Isolation of Nonobese Diabetic Mouse T-Cells That Recognize Novel Autoantigens Involved in the Early Events of Diabetes

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Insulin-dependent diabetes mellitus (IDDM) is thought to result from chronic, cell-mediated, autoimmune islet damage. Our aim was to identify the earliest T-cell autoantigen in IDDM, reasoning that this antigen could be causally involved in the initiation of the disease. Identification of the earliest β-cell-specific autoantigen is extremely important in allowing advances in prevention and treatment of initial events in the development of inflammatory insulitis that precedes β-cell destruction and overt diabetes. Therefore, we analyzed the proliferative responses of peripheral T-cells from young, female nonobese diabetic (NOD) mice to extracts of pancreatic β-cell lines. We were able to demonstrate that T-cells responsive to β-cell antigens exist in the peripheral lymphoid tissue of these mice in the absence of deliberate priming before the manifestation of histologically detectable insulitis. T-cell lines and clones isolated from the peripheral lymphatic tissues of young, unimmunized, female NOD mice were also shown to react with extracts of β-cells. Fractionation of the β-cell extracts showed that these T-cell clones recognized multiple β-cell-specific autoantigens but none of the previously reported putative autoantigens (glutamic acid decarboxylase [GAD]65, GAD67, Hsp65, insulin, ICA 69, carboxypeptidase-H, and peripherin). Thus, we can conclude that these responses are specific for novel β-cell autoantigens. Finally, NOD T-cell proliferative responses were also seen to an extract of human islets suggesting potential shared antigenic determinants between human and mouse β-cells. Our observation that human and murine β-cell-specific antigens are conserved offers the possibility that identification of these murine autoantigens may lead to the discovery of the human homologue. This will pave the way toward effective diagnosis and/or immunotherapy to prevent diabetes. *Diabetes* 43:33–39, 1994

Human insulin-dependent diabetes mellitus (IDDM) is caused by an autoimmune destruction of the pancreatic β-cells (1,2). Nonobese diabetic (NOD) mice spontaneously develop diabetes resembling human IDDM (3), characterized by a progressive lymphocytic infiltration of islets (insulitis) before the manifestation of overt diabetes (hyperglycemia) (4,5). Lymphocyte infiltration of islets can be detected as early as 4–6 weeks of age (6). Although antibodies directed against islet cells appear in the serum during the development of insulitis (7), NOD mouse diabetes (and presumably human IDDM) has been shown to be a T-cell-dependent disease (8–20). Susceptibility to the development of diabetes in mice (and humans) is strongly associated with the major histocompatibility complex (MHC) class II genes: mouse I-Aβ and human DQB (21–24).

This association allows the possibility that a single autoantigen might be recognized leading to infiltrative insulitis with resultant β-cell destruction and IDDM. The autoantigen(s) responsible for triggering the initial attack on the pancreatic β-cells has not yet been identified. Although the actual mediators of β-cell destruction are unknown, two previous reports have described potential targets of T-cell clones: *1*) NOD T-cell clones responded to a 65,000-M_r heat shock protein (Hsp65) and success-

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IDDM, insulin-dependent diabetes mellitus; NOD, nonobese diabetic; MHC, major histocompatibility complex; GAD, glutamic acid decarboxylase; αTCL, α-glucagonoma cell line; PBS, phosphate-buffered saline; HPEC, high-performance electrophoretic chromatography; FCS, fetal calf serum; SDS, sodium dodecyl sulfate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis ^{3}H-TdR, ^{3}H-methyl-thymidine; WCE, whole cell extracts; PCR, polymerase chain reaction.

fully transferred insulitis and transient hyperglycemia to prediabetic NOD mice (20), *2*) a human T-cell clone derived from a newly diagnosed IDDM patient was stimulated by a 38,000-M_r protein derived from insulin secretory granules of rat insulinoma tissue (25). In addition, autoantibodies from IDDM patients and from NOD mice have identified other potential targets of the immune response (glutamic acid decarboxylase [GAD: 65,000 and 67,000 M_r], insulin, Hsp65, carboxypeptidase-H, peripherin [26–34], and ICA 69 [M. Pietropaolo, L. Castano, S. Baba, S. Martin, A. Martin, A. Powers, N. Prochazka, J. Naggert, E.H. Leiter, G.S. Eisenbarth, unpublished observations, islet cell autoantigen 69,000 M_r [ICA 69]. Molecular cloning and characterization of novel diabetes associated autoantigen.)

In an attempt to identify the events involved in the initiation of NOD disease, we studied the spontaneous T-cell proliferative response of peripheral lymphatic tissue from young, (prediabetic) NOD mice to extracts of an insulinoma cell line. T-cell proliferative responses to whole cell extracts (WCE) and subcellular fractions of the insulinoma were seen in NOD mice ≥8-days-old. NOD T-cell proliferative responses were also seen to an extract of human islets suggesting potential shared antigenic determinants between human and mouse β-cells.

