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Alternatively activated macrophages in infection and autoimmunity

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Abstract

Macrophages are innate immune cells that play an important role in activation of the immune response and wound healing. Pathogens that require T helper-type 2 (Th2) responses for effective clearance, such as parasitic worms, are strong inducers of alternatively activated or M2 macrophages. However, infections such as bacteria and viruses that require Th1-type responses may induce M2 as a strategy to evade the immune system. M2 are particularly efficient at scavenging self tissues following injury through receptors like the mannose receptor and scavenger receptor-A. Thus, M2 may increase autoimmune disease by presenting self tissue to T cells. M2 may also exacerbate immune complex (IC)-mediated pathology and fibrosis, a hallmark of autoimmune disease in women, due to the release of profibrotic factors such as interleukin (IL)-1 β , transforming growth factor- β , fibronectin and matrix metalloproteinases. We have found that M2 comprise anywhere from 30% to 70% of the infiltrate during acute viral or experimental autoimmune myocarditis, and shifts in M2 populations correlate with increased IC-deposition, fibrosis and chronic autoimmune pathology. Thus, women may be at an increased risk of M2-mediated autoimmunity due to estrogen's ability to increase Th2 responses.

Keywords

Autoimmunity; Complement; Cytokines; Infection; Macrophage

Introduction

A number of excellent reviews have been written in the past few years describing macrophage activation and polarization [see 1–4]. The aim of this review is to highlight the role of infection and tissue damage in directing macrophage phenotype and the impact polarized macrophage populations have on the initiation and propagation of autoimmune diseases. The influence of sex hormones on macrophage polarization and function will also be examined. Most of the discussion of alternatively activated macrophages in this review will focus on research conducted in animal models.

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1. Classification of macrophages

Macrophages are innate immune cells that play a key role in normal tissue homeostasis, presentation of foreign and self antigens following infection or injury, pathogen clearance, resolution of inflammation and wound healing [1,2,5–7]. Following antigen stimulation immature monocytes emigrate from blood vessels into peripheral tissues where they differentiate into mature macrophages and dendritic cells (DCs) comprising as much as 15% of total cells [1,7]. Macrophages are considered classically activated (M1) when stimulated by interferon (IFN)- γ or lipopolysaccharide (LPS) to release nitric oxide (NO), important for killing intracellular pathogens, and alternatively activated (M2) when stimulated by interleukin (IL)-4 or IL-13 (M2a) to produce IL-10, transforming growth factor (TGF)- β and arginase-1 (Arg1), important for killing extracellular parasites [3]. M2 can be further subdivided to those induced by immune complexes (ICs) and LPS or IL-1 β (M2b) or those induced by IL-10, TGF- β or glucocorticoids (GC) (M2c) [2]. Thus, LPS and IL-1 β , acting through Toll-like receptor (TLR)4 and IL-1R signaling, respectively, are associated with both M1 and M2 responses. Other suppressive or anti-inflammatory macrophages include tumor-associated macrophages (TAM) and “immature” monocyte-like (GR1/Ly6C⁺) or “mature” neutrophil-like (GR1/Ly6G⁺) myeloid-derived suppressor cells (MDSCs). Because IL-4 and IL-13 are not required for the differentiation of TAM or MDSCs, these macrophage populations are usually not referred to as alternatively activated [3]. Keep in mind that NO released from IFN- γ R⁺ M1 down-regulates T cell responses and so M1 can also be considered “suppressive” [8]. Importantly, macrophages are not “permanently differentiated” in the way we consider T helper-type 1 (Th1) or Th2 cells to be, but are “plastic”-able to move from macrophage to DC and back again and from M1 to M2 depending on the local environment [7].

1.1 Classically activated macrophages

Classically activated or M1 macrophages develop in culture in response to stimulation by IFN- γ and LPS. IFN- γ is produced by many cell types including CD8⁺ cytotoxic T lymphocytes, natural killer (NK) and Th1 cells [2]. M1 are characterized by expression of MHC class II and CD86 and their ability to secrete proinflammatory cytokines such as tumor necrosis factor (TNF)- α , IL-1 β , IL-12, IL-18 and the chemokines CCL15, CCL20, CXCL8-11 and CXCL13 [2,3]. M1 efficiently kill intracellular pathogens by endocytosis of bacteria and viruses, production of NO via IFN- γ -inducible NO synthase (iNOS), synthesis of reactive oxygen intermediates (ROI), and by restriction of iron and other nutrients required for bacterial growth and viral replication [2].

1.2 Alternatively activated macrophages

Alternatively activated or M2 macrophages were originally named “alternative” because they could be induced by IL-4 rather than IFN- γ . Monocytes differentiate into M2 in culture in response to IL-4, IL-13, IL-10, TGF- β , GC, ICs, LPS and/or IL-1 β [2]. In general, M2 are distinguished from other macrophage populations by several markers including the IL-4R, mannose receptor (MR/CD206), Arg1, Fizz1 (found in inflammatory zone), PPAR γ (peroxisome proliferator-activated receptor) and Ym1/2 (eosinophilic protein from chitinase family) [3]. IL-4 and IL-13 are produced by mast cells (MCs), Th2 cells and eosinophils, stimulate Arg1 production via STAT6, and upregulate the MR, scavenger receptor (SR)-A and C-type lectins (CLECs) such as MGL1/2. IL-10 is produced by MCs, T and B cells, and macrophages and induces differentiation of monocytes to macrophages while blocking their differentiation to DCs [9].

