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Myeloid-derived suppressor cells are increased in frequency but not maximally suppressive in peripheral blood of Type 1 Diabetes Mellitus patients

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Abstract Type 1 Diabetes Mellitus (T1D) results from the destruction of insulin-producing beta cells in the pancreas by autoreactive T cells. Myeloid derived suppressor cells (MDSCs) are a recently identified immune cell subset that down-regulate T cells. Whether defects in MDSC numbers or function may contribute to T1D pathogenesis is not known. We report here that MDSCs are unexpectedly enriched in peripheral blood of both mice and patients with autoimmune diabetes. Peripheral blood MDSCs from T1D patients suppressed T cell proliferation in a contact-dependent manner; however, suppressive function could be enhanced with *in vitro* cytokine induction. These findings suggest that native T1D MDSCs are not maximally suppressive and that strategies to promote MDSC suppressive function may be effective in preventing or treating T1D.

1. Introduction

Autoimmune T cells mediate the destruction of pancreatic insulin-producing beta cells in the pancreas in Type 1 Diabetes Mellitus (T1D) [1]. Both $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells can transfer autoimmune diabetes to immunodeficient hosts in mouse models [2,3], and T cells are found in inflammatory infiltrates surrounding

Abbreviations: T1D, type 1 diabetes mellitus; MDSC, myeloid-derived suppressor cells; ROS, reactive oxygen species; NO, nitric oxide; PBMC, peripheral blood mononuclear cells; STZ, streptozotocin; LNMMA, N^G-monomethyl-L-arginine; MnTBAP, Mn(III) tetra (4-benzoic acid) porphyrin chloride.

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pancreatic islets in T1D patients [4]. Thus, strategies that down-regulate the autoimmune T cell response may prevent or reverse T1D.

An approach to dampening the autoimmune T cell response is to augment existing immune cell subsets that normally suppress T cell function. Myeloid-derived suppressor cells (MDSCs) are a recently described subset of bone marrow-derived cells that down-regulate T cell responses by a variety of mechanisms [5,6]. This heterogeneous group of cells consists of myeloid progenitor and immature myeloid cells, and can be identified by expression of CD11b and Gr-1 in mice and CD33 and CD11b in humans [6,7]. MDSCs can be further subdivided into monocytic and granulocytic populations with potentially distinct functions [6]. Although MDSC function has mostly been studied in the cancer setting, a role for MDSCs in autoimmunity and other inflammatory settings is now emerging [8–10].

In mouse models of autoimmune diabetes, recent evidence suggests that MDSCs in therapeutic settings can dampen diabetes development. Adoptive transfer of MDSCs derived from mice harboring allogeneic mammary tumor cells partially delays diabetes onset [11]. Additionally, in mice, expansion of MDSCs has been associated with anti-CD20 therapy, a B cell depletion strategy that prevents and reverses diabetes [12]. Although these results suggest that MDSCs may decrease autoimmunity in therapeutic settings, the population dynamics of native MDSCs during spontaneous diabetes development is not known. Furthermore, the role of MDSCs in autoimmunity has been studied almost exclusively in mice [13–15], and the potential role of MDSCs in human T1D has not been explored.

We show here that suppressive MDSCs accumulate in the peripheral blood of diabetic NOD mice and T1D patients. Although MDSCs from T1D patients suppressed T cell proliferation, cytokine induction of MDSCs increased the efficacy of T1D MDSC suppressive function. Suppression of T cells was contact dependent, but independent of reactive oxygen species (ROS) or nitric oxide (NO). Together, these findings suggest that T1D is associated with increased MDSC numbers, but that these MDSCs are not maximally suppressive. Thus, strategies such as cytokine induction that augment MDSC suppressive function may dampen T1D progression.

2. Materials and methods

2.1. Human subjects

Peripheral blood mononuclear cells (PBMCs) were obtained from subjects after written consent was obtained in accordance with the UNC Office of Human Research Ethics. Subject demographics are shown in Supplemental Table 1.

2.2. Mice

Mice were housed in AALAC approved sterile barrier facilities at the University of North Carolina at Chapel Hill's Animal Facility and in accordance with the UNC Office of Animal Care and Use Committee. C57BL/6 and NOD/LtJ (NOD) mice were purchased from The Jackson Laboratory. To induce diabetes, 12–14 week old female C57BL/6 mice were injected intraperitoneal with a single dose of STZ (Sigma) (150 mg/kg in citrate buffer, pH 4.5 [Friesen NT et al. 2004]). Control mice received only citrate buffer. Blood glucose levels were measured two days after injection, and diabetes was confirmed with two consecutive blood glucose readings of >250 mg/dL.

2.3. Flow cytometry

Flow cytometry antibodies: anti-mouse [FITC-Gr-1 (Ly6C/Ly6G), PE-Gr-1 (Ly6G), APC-Ly6C, APC-Cy7- CD11b] and anti-human [biotin-CD33, PeCy7-CD11b, PE-CD3, PE-CD19, PE-CD56 and PB-HLA-DR]. Isolation of human PBMCs was performed using a Ficoll-Hypaque density gradient prior to antibody staining. Isolation of mouse pancreatic islets was performed prior to antibody staining for intra-islet cells [16]. Data were acquired on a Beckman-Coulter (Dako) CyAn ADP and analyzed using Summit or Flowjo software. The proliferation index was calculated using the Flowjo software and is defined as the total number of divisions divided by the number of cells that went into division.

2.4. Isolation of mouse cell populations

Murine $CD11b^+$ cells were magnetically isolated from the spleens of diabetic mice using $CD11b^+$ magnetic microbeads (Miltenyi). The purity of $CD11b^+$ cells was >95%.

Murine $CD11b^+$ $Ly6C^+$ or $Ly6G^+$ cells were isolated by flow sorting. $CD11b^+$ cells isolated by magnetic bead separation were stained with anti-Ly6G and anti-Ly6C antibodies and $CD11b^+$ $Ly6G^-$ $Ly6C^+$ and $CD11b^+$ $Ly6G^+$ $Ly6C^-$ sorted on a Mo-Flo XDP cytometer™. The purity of $CD11b^+$ $Ly6G^-$ $Ly6C^+$ and $CD11b^+$ $Ly6G^+$ $Ly6C^-$ cell populations was >99%.

2.5. Isolation, expansion and purification of human MDSCs

Isolation of PBMCs was performed using a Ficoll-Hypaque density gradient. Isolated PBMCs were incubated for 7 days in GM-CSF (1 ng/mL) and IL-1β (1 ng/mL) to expand MDSC cells, as described [17]. Human $CD33^+$ cells were isolated with biotinylated anti-CD33 antibody and anti-biotin magnetic microbeads (Miltenyi) prior to use in suppression assays.

2.6. Cytochemical analysis

Sorted cells were collected onto Superfrost slide by cytospin and air-dried for 30 min. Cells were then Wright-Giemsa stained as described in [8].

2.7. In vitro suppression assay

Graded numbers of MDSCs were added to 2×10^5 CFSE labeled $CD3^+$ T cells stimulated for 24 h with anti-CD3 (4 μg/mL) (anti-human clone HIT3 or anti-mouse clone 23HC11 Ebioscience) and anti-CD28 (1 μg/mL) (anti-human clone CD28.2 and anti-mouse clone 37.51), and cultured in a flat-bottom 96-well plate at 2×10^5 cells per well. $CD3^+$ T cell cultures without MDSCs served as positive controls. In human studies, third party responders consisted of enriched $CD11b^-CD11c^-CD19^-CD3^+$ T cells from healthy donors. The level of proliferation was assessed by CFSE dilution after