Macrophage Polarization Mini Review

1. History of the terminology of macrophage polarization
   In the 1990s it was discovered that the cytokine interleukin (IL)-4 induced different effects on macrophage gene expression compared to that of interferon (IFN)-gamma and lipopolysaccharide (LPS). In contrast to the classical activation of macrophages by IFN-gamma (Nathan CF et al. 1983), the macrophage gene expression induced by IL-4 was described as “alternative activation” (Stein M et al. 1992, Martinez FO and Gordon S 2014).

   A few years later, in 2000, Mills et al. proposed a new classification of macrophages as either M1 or M2. This terminology originated from an observation of differential macrophage arginine metabolism in various mouse strains with T helper type 1 (Th1) (C57BL/6 mice) and T helper type 2 (Th2) (Balb/c mice) backgrounds. Th1 mice with T cells producing mostly IFN-gamma demonstrated macrophage activation in which nitric oxide (NO) was generated from arginine, versus ornithine production from Th2 mice with T cells producing IL-4 and tumor growth factor (TGF)-beta 1 (Mills CD et al. 2000). This finding led to a consensus within the scientific community that M1 (classically activated) macrophages exhibit inflammatory functions, whereas M2 (alternatively activated) macrophages exhibit anti-inflammatory functions. In 2004, Mantovani et al. further divided M2 macrophages into M2a, M2b, M2c and M2d subtypes based on the applied stimuli and the induced transcriptional changes (Mantovani A et al. 2004, Röszer T 2015).

   The M1/M2 classification of macrophages is now considered an oversimplified approach that does not adequately describe the spectrum of macrophage populations. For instance, the identification of tumor associated macrophages (TAMs), which do not fit nicely into the criteria for M1 or M2 macrophages complicates this system (Qian BZ and Pollard JW 2010). In addition, macrophages expressing T cell receptors (TCR) and CD169 have also been identified.

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2. Phenotype of M1 and M2 macrophage subsets and their functions

Macrophages derived from monocyte precursors undergo specific differentiation depending on the local tissue environment. They respond to environmental cues within tissues such as damaged cells, activated lymphocytes, or microbial products, to differentiate into distinct functional phenotypes. The M1 macrophage phenotype is characterized by the production of high levels of pro-inflammatory cytokines, an ability to mediate resistance to pathogens, strong microbicidal properties, high production of reactive nitrogen and oxygen intermediates, and promotion of Th1 responses. In contrast, M2 macrophages are characterized by their involvement in parasite control, tissue remodeling, immune regulation, tumor promotion and efficient phagocytic activity.

LPS, IFN-gamma and granulocyte-macrophage colony stimulating factor (GM-CSF) polarize macrophages towards the M1 phenotype, which induces secretion of large amounts of cytokines such as IL-1-beta, tumor necrosis factor (TNF), IL-12, IL-18 and IL-23. This helps to drive antigen specific Th1 and Th17 cell inflammatory responses. Phenotypically, M1 macrophages express high levels of major histocompatibility complex class II (MHC II), the CD68 marker, and co-stimulatory molecules CD80 and CD86 (see Table 1). M1 macrophages have also been shown to up-regulate the expression of the intracellular protein suppressor of cytokine signaling 3 (SOCS3), as well as activate inducible nitric oxide synthase (NOS2 or iNOS) to produce NO from L-arginine (MacMicking J et al. 1997, Arnold CE et al. 2014) (Table 1). In disease contexts, M1 macrophages are implicated in initiating and sustaining inflammation, and can therefore be detrimental to health.

In contrast, M2 macrophage activation is induced by fungal cells, immune complexes, helminth infections, complement components, apoptotic cells, macrophage colony stimulating factor (MCSF), IL-4, IL-13, IL-10 and TGF-beta. This activation leads to the secretion of high amounts of IL-10 and low levels of IL-12. Phenotypically M2 macrophages have been characterized as IL-12lowIL-10highIL-1decoyRhighIL-1RAhigh. They are also defined as IL-2low, IL-23low, IL-1betalow and caspase-1low. In addition, they express high levels of scavenger mannose and galactose E-type and C-type receptors (Table 1), and repurpose arginine metabolism to express ornithine and polyamine, which promotes growth.

