

Myeloid-derived Suppressor Cells in Autoimmune Diabetes: Their Anti-diabetic Potential and Mechanism

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Abstract

Autoimmune diabetes is caused by a destruction of pancreatic β -cells by autoreactive immune response, leading to insulin insufficiency/deficiency and hyperglycemia and fatal complications. This disease afflicts up to 10 million people worldwide. There is no cure for autoimmune diabetes. Insulin injection is the only supportive medication, which always accompanies fatality. Apart from replacement therapy using insulin and/or β -cells, immune interventions hold the key to stopping this illness. Myeloid-derived suppressor cells have emerged as a new regulator in harnessing immune response. In this review, we first up-dated the advances on etiology, development and immune interventions of autoimmune diabetes. Next, we highlighted the origin, development, tolerogenic mechanisms of myeloid-derived suppressor cells with an emphasis of the signaling pathways in their development and action. Finally, we summarized and discussed the recent progress in exploring the potential and mechanism of myeloid-derived suppressor cells in autoimmune diabetes. A novel vista on MDSC-based immune intervention with AID development was also discussed.

Keywords: Autoimmune diabetes; MDSC; Immune cells; Immune intervention; MDSC development; Soluble mediators; Cell contact; Mechanism and immune tolerance

Abbreviations: AID: Autoimmune Diabetes; MDSC: Myeloid-Derived Suppressor Cells; NOD: Non-Obese Diabetic; B: Biobreeding LETL: Long Evans Tokushima Lean; APC: Antigen-Presenting Cells; Teff Cells: Effector T Cells; T_{reg} Cells: Regulatory T Cells; MHC II: Histocompatibility Complex Class II; CLTA4: Cytotoxic T-Lymphocyte Antigen 4; IL: Interleukin; IFN: Interferon; TGF: Tumor Growth Factor; Inos: Inducible Nitric Oxide Synthase; NO: Nitric Oxide; TNF: Tumor Necrosis Factor; ROS: Reactive Oxygen Species; CCR2: CC Chemokine Receptor; CCL2: CC Chemokine Ligand; MMP: Matrix Metalloproteinase; TLR: Toll-Like Receptors; IMC: Immature Myeloid Cells; PIR: Paired Immunoglobulin-Like Receptors; ICAM-1: Intercellular Adhesion Molecule 1

Introduction

Autoimmune Diabetes (AID)

Cause, pathogenesis and current therapy of AID: In 2005, the US National Institutes of Health estimated that 23.5 million people, ~ 8% of Americans, suffer from autoimmune diseases with direct health care costs totaling 100 billion dollars annually [1]. Among over 100 autoimmune diseases whose causes are identified, autoimmune diabetes, known as type 1 diabetes, is estimated to afflict as many as 10 million people worldwide [1].

As with other autoimmune diseases, AID is initiated and developed by an interaction of environment, genes and immune system [2]. Environmental factors such as infectious agents, commensal microbiota, sex hormones and diets contribute to the establishment and (re)shaping of the immune system [3-5]. A number of genes such as major histocompatibility complex class II (MHC II), cytotoxic T-lymphocyte antigen 4 (CLTA4), insulin and many others are implicated in the immune response that regulates AID outcome [3].

Over-reactive immune system also occurs in AID patients [2]. As a result of their interplay, AID stems from a loss of insulin-producing pancreatic β -cells caused by infiltrating immune cells, resulting in hypoinsulinemia, hyperglycemia and fatal complications.

So far, there has been no cure for AID. Daily injection of insulin is the only medication. However, this treatment cannot match the naturally precise timing and dosing of insulin secretion of the pancreas in response to hyperglycemia, leading to severe complications, namely, kidney failure, retinopathy, cardiovascular disease, and chronic ulcers [6]. A variety of strategies has been developed, aimed at re-establishing physiological insulin production in diabetic patients [7]. Despite some progress, devising a means capable of restoring self-tolerance or specifically correcting autoimmunity is a crucial step toward reversing AID. In this respect, regulatory T_{reg} and Myeloid-Derived Suppressor (MDSC) have received particular attention [8-10].

