

Myeloid-Derived Suppressor Cells Prevent Type 1 Diabetes in Murine Models

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Effective immunotherapy for type 1 diabetes (T1D) relies on active induction of peripheral tolerance. Myeloid-derived suppressor cells (MDSCs) play a critical role in suppressing immune responses in various pathologic settings via multiple mechanisms, including expansion of regulatory T cells (Tregs). In this study, we investigated whether MDSCs could act as APCs to induce expansion of Ag-specific Tregs, suppress T cell proliferation, and prevent autoimmune T1D development. We found that MDSC-mediated expansion of Tregs and T cell suppression required MHC-dependent Ag presentation. A murine T1D model was established in INS-HA/RAG^{-/-} mice in which animals received CD4-HA-TCR transgenic T cells via adoptive transfer. We found a significant reduction in the incidence of diabetes in recipients receiving MDSC plus HA, but not OVA peptide, leading to 75% diabetes-free mice among the treated animals. To test further whether MDSCs could prevent diabetes onset in NOD mice, nondiabetic NOD/SCID mice were injected with inflammatory T cells from diabetic NOD mice. MDSCs significantly prevented diabetes onset, and 60% of MDSC-treated mice remained diabetes free. The pancreata of treated mice showed significantly lower levels of lymphocyte infiltration in islet and less insulinitis compared with that of the control groups. The protective effects of MDSCs might be mediated by inducing anergy in autoreactive T cells and the development of CD4⁺CD25⁺Foxp3⁺ Tregs. This study demonstrates a remarkable capacity of transferred MDSCs to downregulate Ag-specific autoimmune responses and prevent diabetes onset, suggesting that MDSCs possess great potential as a novel cell-based tolerogenic therapy in the control of T1D and other autoimmune diseases. *The Journal of Immunology*, 2010, 185: 5828–5834.

Type 1 diabetes (T1D; diabetes mellitus) is an insulin-dependent disorder characterized by kidney failure, blindness, heart disease, and chronic ulcers (1). It is now clear that the chronic inflammatory response against specific autoantigens, mainly insulin, leads to the eventual destruction of insulin-producing endocrine β cells. Daily injection of insulin cannot match the naturally precise timing and dosing of insulin secretion of the pancreas in response to hyperglycemia. Even well-managed diabetic patients, therefore, can experience severe treatment side effects and worsening of their disease (2). More recently, a variety of strategies has been developed, aimed at re-establishing physiological insulin production in diabetic patients (3). Despite these advancements, devising a means capable of restoring self-tolerance or specifically down-modulating autoimmunity

remains a critical step toward preventing and/or reversing T1D. In this respect, regulatory T cells (Tregs) have received particular attention (4).

Myeloid-derived suppressor cells (MDSCs) represent a population of myeloid origin with immunoregulatory activity. These cells can function to suppress Ag-specific and nonspecific T cell responses via diverse mechanisms (5–9). Accumulating evidence has implicated a potential broad application of MDSCs as a novel cell-based immunotherapy within the fields of transplantation and autoimmune diseases (10–12). To date, transfer of MDSCs has been shown to be capable of inducing immune tolerance in allogeneic bone marrow transplantation (13), prolonging the survival of allo-skin transplants via inhibitory receptor Ig-like transcript 2 mediated tolerance (14), participating in anti-CD28-mediated tolerance in allo-kidney transplantation (15), and amelioration of symptoms in the inflammatory bowel disease model (10). In this study, we have shown that adoptive transfer of CD115⁺Gr-1⁺ MDSCs plus hemagglutinin (HA) peptides efficiently prevents the onset of HA-specific TCR T cell-induced diabetes in INS-HA/RAG^{-/-} recipient mice. Further, MDSCs prevented diabetes onset in NOD/SCID mice and maintained these mice diabetes-free for the long term.

Materials and Methods

Mice

CD4-HA-TCR transgenic mice (BALB/c, H-2^d), a gift from Dr. Constantine A. Bona (Mount Sinai School of Medicine, New York, NY), express the 14.3.d HA-specific TCR, which recognizes the influenza hemagglutinin (HA, 110–120) of A/PR/8/34 influenza virus in association with I-E^d. INS-HA/RAG^{-/-} mice (B10.D2, H-2^d) express the HA protein in pancreatic β cells under the control of the rat insulin promoter. MHC class I KO, MHC class II KO, and actin-OVA transgenic mice were purchased from

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Abbreviations used in this paper: HA, hemagglutinin; MDSC, myeloid-derived suppressor cell; T1D, type 1 diabetes; Treg, regulatory T cell; WT, wild-type.

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The Jackson Laboratory (Bar Harbor, ME). All animal experiments were performed in accordance with the animal guidelines of the Mount Sinai School of Medicine.

Peptide and Abs

HA peptide (110 SFERFEIFPK 120) and OVA peptide (323 ISQAVHAA-HAEINEAGR 339) were purchased from Washington Biotechnology (Baltimore, MD). All fluorescence-conjugated monoclonal Abs were purchased from eBioscience (San Diego, CA).

