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Chapter

New Tools for Studying Macrophage Polarization: Application to Bacterial Infections

Soraya Mezouar and Jean-Louis Mege

Abstract

Macrophages are tissue immune cells involved in homeostasis and are considered as the first line of defense during bacterial infections. They are resident cells but may be recruited during inflammation and/or infection. Hence, their study is necessary not only to decipher innate immune mechanisms involved in bacterial infections but also to follow infected patients. Among the numerous functions of macrophages, their polarization into microbicidal or permissive cells has been an interesting concept to describe their responses to bacterial aggression. Numerous *in vitro* studies, including ours, have shown the ability of bacteria to induce different patterns of macrophage polarization. However, the studies of patients during infections have produced less convincing results. We propose in this review to take stock of the tools for studying the polarization of macrophages and to show their limits. We make recommendations for using macrophage polarization as a biomarker for measuring severity and response to treatment in bacterial infectious diseases.

Keywords: macrophage, infection, bacterium, polarization, infectious diseases

1. Introduction

Elie Metchnikoff used for the first time the term "macrophage" to describe highly mobile cells able to phagocyte bacteria, which earned him the Nobel Prize in 1908 [1]. During several decades, it was admitted that macrophages are issued from circulating monocytes in homeostatic conditions or after their migration to the tissues following chemokine gradients. More recently, the use of new tools such as genetic fate mapping techniques has shown that most of resident macrophages are of embryonic origin and monocytes contribute to their renewal when homeostasis is impaired [2]. In addition to their role in regulation of tissue development and homeostasis, macrophages actively participate to innate immune defense through the recognition of viruses, bacteria, parasites or fungi [3].

In contrast to lymphoid cells, macrophages are neither antigen specific nor clonally restricted and express a large panel of membrane molecules. The activation ways of macrophages during infection rely on the interaction of pathogen-associated molecular pattern (PAMPs) with pathogen recognition receptors (PRRs) such as toll-like receptors (TLRs), scavenger receptors, C type lectin receptors, or complement receptors [4]. The interaction between PAMPs and PRRs leads to the activation of macrophages including production and secretion of cytokines,

chemokines and lipid mediators, and promotes the uptake of microorganisms and their destruction [5]. Hence, macrophages are at the center of anti-infectious immune response, which includes pathogen recognition, macrophage activation and pathogen elimination [6, 7].

The polarization state of macrophage is characterized by their activation by pathogen ligands and inflammatory molecules. As previously described for T cell subsets with Th1 and Th2 functional dichotomy, M1 and M2 polarization may correspond to downstream effects of T cell polarization [8]. Numerous approaches have been performed to investigate macrophage responses during infection. Among them, the concept of polarization profile has represented a powerful strategy to investigate macrophage activation states during infection [9]. Here, we investigate tools available to study macrophages in a critical point of view and we propose them to assess prognosis and therapeutic response in bacterial infectious diseases.

2. Concept of macrophage polarization

The term of "polarization" corresponds to functional states exhibiting a binary distribution. It was used for the first time in 1986 by Mosmann et al. to characterize two murine T helper lymphocyte sub-populations, i.e., Th1 and Th2 according to their respective stimuli, interferon (IFN)-γ and interleukin (IL)-4 [10]. The concept of macrophage polarization was deduced from the Th1 and Th2 polarization and accounted for the diversity of macrophage activation [8]. Hence, Stein et al. showed that IL-4 stimulated the expression by murine macrophages of the mannose type 1 receptor (MRC1, CD206) associated to enhanced particle uptake and decreased release of tumor necrosis factor (TNF), a potent inflammatory cytokine; these characteristics may be considered as a model of M2 signature [11]. Later, Mills et al. confirmed that Th1 or Th2 lymphocytes led to the polarization of macrophages into M1 (inflammatory) and M2 (immunoregulatory) profiles [8]. Another nomenclature coexisted with M1/M2 polarization: M1 macrophages were also called classically activated macrophages while M2 macrophages exhibited an alternative type of activation [12]. Few authors use now these two terms and the heterogeneity of M2 macrophages do not fit with the category of alternative activation, explaining why we will use only M1 and M2 terms.

As depicted in **Figure 1**, M1 and M2 profiles are induced by specific ligands. M1 profile is elicited by inflammatory cytokines (TNF or IFN-γ), bacterial components such as lipopolysaccharide (LPS) or growth factors including granulocyte-macrophage colony-stimulating factor (GM-CSF). In contrast, Th2 cytokines (IL-4, IL-10 and IL-13) lead to M2 polarization in the same way as IL-33, transforming growth factor (TGF)-β or macrophage colony-stimulating factor (M-CSF), the master growth factor of myeloid lineage. According the way of stimulation, macrophages express several different markers, secrete different mediators and exercise specific functions (**Figure 1**) [13–16]. It is important to note that M2 macrophages are more heterogeneous than M1 macrophages and have been divided into three distinct profiles including M2a, M2b and M2c according their functions as "alternative and repairers" (M2a) or anti-inflammatory regulators (M2b and M2c) [17, 18]. M2a, M2b and M2c macrophages are activated by IL-14 and IL-13, immunes complexes associated with TLRs or glucocorticoids, IL-10 and TGF-β, respectively [13]. In contrast to a general point of view, using mass spectrometry we found that IFN-γ-stimulated macrophages exhibit a proteomic profile distinct from LPS-stimulated macrophages or LPS/IFN-γ-stimulated macrophages even if they are all included in M1 category [19]. The appearance of numerous discrepancies with the concept of M1/M2 dichotomy led scientists working on macrophage polarization to propose a

