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Macrophage diversity in cardiac inflammation: a review

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Abstract

Cardiac inflammatory disease represents a significant public health burden, and interesting questions of immunopathologic science and clinical inquiry. Novel insights into the diverse programming and functions within the macrophage lineages in recent years have yielded a view of these cells as dynamic effectors and regulators of immunity, host defense, and inflammatory disease. In this review, we examine and discuss recent investigations into the complex participation of mononuclear phagocytic cells in the pathology of animal models of myocarditis.

Keywords

myocarditis; macrophage; monocyte; autoimmunity

Introduction

Mononuclear phagocytic cells were characterized over a century ago in pioneering studies by Metchnikoff – heralding the advent of our understanding of cell-mediated immunity (1). In the century since, investigations have described a diversity of related cells falling under the banner of “macrophage”, with activities specialized and adapted for effector and regulatory functions in host defense and immunopathologic disease (2). The predominant paradigm for macrophage biology in innate immunity for the past half-century has been the mononuclear phagocyte system (MPS) model, established by van Furth and others (3). The model holds that macrophages in peripheral tissues arise from blood borne precursors, monocytes, which in turn arise from bone marrow precursors that are distinct from lymphocyte precursors. While the model has undergone extensive revision, refinement and complication, the essential notion is still valid (4). In addition to phagocytosis and other

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microbicidal roles, macrophages are known to also participate in endogenous homeostatic functions, such as scavenging (5).

Macrophage involvement in autoimmune disease, particularly inflammatory autoimmunities, has long been suspected. This review summarizes current understanding of the diverse functions and plasticity of macrophages as applied to addressing these longstanding issues. Current research emphasizes an important role for macrophages in the inflammatory component of cardiovascular diseases. Roles for inflammatory macrophage recruitment have been proposed for hypertensive cardiac remodeling (6), and atherosclerosis (7). Similarly, infectious agents associated with cardiac inflammation elicit macrophage and monocyte invasion of the heart, as reported in Lyme carditis (8), simian immunodeficiency virus carditis (9), human immunodeficiency virus carditis (10, 11), Chagas disease (12), and rheumatic carditis (13). An analysis of autopsy samples from sudden death cases revealed that across several infectious agents associated with myocarditis, macrophage and monocyte infiltration was among the most common findings (14).

Macrophage diversity - T cell- and tumor-derived signals

While commonly considered integral to “innate” host defense, macrophages are also responsive to instructive cues from CD4⁺ T cell-derived signals. Interferon-gamma (IFN γ) was first identified as a potent macrophage-activating factor, inducing peroxide production and microbicidal activity in human peripheral blood monocyte-derived macrophages (15). Macrophages are still regarded as major effectors of Th1-associated host defenses. IFN γ control of macrophage activation is associated with microbicidal and tumoricidal activity (16, 17) through the upregulation of lysozymal proteolytic enzymes and oxygen radicals (18). Macrophages from IFN γ -deficient mice have been shown to be defective in both antigen-presentation and the production of iNOS-dependent nitric oxide (19-21). Martinez and colleagues have categorically proposed a distinction between these “classically-activated” or M1-type macrophages, and those elicited by purely innate recognition of microbial pathogen-associated molecular patterns (22); this “innate activation” phenotype express some inflammatory cytokines, but lack enhanced phagocytotic uptake, and express a different program of scavenging and uptake receptors, such as MARCO (*Scara2*) (23).

Th2-associated cytokines were at first thought to suppress macrophage function, as IL4 downregulated superoxide production and the production of IL1 β (24). The activation of macrophages by IL4 was demonstrated in 1992 by Stein and colleagues, who reported that IL4 induced the expression of macrophage mannose receptor (M ϕ MR, CD206), upregulation of MHC Class II, proliferation, and syncytiation in macrophages (25). Additional transcriptional targets upregulated by IL4 or IL13 signaling in macrophages have since been identified, including the Type A Scavenger receptor (SR-A, CD204), the chitinase-like lectins Ym1/2, Resistin-like alpha (HIMF, FIZZ1, RELM α), and arginase-1 (26-28). The resulting paradigm categorizes T cell-elicited macrophages into two major categories, IFN γ -driven “classically-activated” macrophages, and IL4/IL13-elicited “alternatively-activated” macrophages, thought to be specialized for wound healing, fibrotic and scavenger programs, particularly evident in Th2 responses to helminthic, protozoan and other parasitic infections (29).