M2 are typically associated with Th2 responses and tissue repair where they are thought to increase fibrosis by expressing profibrotic factors such as fibronectin, matrix metalloproteinases (MMPs), IL-1 β and TGF- β . However, a definitive role for M2 in mediating

fibrosis has not yet been established *in vivo* [3]. IL-10 and TGF- β released from macrophages have been found to inhibit LPS/TLR4-mediated production of TNF- α and IL-1 β [10] and downregulate Th1 responses by increasing CD4⁺Foxp3⁺ regulatory T cells (Treg) [11]. M2 are characterized by producing Arg1 rather than iNOS/NO, which makes them highly effective at eliminating parasitic helminth infections but significantly compromises their ability to kill intracellular pathogens [12,13]. However, this distinction between iNOS in M1 vs. arginase in M2 is absent in human macrophages [11]. The MR is upregulated on M2 by IL-4, IL-13 or IL-10 and down-regulated by proinflammatory cytokines [14]. MR signaling inhibits Th1 responses by interfering with TLR/IL-1R-mediated signaling by upregulating IL-1R type II (a decoy receptor) and by secretion of the IL-1R antagonist (IL-1Ra) and IL-10 [3,15]. Importantly, expression on M2 of scavenger receptors and C-type lectins like the MR and DC-SIGN serve not only as pattern recognition receptors for pathogen binding and uptake during infection, but also bind self tissues such as apoptotic cells and oxidized LDL as a part of natural homeostasis and tissue repair [1,16,17]. Thus, M2 are increased in response to many infections, particularly those associated with Th2 responses, and are important in mediating wound healing following tissue damage.

1.3 Myeloid-derived suppressor cells

MDSCs are considered to be an immature population of myeloid cells associated with infections or tumors that are capable of suppressing proinflammatory responses [4,18–20]. Mouse MDSCs have been found to express CD11b, GR1 (Ly6C or Ly6G), F4/80, CD80, STAT3, IL-4R α , Arg1, iNOS, ROS, IL-10, and TGF- β . MDSCs are separated into monocyte-like suppressor cells that express NO (ROS undetectable) and neutrophil-like suppressor cells that express ROS (NO undetectable) [4,11]. Like M2, IL-4 and IL-13 promote MDSC development along with IL-10, TGF- β , IL-1 β and LPS/TLR4. However, MDSCs differ from M2 in that they are dependent on IFN- γ to produce NO, ROS and Treg. Thus, MDSCs display an array of M1 and M2 characteristics further emphasizing the malleable nature of macrophages.

CD11b⁺GR1⁺ MDSCs are present in most cancers in humans and mice and bear many similarities to M2 and TAM [1,4,11]. MDSCs induced by infection or tumors inhibit innate and adaptive immune responses and drive monocytes to a macrophage rather than a DC phenotype [21]. During damage induced by myocardial infarction, Ly-6C^{hi} monocytes were found to digest damaged tissue early after acute injury, while Ly-6C^{lo} monocytes promoted tissue repair by stimulating myofibroblast formation, angiogenesis and deposition of collagen [6]. Thus, MDSCs also appear to be important for wound healing in response to tissue damage. MDSCs are not always observed as immature Ly6C⁺ populations, but can also express markers of mature macrophages such as Ly6G and MHC class I or II [18,22,23]. Thus, a proportion of GR1⁺Ly6G⁺ cells that are identified in studies as neutrophils may in fact be neutrophil-like MDSCs [24]. MDSCs have been shown to directly suppress NK, CD4⁺ and CD8⁺ T cell function via Arg1 and are associated with increased Treg and Th2 responses. For example, influenza virus infection of mice was found to increase MDSCs that inhibited antiviral NK cell responses resulting in increased viral replication [19]. Thus, MDSCs are thought to be induced by infections that are typically cleared by Th1 or Th17 responses as a strategy to evade elimination by the immune system.

1.4 Tumor associated macrophages

Similarly, tumors are able to escape immune system control by secreting anti-inflammatory cytokines such as IL-10, TGF- β and prostaglandins (i.e. PGE2) that direct macrophages to a suppressive phenotype called TAM [2,11,21,22]. TAM directly inhibit CD4⁺ and CD8⁺ T cell-mediated tumor killing in humans and mice via cell-to-cell contact and release of TGF- β and NO [22]. Like MDSCs, TAM express CD11b, GR1, F4/80, NO, Arg1, STAT1, STAT3 and TGF- β [11,25,26]. Only a small number of TAM are needed to shut down tumor-specific T

cell killing, which does not induce global suppression of the immune response. Thus, a patient's immune system may appear to function normally while the tumor grows. TAM express many of the same markers as MDSCs and M2, such as the MR, SR-A and TGF- β , and the term MDSC is often used interchangeably with TAM [4]. Like MDSCs, TAM also express typical M1 markers such as the proinflammatory cytokines TNF- α , IL-1 β and IL-6. However, TAM are generally considered to be poor producers of NO and ROI compared to M1 or MDSCs [2]. Similar to M2 and MDSCs, TAM are able to scavenge cellular debris following tissue damage and are important in angiogenesis, lymphogenesis, tissue remodeling and repair- functions that promote tumor progression and metastasis [27,28]. Thus, TAM, MDSCs and M2 share many features in common such as inhibiting proinflammatory responses, being activated by self-antigens following tissue damage, and mediating extracellular matrix remodeling and wound repair. In the next section, we will describe how suppressive macrophage populations induced by infection and tissue damage (or adjuvant and self-peptides in animal models) play a key role in the development of autoimmune disease.

2. Infection, M2 and autoimmune disease

2.1 Infection and injury in AD

For some time infections have been postulated to play a role in the initiation and/or promotion of autoimmune diseases (ADs). The role of infection has been difficult to substantiate for a number of reasons. Many of the infections suspected to cause ADs are widespread in the population, such as coxsackievirus or cytomegalovirus, and often an active infection can no longer be detected by the time the signs and symptoms of AD appear [29]. Several theories have been proposed to explain mechanistically how infections could induce ADs including persistent infection, molecular mimicry, the bystander effect and the adjuvant effect [30–35]. It is clear that physical injury must occur in target tissues in order to release self antigens, and so infections may be able to damage tissue as well as providing the adjuvant stimulus needed for the development of AD. A better understanding of the innate immune response has revealed that adjuvants like complete Freund's adjuvant (CFA) and pertussis toxin increase inflammation not simply by acting as a depot for self antigens or by increasing vascular permeability, respectively, but by activating the innate immune response via pathogen pattern recognition receptors (PRRs) such as TLR2 and TLR4 [36–40]. It must be emphasized that self-antigens do not induce AD in animal models unless administered at the same time as adjuvant, indicating that simultaneous activation of PRRs by microbial peptides (provided by inoculation of adjuvant or infection) and self peptides is critical for the development of an autoimmune response. TLR4, in particular, plays a key role in certain ADs such as arthritis and myocarditis, perhaps because of its unique ability to respond to/interact with damaged self peptides like heat shock protein (Hsp)90 and HMGB1 from dying cells as well as various bacterial (i.e. LPS) and viral (i.e. RSV, MMTV, CVB3) peptides [29,30,32,41–44].

