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Development of autoreactive diabetogenic T cells in the thymus of NOD mice

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Abstract

Type 1 diabetes results from destruction of pancreatic β cells by β cell-specific autoreactive T cells in the nonobese diabetic (NOD) mouse. Defects in thymic negative selection are thought to result in failure to delete potential β cell-reactive T cells, contributing to the development of autoimmune diabetes. We investigated this possibility by comparing the deletion profile of double-positive (DP) thymocytes in NOD mice with diabetes-resistant strains of mice after anti-CD3 Ab treatment to trigger the TCR-mediated signaling pathway. We found that immature NOD $CD4^+CD8^+$ DP thymocytes have a lower activation threshold than C57BL/6 and Balb/c thymocytes. This was confirmed by showing that NOD DP thymocytes have a higher level of ERK and JNK phosphorylation. The low activation threshold of immature thymocytes resulted in rapid deletion of strongly activated immature DP thymocytes by negative selection, whereas weakly activated immature thymocytes differentiated more efficiently into $CD69^+CD3^{high}$ DP thymocytes by positive selection. SP thymocytes, particularly $CD4^-CD8^+$ T cells that were efficiently generated from activated DP thymocytes, could induce severe insulitis and diabetes in NOD.scid mice. We conclude that the development of autoreactive diabetogenic T cells results from inordinate positive selection due to the low activation threshold of DP thymocytes in NOD mice.

Keywords: Autoimmune diabetes; Cytotoxic T lymphocytes; Immune tolerance; Cell-mediated immunity; Cell differentiation

1. Introduction

Autoimmune disease is generally considered to result from failure to establish self-tolerance. T cell tolerance to self may be established and maintained by clonal deletion, clonal anergy and suppression of autoreactive T cells. The development of the T cell repertoire is shaped through positive and negative selection [1–4]. Low affinity/avidity interactions elicit positive selection, whereas high affinity/avidity interactions elicit negative selection. Clonal deletion of T cells reactive to self antigens by negative selection in the thymus is a major mechanism of central tolerance.

Type 1 diabetes is an autoimmune disease characterized by T cell-mediated destruction of pancreatic β cells [5–7]. Both genetic predisposition and environmental triggers are believed to affect both the immune system

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and the target β cells, resulting in a loss of T cell tolerance to β cells and the consequent development of type 1 diabetes. The nonobese diabetic (NOD) mouse spontaneously develops diabetes with many characteristics similar to human type 1 diabetes [5–9]. Many immunological defects have been reported in NOD mice, including reduced production of inhibitory cytokines [10], defective function of natural killer cells [11], impaired function of antigen-presenting cells due to the expression of the H-2^{g7} haplotype [12,13], reduced expression of cytotoxic T lymphocyte antigen-4 (CTLA-4) [14], reduced number of regulatory $CD25^+CD4^+$ T cells [15], defects in $CD8^+$ T cell peripheral tolerance [16], and hypo-responsiveness of T cells after T cell activation [17].

It has been suggested that defects in central tolerance may result in the failure to delete β cell-reactive T cells in the thymus, contributing to the development of autoimmune diabetes in the NOD mouse. This investigation was initiated to determine whether there are any defects in negative selection of DP thymocytes in NOD mice as compared with diabetes-resistant strains of mice. We found no evidence for defects in negative selection of DP thymocytes in the thymic cortex of NOD mice. In contrast, strongly activated immature DP thymocytes are rapidly deleted by negative selection and weakly activated immature DP thymocytes efficiently differentiate into $CD69^+CD3^{high}$ DP thymocytes by positive selection. However, positively selected SP thymocytes that develop from efficiently activated DP thymocytes, possibly in the corticomedullary junction, may escape deletion, probably due to defects in negative selection in the medulla of the thymus in NOD mice. On the basis of these observations, we suggest that the development of autoreactive diabetogenic T cells results from inordinate positive selection due to the low activation threshold of DP thymocytes and may be further augmented by escaping deletion due to defects in negative selection in the medulla of the thymus in NOD mice.

2. Materials and methods

2.1. Animals

NOD, NOD.scid, C57BL/6, C57BL/6.scid, and Balb/c mice were obtained from The Jackson Laboratory (Bar Harbor, ME). $NOD^{lpr/lpr}$ mice were kindly provided by Dr. M. Lee from the Samsung Medical Research Centre (Seoul, Korea). The animals were bred and maintained under specific pathogen-free conditions.

2.2. Western blot analysis

Cell lysates from total thymocytes or sorted DP thymocytes were prepared, and phosphorylation of ERK and JNK protein was determined with anti-phospho-ERK and anti-phospho-JNK Abs (Cell Signaling Technology, Beverly, MA) by western blot. The membrane was stripped with Re-Blot Plus (Chemicon International, Temecula, CA) and total ERK and JNK protein levels were determined with anti-ERK and anti-JNK Abs (Cell Signaling Technology). Proteins were detected by enhanced chemiluminescence (ECL Plus, Amersham Bioscience). Protein band intensity was determined by scanning with Scion Image (Scion Corp, Frederick, MD). The ratio of phosphorylated ERK and JNK was determined by dividing by the total amount of ERK and JNK, respectively.

2.3. Administration of anti-CD3 Ab into NOD and C57BL/6 mice

Four- to 5-week-old female mice were injected intraperitoneally with various doses of anti-CD3 Ab or isotype IgG (hamster, PharMingen, San Diego, CA) in 200 μl PBS. Animals were killed at various times after Ab treatment and thymocytes were collected.

2.4. Flow cytometric analysis

All Abs used in FACS analysis were purchased from Pharmingen (San Diego, CA); streptavidin-PerCP was obtained from Beckton Dickinson (Mountain View, CA). The cells were incubated with the appropriate fluorochrome-labeled Abs, washed twice with FACS buffer, and analyzed by FACScan. Data files were analyzed using FlowJo software (Tree Star, Inc., San Carlos, CA).

2.5. DNA fragmentation assay and TUNEL staining

Apoptosis of thymocytes was measured by DNA fragmentation as described previously [18]. For TUNEL staining, thymocytes (2×10^6) were stained with PE-labeled anti-CD8 and biotin-labeled anti-CD4 Abs and then with streptavidin-PerCP. After permeabilising the cells with 0.1% Triton X-100 in 0.1% sodium citrate (pH 5.7), the cells were incubated with FITC-labeled TUNEL reagent (In Situ Cell Death Detection Kit, Roche, Laval, PQ, Canada), washed, and analyzed by FACScan.

2.6. Fetal thymic organ culture (FTOC)

Fetal thymic lobes were prepared from C57BL/6 and NOD mice at embryonic day 17.5. Fetal thymic lobes were placed on 0.8 μm polycarbonate filters (Costar, Cambridge, MA), which floated on DMEM supplemented with 12% FCS, 2 mM glutamine, 5×10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin and 100 μg/ml