Investigations in tumor models have provided evidence for a potentially related macrophage phenotype – the “M2” macrophage. The evasive, immunosuppressive tumor microenvironment is associated with the production of IL10, TGF β , glucocorticoids, and vitamin D3, which in turn elicit a poorly activated macrophage phenotype *in situ*, characterized by expression of M ϕ -MR and diminished acquisition of a dendritic cell-like antigen-presenting phenotype (30, 31). Mantovani and colleagues have further termed these variations of alternative-activation of macrophages “M2” phenotypes, respectively (32). These macrophage phenotypes are associated with immunosuppressive and angiogenic functions, elicited by tumors evading immune surveillance (33). Macrophage recruitment to tumors has been found to correlate with poor prognosis, implicating tumor-associated macrophages (TAMs) in critical mechanisms of tumor evasion (34). Recent gene profiling experiments from a breast tumor model have shown that transcriptional profiles among TAMs are surprisingly similar to fetal macrophages, suggesting that the tumorigenic environment adaptively elicits embryonic developmental functions of macrophages as an escape mechanism (35). The complex interactions of macrophages with the tumor microenvironment remain a highly active area of investigation (36).

The relationship of M2 cells and canonical “alternatively-activated” macrophages remains contentious – some authors will use the terms interchangeably, whereas others resist the integration of these models (Refs). The prevailing consensus at present is that these categorizations describe polar simplifications of what is likely to represent a spectrum of macrophage phenotypes *in vivo*, with varying degrees of specialization for microbicidal, surveillance, antigen-presenting, fibrotic, healing, and immunoregulatory functions (37, 38). Moreover, macrophages are thought to be highly plastic, readily programmable integrators of a variety of potentially competing pro-inflammatory, anti-inflammatory, regulatory, and modulatory signaling pathways (39).

Macrophage diversity – different precursors for different functions

In 2003, Geissman and Littman described the phenotypic markers defining circulating monocytic precursors that distinguish subsets of monocytes in mice. Monocytes which express Ly6C and CCR2 migrate to sites of inflammation, whereas monocytes which express CX3CR1, the receptor for CX3CL1/fractalkine, are precursors for partially self-renewing tissue-resident macrophages, which traffic through peripheral tissues under non-inflammatory homeostatic conditions (40). Importantly, these murine populations approximately mirror the major monocytic populations described in humans, particularly with regard to chemokine receptor expression and inflammatory potential (41).

The spleen has been identified as a critical reservoir of monocytic precursors in inflammatory conditions; egress of these precursors has been shown to be under the control of angiotensin II signaling (42). In experimental ischemic heart injury, early recruitment of Ly6C^{hi} inflammatory macrophages is associated with injury, preceding the recruitment of Ly6C^{lo} resident macrophages, which appear to be involved in myocardial healing (43). To further complicate matters, “inflammatory” monocyte populations may have immunosuppressive or regulatory functions; TNF, iNOS-producing dendritic cells (TipDCs) and myeloid-derived suppressor cells (MDSCs) may be derived from these precursor

populations, suggesting that following recruitment, localized microenvironmental signals further tune phenotype and function among MPS-lineage cells (44, 45).

In contrast, Ly6C^{lo} resident-type monocytes have been proposed to be precursors for alternatively-activated macrophages in tissues (46). Extravasated Ly6C^{lo} monocytes bear a transcriptional profile similar to canonical alternatively-activated macrophages (47).

These findings are consistent with the characterization of the osteopetrotic *op/op* mouse, which carries a deleterious mutation in M-CSF (48). The strain mutation is named for a characteristic overgrown skeletal malformity, caused by defective osteoclast development (49, 50). However, M-CSF deficiency is not sufficient to completely ablate all macrophages; selected subsets of macrophages differentiate under the control of GM-CSF (51). GM-CSF has been reported to induce a more inflammatory program of transcriptional regulation than M-CSF, including the induction of IL1, IL6, TNF α , IL12 and IL23. These authors described a protective, immunoregulatory phenotype induced in the presence of M-CSF. Notably, these transcriptional programs are plastic, and are readily interconverted by switching CSF signals (52). Similar plasticity has been reported for macrophage differentiation induced by Th1 and Th2 cytokines (53).

