctive marker of IL-4-activated MΦs, together with the induction of MHC class II antigens, the concept of alternative activation was proposed, stating, "IL-4 in an inflammatory focus would cause recruited macrophages to acquire an entirely different phagocytic receptor and secretory capability compared with macrophages classically

Table 1 Main features of IL-4 and IL-13 gene regulation and cellular effects

Cytokine family	IL-4 and IL-13 are prototypical four-helix bundle short-chain cytokines, a characteristic of ligands of the class 1 cytokine superfamily
Gene location	The gene for IL-4 resides on mouse chromosome 11 and on the long arm of human chromosome 5. The IL-13 gene lies immediately downstream of the IL-4 locus
Gene regulation	Studies of DNAse1 hypersensitivity, DNA methylation, and permissive marks in naive T cells show that specification of the IL-4/IL-13 transcriptional state is driven by an ordered sequence of regulatory element activation/deactivation events. At the naive stage, preactivation of hypersensitivity site V (HSV) suffices to maintain low levels of IL-4 and IL-13 transcriptional permissivity
Receptor	For IL-4, the receptors are type I and type II. For IL-13, the receptors are type II and IL-13Rα2
Effect on B cells	IL-4/IL-13 induce the isotype switch and secretion of IgE by B lymphocytes
Effect on T cells	IL-4 drives the differentiation of naive Th0 lymphocytes into Th2 lymphocytes and can prevent apoptosis in T cells. T lymphocytes lack IL-13 receptors
Effect on MΦs	IL-4/IL-13 enhance the capacity for fluid-phase pinocytosis and endocytosis IL-4/IL-13 inhibit autophagy in MΦs IL-4/IL-13 induce MΦ fusion/giant cell formation IL-4/IL-13 inhibit NO production/increase arginase activity IL-4/IL-13 treatment leads to enhances MHCII expression/antigen presentation IL-4/IL-13 can inhibit macrophage-mediated killing of pathogens IL-4 induces the expression of mediators of tissue remodeling and inhibits phagocytosis of latex beads. Effects of IL-13 on tissue remodeling or phagocytosis have not been determined
Effect on other cells	IL-4/IL-13 promote fibroblast proliferation, and collagen synthesis IL-4/IL-13 augment the expression of adhesion molecules and profibrotic cytokines from fibroblasts IL-4/IL-13 augment the ability of human mesenchymal cells to contract collagen gels IL-4/IL-13 inhibit bone resorption by osteoclasts

activated by IFN-γ treatment or BCG infection" (14).

In the following years, we learned that the phagocytic and secretory profile of MΦs could be further modified by many other self and pathogen-derived signals (15–17). The main stimuli associated with distinctive MΦ phenotypes include glucocorticoids (GC) recognized by the GCR (glucocorticoid receptor), IL-10 recognized by IL-10R1, and immune complexes recognized by the Fc receptor family, among others. The fact that these phenotypes are all alternatives to classical activation and show a partial overlap with the effects of IL-4 and IL-13 has fuelled confusion in the field over the past decade. Since the discovery of activation and its heterogeneity, several classification schemes have been proposed, the most recent of which defines classically activated MΦ (caMΦ) as M1, and the group of non-caMΦ as M2a-c (16). Before discussing the uniqueness of IL-4/IL-13 or IFN-γ activation in relation to other forms of MΦ stimulation, and the classification system in use, we review and clarify current knowledge concerning IL-4-/IL-13-activated MΦs, based on recent findings regarding cytokine production, signaling, and effects on MΦs. In addition, we assess primary evidence for alternative activation in vivo, with emphasis on parasitic disorders and allergy, and discuss where we are in the field, critically analyzing the less developed areas.

IL-4 AND IL-13 RECEPTORS AND SIGNALING

IL-4 and IL-13 Receptors and Adaptors

IL-4 is recognized by the membrane receptor IL-4Rα, a unique member of the common gamma chain (γ_c) family of receptors (18), characterized by conserved structural motifs in the extracellular region (19). These motifs include

GC: glucocorticoids

caMΦ: classically activated macrophage

γ_c: IL-2 receptor common γ-chain

STAT6: signal transducers and activators of transcription protein 6

conserved paired cysteine residues and, in the membrane proximal region, a WSXWS motif required for the conformation of the receptor chain, which mediates specific cytokine binding (20). The IL-4Rα chain binds IL-4 with high affinity (21), leading to dimerization with another protein to form either type I or type II receptors (**Figure 1*a***). In most cells of the hemopoietic lineage, the type I receptor arises by recruitment of the γ_c chain (22, 23). In nonhemopoietic and myeloid hemopoietic cells, IL-4Rα can recruit IL-13Rα1 (24), forming the type II receptor. Dimerization leading to the formation of either type I or type II receptors induces a cytoplasmic signaling cascade that involves the Janus kinase family (25–27), the insulin receptor substrate family (23), and the phosphoinositide 3-kinase (PI3K) pathway. Janus kinase activation leads to phosphorylation of STAT6 (signal transducer and activator of transcription 6), which dimerizes, migrates to the nucleus, and binds to promoters of genes (28) (**Figure 1*b***).

The type II receptor is the main functional receptor for IL-13 (21). In addition, IL-13 is recognized with high affinity by the IL-13Rα2 receptor (29), endowed with a very short intracellular domain. Kawakami and colleagues (29) found that IL-13Rα2 undergoes internalization after ligand binding without causing signaling and proposed a role as decoy receptor. Furthermore, while IL-13 efficiently caused activation of STAT6 protein in cells transfected with the IL-13Rα1 and IL-4Rα chains, IL-13Rα2 inhibited this activation (29). Novel findings challenge the status of the IL-13Rα2 as a decoy receptor in MΦs (30). Fichtner-Feigl and colleagues (30) showed that IL-13 and tumor necrosis factor (TNF) cooperate to induce the expression of IL-13Rα2; IL-13 then signals through IL-13Rα2 to activate an AP-1 variant containing c-jun and Fra-2, which activates the promoter of tumor growth factor (TGF)-β, leading to its production. Prevention of IL-13Rα2 expression, gene silencing, or blockade of IL-13Rα2 signaling led to marked downregulation of TGF-β production in oxazolone-induced colitis and bleomycin-induced lung fibrosis (30). These data suggest that IL-13Rα2 signaling may become functional during prolonged inflammation (30).

Although the signaling of IL-4 and IL-13 has been considered equivalent, there are differences between them in terms of signaling intensity and kinetics. The epithelial carcinoma cell line A549 has been a useful model to study signaling by the common type II receptor, since it expresses IL-4Rα and IL-13Rα1, but not the γ_c or the decoy receptor IL-13Rα2 (31). In this model, IL-4 induced STAT6 phosphorylation at five- to tenfold lower concentrations than IL-13. IL-4 signaling in the human B cell line Ramos, which expresses γ_c chain and IL-4Rα, stimulated tyrosine phosphorylation of STAT6 with a dose-response similar to that observed in A549. At all concentrations, IL-4 rapidly induced tyrosine phosphorylation of STAT6 in A549, reaching plateau levels between 10–15 min of stimulation, whereas the response to IL-13 was substantially slower. The relative delay in STAT6 phosphorylation induced by IL-13 via the type II receptor complex was most apparent at lower concentrations of the cytokines (31).

Interaction Between IL-4/IL-13 Pathway and Other Signaling Pathways

The IL-4/IL-13 cascades depend on and contribute to other signaling pathways, reminiscent of the cross talk between Toll-like receptor (TLR) and IFN pathways. However, since these findings are relatively new, the relation between the pathways is not always clear. Emergent examples are represented by interaction with nuclear receptors such as the peroxisome proliferator-activated receptors, PPARδ and PPARγ and the GCR, but also membrane molecules and cytokines such as galectin-3, IL-10, and IL-21, among others.

PPARδ and PPARγ seem to coordinate the MΦ transcriptional response to IL-4 and IL-13 at the gene transcription level (32–34). Studies with PPARδ$^{-/-}$, PPARγ$^{-/-}$, and PPARδ/γ$^{-/-}$ MΦs reveal that both receptors are required

