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Myeloid-derived suppressor cells in murine AIDS inhibit B-cell responses in part via soluble mediators including reactive oxygen and nitrogen species, and TGF-β

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ABSTRACT

Monocytic myeloid-derived suppressor cells (M-MDSCs) were increased during LP-BM5 retroviral infection, and were capable of suppressing not only T-cell, but also B-cell responses. In addition to previously demonstrating iNOS- and VISTA-dependent M-MDSC mechanisms, in this paper, we detail how M-MDSCs utilized soluble mediators, including the reactive oxygen and nitrogen species superoxide, peroxynitrite, and nitric oxide, and TGF-β, to suppress B cells in a predominantly contact-independent manner. Suppression was independent of cysteine-depletion and hydrogen peroxide production. When two major mechanisms of suppression (iNOS and VISTA) were eliminated in double knockout mice, M-MDSCs from LP-BM5-infected mice were able to compensate using other, soluble mechanisms in order to maintain suppression of B cells. The IL-10 producing regulatory B-cell compartment was among the targets of M-MDSC-mediated suppression.

1. Introduction

Myeloid-derived suppressor cells (MDSCs) are an immunosuppressive subset of cells that arise from myeloid progenitors that fail to terminally differentiate (reviewed in Gabrilovich and Nagaraj (2009) and Youn and Gabrilovich (2010)). Murine MDSCs are Gr-1$^+$CD11b$^+$ and generally lack expression of mature macrophage and dendritic cell markers (Bronte et al., 1998; Gabrilovich et al., 1998). In healthy mice, Gr-1$^+$ CD11b$^+$ cells are present in very small numbers in the spleen and in circulation and mainly reside in the bone marrow. Unlike MDSCs, these immature myeloid cells are less suppressive (Bronte et al., 2000). MDSCs quickly proliferate and migrate from the bone marrow in response to soluble mediators such as cytokines and chemokines that are produced at sites of inflammation and in the tumor milieu (LaFace and Talmadge, 2011).

MDSCs are heterogeneous, but can be classified into two subsets based on extensive studies of suppression of T cells in tumor models. Granulocytic MDSCs (G-MDSCs) are polymorphonuclear and express high levels of Ly6G and low levels of Ly6C (LaFace and Talmadge, 2011; Ostrand-Rosenberg, 2010; Peranzoni et al., 2010). G-MDSCs often utilize reactive oxygen species (ROS) and arginase 1 for their immunosuppressive function, and require antigen-specific interactions with their target T cells, although there is system-to-system variability (Peranzoni et al., 2010; Movahedi et al., 2008; Youn et al., 2008). Monocytic MDSCs (M-MDSCs), are mononuclear and express low levels of Ly6G and high levels of Ly6C (Ostrand-Rosenberg, 2010; Peranzoni et al., 2010; Youn et al., 2008). M-MDSCs tend to utilize inducible nitric-oxide synthase (iNOS) for suppression (Movahedi et al., 2008; Youn et al., 2008), and may also utilize arginase 1 (Youn et al., 2008), although this can vary in different systems. MDSCs are implicated in a wide range of both murine and human pathologies and have been heavily studied in cancer systems as inhibitors of anti-tumor immunity. Both G-MDSCs and M-MDSCs have been reported to be expanded during oncogenesis, with G-MDSCs often showing a larger expansion (Youn et al., 2008). Despite their higher numbers, G-MDSCs tend to be less potent suppressors in tumor models than M-MDSCs on a per cell basis (Youn and Gabrilovich, 2010). MDSC expansion has been correlated with reduced survival among cancer patients (Gabitass et al., 2011). In addition to their role in

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inhibiting anti-tumor immunity, MDSCs have also been suggested to inhibit anti-viral immunity during influenza infection (Jeisy-Scott et al., 2011), murine models of chronic hepatitis B (Chen et al., 2011), vesicular stomatitis virus (Willmon et al., 2011), and certain strains of herpes simplex virus (Walker et al., 2011). Conversely, a protective role for MDSCs has been reported in several autoimmune models, including collagen-induced arthritis (CIA) (Crook et al., 2015), autoimmune hepatitis (Cripps et al., 2010), inflammatory bowel disease (Haile et al., 2008), and experimental autoimmune encephalomyelitis (Ioannou et al., 2012; Zhu et al., 2011).

The role of MDSCs in murine AIDS (MAIDS) has recently been explored by our lab (Green et al., 2013; O'Connor et al., 2015a; Green et al., 2015; O'Connor et al., 2015b). LP-BM5 retrovirus-induced MAIDS is a murine disease syndrome with many features similar to HIV/AIDS, including early activation parameters such as hypergammaglobulinemia, splenomegaly, and lymphadonopathy; dependence on $CD4^+$ T cells for induction; immunodeficiency characterized by loss of $CD4^+$ T-cell function, decreased T- and B-cell responses, and increased susceptibility to infection and death by opportunistic infections; and development of B-cell lymphomas in late stages (Aziz et al., 1989; Casabianca et al., 2003; Tayar et al., 1999). In susceptible hosts, MAIDS pathogenesis is dependent on binding of CD154 (CD40L) on pathogenic $CD4^+$ T cells with CD40 on B cells (Li and Green, 2006; Green et al., 1996; Green et al., 1998; Yu et al., 1999; Green et al., 2004; Green et al., 2001).