Animal models of AID: Since the access to clinical samples of AID patients is always limited, animal models of AID are requisite for pre-clinical studies. Different animal models of AID have been used in pre-clinical research, including chemical-induced diabetic mice, Non-Obese Diabetic (NOD) mice, Biobreeding (BB) rats, Long Evans Tokushima Lean (LETL) rats, New Zealand white rabbits, Chinese hamsters, Keeshond dogs and Celebes black [11]. These

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animal models have provided a rich information about the inherently complex development of this disease [11,12], albeit the fact that certain differences in AID pathogenesis between humans and animals exist [13].

Based on many similar genetic, immunological and pathological features with human patients, NOD mice stand out as the most commonly used model for AID study [14,15]. Using these animal models, more than 400 compounds were demonstrated effective against AID in pre-clinical settings and some of them are in clinical trials [16].

Development and immune intervention with AID: Similar to AID patients, NOD mice can spontaneously develop diabetes (Figure 1) [12]. At the initiation of AID, leukocytes begin to invade the pancreatic islets [17]. This invasion, called insulinitis, gradually induces a loss of pancreatic β -cells and, eventually, gives rise to insulin insufficiency and deficiency, a hallmark of AID [17]. Overwhelming evidence shows that T-cells play a key role in AID development, though B cells, Dendritic Cells (DC), macrophages, NK cells and other immune cells are also implicated [18,19]. During its development, Antigen-Presenting Cells (APC) infiltrate into the inflamed pancreas, capture auto-antigens and move to pancreatic lymph nodes. Upon T-cell receptor engagement by MHC and auto-antigens, effector $T_{(Teff)}$ cells are activated and differentiated into different T-cell subsets. Eventually, these cells go to the pancreas and release interferon (IFN)- α , tumor necrosis factor (TNF)- α and perforin leading to the destruction of β -cells. T_{reg} cells have been recognized as an inhibitory player in AID development. In 2007, MDSC was coined to describe suppressor cells of myeloid origin [20] and, later on, manifested their ability to inhibit AID [9,10]. Accordingly, harnessing immune cells at the aforementioned steps may reduce autoimmunity and β -cell destruction and, in turn, lead to AID alleviation and cure (Figure 1).

MDSC

Origin and development of MDSC: MDSC were found to accumulate in bone marrows, spleens, and tumors in tumor bearing hosts about three decades ago [21,22]. In the last 10 years, research and clinical interest in MDSC has increasingly grown [23-27]. MDSC represent a heterogeneous population of myeloid progenitor cells induced by inflammatory mediators in malignancies, infections, wounds, transplants, and autoimmune disorders [23]. Their composition and percentage vary with diverse pathological conditions [28,29]. However, MDSC of different origins show great suppressive activities [30-32]. MDSC are featured in their morphological, phenotypic, and functional heterogeneity [23,24,28]. Nonetheless, further characterization of MDSC is now limited by their heterogeneous complexity and scarcity of reliable markers [24].

The development of MDSC in different circumstances is not well understood [33]. In physiological conditions, Immature Myeloid Cells (IMC) differentiate from myeloid progenitors and, gradually, mature into dendritic cells, macrophages, and granulocytes/neutrophils upon migrating to the periphery (Figure 2A). In pathological conditions, abundant growth factors associated with diseases stimulate IMC expansion and subsequently, disturb their normal differentiation in bone marrow [34].

Moreover, inflammatory mediators of pathologies can aberrantly drive IMC to activate and polarize into MDSC with different phenotypes [35] (Figure 2B). As a consequence, MDSC emigrate from bone marrow and accumulate in peripheral tissues. The question as to whether MDSC in the periphery, spleens versus tumor sites, hold the same characteristics is not resolved. Based on lineage markers,

MDSC can be classified into $Gr1^+CD11b^+CD115^+Ly6C^+$ monocytic (M)-MDSC and $Gr1^+CD11b^+Ly6G^+$ granulocytic (G)-MDSC in mice [20,36,37]. A consensus in the markers for human MDSC is not apparent. Depending on cancer types, human MDSC are characterized as $CD11b^+CD14^+CD33^+$ or $Lin-HLA-DR-CD33^+$ myeloid cells [38,39].

