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Macrophage Polarisation: A collaboration of Differentiation, Activation and Pre-Programming?

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Abstract

Macrophages (Mϕs) exhibit a sliding scale of functional heterogeneity ranging from pro-inflammatory, immune activatory and anti-tumoral responses to anti-inflammatory, regulatory and pro-tumoral activity. These effector responses are reflected in distinct Mϕ subsets; the M1/classically activated- and M2/alternatively activated subsets. The functional diversity is determined by the combination of Mϕ subset differentiation, activation, signalling and pre-programming in separate monocyte subsets. This diversity in Mϕ subset and functionality is also reflected in mucosal pathologies associated with chronic inflammation (Crohn's disease, chronic periodontitis) and immunosuppression observed in solid tumours (oral squamous cell carcinoma). The relative functional plasticity between these monocytes and Mϕs represents a realistic therapeutic regimen in the treatment of these Mϕ-driven diseases. This review will discuss the research evidence that is suggestive of the manipulation of Mϕ polarisation plasticity through pre-programming, differentiation, activation and tolerisation in the therapeutic intervention for chronic inflammation and solid tumours.

Introduction

Macrophages (Mϕs) are phagocytic cells of the innate immune system that are present in most tissues of the human body. These cells exhibit a wide variety of functional characteristics including phagocytic clearance, microbial killing, antigen processing and presentation, inflammation, anti-inflammatory processes, tissue repair and immune suppression. This diversity of immune functionality is reflected in macrophage subset heterogeneity. Current understanding categorises Mϕ subsets according to activation status (classical or alternative) or differentiation (M1 or M2). There are however, subtle and not-so-subtle differences in Mϕ subsets and their phenotypic markers when comparing murine and human Mϕ systems, which have been described in other seminal reviews [1]. At this stage, clear delineation of murine Mϕ biology from that of humans would weaken the overall understanding of Mϕ biology and, as such, this review considers the combined contribution of murine and human Mϕ research. This review will focus on the functional role of Mϕ subsets driving immune responses with respect to differentiation and activation stimuli encountered in host tissues as part of homeostatic and pathogenic conditions. Finally, macrophage subset effector responses may already be pre-programmed in the monocyte. Current research suggests that differential effector responses are reflected by classical, intermediate and non-classical monocytes. The overall functional impact of tissue macrophages is likely to be reflected by a subtle balance between pre-programmed monocytes, route of Mϕ differentiation and the activation/suppressive signals encountered in the local environment; impacting on distinct Mϕ effector subset polarisation or switching between functional subsets as a consequence of plasticity.

Macrophage subsets and effector phenotypes

Macrophages exhibit a range of functional characteristics which include: 1) sampling of the local environment, 2) killing of pathogens, 3) inflammation, 4) tissue repair, 5) anti-inflammatory responses or immune-suppression, 6) instruction and development of specific adaptive immunity via antigen processing and presentation and 7) mobilisation of other innate cells (Nϕs & NKs) and adaptive cells which amplify responses at site of infectious/injurious challenge [reviewed in 2]. Mϕs are tissue resident cells, whose behaviour is shaped by the very environment that they inhabit. These tissue Mϕs can be replenished either locally via self-renewal/proliferation or from the periphery via bone marrow-derived monocytes [3-6]. It is this localised tissue distribution that makes the Mϕ an efficient and central responding cell, driving rapid responses to pathogenic infection, tissue injury and repair [7,8].

The local tissue environment determines Mϕ effector function as a consequence of a wide variety of activation and differentiation stimuli. This diversity of stimuli results in Mϕ polarisation and the resulting subsets being described as classically or alternatively activated Mϕs, originally described to be activated by IFNγ/LPS and IL4/IL-13 respectively [9,10]. In addition to activation determining Mϕ polarisation and functionality, several groups have described Mϕ subsets to be dependent on differentiation pathways and possibly pre-programmed. Early studies investigating murine immune responses to Leishmania infection demonstrated the C57Bl/6 strain to be resistant (Th_1-mediated CMI predominates) whereas Balb/c mice were susceptible (predominated by a Th_2-mediated humoral response). This variation in response to infection was found to be determined by the Mϕs rather than the T cell subset [11]. This predominance of Mϕ response observed in this study lead to the description of M1 and M2 subsets where M1 Mϕs activated T cells to secrete IFNγ and the resulting stimulation of Tc and positive feedback to M1. In contrast,

M2 Mϕs induced T cells to produce Th_2-like cytokines (IL-4 and TGFβ) resulting in humoral responses and amplification of M2 activity [12-14]. This latter amplification of M2 activity resulting in walling off pathogens, as a consequence of matrix deposition and fibrosis. Thus, specific responses to pathogen infection are Mϕ-determined which help tune and are in-turn finely tuned by T cells. Finally, these M1/Th_1 and M2/Th_2 responses may both occur for optimally dealing with infection simultaneously or at different progression phases of the pathology [15,16]. M1 Mϕs are generally considered to be the predominant subset involved in pathogen killing, hence host defence whereas the M2 subset is associated with repair and maintenance of tissue integrity. Mϕs are pivotal to directing the immune response where M1s drive T cells towards Th_1 cell-mediated immunity (CMI) and M2s towards Th_2-mediated humoral activity.

The host is under constant challenge by a wide variety of pathogens. The macrophage deals with this ever-changing pathogenic challenge by retaining a heterogeneous functionality through a level of fluidity or plasticity. The degree of plasticity between homeostatic M2 Mϕs and M1 Mϕs is a possible explanation for the ever-increasing number of Mϕ subsets described in the literature. Thus far, Mϕ heterogeneity has resulted in the description of classically activated, M1s, alternatively activated, M2a, M2b, M2c, M2d and regulatory Mϕs [17-19]. Such a variety of subsets exhibiting specific functional heterogeneity has yet to be described in vivo. A likely explanation for this apparent variety of subsets/functionality can either is as a consequence of varying proportions of M1 and M2 Mϕs existing as a heterogeneous population or that these different subsets may be intermediates in a sliding scale of plasticity between homeostatic M2 Mϕs and the M1 Mϕ. At this stage of our understanding however, these other subsets cannot be ignored (Table 1). M2a (alternative) and M2c (deactivated) are induced by IL4/IL13 and IL10/TGFβ/glucocorticoids respectively, both express arginase activity that is associated with the more conventional M2 subset. Both of these Mϕ types also express IL-10^{hi} IL-12^{lo} and the scavenger receptor, MR. The M2b (type II) Mϕ is induced by immune complex recognition as well as LPS and IL-1β; this Mϕ expresses a similar cytokine profile but differs from M2a and M2c by virtue of expression of iNOS, normally associated with the M1 Mϕ subset [reviewed in 8]. This expression of iNOS, yet display of an anti-inflammatory cytokine profile may be suggestive that M2b may represent an intermediate "plastic" state between the canonical M1 and M2 subsets. The M2d Mϕ subset was described for an adenosine-mediated switch in phenotype to an M2-like cell. This subset polarisation resulting from the synergistic activation by A2R agonists in combination with agonists of TLR2, TLR4, TLR7 or TLR9; where the new M2d effector subset exhibited a phenotype: IL-10^{hi} $VEGF^{hi}$ $iNOS^{hi}$ $IL12^{lo}$ $TNFα^{lo}$ and elevated Arg-1 expression [20,21]. These Mϕs do not express Ym-1, FIZZ-1 or CD206 but, again, exhibit a phenotype, which falls between the canonical M1 and M2 subsets; whether this Mϕ is proven to exist as a distinct subset or merely an intermediate awaits clarification. In a separate study, rather confusingly, Duluc et al. described an ovarian TAM phenotype, which was also proposed as M2d [22]. This subset was polarised by LIF, IL-6 and OSM and exhibited a regulatory/immunosuppressive phenotype: $CD14^{hi}$ $CD163^{hi}$ $CD80^{lo}$ $CD86^{lo}$ $ILT2^{hi}$ $ILT3^{hi}$ $IL10^{hi}$ $TNFα^{lo}$ $IL12^{lo}$ $CCL18^{hi}$ $PTX3^{lo}$ $CCL1^{lo}$ $CCL17^{lo}$ $CCL22^{lo}$. In addition, this suppressive subset also expressed IDO, VEGF, TGFβ and B7-H4 whereas iNOS and Arg-1 were not detected. In contrast, the A2R/TLR-polarised M2d subset was found to express both iNOS and Arg-1, suggestive that these two Mϕ subsets are phenotypically and functionally distinct.

Additionally, further Mϕ subsets have been reported, especially associated with investigation of inflammatory pathologies such as atherosclerosis. Whether these further subsets present themselves in the context of mucosal pathology awaits characterisation. These atherosclerotic associated subsets include M4, Mox, HA-mac, M(Hb) and Mhem [23,24]. M4 Mϕs have been shown to be distinct from M1 and M2 phenotypes, where polarisation is induced by the atherosclerotic chemokine, Platelet Factor 4 (PF4) or CXCL4, resulting in a phenotype: MR^{hi} $CD36^{lo}$ $CCL22^{hi}$ $TNFSF10/TRAIL^{hi}$ $TNFα^{hi/lo}$ $IL10^{lo}$ $CD86^{hi}$ $MMP7^{hi}$ $MMP12^{hi}$ which is poorly phagocytic [25]. Mox subset refers to a phenotype of oxidised Mϕs found in atherosclerotic lesions. This phenotype is polarised by oxidised phospholipids such as oxLDLs and express the Nrf2-dependent redox-regulated gene product, heme oxygenase-1 (HO-1) and both anti-inflammatory IL-10 and pro-inflammatory IL-1β [26]. The final putative subsets described in the case of atherosclerosis are HA-mac, M(Hb) and Mhem; all of which are polarised by either haem or haemoglobin and express CD163. HA-mac were first described by Boyle et al and were found to be located in the hemmorhagic zones of plaques and defined as $CD163^{hi}$ whereas these Mϕs were low expressors of HLA-DR, thus are relatively poor antigen presenting cells [27]. In addition, HA-macs exhibit anti-oxidant and anti-inflammatory behaviour, where HO-1 and IL-10 are expressed and polarisation is Nrf2-dependent; resulting in tissue repair and a reduced capacity to form foam cells [28,29]. Thus the Mϕs involved in this inflammatory disease, Mox, HA-mac, M(Hb) and Mhem, are polarised by the local environment and express phenotypes that portray both pro- and anti-atherogenic functionality [23,24]. Whether this functional dichotomy exists in so many distinct subsets in other pathologies remains to be illucidated. Currently, the plasticity exhibited between M1 and M2 subsets is clear in the context of mucosal homeostasis and disease states; the existence of M4 and Mox cells may not be involved in mucosal tissues whereas homeostatic and disease-induced intermediates between these two canonical subsets may well parallel these extra subsets described in both tumour- and atherosclerosis-associated Mϕs.

Macrophages can generally be categorised by their ability to metabolise arginine. Mϕs exhibit a specialised biochemical system utilising L-arginine that allows for functional plasticity between M2 homeostatic subset and M1 function, capable of killing of pathogens and pathogen-infected host cells and cancer cells [11,12,30]. Nitric oxide (NO) is produced by Mϕs as part of the innate system's killing response to pathogens. The very nature of innate responses and non-specificity of NO-killing can result in collateral damage to host tissues/cells [31-33]. Thus, Mϕ subsets have been categorised by the expression and activity of the arginine-metabolising enzymes, inducible nitric oxide synthase (iNOS) and arginase-1 (Arg-1). Arg-1 is a signature molecule for the activation state of alternatively activated or M2 Mϕs [34]. It has been found to be important in immunological functionality of myeloid cells [4] and that these Arg-1^{+} expressing cells contribute to T cell energy, preventing activation of effector Th cells [35,36]. Thus, Arg-1 activity has been associated with previously described myeloid suppressor cells [37]. The secretion of Arg-1 and its breakdown products of arginine are linked with Mϕ functions previously described for M2/alternatively activated Mϕs. Extracellular Arg-1 exerts potent anti-inflammatory effects where sustained expression of Arg-1 is hypoinflammatory and is limiting to T cell polarisation via negative regulation of polarising cytokines, IL-6, IL-12p40 subunit (IL-12/IL-23) and IL-10.

