Lung myeloid-derived suppressor cells and regulation of inflammation

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Abstract Myeloid-derived suppressor cells (MDSCs) have been investigated largely in the context of tumor progression. In contrast to the negative connotation of MDSCs in cancer immunity, our laboratory has recently reported on the development and role of pulmonary MDSC-like cells ($CD11b^+Gr1^{int}F4/80^+$) in the regulation of allergic airway inflammation. These regulatory cells were expanded in a TLR4/MyD88-dependent manner and were both phenotypically and morphologically similar to those described in the tumor microenvironment. Although bacterial lipopolysaccharide (LPS) was initially described as an adjuvant in the development of allergic inflammation, subsequent studies showed that this is true only at relatively low doses of LPS. A high dose of LPS was shown to actually suppress eosinophilic airway inflammation. In our efforts to understand the mechanism underlying LPS-mediated suppression of allergic airway disease, we recently showed that LPS induces MDSC-like cells in the lung tissue in a dose-dependent manner, with increased accumulation of the cells at high doses of LPS. In contrast to lung dendritic cells (DCs), the MDSC-like cells did not traffic to the lung-draining lymph nodes, allowing them to act in a dominant fashion over DCs in the regulation of Th2 responses. The MDSC-like cells were found to blunt the ability of the lung DCs to upregulate GATA-3 or to promote STAT5 activation in primed Th2 cells, both transcription factors having critical roles in Th2 effector function. Thus, a complete understanding of the generation and regulation of the lung MDSCs would provide novel options for therapeutic interventions.

Keywords Lung · MDSCs · LPS · Allergic inflammation · Suppression

Introduction

The field of myeloid-derived suppressor cell (MDSC) biology has grown in recent years with MDSCs found to play a regulatory role in a variety of tissue and disease settings. This heterogeneous population of monocytes and granulocytes were first identified in the 1980s as immunosuppressive $CD11b^+$, $Gr-1^+$ myeloid cells in cancer patients [1]. They create a tolerogenic environment via cytokine production, manipulation of L-arginine metabolism, and dysregulation of both CD4 and CD8 T-cell function. Increased tumor growth is often correlated with an increase in MDSC accumulation; however, the mechanisms by which MDSCs are generated via exposure to tumor-secreted factors or how they are recruited to the tumor microenvironment are not well understood [2]. It is clear that MDSCs employ multifaceted mechanisms to mediate immune suppression. Comparable, but not phenotypically identical, cell populations with immunosuppressive function have been identified at mucosal sites such as the intestine where the cells serve to suppress local inflammation [3, 4]. Given that recent studies have

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identified MDSC-like cells in contexts other than cancers [5–7], a better understanding of their generation and function would be important to either dampen or promote their function depending on the disease state. As described below, our study for the first time identified LPS-induced MDSCs in the lung while investigating mechanisms by which high doses of LPS suppress allergic airway disease.

Multi-dimensional role of LPS on allergic inflammation

Bacterial lipopolysaccharide (LPS) (endotoxin) has been shown to have multi-dimensional effects on the development of allergic inflammation and can either cause or prevent allergic airway inflammation depending on the dose, duration, and timing of LPS exposure. Endotoxin has been known to have pro-inflammatory properties. When inhaled, it elicits neutrophilic airway inflammation with accompanying systemic responses such as blood leukocytosis with neutrophilia [8]. In contrast, various epidemiological studies in support of the hygiene hypothesis have shown an inverse association of endotoxin exposure with hay fever and atopic asthma, suggesting that early exposure to endotoxin protects against allergic diseases [9]. Although these initial epidemiological studies showed that early-life farm contact protects against allergic diseases, recent studies have shown that farm contact even later in life not only reduces the incidence of allergic disease but also results in loss of sensitization [10–12]. Taken together, a low dose of LPS in the nanogram range generally increases the risk of asthma due to its adjuvant effect [13], whereas a high dose in the microgram range is protective against allergic airway inflammation as shown in various studies including our own recent study [13–17].

Despite many observations of protective effects of endotoxin on allergic diseases, the underlying molecular mechanisms were not sufficiently explored until recently. The concept of Th1/Th2 cross-regulation was initially invoked to explain the hygiene hypothesis in which stimulation of a strong Th1 response by LPS was presumed to cause immune deviation away from Th2. While undoubtedly IFN-γ produced by Th1 cells does suppress the development of Th2 cells, it is clear that asthma and allergic diseases are caused by complex gene–environment interactions, which cannot all be explained by the mere absence of a strong Th1 immune response in early years [18]. Central to this concept are regulatory T cells (Tregs) balancing both Th1 and Th2 responses. It was shown that Tregs express Toll-like receptor 4 (TLR4), and exposure to endotoxin not only promotes Treg survival and proliferation but also enhances their suppressive functions [19]. In another study, local LPS application in the nasal mucosa of non-atopic children promoted activation and proliferation of T cells resembling Tregs [20]. These studies suggest that LPS-activated Tregs may have the ability to regulate allergic responses. However, LPS-mediated immunosuppressive mechanisms in the context of allergic airway disease were not adequately explored in these studies.