From these data, we can synthesize a model wherein constitutive M-CSF expression controls Ly6C^{lo} resident macrophages at steady-state, maintaining largely homeostatic macrophages such as scavenging and passive surveillance. In inflammatory conditions, such as in the case of infection or an autoimmune lesion, Ly6C^{hi} monocytes expand in response to GM-CSF and are recruited to sites of inflammation. These cells are predisposed to inflammatory, antimicrobial, and cytotoxic functions. Resolution of inflammatory responses allows M-CSF levels to return to predominance, allowing Ly6C^{lo} cells to mediate homeostatic repair and healing from inflammatory insult to the tissue.

Due to the developmental relationships between macrophages and dendritic cells, it may also be worthwhile to consider specialization for antigen-presentation and education of adaptive responses as a separate, perhaps orthogonal function of MPS-lineage cells. It has been recently argued that treating macrophages and dendritic cells as functionally independent lineages obscures the shared ontogeny and functional relationships of these cells (54). Indeed, both cell types have been shown to be able to perform functions commonly assumed to be restricted to the other: dendritic cells are capable of phagocytosis and production of oxygen radicals (55-58), while macrophages are capable of cross-presenting intracellular antigens (59-61). In this review, we confine our attention to macrophage populations.

Monocyte and macrophage infiltration in autoimmune disease

Cells of the monocyte and macrophage lineages comprise a substantial fraction, if not the majority, of infiltrating inflammatory cells in EAE (62-64), EAU (65), CIA (66) and experimental myocarditis (67).

Substantial research into macrophage-mediated autoimmune pathology has been pursued in various models of arthritis. Kelchtermans and colleagues observed that neutralization of

IL17 during the severe CIA of IFN γ R^{-/-} mice diminished the production of GM-CSF in serum, following *in vivo* challenge with α CD3. These authors found no role for IFN γ in direct control of GM-CSF, arguing that GM-CSF is directly regulated by IL17 (68). In a model of streptococcal cell wall-induced arthritis, Plater-Zyberk and colleagues found that neutralization of GM-CSF by mAb blockade synergized with IL17R deficiency in the control of arthritis, indicating that GM-CSF mediates effector functions independent and non-synonymous with IL17 signaling (69). Campbell and colleagues found that treatment of mice with exogenous M-CSF exacerbated the severity and increased the incidence of CIA. The authors also found that neutralization of M-CSF with mAb, or induction of CIA in M-CSF-deficient *op/op* mice resulted in less severe disease (70). This group also described a pathogenic role for GM-CSF in CIA, as GM-CSF^{-/-} mice were protected from CIA, characterized by no change in collagen II autoantibodies and diminished delayed-type hypersensitivity responses (66).

Research into the role of CSFs in other autoimmune diseases varies substantially, according to the specific model in question. In experimental lupus nephritis, *op/op* mice were protected from disease (71). In EAE, GM-CSF^{-/-} mice are protected from disease (72). Enrichment of Ly6C^{hi} inflammatory monocytes by adoptive transfer exacerbated EAE (64). Retroviral transduction of autoreactive T cells with GM-CSF was reported to be sufficient to enhance the severity of EAE (73). A specific role for GM-CSF has been described in the activation of microglia, as a necessary prerequisite for the onset of EAE (74). Reddy and colleagues have described a role for direct GM-CSF signaling in the induction of CD40 and TNF α expression in microglia, the resident macrophage cell type in the CNS (75).

Myocarditis – cellular mediators of autoimmunity, inflammation, and cardiac dysfunction

Cardiac inflammation is among the most common causes of non-congenital, non-ischemic sudden death in otherwise normal, healthy young adults (76, 77). Myocarditis in patients is diagnostically heterogeneous, with limited available treatment options (78, 79). While evidence for a wide variety of etiologies exist for myocarditis, infectious agents – notably various enteroviruses – are among the most common (80). The best studied and characterized model of viral myocarditis is infection with Coxsackievirus B3 (CB3), a common enterovirus (81). Recent evidence suggests persistent expression of viral products subsequent to parvovirus B19 infection may also be a critical determinant of myocarditis (82-84).