Isolation of MDSCs

BALB/c mice bearing syngeneic colon cancer MCA26 and C57BL/6 mice bearing syngeneic Lewis lung carcinoma were used as the source of MDSCs. The detailed Percoll protocol for MDSC isolation has been described previously (6). The fraction II Gr-1 $^{+}$ CD115 $^{+}$ MDSCs were sorted by flow cytometry, and cells with a purity of 98% were used in the INS-HA diabetic model.

Treg expansion and suppression of CD4 $^{+}$ CD25 $^{-}$ T cell proliferation by MDSCs

CFSE-labeled, purified CD4 $^{+}$ CD25 $^{+}$ Tregs and CD4 $^{+}$ CD25 $^{-}$ T cells from naive CD4-HA-TCR transgenic mice (BALB/c background) or CD4 OVA-specific TCR transgenic (OT-II) mice (B6 background) were cocultured with MDSCs, at a 4:1 ratio, in the presence of HA or OVA peptides (0.5 μ g/ml) and IL-2 (100 U/ml) for 4 d. Proliferation was assessed by flow cytometry (CFSE dilution) using fluorochrome-labeled Abs against CD4, CD25, and Foxp3 or isotype controls.

Diabetes model

CD4-HA-TCR transgenic T cells were enriched by T cell enrichment columns (R&D Systems, Minneapolis, MN) and adoptively transferred into INS-HA/RAG $^{-/-}$ mice by tail vein injection (1×10^5 per mouse). Mouse glucose levels were measured daily using a blood glucose meter (Bayer, Pittsburgh, PA) to determine the onset of diabetes. Mice were considered diabetic when their blood glucose level was ≥ 200 mg/dl for two consecutive measurements. Sorted MDSCs (5×10^6 cell/mouse) were transferred twice, on the first and fourth days after T cell transfer.

NOD/SCID diabetic model

CD4 $^{+}$ T cells were purified from the spleens of diabetic NOD females using a CD4 isolation kit. To generate MDSCs, mouse mammary tumor TSA cells (10^6) were implanted into the fat pad of NOD/SCID mice. Two to three weeks later, MDSCs (CD11b $^{+}$ Gr-1 $^{+}$ cells) were positively selected from bone marrow using Ab-conjugated Miltenyi beads (Miltenyi, Auburn, CA). Female NOD/SCID mice aged 6 wk received a tail vein injection of PBS, CD4 $^{+}$ T cells from diabetic NOD mice (2×10^7), and control CD4 $^{+}$ T cells from normal mice plus a single various dose response of MDSCs (0, or 0.5, or 1, or 2×10^7).

Using an Elite glucometer (Bayer), fasting blood glucose concentrations were measured daily starting postinjection (days 0 to 100). Mice with fasting blood glucose concentrations over 200 mg/dl for two consecutive measurements were considered diabetic. To determine the severity of insulinitis, pancreata were examined by H&E staining. At least 10 islets per pancreas were examined to assess the degree of insulinitis. The severity of insulinitis was scored as follows: 0, normal; 1, leukocyte infiltration around islets; 2, <50% leukocyte infiltration; and 3, >50% leukocyte infiltration using published criteria (16).

Histopathological analysis

Pancreatic tissue was fixed in 10% buffered formalin solution and embedded in paraffin. Prepared sections were stained by Mayer's H&E. For each organ, 10 sections were analyzed. Staining for intracellular insulin was performed using polyclonal rabbit anti-insulin (Santa Cruz Biotechnology, Santa Cruz, CA) in conjunction with HRP-goat-anti-rabbit conjugate (Southern Biotechnologies, Birmingham, AL). Additional tissue samples were frozen at -80°C , and frozen sections were cut in stepwise fashion (8 μ m per section). Staining for CD4 T cells in islets was performed using monoclonal anti-CD4 (eBioscience) in conjunction with HRP-goat-anti-mouse conjugate (Southern Biotechnologies).

Cytokine detection by ELISA

The concentrations of mouse IFN- γ , IL-10, and TGF- β in the culture supernatants were measured by ELISA (R&D Systems).

Suppression/proliferation assay

Column-enriched Thy-1 $^{+}$ T cells (1×10^5) from TCR transgenic mice or treated mice were cocultured with HA peptide (5 μ g/ml) alone or in the presence of escalating numbers of CD25 $^{+}$ T cells recovered from treated nondiabetic mice in 96-well microplates. Irradiated (2500 rad) naive splenocytes (5×10^4) were used as APCs. [^3H]Thymidine was added during the last 8 h of 72 h culture.