TLR signaling not only initiates proinflammatory responses, but is key in their regulation. For example, TLR4 signaling is necessary for the development of Treg during acute coxsackievirus B3 (CVB3)-induced myocarditis- an autoimmune model of myocarditis induced by inoculation with infectious virus and heart tissue [42,45]. Increasing evidence indicates that TLR expression on Treg influences their ability to regulate infection and AD [46]. T cell Ig mucin (Tim) receptor-mediated signaling is another important pathway that inhibits proinflammatory responses in AD models like experimental autoimmune encephalomyelitis (EAE) and CVB3-induced myocarditis [47,48]. Increased Tim-3 expression on the surface of Th1 cells binds galectin-9, which is widely expressed on tissues, directing Th1 cells to undergo apoptosis thereby resolving acute inflammation [49]. We found that around 70% of T cells and 30% of macrophages in the heart during acute CVB3-induced myocarditis express Tim-3, allowing rapid elimination of acute inflammation once CVB3 is cleared [42]. We observe that Tim-3⁺F4/80⁺ macrophages also express CD11b and GR1, markers for MDSCs [50].

Importantly, TLR4 and Tim-3 receptor signaling regulate each others expression on MCs and macrophages. In TLR4 deficient mice a significantly higher percentage of MCs, macrophages and T cells express Tim-3 resulting in reduced acute inflammation [42]. In contrast, blocking Tim-3 signaling significantly increases the percentage of MCs and macrophages expressing TLR4 resulting in increased inflammation [42,48]. Knowing the importance of the innate immune response in directing adaptive immunity further emphasizes why concurrent administration of self-antigens and adjuvant is critical for the induction of AD in animal models.

2.2 Infection and M2

Microbes that induce a predominant Th1 or Th17 response include *Mycobacterium* species, *Listeria*, *Leishmania* species, *Trypanosoma cruzi* and *Toxoplasma gondii*. TLR and IFNs synergistically trigger NO production that enhances iNOS in M1 enabling them to clear intracellular infections [13]. In contrast, nematode and trematode worms like *Nippostrongylus*, *Toxocara*, *Schistosoma*, and *Taenia* require an IL-4 or IL-13-driven Th2-type immune response to clear infection, and induce strong M2 responses [3,11,12,51]. Not only do Th2-inducing helminthes such as *Schistosoma mansoni* and *Taenia crassiceps* upregulate M2 markers like Arg1, MR, Fizz1 and Ym1 in macrophages in response to infection, but they also induce expression of CD11b, GR1 and F4/80- markers associated with MDSCs (Table 1). This suggests that macrophages considered as MDSCs due to the expression of CD11b and GR1 could perhaps be classified as M2, and visa versa. Table 1 highlights that upregulation of CD11b and GR1 is a common feature following infection, from Th2-inducing helminthes to Th1/Th17-inducing bacteria and viruses. Thus, MDSCs and M2 are generated following infections that require Th1 and/or Th17-type immune responses for clearance, such as *Mycobacterium tuberculosis* (used to supplement CFA in animal models of AD), *Helicobacter pylori*, *Trypanosoma cruzi*, influenza A and CVB3 (Table 1). For example, infection with *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) was found to suppress NO and to induce Arg1 via TLR2 and MyD88 signaling, pathways usually considered to reflect proinflammatory Th1-type responses [13]. Thus, microorganisms that induce Th1 responses can also direct macrophages to a M2 or MDSC phenotype, perhaps in an attempt to better evade the immune response.

Following infection or injury, there is often a shift from Th1 to Th2-type immunity as the adaptive immune response progresses from acute to chronic. This shift may also be associated with a change in the proportion of M1 to M2/MDSC at sites of inflammation. Several infections where a shift from Th1 to Th2 has been observed include measles virus, HIV, CVB3, *S. mansoni*, *Plasmodium chabaudi* (mouse malaria) and *T. cruzi* infections [13,52,53]. The mechanism allowing the switch from Th1 to Th2 during *S. mansoni* infection was identified as an immature population of monocyte-like MDSCs that expressed CD11b, GR1 and F4/80 [8]. Importantly, these cells could be detected within 2 hours of infection with *S. mansoni* during the innate immune response [8]. The CD11b⁺GR1⁺ macrophages had a mixed M1/M2 phenotype similar to MDSCs in that they were IFN- γ and NO-dependent yet secreted TGF- β and IL-10 [51]. A shift from a Th1 to Th2 response is also observed in the progression of ADs from acute to chronic phases and in the process of wound healing following tissue damage [52,53]. Thus, a shift from M1 to M2/MDSC may be a common feature following infection or tissue damage that is important for resolution of inflammation and wound healing.

Because of the ability of helminth infections to induce M2/MDSCs, inoculation with helminths or helminth-glycoproteins/glycolipids has been proposed as a potential therapy for Th1-mediated ADs and other inflammatory conditions. *S. mansoni* infection was found to inhibit a dextran sodium sulfate (DSS) model of colitis in BALB/c mice [54]. CD11b⁺F4/80⁺ macrophages that secreted IL-10 and TGF- β were found to be responsible for the suppressive

effect. Although M2/MDSCs are able to inhibit acute Th1-mediated inflammation, it is possible that their release of profibrotic agents such as MMPs, IL-1 β and TGF- β could exacerbate the chronic fibrotic pathology associated with many ADs. We will explore this possibility in more detail in the next section.