T-cell clones derived from young, prediabetic NOD mice also responded to the WCE and the same subcellular fractions of the insulinoma as did peripheral lymphocytes but did not respond to purified autoantigens including GAD (65 or 67), ICA 69, carboxypeptidase-H, peripherin, insulin, or Hsp65. These data suggest that novel antigens of islet β-cells are recognized early in NOD disease and that such antigens may be shared between human and mouse β-cells. Identification of these novel autoantigens may lead to immunotherapy and/or early diagnosis of IDDM.

RESEARCH DESIGN AND METHODS

Mice. NOD mice were purchased from Taconic (Germantown, NY). Of the female mice, 80% developed spontaneous diabetes at ≤160 days. BALB/c and C57BL/6 mice were also purchased from Taconic.

Cell lines. The β-insulinoma cell line B23720 was developed from RIP-Tag2 transgenic mice containing SV-40 T antigen under the control of the rat insulin promoter (35). These mice were crossbred with NOD mice. After the eighth backcross, an insulinoma tumor was isolated from one mouse, and an in vitro cell line (B23720) was generated. These insulinoma cells express insulin, peripherin, and class I MHC but do not express GAD 65, GAD 67, or class II MHC molecules (S. Singer, H.O. McDevitt, C. Joliceour, D. Hanahan, and H. Acha-Orbea, unpublished observation).

The α-glucagonoma cell line (αTCL) was generated similarly in transgenic mice containing the SV-40 T-antigen under the control of the glucagon promoter (35), but was not crossed to NOD.

The human neuroblastoma line SY5Y was a gift from Dr. L. Jensen (UCSF, San Francisco, CA).

Human islets. WCE was prepared by sonication of 10^9 cells in 200 μl of phosphate-buffered saline (PBS) and 3 times for 10 s on ice. Human islets were received as a gift from Dr. Ricardi (Pittsburgh, PA).

High-performance electrophoretic chromatography (HPEC) fractionation. B23720 were grown in Dulbecco's modified Eagle medium (Gibco, Grand Island, NY) containing 10% fetal calf serum (FCS) (Gibco). Cytosolic and membrane extracts from this cell line were prepared by sonication followed by a 150,000 *g* speed spin. B23720 cytosolic and membrane extracts were fractionated using the HPEC (ABI, HPEC, model 230A, Foster City, CA) system. A sample containing 400 μg of cell-extract in 7.5 mM tris-phosphate, pH 7.5, 0.25% sodium dodecyl sulfate (SDS), and 15% glycerol was loaded onto a 10% SDS-tris phosphate tube gel (3.5 × 10 cm) and electrophoresed using a trisphosphate buffer system. The proteins were eluted from the bottom of the gel into 7.5 mM tris HCl, pH 7.5. The collected fractions were assayed for protein concentration and then analyzed on a 12.5% SDS-polyacrylamide gel.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) fractionation of β-insulinoma proteins. Two milligrams of the insulinoma cytosolic or membrane extract was electrophoresed on a nonreducing 10% SDS-PAGE and transferred to nitrocellulose. The nitrocellulose was cut into several strips that were sonicated in PBS to generate suspensions of fine particles that were then used in T-cell proliferation assays.

Proliferation assay. Single-cell suspensions from spleens (6–8 mice/group; pooled together) were prepared in complete RPMI-1640 medium supplemented with 5% FCS and 10 U/ml penicillin/streptomycin. Spleen cells were assayed by titrating antigen (0.3–2.0 μg/ml) into microtiter plates containing 1 × 10^6 cells in complete media, in triplicates, and harvesting as described below. For analysis of clones, cultures of 1–3 × 10^5 T-cells and 0.5 × 10^6 irradiated (2,000 R) spleen antigen presenting cells per well were cultured in triplicate with added antigen (0.3–20 μg/ml). The cultures were incubated for 72 h, pulsed with 1 μCi per well ^{3}H-methyl-thymidine (^{3}H-TdR) (Amersham, Arlington Heights, IL), and harvested 16 h later. The incorporated radioactivity was determined using a Betaplate scintillation counter (Pharmacia, LKB, Piscataway, NJ). Results are expressed as mean counts per minute of incorporated ^{3}H-TdR. Standard deviations were ≤10% of the mean.

The insulinoma extracts fractionated by HPEC were used at a protein concentration (final) of 10 μg/ml, and the samples were then diluted 1:10–1:40 to reduce toxicity caused by SDS. Similarly, when insulinoma membranes were fractionated by SDS-PAGE and blotted on nitrocellulose, the strips were sonicated, diluted 1:40–1:1600, and tested in the T-cell assay. All presented experiments were repeated at least 3 times. The presented graphs represent results of ≥3 experiments.

Generation of β-cell-specific T-cell lines and clones. Lymphocytes from spleen or pancreatic lymph nodes taken from 30- to 40-day-old female NOD mice were cultured for 3 days with 10 μg/ml of the insulinoma WCE or membrane extracts in complete RPMI with 5% FCS. Three days later the cells were washed and cultured in complete RPMI medium containing 10% FCS and 20

U/ml recombinant-mouse IL-2 (Genzyme, Cambridge, MA).

The T-cell lines were established by repeated cycles of stimulation as described above and cloned by standard limiting dilution procedures. Growing clones were expanded and tested for antigen recognition in proliferation assays.