It is now appreciated that the M2 terminology encompasses a functionally diverse group of macrophages rather than a unique activation state. Accordingly, M2 macrophages can be further divided into subsets (Table 1), specifically M2a, M2b, M2c and M2d based on their distinct gene expression profiles (Mantovani A et al. 2004, Röszer T 2015). The M2a subtype is elicited by IL-4, IL-13 or fungal and helminth infections. M2b is elicited by IL-1 receptor ligands, immune complexes and LPS whereas M2c is elicited by IL-10, TGF-beta and glucocorticoids. The fourth type, M2d, is elicited by IL-6 and adenosine. M2d macrophages have phenotypic and functional attributes similar to ovarian TAMs but are distinct from M2a-c (Duluc D et al. 2007).

M1 and M2 macrophages have distinct chemokine and chemokine receptor profiles, with M1 secreting the Th1 cell attracting chemokines CXCL9 and CXCL10 and M2 secreting CCL17, CCL22 and CCL24. It has recently been demonstrated that in vitro, macrophages are capable of complete repolarization from M2 to M1, and can reverse their polarization depending on the chemokine environment (Davis MJ et al. 2013). The change in polarization is rapid and involves rewiring of signaling networks at both the transcriptional and translational levels.
Table 1. Classically activated (M1) and alternatively activated (M2) subset phenotypes

<table>
<thead>
<tr>
<th></th>
<th>M1</th>
<th>M2a</th>
<th>M2b</th>
<th>M2c</th>
<th>M2d</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stimulation/activation</strong></td>
<td>IFN-gamma, LPS, GM-CSF</td>
<td>IL-4, IL-13, Fungal and Helminth infection</td>
<td>ICs, IL-1R</td>
<td>IL-10, TGF-beta, GCs</td>
<td>IL-6, LIF, Adenosine</td>
</tr>
<tr>
<td><strong>Marker expression</strong></td>
<td>CD68, CD86, MHC II, IL-1R, TLR2, TLR4, iNOS, SOCS3</td>
<td>CD163, MHC II, SR, MMR/CD206, CD200R, TGM2, DecoyR, IL-1R II, Mouse only: Ym1/2, Fizz1, Arg-1</td>
<td>CD86, MHC II</td>
<td>CD163, TLR1, TLR8</td>
<td>VEGF</td>
</tr>
<tr>
<td><strong>Cytokine secretion</strong></td>
<td>TNF, IL-1beta, IL-6, IL-12, IL-23</td>
<td>IL-10, TGF-beta, IL-1ra</td>
<td>IL-1, IL-6, IL-10, TNF-alpha</td>
<td>IL-10, TGF-beta</td>
<td>IL-10, IL-12, TNF-alpha, TGF-beta</td>
</tr>
<tr>
<td><strong>Chemokine secretion</strong></td>
<td>CCL10, CCL11, CCL5, CCL8, CCL9, CCL2, CCL3, CCL4</td>
<td>CCL17, CCL22, CCL24</td>
<td>CCL1, CCR2</td>
<td>CCL5, CXCL10, CXCL16</td>
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</table>

Adapted from Röszer T 2015 and Duluc D et al. 2007.
Arg-1, arginase-1; FIZZ1, resistin-like molecule-alpha (Relm-alpha); GCs, glucocorticoids; ICs, immune complexes; IL1-ra, IL-1 receptor antagonist; LIF, leukocyte inhibitory factor; TGM2, transglutaminase 2; TGF-beta, transforming growth factor-beta; TNF-alpha, tumor necrosis factor alpha; TLR, Toll-like receptor; MMR (CD206), macrophage mannose receptor; iNOS, inducible nitric oxide synthase; SR, scavenger receptor; SOCS3, suppressor of cytokine signaling 3; VEGF, vascular endothelial growth factor; Ym1 (also known as chitinase-3-like protein-3 (Chi3l3)).

3. Signaling molecules involved in M1/M2 polarization

A network of transcription factors and post-transcriptional regulators are involved in M1/M2 polarization (Sica A and Mantovani A 2012) (Figure 1). Interferon regulatory factor (IRF), signal transducers and activators of transcription (STAT) and suppressor of cytokine signaling (SOCS) proteins all play a role in skewing macrophage function towards either the M1 or M2 phenotype. The IRF/STAT pathways, activated by IFNs and toll-like receptor (TLR) signaling, polarize macrophages to the M1 activation state via STAT1. On the other hand, IL-4 and IL-13 skew macrophages toward the M2 activation state via STAT 6 (Sica A and Bronte V 2007).