The actual mechanistic relationships between viral infection and chronic cardiac inflammatory disease, with particular relevance to progression to heart failure, appears to involve autoimmunity, particularly in the case of CB3 (85-88). In susceptible strains of mice, such as A/J, A.SW, and BALB/c, myocarditis continues well past the clearance of infectious virus due to autoreactive adaptive responses (87, 89, 90). In the same strains of mice, viral infection can be replaced by immunization of animals with cardiac antigen with appropriate adjuvants – this model is termed experimental autoimmune myocarditis (EAM) (91).

Depletion and adoptive transfer studies have unambiguously proven that experimental autoimmune myocarditis in rodents is a CD4⁺ T cell-dependent disease (92, 93). On one

hand, these findings immediately implicate T cell-derived cytokines as critical effectors of autoreactive CD4⁺ T cells. They also further implicate other hematopoietic-origin cells as signaling targets of these cytokines, as downstream cellular effectors of T cell-driven autoimmune cardiac pathology (94).

Infiltration with total CD45⁺ leukocytes peaks at day 21 of EAM (Figure 1a). Our laboratory has previously demonstrated that infiltration of the heart by CD4⁺ T cells negatively correlates with cardiac function, further underscoring the centrality of CD4⁺ T cells in cardiac autoimmunity (67).

Macrophage kinetics in the course of autoimmune heart disease

In autoimmune myocarditis, myeloid-lineage cells have been shown to comprise the bulk of infiltrating leukocytes (94). The majority of these cells express CD11b, indicating their myeloid lineage, but do not all express high levels of Ly6G (one component of the shared epitope Gr1), excluding the possibility that they are neutrophils (67). Similar results were obtained whether BALB/c or A/J mice were studied.

To address the role of macrophages in the natural history of autoimmune myocarditis, we examined the time course of infiltration of various populations of macrophages into the heart during EAM. As a proportion of total leukocytes, macrophages comprise the bulk of cardiac-resident cells in naïve mice (Figure 1b). However, during the course of EAM, influx of other CD45⁺ hematopoietic cells substantially diminishes the proportion of macrophages in the heart relative to other cell types, although MPS-lineage cells remain among the most numerous. As depicted in Figure 1c, infiltration of CD11b⁺F4/80⁺ monocytes precedes the infiltration of macrophages into the heart; the peak of monocyte infiltration occurs on day 14 – indicating monocytes are among the first components of the autoaggressive cardiac infiltrate. Macrophage infiltration appears to peak later, on day 21.

Examining the expression of Ly6C on infiltrating macrophages reveals similar kinetic discontinuity. Cardiac infiltration with Ly6C^{hi} inflammatory macrophages peaked on day 14, while the peak of infiltration with Ly6C^{lo} resident macrophages followed on day 21 (Figure 2).

Macrophage balance in the control of inflammatory cardiac disease

In myocarditis, GM-CSF^{-/-} mice were protected from disease, through a specific defect in the ability of GM-CSF^{-/-} dendritic cells to potentiate pathogenic Th17 responses via IL6 signaling (95). Transfer of M-CSF-derived bone marrow-derived macrophages (BMMφ) during the effector phase of disease was reported to be sufficient for the control of EAM, through a mechanism purported to involve IFNγ-dependent induction of nitric oxide and subsequent apoptosis of autoaggressive T cells (96). This mechanism conflicts with findings that inhibition of iNOS-derived nitric oxide production ameliorates myocarditis in rats (97, 98) and mice (99).

Our laboratory has reported that IL13-deficient mice develop a particularly severe form of myocarditis. In spite of the fact that mouse lymphocytes do not express receptors for IL13, we observed heightened T and B cell responses, suggesting that the protection afforded by

IL13 in EAM is mediated indirectly through an innate compartment. Indeed, we observed fewer heart-infiltrating macrophages expressing markers of alternative activation, raising two non-exclusive possibilities: that alternatively-activated macrophages exert protective functions in EAM, or classically-activated macrophages mediate disease pathophysiology (100).