Quantitative real-time PCR

Total RNA was extracted using TRIzol reagent (Invitrogen). The reverse transcription-PCR (RT-PCR) procedure (One-step RT-PCR kit; Qiagen, Valencia, CA) using primers for Foxp3 and for GAPDH (internal control) has been previously described (9). For quantitative real-time PCR, reverse-transcribed cDNA was amplified by real-time PCR with SYBR Green Universal PCR Mastermix (Bio-Rad, Richmond, CA). Samples were analyzed in duplicate with the IQ-Cycler (Bio-Rad). The normalized signal level was calculated based on the ratio to the corresponding GAPDH signal.

Results

MDSCs can act as APCs for Treg expansion and suppress effector T cell proliferation in vitro

Our laboratory and others have found that MDSCs from tumor-bearing mice can suppress T cell proliferation and induce Treg expansion (5–8, 9, 17). We have reported that MDSC-mediated Treg activation requires the presence of IFN- γ and IL-10 (6). We then studied whether MDSCs can act as APCs to mediate Treg activation and, therefore, lead to the development of a better strategy for cell-based tolerogenic therapy using MDSCs.

To determine whether MDSC-mediated Treg expansion was Ag-dependent and MHC-restricted, CD4 $^{+}$ CD25 $^{+}$ splenic T cells purified from naive OT-II mice were labeled with CFSE and cocultured with wild-type (WT) MDSCs or MHC class I or II KO MDSCs in the presence or absence of OVA peptides, or with MDSCs isolated from tumor-bearing, constitutive actin promoter-driven, OVA-expressing, transgenic mice. Based on FACS analysis for the presence of CFSE-labeled cells (Fig. 1A) on four reproducible and separate experiments, we observed a significantly higher level of CD4 $^{+}$ CD25 $^{+}$ Foxp3 $^{+}$ Treg expansion (i.e., CFSE dilution) in the presence of WT MDSCs and OVA peptide ($80.1 \pm 7.2\%$; Fig. 1B) than in the presence of WT MDSCs alone ($6.2 \pm 4.5\%$) or OVA peptide alone ($46.1 \pm 4.7\%$) (Fig. 1B; *t* test, $p < 0.001$). Treg expansion was similarly mediated by actin-OVA-expressing MDSCs ($67.4 \pm 2.1\%$; $p < 0.001$), which contain endogenous OVA. Furthermore, Treg expansion in the presence of MHC class II KO MDSCs and OVA peptide ($46.9 \pm 6.9\%$) was significantly decreased to levels similar to those observed when cultured in the presence of OVA peptide alone. However, even when cultured with MHC class I KO MDSCs in the presence of OVA peptide, Treg expansion was not significantly decreased ($77.0 \pm 10.0\%$; $p < 0.001$). Taken together, these data suggest that MDSC-mediated Treg expansion is Ag-specific and MHC class II dependent.

We next asked whether MDSC-mediated suppression of naive CD4 $^{+}$ CD25 $^{-}$ OT-II TCR transgenic T cell proliferation was Ag- and/or MHC-restricted. Using the same experimental design described in Fig. 1, we found that suppression of CD4 $^{+}$ CD25 $^{-}$ T cell proliferation was both Ag and MHC class II dependent. We again performed FACS analysis for CFSE-labeled cells (Fig. 2A) in four separate and reproducible experiments and found a significant proportion of CD4 $^{+}$ CD25 $^{-}$ OT-II T cells proliferated in the presence of OVA peptide and irradiated naive splenocytes ($53.5 \pm 5.4\%$) (CT, Fig. 2B). In the presence of WT MDSCs and OVA peptide or actin-OVA MDSCs, the proliferation of CD4 $^{+}$ CD25 $^{-}$ OT-II T cells was significantly suppressed ($9.4 \pm 2.6\%$ and $18.4 \pm 5.0\%$; $p < 0.001$, compared with control splenocytes with OVA