M1 macrophages have been shown to upregulate IRF5, which is critical for M1 polarization and the induction of IL-12, IL-23 and TNF (Krausgruber T et al. 2011) as well as Th1 and Th17 responses. The LPS/TLR4 pathway also plays a role in M1 polarization by activating STAT1-alpha/beta in a MyD88 independent fashion (Toshchakov V et al. 2002). A role for Bruton's tyrosine Kinase (Btk) has also been implicated in macrophage polarization in response to LPS stimulation. Absence of Btk was shown to skew macrophages towards an M2 phenotype, indicating its critical role in M1 polarization (Ni G et al. 2014). Other molecules implicated in the induction of the M1 phenotype state are the G-protein coupled receptor, P2Y(2)R, which plays a role in inducing NO via NOS2 (Eun SY et al. 2014); SOCS3, which activates NF-κB/PI-3 kinase pathways to produce NO (Arnold CE et al. 2014) and the growth and differentiation factor Activin A, which promotes M1 markers and down-regulates IL-10 (Sierra-Filardi E et al. 2011).
Arginase 1 production is a distinct hallmark of M2 macrophages and is transcribed by STAT6, which is downstream of IL-4/IL-13 receptor signaling. Krüppel-like factor 4 (KLF-4) coordinates with STAT6 to induce M2 genes such as Arg-1, Mrc1, Fizz1 and PPARγ, and inhibit M1 genes such as TNFalpha, Cox-2, CCL5 and NOS2 (Figure 2). This is mediated through sequestration of co-activators necessary for NF-κB activation (Liao X et al. 2011). Accordingly, the NF-κB p50 subunit (as homodimers) has been shown to be essential for M2 polarization in vitro and in vivo (Porta C et al. 2009). In addition, the nuclear receptor, peroxisome proliferator-activated receptor γ (PPARγ), has been shown to regulate genes involved in oxidative metabolism and activation of the M2 phenotype (Odegaard JI et al. 2007, Bouhlel MA et al. 2007). The hypoxia inducible factors HIF-1α and HIF-2α also play a role in regulating M1/M2 polarization with HIF-1α regulating NOS2 expression and the M1 state and HIF-2α arginase 1 expression and the M2 state (Takeda N et al. 2010).

Other signaling molecules shown to play a role specifically in M2 polarization include the cytokine IL-21, which mediates M2 polarization by decreasing NOS2 expression and increasing STAT3 phosphorylation (Li SN et al. 2013); IRF4 which negatively regulates TLR signaling in a MyD88 independent manner to drive M2 activation (Satoh T et al. 2010) and BMP-7 which induces M2 polarization in vitro via activation of the SMAD-PI3K-Akt-mTOR pathway (Rocher C et al. 2013).
Table 2. Signaling molecules and genes involved in each macrophage polarization state

<table>
<thead>
<tr>
<th></th>
<th>M1</th>
<th>M2</th>
</tr>
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<tbody>
<tr>
<td><strong>Signaling molecules</strong></td>
<td>STAT1-alpha/beta, IRF5, Btk, P2Y (2)R, SOCS3, Activin A, Hif1-α</td>
<td>STAT6, IRF4, KLF-4, NF-κB p50 homodimers, PPARγ, HIF-2α, IL-21, BMP-7, FABP4, LXR α</td>
</tr>
<tr>
<td><strong>Genes</strong></td>
<td>TNF alpha, Cox-2, CCL5, NOS2</td>
<td>Arg-1, Mrc-1, Fizz1, PPARγ</td>
</tr>
</tbody>
</table>

Adapted from Sica A and Mantovani A 2012 and Chávez-Galán L et al. 2015.

4. Mouse and human macrophages - similarities and differences

Some of the markers used to define M1 and M2 polarization vary between mouse and human macrophages (Martinez FO et al. 2013). For instance, there are no human homologs of the mouse M2 markers Ym1, Fizz1, or arginase-1. However, some molecules such as the multifunctional enzyme transglutaminase 2 (TGM2) serves as conserved markers for both human and mouse M2 macrophages and monocytes (Martinez FO et al. 2013). Identification of human M2 macrophages can now easily be performed by immunohistochemistry double staining techniques using a combination of TGM2, mannose receptor C type 1 (MRC1/CD206), and CD68 antibodies.

The following table highlights some key markers for distinguishing human and mouse M1 and M2 macrophages.