	M1 polarization	M2 polarization
Stimulation	• Proinflammatory cytokine TNF • Lipopolysaccharide *via* TLR-4 • IFN-γ • GM-CSF	• Th2 cytokines: IL-4, IL-10 and IL-13 • Glucocorticoids • IL-33 • TGF-β • G-CSF
Markers and cytokines production	CX3CR1, CCR7, IL-7R, CD80, CD86 IL-1β, IL-12, IL-23, ROS, TNF-α, IL-23	CD209, CD206, CD369, VEGF, Arg-1, CD369 IL-1β, IL-23, IL-10, IL-4, IL-6, TGF-β ↘ IL-12, TNF-α
Functions	• Progression of inflammation • Th1 response • Antimicrobial activities	• Enhanced scavenger receptors • Enhanced mannose receptors • Tissue repair and regeneration • Anti-inflammatory • Would healing • Immunologic homeostasis • Th2 response

Figure 1.
Polarization profile of placental macrophages. Summary of the molecules involved in polarization profiles inducing the expression of several proteins leading to several functions of placental macrophages.

reappraisal based on the type of agonist. Hence, the concept of polarization should include the source of macrophages, the type of activation and a collection of activation markers. We have proposed to adopt a nomenclature related to the agonist: M(IL-4), M(IFN-γ), M(IL-10), M(GC), M(Ig) and M(LPS) [20].

3. Macrophage polarization during bacterial infections

The M1 profile is classically associated with control of bacterial infections but its definition is variable among publications. In some reports, only few inflammatory mediators (cytokines and inducible nitric oxide synthase (iNOS)) are considered whereas, in others, a combination of markers is used with large sets of genes or proteins [16, 21, 22]. In *in vitro* studies, we and others reported that a M1 profile is found in response to several bacterial pathogens including *Salmonella typhimurium*, *Orientia tsutsugamushi*, *Legionella pneumophila*, *Francisella* spp., *Rickettsia montanensis*, *Shigella dysenteriae*, *Bartonella* spp., *Mycobacterium ulcerans*, *Chlamydia spp.* or *Listeria monocytogenes* [16, 23–27]. The M1 profile is not synonymous of cure of infections since inappropriate M1 response to infection may be deleterious to the host. In animal models of sepsis, M1 phenotype is prevailing in animals that died dye? [28]. This paralysis of immune system may be modelized in models of LPS tolerance. Hence, in repeatedly treated macrophages by LPS, a M2 profile of macrophages becomes prevailing in the late phase of sepsis. The addition of IFN-γ produced by NK cells may reprogram macrophages toward a M1 phenotype [29].

On another hand, bacteria such as *Yersinia spp*. [30], *Ehrlichia muris* [31], *Chlamydia pneumoniae* [32], *Borrelia burgdorferi* [33], *Salmonella typhimurium* [34] or *Rickettsia conorii* [35] favor the occurrence of M2 profiles in macrophages.

As example, we reported that macrophages infiltrating lamina propria during Whipple's disease, an infectious disease due to *Tropheryma whipplei*, are clearly polarized toward M2 phenotype [36]. The M2 profile is a source of a relative consensus and consists of a panel of lectin-like molecules, arginase-1 (Arg1) and a lot of immunoregulatory genes and proteins. It is noteworthy that the number of bacteria inducing a M2 profile is more limited than those inducing a M1 profile. This may be related to the fact that antibacterial responses are of Th1-type rather than Th2-type.

The survival and the replication of pathogenic bacteria within macrophages may rely on strategies interfering with their polarization. *Shigella flexneri* escapes to TLR-4 recognition in murine macrophages via the expression of a truncated form of LPS (hypoacetylated) [37]. This strategy leads to a decreased inflammatory response and prevents the development of M1 response. *Staphylococcus aureus* inhibits NF-κB activity in mice, which is associated with an inhibition of the M1 phenotype of macrophages [38]. This may be related to the resistance of the biofilm of *S. aureus* to macrophage invasion through a decreased expression of inflammatory mediators including IL-1β, TNF, iNOS and an increased expression of Arg1, suggesting a M2 reprogramming [39]. *M. tuberculosis* also interferes with the M1 polarization profile of macrophages by inhibiting phagosome maturation and NF-κB activation [40] or the stimulation of the pathway of Wingless-type MMTV integration site family, member 6 (Wnt6), leading to a M2-like polarization [41]. Interestingly, it was reported that during *M. tuberculosis* infection, macrophage population found in granuloma are mainly TCR^+ that were directly involved in the maintain of the granuloma structure in an TNF-dependent manner [42]. Considered as a distinct subpopulation, macrophage TCR^+ were suggested to present specific characteristics and functionalities whose polarization status is not yet known.

