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The plasticity of inflammatory monocyte responses to the inflamed central nervous system

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ABSTRACT

Over the last three decades it has become increasingly clear that monocytes, originally thought to have fixed, stereotypic responses to foreign stimuli, mediate exquisitely balanced protective and pathogenic roles in disease and immunity. This balance is crucial in core functional organs, such as the central nervous system (CNS), where minor changes in neuronal microenvironments and the production of immune factors can result in significant disease with fatal consequences or permanent neurological sequelae. Viral encephalitis and multiple sclerosis are examples of important human diseases in which the pathogenic contribution of monocytes recruited from the bone marrow plays a critical role in the clinical expression of disease, as they differentiate into macrophage or dendritic cells in the CNS to carry out effector functions. While antigen-specific lymphocyte populations are central to the adaptive immune response in both cases, in viral encephalitis a prominent macrophage infiltration may mediate immunopathological damage, seizure induction, and death. However, the autoimmune response to non-replicating, non-infectious, but abundant, self antigen has a different disease progression, associated with differentiation of significant numbers of infiltrating monocytes into dendritic cells in the CNS. Whilst a predominant presence of macrophages or dendritic cells in the inflamed CNS in viral encephalitis or multiple sclerosis is well described, the way in which the inflamed CNS mobilizes monocytes in the bone marrow to migrate to the CNS and the key drivers that lead to these specific differentiation pathways *in vivo* are not well understood. Here we review the current understanding of factors facilitating inflammatory monocyte generation, migration and entry into the brain, as well as their differentiation towards macrophages or dendritic cells in viral and autoimmune disease in relation to their respective disease outcomes.

1. Introduction

Monocytes, macrophages (MΦ), and dendritic cells (DC) are part of the 'mononuclear phagocyte system', also known as the 'reticuloendothelial system', found throughout the body. In normal tissues most MΦ and DC are considered to be 'tissue-resident', populating the tissue early in life, often specifically named. In the CNS, the resident MΦ are microglia. They originate from the yolk sac [1] and are renewed *in situ* [2]. During inflammation, however, monocytes can migrate from the bloodstream into affected tissues, including the CNS, where they differentiate into "infiltrating" MΦ or DC. Whilst these resident and infiltrating cells may play prominent roles in the CNS during viral or autoimmune disease, the methods by which the inflamed CNS induce the mobilisation of monocytes in the bone marrow is poorly understood. Moreover, the signalling events responsible for alternate monocyte or DC differentiation in the local inflammatory environment of the CNS are not well described. As these signalling events dictate the nature and progression of the immune response to CNS pathologies, an understanding of the mechanisms involved is crucial to identify novel targets for immune modulating therapy in these diseases.

Abbreviations: MΦ, macrophage; IFN, interferon; IRF, interferon regulatory factor; WNV, West Nile virus; EAE, experimental autoimmune encephalomyelitis; MS, multiple sclerosis; BM, bone marrow; LN, lymph node; DLN, draining lymph node; TNF, tumour necrosis factor; MDP, macrophage/dendritic cell progenitor; CCL, C-C motif ligand; BBB, blood–brain barrier; IFNAR, IFN-α receptor; cMoP, common monocyte progenitor; GM-CSF, granulocyte/macrophage colony stimulating factor; M-CSF, macrophage colony stimulating factor; CNS, central nervous system; HSC, haematopoetic stem cell; TLR, Toll-like receptor.

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2. Monocytes and CNS pathologies: viral encephalitis and experimental autoimmune encephalomyelitis

2.1. Monocyte subtypes

Monocytes are one of the mononuclear cell types circulating in the blood that are produced in hemopoietic tissues of the bone marrow (BM) throughout life. They can be identified in human and mouse by flow cytometry, using a combination of cell surface markers. Under normal conditions, the entire monocyte population is identified as $CD14^+$ (including $CD14^{lo}$ and $CD14^{hi}$ subpopulations) in humans, and $CD115^+CD11b^+$ in mice and humans. In both species, two principle populations and an 'intermediate' population are identifiable. Classical (also termed 'inflammatory') monocytes are $CD14^{hi}$ $CD16^-$ in humans and $Ly6C^{hi}$ ($CD43^{lo}$ $CCR2^{hi}$ $CX3CR1^{lo}$) in mice and are the major monocyte population in the blood [3,4]. Non-classical (also termed 'patrolling') monocytes, represent a much smaller subset (approximately 10%) of blood monocytes and are $CD14^{lo}$ $CD16^{hi}$ in humans, and $Ly6C^{lo}$ ($CD43^{hi}$ $CCR2^{lo}$ $CX3CR1^{hi}$) in mice [3,4]. The intermediate group is $CD14^{hi}CD16^{hi}$ in humans and $Ly6C^{hi}CD43^{hi}$ population in mice. There are transcriptional similarities between humans and mice comparing their respective subsets, but functionally, mouse classical monocytes appear to be more related to human intermediate monocytes, based on their pro-inflammatory roles [5]. During normal hematopoiesis in mice, the MΦ/DC progenitor (MDP) gives rise to a pre-DC [6] and a recently-described common monocyte progenitor (cMoP), distinguished from the MDP by its downregulated CD135 and upregulated Ly6C, although it still lacks CD11b. The cMoP differentiates into c-kit^- $CD115^+$ $CD11b^+$ $Ly6C^+$ monocytes and in turn into c-kit^- $CD115^+$ $CD11b^+$ $Ly6C^-$ monocytes [7].

