The Suppressive Attitude of Inflammatory Monocytes in Antiviral Antibody Responses

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Abstract

Inflammatory monocytes play important functions in antiviral immune responses, including release of inflammatory cytokines and antigen presentation to T lymphocytes. Depending on the pathological context, these functions might translate into beneficial or detrimental effects in the resolution of the disease. Recent literature has highlighted a role for inflammatory monocytes also in direct suppression of B cell responses. In this review, we will briefly discuss research showing the relationship between inflammatory monocytes and B lymphocytes, its functional consequences on antiviral antibody responses, and possible implications in the design of future vaccination strategies.

Keywords: inflammatory monocytes, nitric oxide, humoral immunity, B cell, suppression

Introduction

VIRAL INFECTIONS ARE usually controlled efficiently by humoral and cellular immune responses. Some viruses, however, have evolved strategies to escape immune control and to establish persistent infections (21,36). In certain cases, immune responses are generated normally, but viruses are not recognizable by immune cells or antibodies: for example, they can mutate or shield their surface antigens, or even hide within cells in a latent mode (5–7,9,28,48,49). In alternative, viruses can actively interfere with activation of B or T lymphocytes, thus inhibiting the generation of adaptive immune responses (28,36). In particular, since high-affinity neutralizing antibodies are very efficient in clearing infections, inhibition of B cell activation and of neutralizing antibody (Ab) responses can be detrimental for viral control. The many and diverse strategies that viruses adopt to actively suppress B cell activation have been reviewed elsewhere (28). We have recently shown that one of these strategies consists in promoting recruitment of suppressive inflammatory monocytes to virus-draining lymph nodes (dLN) (38). In this review we will focus on and discuss studies regarding the relationship between monocytes and B lymphocytes during viral infections.

Circulating Monocytes

Monocytes are an extremely heterogeneous and plastic cell type, and undergo several phenotypic changes when exposed to a certain cytokinic milieu within tissues (3,26,32). They all originate from a common monocytic precursor that

gives rise to two main circulating populations in mice: inflammatory monocytes (which express high levels of CCR2 and Ly6C, and low levels of CX3CR1) and patrolling monocytes (which express high levels of CX3CR1 and do not express CCR2) (44). It has been proposed that in some settings patrolling monocytes could also originate from a phenotypic change of inflammatory monocytes (31). The functions of these two subsets of monocytes differ based on the pathological setting. CCR2⁺ monocytes are thought to play a role in patrolling the endothelium during steady state conditions. Upon infection, they can enter lymphoid organs where they produce an early but very transient burst of inflammatory cytokines, before inflammatory monocytes start their activity. Inflammatory monocytes, on the contrary, are mobilized from the bone marrow (BM) when they sense a gradient of MCP-1 (also called CCL2, a CCR2 ligand) in the blood, a chemokine released upon inflammation and infection and dependent on type I IFN signaling (4,10,42,44). As a result, the number of CCR2⁺ monocytes in the blood, dLN, and some peripheral organs increases. Inflammatory monocytes display a wide range of functions (as both activators and suppressors of the immune response) in different settings (43,44). In the next paragraphs, we will focus on their role during viral infections.

Experimental Approaches to Study the Role of Inflammatory Monocyte Functions

Studies addressing the role of inflammatory monocytes during infections and other pathological settings have been