Expression and purification of recombinant proteins. The cDNA encoding human Hsp65 was cloned by polymerase chain reaction (PCR) using polyA$^+$RNA isolated from a human Epstein Barr Virus–transformed β-cell line (Priess) (36). Postincubation was at 42°C for 2 h. Following reverse transcription, the Hsp65 encoding DNA fragment was amplified by PCR using the primers 5′CGG GGATCCGCCAAAGATGTAAAATTTGGTGCAGATGCC and 5′GTCCTCGAGTTAGAACATGCCACCTCCCATAC CACCTCC (30 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C). The cDNAs encoding for human carboxypeptidase-H, ICA69 (gifts from G. Eisenbarth), and Hsp70 (37, ATCC/clone pH 2.3) were cloned into expression vector pTrc99A (His6) that was constructed by insertion of a synthetic DNA fragment encoding six histidine residues ([CAC]6) into the polylinker of the pTrc99A expression vector (38). The recombinant proteins were tagged with six histidine residues at the NH_2-terminus. Plasmid constructs were transformed into *E. coli*-TG1 (supE hsdΔslac-proAB) F′[traD36proAB + laclqlacZΔM15] and protein expression was induced by addition of IPTG to the culture medium. Bacteria were lysed in 100 mM Tris pH 8.0, 6M GuHCl and insoluble material was removed by centrifugation at 40,000 *g* for 30 min. Recombinant proteins were purified using Ni-NTA-agarose (Qiagen, Chatsworth CA) in the presence of 6 M GuHCl and dialyzed against PBS. Protein concentration was determined using BCA (protein assay reagent) assay (Pierce, Rockford, IL).

Full-length murine cDNAs encoding the two isotypes of GAD (GAD65, GAD67), peripherin, and carboxypeptidase-H were engineered to contain six histidine residues found at the COOH-terminus, thereby allowing affinity purification of each antigen using a Ni^{++}-conjugated resin.

A baculovirus expression system has been used to express the cDNAs as recombinant proteins. Briefly, the histidine-tagged cDNAs were subcloned into the PVL 1393 transfer vector and transfected into Sf9 cells with baculovirus. Recombinant viruses were selected and assessed for protein expression. Selected viral recombinants were then used for large-scale expression of each protein. Pancreatic hormones were purchased from Sigma (Sigma, St. Louis, MO). The rat C-peptide sequence was EVENPQVPQIGGGPEAENIQTIAIEVARQ, and the mouse C-peptide sequence was EVEDPQVEQ IGGSPGDIQTIAIEVARQ (28–31).

RESULTS

Unprimed peripheral lymphocytes from young, female NOD mice of different ages (Fig. 1*A*) were isolated from spleen and stimulated in vitro by whole cell, cytosolic, or membrane extracts from the B23720 (insulinoma) cell line. T-cell proliferation to the WCE of the insulinoma was seen using cells from NOD mice at each age assayed beginning at ≥8 days old (data not shown). To separate the components of the insulinoma WCE recognized by unprimed NOD T-cells, protein fractionation of the WCE was performed based on SDS-PAGE and elution of the fractions into liquid phase by HPEC. Proliferative responses of splenocytes from 17- to 54-day-old female NOD mice were assayed using cytosolic extracts of the insulinoma fractionated by HPEC. Data from 1 of 10 similar assays are presented in Fig. 1*A*. Three major peaks that elicited T-cell proliferation were identified with approximate molecular weights of 30,000–40,000, 50,000, and 55,000–65,000 M_r in the cytosolic fraction of the insulinoma antigen preparation (Fig. 1*A*). The magnitude of the T-cell proliferative response to these fractions increased with age (Fig. 1*A*), but proliferative responses could be seen in female NOD mice as early as 8 days old (Fig. 2*B*).

When insulinoma membrane extracts were fractionated by conventional SDS-PAGE followed by blotting of the proteins on nitrocellulose and the nitrocellulose particles carrying the proteins were assayed by T-cell pro-

