CD4+ β Islet Cell–reactive T Cell Clones That Suppress Autoimmune Diabetes in Nonobese Diabetic Mice

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Summary

We report the isolation of a panel of CD4+ T helper type 1 autoreactive T cell clones from the spleen of unprimed nonobese diabetic mice, a murine model of human insulin-dependent diabetes mellitus. The T cell clones express a diverse repertoire of T cell receptors, three of which recognize β islet cell autoantigen(s). The islet cell–reactive T cell clones inhibit adoptive transfer of insulin-dependent diabetes mellitus and intraislet lymphocytic infiltration. The protective capacity of the T cell clones correlates with their ability to produce a novel immunoregulatory activity that potently inhibits in vitro allogeneic mixed lymphocyte reaction. The partially purified activity significantly inhibited the adoptive transfer of diabetes. Our work provides evidence in support of the existence of T helper type 1, CD4+ T cells reactive to β islet cell autoantigens that have acquired a protective instead of a diabetogenic effector function. These T cells mediate their protective action in part by production of an immunoregulatory activity capable of down-regulating immune responses, and they are likely to represent a population of regulatory T cells that normally plays a role in maintaining peripheral tolerance.

Insulin-dependent diabetes mellitus (IDDM)[1] is a result of autoimmune destruction of the insulin-producing pancreatic β islet cells (for review see reference 1). The β islet autoantigen-specific T cells are implicated to play a key role in the pathogenesis of IDDM (2–6). Susceptibility to IDDM in humans and in nonobese diabetic (NOD) mice is strongly associated with expression of MHC class II β chain that lacks the usual acidic aspartate residue at position 57 (Asp-57) (for review see reference 7). Expression of transgenic class II β chain containing the Asp-57 protected the NOD mice from spontaneous development of IDDM (8–12). Clonal deletion or functional silencing of β islet autoantigen-specific diabetogenic T cells has been proposed as the mechanism whereby these molecules provide protection (4). However, it is now evident that protection by MHC class II is not mediated by these mechanisms (9, 13, 14). Since splenic T cells isolated from NOD mice expressing the protective $A\beta^d$ transgene have the capacity to inhibit adoptive transfer of IDDM, it has been suggested that the mechanism whereby protective MHC class II molecules protect mice from diabetes includes selection in the thymus and/or induction in the periphery T cells capable of suppressing diabetes development (15).

The nature of T cells capable of suppressing autoimmune diabetes is at present poorly understood. Boitard et al. have demonstrated the presence of such T cells in the CD4+, but not CD8+, compartment of spleen cells from 8-wk-old NOD mice (16). T cell clones capable of suppressing IDDM have been isolated by various laboratories (17–20). These T cell clones appear to exhibit properties that are quite different one from another, indicating that a diverse population of regulatory T cells may be involved in maintaining peripheral tolerance. At present, the repertoire of regulatory T cells capable of inhibiting IDDM, their requirements for activation, their cellular targets, T cell receptor diversity, and their molecular basis for protection are poorly understood.

We report here the isolation and characterization of three β islet cell–reactive T cell clones that suppress the development of IDDM. These islet cell–reactive T cells, which have acquired a protective instead of a diabetogenic effector function, may represent part of a regulatory network of T cells that normally play a role in maintaining peripheral tolerance.

[1] *Abbreviations used in this paper:* AMLR, autologous mixed lymphocyte reaction; IDDM, insulin-dependent diabetes mellitus; LPS-blast, LPS-activated splenic blast; MLR, mixed lymphocyte reaction; NOD, nonobese diabetic; SN, supernatant; TCGF, T cell growth factor.

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Materials and Methods

Mice, Cell Lines, Antibodies, and Peptides. Mice were purchased from Taconic Farms, Inc. (Germantown, NY). NOD/Lt mice were bred at the animal facility at Dana-Farber Cancer Institute (Boston, MA). The following mAbs were used: anti-I-$A^{k,r,s,f,g7}$ (10-2.16, [21]); anti-I-A^d (MK-D6, [22]), anti-IL-4 (11b11, [23]); anti-IL-2 receptor (PC 61 5.3; TIB-222; American Type Culture Collection, Rockville, MD); anti-Vβ2 (B20.6, [24]); anti-Vβ8 (F23.1, [25]); and anti-Vβ14 (14-2, [26]). The peptides used, P1 (yTYTVHAA-HAYTYt; small letters denote D amino acids) and P2 (KMKMVH-AAHAKMKM) (27), were synthesized at Dana-Farber Cancer Institute core facility.

Generation and Maintenance of Autoreactive T Cell Clones. T cell clones were derived from unprimed 4-wk-old female NOD mice purchased from Taconic Farms, Inc. Splenic lymphocytes were cultured in a U-bottomed 96-well plate (5×10^5/well). T cell clones NOD-2, NOD-5, NOD-6, and NOD-12 were generated in DMEM supplemented with 10% FCS and 4 U/ml of partially purified rat IL-2 (T cell growth factor [TCGF], Collaborative Research, Inc., Lexington, MA), whereas clones NOD-14 and NOD-21 were established in FCS-free DMEM supplemented with 0.5% NOD serum and 4 U/ml recombinant mouse IL-2 (Genzyme Corp., Cambridge, MA) during the first 2 wk of culture. Surviving T cells were harvested 1 wk later by Ficoll centrifugation, and they were expanded in flat-bottomed 96-well plate using the same culture medium. 1 wk later, the surviving T cells were restimulated with NOD spleen cells using the same culture medium but without IL-2 supplementation. After 4 d of selection in IL-2–free medium, surviving T cells were pooled and were cloned by limiting dilution in culture medium supplemented with 10% FCS and 4 U/ml of rat TCGF and in the presence of freshly isolated irradiated NOD spleen cells (1×10^4 APC/U-bottomed well). T cell clones thus generated were maintained in the T cell medium supplemented with varying concentrations of rat TCGF (4–25 U/ml). They were restimulated with NOD spleen cells at intervals of 2–3 wk.

Proliferation Assays. Proliferation of T cells was measured as incorporation of [^{3}H]thymidine by the indicated number of T cells for 16–20 h after the specified period of stimulation with irradiated (2,000 rad) APC in the presence or absence of appropriate concentrations of antigens, and in the absence of added IL-2. LPS-activated splenic blast (LPS-blast) APC used for FCS-free activation were prepared from spleen cells depleted of T cells by two rounds of incubation with saturating concentrations of purified mAbs specific for Thy-1.2, CD4, and CD8, followed by complement lysis, all in FCS-free DME. The T cell–depleted spleen cells were cultured for 48 h in FCS-free DME supplemented with 0.5% NOD serum and LPS (10 μg/ml). The resultant blasts were washed twice with PBS before use as APC in a proliferation assay. FCS-free mouse IL-2 was prepared from 24-h culture supernatant of NOD spleen cells stimulated with Con A (5 μg/ml) in RPMI 1640 medium supplemented with only 0.5% NOD serum, and partially purified by NH_4SO_4 precipitation and extensive dialysis with PBS. Pancreatic β islets were isolated from NOD mice according to the method of Brunstedt et al. (28) using FCS-free DME medium supplemented with 0.5% NOD serum. The islets were digested to single cells in serum-free DME containing trypsin (0.05% wt/vol) and DNase (2 μg/ml) for 30 min at 37°C with agitation. The islet cells were cultured for 24 h in FCS-free DME/0.5% NOD serum before use.

Assay for Cytokine Production. The IL-2 content of culture supernatants was determined by bioassay using the IL-2–dependent CTLL-20 cell line (29) in the presence and absence of anti–IL-2 receptor (PC61 5.3) and anti–IL-4 (11B11) mAbs (1:10 dilution of hybridoma culture supernatants). 1 U of IL-2 is defined as the concentration that supports half-maximal proliferation of CTLL-20 cells. The IL-4, IFN-γ, and TNF-α concentrations were determined by ELISA assay. All of the antibodies used in the assays were purchased from PharMingen (San Diego, CA), and the assays were performed according to the manufacturer's protocol. The recombinant IL-4 (Schering-Plough, Kenilworth, NJ), IFN-γ (Amgen Biologicals, Thousand Oaks, CA), and TNF-α (Genzyme Corp.) were used as standards for ELISA assays.