2.3 Complement and M2

Another aspect of the immune response that is critical for the initiation and regulation of inflammation following infection is the complement system. Complement and complement receptors (CRs) play a central role in immune defense by initiating the rapid destruction of invading microorganisms, promoting phagocytosis via scavenger and Fc receptors, activating the innate and adaptive immune response, and clearing ICs [55–57]. Complement components activate the innate immune response by responding to bound antibody and PRRs found on pathogens. Activation leads to the formation of complement split products that have potent proinflammatory properties [58,59]. The opsonins C1q, C3b and iC3b interact with CR1, CR3 and CR4 to promote phagocytosis of pathogens. CR1 (CD35) is a receptor for C1q, C3b, C4b and mannose-binding lectin (MBL) - a C-type lectin. CR1 is important for clearing ICs and is a complement regulator with decay-accelerating and membrane cofactor activities in mice [55,59,60]. CR2 (CD21) is the receptor for the cleavage products of C3 including iC3b, C3dg, and C3d. CR2 expression on B cells plays a major role in the induction of antibody responses and has been proposed as the primary mediator of the adjuvant effect of complement, and has been shown to be necessary for disease development in adjuvant-induced models of AD [57, 61,62]. While CR1 and CR2 are encoded on separate genes in man, in the mouse they are derived from the same gene, *Cr2*, so that mice deficient in one receptor are also deficient in the other [63,64]. The β 2 integrin CR3 (CD11b/CD18), also called Mac-1, binds C3b and iC3b opsonized pathogens and is important in mediating their phagocytosis [17]. CR3 is expressed on immature and mature macrophages, neutrophils, DCs and MCs [42,59].

Deficiencies in complement or CRs are known to increase susceptibility to certain infections such as group B *Streptococcus* and lymphocytic choriomeningitis virus (LCMV), highlighting the important role for the complement system in activating protective immunity [65,66]. Complement deficiencies are also associated with the loss of tolerance to self proteins and the development of IC-mediated ADs such as systemic lupus erythematosus (lupus) and myocarditis [60,67–70]. CD11b/CR3 expressed on macrophages was found to generate an inhibitory signal to T cells [7,68]. In a low-dose oral tolerance model, CD11b deficient mice developed significantly increased inflammation, IL-6 and a Th17 response indicating that CD11b reduces inflammation [68,70]. In a separate study, binding of iC3b to CD11b/CR3 was shown to be necessary for the development of oral and peripheral tolerance during delayed-type hypersensitivity (DTH) responses by increasing expression of IL-10 and TGF- β [62,70]. iC3b had to be present during the first 12 hours of antigen presentation to mediate this tolerogenic affect [62]. Although the role of MDSCs or M2 were not specifically examined in these studies, the ability of CD11b⁺ cells to inhibit inflammation and T cell responses and to produce TGF- β and IL-10 suggests that they could be alternatively activated or suppressive macrophages.

We have shown that CR1/2 deficiency significantly increases acute myocarditis, IC-deposition, fibrosis and dilated cardiomyopathy (DCM) at day 10 post infection (pi) following CVB3/ cardiac myosin inoculation [60]. Usually DCM does not develop until around day 35 pi in this model [45], indicating that deficiency in CR1/2 signaling accelerates fibrosis and the development of chronic heart disease. CD11b⁺GR1⁺(Ly6G)F4/80⁺ macrophages expressing the MR, TLR4, Tim-3, IL-4R, caspase-1, IL-1 β and IL-10 were significantly increased in the hearts of CR1/2 deficient mice, indicating that CRs usually inhibit this potentially profibrotic population (Fig. 1). Recently, caspase-1, which cleaves proIL-1 β to its active form, was shown

to regulate the activity of other proteins important for fibrosis including fibroblast growth factor (FGF)-2 and MMP14 [71]. We also observed a significant increase in CD11b⁺GR1⁺F4/80⁺ macrophages at 12 hours pi in the spleen of CR1/2 deficient mice, suggesting that these cells influence the adaptive immune response (D. Fairweather, unpublished data). Recall that a similar observation was made for innate CD11b⁺GR1⁺F4/80⁺ macrophages following *S. mansoni* infection and for tolerance following DTH [8,62]. Thus, CR1/2 signaling not only activates immunity by interacting with pathogen-antibody ICs, but also regulates profibrotic TLR4⁺IL-1 β ⁺ M2.

3. M2 and autoimmune diseases

Although the hallmark of AD is the generation of autoantigen-specific T and B cell responses, macrophages and neutrophils comprise the majority of the infiltrate during the peak of acute inflammation [42,72]. Other factors critical in determining the development of AD include genetic background and sex [31,42,45,53,73]. For example, BALB/c mice develop predominant Th2 and M2 responses following infection or adjuvant inoculation compared to C57BL/6 mice due to their high numbers of MCs, which are an important innate source of IL-4 for differentiation of monocytes and naïve T cells to M2 and Th2 cells [2,3,42,73]. Furthermore, estrogen in female BALB/c mice increases Th2 responses by upregulating GATA3 and inhibiting NF κ B [53,74,75].

3.1 Sex hormones and cell-mediated vs. antibody-mediated ADs

ADs affect approximately 5–8% of the population, making them the third most common disease in the US after cardiovascular disease and cancer. Conservative estimates indicate that almost 80% of the people with ADs are women [76–78]. Although studies of estrogen's effects on immune function have been contradictory, many reports indicate that estrogen not only stimulates antibody production from B cells but also increases IL-4, IL-10 and TGF- β thereby increasing fibrosis while downregulating Th1-type proinflammatory responses [53,79–81]. Although the role of estrogen in macrophage differentiation has not yet been examined, known effects of estrogen on the immune system would be expected to increase M2 and MDSC populations. Recall that M2 and MDSCs express Arg1 and are associated with increased Treg and Th2 responses [3,12,19]. We have found that female BALB/c mice express significantly elevated levels of IL-4 (Th2), a greater percentage of B cells and CD25⁺Foxp3⁺ Treg, and increased expression of Tim-3 on MCs, macrophages and T cells in the heart compared to males with acute CVB3-induced myocarditis [42]. Tim-3 is located in the same region of chromosome 11 (human 5q23-35) as the IL-4 gene cluster that includes IL-4, IL-5, IL-13 and GM-CSF and several other immune-related genes such as IL-12 p40, CD14 (part of the TLR4 complex) and Itk [49]. In mice, chromosome 11 has been linked with susceptibility to type 1 diabetes (*Idd4*) and EAE (*Eae6*) [49]. Comparing males to females, we find that females upregulate the M2 marker Arg1 in the heart during acute CVB3-induced myocarditis as assessed by microarray analysis, as well as other markers of M2 differentiation including Dectin-1, CD169, Galectin-3, CCL2, CCL7, and CXCL3 (D. Fairweather, unpublished data).