In addition to being characterised by arginine metabolism, this dichotomy in Mϕ effector subset functionality can be further defined by a whole plethora of molecules expressed and functional outcomes. In general, M1 Mϕs are iNOS$^+$ hence NO production and anti-microbial functions, they also express high levels of HLA-DR$^+$, co-stimulatory CD86 and IL-12 associated with mediating and polarising Th_1 responses to intracellular-resident pathogens and anti-tumour responses [38-40]. This high-level expression and protection conferred by IL-12p40 extends to this subunit being shared by IL-23, which is also produced by M1s, and plays a pivotal role in the differentiation and activation of Th_{17} cells [41,42]. Additionally, these Mϕs are generally STAT1$^+$ TREM-1$^+$ cells expressing IL-8 (CXCL8) and MCP-1 (CCL2), which are responsible for perpetuation of inflammatory responses through the chemotactic recruitment of neutrophils and monocytes. M1s also produce a wide array of pro-inflammatory cytokines (TNFα, IL-1β, IL-6, IL-18, IL-23), chemokines (CXCL1,2,3,5,8,10, CCL3,4,5,11,17 and 22), matrix metallo-proteinases (MMP-1,-2,-7,-9 and -12), reactive oxygen species (ROS) and pattern recognition receptors (TLR2, TLR4 and TLR5) [18,43-46]. M2 Mϕs, on the other hand, in addition to arginase/ornithine, can be characterised by their expression of the phagocytic scavenger receptor (Mannose Receptor, CD206), TGFβ (immune regulation/suppression), EGF (tissue repair) and VEGF (angiogenesis). This serves to highlight the regulatory and reparative nature of these M2 Mϕs. Additionally, M2s can also produce/secrete IL-1β, IL-6, TNFα, IL-10, MMPs and TIMPs; these cytokines/enzymes are less strongly associated with the M2 phenotype and are also expressed by M1 Mϕs [reviewed in 8; 47,48], although a predominance of anti-inflammatory and regulatory factor production underpins this functionally distinct Mϕ subset. The sharing of expression of effector molecules to a greater or lesser extent between these functionally divergent Mϕ subsets may go some way to explaining the apparent existence of several subsets/intermediates between M1 and M2s.

Cytokines play a fundamental role in both differentiation and activation of M1-like and M2-like Mϕs. The growth factors, M-CSF and GM-CSF have been demonstrated to differentially control Mϕ lineage populations in homeostatic and inflammatory conditions [49]. Indeed, the Th_1-derived cytokines, GM-CSF and IFNγ in combination with inflammatory stimuli such as LPS or TNFα, polarise Mϕs towards the M1 phenotype [50]. Polarisation towards this M1 pro-inflammatory phenotype is also achieved by hypoxic/anoxic environments, β-chemokines and the DAG analogue/PKC activator, phorbol myristate acetate (PMA) [51-54]. On the other hand, Th_2-derived cytokines, IL-4, IL-13 and IL-21 were described to polarise Mϕs to a mannose receptor (CD206)-expressing M2 phenotype [17,55,56]. Extensive research has described many factors, in addition to Th_2-derived cytokines, to be M2-polarising; these include IL-10, TGFϕ, M-CSF, Vitamin D_3 and immune complexes [reviewed in 57], with one of the first polarising studies describing M-CSF-mediated differentiation resulting in the development of Mϕs deficient in IL-12 production [58]. Indeed, the immunosuppressive cytokines TGFβ and IL-10 may be responsible for the observed effects of CD4$^+$CD25$^+$Foxp3$^+$ Tregs in the induction/polarisation of monocytes to alternatively activated M2-like Mϕs [59]. What is relatively unclear is the stage of sensitivity to polarisation and plasticity. It is commonly thought that terminally differentiated cells lose their plasticity, with only intermediates retaining this ability to polarise according to the tissue environment. Of interest are the early studies of Rees and colleagues who described first cytokine exposure to irreversibly determine previously uncommitted Mϕ responses, where the initial cytokine exposure (IFNγ, TNFα, TGFβ, IL-4, IL-6, IL-10) determined Mϕ response to be pro-inflammatory, anti-inflammatory, phagocytic or anti-microbial (NO production) and failed to be modulated by subsequent cytokine exposure [60].

Subset **Function/phenotype**	**M1 classical**	**M2a alternative**	**M2b type II**	**M2c deactivated**
Stimulation/Differentiation	LPS, IFNγ, GM-CSF	L-4 / IL-13	IC, LPS, IL-1β	IL-10, TGFβ, Glucocorticoids
Cytokine expression	TNFα,IL-1β,IL-6,IL-12,IL-18, IL-23, IL-10^{low}	IL-12^{low},IL-23^{low}, TGF β, IL-10^{high}, IL-1Ra, sIL-1R, II decoy	IL-10^{high},IL-12^{low}, IL-23^{low}, TNFα, IL-1β, IL-6	IL-10^{high},IL-12^{low}, IL-23^{low}, TGFβ
Chemokine expression	CCL2,3,4,5,11,17 & 22 CXCL1,2,3,5,8,9,10, 11 & 16	CCL-17, CCL18, CCL-22, CCL-24	CCL-1	CCL-16, CCL-18 CXCL13
Scavenger Receptor expression		SR, MR		MR, CD163
Signalling	STAT-1, STAT-4, SOCS-3	STAT-3		STAT-6
Tryptophan metabolism	iNOS	Arg$^+$	iNOS	Arg$^+$
Function	Anti-microbial Pro-inflammatory Tissue-damage Th_1CMI response Anti-Tumoural	Anti-parasitic Allergic response Tissue repair Th_2 response	Anti-parasitic Allergic response Humoral immun. Th_2 response	Anti-inflammatory Immunoregulation Scavenger Tissue-repair Tumour promotion

Table 1: Macrophage functional phenotypes of defined subsets: M1 classical and M2 alternatively activated phenotypes are characterised according to polarising stimulation/differentiation signals, cytokine and chemokine expression, scavenger/phagocytic receptors, tryptophan metabolism and intracellular signalling molecules. In general iNOS Mφs are M1 and Arg$^+$ Mφs are M2; defining subsets as pro-inflammatory and driving CMI/anti-tumoral responses or anti-inflammatory and driving humoral/regulatory and pro-tumoral responses, respectively. Although M1 classical and M2 alternatively activated subsets are generally acknowledged, no firm evidence exists for the existence of the M2-variants, M2a, M2b and M2c. Note the expression of iNOS by M2b subset; a characteristic more typical of M1 Mφs. It is possible that these additional subsets may represent intermediates between M1 and M2 Mφs. This table has been adapted from [8,10,18,46,80].

The expression and secretion of effector molecules defines the functional responses of M1 and M2 subsets and is integrally-linked to the manner of cell activation [reviewed in 8]. An efficient Mϕ response to an infection will thus include both pathogen/tissue destructive and reparative mechanisms mediated by the activity of both M1 and M2 Mϕs. Central to this development of appropriate Mϕ immune responsiveness is the selective recognition and descrimination of pathogen associated molecular patterns (PAMPs), danger associated molecular patterns (DAMPs) and apoptotic cell associated molecular patterns (ACAMPs). The recognition of apoptotic cells/ACAMPs by Mϕs regulates pro-inflammatory cytokine production and possibly Mϕ polarisation through the induction of TGFβ and PGE_2 [61,62]. Toll-like receptors (TLRs) mediate responsiveness to PAMPs and DAMPs, hence determining appropriate immune response. TLRs mediate anti-viral, anti-bacterial, anti-fungal or anti-parasitic responses through involvement of appropriate receptors, adaptor proteins and either MAPK- NFκB- or IRF-dependent signalling pathways [63]. LPS has been shown to be transduced through TLR4 which results in the activation of ERK-1,2, JNK, p38 MAPKs as well as NFκB and IRF3 which induce a wide variety of immune gene expression including TNFα, IL-1β, IL-6, IL-12, IL-10, MHC II and iNOS. Interestingly, these TLR-mediated signals can be negatively regulated by a wide variety of endogenous inhibitor molecules, which include Myd88s, IRAK-M, IRF4, ST2, TREM2, Tollip, TRIAD3A, p50/p50 NF-κB, suppressor of cytokine signalling 1 (SOCS-1), SOCS-3, SHP1, SHP2 and SIGIRR [64-68]. This range of endogenous inhibitors of TLR signalling becomes more significant when considering the associations of these molecules with regards control of Mϕ polarisation. Alternatively activated, M2-like anti-inflammatory Mϕs have been described to be polarised by IL-4-requiring SHIP degradation and NFκB inhibition [69,70] whereas IRF5 promotes pro-inflammatory Mϕ polarisation and downstream Th_1-Th_{17} responses [71] and SOCS3 expression is essential for classically activated Mϕs [72].

Distinct signalling components regulate Mϕ polarisation

Mϕ polarisation and effector function is governed by a wealth of signal pathways and their component signalling molecules. Such signals, which have been previously described to regulate Mϕ polarisation include: NFκB, PI3K/PTEN, STAT3 and SOCS3. There is a reciprocal relationship between the lipid phosphatase, PTEN (phosphatase and tensin homologue deleted on chromosome ten) and PI3K (phosphoinositide 3-kinase) in the polarisation of Mϕ subsets. PTEN has been shown to regulate the expression of Arg-1 in macrophages, with corresponding downstream modulation of both innate and adaptive immune responses [73]. PTEN antagonises the activity of PI3K where PI3K itself has been demonstrated to function as a negative regulator of pro-inflammatory cytokine production and iNOS expression, activity and production of nitric oxide (NO) [74,75]. PTEN positively regulates TLR-induced IL-6 production; PTEN deletion as well as constitutive activation of PI3K was found to induce Arg-1 expression. This is suggestive that $PTEN^{-ve}$ Mϕs expressed a functional phenotype similar to alternatively activated or M2-like Mϕs in a manner mediated by increased activation of the transcription factors, C/EBPβ and STAT3. IL-10 signalling would appear to be integrally associated with STAT3 and M2 polarisation, where STAT3 activation and IL-10 secretion are linked [76] and the STAT3-inducible cytokines, IL-10 and IL-6, activate Arg-1 expression [77], a key marker of M2/alternatively activated Mϕ polarisation. If STAT3 plays a key role in M2 polarisation, it may represent a potential therapeutic target for the treatment of inflammatory pathology as evidenced by the conditioned STAT3 KO in mouse Mϕs which were refractory to IL-10 effects and spontaneously developed chronic enterocolitis [78,79].

The polarisation of M1 Mϕs is transduced by activation of the transcription factors NFκB and STAT-1 which induce the expression of M1-associated genes with further control of polarisation through the activity of SOCS3 [72]. In addition, the potential for differentiation towards an M2-like subset is prevented via STAT-1 inhibition of activation of the M2-polarising transcription factor, STAT-6 [80], whereas the expressional knock-down (KO) of SOCS3 favours M2 polarisation [72]. Indeed Th_2 cytokines induce Ym-1 expression (a poorly-defined M2-associated molecule in mice) by a STAT6-dependent mechanism [81]. NFκB has been shown to be integral to Mϕ polarisation and effector function; inhibition of which resulted in the development of an anti-inflammatory M2-like Mϕ phenotype [70]. NFκB is also involved in M2 polarisation, where in contrast to p65 NFκB subunit involvement with M1 effector function, M2 polarisation processes are driven by p50 NFκB subunits [82]. The targeting of NFκB would appear to be a promising target for manipulation of Mϕ polarisation and has been the subject of intense efforts in the re-education of tumour-associated macrophages (TAMs), originally described as exhibiting a pro-tumoral M2-like phenotype [83].

Activation of the transcription factor, C/EBPβ is associated with the cAMP-dependent activation of CREB; cascades involving these transcription factors have been demonstrated to initiate M2 Mϕ-specific gene expression and tissue reparative mechanisms [84]. The cAMP-activated factor, CREB, is required for full induction of C/EBPβ [84], which transctivates the Arg-1 gene promoter [85]. As was the case with STAT3, the expression and activity of IL-10 is associated with cAMP-mediated responses; whether this signalling pathway directly modulates polarisation or is an indirect consequence of IL-10 expression requires further investigation. What is clear is that the profiles of pro-inflammatory and anti-inflammatory cytokines are differentially regulated by cAMP in a manner determined by original Mϕ differentiation signals and activation signals in a PKC/cAMP/CREB axis [86]. In addition to these signalling pathways driving Mϕ polarisation, it is probable that monocytes also display a level of polarisation.