Allogeneic Mixed Lymphocyte Reaction (MLR). The following procedure was routinely performed unless otherwise indicated. 5×10^5 nylon wool–purified NOD splenic T cells (responders) were cocultured with 5×10^5 irradiated (2,000 rad) unfractionated spleen cells from DBA/2 mice (stimulators), in the presence or absence of appropriate concentrations of various test reagents, for 116 h. Proliferation of responder T cells was then measured as incorporation of [^{3}H]thymidine during the last 20 h of culture.

Cloning and Sequencing of TCR mRNAs. The TCR α and β chain cDNA molecules were cloned and sequenced by anchored PCR as described previously (30).

Adoptive Transfer of Diabetes. 8-wk-old male or female NOD mice were irradiated (775 rad). Within 2 h, the mice were injected intravenously with pooled splenocytes ($1–2 \times 10^7$) derived from newly diabetic female NOD/Lt mice suspended in 400 μl of sterile PBS. For cotransfer with T cell clones, mice were injected with a mixture of appropriate T cells (1×10^7) and diabetogenic splenocytes ($1–2 \times 10^7$) suspended together in 400 μl of PBS. Animals were kept in pathogen-free conditions. Urine was tested for glucose (Keto-Diastix; Miles, Inc., Kankakee, IL), and glycosuric mice were checked for fasting serum glucose (Chemstrip bG, Accu-check III; Boehringer Mannheim Corp., Indianapolis, IN). Diabetes was diagnosed when permanent fasting glycemia >2.5 g/liter occurred.

Histology. Recipient mice were killed 21 d after spleen cell transfer. Pancreata were excised, fixed in buffered 10% formalin, and processed for paraffin sectioning. Tissue sections (5 μm) were stained with hematoxylin and eosin and microscopically evaluated for the presence of insulitis by using the following classification: 0, islet devoid of lymphocytic infiltration; 1, inflammatory mononuclear cells located peripheral to the islet; 2, mild insulitis, <25% of the islet interior contains lymphocytic inflammatory cells; 3, moderate insulitis, 25–50% of the interior of the islet contains lymphocytes; 4, severe insulitis, 50–100% of the islet interior is infiltrated; ES, end-stage islets devoid of β cells and associated lymphocytic infiltrate. All islets contained in two noncontiguous sections of the pancreas were examined and scored individually.

Results

Cellular Characteristics of Isolated Autoreactive T Cell Clones. Our panel of T cell clones was isolated from the spleen of unprimed 4-wk-old female NOD mice by a procedure that selects for growth of T cells reactive to syngeneic APC. The detailed procedure for generation of the clones is described in Materials and Methods. The T cell clones thus established responded routinely to irradiated (2,000 rad) NOD spleen cells in medium containing 10% FCS in the absence of IL-2. Indirect immunofluorescence staining using appropriate mAbs

Table 1. *Autoreactive T Cell Clones Respond to Syngeneic APC in an I-A^{g7}–restricted Manner*

Stimulator cells	[^{3}H]thymidine incorporation (cpm)					
	Spleen cells (+10% FCS)		LPS-blasts (FCS-free)			
	BALB/c	NOD	BALB/c	NOD	NOD + 10-2.16	NOD + MK-D6
NOD-5	413 ± 120	36,920 ± 2,266	1,866 ± 205	39,540 ± 1,603	3,100 ± 428	31,236 ± 3,300
NOD-6	2,796 ± 731	26,660 ± 4,992	1,536 ± 32	15,090 ± 1,199	2,130 ± 330	16,020 ± 2,065
NOD-14	190 ± 70	12,220 ± 2,163	776 ± 31	9,560 ± 1,361	850 ± 78	10,670 ± 629
NOD-21	1,253 ± 119	92,686 ± 5,435	1,950 ± 100	38,700 ± 4,937	10,030 ± 5,635	45,500 ± 8,122
NOD-2	1,056 ± 261	80,386 ± 9,720	1,110 ± 135	1,576 ± 150		
NOD-12	630 ± 88	74,400 ± 3,497	586 ± 145	876 ± 138		

5×10^4 T cells were incubated with 5×10^5 irradiated (2,000 rad) spleen or T cell–depleted LPS-blast stimulator cells (prepared as described in Materials and Methods) for 44 h. For FCS-free experiment, T cells were washed twice in protein-free DMEM before assay. For anti-Ia experiment, the stimulators were preincubated with the appropriate mAb (1:10 dilution of hybridoma supernatant) for 30 min at 4°C before assay. The cultures were pulsed with 1 μCi of [^{3}H]thymidine during the last 20 h of culture. The values shown are means ± SEM of triplicate cultures.

showed that all the clones were Thy-1.2$^+$, CD4$^+$CD8$^-$, and TCR-α/β^+ (data not shown).

All of the T cell clones (NOD-2, -5, -6, -12, -14, and -21) proliferated vigorously in response to irradiated (2,000 rad) NOD, but not to BALB/c, splenic APC in DME supplemented with 10% FCS (Table 1). They responded poorly to NOD splenic APC in the FCS-free medium (data not shown). However, NOD-5, -6, -14, and -21 proliferated vigorously in response to stimulation with syngeneic NOD but not to BALB/c LPS-blast APC in FCS-free DME supplemented with only 0.5% NOD serum (Table 1). In this experiment, the T cell clones were washed twice with FCS-free medium before the assay, and the LPS-blasts were prepared in the absence of FCS and in the presence of 0.5% NOD serum. T cell clones NOD-2 and NOD-12, which we have subsequently determined to be BSA/I-A^{g7} specific, were not activated by syngeneic LPS-blasts (Table 1). The I-A^{g7}–cross-reactive mAb 10-2.16, but not the irrelevant MK-D6 mAb (I-A^d specific), inhibited the autoreactive responses of T cell clones NOD-5, -6, -14, and -21 (Table 1). We conclude from data presented in Table 1 that NOD-5, -6, -14, and -21 are I-A^{g7}–restricted autoreactive T cell clones because they can proliferate in response to syngeneic LPS-blast APC in the absence of added foreign antigens, and that this response is inhibited by anti–I-A^{g7} mAb.

T cell clones NOD-2 and NOD-12 are not autoreactive but are BSA/I-A^{g7} specific, because they proliferated in a dose-dependent manner to BSA plus NOD splenic APC, and the responses were inhibited by the anti–I-A^{g7}–cross-reactive 10-2.16 mAb (data not shown). In a similar assay, T cell clones NOD-5, -6, -14, and -21 did not respond to BSA (data not shown). Since the ABBOS BSA peptide sequence (position 152–168) has been implicated as a possible trigger of human IDDM (31), we tested NOD-2 and NOD-12 for reactivity to ABBOS peptide. We observed that neither NOD-2 nor NOD-12 was reactive to ABBOS/I-A^{g7} (data not shown).

The lymphokine production profile of the T cell clones is shown in Table 2. Upon activation, T cell clones NOD-2, -5, -12, -14, and -21 showed enhancement of IL-2 and IFN-γ production but not IL-4. The NOD-2, -12, -14, and -21 T cell clones also produced TNF-α. Cross-reactivity of the antimurine TNF-α mAb pairs, used in the TNF-α ELISA assay, with TNF-β is not known (PharMingen technical information). Clone NOD-6 showed increased production of IL-4 but not of IL-2, IFN-γ, and TNF-α. Thus, NOD-6 belongs to the Th2 and the other T cell clones to the Th1 subset of T cells (32).