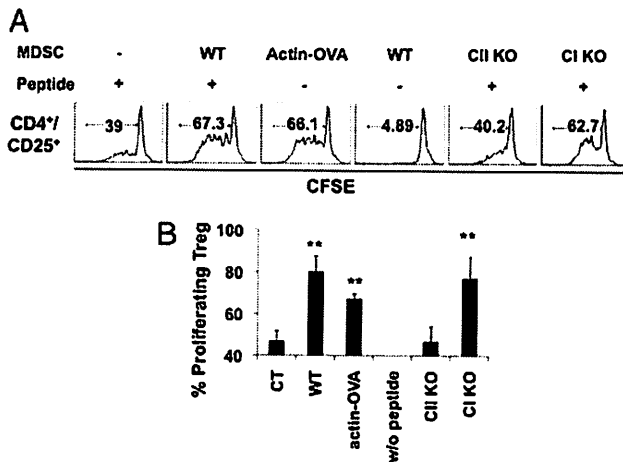


FIGURE 1. MDSC-mediated Treg expansion requires MHC class II-restricted Ag presentation. MDSCs were isolated from tumor-bearing WT, actin-OVA transgenic, MHC class I KO, or MHC class II KO mice. Purified CD4⁺CD25⁺ Tregs from naive OT-II transgenic mice were labeled with CFSE and cocultured with MDSCs at a 4:1 ratio for 4 d in the presence or absence of exogenously added OVA peptides. Subsequently, the cells were stained with fluorochrome-conjugated Abs against CD4, CD25, and Foxp3 or corresponding isotype controls. Proliferation (as demonstrated by dilution of the CFSE signal) of CD4⁺CD25⁺Foxp3⁺ Tregs was assessed by flow cytometric analysis. *A*, Histograms of CFSE-positive cells gated on CD4⁺CD25⁺Foxp3⁺ cells from one of four repeated experiments. *B*, Statistical analysis of the data from four repeated experiments was performed. Results presented are the average percentage of proliferating CD4⁺CD25⁺Foxp3⁺ Tregs from four experiments \pm SD. ** $p < 0.001$ compared with the control group (i.e., OVA peptide without MDSCs).

peptide 53.5%) (Fig. 2*B*). MHC class II-deficient MDSCs lost their suppressive activity, whereas MHC class I-deficient MDSCs remained suppressive (percentage proliferating CD4⁺CD25⁺ OT-II T cells: 67.2 \pm 16.0% versus 17.5 \pm 5.5%).

Collectively, these data indicate that both MDSC-mediated suppression of T cell proliferation and Treg expansion are Ag-dependent and MHC class II-restricted processes.

MDSCs prevent the onset of autoreactive T cell-mediated diabetes

Evaluation of autoimmune responses against natural autoantigens has been impeded due to the polyclonal nature of autoreactive T cells and the lack of traceable markers for *in vivo* studies. Moreover, the results depicted in Figs. 1 and 2 indicate that both MDSC-mediated suppression of T cell proliferation and Treg expansion were both Ag dependent and MHC class II restricted. Therefore, we chose a well-characterized T1D model (18) in which a surrogate autoantigen and Ag-specific TCR transgenic T cells were used to induce diabetes. In this model, T cells isolated from the CD4-HA-TCR transgenic mice were adoptively transferred into INS-HA/RAG^{-/-} mice. For the proof-of-principle study, we used MDSCs isolated from tumor-bearing mice and HA peptide as autoantigen to induce suppression of autoreactive T cells and expansion of autoreactive Tregs.

Consistent with previous reports (18), the onset of diabetes, as determined by high blood glucose levels (≥ 200 mg/dl), was readily detectable 7–10 d after T cell injection. This was true for all treated mice that received MDSCs plus OVA, control cells from Fr.3 cells plus HA, or PBS. However, the incidence of disease was drastically decreased in mice receiving MDSCs plus HA peptides, with 75% of all treated mice remaining diabetes free (Fig. 3; $p < 0.005$). These data strongly imply that transfer of

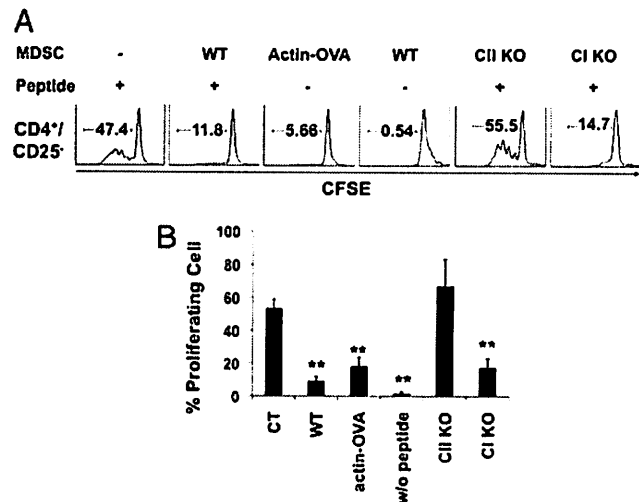


FIGURE 2. MDSC-mediated suppression of CD4⁺CD25⁻ T cell proliferation requires MHC class II-restricted Ag presentation. MDSCs were isolated from tumor-bearing WT, actin-OVA transgenic, MHC class I KO, or MHC class II KO mice. Purified CD4⁺CD25⁻ naive T cells from OT-II transgenic mice were labeled with CFSE and cocultured with MDSCs at a 4:1 ratio or with irradiated naive splenocytes (as APCs) at a 10:1 ratio for 4 d in the presence or absence of exogenously added OVA peptides. Proliferation (CFSE dilution) of CD4⁺CD25⁻ OT-II cells was assessed by flow cytometry. *A*, Histograms of CFSE-labeled cells in the population of CD4⁺CD25⁻ cells from one of the four repeated experiments. *B*, Statistical analysis of the data from four repeated experiments was performed. Results presented are the average percentage of proliferating CD4⁺CD25⁻ T cells \pm SD from four experiments. ** $p < 0.001$ compared with the control group (i.e., OVA peptide without MDSCs).

MDSCs plus HA peptide can effectively suppress the HA-specific T cell-mediated immune response against pancreatic islet cells expressing HA and, thus, prevent the development of diabetes in INS-HA/RAG^{-/-} recipient mice.

