Linkage of Faulty Major Histocompatibility Complex Class I to Autoimmune Diabetes

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Pancreatic islet cells are the targets of an autoimmune response in type I diabetes. In the nonobese diabetic (NOD) mouse model of autoimmune diabetes, expression of major histocompatibility complex (MHC) class I proteins was inversely correlated with diabetes; in this mouse a mutation in the MHC class II–linked gene for the putative MHC class I peptide transporter was also present. Mice deficient in MHC class I expression because they do not produce β_2-microglobulin also developed late onset autoimmune diabetes. In cells from humans with type I diabetes expression of MHC class I was decreased; subsets of prediabetics categorized as most likely to become hyperglycemic also had low MHC class I. T cell responses to self antigens are faulty in diabetics. In sets of genetically identical twins that are discordant for diabetes, the defect appeared to reside with the antigen presenting cell. Thus, a lack of surface MHC class I protein is associated with autoimmune diabetes; the concomitant defect in antigen presentation may impair the development of self tolerance, which could result in autoimmune disease.

TYPE I DIABETES IN HUMANS IS AN AUTOIMMUNE DISEASE IN which lymphocytes infiltrate the pancreas and destroy the β cells of the islets of Langerhans (*1*). It is accompanied by a cellular and humoral immune response to an array of endogenous β cell antigens (*2*). This disease and several other autoimmune diseases are linked to the MHC class II region (*3, 4*). Although this linkage implies genetic elements in the development of hyperglycemia, identical twin sets exist in which only one of the twins becomes diabetic; that is, they are discordant in diabetes. The identity of the specific genes, as well as the mechanisms by which this MHC class II linkage contributes to autoimmunity directed toward islet polypeptides, is not known.

Reduced expression of MHC class I and mutant peptide transporter gene in the NOD mouse. The nonobese diabetic (NOD) mouse is a model of type I diabetes that is characterized by chronic lymphocytic infiltration of the islets, autoantibodies to insulin and some islet cell constituents, and hyperglycemia (*5*). In our colony approximately 86 percent of the females are hyperglycemic by the age of 26 weeks. Antigen presentation is defective in the prediabetic stage and results in depressed T cell reactivity to autologous antigens (*6*). Because MHC class I molecules bind peptides derived from endogenous proteins and present them to T cells, we measured cell surface expression of MHC class I on splenocytes from NOD mice as compared to H-2 congenic and noncongenic mouse strains (Fig. 1). The MHC class I haplotype of the NOD mouse (H-2 locus) is K^d and D^b (*7*). The characteristic cell surface density (mean channel fluorescence, MCF; flow cytofluorometry) of specific MHC class I epitopes and the percentage of positive cells for a 6-week-old female NOD mouse was compared to that of splenocytes from C57BL/6 (*H-2^b*) and BALB/c (*H-2^d*) mice. The density of H-2K^d epitopes on NOD splenocytes was significantly reduced to an MCF of 20.8 compared to the MCF of 104 for the positive control BALB/c mice. As was expected, the negative control mice, C57BL/6, cross-reacted somewhat (33 percent positive) with the monoclonal antibody (MAb) to K^d. In this particular experiment, NOD splenocytes (*H-2K^d*) were 23 percent positive compared to 92 percent positive BALB/c (*H-2K^d*) splenocytes when stained with the MAb SF1-1.1, which is specific for H-2K^d.

The cell surface expression of H-2D^b on NOD splenocytes was also reduced. For example, 53 percent of NOD splenocytes reacted specifically with MAb 5041.16.1, specific for H-2D^b, and were considered positive, compared to 98 percent of splenocytes from C57BL/6 mice (*H-2^b*); the 53 percent of the splenocytes that were positive from the NOD mice should be compared to the 7 percent positive on splenocytes from the negative control BALB/c mice (*H-2K^d*). Thus MHC class I cell surface expression was depressed on lymphocytes from prehyperglycemic NOD mice.