Specific Self Peptides Are Required for Activation of Autoreactive T Cell Clones. We next asked whether specific self peptides are required for autoreactive responses of NOD-5, -6, -14, and -21. We approached this question by examining the effect of peptide P1 on activation of our panel of autoreactive T cell clones. Peptide P1 (yTYTVHAAHAYTYt; small letters denote D amino acids) has been reported to bind I-A^{g7} and to block antigen presentation by I-A^{g7} (27). We observed that peptide P1 inhibited the autoreactive responses of NOD-5, -6, -14, and -21 T cell clones in a dose-dependent manner (Fig. 1). Peptide P1 also inhibited the BSA-specific responses of NOD-2 and 12 T cell clones, which were included as positive controls (Fig. 1). The inhibition was specific, since similar concentrations of peptide P2 (KMKMVHAAHAKMKM), which binds I-A^d but not I-A^{g7} (27), had no effect on the proliferative responses (Fig. 1). We conclude, therefore, that presentation of specific self peptides present on LPS-blasts by I-A^{g7} is required for autoreactive responses of NOD-5, -6, -14, and -21 T cell clones.

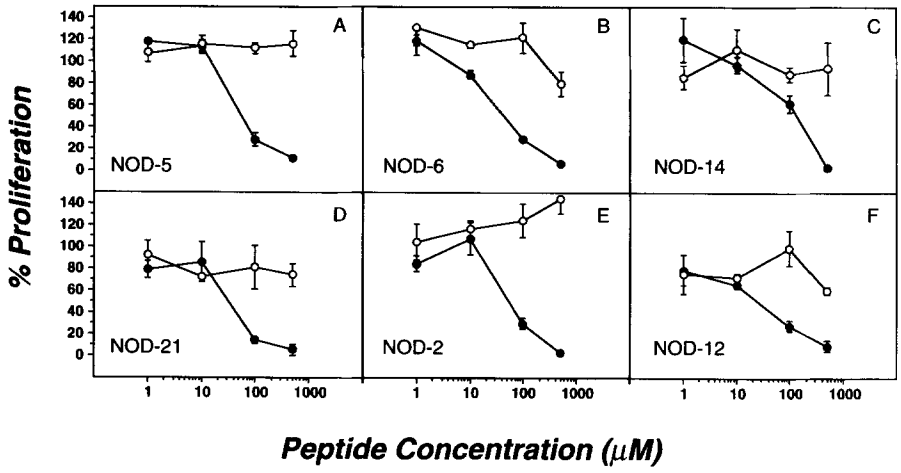

Figure 1. Selective inhibition of autoreactive and BSA-specific proliferative responses of NOD T cell clones by peptide P1 that binds I-A^{g7} (*closed circles*) and not by peptide P2 that binds I-A^{d} but not I-A^{g7} (*open circles*). LPS-activated NOD spleen cell APCs (*A–D*) or unactivated NOD spleen cells (*E, F*) were preincubated for 24 h with the indicated concentrations of peptide P1 or P2 in FCS-free DME supplemented with 0.5% NOD serum before irradiation (2,000 rad) and use as stimulators. T cell clones (5×10^4) were cocultured for 24 h with the pretreated stimulators (5×10^5) in the presence of the indicated concentrations of peptide P1 or P2 in FCS-free DME/0.5% NOD serum medium, and, for *E* and *F*, in the presence of BSA (1 mg/ml). The cultures were pulsed with [^{3}H]thymidine (1 μCi/culture) and harvested 20 h later. 100% proliferation denotes [^{3}H]thymidine incorporation of activated T cells in the absence of P1 or P2. 0% proliferation was background proliferation of T cells stimulated with BALB/c LPS-blasts (*A–D*) or NOD spleen cells in the absence of added BSA (*E, F*). The values for 0% and 100% for *A–F* were: 2,603 ± 228 and 30,063 ± 4,088 (*A*); 1,020 ± 271 and 10,136 ± 1,024 (*B*); 63 ± 32 and 14,866 ± 1,230 (*C*); 875 ± 219 and 11,613 ± 360 (*D*); 895 ± 147 and 32,266 ± 2,967 (*E*); and 1,160 ± 1,018 and 24,870 ± 3,521 (*F*). Values shown are means ± SEM of triplicate experiments.

Three Autoreactive T Cell Clones Are Reactive to β Islet Cell Antigen(s). We tested our panel of autoreactive T cell clones for reactivity to autoantigens present on NOD pancreatic β islet cells. Activation of T cell clones by NOD splenic APC in the presence or absence of NOD islet cells was assessed in FCS-free DME supplemented with 0.5% NOD serum. Fig. 2 shows that NOD-5, -6, -14, and -21 were not significantly activated by NOD splenic APC alone in the FCS-free DME/0.5% NOD serum medium. The addition of 10,000 irradiated (2,000 rad) NOD β islet cells to 5×10^5 NOD splenic APC resulted in enhanced proliferation of NOD-5, -14, and -21 T cell clones. The autoreactive NOD-6 T cell clone, which is similar to NOD-5, -14, and -21 in its responsiveness to LPS-blasts, is not activated by the presence of islet cells.

T Cell Receptor Diversity of Autoreactive T Cell Clones. The repertoire of TCRs expressed by the T cell clones was determined. The TCR-α and -β cDNAs were amplified by anchored PCR and subcloned into a plasmid vector, and the V/J junctional cDNA sequences were determined. The sequences of the in-frame α and β chain V/J junctional CDR-3 region are presented in Fig. 3, *A* and *B*. The surface Vβ expression was confirmed by indirect immunofluorescence using the Vβ2-, Vβ8-, and Vβ14-specific mAbs B20.6, F23.1, and 14-2, respectively. Two of the T cell clones express TCR Vβ8.1 region (NOD-14 and NOD-21), and two express TCR Vα4 region (NOD-6 and NOD-14); otherwise, the Vα, Vβ, Jα, and Jβ segment usage was diverse, and there was neither apparent sequence identity nor selection for particular amino acid residues in the V/J junctional region.

Table 2. *Lymphokine Production by NOD T Cell Clones*

T cell clones	IL-2	IL-4	IFN-γ	TNF
	U/ml	*U/ml*	*U/ml*	*pg/ml*
NOD-5	3.0 ± 0.1	<10.0	86.0 ± 4.9	<30.0
NOD-6	<0.5	158.0 ± 3.2	<8.0	<30.0
NOD-14	2,704.0 ± 40.0	<10.0	307.0 ± 14.0	2,496.0 ± 65.0
NOD-21	8.1 ± 0.1	<10.0	315.0 ± 11.0	1,243.0 ± 124.0
NOD-2	26.0 ± 7.0	<10.0	219.0 ± 11.2	2,237.0 ± 81.0
NOD-12	7.7 ± 2.6	<10.0	275.0 ± 12.5	948.0 ± 157.0

5×10^5 T cells were cultured in the presence and absence of 5×10^6 irradiated (2,000 rad) NOD spleen cells in 1.0 ml cultures. The differences in lymphokine contents of supernatants harvested from 24-h culture of stimulated and unstimulated T cell clones were determined as described in Materials and Methods. Values shown are means ± SEM of triplicate assays.

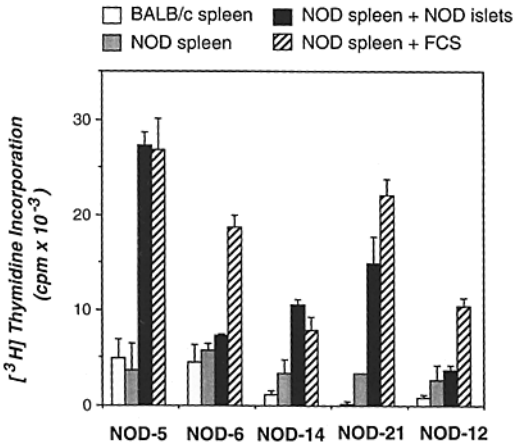

Figure 2. T cell clones NOD-5, -14, and -21 are reactive to β islet cell antigen(s). T cells (5×10^4) were cocultured for 44 h with irradiated BALB/c or NOD spleen cells (5×10^5) in FCS-free medium supplemented with 0.5% NOD serum in the presence or absence of NOD β islet cells (10,000 cells) or 10% FCS. The cells were pulsed with [^{3}H]thymidine (1 μCi/culture) during the last 20 h of culture. Values shown are means ± SEM of triplicate experiments.