performed by adopting different experimental approaches. One of these approaches is the use of cell-depleting monoclonal antibodies (mAb), such as the antigranulocyte receptor-1 (Gr-1) mAb, RB6-8C5 (11,16,38,40). RB6-8C5 binds to Ly6G, which is present on neutrophils, and to Ly6C, which is more widely expressed on neutrophils, inflammatory monocytes, and some lymphocytes. However, since its activity is not targeted only to inflammatory monocytes, the anti-Gr-1 mAb has usually been used in combination with neutrophil-depleting antibodies (11,16,40) or with more specific genetic approaches (38). An alternative way to deplete monocytes is the use of clodronate-containing liposomes. This approach has been mainly developed for highly phagocytic cells such as macrophages, which engulf liposomes and are exposed to their content after lysosomal digestion of the lipid layers (46). However, when clodronate-containing liposomes are injected systemically, a depleting effect on blood monocytes is also observed (37,46). Besides the caveat of multiple cell targets (all highly phagocytic cells), it is important to bear in mind that treatment with clodronate-containing liposomes might have additional confounding effects. It was shown, for example, that clodronates serve as direct adjuvants for B cell activation, and this activity might result in misleading interpretations on the role of monocytes in the context of an immune response (45). Finally, one of the most widely used approaches to study the role of inflammatory monocytes in different contexts is the exploitation of CCR2 as a key marker for their mobilization from the BM to the blood, and in some cases for their migration to peripheral organs (4,10,44). To this end, two genetic models have been developed: the CCR2-deficient and the CCR2-DTR transgenic mice (4,22). In the first model, inflammatory monocytes cannot be mobilized from the BM because they cannot sense the MCP-1 (CCL2) gradient generated upon infection or inflammation. In the second model, CCR2-expressing cells are deleted upon injection of diphtheria toxin. The result of both these approaches is the absence of CCR2⁺ inflammatory monocytes from the blood and dLN during viral infections. Many of the reports that will be discussed in the next paragraphs were conducted by using the latter and more specific experimental approaches.

The Protective Role of Inflammatory Monocytes During Viral Infections

Inflammatory monocytes have been reported to play context-dependent roles during viral infections (Fig. 1). These different roles are most of the time a reflection of their physiological functions, both during innate and during coordination of adaptive immune responses. The first experiments performed with CCR2-deficient animals revealed a profound defect of CD4⁺ T helper 1 (Th1) responses in an antigen-dependent inflammation model. This model was characterized by a lower recruitment of monocytes to the inflammatory site; however, it was not established with certainty whether the impaired IFN γ production by T cells was the result of a diminished antigen presentation by monocytes or to an intrinsic defect in the activation of T cells lacking CCR2 (4). The ability of inflammatory monocytes in promoting Th1 responses was further confirmed in both Complete Freund's Adjuvant (CFA)-adjuvanted OVA vaccination setting and in influenza-infected mice, where mono-

cytes infiltrating the dLN produced the Th1-polarizing cytokine IL-12 (35). In addition, inflammatory monocytes support T cell responses in ways other than priming and polarization of naive CD4 T cells to the Th1 fate. CD8 T cells responses have indeed been described to be supported by inflammatory monocytes due to their ability to present antigen during influenza A virus (IAV) infection (2,13). In another setting of respiratory infection (Poxvirus), inflammatory monocytes were dispensable for generation and clonal expansion of antigen-specific CD8 T cells but affected the persistence of a specific subset of circulating and resident memory CD8 T cells (CXCR3^{hi}CX3CR1^{neg}) (15). In a mouse model of West Nile virus infection, the impaired recruitment of CCR2⁺ monocytes to the brain caused high mortality due to severe encephalitis (29), indicating a beneficial role for inflammatory monocytes, although the exact mechanism was not further investigated. Finally, inflammatory monocytes were found to be highly recruited upon acute alphavirus infection and produced high levels of type I IFN upon sensing of virus infected cells: as a result, CCR2⁺ monocytes contributed to viral clearance and a marked decrease in disease severity (20). In summary, thanks to their ability to produce inflammatory and antiviral cytokines, and to induce T cell activation, recruitment of inflammatory monocytes to the virus-draining LNs is frequently beneficial to the control of the infection. Sometimes, however, specific inflammatory settings might exacerbate some of the inflammatory monocyte functions and render them detrimental for the immune response.