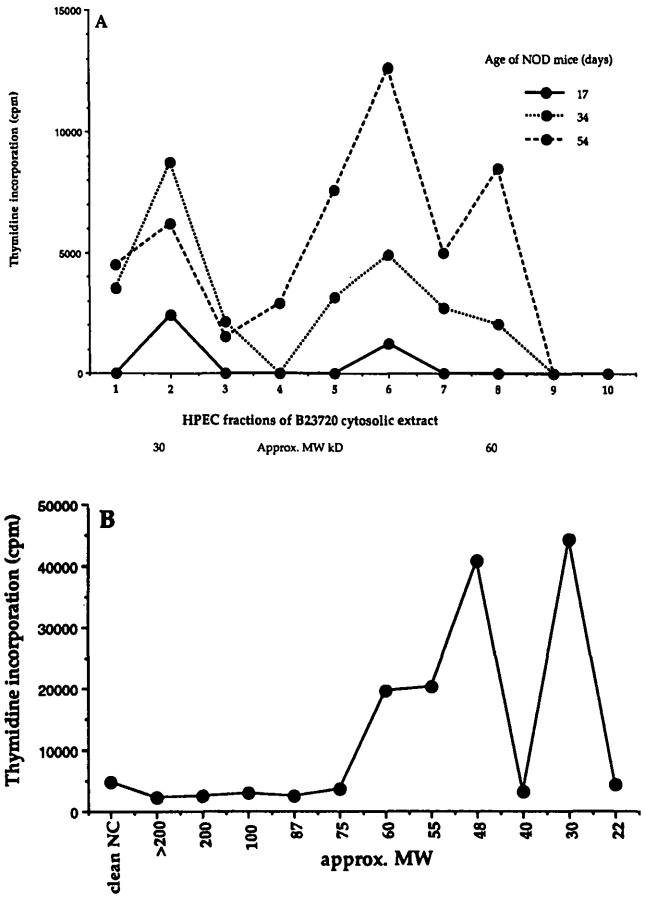

FIG. 1. T-cell response to fractionated β-cell antigens in young NOD mice. *A*: Insulinoma cytosolic extracts fractionated by HPEC (ABI, HPEC, model 230A) were used in a proliferation assay testing the reactivity of splenocytes obtained from 17- to 54-day-old female NOD mice. *B*: Insulinoma membrane extracts, separated by SDS-PAGE and transferred to nitrocellulose, were cut and sonicated to yield small particles that were used in a T-cell proliferation assay to test the response of spleen cells from 24-day-old female NOD mice.

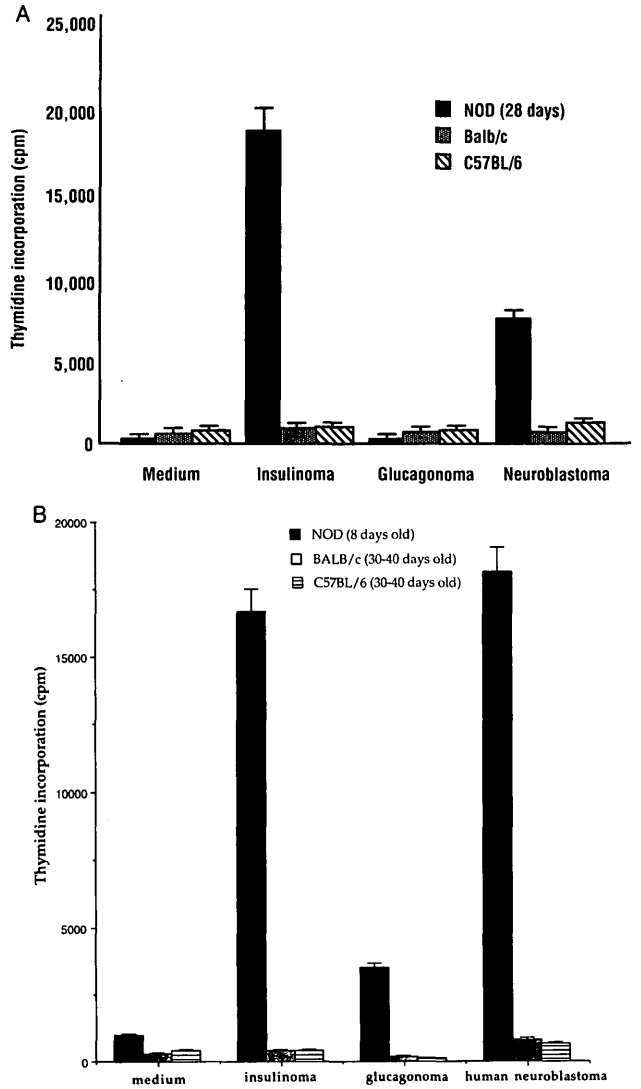

FIG. 2. The T-cell proliferative response to murine insulinoma and human neuroblastoma antigens can be demonstrated in NOD mice and is absent in nondiabetic mouse strains and early NOD T-cell responses to β-cell antigen. *A*: Splenocytes from a 28-day-old female NOD and a 30- to 40-day-old BALB/c or C57BL/6 mice were tested against HPEC fractionated antigen (10 mg/ml) from the insulinoma (B23720), the glucagonoma (αTCL), and human neuroblastoma (SY5Y). HPEC fractions within the size range of 30,000–60,000 M_r were pooled and used in the assay. *B*: Splenocytes from 8-day-old female NOD mice and 30- to 40-day-old BALB/c or C57BL/6 mice were tested as described above.

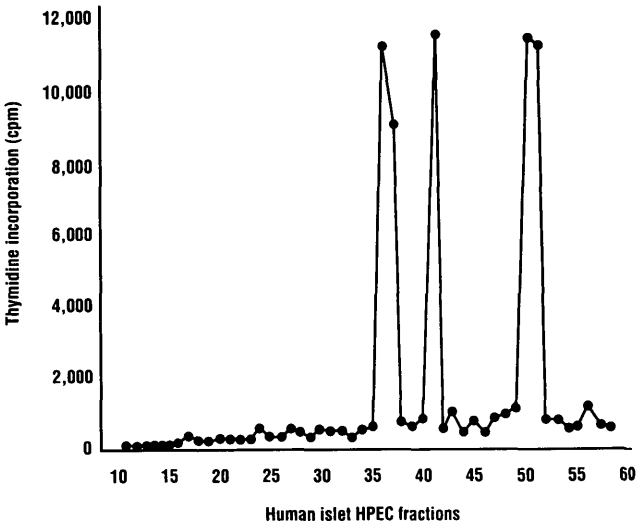

FIG. 3. β-cell-specific antigens are conserved across species. WCE of human islets were fractionated by HPEC and tested in a proliferation assay with unprimed NOD spleen cells from 40-day-old female mice.

liferation using spleen cells from 24-day-old female NOD mice, a similar pattern of T-cell activation was obtained (Fig. 1*B*). Thus, the autoantigens can be detected in the membrane and in the cytosol of the B23720 insulinoma cell line.