Table 3. Human and mouse M1 and M2 phenotype markers

<table>
<thead>
<tr>
<th></th>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human</strong></td>
<td>CD64, IDO, SOCS1, CXCL10</td>
<td>MRC1, TGM2, CD23, CCL22</td>
</tr>
<tr>
<td><strong>Mouse</strong></td>
<td>CXCL9, CXCL10, CXCL11, NOS2</td>
<td>MRC1, TGM2, Fizz1, Ym1/2, Arg1</td>
</tr>
</tbody>
</table>

Adapted from Martinez FO et al. 2013 and Martinez FO and Gordon S 2014.

Arg-1, arginase-1; FIZZ1, resistin-like molecule alpha (Relm-alpha); CCL22, chemokine (C-C motif) ligand 22; MRC1, mannose receptor C type 1; NOS2, nitric oxide synthase 2; SOCS1, suppressor of cytokine signaling 1; TGM2, transglutaminase 2; CXCL, chemokine (C-X-C motif) ligand; IDO, indoleamine 2,3-dioxygenase.

5. Characterization of tumor associated macrophages

Macrophages are one of the major populations of infiltrating leukocytes associated with solid tumors (Gordon S and Taylor PR 2005). They can be recruited to the tumor site from surrounding tissues by the tumor itself through secretion of chemotactic molecules. In addition, monocytes circulating in the blood stream can infiltrate into the tumor microenvironment and mature into TAMs (Kitamura T et al. 2015). Studies in mice have suggested that the tumor infiltrating monocyte pool is primarily Ly-6C<sup>+</sup>CX3CR1<sup>low</sup>, and TAM monocyte precursors are exclusively Ly-6C<sup>+</sup>CX3CR1<sup>high</sup> cells (Movahedi K et al. 2010).

The term TAMs actually describes various macrophage subsets that vary depending on the cytokine balance within the tumor microenvironment. They have been primarily described as having an M2-like phenotype (Sica A et al. 2006) but studies have shown that they can express both M1 and M2 polarization hallmarks (Mantovani A et al. 2006, Allavena P et al. 2008). Notably, switching TAMs to a predominantly M1 phenotype has been proposed as a key anti-cancer immunotherapeutic treatment strategy (Mills CD et al. 2016).
TAMs are known to promote tumor progression and are associated with poor prognosis (Komohara Y et al. 2014). They induce angiogenesis, lymphogenesis, stroma remodeling and immune suppression. They also play a key role in promoting tumor invasion and metastasis (Komohara Y et al. 2015) through secretion of the enzymes plasmin, uPA, matrix metalloproteinases (MMPs) and cathepsin B (Gocheva V et al. 2010, Wang R et al. 2011).

TAMs exhibit a distinct transcriptional profile from M1 and M2 macrophages (Biswas SK et al. 2006) and are phenotypically characterized as CCL2[hi]CCL5[hi]IL-10[hi]. They also express MGL -1, Dectin-1, CD68, CD206, VEGF-A, NOS2, CD81, MHC II and scavenger receptor A (Chávez-Galán L et al. 2015, Röszer T 2015). In addition, they exhibit enhanced IRF-3/STAT-1 activation and defective NF-κB signaling (Biswas SK et al. 2006).

The table below shows a selection of human cancers and the TAM markers used for diagnosis.

### Table 4. Markers of tumor associated macrophages in human cancers

<table>
<thead>
<tr>
<th>Organ</th>
<th>Cancer Type</th>
<th>Markers</th>
<th>Method</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph node</td>
<td>Hodgkin's lymphoma</td>
<td>CD68, CD163</td>
<td>TMA</td>
<td>Harris JA et al. 2012</td>
</tr>
<tr>
<td>Lung</td>
<td>Non-small cell lung cancer (NSCLC)</td>
<td>CD68, CD163, IL-10, iNOS, VEGF, HLA-DR</td>
<td>DIHC</td>
<td>Ohri CM et al. 2011</td>
</tr>
<tr>
<td>Uterus</td>
<td>Endometrial carcinoma</td>
<td>CD163, CD31, H1F-1α</td>
<td>IHC</td>
<td>Espinosa I et al. 2012</td>
</tr>
<tr>
<td>Peripheral lymphoma</td>
<td>Angioimmunoblastic T cell lymphoma</td>
<td>CD68, CD163</td>
<td>DIHC</td>
<td>Komohara ND et al. 2010</td>
</tr>
<tr>
<td>Ovarian and peritoneum</td>
<td>Ovarian carcinoma</td>
<td>CD3, HAM56, B7-H4</td>
<td>DIHC, FIHC, FC</td>
<td>Kryczek I et al. 2006</td>
</tr>
</tbody>
</table>

Adapted from Heusinkveld M and van der Burg SH 2011.