When Too Much Is Too Bad: The Detrimental Role of Monocytes in Infection

As mentioned earlier, CCR2⁺ inflammatory monocytes sometimes display both protective and detrimental functions during viral infections (Fig. 1), as for example in the IAV setting (2,13). Numbers of lung-recruited Ly6C⁺CCR2⁺ inflammatory monocytes usually correlate with the severity of disease during pathogenic IAV infection. These recruited inflammatory monocytes establish a positive feedback loop of type I IFN and CCR2-ligands induction, which promotes further increase of inflammatory monocyte numbers in the lungs. Levels of inflammatory cytokines and of iNOS, as well as mice mortality upon lethal influenza infection, were indeed dramatically decreased in CCR2-KO mice, suggesting a pathogenic role for inflammatory monocytes in this model (30). In another study focused on IAV infection, the cause for increased morbidity and mortality of juvenile IAV-infected mice was again to be ascribed to a massive recruitment of inflammatory monocytes to the lungs. Monocytes were recruited in response to high levels of type I IFN and MCP-1 (CCL2) production, and resulted in a highly damaging inflammasome activation and cytokine storm (8). Interestingly, it was shown that a partial inhibition of monocyte recruitment to the lungs of IAV-infected mice moderated the early mortality caused by exacerbated inflammation. On the contrary, when recruitment of monocytes was completely abrogated, the diminished antigen presentation resulted in impaired CD8 T cells responses and higher viral titers (2,13). These conflicting results could be explained by taking into consideration the fact that different functions of inflammatory monocytes might be performed with a different timing, therefore resulting in opposing effects on the immune

FIG. 1. Protective and detrimental roles of inflammatory monocytes in infection. Inflammatory monocytes (IM) can play both protective (**A**) and detrimental (**B**) roles in viral infections. Their ability to produce Th1-polarizing cytokines ①, present antigen to CD8⁺ T cells ②, promote survival of certain memory CD8⁺ T cell subsets ③, or promote viral clearance through type I IFN and other cytokines production ④ renders IM beneficial for an efficient immune response. On the contrary, an excessive recruitment to the sites of infection followed by exacerbated inflammasome activation and cytokine storm ⑤, ⑥ leads to tissue injury and high mortality. Finally, IM can serve as reservoirs for viral replication ⑦ and support persistence of the viral infection.

response. IAV infection is not the only setting where inflammatory monocytes have been shown to be detrimental to resolution of the disease. Indeed, recruitment of inflammatory monocytes to the brain promotes immune activation that leads to damage of the hippocampus during acute picornavirus infection (23–25). The gradient of MCP-1 (CCL2) responsible for the recruitment of monocytes to the brain was generated by the neuronal cells of the hippocampus (23). Finally, inflammatory monocytes can serve as reservoirs for viral replication, therefore increasing viral titers, as it happens during systemic murine norovirus (MNV) infection (47). In this model, a first wave of viral replication results in a massive release of IL-1 and other inflammatory cytokines that induce recruitment of inflammatory monocytes to the site of infection. These inflammatory monocytes are in turn infected by the virus, therefore amplifying the viral burden

and supporting systemic MNV persistence. In conclusion, while recruitment of inflammatory monocytes to the infected tissues or dLN is a physiological event aimed at supporting immune responses, there might be cases in which an excessive or dysregulated recruitment causes detrimental effects that outbalance the protective ones.

Monocytes Suppress B Cells and Ab Responses

In most of the studies, functions of inflammatory monocytes in either activating or suppressing immune responses were imputed to their characteristics of inflammatory cytokine-producing or antigen-presenting cells. Their ability to affect T cell activation was evaluated extensively, but little was reported on their relationship with B lymphocytes. In one study, for example, CCR2-deficient or CCL2-deficient