Mirroring the nomenclature of type 1 classic activation-like (M1) and type 2 alternative activation-like (M2) macrophages, polarized MDSC can be defined as M1 and M2 cells based on their corresponding phenotypes and functions (Figure 2B). The molecular basis of MDSC development at the stages of expansion, activation, and functional polarization is largely unknown. One signal model was originally proposed to explain the requirement of one of tumor-associated factors for MDSC development.

More recently, this model was evolved into the “two signal model” stating that two distinct tumor-associated mediators are required at the stages of MDSC expansion and activation [33]. Since MDSC development from expansion to activation and functional polarization is a multiple-step process, “multiple signal model” in which multiple factors/signals are necessary for this process should be considered. However, type and mechanism of the pathology-associated factors in pathogenesis of MDSC from hematopoietic progenitors remain mostly unclear.

Functional polarization of MDSC is less studied probably due to the complexity and heterogeneity of MDSC subsets. Compelling evidence support the concept that tumor-associated MDSC predominantly exhibit M2-like phenotypes and immunosuppressive and pro-tumoral activities [28,31,32,34,36,40-42]. However, co-existence of M1 and M2 phenotypes in MDSC was observed in few cases [43].

The M2 M-MDSC were phenotypically characterized by a number of enhanced signature markers such as Interleukin (IL)-10, arginase,