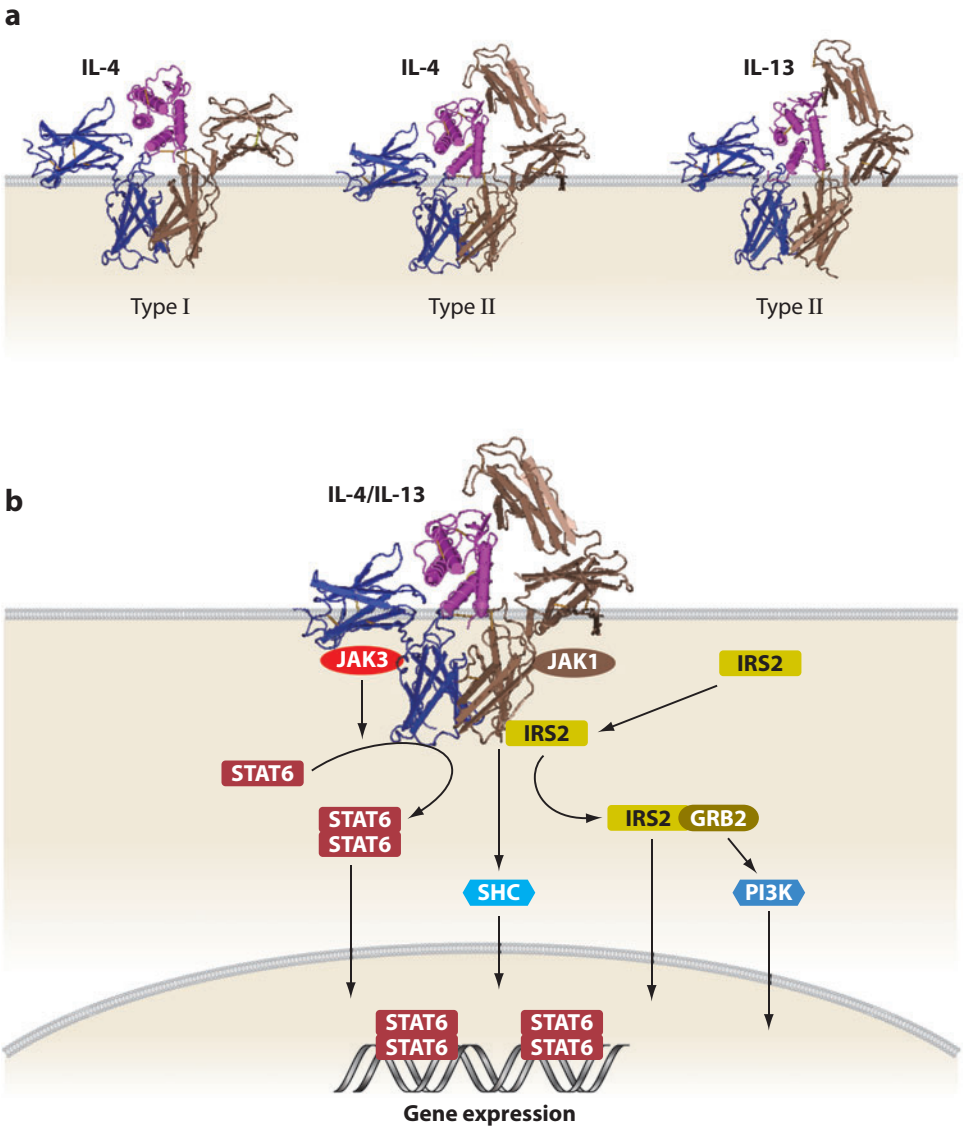

Figure 1

Recognition and signaling machinery of IL-4 and IL-13. (*a*) Recently, LaPorte and colleagues (31) defined the three-dimensional structures of the type I (IL-4Rα/IL-4/γ_c) and type II (IL-4Rα/IL-4/IL-13Rα1 or IL-13Rα1/IL-13/IL-4Rα) ternary signaling complexes (the order shown in parentheses coincides with the order of complex assembly). In these models, the type I complex shows the structural basis for γ_c ability to recognize six different γ_c cytokines, whereas type II complexes show an unusual top-mounted Ig-like domain on IL-13Rα1 for a novel mode of cytokine engagement, perhaps responsible for the reversal in the IL-4 versus IL-13 ternary complex assembly (21). IL-4Rα is depicted in blue for the three models; γ_c and IL-13Rα1 are brown for type I and type II complexes, respectively; and IL-4 and IL-13 are fuchsia. (*b*) Dimerization of the type I and II receptors activates the Janus kinases, Jak1, Jak3, and Jak1, Jak2, Tyk2, respectively (25–27). IRS2, the main member of the insulin receptor substrate family in hemopoietic cells, is also recruited to the receptor complex (23). Phosphorylated IRS activates PI3K and the adaptor Grb2 (18). STAT6 (28) is recruited to the receptor complex and tyrosine phosphorylated. Phosphorylated STAT6 dimerizes, migrates to the nucleus, and binds to promoters of genes (28). Additional signals may be mediated by the recruitment of the adaptor protein Shc.

aaMΦ: alternatively activated macrophage

for optimal expression of alternative activation markers. Importantly, IL-4 induces the production of PPAR ligands in MΦs by induction of the 12/15-lipoxygenase (16). The KO (knockout) mice show that PPARγ regulates primarily metabolic programs in alternatively activated MΦs (aaMΦs), whereas PPARδ is required for the full expression of their immune phenotype, including expression of pattern-recognition receptors (PRRs), and costimulatory molecules and suppression of the MΦ-mediated inflammatory response (32–34).

In contrast to the PPARs, GCs interact with IL-4 in a synergistic fashion, with the combination of stimuli inducing special features. IL-4 per se induces the loss of surface expression of TGF-βRII, whereas simultaneous stimulation with relatively low concentrations of the GC dexamethasone (1×10^{-8} M) suffices to maintain detectable TGF-βRII on the surface (35). Pharmacological concentrations of dexamethasone lead to enhanced surface expression and prolonged TGF-β-mediated signaling. A similar mechanism is responsible for the expression and function of the membrane protein stabilin-1. The combination of GC and IL-4/IL-13 induces an increase and stabilization of the levels of its messenger and protein but, more importantly, activates two distinctive intracellular trafficking pathways for receptor-mediated endocytosis and recycling, resulting in stabilin-1 shuttling between the endosomal compartment and the *trans*-Golgi network. The major role of stabilin-1 seems to be that of a homeostatic scavenger receptor for endogenous signals such as acLDL (acetylated low-density lipoprotein), SI-CLP (chitinase-like protein), and SPARC (secreted protein, acidic, cysteine-rich), the delivery of newly synthesized proteins from biosynthetic to secretory pathways, and perhaps mediation of leukocyte adhesion and transmigration (36).

Other molecules also influence the expression of aaMΦ genes in vivo. Galectin-3 is a membrane molecule that participates in a feedback loop that causes sustained PI3K activation via activation of CD98. siRNA-targeted depletion of galectin-3, murine KO models, and specific galectin-3 inhibitors block the expression of the main aaMΦ markers, thus supporting a role as a key mechanism in IL-4/IL-13 pathways (37). These data confirm the notion that PI3K signaling plays an important role in alternative MΦ activation in vivo. SHIP1 (SH2-containing inositol-5-phosphatase) is a potent negative regulator of the PI3K pathway in hemopoietic cells. MΦs in mice with a targeted deletion of SHIP1 had increased expression of alternative activation markers, a property that appears to be dependent on TGF-β present in vivo (38).

Ablation of the well-known cytokine IL-10 can also inhibit the upregulation of aaMΦ markers in response to African trypanosomiasis (39). The increase in aaMΦ markers coincides with the appearance of IL-10, which could suggest dependence on IL-10. In this IL-10 KO experimental model, of a dozen aaMΦ markers tested, all returned to the levels found in noninfected wild-type (WT) mice. Note that IL-21 receptor KO mice are also deficient in pulmonary aaMΦs following infection by *Nippostrongylus brasiliensis* and the filarial nematode *Schistosoma mansoni* (40). Does this make IL-10, IL-21, and galectin-3 subcomponents of the IL-4 pathway, as with IFN-β for the IFN-γ and TLR4 pathways? Accurate interpretation of these results and critical analysis of the pathways will be essential to understand the contribution of IL-4/IL-13, and other signals, to alternative activation of MΦs in the development of a Th2 immune response, where multiple signals are produced both sequentially and in unison.

THE CELLS BEHIND MΦ ALTERNATIVE ACTIVATION

The main characteristics of IL-4 and IL-13, as well as their sources in inflammation, have long been known (**Table 1**). Several cell types produce IL-4 and IL-13, including conventional $CD4^+$ Th2 and $CD8^+$ T cells (41, 42), NKT cells (43), basophils (44), mast cells (44, 45), and eosinophils (46), which suggests that alternative activation can be of both innate and

acquired origin. Their production is allele restricted in Th2 cells, whereas in innate immune cells the expression is biallelic (47); major epigenetic alterations involving chromatin reorganization and transcription factor activation control their expression. Among other mechanisms, synergistic activation of the 3′ IL-4 and CNS1 (conserved noncoding sequence-1) enhancers during Th2 priming leads to high levels of IL-4 and IL-13 transcriptional permissivity, rather than to transcription itself, which correlates with an enhanced capacity of Th2 cells to express either, or both, cytokines (48). Thus, the studies presented here, although focused on IL-4, possibly also reflect the production of its immediate neighbor, IL-13. This supposition is strengthened by the fact that many of the effects observed in these models, such as fibrosis, mucus secretion, and parasite expulsion, depend on IL-13 rather than IL-4 (49).

Although the origin of the cytokines is broadly known, the precise contribution of each cell type to the bulk of cytokine produced in acute and chronic inflammatory settings remains elusive. Helminthiasis and asthma are the most common Th2 disease models used to study IL-4 and IL-13 production and consequent MΦ alternative activation. The involvement of the Th2 arm of the immune system in tissue repair is less clear, although Th2 cytokines, especially IL-13, have been associated with fibrosis, essential for parasite containment, but also for wound healing. Consequently, relatively few studies have addressed this question. Useful transgenic mice have been developed for components of the IL-4/IL-13 pathways, although only recently has IL-4 expression been associated with green fluorescent protein (GFP) expression: $GFP^+IL\text{-}4^+$ (50) and the 4get mouse (51) (**Table 2**).

IL-4 is produced at very low concentrations under homeostatic conditions in 4get mice. Baseline GFP expression is approximately 1% of spleen, lung, mesenteric, and peripheral lymph node $CD4^+$ cells (51). This unexpected physiologic expression of the IL-4 gene may account for site-restricted alternative activation of MΦs. Among dispersed lung cells, 90% of the GFP^+ cells were $CD4^-$. $IL\text{-}4^+$ cells increased in response to tissue injury as demonstrated by Loke et al. (52), using sterile peritoneal surgery on 4get mice as the model. Post surgery, there was a sharp accumulation of IL-4-expressing cells in the peritoneal cavity of 4get mice, mainly represented by >90% mature $SiglecF^+$ eosinophils. This early IL-4 response of innate origin was of short duration, while in the presence of the filarial nematode *Brugia malayi* it was prolonged, and the cell composition shifted from 90% eosinophils to 50% eosinophils, 40% mast cells, and 10% Th2 cells. As expected, this new infiltrate resulted in progression to a late, increased Th2 response that, however, shared MΦ effector functions with the early response. Experiments done in $RAG^{-/-}$ mice, which lack B or T cells, showed no significant differences from WT animals in the early effector response, supporting its association with cells of innate origin (52).