To demonstrate specificity, the proliferative responses of splenocytes from a 28-day-old NOD, from a 30- to 40-day-old BALB/c, and from C57BL/6 female mice (used as control samples) were compared using HPEC fractionated cytosol preparations from *1*) the NOD insulinoma (B23720), *2*) a murine α-glucagonoma (αTCL), and *3*) a human neuroblastoma (SY5Y). The proliferative response seen in NOD mice to cytosolic proteins of the insulinoma was specific (Fig. 2): *1*) T-cells obtained from the same NOD mice failed to respond to glucagonoma proteins, and *2*) splenocytes from other nondiabetic mouse strains including BALB/c and C57BL/6 did not respond to the insulinoma extract (Fig. 2*A*). Furthermore, when splenocytes from 8-day-old female NOD mice were tested, a spontaneous proliferative response to β-cell but not to α-cell antigens was detected (Fig. 2*B*). Control mice T-cell proliferative responses to alloantigens and mitogens were found to be similar in cells from all mice tested (data not shown). These data suggest that the T-cell proliferative response to the insulinoma is specific and seen only in NOD mice. Moreover, these data demonstrate that T-cells responsive to β-cell antigens exist in the peripheral lymphoid tissue of very young, female NOD mice in the absence of deliberate priming and before manifestation of histologically detectable insulitis.

Because the cytosolic preparation from the human neuroblastoma (SY5Y) also triggered NOD T-cell proliferation (Fig. 2), these results suggest that these neuroendocrine-derived cells may share antigenic determinants with the insulinoma and such determinants may be conserved across species. To further address the potential for β-cell-specific, antigenic determinants shared across species, human islets were obtained from which a WCE was prepared, fractionated by HPEC, and assayed in a NOD T-cell proliferative assay. Human islet cell–derived proteins, with approximate molecular weights of 30,000–40,000, 50,000, and 55,000–65,000 M_r were shown to induce proliferation of T-cells from spleens of 40-day-old, unprimed, female NOD mice (Fig. 3). This result indicates that β-cell-specific antigens may be conserved between humans and mice that can be recognized by unprimed T-cells from NOD mice.

To determine whether proteins contained in the three peaks that correspond to T-cell-stimulating activity (Fig. 1) were the result of a degradation process (1), represented isoforms of the same molecule (2), or represented

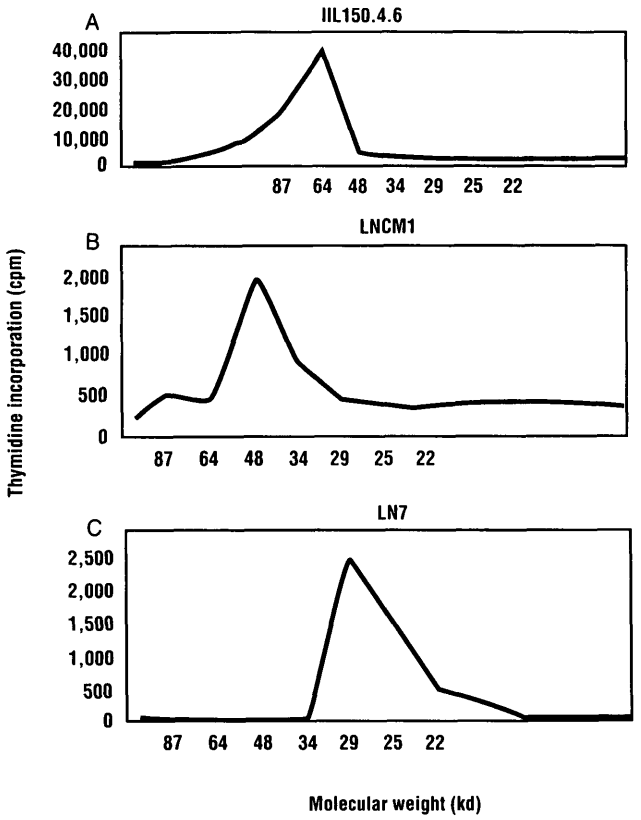

FIG. 4. Existence of several discrete β-cell-specific autoantigens detected by the peripheral T-cell response in the NOD mouse. Proliferative responses of three insulinoma-specific T-cell clones tested with the SDS-PAGE fractionated β-cell extract. (IIL150.4.6 [*A*], $CD4^+$ T-cell cloned from islets; LNCM1 [*B*] and LN7 [*C*], $CD4^+$, $I\text{-}A^{NOD}$ restricted T-cells were cloned from lymph-nodes of 30-day-old female NOD mice).