Coxiella burnetii is the cause of Q fever that targets monocytes and macrophages and macrophage polarization may reflect the different steps of disease progression [43]. *C. burnetii* infects monocytes and macrophages, but only M2 polarization environment favorizes their survival [44]. In this context, *C. burnetii* infection leads to a M2 activation of human macrophages including alveolar and monocyte-derived macrophages (MDMs) [16, 45]. This M2 activation is atypical, characterized by the expression of both M2 (TGF-β, CCL18, Arg1, mannose receptor and IL-1 receptor antagonist) and M1 (IL-6 and IL-18) markers. In contrast, *C. burnetii* elicits M1 profile in monocytes in which bacteria do not replicate but only survive [16]. The deficiency of M1 markers, using $NOS2^{-}/^{-}$ or $IFN\gamma^{-}/^{-}$ mice, leads to bacterial replication whereas *C. burnetii* replication is impaired in $IL\text{-}4^{-}/^{-}$ mice [46]. In patients with Q fever, the polarization state of macrophages is closely dependent on the form of Q fever disease including acute or persistent infection. Our team reported the central role of IL-10 associated with uncontrolled *C. burnetii* replication in macrophages from patients with persistent Q fever [47], as well as the bacterial persistence in transgenic mice with IL-10 overexpression in the macrophage compartment [48]. These results suggest that a M2b (IL-10-dependent) profile is associated with bacterial persistence in patients with persistent Q fever.

4. Models of macrophage polarization and methods of study

The evaluation of macrophage polarization depends on cell type (primary cells *versus* cell lines) and origin (murine *versus* human macrophages). The murine (RAW264.7 and J774) and human (THP-1) cell lines have been largely used to study macrophage polarization but the immaturity of murine cell lines limits

experimental conclusions. The THP-1 cell line is a robust and proliferative cell line that differentiated into "macrophage-like" following phorbol 12-myristate 13-acetate or M-CSF treatment. In contrast to primary macrophages, THP-1 cells are easily transfected to modify genes involved in polarization pathways. Despite these advantages, the THP-1 cell line presents a lack of physiological relevance and should be dedicated to basic research [49]. Rodents provide a convenient model for macrophage studies since all macrophage compartments are accessible. For a long time, peritoneal macrophages have been the gold standard of macrophage studies despite of their great heterogeneity because they were isolated from peritoneal cavity or from exudates in great quantities. As resident peritoneal macrophages are of M2-type, there may be a concern for their use in polarization experiments [2]. Bone marrow-derived macrophages (BMDMs) can be isolated from wild type and transgenic mice and they represent murine macrophage primary cells mostly used for the investigation of macrophage polarization; these cells have the advantage to present low donor variability and to be genetically modifiable [50].

In healthy humans and patients, primary macrophages derived from peripheral blood mononuclear cells (PBMCs) constitute the most practical model, especially to evaluate the polarization profile. Monocytes are isolated from PBMCs using $CD14^{+}$ positive selection and differentiated into monocyte-derived macrophages (MDMs) that are not immortalized and do not proliferate. MDMs are produced in large quantities that allow the evaluation of several polarization markers [51]. Nevertheless, there is large donor variability, and cells from certain donors do not respond to polarizing agonists. This variability among individuals may point to *in vitro* cell isolation techniques or artificial differentiation techniques, which could modify transcriptional profile. Recently, it has been showed that macrophages derived from monocytes issued from human stem cells (embryonic or pluripotent) represent a powerful tool to investigate human macrophage polarization [52]. Takata et al. generated human macrophages from induced pluripotent stem cells (iPSCs): these iPSC-derived primitive macrophages (iMacs) exhibit all the criteria of human MDMs [53]. Besides the differentiation ex vivo of monocytes or stem cells into macrophages, the access to tissue macrophages in humans remains a major pitfall. An indirect strategy to reproduce immune response in tissues consists in the formation of granulomas using PBMCs. We showed that granulomatous macrophages share gene expression signature with IFN-γ-stimulated macrophages and thus exhibit a M1 profile [54, 55]. The development of 3D bioengineered tissue model in which macrophages are in their natural environment will be a strategy for future evaluation [56]. Only some tissue macrophage populations are directly available such as alveolar macrophages obtained from bronchoalveolar lavages (BAL). In addition to ethical restriction of BAL in healthy controls, their purity is a concern for investigators, making standardization almost impossible. Placental macrophages are an original population of macrophages of both maternal and fetal origin. We developed a simple method to isolate and characterize them [57]. As placental macrophages are obtained after delivery, the investigation of their polarization is reserved to retrospective studies. Biopsies of pathological tissues are a source of heterogeneous macrophage populations. Hence, we obtained interesting results about M2 polarization of macrophages in intestinal biopsies of patients with Whipple's disease in which the accumulation of macrophages in lamina propria is a clue for the diagnosis but, again, it is not achievable in healthy subjects. Finally, oncologists have a real expertise in macrophage polarization in tumor-associated macrophages (TAMs) [58]. TAMs were found considered as M2-myeloid population in order to maintain a tolerance in the tumor microenvironment [59–61]. They were considered as a marker for recurrence of cancer [62] and their accumulation in tumor microenvironment is associated with a poor

prognosis. They presented an ability to switch to M1 phenotype during anti-cancer treatment [63] suggesting that this polarization change could constitute a therapeutic approach [64]. We have to learn lessons from results of polarization in TAMs to translate them to bacterial infections.