Fine control of Mϕ polarisation and functionality is likely to be as a result of a complex cross-modulation between distinct signalling pathways rather than singular exclusive subset-specific pathway involvement. This subtlety of signal pathway cross-talk driving Mϕ polarisation is potentially demonstrated by a recent study conducted by Arranz et al, who focussed on the involvement of the Akt/PKB family of serine/threonine protein kinases. PKB/Akt kinases are potentially downstream of PI3K, upstream of p70S6K and regulated by cAMP-dependent signals through the activation of PKC isoforms. This

breadth of pathway cross-talk is indicative of Akt playing a central role in Mϕ polarisation. Indeed, in the case of mouse models of LPS-induced endotoxin shock and dextran sodium sulphate (DSS)-induced colitis, Akt2 KO resulted in M2 Mϕ polarisation and resistance to these inflammatory pathologies whereas Akt1 KO polarised Mϕs towards the M1 subset and an increased sensitivity to induced endotoxin shock and colitis. This polarisation towards M2s as a consequence of Akt2 KO was found to be due to an increased expression of C/EBPβ, a positive regulator of Arg-1 [87]. In addition, tuberous sclerosis complex 1 (TSC1) has been demonstrated to modulate Mϕ polarisation in a manner that is dependent or independent of mTOR, the downstream effector of the p70S6K pathway. TSC1 encourages M2 polarisation in an mTOR-C/EBPβ-dependent manner whereas it suppresses ERK-dependent polarisation towards the M1 subset in an mTOR-independent manner [88].

Macrophage effector function is pre-programmed in monocyte subsets

The effector function of macrophages may already be determined in the monocyte prior to differentiation to the tissue macrophage. The existence of pre-programmed monocyte populations has been suggested in both murine systems and in humans. The following section highlights the existence of functionally distinct monocyte subsets, which are linked to homeostatic and inflammatory environments; just how these subsets fit with the established Mϕ polarisation in health and disease is currently no more than hypothesis but may need to be thoroughly investigated to complete our understanding of Mϕ subsets and functional phenotypes (Figure 1). Two distinct populations of monocytes have been described in mice, on the basis of chemokine receptor expression; a non-inflammatory $CX3CR1^{hi}$ $CCR2^{-}$ subset and an inflammatory $CX3CR1^{lo}$ $CCR2^{+}$ subset [89]. With respect to human monocytes, investigations undertaken by Loems Zeigler-Heitbrock have characterised different subsets, which are dependent on the relative expression of CD16, the FcγRIIIa antibody receptor [90,91] ignored (for surface marker, cytokine and effector phenotype analysis of these monocyte subsets, refer to table 2). The monocyte subsets described are the classical ($CD14^{++}$ $CD16^{-}$ $CD163^{+}$), intermediate ($CD14^{++}$ $CD16^{+}$ $CD163^{+}$) and non-classical ($CD14^{+}$ $CD16^{++}$ $CD163^{-}$) monocytes [92; reviewed in 93], where the intermediate monocytes are thought to represent an intermediate transitional subset between the classical and non-classical monocytes [94]. The classical $CD16^{-}$ monocytes account for 90% of circulatory monocytes whereas $CD16^{+}$ monocytes account for up to 10% whilst at rest [91]. The relative numbers of these pro-inflammatory $CD16^{+}$ monocyte populations have been shown to increase in malignancy and inflammation, rising up to 50% in sepsis and being significantly raised in RA and representing a major source of TNFα [95-98]. These monocytes can also be selectively depleted after either IgG infusion or glucocorticoid therapy [99,100]. The non-classical $CD16^{+}$ monocytes exhibit a distinct functional behaviour where upon stimulation produce higher amounts of TNFα, IL-12 and lower amounts of IL-10, hence have been referred to as pro-inflammatory monocytes [96,101,102]. In addition, these monocyte subsets display differential migratory responses whereby classical monocytes selectively respond to CCL2/MCP-1 and non-classical monocytes are refractory to CCL2 and migrate in response to CX3CL1/Fractalkine [103,104]. Finally, $CD16^{+}$ monocytes also express higher amounts of HLA-DR/Class II MHC and a corresponding greater capacity for antigen presentation, hence T cell activation [91,105]. With respect to these non-classical monocytes, development is determined by the activity of and sensitivity to M-CSF where blockade of the M-CSF-R pathway has been described to selectively reduce $CD16^{+}$ non-classical monocyte numbers [106].

Subset	Classical	Intermediate	Non-Classical
Subset Phenotype	$CD14^{hi}$ $CD16^{-}$	$CD14^{hi}$ $CD16^{lo}$	$CD14^{lo}$ $CD16^{hi}$
Scavenger Receptor expression	$CD163^{+}$	$CD163^{+}$	$CD163^{-}$
Cytokine expression	TNFα, IL-12, IL-10^{higher}		TNFα^{high} IL-12^{high} IL-10^{low}
Chemokine recruitment	CCL2/MCP-1		CX3CL-1/Fractalkine, CCL2-refractory
Antigen Presentation		HLA-DR^{low}	HLA-DR^{high}
Tryptophan metabolism	iNOS	Arg^{+}	iNOS
Function	Anti-microbial Pro-inflammatory Tissue-damage Th_1CMI response Anti-Tumoural	Anti-parasitic Allergic response Tissue repair Th_2 response	Anti-parasitic Allergic response Humoral immun. Th_2 response

Table 2: Monocyte subset functional phenotypes. Classical CD16-negative and CD16-positive non-classical monocytes can be classified according to their functional phenotype of scavenger receptor (CD163), cytokine expression, chemokine responsiveness, antigen presentation capacity (HLA-DR) and arginine metabolism (iNOS or Arg I). The combination of such phenotypes defines monocyte function as pro-inflammatory, CMI-inducing or tissue reparative, induction of humoral immunity. One point to be noted is that the classical and non-classical subsets express iNOS whereas the intermediate monocyte subset expresses arginase. Refer to macrophage table 1 earlier. This table has been adapted from [91,93,94,96,98,101,103,104].

This monocyte system exhibits characteristics, which parallel the macrophage system. Both the monocytes and macrophages exist in two discrete functional phenotypes and exhibit a level of plasticity between these subsets, with the monocytes being described to have a clear intermediate subset between the two potential polar subsets. With the realisation that diseases mediated by Mϕ subsets may be

controlled by the polarisation/plasticity between M1 and M2, comes a further complexity that we are likely to have to consider; the manipulation of the subset of the "macrophage progenitor", the monocyte and how each distinct monocyte subpopulation differentiates to distinct Mϕ effector subsets.

Macrophage subsets and pathology

Macrophages play a predominant role in driving many immunopathological diseases; their pathological function being dictated by the local tissue environment with respect to the balance between polarising activatory, differentiation and suppressive signals. Due to the relative abundance of Mϕ numbers and scientific literature, this section is focussed on the role of Mϕs in the inflammatory pathology of the mouth and intestinal tract and the immunosuppressive pathology associated with tumour associated Mϕs (TAMs) and solid tumours. Mϕs populate both oral and intestinal mucosae in large numbers [107]. In a homeostatic environment, mucosal Mϕs drive tolerogenic mechanisms whereas, at the same time, maintaining an efficient phagocytic response. This homeostatic mucosal tolerance is associated with Mϕs exhibiting an M2-like phenotype, predominated by the expression of anti-inflammatory, suppressive cytokines and phagocytic scavenger receptors (CD36, CD68 and CD206). These tolerogenic Mϕs maintain a state of perpetual readiness required for microbial clearance without inducing a localised hyper-inflammatory state [108-110]. In this homeostatic tolerogenic state, mucosal Mϕs fail to express the pro-inflammatory cytokines (TNFα, IL-1β, IL-6, IL-8, IL-12, IL-18 and IL-23) whereas TGFβ and IL-10 expression is maintained. This tolerised state is further reflected by the lack of expression of CD14/TLRs, FcRs, co-stimulatory molecules (CD40, CD80, CD86) and the pro-inflammatory molecule, TREM-1. Concurrently, there is a marked expression of the regulatory molecules CD33, CD200R and TGFβRI/RII [reviewed in 57]. This homeostatic /tolerogenic function of Mϕs is dysregulated in pathology where mucosal tolerance is broken with respect to inflammatory diseases such as Crohn's disease and Chronic periodontitis and augmented in immune suppression – associated diseases such as colorectal cancer and oral squamous cell carcinoma. These pathologies exhibit mechanisms aligned to M1- or M2-driven responses. In the context of pro-inflammatory diseases, Mϕs exhibit an inflammatory phenotype that is comparable to the M1 subset. These inflammatory Mϕs express a wide variety of effector molecules, which include: PRRs (CD14, TLR2, TLR4, TLR5), FcRs (CD16, CD32, CD64, CD89), HLA-DR, chemokine receptors (CCR5, CXCR4), CRs and the pro-inflammatory markers/cytokines (TREM-1, TNFα, IL-1β, IL-6, IL-18 and CCL20) [57,111-113].

M1-associated pathology: Crohn's disease

Crohn's disease (CD) is an idiopathic inflammatory bowel disease (IBD) that is characterised by transmural skip-lesion-associated inflammatory destruction of the gastro-intestinal tract anywhere from the mouth to the anus. CD is characterised by a dysfunctional innate immune system, which results in inflammatory destruction mediated by a pathogenic axis of Th_1/IL-12 and Th_{17}/IL-23 and the production of IFNγ, TNFα and IL-17 [114]. This chronic inflammatory disease is associated with genetic mutations in bacterial-sensing PRRs: NOD2 mutations have long-since been described to be a feature of CD which resulted in dysregulation of and the augmentation of NFκB-mediated pro-inflammatory cytokine production of TNFα, IL-1γ and IL-12 by mucosal Mϕs [115]. NOD2 has been described to regulate pro-inflammatory signals transduced through TLR2 [116]. Such a breakdown of regulation observed in CD would result in a dysfunctional innate immune response with downstream effects on the adaptive immune system and the commensal microbiota of the gut, which also plays an important role in barrier defences and mucosal tolerance. This total breakdown of barrier integrity and mucosal tolerance, coupled with the bias towards an inflammatory axis of Th_1/IL-12 and Th_{17}/IL-23, results in a mucosal environment low in regulatory cytokines IL10 and TGFβ and high in IL-12p40. This inflammatory environment is conducive to M1-like Mϕ activation/differentiation with the corresponding up-regulation of pro-inflammatory cytokine and co-stimulatory molecule expression [117,118]. The therapeutic targeting of M1 Mϕs or indeed the augmentation of M2-mediated responses may represent a realistic regimen in the control of this chronic inflammatory disease.