FC, flow cytometry; DIHC, double immunohistochemistry; FIHC, fluorescent immunohistochemistry staining; IHC, immunohistochemistry; TMA, tissue microarray.

6. T cell receptor[α] and CD169[α] macrophages

In contrast to the macrophage subsets described above, there is limited data regarding the characteristics of TCR[α] and CD169[α] macrophages. However, their unique traits have fascinated immunologists and further research into their roles in regulating immune function is currently underway.

As the name suggests, TCR expression was originally thought to be exclusive to T cells, however recent studies indicate that other leukocyte subsets such as neutrophils also express the receptor (Puellmann K et al. 2006). Moreover, several studies have reported the presence of both human and mouse TCR[α] macrophages (Chávez-Galán L et al. 2015). These macrophages express the TCR co-receptor CD3 as well as TCRαβ and γδ subtypes (Beham AW et al. 2011, Chávez-Galán L et al. 2015). TNF has been reported to be a key regulator of TCRαβ expression in macrophages (Beham AW et al. 2011), and cholesterol import/export was shown to be an important modulator of expression of the TCRαβ repertoire (Fuchs T et al. 2015). TCRγδ macrophages have been implicated in host defense against bacterial challenge (Fuchs T et al. 2013).
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Both subsets of TCR+ macrophages express molecules shown to be necessary for T cell signaling such as ZAP70, LAT, Fyn and Lck. Furthermore, they demonstrate high phagocytic capacity and secrete the chemokine CCL2. They have also been implicated in inflammatory and infectious diseases (Chávez-Galán L et al. 2015).

CD169+ macrophages represent the other subset of unconventional macrophages. CD169, also known as sialoadhesin or sialic acid binding immunoglobulin-like lectin (Siglec) 1, is the founding member of the Siglec superfamily of proteins. CD169+ macrophages are primarily located in secondary lymphoid organs but redistribute upon immune activation. It is reported that anti-CD169 antibodies label three macrophage populations in mouse secondary lymphoid organs: marginal zone metallophilic (MZM) macrophages in the spleen, and subcapsular sinus (SS) and medullary (Med) macrophages in lymph nodes (Crocker PR and Gordon S 1989, Oetke C et al. 2006). The signaling pathways involved in the activation of this macrophage subset are not fully understood but some aspects of their biological functions have been elucidated. For instance, they do not mediate phagocytosis but are mainly involved in immune regulation rather than steady state homeostasis. Recently, CD169+ macrophages were shown to be capable of antigen presentation to B cells and activation of CD8+ T cells (Martinez-Pomares L and Gordon S 2012). Other molecules that characterize these macrophages include CD11b, MHC II, CD68, CD11c and F4/80, although F4/80 is not expressed on all CD169+ macrophages (Chávez-Galán L et al. 2015).

The current nomenclature for macrophages is complex and the identification of CD169+ and TCR+ macrophages certainly adds to the complexity. Further research is needed to fully understand the exclusive role of these macrophages in disease. The table below summarizes the markers currently used to identify these unique macrophage subsets.

### Table 5. Additional molecular markers for characterizing TCR+ and CD169+ macrophages

<table>
<thead>
<tr>
<th>TCR+</th>
<th>CD169+</th>
</tr>
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<tbody>
<tr>
<td>CD68</td>
<td>CD11b</td>
</tr>
<tr>
<td>ZAP70</td>
<td>MHC II</td>
</tr>
<tr>
<td>LAT</td>
<td>CD68</td>
</tr>
<tr>
<td>Fyn</td>
<td>CD11c</td>
</tr>
<tr>
<td>Lck</td>
<td>F4/80</td>
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</table>

Adapted from Chávez-Galán L et al. 2015.

ZAP70, zeta- chain (TCR) associated protein kinase 70 kDa; LAT, linker of activated T cells, MHC II, major histoocompatibility complex class II.

References


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Movahedi K et al. (2010). Different tumor microenvironments contain functionally distinct subsets of macrophages derived from Ly6C (high) monocytes. Cancer Res 70, 5728-5739.


Ohri CM et al. (2011). The tissue microlocalisation and cellular expression of CD163, VEGF, HLA-DR, iNOS, and MRP 8/14 is correlated to clinical outcome in NSCLC. Plos One 6, e21874.