The evaluation of macrophage polarization needs a set of markers rather than a single molecule. This is exemplified by the use of a scavenger receptor, CD163, as a prototype of M2 marker. Indeed, CD163 is expressed by M1 cells and non-myeloid cells although at lower level [65]. The same comment can be done with iNOS, a M1 marker, also expressed by endothelial cells and arterial wall smooth muscle cells [66]. The development of high-throughput methods (omics technics) has offered the opportunity to provide convenient sets of polarization markers. The transcriptomics methods such as microarray had been a strategy to investigate macrophage polarization since they provide a large panel of transcripts associated with different modes of polarization (**Figure 1**). Martinez et al. reported transcriptomic analysis of activated macrophages: 5.2% and 0.3% of transcripts are associated with M1 and M2 polarization profiles, respectively [14]. Few years later, Xue et al. performed a transcriptomic analysis of human macrophages stimulated by various panels of agonists [67]. They identified nine specific distinct profiles according the agonist used, and a common transcriptomic signature, which was pertinent to isolate a polarized signature in inflammatory and infectious diseases outside of cancer [68]. Some alternative approaches to microarray such as nanostring method uses directly a panel of genes to measure their variation and may be convenient to investigate macrophage polarization [69]. Whatever the method, gene expression data must be controlled by quantitative RT-PCR, a very sensitive technic that needs low amounts of cells [70]. Discrepancies between microarray and quantitative RT-PCR have been often observed in macrophage polarization studies. The emergence of single cell RNA-sequencing (scRNA-seq) method might provide a powerful tool for analysis macrophage populations including their phenotype and therefore their polarization profile. Interestingly, the used of scRNA-seq permitted to show that M2 macrophages express varying levels of Arg1, challenging the dogma that macrophages with M2 profile all express Arg1 [71].

All these methods measure the expression of genes associated with macrophage polarization. This approach does not have the robustness of methods determining the expression of proteins. The enzyme-linked immunosorbent assay (ELISA) has the disadvantage to measure isolated secreted molecules associated with a given profile. The simultaneous measurement of up to 50 proteins using Luminex assays constitutes an interesting option but the cost and the specialized detection equipment represent a disadvantage. We previously investigated macrophage polarization by a proteomic approach using MALDI-TOF mass spectrometry technique combined with gel electrophoresis [19, 72]. This combined approach allows the determination of M1 signature of human macrophages stimulated with IFN-γ, LPS or bacteria. Moreover, different subtypes of M1 and M2 polarized macrophages have been identified using this approach [72].

The flow cytometry and CyTOF techniques offer a better investigation of macrophage phenotypes through the investigation of protein expression at a single cell resolution level. Hence, CyTOF panels have been proposed to measure polarization markers and, combined with high dimensional analysis, CyTOF enables the identification of novel functional macrophage subsets [73]. The emergence of cycling imaging that purposes to stain cells with different cocktail markers after bleaching allows the detection of more than 30 markers at once [74]. Finally, basic methods such as cell morphology could be used to evaluate functional phenotypes of polarized macrophages [75]. Indeed, polarized M2 murine macrophages are more elongated than M1 cells [76, 77].

A new and exciting field of exploring macrophage polarization, the study of metabolic changes, has recently emerged. LPS ligation of TLR-4 elicits a shift to glycolytic metabolism with impaired mitochondrial respiration. Associated with IFN-γ, LPS induces alterations in tricarboxylic acid cycle. In contrast, IL-4 responses are associated with a shift to oxidative metabolism. Hence, the M2 program associates changes in polyamine synthesis and fatty acid oxidation [78, 79].

This huge diversity of methods exploring macrophage activation including macrophage polarization needs to define the conditions of using these methods and the stratification of indications.

5. Recommendations for measuring macrophage polarization in infected patients

The interest of measuring macrophage polarization in patients is to assess activation status of macrophages to stratify them and to measure their response to treatment. The investigation of macrophage polarization in infected patients requires the choice of pertinent cell types and of the method of measurement. Studies with macrophages from healthy controls stimulated *in vitro* with polarizing ligands are needed to collect specific signatures and to standardize those found in patients. When cells are isolated from infected patients, we have to decipher if they are polarized and which agonist is responsible of such activation profile. As a consequence, each signature should contain several molecules for each polarization category and the determination of these different signatures should be easy to perform in biological laboratories. This means that technics for measuring gene expression such as quantitative RT-PCR, phenotyping membrane or intracellular molecules through flow cytometry or molecule secretion using multiplex ELISA should be privileged. Unfortunately, there is no consensus about the content of polarization signature. Some authors used limited number of molecules known to be associated with polarized status of macrophages, other groups including our used signatures obtained from microarray/RNA sequencing data collection. Hence, the comparison of the studies becomes extremely difficult.

The investigation of patients with bacterial infection is limited by accessibility of biological materials in contrast to cancer in whom tissue biopsies are required for the diagnosis. In practice, circulating monocytes, associated or not with lymphocytes, are the major source of myeloid cells. However, it is uncertain that the M1/M2 polarization of tissue macrophages is also found in circulating monocytes. We compared the polarization of monocytes and MDMs from healthy donors in response to canonical agonists of macrophage M1/M2 polarization, IFN-γ and IL-4. While the two cytokines elicit clear polarized profile in MDMs, a similar polarization is observed in early stimulated monocytes and is lost after 24 h of treatment [80]. This observation may account for numerous discrepancies found in several examples of infectious diseases. While *M. tuberculosis* induces a M2 profile in macrophages *in vitro* [81, 82], the study of gene expression in patients suffering from active tuberculosis revealed a signature in which neutrophil and type I IFN are prominent but did not reveal a polarized profile [83]. We draw similar conclusions from our investigation of patients suffering from Q fever. *C. burnetii* interferes with M1 polarization of macrophages leading to an atypical M2 program [16] but the investigation of circulating monocytes using microarray and quantitative RT-PCR as a confirmation did not reveal a polarization in patients with acute or persistent Q fever [80]. These two examples do not invalidate the use of polarization concept in patients with an infectious disease but underlines the necessity to analyze the data according the type of myeloid cells. In addition, the use of macrophages differentiated from

patient monocytes may be biased by the role of M-CSF in the differentiation process that may affect macrophage polarization. It is likely that studying polarization in tissue macrophages such as alveolar macrophages and intestinal macrophages may be more pertinent.