Chronic periodontitis

Chronic periodontitis (CP) is a persistent relapsing-remitting inflammatory disease of the periodontal tissue, which ultimately, if untreated, leads to destruction of the periodontium and resulting tooth loss. Like Crohn's disease, CP is associated with the breakdown of mucosal barrier functionality and tolerance, leading to an uncontrolled inflammatory immune activation response [119]. The observed dysbiosis in the oral microbiota results in the perpetual microbial challenge; one such prominent microbe driving this inflammatory pathology is *Porphyromonas gingivalis* [120,121]. *P. gingivalis* is an intracellular-resident oral bacteria which infects both oral epithelial cells and underlying APCs (DCs and Mϕs). An appropriate host clearing response to such an intracellular pathogen would be to initiate cell-mediated immunity, mediated by Th_1 cells [122,123]. This pathogen however is able to both subvert and suppress appropriate host responses. PG-LPS both exhibits a low endotoxin activity and can mediate its effects through both TLR2 or TLR4 as well as changing the appropriate Th_1-lead response to that of a non-clearing Th_2-mediated humoral response [124-126]. In the case of CP, oral Mϕs exhibit a pro-inflammatory, M1-like cytokine profile: high pro-inflammatory levels (TNFα, IL-1γ, IL-1β, IL-6, IL-8, IL-12, IL-18, IL-32, MCP-1) and low level expression of regulatory cytokines (IL-10) [127]. The Mϕ-driven pathogenic mechanisms that underlie CP is difficult to interpret; *Porphyromonas gingivalis*, a major pathogen associated with CP, induces M1 polarisation whereas subverts the adaptive response to be dominated by Th_2 cells. At the same time, Mϕ subsets have been demonstrated to exhibit a differential sensitivity to endotoxin tolerance (ET); whereby the pro-inflammatory subset, M1 Mϕs, are refractory to ET and the homeostatic M2-like subset was tolerisable [128]. Such tolerisation mechanisms have already been described for the oral mucosa in CP resulting in down-regulation of TLR2, TLR4, TLR5, MD-2, TNFα, IL-1β, IL-6, IL-8 and IL-10 [129]. This selective Mϕ subset-specific sensitivity to ET, coupled with the relapsing-remitting nature of this chronic inflammatory disease, is normally suggestive that inflammation/immune activation is tissue-destructive whereas immune suppression/tolerisation is of benefit to the host via stopping these tissue-destructive mechanisms. Future therapeutic intervention will be reliant on clarification of Mϕ polarisation plasticity, Mϕ subset-specific ET mechanisms and downstream effects on polarisation of T cell responses (Figure 2).

M2-associated pathology: Solid tumours

In addition to the M1 Mϕ subset being integral to driving inflammatory pathology, the M2/alternatively activated subset is associated with suppressive/regulatory mechanisms required for tumorigenesis and progression of solid tumours. High tumour associated macrophage (TAM) numbers have been indicated as a poor prognostic marker in cancers, in particular in squamous cell carcinoma [130]. Indeed, Mϕ depletion (M-CSF gene mutation) in a mouse model of polyoma virus middle T oncoprotein-inducible breast cancer observed a reduced progression of malignant lesions and metastases [131]. The Mϕ has thus become a major focus for the understanding of cancer; it has been shown to play a central role in neoplastic transformation and tumour progression [132]. The established link between chronic inflammation and cancer, for example inflammatory bowel disease (IBD) and colorectal cancer (CRC), is suggestive of the Mϕ playing several roles in tumour development. Which particular Mϕ function is required during each phase of development is indicative that the range of activities may be reflected by plasticity in subset of TAMs. This inflammation-cancer link can be exemplified by the malignant transformation of oral epithelial cells resulting in oral cancer such as oral squamous cell carcinoma (OSCC). The original trigger for cancer or transformation may have been as a consequence of chronic tissue injury induced by an M1-driven inflammatory disease such as Oral Lichen Planus (OLP), where the pro-inflammatory and anti-microbial (ROS/RNS) environment induces mutagenesis and transformation [reviewed in 119].

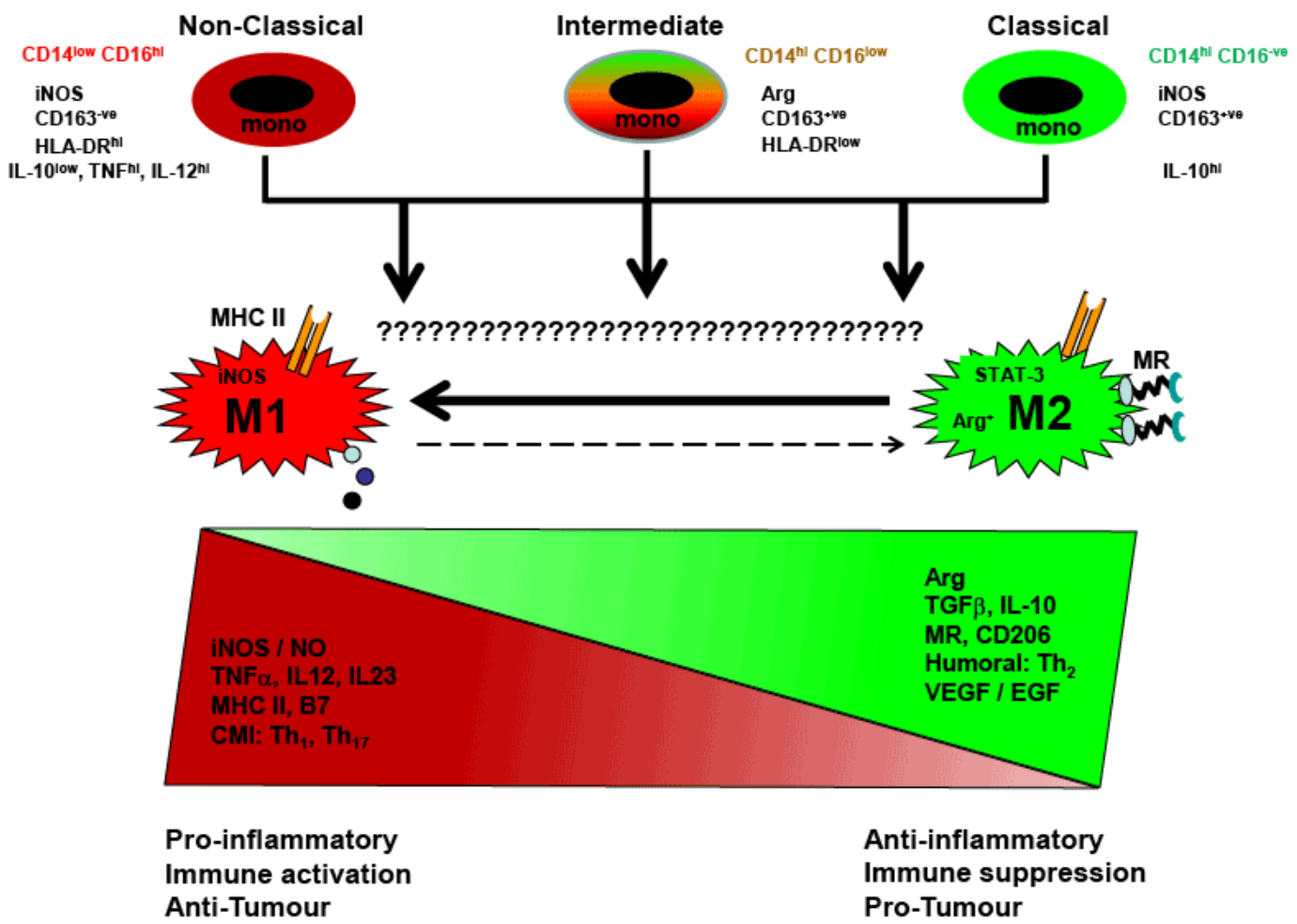

Figure 1: Monocyte pre-programming and macrophage polarisation determine effector function of homeostatic and pathogenic tissue macrophages. Macrophage polarisation and plasticity between M2/homeostatic and M1 subsets determines effector response as anti-inflammatory/regulatory and pro-tumoral versus pro-inflammatory, immune activatory/CMI and anti-tumoral. M1-like effector functionality is indicated as an increasing scale of red colouration whereas M2-like effector functionality is indicated as an increasing scale of green colouration. M1 function is associated with iNOS/NO production, expression of pro-inflammatory cytokines (TNFα, IL-12 and IL-23), hence Th1 and Th17-mediated CMI. Pathogenic association of M1 function is linked with inflammatory diseases such as CD and CP. M2 function is associated with Arg-1 activity, expression of suppressive cytokines, hence Th2 and Treg-mediated responses. Pathogenic association of M2 function is linked with immunosuppressive diseases such as OSCC and localised pro-tumoral environments of solid tumours. Just how pre-programmed monocyte subsets link in to the development of M1- or M2-like Mφ responses is not fully defined and is subject to speculation (hence ????? indicated). Classical CD16-negative monocytes are depicted in green and non-classical CD16-positive monocytes in red. These monocyte subsets correspond to the M1 and M2 subsets with respect to pro-inflammatory and anti-inflammatory/regulatory cytokine production whereas both subsets express iNOS. In contrast, the intermediate monocyte subset expresses Arg-1, normally associated with regulatory, M2-like function. Thus, monocyte subsets do not exactly align with Mφ subsets, but may be representative of a sliding scale of effector functionality determined by a combination of pre-programming, differentiation and activation signals representative of the localised tissue environment in homeostatic, pathogenic challenge or disease status.

OSCC is characterised by a massive cellular infiltrate primarily consisting of MCP-1-recruited monocytes, which in the presence of M-CSF and IL-10, are polarised to an M2-like phenotype of TAMs [130]. These polarised TAMs produce IL-10, EGF, FGF, PDGF and VEGF, which direct advanced stages of tumour progression [133-136]; tumour growth benefitting from the overall immunosuppressive, anti-inflammatory and tissue reparative environment. In a reciprocal manner, the cancer cells also produce TGFβ, IL-10 and M-CSF [134,135]. This environment further benefits the tumour by suppressing pro-inflammatory cytokine production and by inhibiting APC function through the down-regulation of MHC expression and the up-regulation of inhibitory co-stimulatory molecules such as CTLA-4 and B7-H4 [137-139]. TAM contact with malignant cells has indeed been described to result in defective phagolysosomal

interactions hence defective tumour antigen processing and presentation, thus suppressing anti-tumour T cell responses and facilitating tumour survival [140]. In addition, Treg development is encouraged via the M2-like TAM and OSCC cell expression of IL-10 and TGFβ favouring the suppression of host anti-tumour responses [141,142]. Thus, there would appear to be a reciprocal relationship between TAMs and tumour, where the TAMs can modulate tumour survival, growth and development and that the tumour cells can modulate TAM plasticity. Can we limit tumour growth and development by switching M2-like TAMs to an M1-like subset? Theoretically, at first glance, this might be viewed as an attractive option. Practice may be different, given that M1-like TAMs are associated with malignant transformation through chronic inflammatory injury and that the persistent tumour environment may just revert anti-tumoral M1-like Mϕs introduced as a cell-based therapy to the pro-tumoral M2-like TAM. Thus treatment of solid tumours by manipulation of polarisation states/plasticity between M1 and M2 phenotypes may be an inappropriate regimen for the treatment of cancer. What may be more realistic is the manipulation of Mϕ subset sensitivity to tolerisation; selectively suppressing polarised Mϕs, which facilitate tumour development in many different tumour environments.

Manipulation of Macrophage polarisation: the future?

Manipulation of Mϕ polarisation by harnessing differentiation, activation and suppression signals may offer a potentially realistic regimen for the treatment and management of pro-inflammatory (eg. CD or CP), or immune-suppressive, pro-tumour (eg. OSCC) conditions (refer to figure 1). Effective polarisation and modulation of pathological mechanisms are likely to result from the delicate balance of all of these Mϕ-mediating factors, which, if modulated incorrectly may result in exacerbation of disease processes rather than down-regulation. Indeed, in the case of tumours, TAMs are predominated by the pro-tumoral M2-like phenotype. Although experimental over-expression of Mϕ IL-12 increased MHC expression, T cell infiltration and anti-tumour responses [143], attempts to polarise these M2-like TAMs to a cytotoxic anti-tumour M1 subset have resulted in Mϕ polarisation reverting to the suppressive pro-tumoral M2 subset. This is thought to be as a result of the tumour environment expressing a wealth of signals which reverse the polarised "therapeutic" M1 subset to an effector that benefits the tumour. This may be as a consequence of TAM functional heterogeneity where in invasive areas, TAMs encourage cancer cell motility whereas in stromal and perivascular areas TAMs promote metastasis and in avascular, perinecrotic areas hypoxic TAMs stimulate angiogenesis [132,144]. In cancers with a poor prognosis, TAMs accumulate in numbers at sites of hypoxia and necrosis [145-147]. These TAMs respond to hypoxia by up-regulating the expression of HIF-1, HIF-2 and HIF-regulated angiogenic factors [148,149], thus hypoxia may represent a polarising signal which favours pro-tumoral function and an M2-like TAM subset [150]. An additional confounding factor to the understanding of TAM functionality is the characterisation of an additional $CD14^+$ monocyte subset, which expresses Tie-2 (angiopoietin receptor) and is associated with tumour angiogenesis [151]. Upon ligation by angiopoeitin-2, this subset suppresses the release of pro-inflammatory cytokines TNFα and IL-12 via NFκB inhibition by A20-binding inhibitor of NFκB activation-2 (ABIN-2) [151,152]. This may go some way to highlight the requirement to manipulate TLR/NFκB signals in the regulation of TAM plasticity but, in addition, there is a need to fully characterise this tumour-associated Tie-2 expressing monocyte (TEM) subset and where it is placed in the sliding scale of monocyte/macrophage functional plasticity. An alternative approach to manipulating M2 to M1, is to encourage M2 polarisation but to manipulate these pro-tumoral Mϕs to act as Trojan horses, acting as delivery systems for anti-tumour cytotoxic drugs. This very approach is currently being investigated where studies have demonstrated Mϕs to be ideal delivery systems for oncolytic virus, which resulted in the suppression of tumour regrowth and metastasis [153,154].