MDSCs inhibit lymphocyte infiltration and insulinitis

Lymphocyte infiltration and the resultant inflammation is a hallmark feature of diabetes. To better understand the effect of MDSCs on the suppression of diabetes development, we examined the histological changes in the pancreata of treated mice. H&E staining

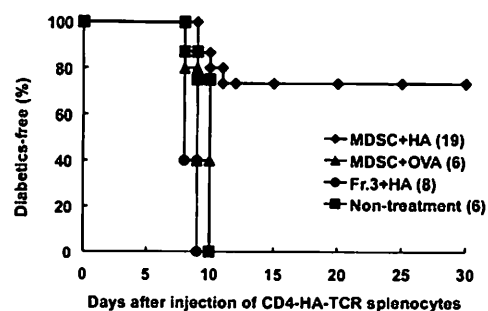


FIGURE 3. Transfer of MDSCs plus autoantigen prevents diabetes onset in treated mice. INS-HA/RAG^{-/-} mice were injected with 1×10^5 CD4-HA-TCR T cells via the tail vein (day 0). Twenty hours after T cell transfer, recipients were injected with MDSCs (5×10^6 /mouse) plus HA ($5 \mu\text{g}/\text{mouse}$, $n = 19$) or OVA peptide ($n = 6$), control Fr. 3 cells with HA peptide ($n = 8$), or PBS (nontreatment, $n = 6$). A repeat injection of the same treatment was given on day 4. Blood glucose levels were measured by blood glucose meter. The data shown are combined from three separate experiments with reproducible results.

indicated those recipients of MDSCs plus HA peptide had intact islets, and the severity of insulinitis in these mice was markedly reduced (Fig. 4, upper left panel). In sharp contrast, examination of nontreated (PBS) mice showed massive peri-islet infiltration of mononuclear cells (Fig. 4, upper right panel). These observations were further verified by assessing CD4 T lymphocyte infiltration. MDSC plus HA peptide recipients showed only minimal staining (Fig. 4, middle left panel), whereas the islets recovered from PBS-treated mice contained large numbers of CD4 T cells (Fig. 4, middle right panel). Furthermore, immunohistochemical staining for insulin revealed strong insulin expression in the intact islets of mice treated with MDSCs plus HA (Fig. 4, lower left panel). On the contrary, insulin production was almost absent from the pancreata of mice that received transferred T cells and PBS (Fig. 4, lower right panel). These results indicate that transfer of MDSCs effectively inhibits the infiltration of T lymphocytes into islets, thus avoiding the harmful inflammatory response against islet cells and destruction of insulin-producing β cells.

MDSCs mediate T cell tolerance

We postulated that the profound suppression of T cell infiltration into the islets of treated mice likely resulted from potent induction of T cell tolerance by MDSCs. To test this idea, we reisolated autoreactive CD4-HA-TCR T cells from treated mice and examined their ability to proliferate in vitro. As shown in Fig. 5A, T cells recovered from mice receiving MDSCs + OVA, control cells from Fr.3 + HA, or PBS proliferated vigorously upon restimulation with HA peptide. In the group treated with MDSCs + HA, T cells isolated from clinically diabetic mice still proliferated to some extent, but those from diabetes-free mice exhibited barely detectable proliferative potential, similar to that of naive mice (without HA-CD4 T cell transfer). The inability of T cells to proliferate in response to specific Ag stimulation suggests that an anergic state was actively induced in the autoreactive T cells from the mice treated with MDSCs + HA.

We also assessed cytokine expression in the culture supernatants harvested from the above T cell proliferation assays. Strikingly,

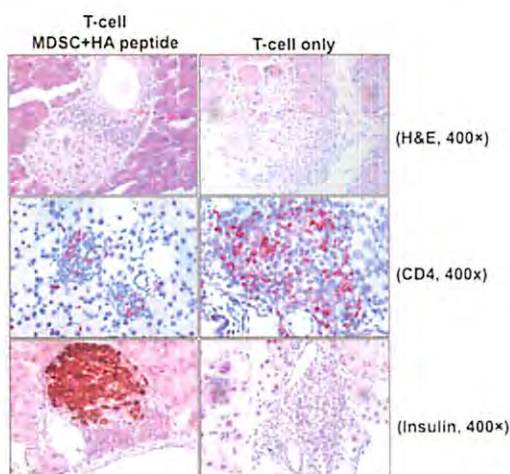


FIGURE 4. Lymphocyte infiltration, islets, and insulin expression in mice treated with MDSCs plus HA peptides. Serial sections of pancreas were prepared from treated mice at 4 wk after cell transfer. Sections were stained by H&E (upper panels; original magnification $\times 400$) and immunohistochemically stained with anti-CD4 (middle panels; original magnification $\times 400$) or rabbit polyclonal anti-insulin (lower panels; original magnification $\times 400$). Left panels, Representative photomicrographs from MDSC + HA peptide-treated diabetes-free mice. Right panels, Representative photomicrographs from PBS-treated diabetic mice.