The biopsies are reserved to severe infections or rare infectious diseases in which they are necessary for the diagnosis as in Whipple's disease. The advantage of such approach has been recently illustrated. In patients with tuberculosis who underwent surgical treatment, the investigation of pulmonary biopsies revealed that M2-like polarization was correlated with multidrug resistance [84]. We are suggesting adopting the guidelines used in oncology to characterize TAMs [66]. The polarization of tissue macrophages may be assessed in histological sections either by isolation of cells and *ex vivo* studies or *in situ*. In this later case, immunohistochemistry (IHC) is the best strategy for studying macrophage polarization. The choice of detection method, immunofluorescence or chromogenic method, is discussed. As most samples are fixed with formalin and embedded in paraffin, the chromogenic method is the most convenient. The limit of IHC is the number of available antibodies. Hence, Jayasingam et al. recommend to use double IHC staining: CD68/iNOS or CD68/HLA-DR for M1 macrophages and CD68/CD163 for M2 macrophages. This is in contradiction with the concept of signature and it is necessary to provide new technological solutions to better characterize macrophage polarization in tissues. For instance, mass spectrometry imaging would be useful to analyze macrophages in tissues as already done in tumors. The development of mass cytometry will be interesting for phenotyping tissue macrophages [85, 86].

The concept of macrophage polarization has reached adulthood. If it is extremely efficient for pathophysiological studies, it needs to be adapted to the requirements of clinical investigations. This requires to follow the guidelines we defined several years ago according each type of agonist instead of too imprecise categories such as M1 or M2 cells. It also requires new technical solutions to directly investigate macrophages within tissues. Finally, we have to propose alternatives to biopsy sampling in infected patients who do not require such aggressive procedure.

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Declaration of interest

The authors declare no competing interests.

Author contributions

S.M. and J.L.M. conceived and wrote the manuscript.

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References

[1] Tauber AI. Metchnikoff and the phagocytosis theory. Nature Reviews. Molecular Cell Biology. 2003;**4**(11):897-901

[2] Epelman S, Lavine KJ, Randolph GJ. Origin and functions of tissue macrophages. Immunity. 2014;**41**(1):21-35

[3] Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. Nature. 2013;**496**(7446):445-455

[4] Gordon S. Pattern recognition receptors. Cell. 2002;**111**(7):927-930

[5] Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. Nature Reviews. Immunology. 2011;**11**(11):723-737

[6] Flannagan RS, Cosío G, Grinstein S. Antimicrobial mechanisms of phagocytes and bacterial evasion strategies. Nature Reviews. Microbiology. 2009;**7**(5):355-366

[7] Weiss G, Schaible UE. Macrophage defense mechanisms against intracellular bacteria. Immunological Reviews. 2015;**264**(1):182-203

[8] Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M1/M2 macrophages and the Th1/Th2 paradigm. Journal of Immunology. 2000;**164**(12):6166-6173

[9] Mills CD, Ley K. M1 and M2 macrophages: The chicken and the egg of immunity. Journal of Innate Immunity. 2014;**6**(6):716-726

[10] Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. Journal of Immunology. 1986;**136**(7):2348-2357

[11] Stein M, Keshav S, Harris N, Gordon S. Interleukin 4 potently enhances murine macrophage mannose receptor activity: A marker of alternative immunologic macrophage activation. The Journal of Experimental Medicine. 1992;**176**(1):287-292

[12] Gordon S. Alternative activation of macrophages. Nature Reviews. Immunology. 2003;**3**(1):23-35

[13] Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends in Immunology. 2004;**25**(12):677-686

[14] Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: New molecules and patterns of gene expression. Journal of Immunology. 2006;**177**(10):7303-7311

[15] Martinez FO, Helming L, Milde R, Varin A, Melgert BN, Draijer C, et al. Genetic programs expressed in resting and IL-4 alternatively activated mouse and human macrophages: Similarities and differences. Blood. 2013;**121**(9):e57-e69

[16] Benoit M, Desnues B, Mege J-L. Macrophage polarization in bacterial infections. Journal of Immunology. 2008;**181**(6):3733-3739

[17] Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. Frontiers in Bioscience. 2008;**13**:453-461

[18] Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nature Reviews. Immunology. 2008;**8**(12):958-969

[19] Ouedraogo R, Daumas A, Capo C, Mege J-L, Textoris J. Whole-cell

MALDI-TOF mass spectrometry is an accurate and rapid method to analyze different modes of macrophage activation. Journal of Visualized Experiments. 2013;**82**:50926

[20] Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage activation and polarization: Nomenclature and experimental guidelines. Immunity. 2014;**41**(1):14-20

[21] Jenner RG, Young RA. Insights into host responses against pathogens from transcriptional profiling. Nature Reviews. Microbiology. 2005;**3**(4):281-294

[22] Nau GJ, Richmond JFL, Schlesinger A, Jennings EG, Lander ES, Young RA. Human macrophage activation programs induced by bacterial pathogens. Proceedings of the National Academy of Sciences of the United States of America. 2002;**99**(3):1503-1508