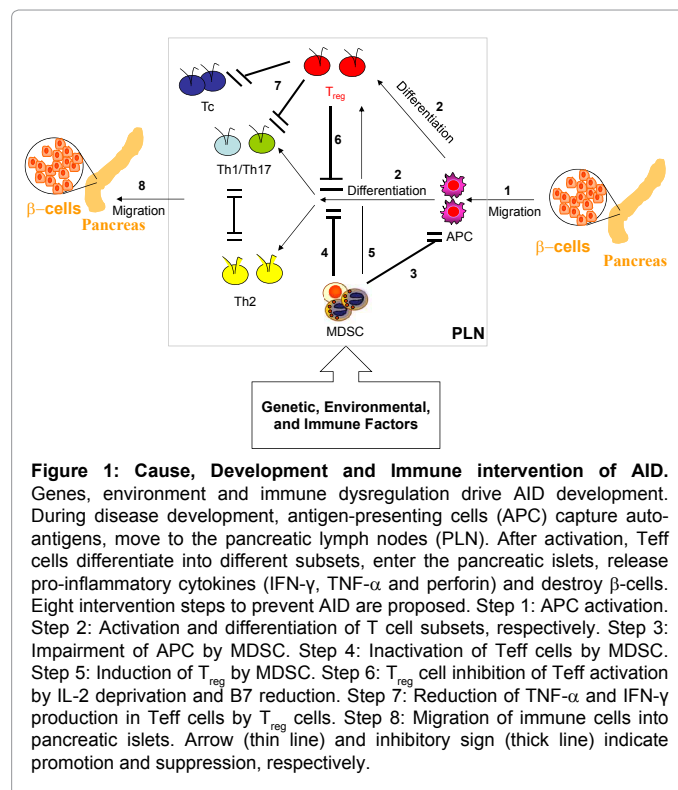
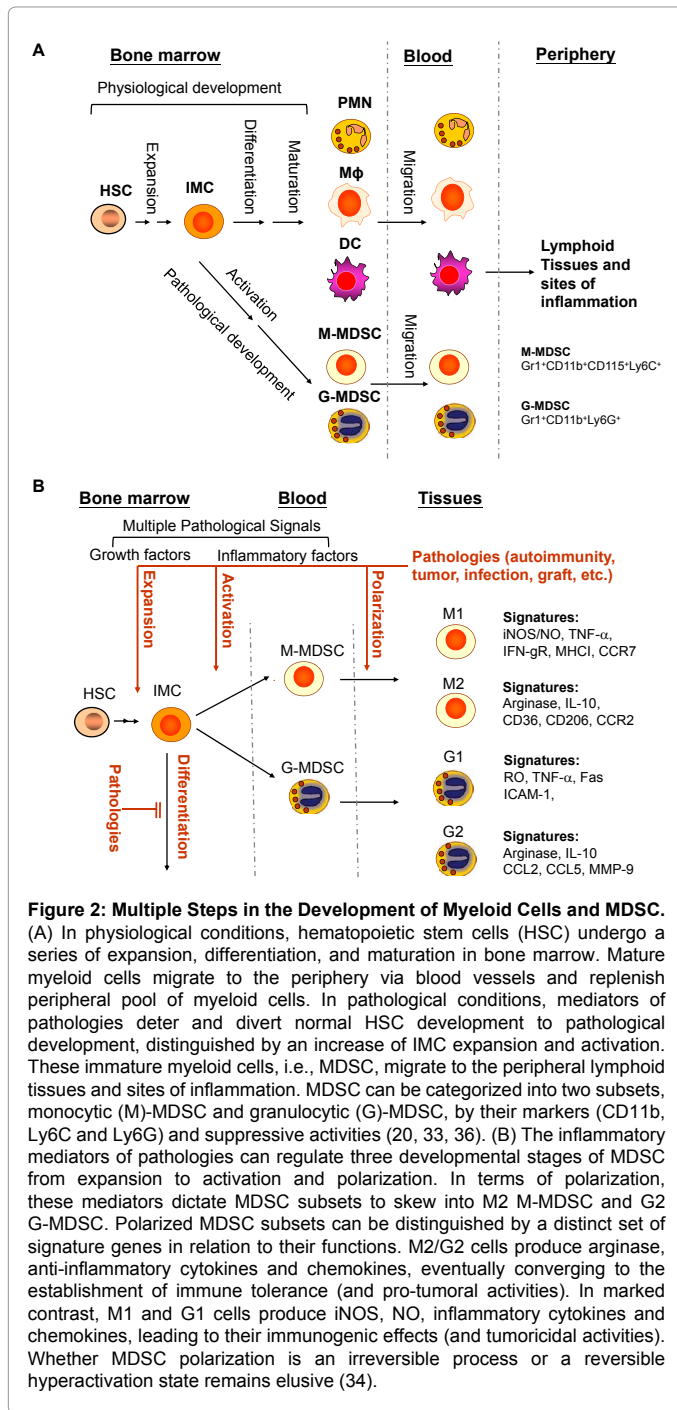


Figure 1: Cause, Development and Immune intervention of AID. Genes, environment and immune dysregulation drive AID development. During disease development, antigen-presenting cells (APC) capture auto-antigens, move to the pancreatic lymph nodes (PLN). After activation, T cell subsets differentiate into different subsets, enter the pancreatic islets, release pro-inflammatory cytokines (IFN- γ , TNF- α and perforin) and destroy β -cells. Eight intervention steps to prevent AID are proposed. Step 1: APC activation. Step 2: Activation and differentiation of T cell subsets, respectively. Step 3: Impairment of APC by MDSC. Step 4: Inactivation of Tc cells by MDSC. Step 5: Induction of T_{reg} by MDSC. Step 6: T_{reg} cell inhibition of Tc activation by IL-2 deprivation and B7 reduction. Step 7: Reduction of TNF- α and IFN- γ production in Tc cells by T_{reg} cells. Step 8: Migration of immune cells into pancreatic islets. Arrow (thin line) and inhibitory sign (thick line) indicate promotion and suppression, respectively.