β Islet Cell–reactive Autoreactive T Cell Clones Suppressed Development of IDDM. To examine the effect of NOD-5 (islet reactive) on spontaneous development of diabetes, we injected 1×10^7 NOD-5 T cells into 4-wk-old unirradiated female NOD mice housed in open cages. They were suitable as recipients because, at 4 wk of age, pancreatic histology showed these mice to be free of intraislet lymphocytic infiltration; hyperglycemia does not develop until after 4 mo of age when kept at our animal facility. Diabetes developed at age 6–7 mo in 66% of control unmanipulated animals, and in 75% of mice when given a single injection of 1×10^7 control nylon wool–purified splenic T cells from 4-wk-old female NOD mice. By contrast, a single intraperitoneal injection of 1×10^7 NOD-5 T cells into 4-wk-old NOD mice completely protected the mice from diabetes for the entire 12 mo of observation (Fig. 4 *A*).

The adoptive transfer procedure is widely accepted as a valid method for rapid assessment of insulitis and diabetes in recipient mice (37). We used this protocol for subsequent testing of T cell clones in vivo. Fig. 4 *B* shows that all control mice receiving 1×10^7 diabetogenic donor spleen cells alone developed overt diabetes within 3 to 4 wk of transfer. In experiment 1, mice receiving a cotransfer of the Th1 islet-reactive NOD-5, NOD-14, or NOD-21 T cell clones remained normoglycemic in the 21-d period of observation before the killing of the animals for pancreatic histology. In contrast, the Th2 autoreactive NOD-6 and the Th1 BSA/I-A^{g7}–specific NOD-2 and NOD-12 T cell clones were not protective. In a second experiment, we observed that NOD-5, NOD-14, and NOD-21 T cell clones significantly inhibited adoptive transfer of diabetes for at least an 8-wk period.

To control for the possibility that BSA-specific T cell clones would not be activated in vivo because of the absence of antigen, we performed an additional experiment whereby the BSA-specific NOD-2 cotransferred mice were given a twice-a-week injection of 1 mg BSA per mouse (Fig. 4 *B*, *NOD-2 Expt. 2*). Injection of BSA had no effect on the kinetics of development of diabetes.

Protective T Cell Clones Inhibited Lymphocytic Infiltration of Pancreatic Islets. The pancreatic islets of recipient mice, ob-

A

CLONE		V	N	J	
		L C A A M G		D R G S A	
NOD-5	Vα10	CTC TGT GCT GCT ATG GG	G	GAT AGA GGT TCA GCC	JαLB2
		Y C A L S	G E	N M G Y K	
NOD-6	Vα4	TAC TGT GCT CTG AGT G	GG GAG	AAC ATG GGC TAC AAA	JαTA19
		Y C A L S	D L G	G G S G N	
NOD-14	Vα4	TAC TGC GCT CTG AGT G	AT CTG GG	T GGG GGC AGT GGC AAC	JαTT11
		F C A A S	S R	N Y G N E	
NOD-21	Vα1	TTC TGC GCA GCA AGC	AGT AGA	AAC TAT GGA AAT GAG	JαTA31

B

CLONE		V	N	D	N	J	
		L C A W S	F A	G T G G		D E R L F	
NOD-5	Vβ14	CTC TGT GCC TGG AGT	TTC GCC	GGG ACA GGG GGC	G	AC GAA AGA TTA TTT	Jβ1-4
		C T C S	A E	R	R	D T Q Y F	
NOD-6	Vβ2	TGC ACC TGC AGT G	CA GAA C	GG A	GG	GAC ACC CAG TAC TTT	Jβ2-5
		F C A S R		D R G		Y G Q Y F	
NOD-14	Vβ8.1	TTC TGT GCC AGC AG		G GAC AGG GG		C TAT GGA CAG TAC TTC	Jβ2-7
		F C A S S Q	I	G G		D Q A E Q	
NOD-21	Vβ8.1	TTC TGT GCC AGC AGT GAT	AT	T GGG GGG G		AC GAT GCT GAG CAG	Jβ2-1

Figure 3. Nucleotide and predicted amino acid sequences of the junctional regions of TCR α chain (*A*) and β chain (*B*) of autoreactive T cell clones NOD-5, -6, -14 and -21. These sequence data are available from EMBL/GenBank/DDBJ under accession numbers U27223–U27230. The assignment of Vβ junctions was based on homology comparison of the determined cDNA sequences with the germline sequences for Vβ14 (33) and Vβ8.1/Vβ8.2/Vβ8.3 (34), and deduced for NOD-6 (Vβ2) by comparison with mRNA sequences available for Vβ2 from GenBank. The assignment of Dβ and Jβ nucleotide sequences was based on comparison of the determined sequences with the published germline Dβ1.1/Dβ2.1 and germline Jβ sequences (35, 36). The assignment for the Vα junctions was deduced from comparison of the determined sequences with all GenBank mRNA sequences available for the particular Vα family. The nomenclature and assignment of Jα sequences were based on the germline TCR α and δ chain locus available from GenBank (MUSTCRA, accession number M64239).