[23] Shaughnessy LM, Swanson JA. The role of the activated macrophage in clearing *Listeria monocytogenes* infection. Frontiers in Bioscience. 2007;**12**:2683-2692

[24] Rottenberg ME, Gigliotti-Rothfuchs A, Wigzell H. The role of IFN-γ in the outcome of chlamydial infection. Current Opinion in Immunology. 2002;**14**(4):444-451

[25] Chacón-Salinas R, Serafín-López J, Ramos-Payán R, Méndez-Aragón P, Hernández-Pando R, Van Soolingen D, et al. Differential pattern of cytokine expression by macrophages infected *in vitro* with different *Mycobacterium tuberculosis* genotypes. Clinical and Experimental Immunology. 2005;**140**(3):443-449

[26] Kiszewski AE, Becerril E, Aguilar LD, Kader ITA, Myers W, Portaels F, et al. The local immune response in ulcerative lesions of Buruli disease. Clinical and Experimental Immunology. 2006;**143**(3):445-451

[27] Jouanguy E, Lamhamedi-Cherradi S, Lammas D, Dorman SE, Fondanèche M-C, Dupuis S, et al. A human IFNGR1 small deletion hotspot associated with dominant susceptibility to mycobacterial infection. Nature Genetics. 1999;**21**(4):370-378

[28] Mehta A, Brewington R, Chatterji M, Zoubine M, Kinasewitz GT, Peer GT, et al. Infection-induced of M1 and M2 phenotypes in circulating monocytes: Role in immune monitoring and early prognosis of sepsis. Shock. 2004;**22**(5):423-430

[29] Bellora F, Castriconi R, Dondero A, Reggiardo G, Moretta L, Mantovani A, et al. The interaction of human natural killer cells with either unpolarized or polarized macrophages results in different functional outcomes. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**(50):21659-21664

[30] Tumitan ARP, Monnazzi LGS, Ghiraldi FR, Cilli EM, Machado de Medeiros BM. Pattern of macrophage activation in *Yersinia*-resistant and *Yersinia*-susceptible strains of mice. Microbiology and Immunology. 2007;**51**(10):1021-1028

[31] Haloul M, Oliveira ERA, Kader M, Wells JZ, Tominello TR, El Andaloussi A, et al. mTORC1-mediated polarization of M1 macrophages and their accumulation in the liver correlate with immunopathology in fatal ehrlichiosis. Scientific Reports. 2019;**9**(1):14050

[32] Buchacher T, Ohradanova-Repic A, Stockinger H, Fischer MB, Weber V. M2 polarization of human macrophages favors survival of the intracellular pathogen *Chlamydia pneumoniae*. PLoS One. 2015;**10**(11):e0143593

[33] Lasky CE, Olson RM, Brown CR. Macrophage polarization during murine *Lyme borreliosis*. Infection and Immunity. 2015;**83**(7):2627-2635

[34] Eisele NA, Ruby T, Jacobson A, Manzanill PS, Cox JS, Lam L, et al. Cell Host & Microbe. 2013;**14**(2):171-182

[35] Curto P, Santa C, Allen P, Manadas B, Simões I, Martinez JJ. A pathogen and a non-pathogen spotted fever group Rickettsia trigger differential proteome signatures in macrophages. Frontiers in Cellular and Infection Microbiology. 2019;**9**:43

[36] Desnues B, Lepidi H, Raoult D, Mege J. Whipple disease: Intestinal infiltrating cells exhibit a transcriptional pattern of M2/alternatively activated macrophages. The Journal of Infectious Diseases. 2005;**192**(9):1642-1646

[37] Paciello I, Silipo A, Lembo-Fazio L, Curcuru L, Zumsteg A, Noel G, et al. Intracellular Shigella remodels its LPS to dampen the innate immune recognition and evade inflammasome activation. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**(46):E4345-E4354

[38] Xu F, Kang Y, Zhang H, Piao Z, Yin H, Diao R, et al. Akt1-mediated regulation of macrophage polarization in a murine model of *Staphylococcus aureus* pulmonary infection. The Journal of Infectious Diseases. 2013;**208**(3):528-538

[39] Thurlow LR, Hanke ML, Fritz T, Angle A, Aldrich A, Williams SH, et al. *Staphylococcus aureus* biofilms prevent macrophage phagocytosis and attenuate inflammation in vivo. Journal of Immunology. 2011;**186**(11):6585-6596

[40] Lugo-Villarino G. Macrophage polarization: Convergence point targeted by *Mycobacterium tuberculosis* and HIV. Frontiers in Immunology. 2011;**2**:43. Available from: http://journal.frontiersin.org/article/10.3389/fimmu.2011.00043/abstract

[41] Schaale K, Brandenburg J, Kispert A, Leitges M, Ehlers S, Reiling N. Wnt6 is expressed in granulomatous lesions of *Mycobacterium tuberculosis* – infected mice and is involved in macrophage differentiation and proliferation. The Journal of Immunology. 2013;**191**(10):5182-5195

[42] Beham AW, Puellmann K, Laird R, Fuchs T, Streich R, Breysach C, et al. A TNF-regulated recombinatorial macrophage immune receptor implicated in granuloma formation in tuberculosis. PLoS Pathogens. 2011;**7**(11):e1002375