GFP: green fluorescent protein

N. brasiliensis, another commonly studied nematode, causes infections typically associated with intestinal pathology, although the host can develop, in addition, a profound pulmonary mucus response (51, 53, 54). Infection of 4get mice with this pathogen showed that eosinophils were the most prevalent cell type, increasing up to 1000 times, with Th2 cells and basophils comprising three and ten times lower cell numbers, respectively (55). After ten days of infection, 40% of the total $CD4^+$ T cells in the lung spontaneously expressed GFP. Importantly, $CD4^+$ T cells in spleen and draining mesenteric lymph nodes were 15%, with a significant 5% $CD4^+$ cell accumulation in nondraining peripheral lymph nodes (51). In response to the nematode *Heligmosomoides polygyrus*, the cell infiltrate was similar to that induced by *N. brasiliensis*. In this study, the frequency of $CD4^+/GFP^+$ Th2 cells increased in peripheral blood lymphocytes and in all lymphoid organs analyzed, including the draining mesenteric lymph nodes, spleen, and Peyer's patches, but also in some tertiary sites such as the lung (56). These results show that IL-4

Table 2 Principal animal models developed to study the signaling and effects of IL-4 and IL-13[1]

Molecule	Transgenesis details	Alternative activation phenotype	References
IL-4 KO	*IL4*^{tm1Cgn}—Disruption by insertion of vector into the first exon of the gene *IL4*^{tm1Kopf}—Disruption by insertion of vector into the third exon of the gene *IL4*^{tm1Nnt}—Disruption caused by insertion of vector into exon 3 of the gene	Normal expulsion of *N. brasiliensis* Impaired induction of pulmonary granuloma formation in response to *S. mansoni* eggs Increased resistance to *Leishmania major* infection relative to BALB/c control mice Exacerbated EAE Conflicting data regarding eosinophilia in asthma	152, 153
IL-13 KO	*IL13*^{tm2Anjm}—Disruption caused by insertion of vector into exon 3	Failure to expel *N. brasiliensis* Impaired induction of pulmonary granuloma formation in response to *S. mansoni* eggs Decreased fibrosis in response to injury	154
IL-4/IL-13 KO	*IL4/IL13*^{tm3Anjm}—Deletion of a 15-kb region from exon 3 of the Il13 locus to intron 3 of the IL4 locus	Failure to expel *N. brasiliensis* Induction of pulmonary granuloma formation in response to *S. mansoni* eggs is abolished Decreased fibrosis in response to injury	154
IL-4Rα KO	*IL4ra*^{tm1Fbb}—Exons 7 through 9 replaced with a single loxP site *IL4ra*^{tm1Sz}—Insertion of vector replaced exons 7–9 of the gene	Failure to expel *N. brasiliensis* Increased resistance to *L. major* infection relative to BALB/c control mice Decreased granulomatous pathology, decreased fibrosis, and increased mortality after infection with *S. mansoni* Attenuated asthma phenotype	123
STAT6 KO	*Stat6*^{tm1Aki}—The 3 exons encoding the SH2 domain were replaced *Stat6*^{tm1Gru}—The region encoding the SH2 domain was replaced *Stat6*^{tm1Jni}—Disruption was caused by insertion of vector into the first coding exon	Failure to expel *N. brasiliensis* Abolition of eosinophilia in asthma	155
GFP^{+}IL-4^{+}	Hu-Li and colleagues engineered a gene-targeted mouse in which the first exon and 178 nucleotides of the first intron of the IL-4 gene were replaced by the green fluorescence protein (GFP)	In heterozygous mice, no impairment of the type II response required for *OVA* allergic airway inflammation despite gene disruption	50
4get	Mohrs et al. designed a bicistronic IL-4 reporter in which the GFP was introduced without deleting IL-4, designated IL-4/GFP-enhanced transcript mice (4get)	IL-4 response appears fully conserved	51
LysMCreIL-4Rα$^{-/flox}$	*IL4ra*^{tm2Fbb}—Exons 7–9 floxed by insertion of loxP sites Intercross between hemizygous IL-4Rα$^{-/flox}$ mice (bearing one floxed and one disrupted IL-4Rα allele) and transgenic LysM^{Cre} mice on an IL-4Rα$^{-/-}$ background	Increased susceptibility to infection with *S. mansoni*: increased mortality (associated with increased Th1 cytokines, hepatic and intestinal histopathology, increased NO and sepsis) but normal fibrosis Increased resistance/delayed disease progression in cutaneous leishmaniasis	132

[1]Further information can be found in the MGI website at **http://www.informatics.jax.org.**

can be produced outside the primary inflammatory locus, thus possibly affecting the activation of widely distributed MΦs in secondary and even tertiary organs. While in 4get mice the predominant myeloid cell making IL-4 is the eosinophil, the same pathogen in GFP$^+$IL-4$^+$ showed that basophils were far and away the most numerous IL-4-producing cells (57). The nature of these differences remains to be defined.

GFP$^+$IL-4$^+$ mice were also used by Chen and colleagues (47) to study the production of IL-4 in an OVA-induced lung allergy model. Despite the gene disruption in GFP$^+$IL-4$^+$ heterozygous mice, the type II response required for *OVA* allergic airway inflammation was not impaired. The authors found accumulation of GFP$^+$ cells, and thus of IL-4-positive cells, in the bronchoalveolar lavage, where the CD4$^+$GFP$^+$ cells were twice the number of CD4$^-$GFP$^+$ cells. Among the CD4$^-$GFP$^+$ population, 93% of the cells were eosinophils, 4% monocyte-like cells, and 2.6% blast-like cells. In the Th2 response induced by alum adjuvants, the IL-4 reporter signal was restricted to CD4$^+$ T cells in draining lymph nodes throughout the duration of the experiments, without apparent involvement of innate immune sources (58) (see **Table 3**).

Kang and colleagues (59) demonstrated that in addition to hemopoietic cells, adipocytes are a source of Th2 cytokines, especially IL-13. In 3T3-L1 adipocyte cell lines, expression of IL-13 is dramatically induced upon differentiation from preadipocytes to adipocytes, with similar results in differentiated human adipocytes. IL-4 was also induced in differentiated adipocyte cell lines but was undetectable in human adipocytes under the same conditions. ELISAs quantifying IL-13 and IL-4 concentrations showed that IL-13 was tenfold and fourfold higher than IL-4 in media from 3T3-L1 adipocytes and primary adipocytes, respectively. The IL-13 produced by adipocytes is relevant to the homeostasis of adipose tissue and regulation of diseases, such as control of insulin resistance associated with the metabolic syndrome.

EFFECTOR FUNCTIONS OF ALTERNATIVELY ACTIVATED MΦS IN THE TH2 IMMUNE RESPONSE

The connection of classical activation with the well-understood Th1 response, first demonstrated at cellular levels and then in molecular detail, soon made it broadly accepted. Alternative activation has remained ill defined for many years, perhaps influenced by the fact that in contrast to classical activation, it started as an in vitro paradigm, and for years little was done to understand the effector function of MΦs in vivo. The goal of the Th2 response is the elimination and control of infection by extracellular pathogens such as helminths. The size of these pathogens impedes their phagocytosis, and thus MΦs and dendritic cells (DCs) developed other functions to sample, present their antigens, and perhaps eliminate them. It has been hypothesized that Th2 immune responses control helminth infection by challenging and directly causing damage to the parasites, which in turn deviate their priorities from reproduction to defense; intense mucus production and increased muscle motility promote physical expulsion of the invading organisms. How MΦs contribute to this response should

Table 3 Summary of IL-4 and IL-13 cellular sources in diverse disease models

Cell type	Basal	Injury	Helminths	Asthma
Eosinophils	+	+++	++++++	+++
Basophils	?	?	+++	?
Th2 cells	+	–	+++	++++++
Mast cells	?	?	?	?

be a direct reflection of the changes induced by IL-4 and IL-13 in their general functions and gene expression programs, as discussed in the next section.

Direct Effects of IL-4 on MΦ Function: Phagocytosis, Endocytosis, and Autophagy

Similarly to IFN-γ, IL-4 and IL-13 modify the function of MΦs at various levels, with some mechanisms strictly dependent on direct signaling and others also requiring de novo gene expression. In contrast to IFN-γ, which increases phagocytosis via selected receptors, IL-4, through direct PI3K activation, increases the capacity for fluid-phase pinocytosis, MRC1-dependent endocytosis, as well as MRC1-independent micro- and macropinocytosis (60, 61). At the cellular level, IL-4 increases tubular vesicle formation at the pericentriolar region, associated with trafficking of recycling and early endosomes, concurrent with decreased particle sorting to lysosomes (60). The combination of IL-4 with PGE2 increases the size of early endosomes, endoplasmic reticulum, and Golgi compartments, in parallel with MRC1-mediated and fluid-phase endocytosis (62). IL-4 in combination with GM-CSF also affects lamellipodia formation in MΦs via Rac-1 activation, without consequences for the uptake of latex beads (63). In contrast, another study shows that phagocytosis of latex beads is decreased in aaMΦ (64). The endosomal stimulation by IL-4 may support increased antigen uptake and presentation of soluble extracellular antigens, providing a mechanism for cytokine-dependent enhancement of antigen uptake in bystander MΦs (60). Increased sampling of the extracellular environment by MΦs and DCs is crucial for MHC class II presentation of pathogens, such as helminths, that cannot enter the phagocytic pathway.

Autophagy, a process that resembles phagocytosis, is a catabolic mechanism involving the degradation of a cell's own components through its lysosomal machinery. Autophagy is important for innate immunity against intracellular pathogens such as *Mycobacterium tuberculosis* (65). IL-4 inhibits autophagosome formation induced by starvation and reduces the number of IFN-γ-induced autophagic vacuoles (65). Importantly, during mycobacterial infection, alternative activation of MΦs does not affect phagosome maturation, but inhibits autophagy-dependent maturation and killing of mycobacteria (66). IL-4 may spare investing energy and resources in presentation of intracellular antigens in the antihelminthic response.

IL-4 and MΦ Fusion

IL-4 and IL-13 induce MΦ fusion with formation of multinucleated giant cells in vitro (67). This phenomenon can be observed during granulomatous responses induced by infection with several pathogens, e.g., *S. mansoni* and *M. tuberculosis*, and is also present during the foreign body reaction. MΦ fusion and therefore multinucleation is also the basis of the formation of osteoclasts induced by RANKL and M-CSF (49, 50). While osteoclasts have the capacity to degrade bone, we can only speculate that IL-4- and IL-13-induced polykaryons may acquire the ability to degrade large components that cannot otherwise be internalized by individual MΦs. This may be important for the degradation of large extracellular parasites and foreign materials such as implants. However, MΦ fusion could have other functional consequences such as complementation of the properties of individual MΦs and enhanced/decreased antigen presentation, pathogen killing, or secretory activity.