Macrophage infiltration was increased in the enhanced acute viral myocarditis of complement receptor 1/2-deficient mice following infection with CB3 (101). A role for differential activation of macrophages in the inflamed heart has been associated with the sex differences observed in cardiotropic CB3 infection in BALB/c mice – where male mice are more susceptible to increased cardiac inflammation. Li and colleagues reported that intracardiac macrophages from CB3-infected male mice preferentially expressed markers associated with classical or M1 activation, including iNOS, IL12, TNF α , and CD16/32, whereas heart-infiltrating macrophages in females bore a transcriptional profile consistent with alternative or M2 activation, characterized by arginase 1, IL10, and M ϕ -MR (102).

Frisancho-Kiss and colleagues orchietomized male mice prior to infection with CB3, which diminishes the severity of resulting viral myocarditis with no change in cardiac viral titers – indicating this effect of complement signaling affects cardiac inflammation independently of antiviral immunity. This sex hormone-dependent protection from disease was associated with expansion of an “M2”-like F4/80⁺Gr1⁺ macrophage population, which coexpressed increased levels of TIM3, and decreased IL1 β and IL10 (103, 104).

Chemokines associated with the recruitment of various MPS-lineage populations have similarly been described as effectors of autoimmune pathophysiology. Through bone marrow chimerization experiments, Mildner and colleagues found that CCR2 expression in the myeloid compartment enables the recruitment of Ly6C^{hi} inflammatory monocytes to the CNS in EAE (105). Blockade of the CCR2 ligands CCL2/MCP1 or CCL3/MIP1 α ameliorated autoimmune myocarditis, further underscoring that inflammatory Ly6C^{hi} monocytes serve as precursors for effectors of cardiac inflammation (106).

Interactions of macrophages and other inflammatory cells

While some macrophages are clearly heart-resident (Figure 1), macrophages are also recruited to the inflamed heart in a CD4⁺ T cell-dependent manner. As shown in our kinetic studies, Ly6C^{hi} “inflammatory” monocytes are among the first immigrants into the inflamed heart in EAM (Figure 2a). Moreover, intracardiac macrophages upregulate ICAM1 concurrent with the downregulation of M ϕ MR/CD206 (Figure 2b), indicating a switch from a native “alternatively-activated” macrophage environment, to an inflammatory “classically-activated” state over the course of disease. The biphasic recruitment of MPS subpopulations suggests that MPS involvement in EAM pathophysiology is discretely regulated by interactions with other cell types, most notably autoreactive CD4⁺ T cells. However, data from a variety of disease models suggest that relevant MPS crosstalk is not limited to the T cell compartment.

Treatment of mice infected with CB3 with alpha-galactosylceramide, a potent synthetic agonist of invariant NKT cells, reduced viral replication and cardiac inflammation,

associated with expansion of splenic CD11b⁺F4/80⁺ cells, pointing to some measure of regulation between NKT cells and the MPS (107). IL6-deficient mice failed to control acute CB3 viremia, leading to increased cardiac infiltration during post-viral chronic myocarditis (108).

There is also evidence that hematopoietic cells, with some measure of “stemness”, are involved in complex regulation of cardiac inflammation. Kania and colleagues have reported a Prominin/CD133⁺ heart-resident pluripotent hematopoietic cell type with the ability to differentiate into myeloid cell types, including macrophages, or cells that resembled cardiomyocytes both *in vitro* and *in vivo*. Intravenous transfer of these cells into mice with EAM limited the development of disease through a mechanism dependent on IFN γ . These authors proposed that microenvironmental signals dictate the lineage commitment of these cells, whether to exert TGF β -dependent fibrosis through differentiation into a fibroblast-like cell, or protective effects as a nitric oxide-producing macrophage (109, 110).

In contrast, in a mouse model of myocardial infarction, transfer of bone marrow cells led to increased trafficking of macrophages to the heart, associated with increased expression of inflammatory markers and improvement of cardiac functional parameters (111). Together, these data present a novel field of inquiry – the exciting proposition that MPS-lineage cells participate in the control of stem cell renewal and differentiation, and that manipulation of these pathways may present an attractive regenerative therapeutic target for a host of cardiac diseases.