[43] Eldin C, Mélenotte C, Mediannikov O, Ghigo E, Million M, Edouard S, et al. From Q fever to *Coxiella burnetii* infection: A paradigm change. Clinical Microbiology Reviews. 2017;**30**(1):115-190

[44] Amara AB, Bechah Y, Mege J-L. Immune response and *Coxiella burnetii* invasion. Advances in Experimental Medicine and Biology. 2012;**984**:287-298

[45] Dragan AL, Voth DE. *Coxiella burnetii*: International pathogen of mystery. Microbes and Infection. April 2019;**22**(3):100-110

[46] Fernandes TD, Cunha LD, Ribeiro JM, Massis LM, Lima-Junior DS, Newton HJ, et al. Murine alveolar macrophages are highly susceptible to replication of *Coxiella burnetii* phase II *in vitro*. Infection and Immunity. 2016;**84**(9):2439-2448

[47] Honstettre A, Imbert G, Ghigo E, Gouriet F, Capo C, Raoult D, et al. Dysregulation of cytokines in acute Q fever: Role of interleukin-10 and tumor necrosis factor in chronic evolution of Q fever. Journal of Infectious Diseases. 2003;**187**(6):956-962

[48] Meghari S, Bechah Y, Capo C, Lepidi H, Raoult D, Murray PJ, et al. Persistent *Coxiella burnetii* infection in mice overexpressing IL-10: An efficient model for chronic Q fever pathogenesis. PLoS Pathogens. 2008;**4**(2):e23

[49] Baxter EW, Graham AE, Re NA, Carr IM, Robinson JI, Mackie SL, et al. Standardized protocols for differentiation of THP-1 cells to macrophages with distinct M(IFNγ + LPS), M(IL-4) and M(IL-10) phenotypes. Journal of Immunological Methods. 2020;**478**:112721

[50] Pineda-Torra I, Gage M, de Juan A, Pello OM. Isolation, culture, and polarization of murine bone marrow-derived and peritoneal macrophages. Methods in Molecular Biology. 2015;**1339**:101-109

[51] Jin X, Kruth HS. Culture of macrophage colony-stimulating factor differentiated human monocyte-derived macrophages. Journal of Visualized Experiments. 2016;**112**:54244

[52] van Wilgenburg B, Browne C, Vowles J, Cowley SA. Efficient, long term production of monocyte-derived macrophages from human pluripotent stem cells under partly-defined and fully-defined conditions. PLoS One. 2013;**8**(8):e71098

[53] Takata K, Kozaki T, Lee CZW, Thion MS, Otsuka M, Lim S, et al. Induced-pluripotent-stem-cell-derived primitive macrophages provide a platform for modeling tissue-resident macrophage differentiation and function. Immunity. 2017;**47**(1):183-198.e6

[54] Faugaret D, Ben Amara A, Alingrin J, Daumas A, Delaby A, Lépolard C, et al. Granulomatous response to *Coxiella burnetii*, the agent of Q fever: The lessons from gene expression analysis. Frontiers in Cellular and Infection Microbiology. 15 December 2014;**4**:172. Available from: http://journal.frontiersin.org/article/10.3389/fcimb.2014.00172/abstract

[55] Delaby A, Gorvel L, Espinosa L, Lépolard C, Raoult D, Ghigo E, et al. Defective monocyte dynamics in Q fever granuloma deficiency. The Journal of Infectious Diseases. 2012;**205**(7):1086-1094

[56] Roh TT, Chen Y, Paul HT, Guo C, Kaplan DL. 3D bioengineered tissue model of the large intestine to study inflammatory bowel disease. Biomaterials. 2019;**225**:119517

[57] Mezouar S, Ben Amara A, Chartier C, Gorvel L, Mege J-L. A fast and reliable method to isolate human placental macrophages. Current Protocols in Immunology. 2019;**125**(1):e77

[58] Tamura R, Tanaka T, Yamamoto Y, Akasaki Y, Sasaki H. Dual role of macrophage in tumor immunity. Immunotherapy. 2018;**10**(10):899-909

[59] Mantovani A, Schioppa T, Porta C, Allavena P, Sica A. Role of tumor-associated macrophages in tumor progression and invasion. Cancer Metastasis Reviews. 2006;**25**(3):315-322

[60] Sica A, Schioppa T, Mantovani A, Allavena P. Tumour-associated macrophages are a distinct M2 polarized population promoting tumour progression: Potential targets of anti-cancer therapy. European Journal of Cancer. 2006;**42**(6):717-727

[61] Allavena P, Sica A, Garlanda C, Mantovani A. The Yin-Yang of tumor-associated macrophages in neoplastic progression and immune surveillance. Immunological Reviews. 2008;**222**(1):155-161

[62] Suriano F, Santini D, Perrone G, Amato M, Vincenzi B, Tonini G, et al. Tumor associated macrophages

polarization dictates the efficacy of BCG instillation in non-muscle invasive urothelial bladder cancer. Journal of Experimental & Clinical Cancer Research. 2013;**32**(1):87

[63] Chen D, Xie J, Fiskesund R, Dong W, Liang X, Lv J, et al. Chloroquine modulates antitumor immune response by resetting tumor-associated macrophages toward M1 phenotype. Nature Communications. 2018;**9**(1):873

[64] Mantovani A, Germano G, Marchesi F, Locatelli M, Biswas SK. Cancer-promoting tumor-associated macrophages: New vistas and open questions. European Journal of Immunology. 2011;**41**(9):2522-2525