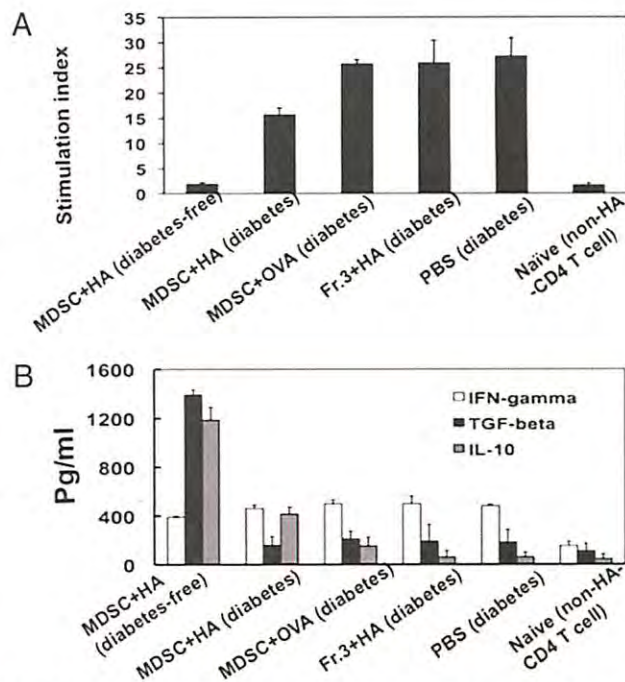


FIGURE 5. Proliferation and production of inhibitory cytokines by autoreactive T cells recovered from treated diabetes-free mice. *A*, A comparison of the proliferative capacity of T cells recovered from the different groups at 30 d posttreatment. Purified T cells and irradiated naive splenocytes (as APCs) were cocultured in the presence of HA peptide ($5 \mu\text{g/ml}$). [^3H]Thymidine ($1 \mu\text{Ci/well}$) was added for the last 8 h of a 72 h culture. Stimulation index is calculated as cpm in the presence of HA divided by cpm in the absence of HA ($n = 3$ replicates). *B*, Cytokine production by autoreactive T cells. Cytokine concentrations in the supernatants, harvested from the same cultures as described in *A*, were measured by ELISA ($n = 3$ replicates).

although there was no substantial difference in IFN- γ production between all HA-CD4 T cell transferred groups, large amounts of IL-10 and TGF- β were detected only in samples from diabetes-free mice treated with MDSCs + HA (Fig. 5B; $p < 0.001$). This indicates that regulatory, rather than inflammatory, cytokines were generated in vivo in the presence of MDSCs, and, hence, these factors may play a critical role in the induction of T cell tolerance by MDSCs.

MDSCs induce Treg development

Previous studies from our group and others have demonstrated that MDSCs exhibit an exceptional ability to activate and expand Tregs (6, 17), a cell type shown to be a key component in the regulation of T1D and other autoimmune responses. To assess the potential involvement of Tregs in the control of T1D by MDSCs, Treg development in secondary lymphoid organs (i.e., spleen) was evaluated. As shown in Fig. 6A, T cells recovered from diabetes-free mice receiving MDSCs and HA peptide contained a significantly higher percentage (35.5%) of Foxp3 $^+$ CD4 $^+$ CD25 $^+$ T cells compared with that of T cells recovered from diabetic mice (15.0%) receiving the same treatment or control treatments. We further confirmed these findings by examining Foxp3 gene expression by both RT-PCR (Fig. 6B, upper panel) and real-time RT-PCR (Fig. 6B, lower panel), which demonstrated that the highest level of Foxp3 gene expression was detected in non-diabetic mice that received MDSC and HA treatment (lane 1). These results suggest that transfer of HA peptide pulsed MDSCs induced a considerable proportion of autoreactive T cells to become Tregs.