In ADs that are more prevalent in males, such as myocarditis, acute inflammation is characterized by increased numbers of macrophages and neutrophils and a more Th1/Th17-type immune response [42,53]. In genetically susceptible mouse strains (i.e. BALB/c and A/J) the acute phase progresses to a chronic phase with increased autoantibodies and fibrosis indicating a switch from a Th1 to a Th2-type response [45,53]. In contrast, a dominant Th2 response initiates disease in females and predisposes them to increased acute and chronic antibody/IC-mediated tissue damage and fibrosis, characteristics that are more prevalent in women with ADs such as lupus and arthritis. Thus, in general testosterone appears to increase cell-mediated pathology in males while estrogen increases antibody-mediated, fibrotic pathology in females. However, it is important to realize that both male and female mice mount

a “mixed” Th1/Th2/Th17 response to infection or adjuvant [50,53,82]. In a similar manner we would anticipate a mix of M1 and M2 macrophages in the infiltrate during AD (Fig. 2) [50, 82]. During acute experimental autoimmune myocarditis (EAM) nearly 70% of the myocardial infiltrate are alternatively activated based on expression of SR-A (CD204), around 10% of the CD11b⁺ infiltrate express both the MR (CD206) and SR-A (CD204) (data not shown), while about 15% are CD11b⁺ (CD204^{neg} and CD206^{neg}) M1 (Fig. 2A) [82]. In contrast, during acute CVB3 myocarditis approximately 70% of the F4/80⁺ macrophages in the heart are GR1^{neg}CD11b⁺ M1 compared to around 30% GR1⁺CD11b⁺ M2/MDSC (Fig. 2B) [50]. Following gonadectomy of male BALB/c mice, M2 levels increase from around 30% to nearly 50% resulting in decreased acute CVB3 myocarditis [50]. These findings indicate that fluctuating levels of M1 to M2 influence autoimmune heart disease.

Regardless of sex, BALB/c and A/J mice develop dominant Th2 responses with more MCs and M2 than C57BL/6 mice [3,73]. Signaling via IL-4R α has been shown to induce arginase and a M2 phenotype in BALB/c mice [83]. We were initially surprised to find that it was GR1⁺F4/80⁺ M2/MDSC rather than GR1^{neg}F4/80⁺ M1 that expressed TLR4, Tim-3 and IL-1 β in the heart of males with acute CVB3-induced myocarditis (Fig. 2C) [50]. This population also expresses the IL-4R and IL-10 (Fig. 2C) and could be similar to the M2b described by Martinez et al. [2]. Our findings suggest that TLR4⁺caspase-1⁺IL-1 β ⁺ M2 could contribute to the pathogenesis of AD by increasing fibrosis (Fig. 1). Thus, an elevated Th1-type M1 compared to M2 response to CVB3/cardiac myosin in BALB/c males increases cell-mediated inflammation but enables clearance of the virus (Fig. 2) [42,50]. However, elevated TLR4⁺caspase-1⁺IL-1 β ⁺ M2 in males promote chronic fibrosis and DCM (Fig. 1) [60]. Women are known to develop strong Th2 responses and elevated antibodies in response to infections [53]. If women develop increased numbers of TLR4⁺Fc γ R⁺IL-1 β ⁺ M2 (M2b) following infection resulting in more IC deposition and fibrosis- the hallmark of AD pathology in females- this could explain, at least in part, why women are at an increased risk of developing ADs [2,53]. There is some evidence to support the idea that estrogen increases IL-1 β and M2 responses. Estrogen was shown to increase expression of MCP-1/CCL2, which is a marker of M2 [3,84]. In a separate study, MCP-1 was found to be important for inflammatory recruitment in EAM increasing IL-4 and IL-1 β levels in the heart [85]. However, the role of M2 cells was not specifically examined in the study.

3.2 Evidence for M2 in ADs

There are few studies specifically examining the role of M2 or MDSCs in the development of ADs. Most experimental models of AD, such as EAE, collagen-induced arthritis (CIA) and EAM, use CFA supplemented with *M. tuberculosis* to induce disease and are considered to be Th1 and/or Th17-mediated [53]. However, IL-4 and IL-13-induced Th2 responses are necessary for the development of several ADs usually considered to be mediated by Th1 or Th17 responses such as EAE, EAM and CVB3-induced myocarditis [82,86–90]. Many PRRs that are present on M2 and MDSCs, such as MR, Dectin-1, CD163, DC-SIGN and MGL-1/2, are able to recognize both foreign and self antigens suggesting a possible role for alternatively activated suppressor macrophages in the pathogenesis of AD [1].

3.3 ADs more prevalent in Females

Multiple sclerosis—Multiple sclerosis affects women about 2 times more frequently than men [53]. EAE, an animal model of multiple sclerosis, is induced in many species including mice by inoculation of an equal volume of self-tissue such as whole myelin homogenates, MBP, MOG or PLP with CFA containing *M. tuberculosis* boosted with *Bordetella pertussis* [91,92]. The acute phase of EAE has been associated with a Th1 and/or Th17 response and the chronic phase with a Th2 response, TGF- β and IL-10. Immunization with MOG in particular induces both cellular and antibody-mediated pathology and chronic EAE in female C57BL/6

mice [91]. Although Th2 responses have been shown to protect against acute inflammation in EAE in mice, they have been found to severely aggravate disease in primates [91]. IFN- γ deficient mice develop more severe EAE, which is thought to be due to increased IL-17 levels (IL-17 deficient mice have reduced EAE indicating that IL-17 increases disease) [92]. However, IL-4 levels are also increased in IFN- γ deficient mice and are believed to be responsible for increasing disease in CVB3 myocarditis and EAM models [86,88,90].