[65] Hu JM, Liu K, Liu JH, Jiang XL, Wang XL, Chen YZ, et al. CD163 as a marker of M2 macrophage, contribute to predicte aggressiveness and prognosis of Kazakh esophageal squamous cell carcinoma. Oncotarget. 2017;**8**(13):21526-21538

[66] Jayasingam SD, Citartan M, Thang TH, Mat Zin AA, Ang KC, Ch'ng ES. Evaluating the polarization of tumor-associated macrophages into M1 and M2 phenotypes in human cancer tissue: Technicalities and challenges in routine clinical practice. Frontiers in Oncology. 2020;**9**:1512

[67] Xue J, Schmidt SV, Sander J, Draffehn A, Krebs W, Quester I, et al. Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. Immunity. 2014;**40**(2):274-288

[68] Hussell T, Bell TJ. Alveolar macrophages: Plasticity in a tissue-specific context. Nature Reviews. Immunology. 2014;**14**(2):81-93

[69] Ayaub EA, Tandon K, Padwal M, Imani J, Patel H, Dubey A, et al. IL-6 mediates ER expansion during hyperpolarization of alternatively activated macrophages. Immunology and Cell Biology. 2019;**97**(2):203-217

[70] Spiller KL, Wrona EA, Romero-Torres S, Pallotta I, Graney PL, Witherel CE, et al. Differential gene expression in human, murine, and cell line-derived macrophages upon polarization. Experimental Cell Research. 2016;**347**(1):1-13

[71] Arlauckas SP, Garren SB, Garris CS, Kohler RH, Oh J, Pittet MJ, et al. Arg1 expression defines immunosuppressive subsets of tumor-associated macrophages. Theranostics. 2018;**8**(21):5842-5854

[72] Ouedraogo R, Flaudrops C, Ben Amara A, Capo C, Raoult D, Mege J-L. Global analysis of circulating immune cells by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. PLoS One. 2010;**5**(10):e13691

[73] Roussel M, Bartkowiak T, Irish JM. Picturing polarized myeloid phagocytes and regulatory cells by mass cytometry. Mass Cytometry. 2019;**1989**:217-226. DOI: 10.1007/978-1-4939-9454-0_14

[74] Giedt RJ, Pathania D, Carlson JCT, McFarland PJ, del Castillo AF, Juric D, et al. Single-cell barcode analysis provides a rapid readout of cellular signaling pathways in clinical specimens. Nature Communications. 2018;**9**(1):4550

[75] Marklein RA, Lam J, Guvendiren M, Sung KE, Bauer SR. Functionally-relevant morphological profiling: A tool to assess cellular heterogeneity. Trends in Biotechnology. 2018;**36**(1):105-118

[76] Rodell CB, Arlauckas SP, Cuccarese MF, Garris CS, Li R, Ahmed MS, et al. TLR7/8-agonist-loaded nanoparticles promote the polarization of tumour-associated

macrophages to enhance cancer immunotherapy. Nature Biomedical Engineering. 2018;**2**:578-588

[77] Nishio M, Urakawa N, Shigeoka M, Takase N, Ichihara Y, Arai N, et al. Software-assisted morphometric and phenotype analyses of human peripheral blood monocyte-derived macrophages induced by a microenvironment model of human esophageal squamous cell carcinoma: Image analysis of human PBMC-derived macrophages. Pathology International. 2016;**66**(2):83-93

[78] Phan AT, Goldrath AW, Glass CK. Metabolic and epigenetic coordination of T cell and macrophage immunity. Immunity. 2017;**46**(5):714-729

[79] Caputa G, Castoldi A, Pearce EJ. Metabolic adaptations of tissue-resident immune cells. Nature Immunology. 2019;**20**(7):793-801

[80] Mehraj V, Textoris J, Ben Amara A, Ghigo E, Raoult D, Capo C, et al. Monocyte responses in the context of Q fever: From a static polarized model to a kinetic model of activation. The Journal of Infectious Diseases. 2013;**208**(6):942-951

[81] Huang Z, Luo Q, Guo Y, Chen J, Xiong G, Peng Y, et al. *Mycobacterium tuberculosis*-induced polarization of human macrophage orchestrates the formation and development of tuberculous granulomas *in vitro*. PLoS ONE. 2015;**10**(6):e0129744

[82] Roy S, Schmeier S, Kaczkowski B, Arner E, Alam T, Ozturk M, et al. Transcriptional landscape of *Mycobacterium tuberculosis* infection in macrophages. Scientific Reports. 2018;**8**(1):6758

[83] Berry MPR, Graham CM, McNab FW, Xu Z, Bloch SAA, Oni T, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature. 2010;**466**(7309):973-977

[84] Cho HJ, Lim Y-J, Kim J, Koh W-J, Song C-H, Kang M-W. Different macrophage polarization between drug-susceptible and multidrug-resistant pulmonary tuberculosis. BMC Infectious Diseases. 2020;**20**(1):81

[85] Maby P, Corneau A, Galon J. Phenotyping of tumor infiltrating immune cells using mass-cytometry (CyTOF). Methods in Enzymology. 2020;**632**:339-368

[86] Mrdjen D, Pavlovic A, Hartmann FJ, Schreiner B, Utz SG, Leung BP, et al. High-dimensional single-cell mapping of central nervous system immune cells reveals distinct myeloid subsets in health, aging, and disease. Immunity. 2018;**48**(2):380-395.e6