Not all female NOD mice become hyperglycemic; penetrance is approximately 85 percent in our colony. We compared MHC class I expression on six adult (6-month-old) NOD females with normal blood sugar to their hyperglycemic littermates (Fig. 1, G and H). The peripheral blood from the unaffected littermates had a normal proportion of MHC class I positive cells that, when analyzed by flow cytometry, consistently formed a peak in the histogram. The hyperglycemic peripheral blood lymphocytes (PBLs) had many more dull cells, and the narrow peak of fluorescence was absent. Thus the decreased level of lymphocyte MHC class I expression in the NOD females appears to correlate with clinical hyperglycemia.

The genetic locus most strongly associated with diabetes susceptibility in both humans and NOD mice is closely linked to the MHC class II genes, not to the MHC class I genes (*3, 8–13*). Nevertheless, several gene products in addition to MHC class I and β_2-microglobulin are required for normal MHC class I expression, including proteases that generate peptide antigens and peptide-binding molecular chaperones. A cluster of genes in the MHC class II region influences, in trans, the expression of MHC

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class I antigens on the cell surface (*14*). The gene products regulate the availability of peptides that bind to and presumably stabilize MHC class I molecules, allowing proper folding and assembly of class I on the cell surface (*15–21*). The proteins encoded by these MHC class II–linked genes are homologous to the multiple drug resistance (MDR) class of transmembrane proteins, and are presumed to encode subunits of an adenosine triphosphate (ATP) dependent peptide transporter of the endoplasmic reticulum. The mouse homologs, *HAM1* and *HAM2* have polypeptide products that share 77 percent identity and that probably function as homo- or heterodimers, by analogy to other members of this gene family.

Because diabetes susceptibility in the NOD mouse is linked to the MHC class II region, we used a cDNA probe that encoded the human homolog of *HAM*, *RING4*, to examine the expression of the *HAM* in splenocytes from the NOD mouse (Fig. 2A). The amount of MHC class I peptide supply gene mRNA was reduced in mRNA prepared from NOD splenocytes in comparison to the amount in splenocyte mRNA prepared from C57BL/6 and BALB/c mice.

In search of a mutation in the HAM region, we used 20 different restriction enzymes to digest splenic genomic DNA from NOD mice, MHC identical mice (B10.D2 and BALB/c), and nonidentical mice (C57BL/6). In the mouse, the MHC-linked transporter genes are located in approximately a 200-kb region between *H-2K(Pb)*