The potential benefits or costs of fusion and the contribution of IL-4/IL-13 to MΦ fusion in vivo remain largely unknown. Given that alternative activation induced by IL-4 and IL-13 does not induce giant cell formation in all situations, we can postulate that extra signaling pathways are required, which could originate from the recognition by MΦs of foreign materials such as schistosomal eggs, and mycobacteria, among others (68, 69). In line with this hypothesis, adhesion to a permissive substratum but also the inflammatory status of the MΦs are

important determinants of efficient IL-4-induced giant cell formation (69). The molecular mechanism of MΦ fusion induced by IL-4 is also unknown; however, it is clear that alternative activation leads to the induction of fusogenic molecules on MΦs (69). In fact, recent reports identify in mouse the seven-transmembrane receptor DC-STAMP (dendritic cell–specific transmembrane protein) and Cadherin-E as IL-4-induced fusogenic molecules (64, 70).

IL-4 and IL-13 Gene Signatures in MΦs: A Species Matter

Although the signaling cascade induced by IL-4/IL-13 modulates key effector functions of MΦs such as endocytosis, those effects cannot be compared with the repercussions of de novo IL-4-associated gene programs (16, 71–74). These gene expression programs determine to a great extent the altered functions acquired by aaMΦs. In the past decade, information about IL-4 effects on MΦ gene expression has grown exponentially owing to the sequencing of the complete human and murine genomes and the establishment of tools to assess gene expression at full genomic level. Efforts by several investigators have yielded comprehensive gene profiles of IL-4- and IL-13-stimulated MΦs, providing novel candidate markers for alternative activation while clarifying differences among species and between these cytokines and other inflammatory factors (16, 71–74). Importantly, although the general functions and behavior of murine and human aaMΦs are expected to be highly conserved, our own unpublished data suggest that the genes modulated by IL-4 in each species are different to some extent.

Quantitatively, the changes in the MΦ transcriptome induced by IL-4 and IL-13 alone are more restricted than the gene expression programs induced by LPS or LPS in combination with IFN-γ, but similar in magnitude to the effect of IL-10, IFN-γ alone, oxidized LDL, and GCs (16). Qualitatively, although distinctive, the nature of IL-4 and IL-13 profiles at times overlaps with those of other cytokines, e.g., IL-10 and GC (16). Before discussing the main gene signatures associated with IL-4 and IL-13, note that most of our current knowledge of aaMΦ gene transcription originates from studies carried out in murine models, whereas human studies are scanty (**Table 2**). Thus, it will be crucial to keep updating this subject as further evidence is produced.

Among the group of genes extensively studied in mouse MΦs, arginase 1 (ARG1) is a prototypic alternative activation marker. The expression of ARG1 induces a shift of arginine metabolism from the IFN-γ-induced production of NO via iNOS toward production of ornithine and polyamines, which are important for wound healing (75). Similar to IFN-γ-driven tryptophan depletion, arginine depletion in IL-4-treated MΦs can also lead to inhibition of T cell proliferation (76). Although homologs exist in humans, both ARG1 and iNOS induction are confined mainly to murine MΦs (77, 78). Other murine restricted markers lack homologs in humans including YM1 and YM2 (CHI3l3 and CHI3l4), members of the chitinase family (79, 80), and FIZZ1 (found in inflammatory zone 1, RETNLA) (80, 81). YM1 and YM2 are members of the glycosyl hydrolase 18 family, which includes other hydrolases such as the acidic mammalian chitinase (AMCase), an enzyme able to hydrolyze chitin, a polymer of *N*-acetylglucosamine. Chitin is not expressed in mammalian systems but is abundant in the structural coat of fungi, the exoskeleton of many arthropods, and parasitic nematodes. Both YM1 and YM2 lack chitinase activity, and although it has been hypothesized that they function as lectin binding proteins or cytokines, no clear evidence exists in support of this function.

Human MΦs also have restricted expression of alternative activation markers. An interesting gene signature present in human but not in mouse aaMΦs is that of nucleotide G protein–coupled receptors (GPCRs). IL-4 activation is characterized by the expression of a restricted group of GPCRs, five of which constitute nucleotide and sugar nucleotide

receptors: GPR86, GPR105 (also P2Y14), P2Y8, P2Y11, and P2Y12 (16). Three of these, P2Y12, GPR105, and GPR86, are linked on chromosome 3. Preliminary data suggest that nucleotides and sugar nucleotides are released during cell injury and perhaps by pathogens, and thus IL-4 can make MΦs responsive to a whole new set of environmental signals (82, 83).

One postulate of the concept of MΦ alternative activation is the acquisition of an entirely different phagocytic receptor repertoire. Of all the murine and human alternative activation–associated receptors, MRC1 has been most comprehensively studied. It is detected in most tissue MΦs but also in hepatic and lymphatic endothelia, as well as in glomerular mesangial cells. MRC1 was identified because of its ability to recognize endogenous glycoproteins, such as lysosomal hydrolases, but MRC1 also mediates the uptake of mannosylated antigens, cell-cell contact through its interaction with L-selectin, binding and uptake of agalactosyl IgG, and importantly, pathogen recognition. MRC1 interacts with a wide range of microorganisms including the fungus *Candida albicans* and several species of mycobacteria and *Leishmania* (84). Current data suggest a role for MRC1 in cell signaling, perhaps associated mainly with inhibition of IL-12 production in response to endotoxin and production of the antiinflammatory cytokines IL-10 and IL-1Ra (IL-1β receptor antagonist) and of the nonsignaling IL-1RII (IL-1β decoy receptor) (85).

In addition to upregulating MRC1 expression, IL-4 and IL-13 regulate a broad set of phagocytic receptors. IL-4 and IL-13 upregulate MΦ scavenger receptor 1 (MSR1) with clear roles in pathogen recognition and lipoprotein clearance, the C-type lectin-like receptor Dectin-1, with specificity for β-1,3- and β-1,6-linked glucans, found in fungi and some bacteria, and a known role in TLR2 signaling (85). DC-SIGN, also an alternative activation marker, was first identified through its binding to ICAM-2 and 3, facilitating antigen presentation and costimulation (85). This receptor plays a role in innate recognition of a plethora of pathogens including *M. tuberculosis*, *C. albicans*, *Helicobacter pylori*, *Leishmania mexicana*, and *S. mansoni*. Less well described markers of human MΦ alternative activation are DCIR and DCL-1 (71, 86–89). IL-4 and IL-13 induce the expression of MGL1/2 (CLEC10A in humans) and CD23 in both species. Functionally, murine MGL has been documented to be involved in the recognition and endocytosis of galactosylated glycoproteins (90, 91), where MGL1 binds preferentially to Lewis X moieties and MGL2 shows specificity for α-GalNAc or β-GalNAc residues (92). MGL1 has been implicated in MΦ homing, granuloma maintenance (93–96), and suppression of proinflammatory cytokines (97).

The other postulate of the alternative activation concept is the acquisition by MΦs of a secretory repertoire entirely different from that of classical activation. It is well accepted that IL-4 and IL-13 mediate repression of proinflammatory cytokines and induction of an antiinflammatory milieu (16). In addition to antagonizing the secretion of IL-12, IL-1β, TNF, and IL-8 induced by classical activation and other proinflammatory factors (11, 98), IL-4 and IL-13 have other means to downregulate a pro-Th1 response. In humans, aaMΦs express less IL-1β but also more IL-1RII and IL-1Ra (99). Downregulation of caspase-1, the enzyme responsible for the activation of IL-1β, contributes to downregulation of its secretion (72); importantly, this system also processes IL-18. However, this property may change in certain conditions, in which IL-4 may increase the production of inflammatory cytokines (100). Human and murine aaMΦs secrete a number of fibrogenic factors such as fibronectin 1 (FN1) and matrix associated protein βIG-H3 (74, 101), and the coagulation factor XIII (F13A1) (102), which bears transglutaminase activity. They also produce insulin-like growth factor 1 (IGF1) (71, 103) and platelet-derived growth factor C, which provide signals for tissue proliferation and repair (101).

One function of IFN-γ is amplification of the inflammatory infiltrate, through production of selected chemokines. Importantly, one

of the stronger gene signatures also found in IL-4- and IL-13-activated MΦs is that of a distinct set of chemokines (71). Compared with other stimuli, IFN-γ and IL-4/IL-13 are the strongest for selective chemokine induction (71). IL-4 upregulates a group of six chemokines in human MΦs, including CCL13, CCL14, CCL17, CCL18, CCL22, and CCL24. Three of the upregulated human chemokines lack murine orthologs: CCL14 (only human), CCL18 (pseudogene in the mouse), and CCL23 (low homology to murine CCL6), whereas CCL17 and CCL24, which do have murine orthologs, are exclusively upregulated in humans. In mouse MΦs, IL-4 induces CCL2, which represents the homolog of human CCL13. Information about regulation of other murine chemokines is lacking. The chemokines produced by both species target a conserved set of chemokine receptors: CCR1, CCR2, CCR3, and CCR4. CCR1 and CCR2 are mainly expressed by monocytes, basophils, monocytes, and memory T cells, whereas CCR3 characterizes Th2 cells, eosinophils, and basophils, and CCR4 characterizes only Th2 cells. Furthermore, several aaMΦ CC-chemokines act as natural antagonists of the IFN-responsive chemokine CXCL10, competing with moderate affinity for the binding to CXCR3 (104). CC-chemokines such as CCL2 and CCL13 not only attract selected leukocytes, but also dampen the accumulation of mRNA for IL-12 (105) and act as activators of eosinophil effector functions (106). The role of this set of chemokines is well documented in Th2 human and murine diseases such as the immune response to *Leishmania major* (107), eosinophil-rich interstitial lung granulomas induced by antigens of *S. mansoni* eggs (108), and in asthmatic lungs (109). A species-specific diagram of the main alternative activation gene markers is shown in **Figure 2**.