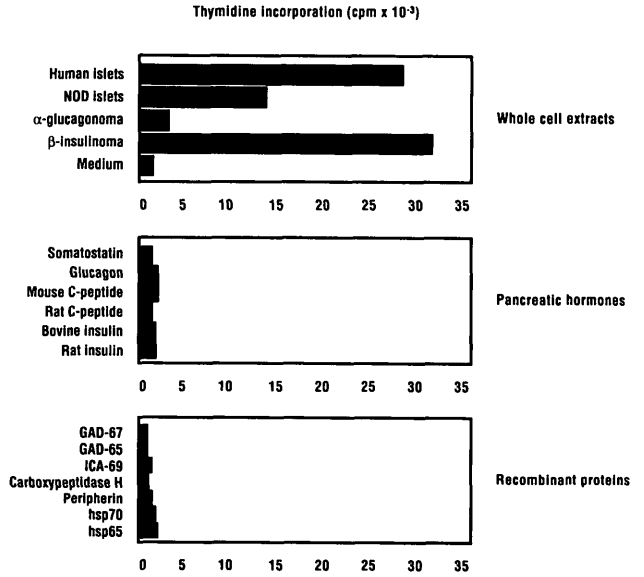

FIG. 5. Antigen specificity of T-cell line and clones from young, female NOD mice (representative graph). The antigen specificity of insulinoma-specific T-cell line was tested in a proliferation assay using whole-cell extracts of islets, insulinoma, and pancreatic hormones (*upper* and *middle* panel). T-cell hybridomas generated from the above T-cell line were assayed with recombinant proteins in cytokine (IL-2) release assays. (The assays were performed with a protein concentration of 10μg/ml).

different antigens (3), a panel of insulinoma-specific T-cell clones was generated and tested against the SDS-PAGE fractionated insulinoma extract. Insulinoma-specific T-cell lines established from lymph node cells of 30-day-old female NOD mice selected on insulinoma WCE exhibited a profile of T-cell reactivity similar to unprimed NOD lymphocytes, i.e., responses corresponding to the three peaks of activity within the molecular weight range of 30,000–60,000 M_r (data not shown). The clones were generated following two cycles of antigenic restimulation with insulinoma WCE. The T-cell lines were cloned by limiting dilution. Different T-cell clones ($CD4^+$, $I\text{-}A^{NOD}$ restricted) proliferated in response to either the 30,000-M_r fraction or to the 50,000-M_r fraction of insulinoma antigen (Fig. 4*C* and *B*, respectively). These T-cell clones were shown to accelerate destructive insulitis upon adoptive transfer into 14-day-old NOD mice (data not shown) suggesting their relevance to the disease process. A third β-cell-specific, $CD4^+$- and $I\text{-}A^{NOD}$-restricted T-cell clone was isolated from islets of 30-day-old NOD mice previously infused with diabetic T-cells isolated from spleens. This islet-infiltrating T-cell clone was shown to be stimulated by the 65,000-M_r fraction of insulinoma antigen (Fig. 4*A*). These data suggest that the insulinoma proteins contained within the peaks of T-cell activity, against WCE, probably consist of discrete proteins. Furthermore, these data suggest that T-cells from young, prediabetic, female NOD mice react with more than one insulinoma antigen.

To determine whether T-cells from unprimed, female NOD mice were responding to previously identified candidate autoantigens, a T-cell line generated from pooled lymphocytes from eight 30-day-old, female NOD mice and the three T-cell clones (Fig. 4) were challenged with a panel of antigens including cell extracts (islets, insulinoma, and glucagonoma), pancreatic hormones (insulin [28–31], glucagon, and somatostatin), proinsulin peptides (28–31), and recombinant proteins (Hsp65 [20, 32], carboxypeptidase-H [33], peripherin [34], GAD65, GAD67 [26,27], and ICA69). All tested T-cell clones and the line from 30-day-old female NOD mice were vigorously stimulated by β-cell extracts (islet and insulinoma) but not by α-cell extracts, pancreatic hormones, or any of the above mentioned recombinant proteins (Fig. 5). Spontaneous T-cell proliferative responses of NOD lymphocytes to the recombinant proteins Hsp65, peripherin, GAD65, GAD67, and carboxypeptidase-H have been demonstrated although not earlier than 4 weeks of age (R. Tisch, unpublished observations).

DISCUSSION

Data presented in this study suggest that young, female NOD mice ≥8 days old have peripheral autoreactive T-cells that recognize novel islet cell antigens in the absence of antigen priming and preceding the onset of histopathologically demonstrable insulitis. Furthermore, these autoreactive, NOD T-cells recognize human islet cell extracts suggesting conservation of antigenic determinants between human and murine islet β-cells. The

autoreactive, T-cell proliferative responses observed can be seen using splenocytes from very young (8-day-old), female NOD mice (Fig. 2*B*). The inability of peripheral lymphocytes from young NOD mice to recognize conventional autoantigens including GAD65, GAD67, ICA69, Hsp65, carboxypeptidase H, and peripherin, and the inability of such T-cells to respond to insulin, suggests that recognition of novel β-cell autoantigens might precede β-cell damage with resultant release and recognition of these putative autoantigens. These data are consistent with the unpublished observation suggesting that peripheral lymphocytes from female NOD mice begin to recognize these conventional autoantigens between 4 and 5 weeks of age (R. Tisch, unpublished observation).

