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Apoptotic Non- β Cells Suppress β Cell Antigen-Reactive T Cells and Induce β Cell Antigen-Specific Regulatory T Cells: Transfusion of Apoptotic Splenic Stromal Cells Prevents Type 1 Diabetes

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Abstract

Steady-state cell apoptosis plays an important role in maintenance of self-tolerance. Based on this notion, the use of apoptotic cells to restore self-tolerance to β cell antigens is a rational approach to type 1 diabetes (T1D) prevention. Our previous study demonstrated that transfusion of apoptotic β cells induced immune tolerance to β cell antigens in NOD mice. However, concerned about the limited β cell source for future clinical applications, we attempted in the present study to develop a more practical approach for T1D prevention using apoptotic non- β cells. We found that UVB-irradiation-induced apoptotic NOD splenic stromal cells significantly suppressed β cell antigen-specific T cell proliferation *in vitro* and *in vivo*. Furthermore, TCR-transgenic CD4⁺ T cells primed by the antigens to which they were specific in the presence of UVB-irradiated stromal cells were rendered unresponsive to the antigen restimulation, a result that was partially attributed to the induced IL-10-producing regulatory T cells. Of more interest, transfusion of UVB-irradiated stromal cells appeared to induce β cell antigen-responding IL-10-producing regulatory T cells *in vivo*. Most importantly, transfusion of UVB-irradiated stromal cells effectively prevented T1D in NOD mice, which is consistent with these findings. This study suggests that it is possible to use apoptotic non- β cells such as peripheral blood mononuclear cells to induce β cell antigen-specific tolerance, thereby preventing T1D in humans.

Keywords

type 1 diabetes; apoptosis; immune tolerance; regulatory T cells

Introduction

Type 1 diabetes (T1D) is an autoimmune disease mediated by β cell antigen-specific T cells.¹ The breakdown of T cell self-tolerance is a major cause.² Therefore, with regard to T1D prevention, it is always ideal and highly desirable to restore self-tolerance. On the basis of the notion that steady-state cell apoptosis is associated with maintenance of self-tolerance,³ and the evidence that induction of limited β cell apoptosis *in vivo* prevents T1D in young NOD mice,⁴ we effectively prevented T1D in NOD mice using *ex vivo*-induced apoptotic β cells by

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Conflicts of Interest

The authors declare no conflicts of interest.

inducing β cell antigen-specific tolerance.⁵ Nevertheless, because of the limited β cell source for future clinical applications, it is necessary to develop more practical apoptotic cell-based approaches for T1D prevention.

It is reported that infusion of apoptotic splenocytes along with administration of an antigen can induce this antigen-specific tolerance.⁶ Thus, it is possible that infusion of apoptotic non- β cells, such as splenocytes or peripheral blood mononuclear cells, would induce β cell antigen-specific immune tolerance in the case of endogenous exposure of β cell antigens during autoimmunity in NOD mice. In the present study, we demonstrated that UVB-irradiation-induced apoptotic NOD splenic stromal cells remarkably suppressed β cell antigen-specific T cells and substantially prevented T1D in NOD mice by inducing IL-10-producing regulatory T cells.

Materials and Methods

Mice

Female NOD mice were purchased from Jackson Laboratory. NOD.BDC2.5 breeders were kindly provided by Dr. David Serreze (Jackson Laboratory). All the mice were kept in our department SPF mouse colony. All animal studies were approved by IACUC of the University of Florida.

***In Vitro* Assay for UVB-Irradiated Stroma-Induced β Cell Antigen-Specific T Cell Suppression**—CD4⁺ T cells (1×10^5) were stimulated for 4 days with purified NOD splenic dendritic cells (DCs) (1×10^4) pulsed with mimotope peptide 1040–55⁷ in the presence of different doses of UVB-irradiated stromal cells (1×10^5 , 5×10^4 , 2.5×10^4 , 1.25×10^4 , 6.25×10^3 , 0). Then, 1 μ Ci [³H]-thymidine was added to the cultures and incubated for an additional 16 hours. The incorporation of [³H]-thymidine was measured by liquid scintillation counting.

***In Vivo* Assay of Suppression of β Cell Antigen-Specific T Cells Induced by UVB-Irradiated Stroma**—Eight-week-old NOD mice received intravenous injection of 1×10^7 CFSE-labeled BDC2.5 splenocytes along with 1×10^6 UVB-irradiated stromal cells or PBS. Four days after the injection, the mice were killed and the proliferation of CFSE⁺ CD4⁺ T cells was examined by flow cytometry.

