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Infusion of UVB-treated splenic stromal cells induces suppression of β cell antigen-specific T cell responses in NOD mice

Chang-Qing Xia*, Yushi Qiu, Rui-Hua Peng, Jeannette Lo-Dauer, Michael J. Clare-Salzler

Department of Pathology, Immunology and Laboratory Medicine, University of Florida, P.O. Box 100275, 1600 SW Archer Road, Gainesville, FL 32610, United States

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Abstract

Our previous study has demonstrated that transfusion of UVB-irradiation-induced apoptotic β cells effectively prevents type 1 diabetes (T1D) in non-obese diabetic (NOD) mice. However, the limitation of β cell source would preclude the clinical application of this approach. Therefore, in the present study, we have attempted to establish a more practical approach by utilizing apoptotic non-β cells to prevent T1D. We find that apoptotic splenic stromal cells significantly suppress β cell antigen-reactive T cell proliferation *in vitro* and *in vivo*. Moreover, β cell antigen-specific T cells primed by β cell antigens in the presence of apoptotic stromal cells have markedly reduced responsiveness to the re-stimulation of the same β cell antigen. We also find that β cell antigen-specific IL-10-producing CD4+ T cells are induced in the presence of apoptotic splenic stromal cells. As expected, transfusion of apoptotic stromal cells effectively protected NOD mice from developing T1D. Furthermore, the proliferation of adoptively transferred β cell antigen-specific TCR-transgenic T cells in pancreatic draining lymph nodes is markedly suppressed in UVB-stroma-treated mice, indicating that UVB-stroma treatment induces immune tolerance to multiple β cell antigens. This study provides an effective and convenient approach for managing T1D by utilizing apoptotic non-β cells.

Keywords: Apoptosis; Immune tolerance; Type 1 diabetes; Regulatory T cell; Cell therapy

1. Introduction

Steady state cell apoptosis is a physiological process during which phagocytes immediately process apoptotic cells without causing inflammation and maintain self-tolerance [1,2]. Evidence has shown that impaired function of phagocytosing apoptotic cells is associated with development of autoimmune diseases [1–5], suggesting that processing of apoptotic cells through phagocytosis plays an important role in the maintenance of self-tolerance. It has been documented that steady state apoptotic cells phagocytosed by dendritic cells can render dendritic cells to be tolerogenic and subsequently tolerize CD4+ T cells and CD8+ T cells through direct antigen presentation and antigen cross-presentation, respectively [6]. It has also been shown that steady state apoptotic cells can trigger phagocytes to secrete immunosuppressive cytokines, such as IL-10 and TGF-β [7,8], which in turn facilitate the development of regulatory T cells [9]. Due to this unique relationship between apoptotic cells and immune tolerance, apoptotic cells have been employed with success in inducing immune tolerance to allogeneic antigens [10] and other exogenous antigens [11].

We have utilized ultraviolet B (UVB) irradiation-induced apoptotic immature dendritic cells to successfully induce immune tolerance across major histocompatibility complex (MHC) barriers [10]. Recently, we effectively induced β cell antigen-specific immune tolerance in the autoimmune diabetes mouse model, non-obese diabetes (NOD) mouse by transfusion of UVB-irradiated apoptotic β cells [12]. We found that three weekly transfusions of UVB-irradiated apoptotic β cells significantly delayed and prevented T1D in NOD mice when these mice were treated at 10 weeks of age (late stage of

* Corresponding author. Tel.: +1 352 846 0585; fax: +1 352 392 5393.
E-mail address: xia@pathology.ufl.edu (C.-Q. Xia).

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insulitis). Nevertheless, limited β cell sources in humans deter the extensive application of this approach in T1D management. Although the studies on stem-cell-derived insulin-producing cells bring hope for this approach in clinical applications, there are still a variety of issues to be resolved in making stable insulin-producing cells [13]. Thus, it is necessary to find alternative techniques in utilizing apoptotic cells to induce β cell antigen-specific T cell tolerance. Recently, it was reported that intravenous injection of apoptotic syngeneic splenocytes along with administration of a hapten, 2,4,dinitrofluorobenzene (DNFB) to the skin induced immune tolerance specific to DNFB [11]. This study suggests that it is possible to utilize apoptotic non-β cells, such as splenocytes or peripheral blood mononuclear cells to induce immune tolerance to β cell antigens with the exposure of endogenous β cell antigens during the process of autoimmunity, thereby preventing autoimmune diabetes.