Tie-2, CD36, CD206, IL-4R and CC chemokine receptor 2 (CCR2) [42]. Inhibitor of SHP-1/2, NSC87877, could reverse M2-like phenotypes of M-MDSC to M1-like phenotypes [42]. M1-polarized MDSC had an elevation of signature markers such as inducible Nitric Oxide Synthase (iNOS), nitric oxide (NO), TNF- α , IFN- γ R, MHC I and CCR7 [42]. G-MDSC in tumors shares the same markers, CD11b⁺Ly6G⁺, and immunosuppressive activities as splenic G-MDSC. However, they sometimes may not be regarded as the same cells [44,45]. Like M1/M2 MDSC, G2 G-MDSC is a major population found in tumor bearing mice. G2 cells up-regulate the expression of arginase, CC Chemokine Ligand 2 (CCL2), CCL5 and Matrix Metalloproteinase 9 (MMP-9). In

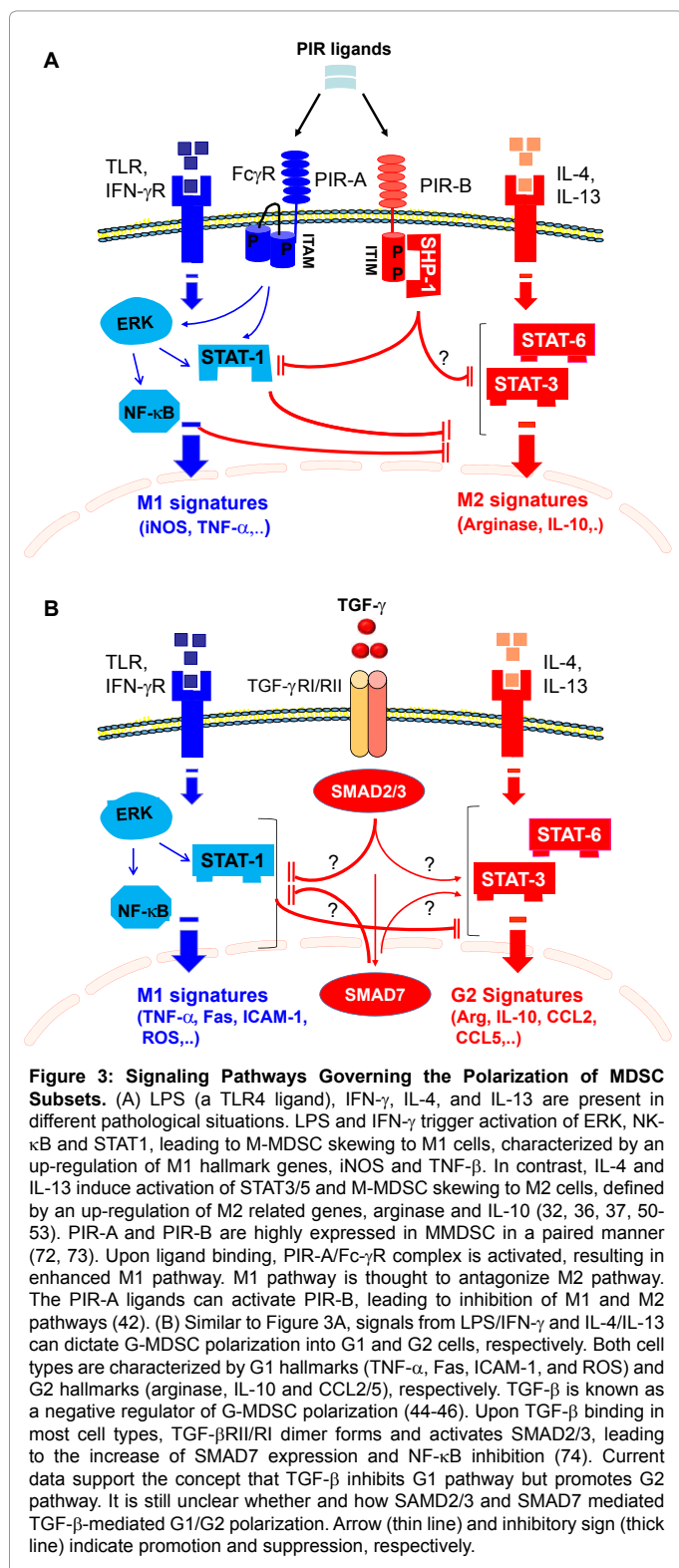
contrast, G1 cells show elevated expression levels of TNF- α , Fas, and Intercellular Adhesion Molecule 1 (ICAM-1). [44,46].