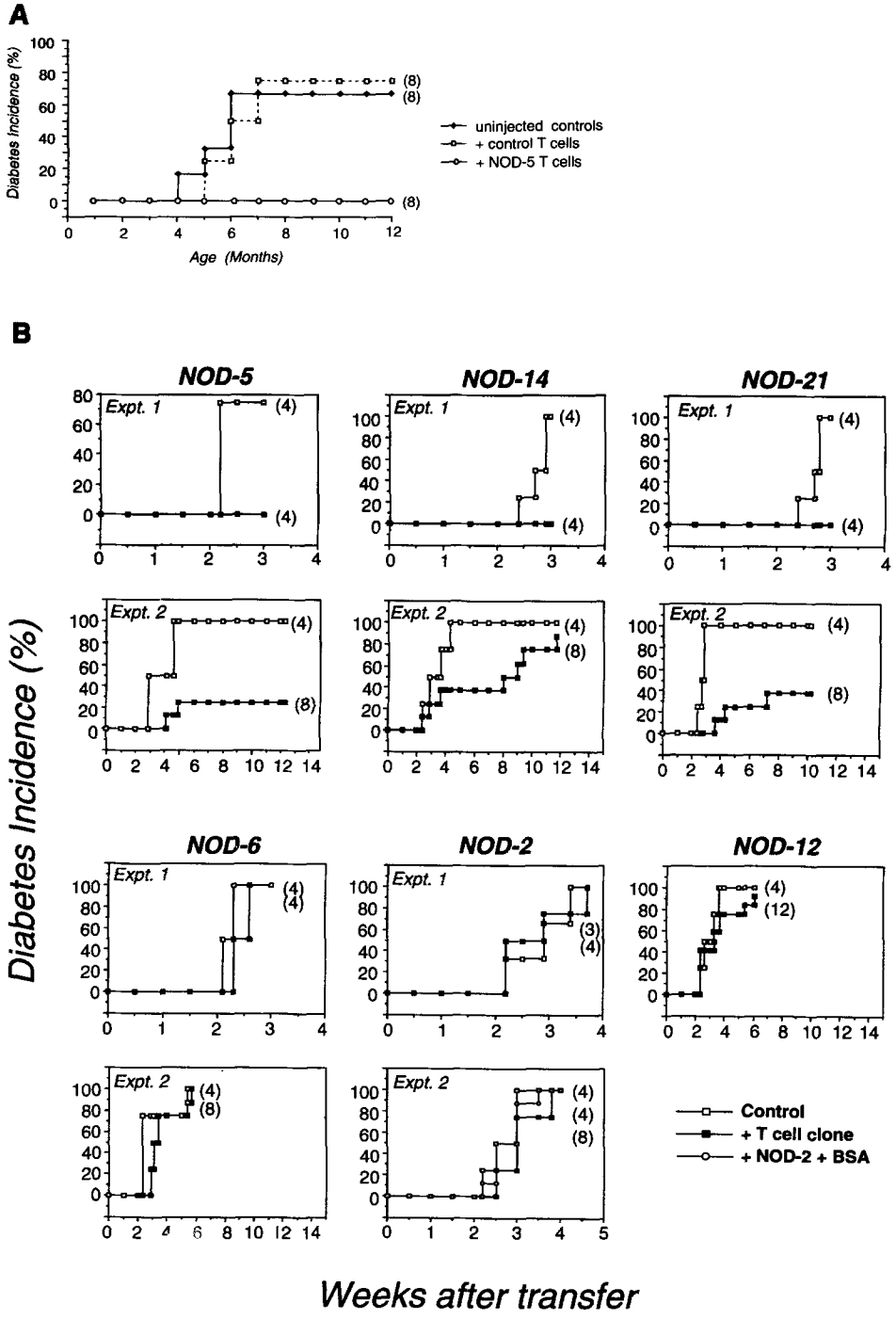

Figure 4. The effect of in vivo inoculation autoreactive T cell clones on development of IDDM. (A) Effect of NOD-5 T cell clone on spontaneous development of diabetes when injected into young mice. 4-wk-old unirradiated female NOD mice were given a single intraperitoneal injection of 1 × 10^7 resting and pathogen-free NOD-5 T cells (*open circles*). Control animals received either no T cell injection (*closed diamonds*) or injection of 1 × 10^7 nylon wool-purified splenic T cells from 4-wk-old female NOD mice (*open squares*). Animals were kept in open cages and were monitored for onset of diabetes. (B) Effect of cotransfer of NOD T cell clones on adoptive transfer of diabetes. 8-wk-old female or male NOD mice were irradiated (775 rad) 2–4 h before intravenous injection of 1 × 10^7 unfractionated pooled spleen cells from newly diabetic female NOD/Lt mice, with (*closed squares*) or without (*open squares*) cotransfer of 1 × 10^7 NOD T cell clones that had been activated with syngeneic spleen cells 2 wk before the experiment. Animals were kept in pathogen-free condition and were monitored for onset of diabetes at the indicated intervals.

tained 3 wk after adoptive transfer, were scored for severity of insulitis according to a standard grading system (see Materials and Methods). Table 3 shows the effect of cotransfer of the T cell clones on development of insulitis. All of the islets of mice receiving donor diabetic spleen cells alone exhibited an advanced degree of insulitis (grade 3, 4, and end stage as described in Materials and Methods). By contrast, most (>70%) of the islets in NOD-5, NOD-14, and NOD-21 coinjected animals were normal or lightly infiltrated in the periphery (grade 0, 1, and 2). Nonprotective autoreactive NOD-6 and BSA/I-A^{g7}–specific NOD-12 T cell clones did not prevent lymphocytic infiltration of β islets, because >75% of the islets examined showed severe insulitis (grade 3 and 4).

Protective T Cell Clones Secrete an Activity That Inhibits In Vitro Allogeneic MLR. IL-2–free culture supernatants (SN) were generated as follows. The T cell clones (1 × 10^6) were activated with 5 × 10^6 irradiated (2,000 rad) NOD splenic

Table 3. *Effect of Cotransfer of T Cell Clones on Adoptive Transfer of Insulitis*

Treatment	Number of mice	Histological grades						Total number of islets
		0	1	2	3	4	ES	
		Percentage of islets						
Experiment 1								
Donor spleen alone	2	0	0	0	6	25	69	16
+ Control T cells*	2	0	0	0	45	23	32	31
+ NOD-5	2	38	26	12	10	14	0	81
+ NOD-12	2	16	0	3	34	41	6	32
Experiment 2								
Donor spleen alone	4	0	0	0	0	31	68	74
+ NOD-5	4	57	24	6	7	3	4	158
+ NOD-12	4	0	0	0	1	45	53	82
Experiment 3								
Donor spleen alone	4	0	0	3	11	54	32	74
+ NOD-14	4	35	19	16	19	6	5	113
+ NOD-21	4	60	24	7	5	0	5	104
Experiment 4								
Donor spleen alone	4	0	0	3	9	66	22	32
+ NOD-6	4	0	1	1	21	67	11	101

8-wk-old male and female NOD mice were irradiated (775 rad) and received an intravenous injection of 1×10^7 spleen cells pooled from newly diabetic female NOD mice with or without a coinjection of 1×10^7 of appropriate T cell clones. Mice were killed 3 wk after cell transfers and pancreatic sections were examined and individual islets were graded for degree of insulitis. Grade 0, islet devoid of lymphocytic infiltration; 1, inflammatory mononuclear cells located peripheral to the islet; 2, mild insulitis, <25% of the islet interior contains lymphocytic inflammatory cells; 3, moderate insulitis, 25–50% of the interior of islet contains lymphocytes; 4, severe insulitis, 50–100% of the islet interior is infiltrated; ES, end stage islets devoid of β cells and associated lymphocytic infiltrate.

* Control T cells are nylon wool–purified splenic T cells derived from 4-wk-old female NOD mice.

APC in 1 ml of DME supplemented with 10% FCS. 48 h later, the activated T cells were separated from dead APC by Ficoll density centrifugation. They were incubated at a density of 1×10^6/ml for another 24 h in DMEM supplemented with 10% FCS without added IL-2. We have determined in a separate time course experiment that our panel of T cell clones produced IL-2 only during the first 24 h after activation. Thus, SN collected between 48 and 72 h after activation contained an insignificant amount of IL-2. The serially diluted SN were assayed for their ability to inhibit allogeneic MLR.

We observed a dose-dependent inhibition of allogeneic MLR by NOD-5, NOD-14, and NOD-21 SN (Fig. 5). SN harvested from similarly activated nonprotective autoreactive NOD-6 or BSA-specific NOD-2 and NOD-12 T cell clones were inactive (Fig. 5). The NOD-5 SN is the most potent of the three SN in inhibiting allo-MLR, followed by NOD-21 SN and then NOD-14 SN. This order of relative potencies of culture supernatants correlated with the protective capacities of the respective T cell clones; that is, NOD-5 is the most protective of the three, followed by NOD-21 and NOD-14.

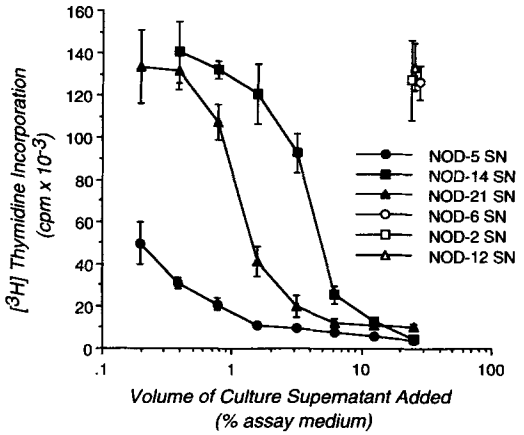

Figure 5. Protective T cell clones secrete an activity that inhibits allogeneic MLR. Culture supernatant harvested from culture of the indicated T cell clones, prepared as described in Results, were serially diluted. 50 μl of the diluted samples were added to a standard allogeneic MLR assay described in Materials and Methods. The cultures were pulsed 96 h later with [^{3}H]thymidine (1 μCi/culture) for 20 h. Values shown are means ± SEM of triplicate cultures.

We have examined NOD-5 SN for possible contamination by pathogens that could cause nonspecific growth inhibition. Several batches of active NOD-5 SN have been determined to be free of endotoxin (limulus amebocyte lysate test was performed by Associates of Cape Cod, Inc., Woods Hole, MA), and bacteria (culture supernatant was tested by Dana-Farber Cancer Institute hospital microbiology laboratory). We have also verified that NOD-5 T cell clone cultured in antibiotic-free medium for >28 d is free of mycoplasma (mycoplasma test kit, Gibco BRL, Gaithersburg, MD).