CD11b⁺ macrophages are major effector cells in EAE associated with Th1 responses in C57BL/6 females [91,93]. EAE in BALB/c mice induces splenic CD11b⁺ suppressor cells that express GR1 at day 10 to 14 post inoculation [94]. The most potent population of CD11b⁺GR1⁺F4/80⁺ suppressor cells in the spleen were identified as immature Ly-6C^{hi}CD11b⁺ cells that inhibited CD4⁺ and CD8⁺ T cells via IFN- γ -mediated NO production. The cells did not express Arg1, but could produce arginase when treated with IL-4 in culture. During peak, acute EAE at day 14 MSDCs comprised approximately 30% of the inflammatory cells in the CNS, reminiscent of the 30% CD11b⁺GR1⁺F4/80⁺ population observed in the heart during CVB3 myocarditis (Fig. 2B).

The most convincing evidence for a role for M2 in EAE comes from a study by Ponomarev and colleagues showing that CD11b⁺ microglial cells isolated from the CNS during disease expressed IL-4 and Ym1 but not NO, indicating that they were M2 [89]. IL-4 was shown to increase Ym1 expression in microglial cell cultures, and IL-4 or IL-4R deficient microglial cells did not produce Ym1. The investigators concluded that M2 can be pathogenic and not just protective.

Arthritis—The incidence of rheumatoid arthritis is higher in women than men (3:1) [53]. In experimental models of arthritis including CIA, the K/BxN mouse rheumatoid arthritis model and Lyme disease arthritis induced by infection with the spirochete *Borrelia burgdorferi*, recruitment of GR1^{+/hi} neutrophils to the joint was found to be necessary for disease development in BALB/c compared to C57BL/6 mice [95]. Additionally, gene expression profiles of peripheral blood cells from patients with new-onset systemic juvenile idiopathic arthritis revealed that patients with disease had increased markers for M2 and genes involved in the TLR/IL-1R signaling pathways [96]. Although sex hormones do not appear to be a factor in disease pathogenesis in juvenile patients, this study suggests a pathologic role for upregulation of TLR/IL-1R and M2 macrophages.

Lupus—SLE occurs in women compared to men at a ratio of 9:1 [53]. In a tetramethylpentadecane (TMPD) model of lupus, mice develop autoantibodies against dsDNA and small nuclear RNPs resulting in IC-mediated glomerulonephritis and arthritis [97]. Increased IFN-stimulated genes correlate with active disease and precede the development of disease in mice. TMPD was found to increase a population of CD11b⁺Ly6C^{hi} immature monocytes that express type I IFNs and whose numbers directly correlate with the production of lupus autoantibodies. This study points to an increase in innate proinflammatory markers and suppressive macrophages associated with IC-mediated pathology.

3.4 ADs more prevalent in Males

Myocarditis—The incidence and severity of heart disease, including myocarditis and DCM, is higher in men [98]. The severity of inflammation is also increased in males in murine models of CVB3-induced myocarditis and EAM [42,82,99,100]. CD11b⁺ monocytes are the major infiltrate during EAM at day 21 post inoculation and CVB3 myocarditis at day 10 post infection in BALB/c mice [42,72]. In EAM approximately 70% of the CD11b⁺F4/80⁺ macrophage infiltrate is alternatively activated based on expression of SR-A (CD204), while only around 30% of the CD11b⁺GR1⁺F4/80⁺ macrophages are M2 based on MR (CD206) expression

during CVB3 myocarditis (Fig. 2A,B) [50,82]. IL-13 deficiency in EAM significantly decreases the percentage of CD204⁺CD206⁺F4/80⁺M2 in the heart indicating that IL-13 is partially responsible for driving M2 differentiation in acute myocarditis [82]. In a separate study, IL-17 was shown to increase recruitment of CD11b⁺GR1⁺F4/80⁺ macrophages to the heart during EAM, which were able to suppress Th17 cells by an IFN- γ -dependent NO mechanism [101]. The investigators considered the cells to be mature macrophages due to low level MHC class II expression. The suppressor cells upregulated NOS2 when stimulated in culture with IFN- γ and LPS. Although the requirement for IFN- γ could indicate M1, the expression of CD11b and GR1 on these cells suggests the possibility that they are MDSCs (Table 1). Thus, IL-17 may promote autoimmune heart disease by increasing M2/MDSC populations. We also observe an increased Th17 response in CR1/2 deficient mice during acute CVB3-induced myocarditis (unpublished data) as well as increased CD11b⁺GR1⁺F4/80⁺ M2/MDSCs (Fig. 1). We have shown that M2/MDSCs are the primary macrophages expressing TLR4 and Tim-3 and that cross-talk by these receptors regulates the severity of CVB3 myocarditis in BALB/c males (Fig. 2C) [41,42,48,50]. Furthermore, regulation by CR1/2 signaling decreases the percentage of TLR4⁺caspase-1⁺IL-1 β ⁺ M2/MDSCs in the heart during acute CVB3 myocarditis thereby reducing chronic fibrosis and DCM (Fig. 1). Thus, maintaining an appropriate ratio of M1 to M2 in the heart following infection is critical for elimination of pathogens and regulation of inflammation and wound healing without the negative consequences of fibrotic pathology.

4. Concluding Remarks

Alternatively activated macrophages are induced by the Th2-type cytokines IL-4 and IL-13 and are well known for their ability to inhibit proinflammatory responses by the release of IL-10 and TGF- β . M2 and MDSCs are also induced in response to viral and bacterial infections usually associated with Th1 or Th17-type responses, such as CVB3, *M. tuberculosis* and CFA supplemented with *M. tuberculosis* and pertussis toxin that is used to induce AD in animal models (Table 1). Recent findings in animal models suggest that M2 and/or MDSCs exacerbate autoimmune disease by presenting self antigens taken up by scavenger receptors in response to tissue damage and by contributing to fibrosis and IC-mediated pathology. We were surprised to discover that the “proinflammatory” TLR4⁺caspase-1⁺IL-1 β ⁺ macrophages that are increased in the heart during acute CVB3-induced myocarditis and EAM were M2 rather than M1 cells (Fig. 2) [50,82]. CR1 is critical for clearing ICs, and CR1/2 deficient mice have increased IC deposition, fibrosis and TLR4⁺ caspase-1⁺IL-1 β ⁺ M2 in the heart during acute CVB3-induced myocarditis (Fig. 1) [60]. Evidence is beginning to emerge that M2 may also be pathogenic in EAE, arthritis and lupus. However, few studies of AD have specifically examined the role of M2 or MDSCs in the pathogenesis of disease. Realizing that proinflammatory receptors such as TLR4 and the IL-1R drive M2 differentiation indicates that an evaluation of the role of M1 vs. M2 macrophages in the pathogenesis of autoimmune disease is needed. As noted by several other contributors to this special issue, we are pleased to include this paper in the issue devoted to the recognition of Noel Rose. We note that this is part of the series of the Journal of Autoimmunity dedicated to major fields in autoimmunology [110–113]. Noel Rose has contributed enormously to many fields in autoimmunity and including his major efforts in developing the American Autoimmune Related Diseases Association (AARDA) [114–119].