Polarization of MDSC involves an array of signaling cascades, leading to their acquisition of phenotypes and functionalities. Several studies showed that IFN- γ could induce iNOS expression whilst IL-4 or IL-13 increased arginase expression in MDSC as well as macrophages [4-50]. Furthermore, activation from Toll-Like Receptors (TLR), IFN- γ R, IL-4R, IL-13R could modify MDSC function [32,36,37,50-53]. Consistently, membrane receptors such as TLR, Interferon- γ Receptor (IFN- γ R), Interleukin-4 Receptor (IL-4R) and IL-10R have been reported to participate in the function and expression of inducible Nitric Oxide Synthase (iNOS), Tumor Necrosis Factor (TNF)- α , M1 hallmarks, and arginase, M2 hallmarks [42,44]. Besides, studies in tumor-bearing hosts using pharmacological intervention and/or genetic ablation revealed that paired immunoglobulin-like receptors (PIR) and Tumor Growth Factor B Receptor (TGF- β R) modulate the polarization of M-MDSC and G-MDSC, respectively (Figures 3A and 3B) [42,44]. Therefore, ligands, receptors, and downstream mediators of the PIR-B and TGF- β R pathways are potential targets for manipulation of functional phenotypes of MDSC that can be used for treatment of autoimmunity, cancer and other diseases. More information on the molecular basis of MDSC polarization and related functional changes is required for their further clinical applications.

Multiple mechanisms of MDSC in immune regulation: MDSC are one of the pivotal regulators of innate and adaptive immunity. They act as a “hub” to link and cross-talk with other immune cells in favor of immune tolerance in order to maintain disease progression and persistence. The details regarding the coordinated regulation of MDSC and other immune cells were summarized in Figure 4A [38,54,55]. MDSC exert immune suppression by cross communication with T cells, NK cells, DC, macrophages, and other immune cells via cell contact (MHC/peptide/TCR, CD28) and soluble mediators (Reactive Oxygen Species (ROS), NO, IL-10, TGF- β) [54,56,57]. MDSC can impair DC functions by decreasing maturation, antigen uptake and migration and skewing DC cytokine profile from inflammatory phenotype to anti-inflammatory one [58]. Additionally, MDSC interact with macrophages. MDSC diminish inflammation by down-regulating macrophage production of IL-12, IL-6 and MHC II. This down-regulation appears to require IL-10 and cell contact [54]. MDSC also suppress development and function of NK cells and this suppression can be enhanced by inflammation [59,60]. As far as T cells are concerned, MDSC can induce T_{eff} cell inactivation and apoptosis [61-65] and expand T_{reg} cells [9,32,36,66-69]. T cell suppression and T_{reg} expansion by MDSC are cell contact-, NO- and/or arginase-dependent [36,61,70-74]. M2-like M-MDSC possess higher abilities to suppress T_{eff} cell activation and proliferation than M1-like counterparts in the co-culture of T cells with M-MDSC and in vivo [42]. Moreover, M-MDSC with M2 functional phenotype possess higher potency in T_{reg} expansion than those with M1 phenotype in vitro and in vivo [42]. M2 M-MDSC-induced T_{reg} increase seemed to be IL-10, IL-4 and IL-13-mediated arginase-dependent [42]. GMDSC could inhibit CD8 T cell activity in tumor-bearing hosts [44]. However, the ability of G-MDSC to induce T_{reg} expansion is not corroborated. Overall, MDSC with M2 functional phenotype induce higher immune tolerance than those with M1 phenotype.