LPS-driven accumulation of lung MDCS-like cells (CD11b+Gr1int cells): heterogenous family of myeloid cells

Myeloid-derived suppressor cells (MDSCs) represent a heterogeneous cell population consisting of various myeloid precursor cells and are characterized by their ability to suppress various T-cell functions [21]. All MDSCs express the surface markers CD11b and Gr1. Although most studies have defined MDSCs as CD11b$^+$Gr1$^+$ cells having suppressive activity, one of the major hallmarks of MDSCs is their phenotypic, morphological, and functional heterogeneity. They have features of both monocytes/macrophages and neutrophils. Identification of MDSC-specific cell surface molecules that would allow specific depletion of these cells would be a long-awaited breakthrough in the field that is needed to further our knowledge about their specific role in various disease conditions.

While MDSCs have been largely investigated in the context of tumor growth and development, recent studies have now shown that MDSC-type cells can play a very important regulatory role in controlling inappropriate inflammatory immune responses in autoimmune diseases in contrast to their negative role in cancer immunity [5]. In our attempt to understand the protective role of LPS in allergic inflammation in the airways, we have recently shown that repeated exposure to a relatively high dose of LPS (up to 10 μg) promotes an increase in CD11b$^+$Gr-1^{int}F4/80$^+$ cells in the lung that closely resemble MDSCs [17]. MDSCs isolated from tumor sites have also been shown to express F4/80 [22]. Further characterization of these LPS-induced MDSC-like cells revealed that other molecules like CD115 (M-CSF receptor), CD124 (IL-4 receptor α chain), and CD62L, which have been shown to be expressed by MDSCs [21], were not expressed by the lung MDSC-like cells [17]. Morphological characterization of these LPS-induced MDSC-like cells revealed a heterogeneous population of immature myeloid cells similar to the Gr1$^+$CD11b$^+$ cells observed in murine models of cancer and trauma [22, 23]. The phenotype of MDSCs and the mechanisms of suppression employed by the cells can vary significantly depending on whether LPS or a tumor-associated factor is the inducing agent making it difficult to derive a one-to-one correspondence between all MDSC-like cells. However, given that the LPS-induced CD11b$^+$Gr-1^{int}F4/80$^+$ cells shared many similarities with

MDSCs, hereafter they are referred to as lung MDSCs as was also described in a recent review [24].

Regulation of accumulation of lung MDSCs

The accumulation of MDSC-type cells is thought to be dependent on a number of factors such as VEGF, IL-6, GM-CSF, M-CSF, stem cell factor, cyclooxygenase-2, prostaglandins, TLR ligands, and members of the S100 protein family [25–30]. Our study has demonstrated the expansion of $CD11b^+Gr1^{int}F4/80^+$ cells in a dose-dependent manner following LPS administration [17]. The expansion of these cells was greatly blunted in MyD88-deficient mice, suggesting dependence on this adaptor protein for their accumulation. Additionally, using GFP to track cells in vivo, we showed that a lineage negative bone marrow progenitor population when introduced intravenously into mice has the ability to differentiate into MDSCs in the lung after intratracheal delivery of LPS [17]. This finding is in agreement with the report of von Andrian and colleagues who identified hematopoietic stem and progenitor cells (HSPCs) in extramedullary tissues including the lung and showed their ability to differentiate into CD11c+ cells in the presence of LPS in which a fraction of the cells also expressed Gr1 [31]. At the time, it was speculated that the purpose of LPS-induced differentiation into myeloid cells was to boost immune surveillance at the appropriate site although this idea was not experimentally interrogated. It is important to note that a number of other studies have also documented the expansion of CD11b+Gr1+ cells in response to repeated LPS exposures [32–34]. For example, CD11b+Gr1+ cells were identified in the context of microbial sepsis which caused immune suppression by promoting Th2 polarization contrasting with the ability of lung MDSCs to suppress Th2 effector function [32]. Collectively, these findings illustrate that the same agent, LPS, can induce MDSC-like cells in different organs that possess very different functions (Fig. 1).

CD11b+Gr1+ MDSC-like cells are present in the bone marrow under homeostatic conditions. Furthermore, the generation of suppressive MDSC-like cells in culture has been demonstrated under low or high GM-CSF conditions [35]. We demonstrated the in vitro generation of CD11b+Gr1int from lineage negative bone marrow progenitors in the presence of LPS and GM-CSF [17]. While the presence of GM-CSF alone favors myeloid DC differentiation, the addition of LPS blocks DC generation in favor of MDSC-like cells. In a similar manner, the combination of GM-CSF and inflammatory cytokines such as IL-6 or IL-1β favors the generation of MDSCs with T-cell suppressive capacity [30]. Suppressors of cytokine signaling (SOCS) proteins have been shown to play a role in this

block in DC differentiation presumably for the purpose of favoring macrophage differentiation under conditions where innate host defense is needed for protection [36]. It is also critical to recognize the central role of STAT3 as a regulator of MDSC accumulation and expansion [37]. Indeed, S100A9, which is regulated via STAT3, has been shown to favor MDSC differentiation [27]. Reciprocally, modulation of STAT3 via miR-17-5p and miR-20a impairs the suppressive potency of MDSCs [38].