In addition to the manipulation of polarising activation and differentiation signals, Mϕ polarisation to distinct functional subsets is likely to be determined by suppressive signals or tolerisation (Figure 2). ET was first described by the observation that LPS pre-treatment rendered innate immune cells refractory to activation upon LPS re-challenge. ET has since been shown to occur in Mϕs for a range of cytokine (TNFα, IL-1β) and TLR-mediated (LPS, LTA, PGN, Flagellin) signals [reviewed in 155]. The suppression of Mϕ functionality could beneficially inhibit harmful inflammatory responses whereas at the same time benefit infectious microbes, thus allowing for a favourable environment for the pathogen to recoup its numbers through growth. In the case of the oral pathogen Porphyromonas gingivalis, associated with chronic periodontitis, Mϕ subsets were differentially sensitive to PG-LPS-induced ET, where M2s were sensitive to ET and M1s were refractory [128]. As suggested earlier in the context of TLR-mediated signalling, many endogenous suppressors exist which can suppress TLR-mediated activatory or polarising responses. In addition to the endogenous suppressors (MD2, Tollip, IRAK-M, Myd88s, TRIAD3A, SIGIRR), many other suppressive molecules play a role in regulating Mϕ responses. These include CD200R, CD47/SIRP1α, Siglecs 3-10, CD32 to name but a few. Ligation of CD200R has been demonstrated to induce immunosuppressive activity and suppress pro-inflammatory cytokine production in models of chronic inflammation such as collagen-induced arthritis [156-158 and reviewed in 159]. CD47-SIRP1α ligation also exhibits a suppressive activity by down-regulating IL-12 production [160]; this response may be reflective of suppression of activity or may alter polarisation status of the Mϕ from M1 to M2. The targeting/augmentation of such suppressive molecules may represent a realistic approach in suppressing chronic inflammatory diseases such as Crohn's disease and chronic periodontitis but may also facilitate control of Mϕ polarisation in the treatment of solid tumours.

A recurring theme that presents itself in every aspect of the Mϕ story is the ability to recognise immunoglobulin or immune complexes (ICs) through the responsiveness of Mϕ FcRs. FcRs and their ligation would appear to be involved in monocyte subset responses, Mϕ subset polarisation through activation and differentiation and through the induction of suppressive/regulatory responses. CD16 (FcγRIIIa) is expressed by both Mϕs and the non-classical subset of monocytes. Activation by immune complexes or immunoglobulin results in an alternatively activated M2-like phenotype through the activation of ITAMs present in the intracellular cytoplasmic signalling domain [161], however CD16-ligation has also been shown to induce Mϕ TNFα production [162].

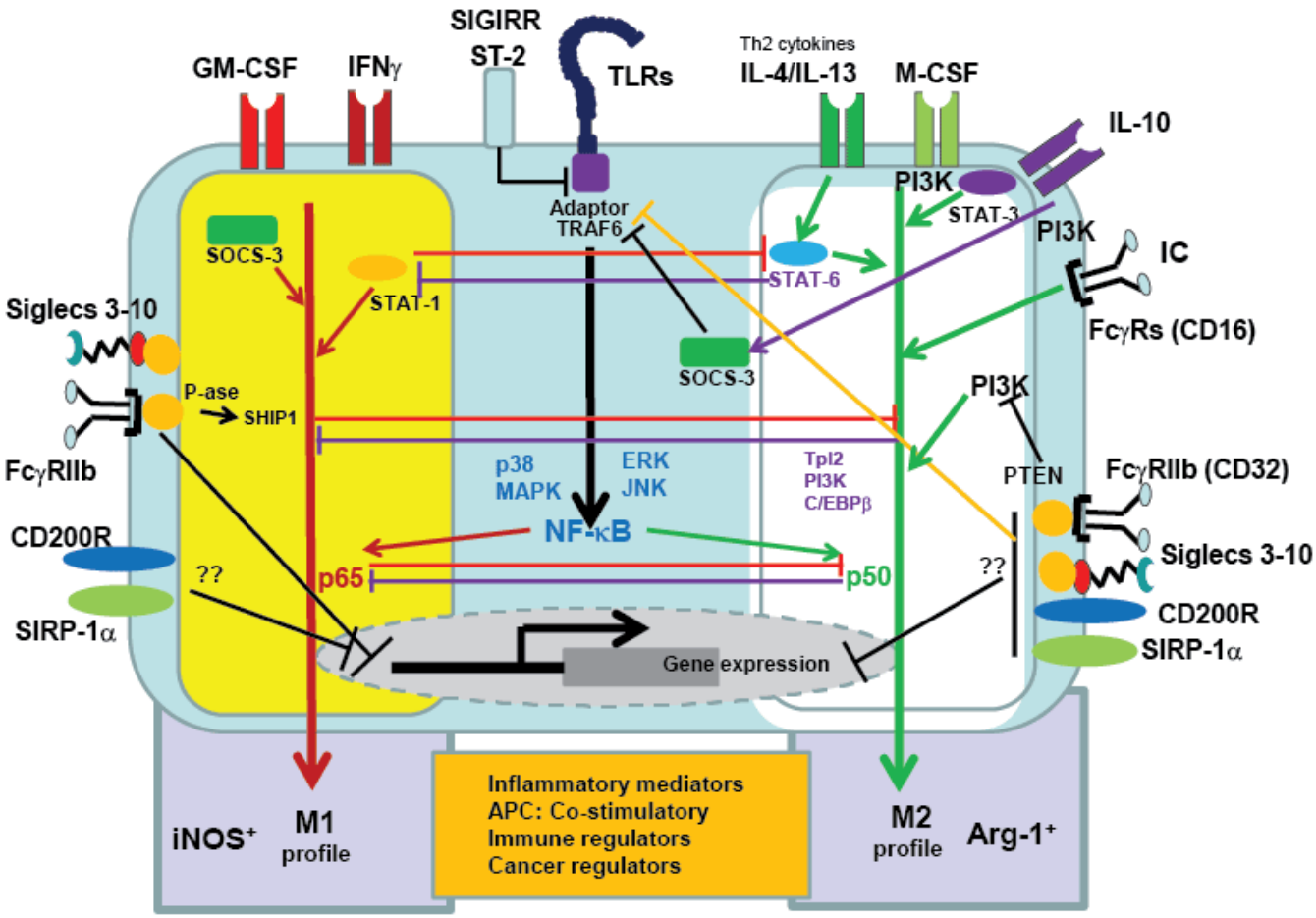

Figure 2: Manipulation of macrophage polarisation: the future? Macrophage polarisation, plasticity and effector phenotype can be determined by the manipulation of differentiation, activation and suppression/tolerogenic signals. Such harnessing of Mφ effector phenotypes may prove beneficial to the future treatment of both inflammatory mucosal diseases such as CP and CD and immune suppressive mucosal diseases such as OSCC. Mφ polarisation can be manipulated or initiated towards the pro-inflammatory, anti-tumour M1 subset (yellow box and red arrows) by a range of receptors, which include GM-CSF-R, IFNγ-R and TLR activation (middle blue box) and signalling intermediates such as STAT-1, p65 NF-κB and SOCS3. Conversely, the polarisation towards the anti-inflammatory, pro-tumour M2 subset is indicated by the white box and green arrows. M2 polarisation can be initiated by the receptors to the Th_2-derived cytokines, IL-4/IL-13, M-CSF-R, FcγRs as well as IL-10R and the TGFβR (not indicated on figure). The signalling intermediates associated with M2 Mφs include STAT3, STAT6, PI3K and p50 NF-κB. Cross-regulation is indicated by inhibitory (blunted) lines, which are coloured purple to indicate M2 suppression of M1 polarisation (by STAT6, Tpl2, PI3K, p50 NF-κB and the cAMP-modulated C/EBPβ) and red to indicate M1 suppression of M2 polarisation (by STAT1, p65 NF-κB and the ITIM-recruited phosphatase (P-ase), SHIP1). In addition, Mφ activation/polarisation signals can be suppressed by a variety of exogenous and endogenous negative regulators, described in ET mechanisms. These include signal suppression by SHIP1 through ligation of siglecs 3-10 and FcγRIIb and ligation of the negative regulatory receptors, CD200R and SIRP1α. PI3K has been suggested to polarise towards the M2 subset, activation of its endogenous negative phosphatase regulator, PTEN, will indirectly bias polarisation towards M1 Mφs. Finally, activation of Mφ function by TLRs can be suppressed by inhibiting adaptor protein association and transduction of signals to TRAF6 and downstream effectors such as NF-κB and MAPKs via membrane-associated exogenous signalling through SIGIRR, ST-2, FcγRIIb, Siglecs, CD200R, SIRP1α and endogenous-associated negative regulation through IL-10-induced SOCS3. Further investigation regarding the fine balance between signals transduced through CD16 (FcγRIII) and CD32 (FcγRIIb) may highlight the contribution of the different monocyte subsets (described by their high or low expression of CD16) to M1/M2 polarisation.

Thus FcγR-mediated responses can drive both activatory and suppressive responses in inflammatory pathologies such as CIA; such observations creating a rationale for FcγR-mediated targeting in the treatment of such inflammatory diseases as RA [163]. FcγR-ligation also suppresses IL-12 transcription, hence inhibiting M1/Th_1 responses and favouring M2-like responses [164]. The relative differential responses mediated through ITAM- and ITIM-containing FcγRs in both M1 and M2 Mϕ subsets may indicate the refined use of

IC-FcγR signalling in the treatment of inflammatory pathologies. The use of in vitro immunoglobulin (IVIG) has been adopted for the treatment of inflammation and autoimmunity [165,166]; whether this is as a consequence of activation of ITAM-containing FcγRs or regulatory responses through suppression by SHP-1/SHIP phasphatase-recruiting, ITIM-containing FcγRs such as FcγRIIb (CD32) remains to be clarified. Thus, IVIG can potentially be used to either suppress pathogenic Mϕ-driven responses or can deviate Mϕ responses to a more protective, less pathogenic mechanism. The relative balance of signals transduced through ITAM- and ITIM-bearing receptors would appear to have a direct effect on Mϕ functionality; this has been clearly demonstrated whereby SHIP activity has been shown to repress the generation of alternatively activated, M2-like Mϕs thus favouring a pro-inflammatory M1/Th_1 axis of Mϕ functionality [167]. Another family of receptors which both positively and negatively regulate Mϕ responses through ITAM and ITIM activity is the sialic acid binding Ig-like lectins, Siglec family [168]. CD33-like ITIM-bearing siglecs are expressed by Mϕs [169]. These siglecs exhibit suppressive functionality but, in addition, may play a prominent role in Mϕ polarisation. This is supported by siglec 9 enhancing Mϕ IL-10 production [170] whereas CD33 responses are blocked by SOCS3 [171], which also targets siglec 7 for proteosomal degradation [172]. Thus CD33-like siglecs may be involved in polarisation of M2-driven responses and are blocked in M1-SOCS3 expressing Mϕs (see overview diagram of manipulation of Mϕ polarisation, (Figure 2).