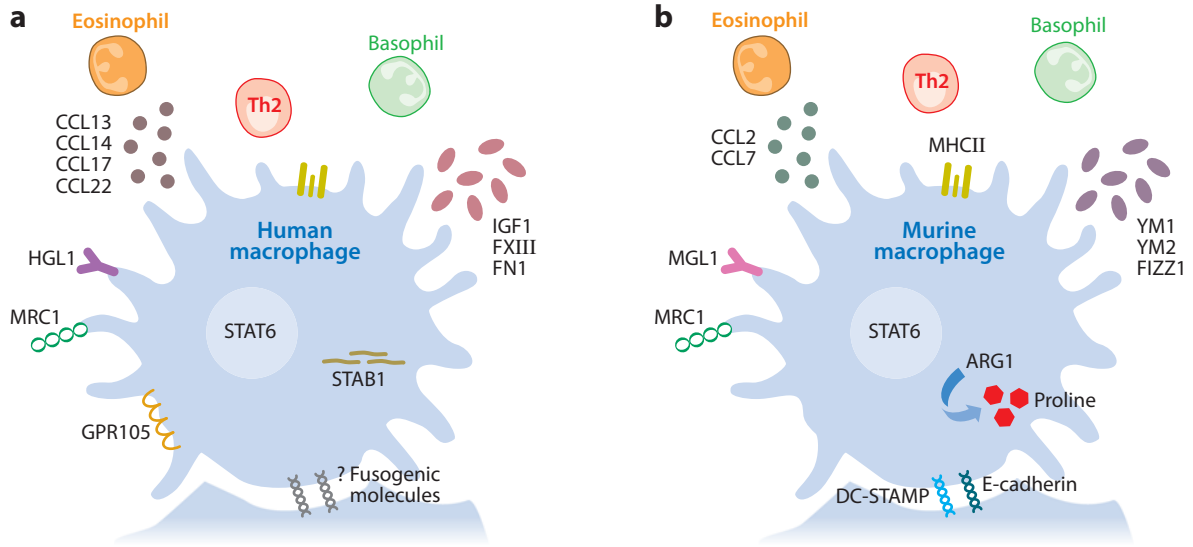

Figure 2

Human and murine macrophage alternative activation markers. MΦ alternative activation is associated with changes in MΦ function and distinctive gene signatures. Although the alternative activation markers for human (*a*) and murine (*b*) MΦs have long been considered analogous, there are differences between species. IL-4 and IL-13 induce genes conserved in both species such as MHC class II (MHCII) and MRC1, as well as divergent ones such as ARG1, YM1 in mouse, and GPR105 in humans. Despite gene evolution, there is conservation of function, i.e., different chemokines attract similar cellular infiltrates in both species. Hitherto, fusogenic molecules have been identified only in mouse. The divergences between species and their functional repercussion require further investigation.

APC: antigen-presenting cell

BONA FIDE ALTERNATIVELY ACTIVATED MΦS IN VIVO

Th2 responses are mainly directed toward extracellular parasites such as helminths and some protozoa, while creating optimal conditions for proliferation of pathogens whose elimination requires a Th1 response. Intracellular pathogens have, as a result, created intricate mechanisms to promote Th2 responses and avoid Th1 immune control. Genetic and environmental factors also affect Th2 responses, inducing a set of allergic diseases. The MΦs present in these pathologies represent bona fide examples of aaMΦs. Other non-Th2 pathologies are characterized by MΦs with very specific phenotypes, sometimes with suppressive phenotype and markers in common with IL-4- and IL-13-treated MΦs, such as endotoxin-tolerant MΦs, and tumor-associated MΦs (TAMs), among others. As a consequence, they have also been called alternatively activated MΦs, but they may not share all their features, defined above.

Necessary Alternative Activation

Helminths are parasitic worms that represent the most common infectious agent in developing countries, affecting close to three billion people. Helminths comprise two phyla: nematodes and platyhelminths, further divided into cestodes and trematodes. Protection against both phyla depends on IL-4 and IL-13. Several murine models have been used to study the immune response to gastrointestinal nematodes and the contribution and phenotype of the participating MΦs (**Table 4**).

Experimental infection by *N. brasiliensis* is performed subcutaneously, and as the parasites mature, they migrate and enter the lungs where they induce strong, polarized Th2 responses in situ and in lung-associated lymph nodes (51, 53, 54). Lung pathology is associated with a strong induction of alternative activation in alveolar MΦs, as shown by elevated expression of YM1 and FIZZ1 (40, 54). In this model, aaMΦs do not participate in worm expulsion following primary infection (110, 111), while examination of lung pathology in response to migrating larvae from *N. brasiliensis* and also in the memory response to another nematode, *H. polygyrus*, demonstrates a clear contribution of aaMΦs (111). Chronic induction of aaMΦs drives lung fibrosis, consistent with the wound-healing functions of aaMΦs induced by the filarial nematodes *B. malayi*, and also drives inhibition of inappropriate $CD4^+$ proliferative responses in the later stages of *Litomosoides sigmodontis* (112, 113). Though mice are usually able to resolve primary gastrointestinal nematode infections, reinfection with *H. polygyrus* has shown aaMΦs to be critical for induction of effective memory responses to secondary infection (111). This finding is in agreement with the requirement of antigen-presenting cells (APCs), and specifically of

Table 4 In vivo evidence for MΦ alternative activation

Pathogen	Model/host	aaMΦ marker	References
Brugia malayi	Mouse	Ym1, Fizz1, Arg1	52, 73
Schistosoma mansoni	Mouse	Ym1, Fizz1, Arg1	51, 75
Fasciola hepatica	Mouse/sheep	Ov-YM1.Mu-ARG1, chitinase activity	156, 157
Taenia crassiceps	Mouse	Ym1, Fizz1, Arg1, Mrc1	119, 158
Trichinella spiralis	Guinea pig	Arg1	159
Nippostrongylus brasiliensis	Mouse	Ym1, Fizz1, Arg1, Mrc1	160
Heligmosomoides polygyrus	Mouse	Ym1	161
Hymenolepis diminuta	Mouse	Arg1, Fizz1	162
Toxocara canis	Mouse	IL-10, TGF-β, IL-12 Null	163
Trichuris muris	Mouse	Fizz1, Mrc1	164

Abbreviations: ov-ovine, mu-murine.

MΦ-derived cytokines, as secondary signals for T cell activation. In contrast to the data for *N. brasiliensis*, in this model memory $CD4^+$ lymphocytes induce a rapid recruitment of aaMΦs to the intestine, where MΦ and arginase depletion inhibits the host's ability to expel worms.

The other major group of helminths are trematodes and cestodes. Experimental infection of mice with the trematode *S. mansoni* results in a Th2 cytokine response important for effective formation of granulomas surrounding parasite eggs, and also expulsion of eggs in the feces (114, 115). *S. mansoni*–infected IL-4R$\alpha^{-/-}$ and LysM^{Cre}IL-4R$\alpha^{-/flox}$ mice are unable to survive acute schistosomiasis, since Th2 progression confers aaMΦ-dependent protection; their absence results in a Th1 response, as seen in IL-4R$\alpha^{-/-}$ mice (110). In this setting, MΦs express the alternative activation markers ARG1, YM1, and FIZZ1. It has been proposed that aaMΦs mediate repair of tissue damage and dampen excessive inflammation resulting from traversal of parasite eggs across the intestine, and are therefore important for host survival from acute schistosomiasis (110). Similar mechanisms may operate in humans, where infection by *S. mansoni* correlates with production of TNF-α, IL-4, and IL-5. A positive correlation is found between cytokine secretion and severity of the disease, measured as periportal fibrosis versus intestinal pathology (116). Infection of mice with the cestode *Taenia crassiceps* induces a mixed Th1/Th2 immune response (117), and unlike the case of infection with the other helminths discussed, caMΦs play a major role in clearing *T. crassiceps* infection (118). However, when the infection progresses to a chronic stage, Th2 cytokine dominance results in aaMΦ recruitment that controls excessive inflammatory responses and increased $CD4^+$ apoptosis (119–122).

Unwanted Alternative Activation

Asthma is a chronic inflammatory disease of the airways characterized by IgE production, goblet cell metaplasia, airway smooth muscle proliferation and hyperplasia, and airway hyperresponsiveness, together with enhanced expression of selected cytokines and chemokines. There is overwhelming evidence that chronic allergic inflammatory processes depend on expression of the Th2 cytokines IL-4, IL-5, IL-9, and IL-13 (123, 124), highly expressed in airway tissues from asthmatic patients. In murine models of asthma, alveolar MΦs express the alternative activation markers Ym1, FIZZ1, and ARG1 (47, 125). There are other congenital noninfectious autoimmune and idiopathic inflammatory diseases, with increased levels of IL-4 and IL-13, generally accompanied by eosinophilia, impaired cellular responses, and increases in one or more B cell activities, such as hypergammaglobulinemia, autoantibody production, or particularly increased IgE production. Examples are Ommen's syndrome, the hypereosinophilic syndrome (HES), Kimura's disease, and Job's and Wells' syndromes. Unfortunately, as for most human-related pathologies, no studies address their association with alternative activation of MΦs.

Viruses benefit from a Th2 environment, and a remarkable example is that of dengue virus, a mosquito-borne flavivirus that can cause hemorrhagic fever in humans. This virus is transmitted to humans by the mosquito *Aedes aegypti*; its salivary factors induce a shift in cytokine expression with downregulation of the type I and type II IFN response and enhanced expression of Th2 cytokines (126). These Th2 cytokines not only dampen the Th1 response required for viral elimination but also induce MRC1, which binds all four serotypes of dengue virus and can be exploited by the virus to infect MΦs (127). Murine gamma herpes virus 68 (MHV-68), from the same family of the human Kaposi's sarcoma–associated herpesvirus and Epstein-Barr virus, induces latent infection in lymphoid tissue in IFN-γR KO. In these mice, infection results in transient fibrosis in multiple organs, with the spleen severely affected (128). Fibrosis is preceded by infiltration of germinal centers by different subsets of splenic MΦs. These MΦs express high levels of ARG1 and murine MΦ

markers F4/80, ER-TR9, and MOMA-1. Other genes upregulated are FIZZ1, tissue inhibitor of metalloproteinase-1, matrix metalloproteinase-12, FN1, and F13A1. This system provides an important model for studying the pathogenesis of fibrosis initiated by a latent herpesvirus infection and may explain complications that arise in the event of unwanted Th2 responses.