Partially Purified Inhibitory Activity Suppressed Adoptive Transfer of IDDM. We next examined the effect of the partially purified allogeneic MLR inhibitory activity, produced by the protective NOD-5 T cell clone, on adoptive transfer of IDDM in NOD mice. The IL-2–free SN prepared as described for Fig. 5 were partially purified to (*a*) concentrate them into a smaller volume suitable for injections, and (*b*) remove other cytokines, such as TNF, IFN-γ, TGF-β, and IL-10. The culture SN were concentrated by precipitation with ammonium sulphate solution (50% saturated). The precipitates were resuspended in one-hundredth of original volumes of PBS and dialyzed extensively against PBS. The dialyzed materials were then passed through heparin-Sepharose column (Pharmacia Biotech, Inc., Piscataway, NJ), which does not bind the NOD-5 inhibitory factor but binds and removes TNF, IFN-γ, TGF-β, and IL-10 (38–40). The column eluate was the "partially purified SN" used in the adoptive transfer experiment described below. We have verified that the 100-fold concentrated and partially purified SN was ~50-fold more potent than the starting material in inhibiting allogeneic MLR (data not shown).

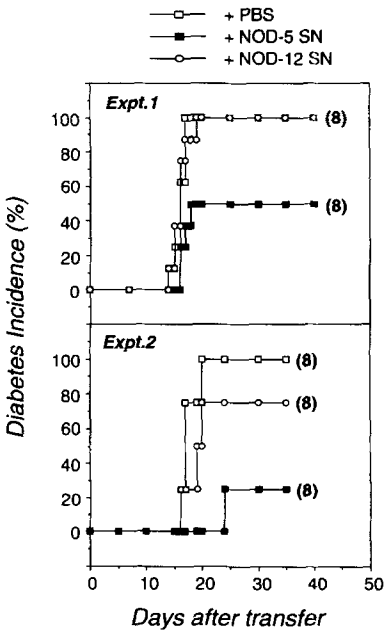

Figure 6. Partially purified NOD-5 culture supernatant inhibits adoptive transfer of IDDM. 8-wk-old female or male NOD/Lt mice were irradiated (775 rad) 2 h before intravenous injection of 2 × 10^7 unfractionated pooled spleen cells from newly diabetic female NOD/Lt mice, with or without cotransfer of 300 μl of partially purified NOD-5 SN or NOD-12 SN. At weekly intervals, the recipient mice received intravenous injection of the same volume of partially purified SN. Animals were kept in pathogen-free condition and were monitored at the indicated intervals for onset of diabetes.

The partially purified NOD-5 or NOD-12 SN (300 μl each), which is equivalent to a starting volume of 3 ml culture SN, was coinjected with 2 × 10^7 donor diabetogenic spleen cells intravenously into preirradiated (775 rad) recipient NOD mice. At weekly intervals, the mice received intravenous injections of the same volume of partially purified SN. In two separate experiments, we observed that injection of the partially purified NOD-5 SN but not NOD-12 SN significantly protected NOD mice from adoptive transfer of IDDM (Fig. 6). The degree of protection was not as impressive as cotransfer of NOD-5 T cell clones as shown in Fig. 4 *B*, suggesting that component(s) other than the secreted inhibitory factor may play additional roles in the protective action of NOD-5. Alternatively, the dose and frequency of injection of the partially purified NOD-5 SN may not have been optimal for protection.

Discussion

Our study was initiated by the hypothesis that autologous MLR (AMLR), or syngeneic MLR, which is present in all normal individuals, may represent interactions among T lymphocytes and other immunological cells that regulate immune responses in the absence of foreign antigens. The frequent association of impaired AMLR with autoimmune diseases in humans (reviewed in 41) and in mice (42) further supports the hypothesis that "AMLR-competent" T cells may play a role in maintaining peripheral T cell tolerance to self antigens. With this hypothesis in mind, we attempted to isolate "AMLR-competent" T cell clones, which we defined initially as T cell clones that are capable of responding to syngeneic APC in the absence of added foreign antigens. We chose to do this isolation in a relevant autoimmune animal model (NOD mouse) that manifested deficient AMLR (43, 44).

We have succeeded in isolating four "AMLR-competent" T cell clones from the spleens of unprimed NOD mice. These T cell clones can be activated by stimulation with (*a*) NOD splenic APC in the presence of 10% FCS; (*b*) NOD LPS-stimulated splenic blasts in the absence of FCS; and (*c*) NOD splenic APC plus β islet cells in the absence of FCS for three of the clones. We postulate that LPS-activated B cells and macrophages express high levels of self antigens, which are required for recognition by autoreactive T cell clones. FCS may be required because it contains factors that activate these APC, a possibility that has been discussed by Clayberger et al. (45). We have narrowed antigen specificity of clones NOD-5, -14, and -21 to self antigens expressed on NOD β islet cells. The islet antigen(s) recognized by these T cell clones are likely to be expressed on LPS-blasts and FCS-stimulated APC as well, since these APC are capable of activating the T cell clones in the absence of islet cells.

The autoreactive T cell clones NOD-5, -6, -14, and -21 express TCR bearing Vβ14, Vβ2, Vβ8.1, and Vβ8.1 regions, respectively. There was no predominant selection for use of any particular Vα or Vβ segment by our panel of autoreac-

tive T cell clones, indicating that I-A^{g7} restriction is not associated with preferential usage of a particular V region segment. There was no apparent selection for specific amino acid residues within the V/J junctional region. The presence of selective junctional amino acid usage would have implications for a restricted self peptide specificity (46). The lack of restricted V region TCR sequences suggests the TCR may recognize either a diverse set of target antigens or different epitopes of a single target antigen. We need to know the self peptides recognized by our panel of TCR to discriminate between these two possibilities.

We have shown that the β islet cell–reactive NOD-5, NOD-14, and NOD-21 T cell clones significantly inhibited adoptive transfer of IDDM. The protective capacity of these Th1 T cell clones is not correlated with production of IL-2, TNF, or IFN-γ. These cytokines are also produced by the BSA-specific NOD-2 and NOD-12 T cell clones, which were not protective even when BSA was coadministered to the recipient mice. Depending on the age and route of administration, TNF-α and IFN-γ have been reported to exacerbate or inhibit IDDM development in NOD mice (47–50). Our panel of Th1 T cell clones does not exacerbate disease and the BSA-specific Th1 T cell clones do not inhibit disease. These phenomena suggest that the in vivo level of TNF-α and IFN-γ produced by the inoculated T cell clones may be inadequate to affect disease development. The level of TNF-α and IFN-γ production may be limited by the concentrations of the activating autoantigens available in vivo. IL-2, IFN-γ, and TNF-α lymphokines are produced by our panel of Th1 T cell clones only during the first 24 h after activation. In contrast, production of the allo-MLR inhibitory factor by the protective T cell clones persisted for 3 wk after activation (Akhtar, I., and K.-N. Tan, manuscript in preparation). Therefore, the in vivo effect of the protective Th1 T cell clones is likely to be dominated by the allo-MLR inhibitory factor.

We have shown that protective capacity of these T cell clones correlates with (*a*) their reactivity to β islet cell antigen(s), and (*b*) their production of an activity that potently suppresses allogeneic MLR in vitro. The β islet cell reactivity may dictate the specificity of action of the protective T cell clones. We observed that protective T cell clones inhibited intraislet but not periislet lymphocytic infiltration. It is possible that the protective islet-reactive T cell clones may migrate to and reside near the islets, thereby inhibiting intraislet lymphocytic infiltration of diabetogenic lymphocytes. This possibility remains to be verified. With regard to production of a factor that inhibits allogeneic MLR, our panel of protective autoreactive T cell clones resembles the contemporary definition of suppressor T cells, in that they produce factor(s) capable of down-regulating immune responses. The partially purified inhibitory activity secreted by NOD-5, which was first depleted of IL-2, IFN-γ, TNF, TGF-β, and IL-10, retained its ability to protect mice from adoptive transfer of IDDM. The biological and biochemical characteristics of this activity will be reported elsewhere (Akhtar, I., and K.-N. Tan, manuscript in preparation). Identification of the molecular structure of this factor, presently in progress, should allow us to further delineate the mechanism of action of this molecule.