The NOD autoreactive T-cell response to SDS-PAGE or HPEC fractionated β-cell, cytosolic, or membrane extracts suggested that multiple β-cell-specific autoantigens are recognized early in NOD disease (Fig. 1). Furthermore, when isolated T-cell clones from young, female NOD mice were assayed with these antigen fractions, each responded to one of the different fractions, which suggests that the different peaks triggering T-cell reactivity were different proteins. The results we have described support the possibility that there are multiple and distinct species of β-cell-specific antigens recognized in the early inflammatory events of NOD disease and suggest that autoreactive T-cells can be identified in very young, prediabetic, female NOD mice that recognize these multiple β-cell antigens.

Knowledge of the autoantigen(s) recognized by T-cells before overt diabetes would be important for certain forms of effective immunotherapy. More importantly, these data suggest that the autoantigens first identified leading to inflammatory insulitis may not be those that have previously been characterized as autoantigens by assaying antibodies and/or T-cells from overtly diabetic humans and/or mice. Our results support the possibility that early recognition of novel β-cell-specific autoantigens leads to inflammatory insulitis with resultant β-cell destruction and the release of β-cell products whose recognition then results in the production of the more conventional autoantibodies and T-cell proliferative responses to antigens such as GAD65, GAD67, Hsp65, etc. If this hypothesis is correct, it would suggest that identification of these early β-cell-specific autoantigens is extremely important in allowing advances in prevention and treatment of early events in the development of inflammatory insulitis that precedes β-cell destruction and overt diabetes. Our observation that β-cell-specific antigens are conserved between humans and mice offers the possibility that identification of these murine autoantigens may lead to the discovery of the human homologue and pave the way toward effective diagnosis and/or immunotherapy to prevent diabetes.

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REFERENCES

1. Eisenbarth GS: Type I diabetes mellitus: a chronic autoimmune disease. *N Engl J Med* 314:1360–68, 1986
2. Rossini AA, Mordes JP, Like AA: Immunology of insulin-dependent diabetes mellitus. *Annu Rev Immunol* 3:289–92, 1985
3. Makino S, Kunimoto K, Muraoka Y, Mizushima Y, Katagiri, K, Tochino, Y: Breeding of a non-obese diabetic strain of mice. *Exp Anim* 29:1–14 1980
4. Gepts W, Lecompte PM: The pancreatic-islets in diabetes. *Am J Med* 70:105–15, 1981
5. Kataoka S, Satoh J, Fujiyi H, Toyota T, Suzuki R, Itoh K, Kumagai K: Immunological aspects of the nonobese diabetic (NOD) mouse-abnormalities of cellular immunity. *Diabetes* 32:247–53, 1983
6. Hurtenbach U, Maurer C: Type I diabetes in NOD mice is not associated with insulin-specific, autoreactive T-cells. *J Autoimmunity* 2:151–61, 1989
7. Maruyama T, Takei I, Taniyama M, Kataoka K, Matsuki S: Immunological aspect of nonobese diabetic mice: immune islet cell killing mechanism and cell mediated immunity. *Diabetologia* 27:121–29, 1984
8. Chatenoud L, Ferran C, Bach JF: Anti-CD3 treatment of autoimmune patients. *Lancet* ii:164–66, 1989
9. Hayward A, Schreiber M: Neonatal injection of CD3 antibody into nonobese diabetic mice reduces the incidence of insulitis and diabetes. *J Immunol* 143:1555–64, 1989
10. Feutren G, Assan RB, Karsenty G, Durostu H, Sirmai J, Papoz L, Vexiau P, Rodier M, Lallemand A: Cyclosporin increases the rate and length of remission in insulin-dependent diabetes of recent onset-results of a multicenter double blind trial. *Lancet* ii:119–24, 1986
11. Ogawa M, Maruyama T, Haregawa T, Kanaya T, Kobayashi F, Tochino Y, Uda H: The inhibitory effect of neonatal thymectomy on the incidence of insulitis on non-obese diabetic (NOD) mice. *Biomed Res* 6:103–108, 1985
12. Mori Y, Suku M, Okudaira H, Matsuba I, Tsuruoaka A, Saski A, Yokoyama H, Tanase T, Shida T, Nishimurra M, Terada E, Ikeda Y: Preventive effects of cyclosporin on diabetes in NOD mice. *Diabetologia* 29:244–47, 1986
13. Tarui S, Tochino Y, Nonaka K (Eds.): *Insulitis and Type I Diabetes, Lessons From NOD Mouse*. Tokyo, Academic Press, 1986, p. 143–64
14. Shizuru JA, Taylor-Edwards C, Banks BA, Gregory AK, Fathman CG: Immunotherapy of the nonobese diabetic mouse treatment with an antibody to T-helper lymphocytes. *Science* 240:659–62, 1988
15. Kelley VE, Gaulton GN, Hattori M, Ikegami H, Eisenbarth G, Strom TB: Anti-interleukin 2 receptor antibody suppresses murine diabetic insulitis and lupus nephritis. *J Immunol* 140:59–68, 1988
16. Wicker LS, Miller BJ, Mullen Y: Transfer of autoimmune diabetes mellitus with splenocytes from nonobese diabetic (NOD) mice. *Diabetes* 35:855–59, 1986
17. Benedelac A, Carnaud C, Boitard C, Bach JF: Syngeneic transfer of autoimmune diabetes from diabetic NOD mice to healthy neonates: requirement for both L3T4$^+$ and LyT-2$^+$ cells. *J Exp Med* 166:823–31, 1987
18. Reich EP, Sherwin RS, Kanagawa O, Janeway CA: An explanation for the protective effect of the MHC II I-E molecule in murine diabetes. *Nature* 341:326–30, 1989
19. Haskins K, Portas M, Bergman B, Lafferty K, Bradley B: Pancreatic islet specific T-cell clones form nonobese diabetic mice. *Proc Natl Acad Sci USA* 86:8000–8008, 1989
20. Elias D, Markovits D, Reshef T, van der Zee R, Cohen IR: Induction and therapy of autoimmune diabetes in the nonobese diabetic (NOD/Lt) mouse by a 65 kD heat shock protein. *Proc Natl Acad Sci USA* 87:1576–84, 1990
21. Hattori M, Buse JB, Jackson RA, Glimcher L, Dorf ME, Minami M Makino S, Moriwak K, Kuzuya H, Imura H, Stauss WM, Seidman JG, Eisenbarth GS: The NOD mouse: recessive diabetogenic gene in the major histocompatibility complex. *Science* 231:733–37, 1986
22. Acha Orbea H, McDevitt HO: The first external domain of the