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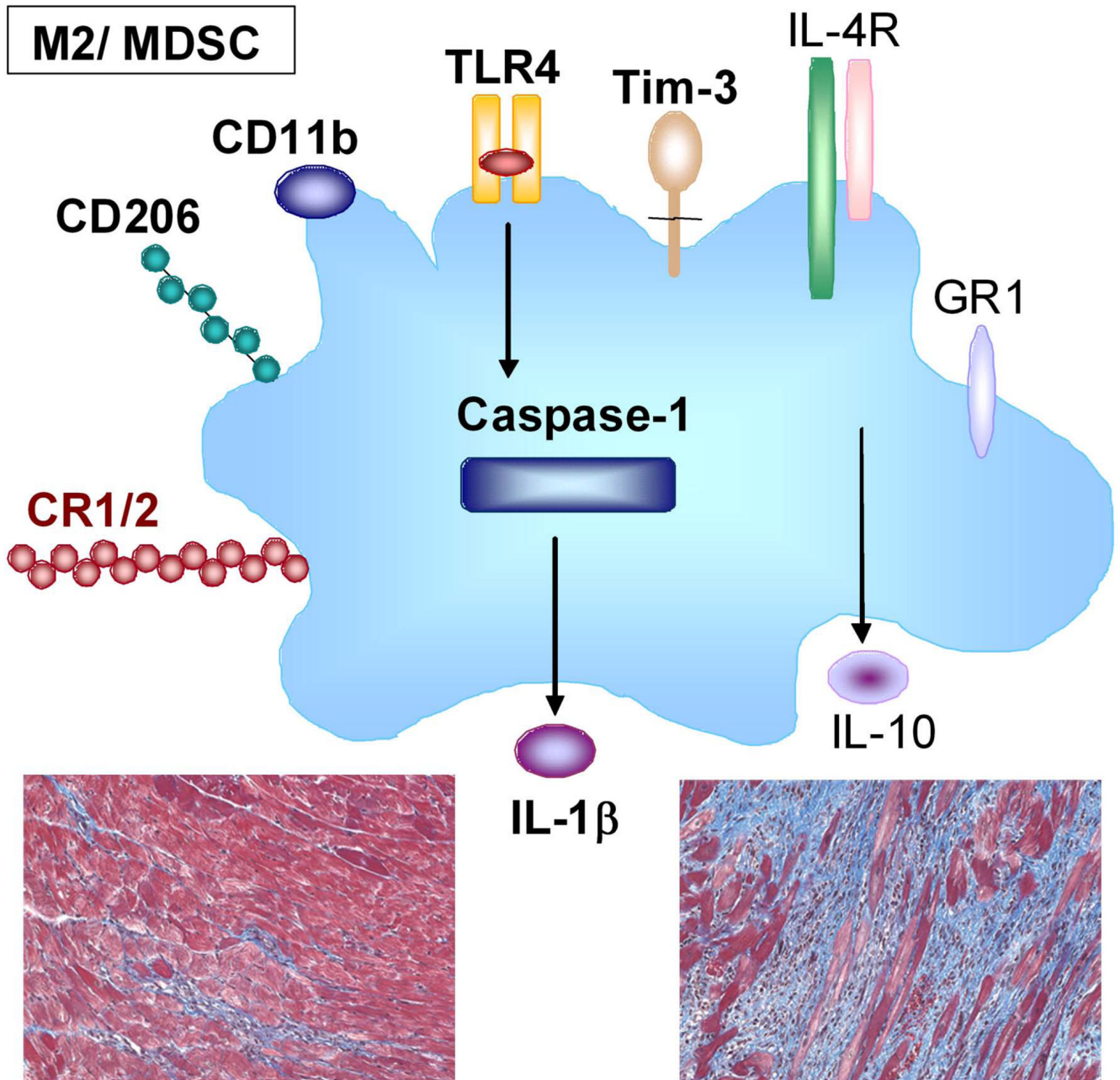


Figure 1.

Complement receptor 1/2 (CR1/2) deficiency increases fibrosis and alternatively activated macrophages in the heart during acute coxsackievirus B3 (CVB3) myocarditis. Male A/J mice deficient in CR1/2 (red lettering) upregulate the mannose receptor (CD206), CD11b (CR3), TLR4, Tim-3, caspase-1 and IL-1 β (bold lettering) on/in CD11b⁺GR1⁺F4/80⁺ macrophages assessed by flow cytometry. Expression levels of GR1, IL-4R and IL-10 (regular lettering) do not change in/on CR1/2 deficient macrophages. The presence of the mannose receptor suggests that these cells are alternatively activated M2, while the presence of CD11b, GR1 and F4/80 indicates they could be myeloid derived suppressor cells (MDSC). CR1/2 deficient mice have increased numbers of M2/MDSC and significantly increased IC deposition and fibrosis during

acute CVB3 myocarditis (photos: left, WT; right, CR1/2 deficient) [60]. Blue in photos depicts collagen deposition by Trichrome staining in the heart.