The reason for development of our panel of protective T cell clones into a Th1 lineage is at present unclear. Autoimmunity is usually attributed to Th1 cells, whereas Th2 responses are regarded as protective (51). Furthermore, recent observations indicate that susceptibility or resistance to spontaneous autoimmunity in different strains of mice is influenced by a non–MHC-encoded polymorphism that dictates predisposition of T cells toward preferential differentiation to Th1 versus Th2 phenotype, with Th2 being protective and Th1 pathogenic (52). Our panel of protective autoreactive T cell clones is Th1 instead of Th2 and therefore appears to contradict these expectations. However, there are other reports that disagree with the simple prediction that Th1 is pathogenic and Th2 is protective. For example, Wogensen et al. have shown that transgenic expression of IL-10 in β islet cells, which promoted a Th2-like lymphokine pattern of islet-infiltrating cells, accelerated the onset of diabetes and increased its prevalence. The authors suggested that β cell destruction in NOD mice may therefore sometimes be a Th2-mediated event (53). Additionally, recent observations indicate that the inflammatory foci in NOD mice are dominated by a Th2 cytokine response (54). It is likely that β cell destruction and T cell suppression of autoimmunity may each involve a more complex interplay between Th1 and Th2 cells than is currently conceived. The respective roles of these subsets may vary depending on the stage of the disease and on the site of action. Our data provide clear evidence in support of the existence of Th1 β islet cell–reactive T cells that have acquired a protective effector function. They mediate their protective action in part by production of an activity capable of down-modulating immune responses. We suggest that this type of $CD4^+$ autoreactive T cells may form part of a regulatory network of T cells that normally plays a role in maintaining peripheral tolerance.

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References

1. Castano, L., and G.S. Eisenbarth. 1990. Type I diabetes: a chronic autoimmune disease of human, mouse, and rat. *Annu. Rev. Immunol.* 8:647–679.
2. Haskins, K., and M. McDuffie. 1990. Acceleration of diabetes in young NOD mice by $CD4^+$ islet specific T cell clone. *Science (Wash. DC).* 249:1433–1436.
3. Nakano, N., H. Kikutani, H. Nishimoto, and T. Kishimoto. 1991. T cell receptor V gene usage of islet β cell–reactive T cells is not restricted in non-obese diabetic mice. *J. Exp. Med.* 173:1091–1097.
4. Reich, E.-P., R.S. Sherwin, O. Kanagawa, and C.A. Janeway. 1989. An explanation for the protective effect of the MHC class II I-E molecule in murine diabetes. *Nature (Lond.).* 341:326–329.
5. Tisch, R., X.-D. Yang, S.M. Singer, R.S. Libau, L. Fugger, and H.O. McDevitt. 1993. Immune response to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mice. *Nature (Lond.).* 366:72–75.
6. Kaufman, D.L., M. Calre-Salzler, J. Tian, T. Forsthuber, G.S.P. Ting, P. Robinson, M.A. Atkinson, E.E. Sercarz, A.J. Tobin, and P.V. Lehmann. 1993. Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in mature insulin-dependent diabetes. *Nature (Lond.).* 366:69–72.
7. Todd, J.A. 1990. Genetic control of autoimmunity in type 1 diabetes. *Immunol. Today.* 11:122–129.
8. Nishimoto, H., H. Kikutani, K.-I. Yamamura, and T. Kishimoto. 1987. Prevention of autoimmune insulitus by expression of I-E molecules in NOD mice. *Nature (Lond.).* 328: 432–434.
9. Bohme, J., B. Schuhbaur, O. Kanagawa, C. Benoist, and D. Mathis. 1990. MHC-linked protection from diabetes dissociated from clonal deletion of T cells. *Science (Wash. DC).* 249:293–295.
10. Miyazaki, T., M. Uno, M. Uehira, H. Kikutani, T. Kishimoto, M. Kimoto, H. Nishimoto, J. Miyazai, and K.-I. Yamamura. 1990. Direct evidence for the contribution of the unique $I\text{-}A^{NOD}$ to the development of insulitis in non-obese diabetic mice. *Nature (Lond.).* 345:722–724.
11. Slattery, R.M., L. Kjer-Nielsen, J. Allison, B. Charlton, T. E. Mandel, and J.F. Miller. 1990. Prevention of diabetes in non-obese diabetic $I\text{-}A^K$ transgenic mice. *Nature (Lond.).* 345:724–726.
12. Lund, T., L. O'Reily, P. Hutchings, O. Kanagawa, E. Simpson, R. Gravely, P. Chandler, J. Dyson, J.K. Picard, A. Edwards, et al. 1990. Prevention of insulin-dependent diabetes mellitus in nonobese diabetic mice by transgenes encoding modified I-A beta-chain or normal I-E alpha-chain. *Nature (Lond.).* 345:727–729.
13. Katz, J.D., B. Wang, K. Haskins, C. Benoist, and D. Mathis. 1993. Following a diabetogenic T cells from genesis to pathogenesis. *Cell.* 74:1089–1100.
14. Slattery, R.M., J.F.A.P. Miller, W.R. Heath, and B. Charlton. 1993. Failure of a protective major histocompatibility complex class II molecule to delete autoreactive T cells in autoimmune diabetes. *Proc. Natl. Acad. Sci. USA.* 90:10808–10810.
15. Singer, S.M., R. Tisch, X.-D. Yang, and H.O. McDevitt. 1993. An Ab^d transgene prevents diabetes in nonobese diabetic mice by inducing regulatory T cells. *Proc. Natl. Acad. Sci. USA.* 90:9566–9570.
16. Boitard, C., R. Yasunami, M. Dardenne, and J.-F. Bach. 1989. T cell–mediated inhibition of the transfer of autoimmune diabetes in NOD mice. *J. Exp. Med.* 169:1669–1680.
17. Reich, E.-P., D. Scaringe, J. Yagi, R.S. Sherwin, and C.A. Janeway. 1989. Prevention of diabetes in NOD mice by injection of autoreactive T cells. *Diabetes.* 38:1647–1651.
18. Pankewycz, O., J.-X. Guan, and J.F. Benedict. 1993. A protective NOD islet infiltrating $CD8^+$ T cell clone, I.S. 2.15 has in-vitro immunosuppressive properties. *Eur. J. Immunol.* 22: 2017–2023.
19. Chosich, N., and L.C. Harrison. 1993. Suppression of diabetes mellitus in the non-obese diabetic mouse by an autoreactive (anti-$I\text{-}A^{g7}$) islet derived $CD4^+$ T cell line. *Diabetologia.* 36:716–721.
20. Utsugi, T., M. Nagata, T. Kawamura, and J.-W. Yoon. 1994. Prevention of recurrent diabetes in syngeneic islet-transplanted NOD mice by transfusion of autoreactive T lymphocytes. *Transplantation (Balt.).* 57:1799–1804.
21. Oi, V.T., P.P. Jones, J.W. Goding, L.A. Herzenberg, and L.A. Herzenberg. 1978. Properties of monoclonal antibodies to mouse Ig allotypes, H-2 and Ia antigens. *Curr. Top. Microbiol. Immunol.* 81:115–120.
22. Kappler, J.W., B. Skidmore, J. White, and P. Marrack. 1981. Antigen-inducible, H-2 restricted, interleukin 2–producing T cell hybridomas. Lack of independent antigen and H-2 recognition. *J. Exp. Med.* 153:1198–1214.
23. Ohara, J., and W.E. Paul. 1985. Production of a monoclonal antibody to and molecular characterization of B-cell simulatory factor-1. *Nature (Lond.).* 315:333–336.
24. Necker, A., N. Rebai, M. Matthes, E. Jouvin-Marche, P.-A. Cazenave, P. Swarnworawong, E. Palmer, H.R. MacDonald, and B. Malissen. 1991. Monoclonal antibodies raised against engineered soluble mouse T cell receptors and specific for Vα8-, Vβ2- or Vβ10-bearing T cells. *Eur. J. Immunol.* 21:3035–3040.
25. Staerz, U.D., H.-G. Rammensee, J.D. Benedetto, and M.J. Bevan. 1985. Characterization of a murine monoclonal antibody specific for an allotypic determinant on T cell antigen receptor. *J. Immunol.* 134:3994–4000.
26. Liao, N.-S., J. Maltzman, and D.H. Raulet. 1989. Positive selection determines T cell receptor Vβ14 gene usage by $CD8^+$ T cells. *J. Exp. Med.* 170:135–143.
27. Hurtenbach, U., E. Lier, L. Adrioni, and Z.A. Nagy. 1993. Prevention of autoimmune diabetes in nonobese diabetic mice by treatment with a class II major histocompatibility complex blocking peptide. *J. Exp. Med.* 177:1499–1504.
28. Brunstedt, J., J.H. Nielsen, and A. Lernmark. 1985. Isolation of islets from mice and rats. *In* Methods in Diabetes Research. J. Larner and S.L. Pohl, editors. John Wiley & Sons, Inc., New York. I:245–265.
29. Baker, P.E., S. Gillis, and K.A. Smith. 1979. Monoclonal cyto-