In conclusion, Mϕ-driven immune responses would appear to be controlled by the polarisation of specific effector phenotype being expressed and by its level of plasticity and reversibility. The plasticity, hence Mϕ subset, can thus be determined by the tissue environment [173]. Thus plasticity is regulated by a wealth of activation, differentiation and suppression signals to be found in the tissue environment. Pro-inflammatory and anti-inflammatory Mϕs are clearly inter-convertible [174] and this plasticity can be controlled by FcγR ligation which can reverse LPS toxicity [175], axis of IKK/NFκB activation [176], IL-4-induced SHIP degradation [69] and relative cytokine environments. Macrophage polarisation is thus truly a collaboration of differentiation, activation, suppression and pre-programming; further characterisation of which will open up a world of therapeutic regimens for the treatment of chronic inflammatory disease and cancer.

References

1. Gordon S, Taylor PR (2005) Monocyte and macrophage heterogeneity. Nat Rev Immunol 5: 953-964.
2. Mosser DM, Edwards JP (2008) Exploring the full spectrum of macrophage activation. Nat Rev Immunol 8: 958-969.
3. Varol C, Yona S, Jung S (2009) Origins and tissue-context-dependent fates of blood monocytes. Immunol Cell Biol 87: 30-38.
4. Jenkins SJ, Ruckerl D, Cook PC, Jones LH, Finkelman FD, et al. (2011) Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation. Science 332: 1284-1288.
5. Epelman S, Lavine KJ2, Randolph GJ3 (2014) Origin and functions of tissue macrophages. Immunity 41: 21-35.
6. Ginhoux F, Jung S (2014) Monocytes and macrophages: developmental pathways and tissue homeostasis. Nat Rev Immunol 14: 392-404.
7. Adams DO, Hamilton TA (1989) The activated macrophage and granulomatous inflammation. Curr Top Pathol 79: 151-167.
8. Foey AD (2014) Macrophages – masters of immune activation, suppression and deviation. InTech Publishing, Rijeka, Croatia. ISBN 978-953-51-1374-4.
9. Anderson CF, Mosser DM (2002) A novel phenotype for an activated macrophage: the type 2 activated macrophage. J Leukoc Biol 72: 101-106.
10. Gordon S (2003) Alternative activation of macrophages. Nat Rev Immunol 3: 23-35.
11. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM (2000) M-1/M-2 macrophages and the Th1/Th2 paradigm. J Immunol 164: 6166-6173.
12. Nathan CF (1987) Secretory products of macrophages. J Clin Invest 79: 319-326.
13. Mills CD (2001) Macrophage arginine metabolism to ornithine/urea or nitric oxide/citrulline: a life or death issue. Crit Rev Immunol 21: 399-425.
14. Jenkins SJ, Allen JE (2010) Similarity and diversity in macrophage activation by nematodes, trematodes, and cestodes. J Biomed Biotechnol 2010: 262609.
15. Murray PJ, Wynn TA (2011) Obstacles and opportunities for understanding macrophage polarization. J Leukoc Biol 89: 557-563.
16. Murray PJ, Wynn TA (2011) Protective and pathogenic functions of macrophage subsets. Nat Rev Immunol 11: 723-737.
17. Stein M, Keshaw S, Harris N, Gordon S (1992) Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. J Exp Med 176: 287-292.
18. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, et al. (2004) The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol 25: 677-686.
19. Fleming BD, Mosser DM (2011) Regulatory macrophages: setting the threshold for therapy. Eur J Immunol 41: 2498-2502.
20. Pinhal-Enfield G, Ramanathan M, Hasko G, Vogel SN, Salzman AL, et al. (2003) An angiogenic switch in macrophages involving synergy between Toll-like receptors 2, 4, 7, and 9 and adenosine A(2A) receptors. Am J Pathol 163: 711-721.
21. Ferrante CJ, Pinhal-Enfield G, Elson G, Cronstein BN, Hasko G, et al. (2013) The adenosine-dependent angiogenic switch of macrophages to an M2-like phenotype is independent of interleukin-4 receptor alpha (IL-4RÎ±) signaling. Inflammation 36: 921-931.
22. Duluc D, Delneste Y, Tan F, Moles MP, Grimaud L, et al. (2007) Tumor-associated leukemia inhibitory factor and IL-6 skew monocyte differentiation into tumor-associated macrophage-like cells. Blood 110: 4319-4330.
23. Colin S, Chinetti-Gbaguidi G, Staels B (2014) Macrophage phenotypes in atherosclerosis. Immunol Rev 262: 153-166.
24. De Paoli F, Staels B, Chinetti-Gbaguidi G (2014) Macrophage phenotypes and their modulation in atherosclerosis. Circ J 78: 1775-1781.
25. Gleissner CA, Shaked I, Little KM, Ley K (2010) CXC chemokine ligand 4 induces a unique transcriptome in monocyte-derived macrophages. J Immunol 184: 4810-4818.
26. Kadl A, Meher AK, Sharma PR, Lee MY, Doran AC, et al. (2010) Identification of a novel macrophage phenotype that develops in response to atherogenic phospholipids via Nrf2. Circ Res 107: 737-746.
27. Boyle JJ, Harrington HA, Piper E, Elderfield K, Stark J, et al. (2009) Coronary intraplaque hemorrhage evokes a novel atheroprotective macrophage phenotype. Am J Pathol 174: 1097-1108.
28. Boyle JJ, Johns M, Lo J, Chiodini A, Ambrose N, et al. (2011) Heme induces heme oxygenase 1 via Nrf2: role in the homeostatic macrophage response to intraplaque hemorrhage. Arterioscler Thromb Vasc Biol 31: 2685-2691.
29. Boyle JJ, Johns M, Kampfer T, Nguyen AT, Game L, et al. (2012) Activating transcription factor 1 directs Mhem atheroprotective macrophages through coordinated iron handling and foam cell protection. Circ Res 110: 20-33.
30. Mills CD, Shearer J, Evans R, Caldwell MD (1992) Macrophage arginine metabolism and the inhibition or stimulation of cancer. J Immunol 149: 2709-2714.

31. Hibbs JB Jr, Taintor RR, Vavrin Z, Rachlin EM (1988) Nitric oxide: a cytotoxic activated macrophage effector molecule. Biochem Biophys Res Commun 157: 87-94.
32. Albina JE, Caldwell MD, Henry WL Jr, Mills CD (1989) Regulation of macrophage functions by L-arginine. J Exp Med 169: 1021-1029.
33. Garside P, Hutton AK, Severn A, Liew FY, Mowat AM (1992) Nitric oxide mediates intestinal pathology in graft-vs.-host disease. Eur J Immunol 22: 2141-2145.
34. Gordon S, Martinez FO (2010) Alternative activation of macrophages: mechanism and functions. Immunity 32: 593-604.
35. Makarenkova VP, Bansal V, Matta BM, Perez LA, Ochoa JB (2006) CD11b+/Gr-1+ myeloid suppressor cells cause T cell dysfunction after traumatic stress. J Immunol 176: 2085-2094.
36. Munder M, Schneider H, Luckner C, Giese T, Langhans CD, et al. (2006) Suppression of T-cell functions by human granulocyte arginase. Blood 108: 1627-1634.
37. Highfill SL, Rodrigues PC, Zhou Q, Goetz CA, Koehn BH, et al. (2010) Bone marrow myeloid-derived suppressor cells (MDSCs) inhibit graft-versus-host disease (GvHD) via an arginase-1-dependent mechanism that is up-regulated by interleukin-13. Blood 116: 5738-5747.
38. Mosser DM (2003) The many faces of macrophage activation. J Leukoc Biol 73: 209-212.
39. Louis J, Himmelrich H, Parra-Lopez C, Tacchini-Cottier F, Launois P (1998) Regulation of protective immunity against Leishmania major in mice. Curr Opin Immunol 10: 459-464.
40. Hölscher C, Atkinson RA, Arendse B, Brown N, Myburgh E, et al. (2001) A protective and agonistic function of IL-12p40 in mycobacterial infection. J Immunol 167: 6957-6966.
41. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B (2006) TGF-β in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity 24: 179-189.
42. Stockinger B, Veldhoen M (2007) Differentiation and function of Th17 T cells. Curr Opin Immunol 19: 281-286.
43. Gibbs DF, Warner RL, Weiss SJ, Johnson KJ, Varani J (1999) Characterization of matrix metalloproteinases produced by rat alveolar macrophages. Am J Respir Cell Mol Biol 20: 1136-1144.
44. Gibbs DF, Shanley TP, Warner RL, Murphy HS, Varani J, et al. (1999b) Role of matrix metalloproteinases in models of macrophage-dependent acute lung injury. Evidence for alveolar macrophage as source of proteinases. Am J Resp Cell Mol Biol 20: 1145-1154.
45. Chizziolini C, Rezzonico R, De Luca C, Burger D, Dayer J-M (2000) Th2 cell membrane factors in association with IL-4 enhance matrix metalloproteinase-1 (MMP-1) while decreasing MMP-9 production by granulocyte-macrophage colony stimulating factor-differentiated human monocytes. J Immunol 164: 5952-5960.
46. Van Ginderachter JA, Movahedi K, Hassanzadeh Ghassabeh G, Meerschaut S, Beschin A, et al. (2006) Classical and alternative activation of mononuclear phagocytes: picking the best of both worlds for tumor promotion. Immunobiology 211: 487-501.
47. Mills CD, Ley K (2014) M1 and M2 macrophages: the chicken and the egg of immunity. J Innate Immun 6: 716-726.
48. Baay M, Brouwer A, Pauwels P, Peeters M, Lardon F (2011) Tumor cells and tumor-associated macrophages: secreted proteins as potential targets for therapy. Clin Dev Immunol 2011: 565187.
49. Lenzo JC, Turner AL, Cook AD, Vlahos R, Anderson GP, et al. (2012) Control of macrophage lineage populations by CSF-1 receptor and GM-CSF in homeostasis and inflammation. Immunol Cell Biol 90: 429-440.
50. Verreck FA, de Boer T, Langenberg DM, van der Zanden L, Ottenhoff TH (2006) Phenotypic and functional profiling of human proinflammatory type-1 and anti-inflammatory type-2 macrophages in response to microbial antigens and IFN-gamma- and CD40L-mediated costimulation. J Leukoc Biol 79: 285-293.
51. Albina JE, Henry WL Jr, Mastrofrancesco B, Martin BA, Reichner JS (1995) Macrophage activation by culture in an anoxic environment. J Immunol 155: 4391-4396.
52. Aliberti J, Machado FS, Souto JT, Campanelli AP, Teixeira MM, et al. (1999) chemokines enhance parasite uptake and promote nitric oxide-dependent microbiostatic activity in murine inflammatory macrophages infected with Trypanosoma cruzi. Infect Immun 67: 4819-4826.
53. Kielian MC, Cohn ZA (1981) Phorbol myristate acetate stimulates phagosome-lysosome fusion in mouse macrophages. J Exp Med 154: 101-111.
54. Green SP, Phillips WA (1994) Activation of the macrophage respiratory burst by phorbol myristate acetate: evidence for both tyrosine-kinase-dependent and -independent pathways. Biochim Biophys Acta 1222: 241-248.
55. de Waal Malefyt R, Figdor CG, Huijbens R, Mohan-Peterson S, Bennett B, et al. (1993) Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes. Comparison with IL-4 and modulation by IFN-gamma or IL-10. J Immunol 151: 6370-6381.
56. Pesce J, Kaviratne M, Ramalingam TR, Thompson RW, Urban JF Jr, et al. (2006) The IL-21 receptor augments Th2 effector function and alternative macrophage activation. J Clin Invest 116: 2044-2055.
57. Foey AD (2012) Mucosal macrophages: phenotype and functionality in homeostasis and pathology. Nova Science Publishers Inc., New York, USA 4: 121-146.
58. Smith W, Feldmann M, Londei M (1998) Human macrophages induced in vitro by macrophage colony-stimulating factor are deficient in IL-12 production. Eur J Immunol 28: 2498-2507.
59. Tiemessen MM, Jagger AL, Evans HG, van Herwijnen MJ, John S, et al. (2007) CD4+CD25+Foxp3+ regulatory T cells induce alternative activation of human monocytes/macrophages. Proc Natl Acad Sci U S A 104: 19446-19451.
60. Erwig LP, Kluth DC, Walsh GM, Rees AJ (1998) Initial cytokine exposure determines function of macrophages and renders them unresponsive to other cytokines. J Immunol 161: 1983-1988.
61. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, et al. (1998) Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-?, PGE2 and PAF. J Clin Invest 101: 890-898.
62. Savill J, Dransfield I, Gregory C, Haslett C (2002) A blast from the past: clearance of apoptotic cells regulates immune responses. Nat Rev Immunol 2: 965-975.
63. O'Neill LA, Fitzgerald KA, Bowie AG (2003) The Toll-IL-1 receptor adaptor family grows to five members. Trends Immunol 24: 286-290.
64. O'Neill LA (2008) When signaling pathways collide: positive and negative regulation of toll-like receptor signal transduction. Immunity 29: 12-20.
65. Negishi H, Ohba Y, Yanai H, Takaoka A, Honma K, et al. (2005) Negative regulation of Toll-like-receptor signaling by IRF-4. Proc Natl Acad Sci U S A 102: 15989-15994.
66. Honma K, Udono H, Kohno T, Yamamoto K, Ogawa A, et al. (2005) Interferon regulatory factor 4 negatively regulates the production of proinflammatory cytokines by macrophages in response to LPS. Proc Natl Acad Sci U S A 102: 16001-16006.
67. Brint EK, Xu D, Liu H, Dunne A, McKenzie AN, et al. (2004) ST2 is an inhibitor of interleukin 1 receptor and Toll-like receptor 4 signaling and maintains endotoxin tolerance. Nat Immunol 5: 373-379.
68. Wald D, Qin J, Zhao Z, Qian Y, Naramura M, et al. (2003) SIGIRR, a negative regulator of Toll-like receptor-interleukin 1 receptor signaling. Nat Immunol 4: 920-927.
69. Wilson HM, Chettibi S, Jobin C, Walbaum D, Rees AJ, et al. (2005) Inhibition of macrophage nuclear factor-kappaB leads to a dominant anti-inflammatory phenotype that attenuates glomerular inflammation in vivo. Am J Pathol 167: 27-37.
70. Weisser SB, McLarren KW, Voglmaier N, van Netten-Thomas CJ, Antov A, et al. (2011) Alternative activation of macrophages by IL-4 requires SHIP degradation. Eur J Immunol 41: 1742-1753.
71. Krausgruber T, Blazek K, Smallie T, Alzabin S, Lockstone H, et al. (2011) IRF5 promotes inflammatory macrophage polarization and TH1-TH17 responses. Nat Immunol 12: 231-238.