Potential and mode of action of MDSC in suppressing AID

MDSC have emerged as one of key immune regulators, raising a hypothesis that MDSC can treat AID and other autoimmune diseases. This hypothesis was first assessed in mouse models of AID as evidenced



by two seminal studies [9,10]. One study from our group, for the first time, demonstrated that MDSC isolated from tumor-bearing mice mediated T_{reg} induction or T_{eff} suppression dependently on a MHC II-dependent antigen presentation [9]. The mechanism of action of MDSCs is via secretion of anti-inflammatory cytokines (TGF- β and

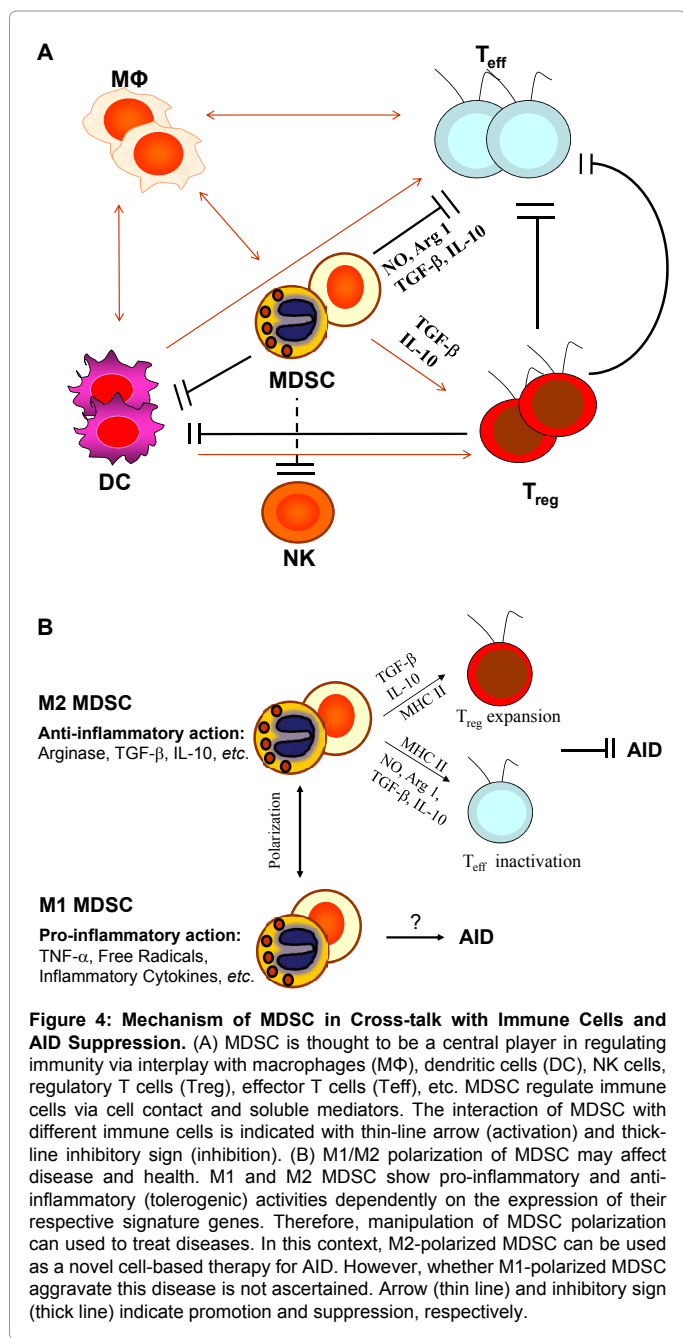
IL-10), induction of CD4⁺CD25⁺Foxp3⁺ T_{reg} or suppression of T_{eff} proliferation that are beneficial for creating host immune tolerance [9]. To understand the role of MDSCs in murine diabetes models, we showed that adoptive transfer of MDSCs reduced diabetes by 75% compared with control group in RIP-HA/Rag2^{-/-} mice [9]. Moreover, the protective role of MDSCs in NOD/SCID mice was investigated [9]. NOD/SCID mice were injected with diabetogenic T cells from diabetic NOD mice in the presence of MDSCs. Consistently, protective efficacy of MDSCs is dose-dependent and single dose treatment of MDSCs showed significant long-term protection, i.e. 60% remained diabetes free over 14-week observation [9]. The overall data prove the concept that MDSCs can suppress AID via regulation of T cell-mediated tolerance. It is worth mentioning that the MDSC were characterized as M2 MDSC. Later on, the other study confirmed the function of MDSC in AID development. They first showed that temporary B-cell depletion by anti-hCD20 antibody increased CD11b⁺Gr1⁺ splenocytes by 6% in h-CD20/NOD transgenic mice [10].