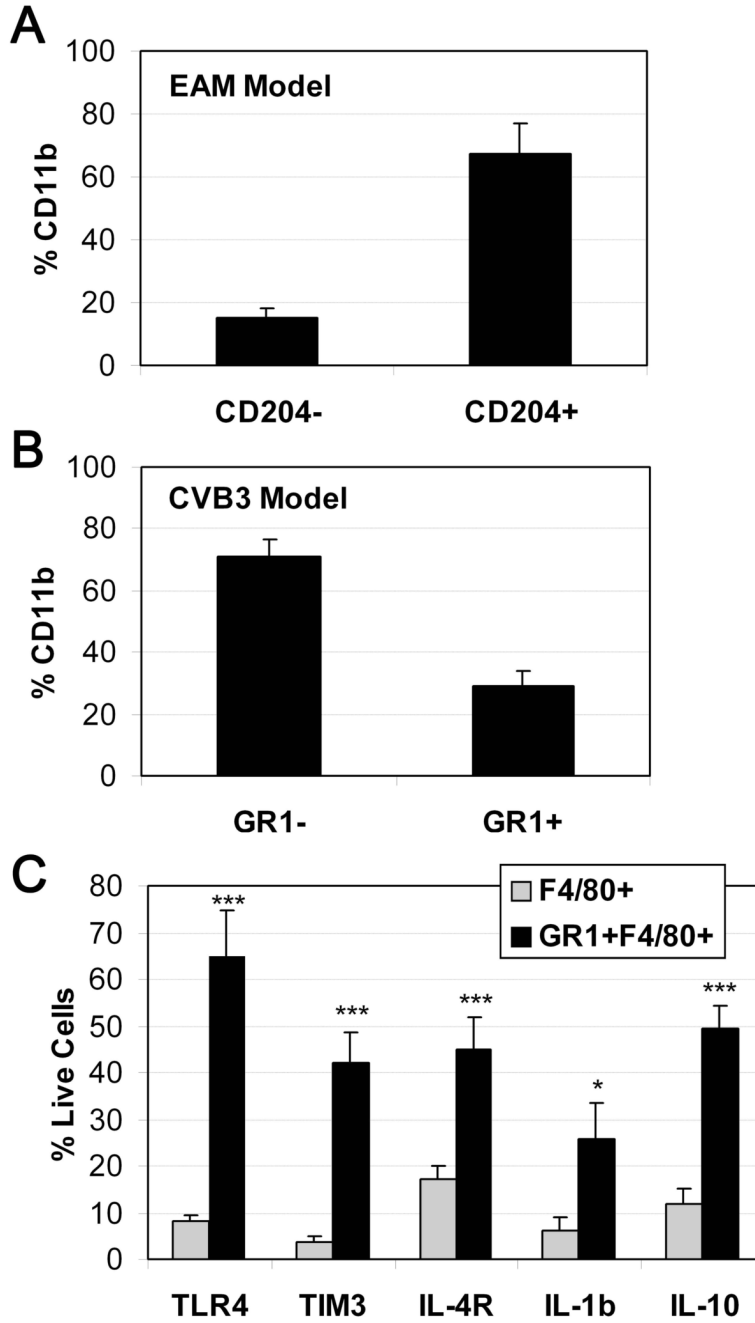


Figure 2. Alternatively activated macrophages in autoimmune myocarditis. A) Experimental autoimmune myocarditis (EAM) was induced using the myocarditogenic peptide MyHC $\alpha_{614-629}$ derived from the murine cardiac myosin heavy chain emulsified in complete Freund's adjuvant on days 0 and 7 supplemented with *Mycobacterium tuberculosis* and pertussis toxin. Percent scavenger receptor CD204^{neg}CD11b⁺ vs. CD204⁺CD11b⁺ F4/80⁺ macrophages were assessed in the heart during acute EAM in BALB/c male mice at day 21 post inoculation by flow cytometry [82]. B) BALB/c male mice received 10³ plaque forming units of coxsackievirus B3 (CVB3) with cardiac myosin on day 0 and the presence of GR1^{neg}CD11b⁺ vs. GR1⁺CD11b⁺ F4/80⁺ macrophages was examined from the heart at day

10 post infection by flow cytometry [50]. C) Similarly, expression levels of TLR4, Tim-3, IL-4R, IL-1 β and IL-10 were compared between GR1⁻F4/80⁺ M1 vs. GR1⁺F4/80⁺ CD11b⁺ M2 macrophages obtained from the heart at day 10 during acute CVB3 myocarditis [50]. * $p < 0.05$, *** $p < 0.001$.

Table 1

Induction of MDSCs and M2 following infection

Infection	MDSC	M2	References
Helminths/extracellular parasitic worms: Require Th2 responses to clear infection			
<i>Schistosoma mansoni</i>	CD11b ⁺ GR1 ⁺ F4/80 ⁺ NO	Arg1,MR,Fizz1,Ym1, IL-10,TGFb	[3,8,11]
<i>Taenia crassiceps</i>	CD11b ⁺ GR1 ⁺ NO	Arg1,MR,Fizz1,Ym1	[3,11]
Bacteria: Require Th1 or Th17 response to clear infection			
<i>Porphyromonas gingivalis</i>	CD11b ⁺ GR1 ⁺	unknown	[11]
<i>Mycobacterium tuberculosis</i>	CD11b ⁺ iNOS	Arg1,MR,IL10,IL-1Ra	[13,15,38,102]
<i>Mycobacterium bovis</i> (BCG)	CD11b ⁺ GR1 ⁺	Arg1	[13,103]
<i>Helicobacter pylori</i>	CD11b ⁺	Arg2	[13,104,105]
Viruses: Require Th1 response to clear infection			
Influenza A	CD11b ⁺ NOS	Arg1	[19,106]
Coxsackievirus B3	CD11b ⁺ GR1 ⁺ F4/80 ⁺	Arg1,MR,Ym1,IL-10,IL-1	[50]
Other:			
<i>Candida albicans</i>	CD11b ⁺ GR1 ⁺ CD80 ⁺	MR,Dectin-1	[23,107,108]
<i>Trypanosoma cruzi</i>	CD11b ⁺ GR1 ⁺ iNOS/NO	Arg1	[11,18,109]
<i>Toxoplasma gondii</i>	CD11b ⁺ GR1 ⁺ F4/80 ⁺ iNOS	Arg1	[13,20]