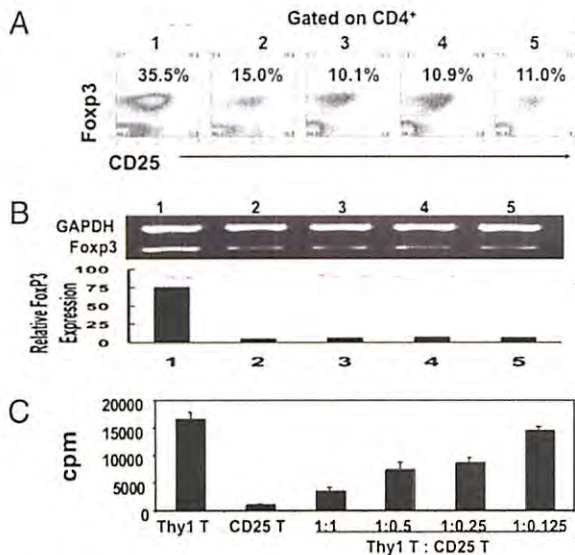


FIGURE 6. Increased percentage of Tregs in diabetes-free mice treated with MDSC plus HA peptide. *A*, Frequency of CD25⁺Fopx3⁺ cells within the gated splenic CD4⁺ T cell population. Splenocytes recovered from various treatment groups were analyzed for the presence of Tregs by intracellular staining for Fopx3 at day 30 after treatment. *B*, Fopx3 gene expression in total recovered splenic T cells. Total RNA was extracted from splenic T cells from the different groups, and the Fopx3 mRNA level was assessed by RT-PCR (upper panel) or real-time PCR (lower panel). Serial numbers in *A* and *B*: 1, nondiabetic mice receiving MDSCs + HA; 2, diabetic mice receiving MDSCs + HA; 3, mice receiving MDSCs + OVA; 4, mice receiving control Fr.3 cells + HA; 5, mice receiving PBS. *C*, Suppressive activity of MDSC-induced Tregs. CD25⁺ T cells isolated from diabetes-free mice that had received MDSCs + HA and Thy-1⁺ T cells purified from naive CD4-HA-TCR transgenic mice were cultured, alone or in the presence of HA peptide (5 μ g/ml), with irradiated splenocytes as APCs at various ratios. [³H]Thymidine (1 μ Ci/well) was added for the last 8 h of the 72 h culture.

We then aimed to test whether MDSC-induced Tregs possess suppressive activity. T cells purified from naive CD4-HA-TCR transgenic mice and CD25⁺ T cells isolated from diabetes-free

mice receiving MDSC plus HA were cultured alone or in the presence of HA peptide at various ratios. The results indicate that the CD25⁺ T cells from nondiabetic mice mediated potent suppression of Ag-specific T cell proliferation in a dose-dependent manner (Fig. 6C).

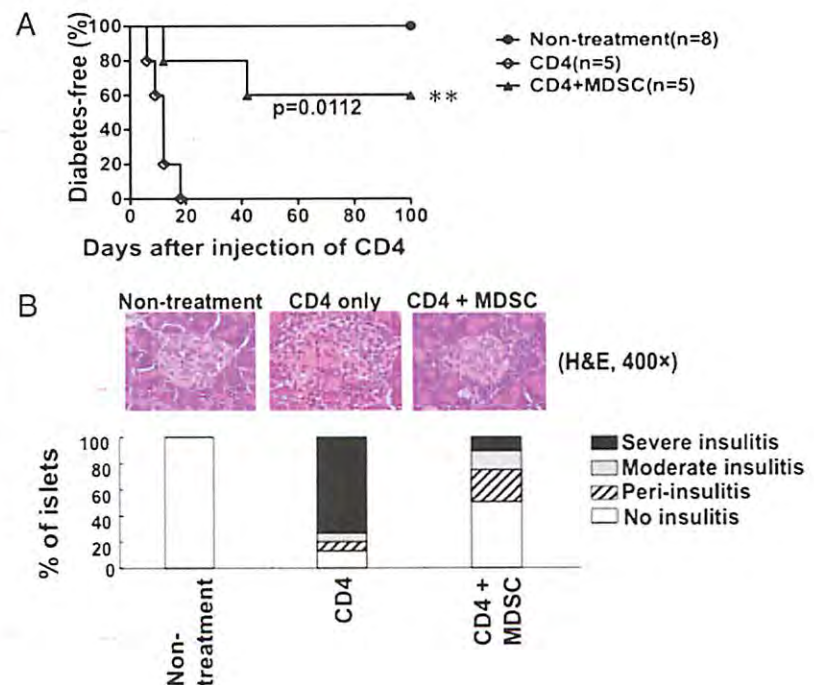
Effects of MDSCs on the development of diabetes in NOD/SCID mice

To test whether MDSCs could affect the development of T1D in another murine model, NOD/SCID mice were adoptively transferred with PBS, splenic CD4⁺ T cells isolated from NOD diabetic mice, or splenic CD4⁺ T cells from NOD diabetic mice along with MDSCs. NOD/SCID mice treated with PBS did not develop diabetes (nontreatment, Fig. 7A). In sharp contrast, NOD/SCID mice, which received autoreactive CD4⁺ T cells from diabetic NOD mice, did become diabetic around day 10, as evidenced by fasting blood glucose levels over 200 mg/dl (CD4, Fig. 7A). However, a single adoptive transfer of MDSCs effectively decreased the rate of diabetes development (CD4 + MDSC, Fig. 7A), and 60% of the animals remained diabetes-free as long as 100 d posttreatment. We also assessed the extent of leukocyte infiltration, a hallmark of insulinitis, in the above mice at 15 d post-injection. We consistently found that adoptive transfer of CD4⁺ T cells, versus PBS control transfer, significantly increased the extent of leukocyte infiltration into the pancreata of NOD/SCID mice (nontreatment versus CD4, Fig. 7B). In contrast, inclusion of MDSCs remarkably decreased the extent of leukocyte infiltration (Fig. 7B). These findings regarding dysregulated glucose and insulinitis suggest that MDSCs can suppress autoreactive T cells and thereby prevent T1D in the NOD system. As shown in Supplemental Fig. 1, this protective effect was maintained even at lower MDSC doses.