In fungal infections, such as the well-described Candidiasis, Th1 responses correlate with host reactivity and asymptomatic or mild forms of the infection, in contrast to the correlation between Th2 cell responses, severe disease, and the establishment of fungal allergies (129). A novel example is *Cryptococcus neoformans*–induced bronchopulmonary disease, in which IL-4- and IL-13-mediated immune mechanisms exacerbate disease. Intranasal infection with *C. neoformans* of IL-4Rα1, IL-4, and IL-13 KO animals and of IL-13 transgenic and WT mice showed that lack of the receptor or of the cytokine correlates with significantly milder pathology, whereas overexpression was correlated with higher fungal brain burden and consequent cerebral lesions. Furthermore, IL-13^{+} transgenic mice harbored large pseudocystic lesions in the CNS parenchyma, bordered by voluminous foamy aaMΦs containing intracellular cryptococci, without significant microglial activation. In WT mice, aaMΦs tightly bordered pseudocystic lesions, and these mice, in addition, showed microglial cell activation. As expected in resistant IL-4$^{-/-}$, IL-13$^{-/-}$, and IL-4Rα$^{-/-}$ mice, no alternative activation markers were discernible. Microglial cells of all mouse genotypes neither internalized cryptococci nor expressed markers of alternative activation, although they displayed similar IL-4Rα expression as MΦs. These data provide the first evidence of the development of aaMΦ in a CNS infectious disease model and point to distinct roles of MΦs versus microglia in the CNS immune response against *C. neoformans* (130).

Protozoans constitute a group of pathogens that appear to require Th1 responses to be killed. In murine models, for example, successful resolution of cutaneous infection with the protozoan *L. major* depends on the host launching an effective Th1 response, enabling caMΦs to phagocytose and kill the parasites (131–133). In the Th2-biased BALB/c, mice develop fatal progressive disease within 3 months of infection. During this time period, deletion of either IL-4 or its receptor made it possible to contain infection, with reduced footpad swelling and parasite load, moderate histopathology, and reduced Th1 response (134). However, these mutant mice were not able to heal as completely as the infected Th1-biased C57BL/6 mice, indicating that additional factors are necessary for subsequent healing and elimination of the pathogen. In longer infection time points such as 6 months, IL-4Rα$^{-/-}$ mice developed progressive disease with massive footpad swelling, ulcerative and necrotic lesions with subsequent destruction of connective tissue and bones, as well as dissemination into organs and consequent mortality. In striking contrast, IL-4$^{-/-}$ mice maintained control of infection on a moderate level but were unable to clear the pathogen, suggesting that IL-13 and not IL-4 plays a protective role during chronic leishmaniasis (135).

In general, alternative activation induced by Th2 responses against helminth infections, asthma, and other pathologies can modulate the immune response in a "bystander" fashion, perhaps explaining the high rate of concomitant infections in developing countries, where secondary infections of protozoan may be facilitated. Epidemiologic studies show common coinfections of helminth and protozoa in humans, including *Schistosoma*/leishmaniasis, *Litomosoides*/leishmaniasis, *Taenia*/leishmaniasis, *Taenia*/trypanosomiasis, filaria/malaria, or helminth/tuberculosis (136).

Non-Th2-Related Alternatively Activated MΦs: Do They Exist?

Other forms of MΦ phenotypes have been categorized as aaMΦ. These include MΦs present during tissue repair and those of several chronic pathologies such as cancer and atherosclerosis. aaMΦs are expected to have strong profibrotic

properties, as demonstrated in studies with IL-4 and IL-13 overexpression. However, no clear data show a direct correlation between these diseases and tissue repair. An interesting finding derives from a sterile tissue injury model study by Loke et al. (52), briefly discussed above. In this model, sterile sham surgery induces a wave of innate cells, mainly eosinophils, able to secrete IL-4. IL-4 secretion correlates with MΦ expression of the alternative activation markers ARG1 and YM1, among others. As such, it was proposed that activation driven by innate immune cells, may be an essential component in tissue repair. Further data are required to strengthen these observations.

TAMs have also been described as aaMΦ (137, 138). No consistent evidence connects IL-4 or IL-13 with tumor progression or the tumor microenvironment, indicating that other cytokines and mechanisms may influence the phenotype of TAMs. In murine and human TAMs, increased levels of p50, the inhibitory subunit of NF-κB, reduce the production of MyD88-dependent IL-12p70 and TNF (139, 140). Importantly, MyD88-independent IRF-3, which induces IL-10 and chemokines, is preserved, leading to dampening of the inflammation. The tumor microenvironment is essential for maintenance of this phenotype, which is lost in culture after 24 h. In accordance with the defects in the NF-κB pathway, TAMs express high levels of IL-10, Dectin-1, MGL1, TGF-β, and SRA; high levels of CXCL10, CXCL9, and other IFN-responsive genes; and low levels of IL-12, TNF-α, and NOS2 (139, 140). Thus, TAMs represent a unique MΦ population, without a clear correlation with IL-4 or IL-13 stimulation. TAMs are characterized by expression of genes in common with aaMΦs, probably the same genes that IL-4 modulates to control inflammation, but also coexpress IFN-γ-inducible chemokines and other genes (139).

Atherosclerotic plaques are characterized by a prominent MΦ infiltrate. These MΦs display a foamy appearance, owing to accumulation of intracellular lipid droplets. The role of IL-4 in atherosclerosis has been investigated in several mouse models; however, the correlation between these cytokines and the disease is not clear. While exogenous IL-4 has protective or deleterious effects, depending on the mouse model used, most KO models for IL-4 components tend to develop milder disease (141, 142). The IL-$4^{-/-}$/ApoE$^{-/-}$-mice have a significant temporary reduction in the aortic root plaque area compared with ApoE$^{-/-}$-mice. Immune cell–specific deficiency of IL-4 in hypercholesterolemic, cholate-fed female LDLR$^{-/-}$ mice provokes a reduction in atherosclerosis in the arch/thoracic regions of the aorta, but not in the aortic root. Accelerated fatty streak formation induced by heat shock protein 65 or *M. tuberculosis* is reduced in IL-$4^{-/-}$ mice compared with lesions in WT mice. Taken together, the majority of findings would suggest a proatherogenic role for endogenous IL-4; this effect appears to be greatly influenced by the vascular site and the disease stage. Importantly, although IL-4 may play a role in the disease itself, in vitro studies of human and mouse foam cell MΦ models do not show a significant overlap between the gene expression profile of these cells and aaMΦs.

MΦ ACTIVATION REVISITED

MΦs appear early in development, occupying strategic positions in different organs, and they persist throughout adult life. As part of their innate immune role, they act as sentinels in tissues, encountering pathogens and orchestrating the attraction and development of more complex acquired responses. MΦs are not the only effector cells of the immune system; DCs, neutrophils, basophils, eosinophils, and T and B cells all participate in the defense against pathogens. As such, the view of the immune response as mere interaction between the APC and the T cell is somewhat limited. DCs and MΦs recognize pathogen or injury signals and then provide signals to initiate the inflammatory response. This initial interaction between pathogen-conserved moieties and the MΦ induces a primed state in MΦs. The receptors for this initial activation include mainly PRRs for PAMPs, such as TLRs, NALPs, and other

Table 5 Macrophage signaling receptors in the context of Th1 or Th2 responses

Selected receptors	Role	Ligands	IL-12/IL-10 balance contribution
		Interleukin-1 and Toll-Like Receptor Superfamily	
		• TLRs are the best characterized signal-generating receptors among the PRRs; they initiate key inflammatory responses and also shape adaptive pro-Th1 immunity • TLRs initiate shared and distinct signaling pathways by recruiting different combinations of the TIR domain–containing adaptor molecules: MyD88, TIRAP (MAL), TRIF (TICAM1), and TRAM • Strong first stimulation of the TLR can induce TLR-signaling inhibitors, such as MyD88s (a splice variant of MyD88) and IRAKM, and as a result inhibit signaling by the second TLR stimulation until the inhibitors disappear, whereas weak sequential TLR stimulations can boost the signaling and thus the immune response	
TLR4	Critical for host defense against Gram-negative bacteria in both mice and humans	LPS from Gram-negative bacteria, mannan from *Candida albicans*, GIPLs from *Trypanosoma*, viral envelope proteins from RSV and MMTV	TLR4 stimulation induces in MΦ the expression of inflammatory cytokines such as IL-6, type I IFNs, TNF-α, and importantly IL-12. However, context-dependent TLR4-PI3K stimulation may induce IL-10 and inhibit IL-12 in DCs
TLR1, 2, 6	TLR2 is essential for host defense against Gram-positive bacteria. This receptor collaborates with other TLRs or non-TLR PRRs, and this collaboration can also modulate signaling outcomes	TLR1: triacyl lipopeptides from bacteria and mycobacteria. TLR2: LTA from Gram-positive bacteria, yeast zymosan, lipopeptides (Pam3CSK4, MALP2), lipoarabinomannan from mycobacteria TLR6: diacyl lipopeptides from Mycoplasma, LTA from Gram-positive bacteria, yeast zymosan	In DCs, prolonged ERK activation and the subsequent, enhanced induction of c-Fos (an AP-1 component) upon TLR2 stimulation seems to play a significant role in the production of a high level of IL-10 and little IL-12
		NOD-Like Receptor Family	
		• NLRs comprise a family of cytosolic proteins that play a pivotal role in the recognition of intracellular PAMPs, mediating protective immune responses elicited by intracellular pathogens or endogenous danger signals, and act in synergy with various TLRs to enhance immune responses in APCs	
NOD1, 2	NOD1: acts as a sensor for Gram-negative bacteria NOD2: acts as a general sensor for most bacteria	NOD1: recognizes the peptide γ-D-glutamyl-meso-diaminopimelic acid, and mainly acts as a sensor for Gram-negative bacteria NOD2: recognizes muramyl dipeptide MDP, NAG-NAM-L-alanyl-isoglutamine	NOD1 and NOD2 act in synergy with TLR3, 4, and 9 in human DCs to induce IL-12p70 production and promote Th1 cell differentiation

(*Continued*)

Table 5 (*Continued*)