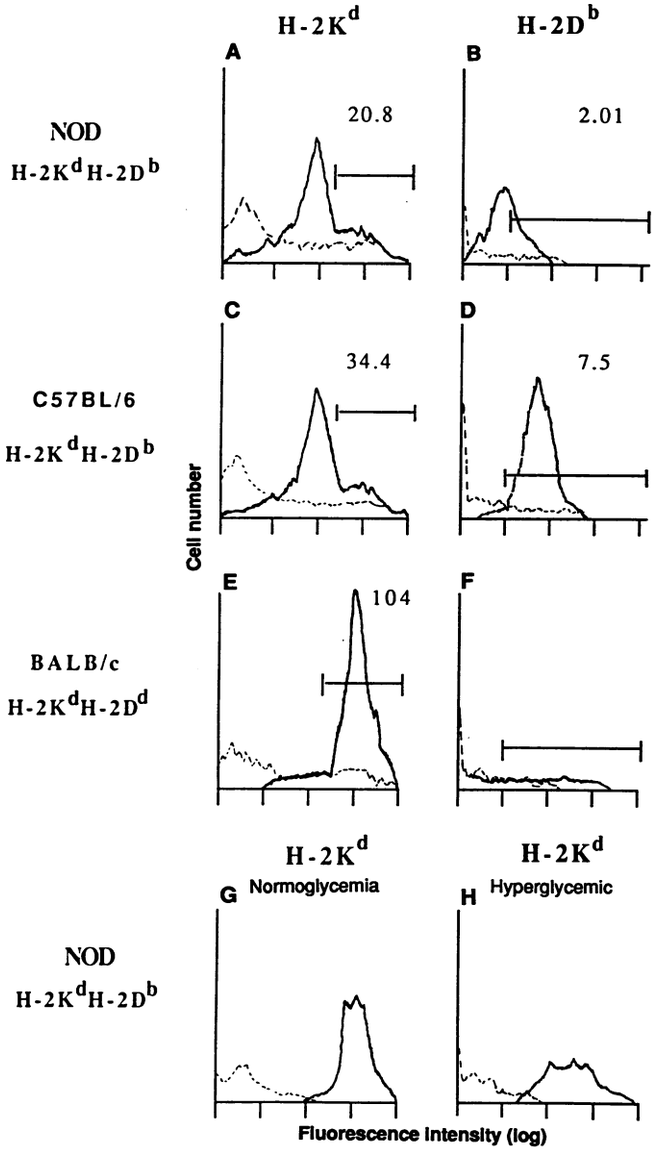

Fig. 1. Flow cytometric analysis of MHC class I on lymphocytes from diabetic and normoglycemic mice. Six-week-old NOD splenocytes (**A**) and (**B**) were compared to C57BL/6 (**C**) and (**D**) and BALB/c (**E**) and (**F**) splenocytes with a MAb to H-2K^d (SF9-9.9; Pharmingen, San Diego, CA) in (A), (C), and (E) and with a MAb to H-2D^b (5041.16.1; Accurate Chemical, Westbury, NY) in (B), (D), and (F). Negative control histograms (a nonbinding first MAb) are shown with dotted lines. Mean channel fluorescence is indicated in each panel. The horizontal line bar indicates the channels considered positive. Splenocytes from 27-week-old female NOD mice that were normoglycemic (**G**) or hyperglycemic (**H**) were analyzed for H-2K^d (MAb 9010A; Accurate Chemical, Westbury, NY). Positive cells were detected with fluorescein-isothiocyanate–conjugated goat antibody to mouse or rat immunoglobulin on a Coulter Electronic Epics Elite flow cytometer.