Conclusion

The delicate balance of subpopulations within the macrophage compartment is increasingly appreciated within a diverse range of infectious, tumor, and autoimmune disease models. Pleiotropic functions of macrophages, beyond widely-acknowledged microbicidal and phagocytotic roles, participate in the pathophysiology and regulation of disease, including inflammatory heart disease. As further investigation yields insight into the functional complexity of these cells, it seems increasingly likely that these findings may provide novel tools and targets for modulation of the pathobiology of these diseases.

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Figure 1.

Monocyte and macrophage infiltration into cardiac tissue in EAM. A) Time course of absolute enumeration of total CD45⁺ leukocytes. B) Representative gating of monocytes and macrophages from heart-infiltrating CD45⁺ leukocytes. C) Absolute enumeration of CD11b⁺F4/80⁻CD45⁺ monocytes and CD11b⁺F4/80⁺CD45⁺ macrophages. Individual data points represent individual wild type female BALB/c mice immunized on days 0 and 7 with 100 µg/mL MyHC_{α614-629} in supplemented CFA, as previously described (91). Heart-infiltrating leukocytes were isolated as previously described (67). Bars indicate mean of each group. Asterisks denote significant *t*-test statistics, compared to day 0 (*, *p* < 0.05; **, *p* < 0.005).

Figure 2.

Cardiac infiltration by macrophage subpopulations in EAM. A) Time course of absolute enumeration of $\text{Ly6C}^{\text{hi}}\text{CD11b}^+\text{F4/80}^+\text{CD45}^+$ “inflammatory” macrophages and $\text{Ly6C}^{\text{lo}}\text{CD11b}^+\text{F4/80}^+\text{CD45}^+$ “resident” macrophages. B) Expression of markers of classical and alternative activation on intracardiac $\text{CD11b}^+\text{F4/80}^+\text{CD45}^+$ -gated macrophages during EAM. Individual data points represent individual wild type female BALB/c mice. Bars indicate mean of each group. Asterisks denote significant *t*-test statistics, compared to day 0 (*, $p < 0.05$; **, $p < 0.005$).

Table I

Models of macrophage differentiation

Macrophage population	Inducing signals	Functions	Markers	Soluble Products	References
Classically-activated (M1) macrophages	IFN γ TLR2 TLR4 TNF α IL1 IL6	Reactive oxygen production Phagocytosis Bactericide Antigen presentation Costimulation	Inducible nitric oxide synthase (<i>Nos2</i> , iNOS); MHC Class II; CD80 (B7.1), CD86 (B7.2)	CCL2 (MCP1) CCL3 (MIP1 α) CCL10 (IP10) IL1 β IL12p70 IL23 TNF α	(37, 112, 113)
Alternatively-activated (M2a) macrophages	IL4 IL13	Fibrosis Wound healing Scavenging T cell suppression	Arginase 1 (<i>Arg1</i>); Macrophage mannose receptor (<i>Mrc1</i> , M ϕ -MR, CD206); Macrophage scavenger receptor (<i>Msr1</i> , SR-A, CD204); Fc ϵ RII (<i>Fcer2</i> , CD23); YMI (<i>Chi3l3</i>); YM2 (<i>Chi3l4</i>); RELM α (<i>Relmla</i> , FIZZ1, HIMF); MHC Class II	IL1ra CCL17 (TARC, A17) CCL18 (AMAC1, MIP4, PARC) Fibronectin	(29, 37)
Type II, Regulatory (M2b) macrophages	IL1/TLR + immune complexes	T cell Suppression Inflammatory resolution	Sphingosine kinase 1 (SPHK1); LIGHT (TNFSF14)	CCL1 (I-309) IL10	(22, 114)
(M2c) macrophages	IL10 TGF β glucocorticoids	T cell Suppression Angiogenesis Tumor evasion	Arginase 1 (<i>Arg1</i>); B7-H4 (<i>Vttn1</i>); Pentraxin 3; Regulator of G-protein signaling-16 (RGS16); Suppressor of cytokine signaling-3 (SOCS3)	IL10 VEGF	(115-117)

Continually evolving nomenclature systems complicate the already confusing overlap between putative macrophage subpopulations. This table, while not meant to be comprehensive or, encapsulates the most commonly referenced subsets, with deference towards their original nomenclature. Those proposed by Mantovani, *et al.* are in parentheses (115).