72. Liu Y, Stewart KN, Bishop E, Marek CJ, Kluth DC, et al. (2008) Unique expression of suppressor of cytokine signaling 3 is essential for classical macrophage activation in rodents in vitro and in vivo. J Immunol 180: 6270-6278.
73. Sahin E, Haubenwallner S1, Kuttke M1, Kollmann I1, Halfmann A2, et al. (2014) Macrophage PTEN regulates expression and secretion of arginase I modulating innate and adaptive immune responses. J Immunol 193: 1717-1727.
74. Díaz-Guerra MJ, Castrillo A, Martín-Sanz P, Boscá L (1999) Negative regulation by phosphatidylinositol 3-kinase of inducible nitric oxide synthase expression in macrophages. J Immunol 162: 6184-6190.
75. Fukao T, Koyasu S (2003) PI3K and negative regulation of TLR signaling. Trends Immunol 24: 358-363.
76. Günzl P, Bauer K, Hainzl E, Matt U, Dillinger B, et al. (2010) Anti-inflammatory properties of the PI3K pathway are mediated by IL-10/DUSP regulation. J Leukoc Biol 88: 1259-1269.
77. Haskó G, Kuhel DG, Marton A, Nemeth ZH, Deitch EA, et al. (2000) Spermine differentially regulates the production of interleukin-12 p40 and interleukin-10 and suppresses the release of the T helper 1 cytokine interferon-gamma. Shock 14: 144-149.
78. Riley JK, Takeda K, Akira S, Schreiber RD (1999) Interleukin-10 receptor signaling through the JAK-STAT pathway. Requirement for two distinct receptor-derived signals for anti-inflammatory action. J Biol Chem 274: 16513-16521.
79. Takeda K, Clausen BE, Kaisho T, Tsujimura T, Terada N, et al. (1999) Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. Immunity 10: 39-49.
80. Sica A, Bronte V (2007) Altered macrophage differentiation and immune dysfunction in tumor development. J Clin Invest 117: 1155-1166.
81. Welch JS, Escoubet-Lozach L, Sykes DB, Liddiard K, Greaves DR, et al. (2002) TH2 cytokines and allergic challenge induce Ym1 expression in macrophages by a STAT6-dependent mechanism. J Biol Chem 277: 42821-42829.
82. Porta C, Rimoldi M, Raes G, Brys L, Ghezzi P, et al. (2009) Tolerance and M2 (alternative) macrophage polarization are related processes orchestrated by p50 nuclear factor kappaB. Proc Natl Acad Sci U S A 106: 14978-14983.
83. Hagemann T, Lawrence T, McNeish I, Charles KA, Kulbe H, et al. (2008) "Re-educating" tumor-associated macrophages by targeting NF-kappaB. J Exp Med 205: 1261-1268.
84. Ruffell D, Mourkioti F, Gambardella A, Kirstetter P, Lopez RG, et al. (2009) A CREB-C/EBPbeta cascade induces M2 macrophage-specific gene expression and promotes muscle injury repair. Proc Natl Acad Sci U S A 106: 17475-17480.
85. Pauleau AL, Rutschman R, Lang R, Pernis A, Watowich SS, et al. (2004) Enhancer-mediated control of macrophage-specific arginase I expression. J Immunol 172: 7565-7573.
86. Foey AD, Brennan FM (2004) Conventional protein kinase C and atypical protein kinase Czeta differentially regulate macrophage production of tumour necrosis factor-alpha and interleukin-10. Immunology 112: 44-53.
87. Arranz A, Doxaki C, Vergadi E, Martinez de la Torre Y, Vaporidi K, et al. (2012) Akt1 and Akt2 protein kinases differentially contribute to macrophage polarization. Proc Natl Acad Sci U S A 109: 9517-9522.
88. Zhu L, Yang T, Li L, Sun L, Hou Y, et al. (2014) TSC1 controls macrophage polarization to prevent inflammatory disease. Nat Commun 5: 4696.
89. Geissmann F, Jung S, Littman DR (2003) Blood monocytes consist of two principal subsets with distinct migratory properties. Immunity 19: 71-82.
90. Ziegler-Heitbrock HW, Passlick B, Flieger D (1988) The monoclonal antimonocyte antibody My4 stains B lymphocytes and two distinct monocyte subsets in human peripheral blood. Hybridoma 7: 521-527.
91. Passlick B, Flieger D, Ziegler-Heitbrock HW (1989) Identification and characterization of a novel monocyte subpopulation in human peripheral blood. Blood 74: 2527-2534.
92. Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, et al. (2010) Nomenclature of monocytes and dendritic cells in blood. Blood 116: e74-80.
93. Ziegler-Heitbrock L (2014) Monocyte subsets in man and other species. Cell Immunol 289: 135-139.
94. Wong KL, Tai JJ, Wong WC, Han H, Sem X, et al. (2011) Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets. Blood 118: e16-31.
95. Fingerle G, Pforte A, Passlick B, Blumenstein M, Ströbel M, et al. (1993) The novel subset of CD14+/CD16+ blood monocytes is expanded in sepsis patients. Blood 82: 3170-3176.
96. Belge KU, Dayyani F, Horelt A, Siedlar M, Frankenberger M, et al. (2002) The proinflammatory CD14+CD16+DR++ monocytes are a major source of TNF. J Immunol 168: 3536-3542.
97. Kawanaka N, Yamamura M, Aita T, Morita Y, Okamoto A, et al. (2002) CD14+,CD16+ blood monocytes and joint inflammation in rheumatoid arthritis. Arthritis Rheum 46: 2578-2586.
98. Ziegler-Heitbrock L (2007) The CD14+ CD16+ blood monocytes: their role in infection and inflammation. J Leukoc Biol 81: 584-592.
99. Fingerle-Rowson G, Angstwurm M, Andreesen R, Ziegler-Heitbrock HW (1998) Selective depletion of CD14+ CD16+ monocytes by glucocorticoid therapy. Clin Exp Immunol 112: 501-506.
100. Siedlar M, Strach M, Bukowska-Strakova K, Lenart M, Szaflarska A, et al. (2011) Preparations of intravenous immunoglobulins diminish the number and pro-inflammatory response of CD14+CD16++ monocytes in common variable immunodeficiency (CVID) patients. Clin Immunol 139: 122-132.
101. Frankenberger M, Sternsdorf T, Pechumer H, Pforte A, Ziegler-Heitbrock HW (1996) Differential cytokine expression in human blood monocyte subpopulations: a polymerase chain reaction analysis. Blood 87: 373-377.
102. Szaflarska A, Baj-Krzyworzeka M, Siedlar M, Weglarczyk K, Ruggiero I, et al. (2004) Antitumor response of CD14+/CD16+ monocyte subpopulation. Exp Hematol 32: 748-755.
103. Weber C, Belge KU, von Hundelshausen P, Draude G, Steppich B, et al. (2000) Differential chemokine receptor expression and function in human monocyte subpopulations. J Leukoc Biol 67: 699-704.
104. Ancuta P, Rao R, Moses A, Mehle A, Shaw SK, et al. (2003) Fractalkine preferentially mediates arrest and migration of CD16+ monocytes. J Exp Med 197: 1701-1707.
105. Grage-Griebenow E, Zawatzky R, Kahlert H, Brade L, Flad H, et al. (2001) Identification of a novel dendritic cell-like subset of CD64(+) / CD16(+) blood monocytes. Eur J Immunol 31: 48-56.
106. Korkosz M, Bukowska-Strakova K, Sadis S, Grodzicki T, Siedlar M (2012) Monoclonal antibodies against macrophage colony-stimulating factor diminish the number of circulating intermediate and nonclassical (CD14(++)CD16(+)/CD14(+)CD16(++)) monocytes in rheumatoid arthritis patients. Blood 119: 5329-5330.
107. Hume DA, Allan W, Hogan PG, Doe WF (1987) Immunohistochemical characterisation of macrophages in the liver and gastrointestinal tract: expression of CD4, HLA-DR, OKM1 and the mature macrophage marker 25F9 in normal and diseased tissue. J Leukocyte Biol 42: 474-484.
108. Smith PD, Ochsenbauer-Jambor C, Smythies LE (2005) Intestinal macrophages: unique effector cells of the innate immune system. Immunol Rev 206: 149-159.
109. Schenk M, Mueller C (2007) Adaptations of intestinal macrophages to an antigen-rich environment. Semin Immunol 19: 84-93.
110. Platt AM, Mowat AM (2008) Mucosal macrophages and the regulation of immune responses in the intestine. Immunol Lett 119: 22-31.
111. Rogler G, Hausmann M, Spöttl T, Vogl D, Aschenbrenner E, et al. (1999) T-cell co-stimulatory molecules are upregulated on intestinal macrophages from inflammatory bowel disease mucosa. Eur J Gastroenterol Hepatol 11: 1105-1111.

112. Smith PD, Smythies LE, Mosteller-Barnum M, Sibley DA, Russell MW, et al. (2001) Intestinal macrophages lack CD14 and CD89 and consequently are down-regulated for LPS- and IgA-mediated activities. J Immunol 167: 2651-2656.

113. Smythies LE, Sellers MT, Clements RH, Mosteller-Barnum M, Meng G, et al. (2005) Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity. J Clin Invest 115: 66-75.

114. Strober W, Zhang F, Kitani A, Fuss I, Fichtner-Feigl S (2010) Proinflammatory cytokines underlying the inflammation of Crohn's disease. Curr Opin Gastroenterol 26: 310-317.

115. Maeda S, Hsu LC, Liu H, Bankston LA, Iimura M, et al. (2005) Nod2 mutation in Crohn's disease potentiates NF-kappaB activity and IL-1beta processing. Science 307: 734-738.

116. Netea MG, Kullberg BJ, de Jong DJ, Franke B, Sprong T, et al. (2004) NOD2 mediates anti-inflammatory signals induced by TLR2 ligands: implications for Crohn's disease. Eur J Immunol 34: 2052-2059.