Selected receptors	Role	Ligands	IL-12/IL-10 balance contribution
		C-Type Lectin Receptor Family	
	• The C-type lectins recognize specific pathogen-associated carbohydrate structures • C-type lectins were originally named to reflect the special importance of Ca^{2+} in carbohydrate binding		
MRC1	MRC1 displays Ca^{2+}-dependent lectin activity toward terminal mannose and acts as general sensor for different intracellular parasites and phagocytic receptors	Endogenous and exogenous ligands bearing mannose, fucose, N-acetyl glucosamine, and sulfated sugars	Stimulation with the anti-mannose receptor antibody blocks induction of the IL-12
Dectin-1	Appears to deal primarily with fungal infection although it may also recognize other ligands	Has specificity for β-1,3- and -1,6-linked glucans found in fungi, plant cell walls, and some bacteria but does not have affinity for monosaccharides or glucans with different linkages	Dectin-1 and TLR2 collaborate synergistically to downregulate proinflammatory cytokines such as TNF-α and/or IL-12 and induce IL-10 production
		Nuclear Receptors	
	• Many nuclear receptors such as glucocorticoid receptor (GR), PPARs, and LXRs have strong antiinflammatory functions that are mediated by negatively regulating the expression of certain inflammatory responsive genes • Ligand-dependent transrepression is thought to be the major mechanism for such gene regulation		
VDR	Vit D3 is essential for calcium and phosphorus homeostasis	$1,25_{OH}2D3$	VDR stimulation inhibits IL-12 production by interfering with NF-κB signaling
RXR	Vit A is a known potentiator of the immune system	9-*cis*-RA, FAs, methoprene acid, DHA	Retinoic acid enhances the production of IL-10 while reducing IL-12 secretion
		Complement Receptors	
	• The complement inflammatory cascade is part of the phylogenetically ancient innate immune response and is crucial for our natural ability to ward off infection • It is important for defense against microbial infections because it triggers the generation of a membranolytic complex (C5b9 complex) at the surface of the pathogen and C fragments (named opsonins, i.e., C1q, C3b, and iC3b) that interact with C cell surface receptors (CR1, CR3, and CR4) to promote phagocytosis • Soluble C anaphylatoxins (C4a, C3a, and C5a) greatly control the local proinflammatory response through chemotaxis and activation of leukocytes		
gC1qR	gC1qR plays a pivotal role in the regulation of inflammatory and antiviral T cell responses	C1q	C1q can downregulate the TLR4-mediated production of IL-12 but not of other inflammatory cytokines such as IL-6 or TNF-α in human monocytes
C5aR	C5a is a potent chemoattractant for neutrophils and monocytes, and signaling is mediated by a 7TM GPCR	C5a	Selectively suppresses TLR4-mediated expression of IL-12 family members IL-12, IL-23, and IL-27, but not of other cytokines such as TNF-α or IL-10

Table 6 Comparison between the best-described MΦ activation phenotypes

	Receptor complex stimulated					
Function	**IL-4RI, II**	**IL-10R**	**GCR**	**TLR4**	**FcR$^+$TLR4**	**IFN-γ$^+$TLR4**
Phagocytosis/ endocytosis	IL-4 increases the expression of MRC1, SR-A, Dectin-1, DC-SIGN, DCIR, DCL-1, and CLECSF13. IL-4/IL-13 increases endocytosis of mannosylated ligands and other receptor-mediated endocytic processes, and downregulates latex bead phagocytosis.	IL-10 increases the expression of FcγRI, II, and III, Marco, and CD163. Exposure of monocytes to a combination of IL-10 and IL-4 results in a synergistic effect on CR-mediated ingestion without membrane expression changes. Anti-IL-10 mAb significantly reduces monocytes' capacity to ingest IgG- or C3b/C3bi-coated particles, suggesting a role for IL-10.	GCs increase the expression of MRC1 and CD163. GCs increase phagocytosis of latex beads, complement-mediated endocytosis, as well as phagocytosis of apoptotic cells.	TLR signaling enhances phagosome maturation, regulates the expression of phagocytic receptors, such as SRA and MARCO, and induces activating Fcγ receptor expression.	No information regarding phagocytosis was found. However, these macrophages are well-known producers of IL-10, which may recreate a prophagocytic profile.	Increases complement secretion and complement receptor surface expression on mononuclear phagocytes, enhancing complement-mediated phagocytosis.
Antigen presentation	Increases MHC class II and presentation to Th2 cells	Inhibits presentation through downregulation of MHC class II and costimulatory molecules	GCs inhibit antigen processing and presentation by several mechanisms, including proteasome suppression	Increases antigen presentation through upregulation of costimulatory molecules. TLRs have a critical role in selecting antigens for presentation via MHC class II molecules after the phagocytosis of particles	Type II MΦs express high levels of costimulatory molecules and are efficient APC to Th2 cells	Increases class I and II antigen-presenting complexes and costimulatory molecules. Also stimulates de novo synthesis of the immunoproteasome. MHC class II loading is increased by synthesis of cathepsins B, H, and L

Production of chemokines	Induces chemokines such as CCL2, CCL13, and CCL17, which coordinate the recruitment of eosinophils, basophils, and some polarized Th2 cells, through activity on CCR3 and is involved in proangiogenic networks	Increases production of CXCL13, CXCL4, CCL23, and CCL18	Inhibits production of the Th1 chemokines CXCL9, CXCL10, CXCL11, CCL5, and CCL24	Directly increases the production of CCL2, CCL3, and CCL5. Induces CXCL9, CXCL10, and CXCL11 through IFN-β production	Increases production of CCL1, CXCL3, and CCL20	Increases production of CXCL9, CXCL10, CXCL11, and CCL2-5 and is known to recruit monocytes, Tc, and Th1 cells
Cytokine production	Induces IL-1Ra, IL-10, TGF-β and antagonizes LPS- and IFN-responsive chemokines	Induces IL-10 and antagonizes most LPS- and IFN-responsive chemokines	Induces IL-10 and TGF and antagonizes most LPS-, IL-4- and IFN-responsive chemokines	Induces IFN-β, IL-6, TNF-α, IL-1β	Induces switch from IL-12 to IL-10 production	IL-12, IL-6, TNF-α, IL-1β, IL-15
Microbicidal properties	Decreases microbicidal functions in MΦs	Decreases microbicidal functions in MΦs	Decreases microbicidal functions in MΦs	Induces NADPH oxidase complex (ROS generation)	Not determined	Induces ROS and RNI generation. Other induced mechanisms are tryptophan depletion by IDO
Cell fusion	Induces cell fusion	Does not induce fusion	Does not induce fusion	Does not induce fusion	Not determined	Does not induce fusion

surface and cytosolic receptors. The best known PRRs are often responsible for an innate pro-Th1 form activation of MΦs based on the selective expression of IL-12, decreased IL-10 levels, and expression of chemokines and other factors that mediate recruitment and activation of selective accessory cells, as NK cells, eosinophils, and basophils, among others (**Table 5**). Little is known about PRRs that initiate Th2 responses, although a few novel results demonstrate the presence of pro-Th2 PAMPs and TLRs in parasites and MΦs, respectively.

With the recruitment of IFN-γ- or IL-4 secreting cells of the innate system, conditions are created for the further development of either a Th1 or a Th2 milieu. The appearance in the inflammatory focus of either of these cytokines induces either classical or alternative activation, first in an innate manner (given the origin of the cytokine) and subsequently in an acquired process (**Figure 3**). For T cells, a two-signal activation model is currently accepted, in which the first signal originates from ligation of the T cell receptor (TCR) by APC MHC-peptide and the second signal, in the form of a cytokine, is provided by the APC, MΦs, or DCs (**Figure 3**). Thus, T cell and MΦ activation seems to occur in an interdependent two-step fashion. This staged activation may also apply to DCs. Significantly, in addition to IFN-γ or IL-4/IL-13 signal recognition complexes, there is a group of MΦ signaling receptors whose ligation also leads to MΦ activation. These receptors respond to a wide variety of stimuli of both innate and acquired origin, including the cytokines IL-10, IL-1, IL-6, TNF, IL-17, and

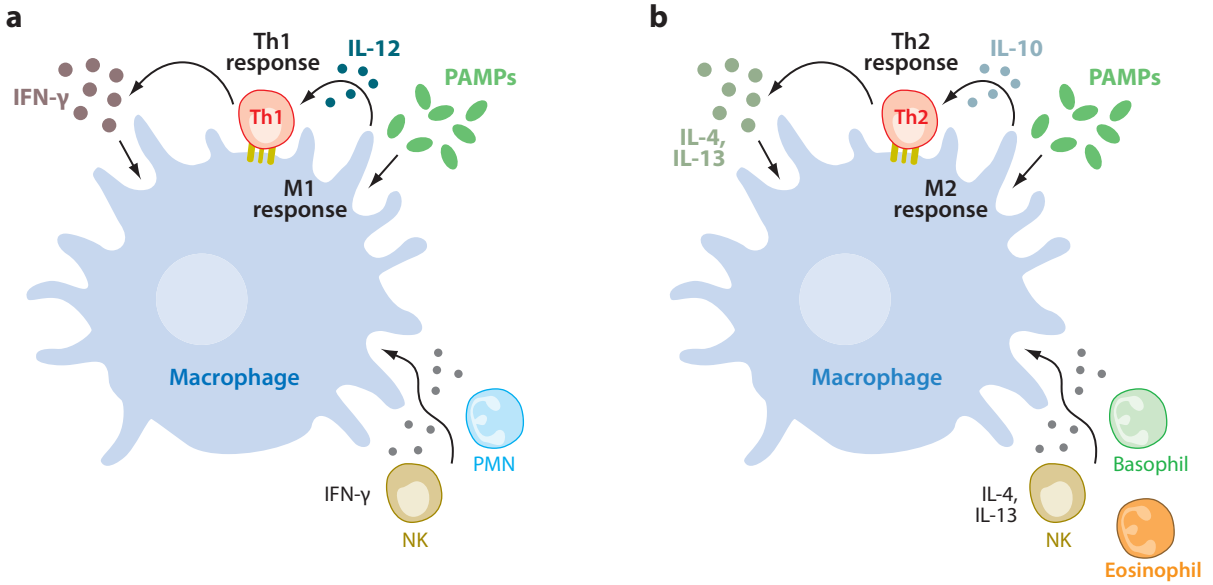

Figure 3

Two-signal macrophage activation model in the context of the immune response. MΦ full activation requires two major signals, recognition of PAMPs by PRRs and stimulation with IFN-γ or IL-4, in a cycle that determines the development of specificity and effector functions in the immune response. For Th1 responses (*a*), PRR-induced chemokines attract IFN-γ-producing innate immune NK cells and also naive T lymphocytes. Innate IFN-γ and PAMPs induce a first wave of classical activation in MΦs, stimulating IL-12 secretion, an important signal for Th1 activation. With Th1 activation, greater levels of IFN-γ induce long-lasting classical activation of MΦs while a full cytotoxic T cell response is mounted. For Th2 responses (*b*), uncharacterized PRRs induce the recruitment of IL-4 and IL-13, producing innate immune cells such as eosinophils and basophils and naive T lymphocytes. IL-4 and IL-13 produced by innate immune cells induce a first wave of innate alternative activation in MΦs but also provide the secondary signal for Th2 development. Innate IL-4 and PAMPs may synergize to stimulate IL-10 secretion from MΦs, which provides a signal for Th1 repression. IL-10 produced by MΦs may also induce the development of repressor T cells, which oppose Th1 activation.