Suppression of immune effector functions by lung MDSCs

MDSCs are well known for their ability to inhibit T-cell proliferation and immune responses. Our study showed that repeated exposure of mice to LPS promotes the expansion of MDSCs in the lung tissue. However, these MDSCs were barely detectable in the lung-draining lymph nodes (LNs). This caused selective enrichment of the MDSCs over dendritic cells (DCs) in the tissue since migratory DCs are induced by LPS to migrate to the draining LNs for antigen presentation to LN T cells [17]. In our efforts to further understand the function of LPS-induced lung MDSCs, we demonstrated a previously unappreciated role of MDSCs in suppressing Th2-cell activation. Our study showed that LPS-induced lung MDSCs suppress the ability of lung DCs to promote Th2 cytokine production, upregulate GATA-3, or induce STAT5 activation in primed Th2 cells, both transcription factors being critical in Th2 effector function [39–41]. Since STAT5 activation promotes T-cell viability [42, 43], it is possible that lung MDSCs impair Th2-cell survival thereby reducing the size of the memory T-cell pool [33, 44].

Mechanism of suppression of immune functions by MDSCs

MDSCs utilize various mechanisms to suppress T-cell functions including modulation of ʟ-arginine amino acid metabolism through expression of Arginase 1, production of nitric oxide, peroxynitrite, and reactive oxygen species, induction of loss of CD3ξ signaling in T cells and T-cell apoptosis [21, 45–49]. MDSCs can also block T-cell activation by sequestering cystine and thus limiting the availability of cysteine, an essential amino acid for T-cell activation and function [50]. Various other mechanisms used by MDSCs to suppress immune responses have also been suggested, which include upregulation of cyclooxygenase 2 and prostaglandin E2 [51], secretion of TGF-β [52], and induction of Tregs [53, 54]. Our study showed that the IL-10/Arg1 axis is involved in the suppressive
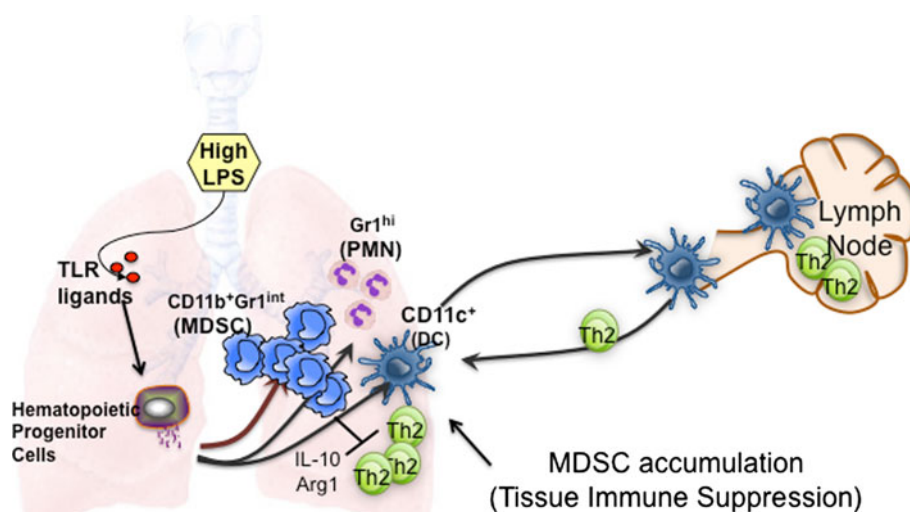
Fig. 1 Schematic depicting regulation of lung MDSC accumulation. A relatively high dose of LPS promotes accumulation of CD11b$^+$Gr1^{int}F4/80$^+$ MDSC-like cells in the lung tissue from hematopoietic progenitor cells. In contrast to activated lung DCs, these cells do not traffic to the draining lymph nodes, which causes an enrichment of these cells relative to DCs in the tissue. Molecules expressed by the CD11b$^+$Gr1^{int}F4/80$^+$ cells such as Arginase 1 and IL-10 suppress reactivation of effector Th2 cells by the resident DCs

activity of lung MDSCs on Th2 cells [17]. In another study, HO-1 was implicated in the suppression of alloreactive responses by LPS-induced MDSCs [33]. Considering the diversity of immune signals to which MDSCs are exposed in different biological contexts, it is expected that the mechanism of suppression by MDSCs induced by various agents would also vary. Given the fact that the accumulation of MDSCs is influenced by different stimuli and a variety of suppressive mechanisms can be induced in MDSCs, it is important to critically evaluate the function of these cells in every situation before targeting them for immunotherapy.

Concluding remarks

In recent years, MDSCs have received significant attention because of their immunosuppressive functions. However, due to their heterogeneous nature, exploitation of MDSCs therapeutically has been a formidable challenge. Our studies show that continuous exposure to LPS suppresses allergic airway disease by inducing lung MDSCs [17]. However, additional research is necessary to understand the immuno-modulatory properties of lung MDSCs, which would provide new avenues to either promote or delete these cells for disease-specific immunoregulation.

Conflict of interest The authors declare no conflicts of interest.

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