mice were immunized in the footpad with an adjuvanted-OVA solution, and Ab responses evaluated several days later. Vaccination induced recruitment of CCR2⁺ monocytes in the dLN of WT control mice, but not in those of KO mice; interestingly, the absence of inflammatory monocytes in the dLN correlated with higher anti-OVA Ab titers (34). However, inflammatory monocytes were shown to directly suppress T cell proliferation *in vitro*, but no direct effect on B cell activation was evaluated. In a similar study, a small CCR2 inhibitor (RS102895) administered every 6 h in concomitance with a dominant influenza antigen inhibited inflammatory monocyte recruitment to the dLN, and resulted in higher T cell and Ab responses 12 days after immunization (33). Finally, research aimed at evaluating Ab responses to vaccination in HIV-infected individuals showed that higher frequencies of inflammatory monocytes were correlated to lower Ab responses to influenza. Also in this case, monocytes were shown to suppress CD4 T cell proliferation *in vitro* (18). All the above mentioned reports indicate a strong inverse correlation between frequency of inflammatory monocytes and levels of Ab responses. However, the implicit proposed mechanism in these studies is a defect of T cell help to B lymphocytes, not a direct suppression of B cell responses by monocytes (Fig. 2). Interestingly, the idea that monocytes might directly inhibit B cells was first proposed in a research showing the existence of radio-

resistant nonadherent mononuclear cells that suppress B cell differentiation *in vitro* (27). In a more recent study, Ab-secreting cells (ASC) in the draining LN of immunized mice were found to interact with myeloid cells. Depletion of these myeloid cells (which expressed CCR2) resulted in higher ASC proliferation and Ab production. Depletion of granulocytes or dendritic cells (DC) did not show a similar effect, suggesting that the population involved in inhibiting ASC proliferation and survival were monocytes or macrophages (17).

We have recently shown that CCR2⁺ monocytes interact with virus-specific B cells and actively induce their apoptosis in the context of lymphocytic choriomeningitis virus (LCMV) infection. LCMV induced a prolonged type I IFN signature that resulted in recruitment of inflammatory monocytes to the dLN. The peak of recruitment was reached 3 days after infection, a time-point when LCMV-specific B cells were confined at the interfollicular areas. Inflammatory monocytes were shown to be interacting with B cells, and their depletion led to recovery of B cell numbers and Ab responses (38).

The Role of Nitric Oxide in Suppression of Ab Responses

One important question regards the mechanisms whereby inflammatory monocytes interfere with B cell survival and

FIG. 2. Proposed mechanisms for monocyte-mediated B cell responses suppression. Inflammatory monocytes (IM) can inhibit production of antiviral antibodies through different mechanisms. Direct interaction of IM with Ag-specific B cells leads to NO-mediated B cell apoptosis ①. *In vitro* studies have shown that IM can also suppress T cells, thus undermining their help for efficient B cell activation and antibody production ②. Finally, IM-derived NO affects BAFF production by other cells, limiting survival and proliferation of activated B cells ③. BAFF, B cell activating factor; NO, nitric oxide.

proliferation. Our results showed that NO, one of the main molecules produced by inflammatory monocytes, was involved in B cell apoptosis (Fig. 2). Indeed, the mere presence of inflammatory monocytes in the dLN was not sufficient to inhibit B cell responses, if these monocytes were not able to produce NO (38). Previous research had already suggested a role for NO in suppression of Ab responses to sheep red blood cells (1,14). In those studies, NO was produced by macrophages upon stimulation of splenocytes with IFN γ , and this effect was reverted upon use of a NOS inhibitor. So what is the biological link between NO and B cell suppression? One possible mechanism is the ability of NO to inhibit B cell activating factor (BAFF) production (Fig. 2). In fact, it was shown that T1D and TD Ab responses were increased in NOS-deficient mice, and this correlated with higher BAFF levels (19). BAFF was produced by inflammatory monocytes, among other cell types. A NOS2 inhibitor upregulated the levels of BAFF produced by *in vitro* cultured bone marrow-derived DC (BMDC). However, the fact that, *in vivo*, depletion of inflammatory monocytes (the potential source of both NO and BAFF) induced higher Ab titers, suggested that in this system BAFF needed for B cells survival and Ab production was produced by other cell types, possibly CCR2⁻ monocytes or other myeloid cells.