Discussion

In recent years, MDSCs have garnered increased interest. Previous studies have shown that MDSCs may be involved in down-modulating alloimmune responses in a variety of allogeneic transplant models (11–15). In this study we provide firm evidence

FIGURE 7. MDSCs suppress the diabetogenic effect of autoreactive CD4⁺ T cells in NOD/SCID mice. *A*, NOD/SCID mice received a dose of PBS (nontreatment), autoreactive total CD4⁺ T cells, 2×10^7 (CD4) from NOD diabetic mice, and autoreactive CD4⁺ T cells plus MDSCs, 2×10^7 (CD4 + MDSC). Fasting blood glucose levels were monitored from days 0 to 100. Number of mice in each group is indicated in parentheses. In the diabetes incidence experiment of *A*, comparisons were made between several groups, including nontreatment versus CD4 T cell alone ($p = 0.00015$), CD4 alone versus CD4 + MDSC ($p = 0.0112$), and nontreatment versus CD4 + MDSC ($p = 0.0584$). *B*, Representative mice from the same treatment groups were sacrificed on day 15. Pancreatic tissue was stained with H&E. Representative images of the pancreatic slides are shown (upper panel; original magnification $\times 400$). The severity of insulinitis in the pancreata of each group was microscopically assessed and scored as no insulinitis (clear bars), peri-insulinitis (hatched), moderate insulinitis (<50% leukocyte infiltration, gray), and severe insulinitis (>50% leukocyte infiltration, black) (lower panel).



that in vitro MDSC-mediated suppression of CD4⁺CD25⁻ T cell proliferation and Treg expansion were Ag-dependent, MHC class II-restricted processes, and that MDSCs were effective at perturbing the autoimmune response in the two diabetic animal models tested. Using a transgenic murine model of autoimmune diabetes, we demonstrated that injection of CD115⁺Gr-1⁺ MDSCs and HA peptide, a surrogate autoantigen, could efficiently protect INS-HA/RAG^{-/-} mice from HA-specific TCR T cell-mediated diabetes in an Ag-dependent fashion. In addition, we observed markedly reduced lymphocyte infiltration and insulinitis in the islets of the MDSC-treated mice. We also confirmed the ability of MDSCs to prevent the natural autoimmune T cell response in NOD/SCID diabetic mice. These findings suggest that MDSCs may have great translational implications for treating T1D and other autoimmune diseases.

The protective effects afforded by MDSC treatment include hypoproliferation of autoreactive T cells and dramatically decreased infiltration of T lymphocytes into pancreatic islets in the diabetes-free treated mice. The latter likely results from an early inhibition of HA-specific T cell expansion in secondary lymphoid organs, as well as specific target organs (pancreas). This inference is supported by previous studies documenting a profound suppression of activated T cell proliferation by MDSC transfer in both tumor (6, 19) and allogeneic bone marrow transplantation models (13). Given the fact that peri-islet infiltration occurs prior to β cell destruction, as observed in NOD mice and TCR-transgenic models (20), the suppression of autoreactive T cell responses by transferred MDSCs may account for the suppression of the autoimmune response and prevention of T1D onset.

Tregs are widely recognized as crucial players in the development of T1D. Previous reports have shown that defects in the frequency and/or function of Tregs are closely associated with the onset or progression of T1D in both NOD mice (21–23) and human patients with T1D (24, 25). Moreover, Treg-based therapies have been under extensive investigation. Two primary strategies for the therapeutic application of Tregs include in vivo induction (26, 27) and in vitro expansion (28, 29). Compared with the currently available approaches, MDSCs, which can be efficiently derived from embryonic and hematopoietic stem cells in vitro (13), may offer a unique opportunity for specific immune intervention in T1D and other autoimmune diseases by inducing Ag-specific Tregs, which are able to suppress directly autoreactive T cells.

Most interestingly, using the NOD/SCID model, we have demonstrated that a one-time injection of MDSCs can provide long-term protection against the onset of T1D. We and others have shown that monocytic MDSCs may play a key role in preventing graft-versus-host disease in allo-organ transplantation (12, 13). Using our Gr-1⁺CD11b⁺ MDSC population, we observed a significant treatment effect even at doses as low as 1×10^7 /mouse and with only a diminutive monocytic population (4–6%; Supplemental Fig. 2). We continue to investigate the relative potency of monocytic versus granulocytic MDSCs. Quantifying the relative abilities of monocytic and granulocytic MDSCs to provoke immune suppression and give rise to Ag-specific immune tolerance is an area of continued investigation.

In conclusion, our findings clearly indicate that transfer of MDSCs conferred an exceptional capability to establish immune tolerance to self-Ag and protect against the onset of autoimmune T1D.

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Disclosures

The authors have no financial conflicts of interest.

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