117. Bouma G, Strober W (2003) The immunological and genetic basis of inflammatory bowel disease. Nat Rev Immunol 3: 521-533.

118. Hardy H, Harris J, Lyon E, Beal J, Foey AD (2013) Probiotics, prebiotics and immunomodulation of gut mucosal defences: homeostasis and immunopathology. Nutrients 5: 1869-1912.

119. Merry R, Belfield L, McArdle P, McLennan A, Crean S, et al. (2012) Oral health and pathology: a macrophage account. Br J Oral Maxillofac Surg 50: 2-7.

120. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr (1998) Microbial complexes in subgingival plaque. J Clin Periodontol 25: 134-144.

121. Ezzo PJ, Cutler CW (2003) Microorganisms as risk indicators for periodontal disease. Periodontol 2000 32: 24-35.

122. Yilmaz O, Watanabe K, Lamont RJ (2002) Involvement of integrins in fimbriae-mediated binding and invasion by Porphyromonas gingivalis. Cell Microbiol 4: 305-314.

123. Jotwani R, Cutler CW (2004) Fimbriated Porphyromonas gingivalis is more efficient than fimbria-deficient P. gingivalis in entering human dendritic cells in vitro and induces an inflammatory Th1 effector response. Infect Immun 72: 1725-1732.

124. Pulendran B, Kumar P, Cutler CW, Mohamadzadeh M, Van Dyke T, et al. (2001) Lipopolysaccharides from distinct pathogens induce different classes of immune responses in vivo. J Immunol 167: 5067-5076.

125. Darveau RP, Pham TT, Lemley K, Reife RA, Bainbridge BW, et al. (2004) Porphyromonas gingivalis lipopolysaccharide contains multiple lipid A species that functionally interact with both toll-like receptors 2 and 4. Infect Immun 72: 5041-5051.

126. Holden JA, Attard TJ, Laughton KM, Mansell A, O'Brien-Simpson NM, Reynolds EC (2014) Porphyromonas gingivalis lipopolysaccharide weakly activates M1 and M2 polarised mouse macrophages but induces inflammatory cytokines. Infect Immun 82: 4190-4203.

127. Barksby HE, Nile CJ, Jaedicke KM, Taylor JJ, Preshaw PM (2009) Differential expression of immunoregulatory genes in monocytes in response to Porphyromonas gingivalis and Escherichia coli lipopolysaccharide. Clin Exp Immunol 156: 479-487.

128. Foey AD, Crean S (2013) Macrophage subset sensitivity to endotoxin tolerisation by Porphyromonas gingivalis. PLoS One 8: e67955.

129. Muthukuru M, Jotwani R, Cutler CW (2005) Oral mucosal endotoxin tolerance induction in chronic periodontitis. Infect Immun 73: 687-694.

130. Koide N, Nishio A, Sato T, Sugiyama A, Miyagawa S (2004) Significance of macrophage chemoattractant protein-1 expression and macrophage infiltration in squamous cell carcinoma of the esophagus. Am J Gastroenterol 99: 1667-1674.

131. Lin EY, Nguyen AV, Russell RG, Pollard JW (2001) Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. J Exp Med 193: 727-740.

132. Lewis CE, Pollard JW (2006) Distinct role of macrophages in different tumor microenvironments. Cancer Res 66: 605-612.

133. Lalla RV, Boisoneau DS, Spiro JD, Kreutzer DL (2003) Expression of vascular endothelial growth factor receptors on tumor cells in head and neck squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 129: 882-888.

134. Sica A, Allavena P, Mantovani A (2008) Cancer related inflammation: the macrophage connection. Cancer Lett 267: 204-215.

135. Kuropkat C, Dünne AA, Plehn S, Ossendorf M, Herz U, et al. (2003) Macrophage colony-stimulating factor as a tumor marker for squamous cell carcinoma of the head and neck. Tumour Biol 24: 236-240.

136. Wang F, Arun P, Friedman J, Chen Z, Van Waes C (2009) Current and potential inflammation targeted therapies in head and neck cancer. Curr Opin Pharmacol 9: 389-395.

137. Kundu N, Fulton AM (1997) Interleukin-10 inhibits tumor metastasis, downregulates MHC class I, and enhances NK lysis. Cell Immunol 180: 55-61.

138. de Waal Malefyt R, Haanem J, Spits H, Roncarolo MG, te Velde A, et al. (1991) Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. J Exp Med 174: 915-924.

139. Kryczek I, Zou L, Rodriguez P, Zhu G, Wei S, et al. (2006) B7-H4 expression identifies a novel suppressive macrophage population in human ovarian carcinoma. J Exp Med 203: 871-881.

140. Balm FJ, Drexhage HA, von Blomberg ME, Snow GB (1982) Mononuclear phagocyte function in head and neck cancer: NBT-dye reduction, maturation and migration of peripheral blood monocytes. Laryngoscope 92: 810-814.

141. Young MR, Wright MA, Lozano Y, Matthews JP, Benefield J, et al. (1996) Mechanisms of immune suppression in patients with head and neck cancer: influence on the immune infiltrate of the cancer. Int J Cancer 67: 333-338.

142. Levings MK, Bacchetta R, Schulz U, Roncarolo MG (2002) The role of IL-10 and TGF-beta in the differentiation and effector function of T regulatory cells. Int Arch Allergy Immunol 129: 263-276.

143. Satoh T, Saika T, Ebara S, Kusaka N, Timme TL, et al. (2003) Macrophages transduced with an adenoviral vector expressing interleukin 12 suppresses tumour growth and metastasis in a preclinical metastatic prostate cancer model. Cancer Res 63: 7853-7860.

144. Biswas SK, Sica A, Lewis CE (2008) Plasticity of macrophage function during tumor progression: regulation by distinct molecular mechanisms. J Immunol 180: 2011-2017.

145. Leek RD, Lewis CE, Whitehouse R, Greenall M, Clarke J, et al. (1996) Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. Cancer Res 56: 4625-4629.

146. Burton JL, Wells JM, Corke KP (2000) Macrophages accumulate in avascular, hypoxic areas of prostate tumours: implications for the targeted therapeutic gene delivery to such sites. J Pathol 192: 8A.

147. Negus RP, Stamp GW, Hadley J, Balkwill FR (1997) Quantitative assessment of the leukocyte infiltrate in ovarian cancer and its relationship to the expression of C-C chemokines. Am J Pathol 150: 1723-1734.

148. Burke B, Tang N, Corke KP, Tazzyman D, Ameri K, et al. (2002) Expression of HIF-1alpha by human macrophages: implications for the use of macrophages in hypoxia-regulated cancer gene therapy. J Pathol 196: 204-212.

149. White JR, Harris RA, Lee SR, Craigon MH, Binley K, et al. (2004) Genetic amplification of the transcriptional response to hypoxia as a novel means of identifying regulators of angiogenesis. Genomics 83: 1-8.

150. Murdoch C, Muthana M, Lewis CE (2005) Hypoxia regulates macrophage functions in inflammation. J Immunol 175: 6257-6263.

151. De Palma M, Venneri MA, Galli R, Sergi Sergi L, Politi LS, et al. (2005) Tie-2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumour vessel formation and a mesenchymal population of pericyte progenitors. Cancer Cell 8: 211-226.

152. Bingle L, Lewis CE, Corke KP, Reed MW, Brown NJ (2006) Macrophages promote angiogenesis in human breast tumour spheroids in vivo. Br J Cancer 94: 101-107.

153. De Palma M1, Lewis CE (2013) Macrophage regulation of tumor responses to anticancer therapies. Cancer Cell 23: 277-286.

154. Muthana M, Rodrigues S, Chen YY, Welford A, Hughes R, et al. (2013) Macrophage delivery of an oncolytic virus abolishes tumor regrowth and metastasis after chemotherapy or irradiation. Cancer Res 73: 490-495.

155. Biswas SK, Lopez-Collazo E (2009) Endotoxin tolerance: new mechanisms, molecules and clinical significance. Trends Immunol 30: 475-487.

156. Gorczynski RM, Cattral MS, Chen Z, Hu J, Lei J, et al. (1999) An immunoadhesin incorporating the molecule OX-2 is a potent immunosuppressant that prolongs allo- and xenograft survival. J Immunol 163: 1654-1660.

157. Gorczynski RM, Chen Z, Yu K, Hu J (2001) CD200 immunoadhesin suppresses collagen-induced arthritis in mice. Clin Immunol 101: 328-334.

158. Simelyte E, Criado G, Essex D, Uger RA, Feldmann M, et al. (2008) CD200-Fc, a novel antiarthritic biologic agent that targets proinflammatory cytokine expression in the joints of mice with collagen-induced arthritis. Arthritis Rheum 58: 1038-1043.

159. Barclay AN, Wright GJ, Brooke G, Brown MH (2002) CD200 and membrane protein interactions in the control of myeloid cells. Trends Immunol 23: 285-290.

160. Armant M, Avice MN, Hermann P, Rubio M, Kiniwa M, et al. (1999) CD47 ligation selectively downregulates human interleukin 12 production. J Exp Med 190: 1175-1182.

161. Ravetch JV, Bolland S (2001) IgG Fc receptors. Annu Rev Immunol 19: 275-290.

162. Abrahams VM, Cambridge G, Lydyard PM, Edwards JC (2000) Induction of tumor necrosis factor alpha production by adhered human monocytes: a key role for Fcgamma receptor type IIIa in rheumatoid arthritis. Arthritis Rheum 43: 608-616.

163. Kleinau S, Martinsson P, Heyman B (2000) Induction and suppression of collagen-induced arthritis is dependent on distinct fcgamma receptors. J Exp Med 191: 1611-1616.

164. Grazia Cappiello M, Sutterwala FS, Trinchieri G, Mosser DM, Ma X (2001) Suppression of Il-12 transcription in macrophages following Fc gamma receptor ligation. J Immunol 166: 4498-4506.

165. Samuelsson A, Towers TL, Ravetch JV (2001) Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. Science 291: 484-486.

166. Bruhns P, Samuelsson A, Pollard JW, Ravetch JV (2003) Colony-stimulating factor-1-dependent macrophages are responsible for IVIG protection in antibody-induced autoimmune disease. Immunity 18: 573-581.

167. Rauh MJ, Ho V, Pereira C, Sham A, Sly LM, et al. (2005) SHIP represses the generation of alternatively activated macrophages. Immunity 23: 361-374.

168. Crocker PR, Varki A (2001) Siglecs, sialic acids and innate immunity. Trends Immunol 22: 337-342.

169. Lock K, Zhang J, Lu J, Lee SH, Crocker PR (2004) Expression of CD33-related siglecs on human mononuclear phagocytes, monocyte-derived dendritic cells and plasmacytoid dendritic cells. Immunobiol 209: 199-207.

170. Ando M, Tu W, Nishijima K, Iijima S (2008) Siglec-9 enhances IL-10 production in macrophages via tyrosine-based motifs. Biochem Biophys Res Commun 369: 878-883.

171. Orr SJ, Morgan NM, Elliott J, Burrows JF, Scott CJ, et al. (2007) CD33 responses are blocked by SOCS3 through accelerated proteasomal-mediated turnover. Blood 109: 1061-1068.

172. Orr SJ, Morgan NM, Buick RJ, Boyd CR, Elliott J, et al. (2007) SOCS3 targets Siglec 7 for proteasomal degradation and blocks Siglec 7-mediated responses. J Biol Chem 282: 3418-3422.

173. Stout RD, Jiang C, Matta B, Tietzel I, Watkins SK, et al. (2005) Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. J Immunol 175: 342-349.

174. Xu W, Zhao X, Daha MR, van Kooten C (2013) Reversible differentiation of pro- and anti-inflammatory macrophages. Mol Immunol 53: 179-186.

175. Gerber JS, Mosser DM (2001) Reversing lipopolysaccharide toxicity by ligating the macrophage Fc gamma receptors. J Immunol 166: 6861-6868.

176. Fong CH, Bebien M, Didierlaurent A, Nebauer R, Hussell T, et al. (2008) An antiinflammatory role for IKKbeta through the inhibition of "classical" macrophage activation. J Exp Med 205: 1269-1276.

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