lytic T cell lines. *J. Exp. Med.* 149:273–278.
30. Lipes, M.A., A. Rozenzweig, K.-N. Tan, G. Tanigawa, D. Ladd, J.G. Seidman, and G.S. Eisenbarth. 1993. Progression to diabetes in nonobese diabetic (NOD) mice with transgenic T cell receptors. *Science (Wash. DC).* 259:1165–1169.
31. Karjalainen, J., J.M. Martin, M. Knip, J. Ilonen, B.H. Robinson, E. Savilahti, H.K. Akerblom, and H.M. Dosh. 1992. A bovine serum albumin peptide as a possible trigger of insulin-dependent diabetes mellitus. *N. Engl. J. Med.* 327:302–307.
32. Mosmann, T.R., and R.L. Coffman. 1989. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* 7:145–173.
33. Malissen, M., C. McCoy, D. Blanc, J. Trucy, C. Devaux, A.-M. Schitt-Verhulst, F. Fitch, L. Hood, and B. Malissen. 1986. Direct evidence for chromosomal inversion during T-cell receptor beta-gene rearrangements. *Nature (Lond.).* 319:28–32.
34. Chou, H.S., S.J. Anderson, M.C. Louie, S.A. Godambe, M.R. Pozzi, M.A. Behlke, K. Huppi, and D.Y. Loh. 1987. Tandem linkage and unusual RNA splicing of the T-cell receptor beta-chain variable-region genes. *Proc. Natl. Acad. Sci. USA.* 84: 1992–1996.
35. Barth, R.K., B.S. Kim, N.C. Lan, T. Hunkapiller, N. Sobieck, A. Winoto, H. Gershenfeld, C. Okada, D. Hansburg, I.L. Weissman, and L. Hood. 1985. The murine T cell receptor employs a limited repertoire of expressed Vβ gene segments. *Nature (Lond.).* 316:517–523.
36. Gascoigne, N.R., Y.-H. Chien, D.M. Becker, J. Kavaler, and M.M. Davis. 1984. Genomic organization and sequence of T cell receptor beta-chain constant- and joining-region genes. *Nature (Lond.).* 310:387–391.
37. Wicker, L.S., B.J. Miller, and Y. Mullen. 1986. Transfer of autoimmune diabetes mellitus with splenocytes from nonobese diabetic (NOD) mice. *Diabetes.* 35:855–860.
38. Lantz, M., H. Thysell, E. Nisson, and I. Olson. 1991. On the binding of tumor necrosis factor (TNF) to heparin and the release in vivo of the TNF-binding protein I by heparin. *J. Clin. Invest.* 88:2026–2031.
39. Lortat-Jacob, H., and J.-A. Grimaud. 1991. Interferon gamma c-terminal function: new working hypothesis. Heparan sulfate and heparin, new targets for IFN-gamma, protect, relax the cytokine and regulate the activity. *Cell. Mol. Biol. (Oxf.).* 37:253–260.
40. McCaffrey, T.A., D.J. Falcone, and B. Du. 1992. Transforming growth factor β-1 is a heparin-binding protein: identification of putative heparin-binding regions and isolation of heparins with varying affinity for TGFβ-1. *J. Cell. Physiol.* 152:430–440.
41. Weksler, M.E., C.E. Moody, and R.W. Kozak. 1981. The autologous mixed lymphocyte reaction. *Adv. Immunol.* 31:271–312.
42. Glimcher, L.H., A.D. Steinberg, S.B. House, and I. Green. 1980. The autologous mixed lymphocyte reaction in strains of mice with autoimmune disease. *J. Immunol.* 125:1832–1888.
43. Serreze, D.V., and E.H. Leiter. 1988. Defective activation of T suppresor cell function in nonobese diabetic mice. *J. Immunol.* 140:3801–3807.
44. Baxter, A.G., M.A. Adams, and T.E. Mandel. 1989. Comparision of high-and-low diabetes incidence NOD mouse strains. *Diabetes.* 38:1296–1300.
45. Clayberger, C., R.H. Dekruyff, and H. Cantor. 1984. Immunoregulatory activities of autoreactive T cells: an I-A-specific T cell clone mediates both help and suppression of antibody responses. *J. Immunol.* 132:2237–2243.
46. Jorgensen, J.L., U. Esser, B.F. De St. Groth, P.A. Reay, and M.M. Davis. 1992. Mapping T-cell receptor-peptide contacts by variant immunization of single-chain transgenics. *Nature (Lond.).* 355:244–230.
47. Jacob, C.O., S. Aiso, S.A. Michie, H.O. McDevitt, and H. Acha-Orbea. 1990. Prevention of diabetes in nonobese diabetic mice by tumor necrosis factor (TNF): similarities between TNF-alpha and interleukin 1. *Proc. Natl. Acad. Sci. USA.* 87:968–972.
48. Yang, X.D., R. Tisch, S.M. Singer, Z.A. Cao, R.S. Liblau, R.D. Schreiber, and H.O. McDevitt. 1994. Effect of tumor necrosis factor α on insulin-dependent diabetes mellitus in NOD mice. I. The early development of autoimmunity and the diabetogenic process. *J. Exp. Med.* 180:995–1004.
49. Campbell, I.L., L. Oxbrow, and L.C. Harrison. 1991. Reduction in insulitis following administration of IFN-gamma and TNF-alpha in the NOD mouse. *J. Autoimmun.* 4:249–262.
50. Debray-Sachs, M., C. Carnaud, C. Boitard, H. Cohen, I. Gresser, P. Bedossa, and J.F. Bach. 1991. Prevention of diabetes in NOD mice treated with antibody to murine IFN gamma. *J. Autoimmun.* 4:237–248.
51. Mason, D., and D. Fowell. 1992. T-cell subsets and autoimmunity. *Curr. Opin. Immunol.* 4:728–732.
52. Scott, B., R. Liblau, S. Degermann, L.A. Marconi, L. Ogata, A.J. Caton, H.O. McDevitt, and D. Lo. 1994. A role for non-MHC genetic polymorphism in susceptibility to spontaneous autoimmunity. *Immunity.* 1:73–82.
53. Wogensen, L., M.-S. Lee, and N. Sarvetnick. 1994. Production of interleukin 10 by islet cells accelerates immune-mediated destruction of β cells in nonobese diabetic mice. *J. Exp. Med.* 179:1379–1384.
54. Anderson, J.T., A.J. Cornelius, A.J. Jarpe, W.E. Winter, and A.B. Peck. 1993. Insulin-dependent diabetes in NOD mouse model II. β cell destruction in autoimmune diabetes is a Th2 and not Th1 mediated event. *J. Autoimmun.* 15:113–122.