LP-BM5 infection results in a suppressive M-MDSCs population characterized by our lab (Green et al., 2013). These M-MDSCs suppress T cells *ex vivo* in an iNOS-dependent manner and also suppress B cells in a partially iNOS-dependent manner (Green et al., 2013). Suppression of B cells was also partially dependent on the novel negative checkpoint regulator, V-domain Ig suppressor of activation (VISTA) (Green et al., 2015). To our knowledge, our report was the first detailing MDSC (specifically monocytic)-mediated suppression of B cells, in particular in a retroviral system. Previous studies discussed natural suppressor cells from murine bone marrow or neonatal spleens, which were reported to be capable of suppressing B cell responses in an iNOS-dependent manner (Schreiber and Forman, 1993; Angulo et al., 1995; Maier and Holda, 1987). However, whether this population of cells included true MDSCs as now defined, is unknown, as the only phenotypic descriptions indicated that the cells are non-adherent, low-density and Thy- and Ig-negative (Schreiber and Forman, 1993; Angulo et al., 1995; Maier and Holda, 1987). Since then, to our knowledge, three groups have shown MDSC-mediated suppression of B cells in autoimmune models. One group found that MDSCs induced during CIA are also capable of suppressing B cells in an iNOS-dependent manner (Crook et al., 2015), and yet another reported that MDSCs could inhibit proliferation of B cells in experimental autoimmune myasthenia gravis (EAMG) via iNOS and arginase (Li et al., 2014). A third group found that MDSC-injection into lupus mice induced suppression of effector B cell population, including germinal cells and plasma cells, via iNOS while simultaneously increasing the proportion of regulatory B cells (Park et al., 2016). Additionally, MDSCs have been identified as inhibitors of B-cell lymphopoiesis in the bone marrow during obesity and aging (Kennedy and Knight, 2015). Although we are unaware of any studies evaluating suppression of B cells by HIV-derived MDSCs, MDSCs capable of suppressing both antigen-specific and non-specific $CD8^+$ T-cell responses were increased in HIV patients, supporting our findings in the LP-BM5 retroviral system, with MDSC-frequencies correlating with clinical parameters such as decreased $CD4^+$ T-cell frequency and increased viral load (Vollbrecht et al., 2012).

Inducible nitric oxide synthase (iNOS) catalyzes the production of nitric oxide (NO) from L-arginine and O_2 (iNOS Signaling. SABiosciences, 2012). In addition to its function as a proinflammatory mediator and its ability to inhibit viral replication (iNOS Signaling. SABiosciences, 2012), NO can also inhibit immune responses and promote chronic infection (Burrack and Morrison, 2014). While our previous work indicates that iNOS accounts for approximately half of the M-MDSC-mediated suppression of B cells, and that VISTA also plays a major role in this suppression (Green et al., 2015), the existence and identity of other suppressive mechanism (s) active against B-cell targets are unknown (Green et al., 2013). In this LP-BM5 retroviral system, suppression of B cells was independent of: arginase 1, another common suppressive mechanism utilized by MDSCs, as well as PD-1/PD-L1 interactions, IL-10, and indoleamine 2,3-dioxygenase (IDO) activity (Green et al., 2013; O'Connor and Green, 2013). Other mechanisms of suppression utilized by either MDSC subset in their inhibition of T-cell responses in different disease settings can include membrane-bound or soluble transforming growth factor β (TGF-β) (Li et al., 2009; Yang et al., 2008; Li et al., 2012), cysteine depletion (Srivastava et al., 2010), ROS production (Kusmartsev et al., 2004; Corzo et al., 2009; Schmielau and Finn, 2001; Szuster-Ciesielska et al., 2004), prostaglandin-E2 (Sarkar et al., 2007; Sharma et al., 2005; Rodriguez et al., 2005), induction of regulatory T cells (Serafini et al., 2008; Huang et al., 2006; Pan et al., 2010), and down-regulation of L-selectin expression (Ostrand-Rosenberg, 2010). As MDSC-mediated suppression of B cells is understudied, it is not clear whether these and/or other potential suppressive pathways, such as adenosine production (Kumar and Sharma, 2009) and stimulation of the inhibitory receptors FcγRIIβ (CD32) (Malbec et al., 1999; Amigorena et al., 1992; Muta et al., 1994; Nimmerjahn and Ravetch, 2008) and CD22 (Hanasaki et al., 1994; Rudge et al., 2002; Smith et al., 1998; Doody et al., 1995; Chan et al., 1998), or CD72 (Parnes and Pan, 2000), are involved in M-MDSC suppression of B-cell targets.

Given the scarcity of studies examining MDSC-mediated suppression of B cells, we utilized the LP-BM5 retroviral system to characterize the mechanism(s) in addition to NO production and VISTA that are used by M-MDSCs to suppress B cells. The following work began with triaging experiments to determine if suppression was contact-dependent and if soluble mediators were involved. Antioxidants, inhibitors, antibodies, and other methods were utilized to block potential reactive nitrogen or oxygen species, soluble TGF-β, and downstream mediator-dependent mechanisms. The effects of genetic ablation of major suppressive mechanisms, as well as the effect of M-MDSCs on regulatory, IL-10-producing B cells, were also examined. These data, and in particular the concept of MDSC plasticity in molecular mechanisms of inhibition, may have a number of potential clinical implications relevant to MDSCs present in a variety of human disease settings.

2. Materials and methods

2.1. Mice

7-week-old male C57BL/6 (B6) mice were purchased from the National Cancer Institute (NCI, Bethesda, MD) or Charles River (Wilmington, MA). B6-background 10BiT (Thy1.1 gene under the control of IL-10 promoter) (Maynard et al., 2007) and VISTA knockout mice were generous gifts from the laboratories of Lloyd Kasper and Randolph Noelle, respectively (Geisel School of Medicine at Dartmouth, Lebanon, NH). B6-background iNOS knockout, purchased from Jackson Lab (Farmington, CT), and VISTA knockout mice were crossed to create F_2-generation-derived VISTA/iNOS double knockout breeders with assistance by the Dartmouth Immunology COBRE (Geisel School of Medicine at Dartmouth,