Prevention of T1D—Eight-week-old female NOD mice were treated with three weekly transfusions of UVB-irradiated stroma or PBS. All the mice were then monitored for diabetes by weekly measurement of urine glucose levels. Once urine glucose became positive, the positive mice were followed up daily by measuring blood glucose levels. Diabetes was diagnosed when blood glucose levels were greater than 250 mg/dL for two consecutive days. All the mice were followed up until they were 28 weeks of age. The comparison between the two groups was conducted using the log-rank test.

***In Vivo* Assessment of β Cell Antigen-Specific Tolerance Induced by UVB-Irradiated Stroma**—These experiments were performed according to the method described in our previously published study.⁵

Results and Discussion

First, we tested the effect of UVB-irradiated stromal cells on β cell antigen-specific T cells. We isolated CD4⁺ T cells from NOD.BDC2.5 mice with transgenic TCR on CD4⁺ T cells recognizing a β antigen. The mimotopes for this transgenic TCR have been described,⁷ such as the peptide 1040–55 utilized in this study. We found that the proliferation of CD4⁺ T cells stimulated by NOD splenic DCs pulsed with 1040–55 peptides was markedly suppressed by

UVB-irradiated stroma. High doses of UVB-irradiated stroma almost completely shut off the T cell proliferation. Of interest, the presence of UVB-irradiated stroma did not affect the short-term activation of T cells because 24 hours after incubation, the T cell activation marker CD69 and CD25 had similar upregulation with or without UVB-irradiated stroma. This finding suggests that T cell growth is arrested after activation in the presence of apoptotic stromal cells. This state may facilitate the formation of immune tolerance.⁸

Indeed, our further studies demonstrated that in the presence of UVB-irradiated stroma, β cell antigen-specific TCR-transgenic CD4⁺ T cells primed with β cell antigens were induced with immune tolerance to these β antigens, showing that the induced tolerant T cells almost lost responsiveness to the restimulation of the same antigens. In dissecting the mechanisms underlying this tolerance state, we saw a dramatic increase of IL-10-producing T cells in the culture with the presence of UVB-irradiated stroma. However, IL-10 production appeared to only partially contribute to the induced T cell tolerance because the neutralizing anti-IL-10 antibodies only partially reversed T cell responsiveness. T cell anergy or other regulatory T cells, such as TGF- β -producing Th3 and Foxp3⁺ adaptive Tregs might also be induced, which will need to be further elucidated.

Given these exciting results, it became of interest to investigate whether UVB-irradiated stromal cells were able to prevent T1D. We treated 8-week-old NOD mice with three weekly intravenous injections of 1×10^5 UVB-irradiated stroma or PBS, and then followed the animals for evidence of onset of diabetes until they reached 28 weeks of age. We found that infusion of UVB-irradiated stroma significantly prevented T1D, demonstrating that by 28 weeks the diabetes rate in mice treated with UVB-irradiated stroma was significantly lower than it was in PBS-treated mice (42% versus 80%, $P = 0.02$).

To determine whether infusion of UVB-irradiated stroma induced β cell antigen-specific regulatory T cells, we stimulated splenocytes from mice treated with UVB-irradiated stroma or PBS-treated mice with insulin B9–23 peptides or NOD β cell line (NIT1) cell lysates. We found significantly higher IL-10 production by splenocytes from mice treated with UVB-irradiated stroma compared with the mice treated with PBS. There were comparable levels of IFN- γ production between two groups and undetectable IL-4. These results suggest that β cell antigen-specific IL-10-producing Tr1 cells are induced by the treatment of UVB-irradiated stromal cells. To further confirm that β cell antigen-specific regulatory T cells were induced, we intravenously injected CFSE-labeled NOD.BDC2.5 splenocytes into mice treated with UVB-irradiated stroma or PBS. Four days after the cell injection, using flow cytometry, we detected the proliferation of CD4⁺ T cells in pancreatic draining lymph nodes. We found that the proliferation of BDC2.5 CD4⁺ T cells was largely suppressed in mice treated by UVB-irradiated stroma to those treated by PBS (proliferating CD4⁺ T cells in total CFSE-labeled CD4⁺ T cells: 38% vs. 66%), indicating that in mice treated by UVB-irradiated stroma β cell antigen-specific immunosuppressive regulatory T cells were induced and thus immune tolerance to multiple β cell antigens might be formed.

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