IL-25. Stimuli such as IL-10, GC, and Fc receptor ligands induce responses biased toward a Th2 profile, and they have been grouped as part of alternative activation. The phenotype induced in MΦs by this last group of stimuli overlaps partially with that of IL-4 or IL-13, but only in limited genes and features (**Table 6**).

Confusion arises from the fact that the term "activation" may be correctly used at both an immune response level, as we use it throughout this manuscript, and from a wider MΦ-centered point of view. In the first application, activation is seen as the acquisition of resistance against specific pathogens, in response to clearly discernible arms of acquired immunity, Th1 and Th2 responses (**Figure 3**), whereas in the second case, it is considered as any stimulus that moves MΦs from their resting condition (**Table 5**). At the level of the immune response are Th1, Th2, Th17, and a set of immune-suppressive T cells, whose main characteristic is their mutual exclusion (143). These immune response specificity–determining cytokines have evolved to deal with either activation against specific pathogens or deactivation of the immune response and resolution. As such, it is natural to suppose that they induce four main effector phenotypes in MΦs. While classical and alternative activation of MΦs and their co-T cell Th1 and Th2 partners are in part understood, the role of IL-17 and other polarized T cell products in MΦs remains largely unknown. The other stimuli are also important because they play a definitive role in modification of the MΦ phenotype in the course of an immune response and may be abundant in specific pathologies; however, they do not represent an immunologic equivalent to the Th cytokines.

In 1999, Goerdt and colleagues (144) proposed a classification of activation phenotypes based on grouping all activators other than IFN-γ and LPS/microorganisms into a common alternative activation group. This classification overlooks important immunological differences in the response to modulators such as IL-4, IL-13, IL-10, glucocorticoids (GC) and TGF. For this reason, in 2002 an extended classification was proposed in which M1 polarization included the classical activation, whereas M2 polarization was subdivided into M2a, corresponding to aaMΦ; M2b, corresponding to type II–activated MΦs; and M2c, which includes heterogeneous MΦ deactivation stimuli (145). The classification took into account the production of IL-10 or IL-12, which by themselves are not enough to categorize the phenotypes. Nevertheless, the classification M1/M2 is useful because it avoids using the terms alternative and classical, which can create confusion. In addition, M1 and M2 are mnemonic and provide a clear link to the T cell response. We suggest keeping classical/M1 or alternative/M2 as synonyms for those categories, and use the ligand/ligands to identify the other phenotypes until a consensus is reached in the community. We strongly suggest avoiding the grouping of stimuli other than IL-4 and IL-13 under the M2/alternative group.

CONCLUDING REMARKS

We have reviewed recent information regarding the effects of IL-4 and IL-13 and have summarized the main evidence while analyzing the robustness of alternative activation of MΦs as an immunological paradigm. IL-4 and IL-13 can be actively produced by both innate and acquired immune system cells. Their in vivo production may not be restricted to the primary inflammatory locus. Production of IL-4 by innate cells allows induction of aaMΦs prior to the secondary immune response. The innate production of IL-4 is important for tissue repair and complements IL-4 production by acquired immune system cells in the control of specific pathogens.

IL-4 and IL-13 pathways have convergent and divergent features, accounting for differences and similarities in the phenotype induced in MΦs. Even the identical type II receptor heterodimer, coupled to the same intracellular signaling molecules, responds to IL-4 and IL-13 with different signaling potencies and kinetics. It is the balance between activation of IL-4 type I and type II receptors and IL-13Rα2 signaling

cascades and their relation with other autocrine, paracrine, and cell-cell contact pathways that determines the phenotype of aaMΦs.

IL-4 and IL-13 modify the function of MΦs at various levels, with some mechanisms strictly dependent on direct signaling and others also requiring de novo gene expression. Gene expression studies have demonstrated that the effect of IL-4 or IL-13 in MΦs is not restricted to isolated genes, but instead involves specific and distinguishable gene signatures. In all, the number of well-characterized specific alternative activation markers does not exceed a dozen. Although the general functions and behavior of murine and human aaMΦs are thought to be preserved, the genes required for such functions are to some extent different. IL-4 and IL-13 induce varied effects on human and murine MΦs.

The functional significance of genes induced by IL-4 and IL-13 is difficult to interpret owing to the restricted information available. However, these cells clearly play a key role in the attraction of cells to inflammatory foci, suppression of Th1 responses, sampling of the microenvironment by endocytosis, and orchestration of tissue repair. Their role in pathogen killing is less clear. The combination of cytoskeletal changes induced by IL-4 and the receptor repertoire of IL-4-treated MΦs makes possible an altered phagocytic and secretory capacity in response to viruses, bacteria, fungi, and helminths, not always with positive consequences for the MΦ or host.

FUTURE ISSUES

The paradigm of alternative activation of MΦs now emerges with greater clarity. The development of novel transgenic mice, high-throughput gene expression and protein-detection techniques, infection models, and other advances have improved our understanding of the Th2 response, the cells that participate in it, and their cross talk, as well as the effector functions of aaMΦs.

Human and murine MΦs, although having functions in common, clearly express different genes in response to both IL-4 and IL-13. Given the feasibility and versatility of mouse models, most of our knowledge derives from them and not from human studies. Considering the differences between species, we must investigate human alternative MΦ activation in more depth, both in vitro and in vivo. Validation of specific markers, gene silencing, and transfection of human primary MΦs may become powerful tools for this purpose.

Initiation of the Th1 response, the link between microbial PAMPs and production of proinflammatory cytokines, and the killing capacity of caMΦs are more or less well understood. However, little is known about the extracellular parasite PAMPs, their recognition by APCs, and the link to initiation of the Th2 response and cytokine production. Similarly, many of the genes and functions of aaMΦs, especially their antiparasitic properties, are still unidentified. Further studies are required to understand host defense against such parasites.

Accumulating evidence suggests that MΦs play major roles in pathology, and several of their products and effector functions make them ideal therapeutic targets. We have reviewed a number of diseases that result from dysregulation of the Th2 response. Similarly, many diseases arise from dysregulation of the Th1 response. To date, the therapeutic interventions that target MΦs rather than their products, e.g., TNF, are limited (146), although a number of MΦ specific drugs are emerging. Cell therapy is also a future possibility, and studies are beginning to assess the beneficial effects of ex vivo MΦ activation and transfusion, with encouraging results (147, 148).

SUMMARY POINTS

1. The finding of a MΦ classical activation, induced by IFN-γ in response to infection with unrelated intracellular pathogens, represents one of the turning points in MΦ biology

and innate immunology, given that it demonstrated the presence of a stimulus-dependent but antigen-nonspecific defense program, true for the diverse forms of MΦ responses found hitherto.

2. The discovery that IL-4 induced MHC class II and MRC1 in MΦs led to the original definition of alternative activation. A current view has replaced the "on/off" model of MΦ activation in pathogen defense with a more complex one in which MΦs exist in resting, classically, or alternatively activated forms.
3. The concepts of classical and alternative MΦ activation involving IFN-γ and IL-4 refer to the acquisition of resistance against specific pathogens in response to clearly discernible arms of the acquired immunity: Th1 and Th2 responses.
4. The pathways of IL-4 and IL-13 have convergent and divergent features, which account for differences and similarities in the phenotype induced in MΦs. It is the balance between activation of IL-4 type I and type II receptors and IL-13Rα2 signaling cascades in relation to other autocrine, paracrine, and cell-cell contact–induced pathways that finally determines the phenotype of aaMΦs.
5. IL-4 and IL-13 can be produced by both innate and acquired immune system cells. Their in vivo production may not be restricted to the primary inflammatory locus.
6. Innate production of IL-4 is likely to be important for tissue repair and to complement IL-4 production by acquired immune cells in the control of specific pathogens.
7. Gene expression studies have demonstrated that the effect of IL-4 or IL-13 in MΦs is not restricted to isolated genes, but instead involves specific and distinguishable gene signatures. These gene signatures depend on the genetic background, maturation, and activation state of cells.
8. Although the general functions and behavior of murine and human aaMΦs are thought to be preserved, the genes required for such functions are to some extent different.
9. Phagocytic receptors expressed by aaMΦs differ from those expressed by caMΦs. Although not fully demonstrated, a common dual feature of these receptors seems to be pathogen and self recognition, leading to increased ligand endocytosis, but also interaction with T cells and negative immune modulation.
10. aaMΦs play a key role in the attraction of other cells to inflammatory foci, suppression of Th1 responses, sampling by endocytosis of the microenvironment, and orchestration of tissue repair.
11. There is an unaccounted degree of divergence between species, still to be determined.
12. Confirmation of aaMΦ markers in studies regarding Th2 responses has become a necessary readout to link cytokine production to the development of immune effector functions.
13. Presence of alternatively activated macrophages has been confirmed in several helminthic and allergic disease models.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We would like to thank all the colleagues who provided information for this review. Fernando O. Martinez Estrada and Laura Helming were supported by grants from the MRC, United Kingdom.

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