nonobese diabetic mouse class II I-AB chain is unique. *Proc Natl Acad Sci USA* 84:2435–43, 1987
23. Todd JA, Bell JI, McDevitt HO: HLA-DQb gene contributes to susceptibility and resistance to insulin dependent diabetes mellitus. *Nature* 329:599–603, 1987
24. Eisenbarth GS: Immunogenetics of endocrine disorders. *Immunol Today* 6:345–47, 1985
25. Roep BO, Arden SD, P de Vries RR, Hutton JC: T-cell clones from a type I diabetes patient respond to insulin secretory granule proteins. *Nature* 345:632–34, 1990
26. Baekkeskov S, Aanstoot H-T, Christgau S, Reetz A, Solimena M, Cascalho M, Folli F, Richter-Olsen H, Camilli P-D: Identification of the 64k autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature* 347: 151–55, 1990
27. Baekkeskov S, Nielsen JH, Marner B Bilde T, Ludvigsson J, Lernmark A: Autoantibodies in newly diagnosed diabetic children immunoprecipitate human pancreatic islet cell proteins. *Nature* 167:298–302, 1982
28. Jackson R, Ziegler R, Castano L, Eisenbarth GS: Insulin autoantibodies-linear correlation with progression of type I diabetes. *Clin Res* 38:408–18, 1990
29. Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, Raghu PK, Pacquette TL: Insulin autoantibodies in insulin dependent diabetes before insulin treatment. *Science* 222:1337–41, 1983
30. Markussen J, Sundby F: Rat-proinsulin C-peptides. *Eur J Biochem* 25:152–62, 1972
31. Blume N: Peptide antisera for proinsulin C-peptide. *Biomed Biochim Acta* 49:1247–51, 1990
32. Elias D, Reshef T, Birk OS, van der Zee R, Walker MD, Cohen IR: Vaccination against autoimmune mouse diabetes with a T-cell epitope of the human 65 kD heat shock protein. *Proc Natl Acad Sci USA* 88:3088–96, 1991
33. Castano L, Russo E, Zhou L, Lipes M, Eisenbarth GS: Identification and cloning of a granule autoantigen (carboxypeptidase-H) associated with type I diabetes. *Clin Endocrinol Metab* 73:1197–201, 1991
34. Escurat M, Djabali K, Huc C, Landon F, Becour TC, Boitard C: Origin of the β-cells of the islets of langerhans is further questioned by the expression of neuronal intermediate filament proteins, peripherin and NF-l, in the rat insulinoma RIN5f cell-line. *Dev Neurosci* 13:424–32, 1991
35. Hanahan, D: Heritable formation of pancreatic β-cell tumors in transgenic mice expressing recombinant insulin/simian virus 40 oncogenesis. *Nature* 315:115–19, 1985
36. Gorga JC, Horejsi V, Johnson DR, Raghupathy R, Strominger JL: Purification and characterization of class-II histocompatibility antigens from a homozygous human B-cell line. *J Biol Chem* 262: 16087–94, 1987
37. Wu B, Hunt C, Marimoto R: Structure and expression of the human gene encoding major heat shock protein HSP70. *Mol Cell Biol* 5:330–41, 1985
38. Aman E, Ochs B, Abel KJ: Tightly regulated tac promoter vectors useful for the expression of unfused and fused proteins in escherichia-coli. *Gene* 69:301–15, 1988