The identification of NO as a potential candidate for suppression of Ab responses could lead to an improvement of vaccination strategies, by implementing the design of new adjuvants. For example, immunization (with OVA or *B. anthracis* antigens) associated to cyclic dinucleotide (CDN) adjuvants induced higher Ab titers 14 and 28 days after vaccination. After 48 h of *in vitro* stimulation, BMDC (and also bone marrow-derived macrophages [BMMs]) cultured with CDNs produced much lower amounts of NO and higher levels of BAFF than those cultured with TLRs. Treatment with a NOS2 inhibitor increased Ab titers in OVA+TLR, but not in OVA+CDN-immunized mice. In addition, aged mice immunized with Ag+CDN were characterized by higher levels of BAFF and Ab titers than those immunized with Ag+TLRs (12). In a similar study, pentablock copolymer micelles were used as adjuvants in association with OVA antigen to immunize mice. They were able to induce high levels of anti-OVA Ab 2 and 4 weeks after immunization. Stimulation of BMDC and BMMs with micelle-adjuvanted antigen induced a nonconventional maturation profile on antigen-presenting cells (APC), with low if no production of inflammatory cytokines and upregulation of costimulatory molecules. Interestingly, also the metabolic profile of micelle-stimulated APC was different, with no production of NO and reactive oxygen species (ROS) (41). Therefore, the design of adjuvants able to induce an “alternative” maturation of APC characterized by low or no production of NO might benefit Ab responses to vaccination.

Concluding Remarks

In this short review, we have discussed studies focused on the role of inflammatory monocytes in suppression of immune responses, with a particular attention to B cell responses. The concept that inflammatory monocytes interact with antiviral B cells and directly inhibit their activation is relatively new, despite a number of reports showing an in-

verse correlation between recruitment of monocytes and levels of antiviral antibodies (18,33,34). More recent findings have established a direct connection between inflammatory monocytes and B cells, thanks also to the use of cutting edge imaging techniques and fluorescent reporter models that allow for visualization of these interactions (38,39). Since the suppressive role of inflammatory monocytes in Ab responses seems to be mediated partially through direct interactions with B cells and partially through inhibition of T cell mediated help to B cells, it will be important in the future to exploit imaging techniques to elucidate the role that inflammatory monocytes might play on B–T cell interactions.

Some important questions arise with regard to the emerging role of inflammatory monocytes in B cell suppression. Inflammatory monocytes are recruited in response to different viral infections; however, they might not suppress B cell responses in all settings. We have observed for example that monocytes recruited upon VSV infection do not inhibit generation of VSV-specific antibodies [(38) and unpublished observations]. One possibility is that these differences might be imputed to a different timing of recruitment or persistence of these monocytes in the dLN. Another possible explanation could be that inflammatory monocytes might change their phenotype once recruited in the infected organs or dLN: their highly plastic nature might render them susceptible to the inflammatory environment and drive their differentiation to more activator or suppressor cells, depending on the viral infection. Future studies aimed at determining the molecular identity and gene expression profile of different subsets of inflammatory monocytes in the dLN or infected organs might shed some light on their role in different contexts. Also, clarification of which cell types (hematopoietic, stromal, or both) are induced to produce CCR2 ligands during different viral infections might add more useful information. In the meantime, it is encouraging to see that the existing studies on inflammatory monocytes and their detrimental effects due to NO production have paved the way to the first attempts in designing new more effective adjuvants for Ab responses (12,41). These new adjuvants might be useful for vaccination against infections that usually tend to establish persistency and are characterized by a lack of strong and early neutralizing Ab responses (21). Indeed, while completely inhibiting recruitment of inflammatory monocytes to the site of infection might not be the optimal approach, due to loss of both protective and detrimental effects, acting through molecules that address only the suppressive phenotype of monocytes could be the winning strategy.

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