Next, they found that these myeloid cells inhibited T cell proliferation in vitro in a NO- and cell contact dependent fashion, suggesting that this subset had MDSC characteristics [10]. Strikingly, they were able to employ one single dose of anti-Gr1 antibody (RB6-8C5 clone) to induce a significant expansion of CD11b⁺Gr1⁺ cells in NOD mice whose diabetic incidence was reduced by ~40%. Besides, anti-TGF- β neutralizing antibody almost abolished the reduction of diabetic incidence in NOD mice, suggesting the implication of TGF- β in the function of CD11b⁺Gr1⁺ cells. The CD11b⁺Gr1⁺ cells showed perfect traits of MDSC as evidenced by in vitro T_{eff} suppression and T_{reg} induction assays [10]. Taken together, MDSC suppress AID via multiple mechanisms involving T_{eff} inactivation, T_{reg} induction, cell contact and soluble mediators (TGF- β , IL-10, NO, etc.) (Figure 4B). Besides, MDSC polarization could affect a potency level of MDSC in AID prevention and/or therapy.

Several lines of evidence have proved the principle indicating a great potential of MDSC-based strategy for AID prevention in mouse models [9,10]. Clearly, immunotherapy with MDSC underscores the establishment of long-term immune tolerance before a complete destruction of remaining β -cells or β -cell replacement/regeneration in hosts, leading to the AID cure. However, such immunotherapy is a double-edged sword. On one hand, it can suppress aberrant autoimmunity. On the other hand, this therapy may increase the risk of infections and malignancy. Ideally, manipulating MDSC to establish antigen-specific immune tolerance can minimize the above risk, which was proven possible in the mouse model [9,10]. MDSC-based immunotherapy for AID from bench side to bed side needs to overcome several hurdles, i.e., reliable source of human MDSC, in vivo establishment of auto-antigen-specific immune tolerance by MDSC and re-establishment of MDSC induced immune tolerance after loss. Before fully exploiting MDSC for AID, more questions remain to be addressed, whether or not MDSC exert their action on macrophages, DC, B and NK cells in AID protection, the mechanism by which MDSC induce T_{reg} cells, whether MDSC are effective for AID therapy, cost-effective way of producing enough and safe MDSC for clinical trials, the relationship of MDSC polarization and AID prophylaxis/therapy and impact of MDSC on β -cell function.

Concluding Remarks

AID is an autoimmune endocrine disorder with premature death. Mounting data have clearly pointed to a critical role of MDSC in autoimmune diabetes. Although some advances have been made in



understanding MDSC development from expansion, activation to polarization stages in recent years, relatively little is known about the multi-stage process. Here, we brought up an evolving concept of the multiple signal model in regulating MDSC development. Moreover, the signaling cascades involving PIR and TGF-β receptors were discussed for the polarization of M-MDSC and G-MDSC, respectively. Control over this polarization might have an impact on the clinical potential of MDSC in AID therapy. A special emphasis was placed on recent progress in understanding the therapeutic potential and mechanism of action of MDSC in AID. A new view on MDSC-based interference with AID development was also discussed.

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