In the present study, we attempted to determine the effect of UVB-irradiated splenic stromal cells derived from NOD mice on induction of immune tolerance to β cell antigens as well as on T1D prevention. We found that UVB-irradiated NOD splenic stromal cells significantly suppressed β cell antigen-reactive T cell response and induced IL-10-producing CD4+ T cells. Three weekly transfusions of UVB-irradiated apoptotic stromal cells significantly prevented NOD mice from developing T1D.

2. Materials and methods

2.1. Animals

Female NOD and NOD-SCID mice were purchased from the Jackson Laboratory. NOD.BDC2.5 mouse breeders were kindly provided by Dr. David Serreze (Jackson Laboratory). All mice were housed in a specific pathogen-free facility of the Mouse Colony of the Department of Pathology, Immunology and Laboratory Medicine at the University of Florida. Animal studies were performed in accordance with the guidelines of University of Florida Institutional Animal Care and Use Committee.

2.2. Culture media and reagents

RPMI 1640 supplemented with 10% fetal calf serum (Cambrex Bio Science, Walkersville, MD) was used for the culture of splenic stromal cells and *in vitro* T cell functional studies. HL-1 media (Cambrex Bio Science, Walkersville, MD) were used for the culture of splenocytes stimulated with β cell antigens. Mouse IL-4, IL-10 and IFN-γ Luminex kits were purchased from Upstate (Temecula, CA). Fluorescence-conjugated antibodies (anti-IFN-γ and anti-IL-10) and isotype control antibodies, Annexin-V-FITC, leukocyte activation cocktail kits, intracellular cytokine staining kits and dead cell removal kits were purchased from BD-PharMingen (San Diego, CA). The fluorescent dye, CFSE was obtained from Invitrogen Molecular Probes (Eugene, OR). Peptides B9-23 (sequence: SHLVEALYLVCGERG) and BDC2.5 TCR-specific mimotope 1040-55 (sequence: RVRPLWVRME) were synthesized by Peptide International (Louiseville, KY). The purity of these peptides was in the range of 95–97%. Mouse splenic CD4+ T cell isolation kits were purchased from Stem Cell Biotech (Vancouver, Canada). CD11c-microbeads were obtained from Miltenyi (Auburn, CA).

2.3. NOD splenic stromal cell culture

NOD splenic stromal cell line was generated using the method as previously described [14] with some modifications. In brief, whole splenocytes from four-week-old mice without any cell depletion and enrichment were cultured in six-well culture plates in RPMI 1640–10% FCS at 37 °C with 100% humidity and 5% CO_2. After two to three weeks, when the stromal cells had formed a monolayer with 80% confluence, the cells were dispersed with 0.25% trypsin containing 5 mM EDTA. The stromal cells were maintained in long-term culture in RPMI 1640–10% FCS by weekly passage to new plates.

2.4. Preparation of UVB-irradiated stromal cells

The stromal cell line was maintained in culture with RPMI–10%FCS media. Stromal cells were harvested after incubation with 0.25% trypsin–5 mM EDTA for 5 min at room temperature. Cells were washed twice with PBS and resuspended in 0.5 ml of PBS. Then, the cell suspension was placed in a 3-cm Petri dish and irradiated with UVB (1200 mJ/cm^2) for 3 min. After irradiation, the cells were harvested and enumerated using a hemacytometer under a microscope. The UVB-irradiated cells were immediately placed on ice until injection. The sensitivity of stromal cells to UVB-irradiation-induced apoptosis was the same as that of NIT1 cells, an NOD β cell line used in our previous study [12]. We consistently found that >90% cells became apoptotic after 24 h incubation in media post UVB-irradiation.

2.5. Cell isolation

Mouse CD4+ T cells were isolated by negative selection using StemCellSep™ kits following instructions from the manufacturer. The purity of CD4+ T cells was in the range of 95–97%. Splenic DCs were purified by positive selection using CD11c-microbeads according to instructions from the manufacturer. The purity of CD11c+ cells was in the range of 90–95%.

2.6. T cell suppression by UVB-stromal cells

NOD.BDC2.5 splenic CD4+ T cells (1×10^5) were stimulated with purified NOD splenic dendritic cells (1×10^4) pulsed with β cell antigenic mimotope 1040-55 in the presence of different concentrations of UVB-irradiated stromal cells as indicated for four days. Then, ^{3}H-thymidine (1 μCi/well) (Amersham Biosciences) was added to the cultures for an additional 16 h. Cells were washed and harvested onto