$Ly6C^{hi}$ monocytes are generated in the BM and during homeostasis they likely emigrate, but eventually give rise to $Ly6C^{lo}$ ($CX3CR1^{hi}$) monocytes [3]. Under homeostatic conditions, $Ly6C^{lo}$ $CX3CR1^{hi}$ monocytes patrol the luminal side of vascular endothelium in a programmatic, albeit peripatetic manner [8]. During inflammation, however, $Ly6C^{hi}$ monocytes emigrate from the BM along the CCR2–CCL2 axis into foci of tissue inflammation, differentiating into inflammatory MΦ, TipDC, or inflammatory DC, which may then migrate to the draining lymph node (DLN), presumably transporting antigen acquired on the way [5,9]. While microglia are normally self-renewing [2], they may be supplemented and/or replenished by infiltrating $Ly6C^{hi}$ monocytes during CNS infection and/or irradiative inflammation [10,11] and these immigrants become $Ly6C^{lo}$ on entry into inflamed tissue [5,9]. $Ly6C^{lo}$ monocytes are also recruited in later stages of inflammation where they are involved in tissue repair. These cells typically differentiate into M2, *i.e.*, anti-inflammatory, MΦ, supporting healing in the injured spinal cord [12]. However, the developmental connection between these 2 phenotypically similar but often temporally disparate populations is not completely clear.

2.2. Induction of bone marrow monocyte responses

The mechanisms by which CNS inflammation induces monocyte mobilisation in the BM are not well understood. The recruitment of BM monocytes during inflammation appears to depend on two initiating events: induction of emigration of existing BM monocytes into the circulation, and generation of new monocytes in the BM to replace the diminished population, which subsequently contribute to the emigrating monocyte population. Monocyte generation relies on two processes, the 'pull' of a diminished downstream population, and/or the 'push' or direct stimulation of hematopoietic stem cells (HSC) or other progenitors. This process has been reviewed in depth elsewhere [13], but relevant factors are considered here.

2.2.1. Emigration of monocytes from the bone marrow

CCL2 is crucial for $Ly6C^{hi}$ inflammatory monocyte emigration from the BM [14]. Recently, BM stromal cells, but not HSC, were shown to secrete CCL2 in response to low levels of circulating Toll-like receptor (TLR) ligands [5]. This induced monocyte migration towards the vascular sinuses, and was dependent on myeloid differentiation primary response protein 88 (MyD88) (involved in responses to TLR ligand binding in all but TLR-3 [15]), but was independent of TNF and type-I interferon (IFN) expression. As the CCL2-expressing cells also expressed various TLR, it was suggested that these cells function to detect infection and rapidly induce monocyte emigration into the circulation.

2.2.2. Generation of new monocytes by myeloid progenitors

Whilst this provides some insight into the mechanisms of monocyte emigration, it does not explain the 'push' signal for monocyte production. This signal is presumably provided by direct inflammatory stimulation of HSC or other progenitors, inducing increased differentiation of self-renewing HSC into downstream progenitors [13]. Direct inflammatory modulation of HSC (which are $CCR2^+$) by circulating TLR has been described, inducing such differentiation [16]. Furthermore, soluble immune-mediators may induce BM changes; Seo et al. showed that IFN-α signalling to HSC was required for the generation of $Ly6C^{hi}$ monocytes in a model of viral pneumonia [17] and type I IFN, produced in the spleen in response to infection with *Listeria monocytogenes*, promoted monocyte emigration from the BM [16]. Interestingly, mice deficient in either MyD88 or IFN-α receptor (IFNAR) still had significant monocyte migration from the BM to the site of inflammation, whereas mice deficient in both did not [16]. Interestingly, in these studies, IFN-γ did not play an important role in monocytopoiesis. However, in a model of chronic *Mycobacterium avium* infection, IFN-γ, but not IFN-α, was found to activate HSC, resulting in differentiation into downstream myeloid and lymphoid progenitors replacing the diminished populations [18,19]. On the other hand, in experimental autoimmune encephalomyelitis (EAE), GM-CSF produced by CNS cells triggers monocyte mobilisation and emigration from the BM [20].

2.2.3. Differential pathways of monocyte mobilisation

In CNS-initiated mobilisation of the BM, it is unclear if there is a difference in initiating events that predisposes to the preferential differentiation of monocytes towards a MΦ or DC phenotype, prior to entering the CNS. The fact that TLR, type-I and -II IFN, and GM-CSF each induce monocyte emigration and the production of monocytes from progenitors in different situations may explain some differences in the manner of BM mobilisation by different CNS pathologies. Monocyte infiltration during viral encephalitis is rapid, with lethality in animal models occurring within days of initial infiltration [10,21]. The pathogenesis of EAE, on the other hand, is chronic and/or relapsing and depends on T cell responses against myelin proteins that are initially induced by DC [22]. Nevertheless, a potential connection exists between viral and auto-immune causes of CNS infiltration by monocytes. In multiple sclerosis (MS), there is evidence to suggest that reactivity against myelin proteins may occur following CNS viral infection, if anti-viral responses cross-react with myelin proteins [23]. The initial presence of viral RNA or DNA in the circulation could certainly induce monocyte emigration from the BM, via binding of intracellular RNA by TLR within BM stromal cells [24], and TLR stimulation or direct infection of HSC could induce downstream differentiation of these cells [13]. However, although TLR ligands may be present initially, recurrent episodes of MS would have few if any virus-associated

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