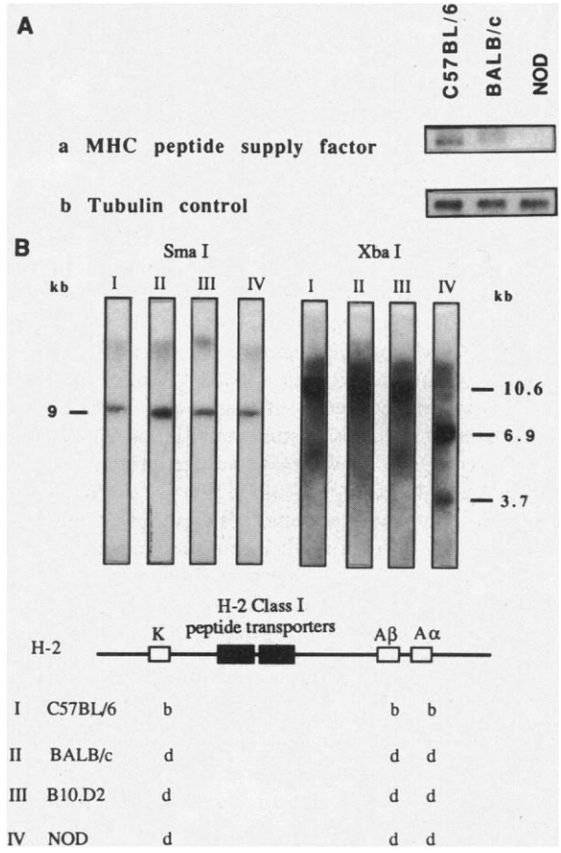

Fig. 2. (**A**) Northern (mRNA) blot of mRNA from splenocytes of C57BL/6, BALB/c, and NOD mice. mRNA abundance of a peptide transporter in murine splenocytes (a) compared to tubulin control (b). Splenic poly(A)$^+$ mRNA was prepared from 6- to 7-week-old mice, 5 μg were separated by agarose gel electrophoresis, the mRNA was transferred to nylon membranes, and the blot was hybridized with a cDNA for *RING4*, the human homolog of the murine peptide transporter genes *HAM1* and *HAM2*. The membrane was then stripped and rehybridized with a cDNA for tubulin, which confirmed equal mRNA loading. Autoradiography was for 4 days for RING4 and 24 hours for tubulin. Methods were as described (*32*). (**B**) Restriction fragment length polymorphism of a murine peptide transporter gene. Genomic DNA (5 mg) from NOD mice and several control strains was digested with 20 restriction enzymes and transferred to GeneScreen Plus membranes (NEN) after agarose electrophoresis. The blot was hybridized with a RING4 cDNA, washed for 1 hour in 2× SSC at 37°C, and washed for 1 hour in 0.1× SSC at 65°C. Nineteen of the 20 enzymes gave identical restriction patterns in all four mouse strains examined; the pattern with Sma I is representative. Digestion with Xba I gave a pattern unique to the NOD mouse in more than five separate experiments.

and *H-2I-A(Ob)*. The digestion products were analyzed by hybridization of DNA (Southern blots) with the *RING4* probe. In 19 of the 20 digests, no differences in the patterns were observed between NOD and the MHC identical and nonidentical strains. In contrast, DNA digested with Xba I consistently showed a pattern characteristic only of the NOD, a loss of the normal 10.6-kb fragment with the appearance of two new fragments at 6.9-kb and 3.7-kb (Fig. 2B). These results suggest the existence of a possible small deletion or point mutation within the peptide transporter gene region of the NOD mouse. This mutation, together with the low mRNA expression from the transporter gene in the NOD splenocytes, implicates this gene as a candidate both for the genetic basis of impaired MHC class I expression and as the MHC class II–linked gene that confers diabetes susceptibility in the NOD mouse.

Autoimmune diabetes in β_2-microglobulin–deficient mice. The defective expression of MHC class I molecules in the NOD mouse could be incidental to the autoimmune state, perhaps reflecting a poorly understood homeostatic response to active autoimmunity, or it might itself be the cause of failure to establish tolerance to self antigens, thereby providing the necessary milieu for the development of autoimmunity. The opportunity to test whether the deficient expression of MHC class I is potentially pathogenic for type I diabetes is provided by the existence of mice rendered deficient in β_2-microglobulin by deletion of both alleles through homologous recombination (*22, 23*). These mice express virtually no H-2K and exhibit low expression of H-2D on their cell surfaces. Blood glucose and body weight were examined in ten homozygous β_2-microglobulin–deficient mice that were more than 18 months of age and compared to measurements carried out on heterozygous and homozygous normal littermates, which expressed varying or normal amounts, respectively, of MHC class I molecules. All ten mice with homozygous β_2-microglobulin–deficiency were mildly hyperglycemic in the nonfasting state [blood glucose of 360 ± 50 mg/dl (mean ± SD)], whereas their ten littermates that expressed β_2-microglobulin were not (blood glucose of 76 ± 8 mg/dl). The β_2-microglobulin–deficient mice also had lower body weights after a year and one-half of age, when compared to the control mice (21.9 ± 3.8 and 37.3 ± 5.6 g, respectively). One heterozygous littermate also became hyperglycemic at 20 months of age. Increased water consumption perhaps due to glycosuric polyuria, was observed in the MHC class I deficient mice.

Microscopic examination of the pancreas revealed islets of Langerhans surrounded by lymphocytic infiltrates in the homozygous-deficient mice (Fig. 3); no such infiltrates were observed in the pancreas of a normal hemizygous animal. Assays of sera (at dilutions of 1:20) from seven diabetic, homozygous β_2-microglobulin–deficient mice by indirect immunofluorescence on frozen sections of mouse pancreas revealed antibodies to the islet in six of the seven β_2-microglobulin–deficient sera compared to BALB/c sera which showed no antibodies; this result provides further evidence for the autoimmune nature of the diabetes seen in these homozygous β_2-microglobulin–deficient mice.

The onset of hyperglycemia in the β_2-microglobulin–deficient mice appears to be delayed beyond a year and one-half and is milder than the severe hyperglycemia in the NOD mouse that causes death within days of onset. Nevertheless, these observations suggest that interruption of MHC class I expression, in mice not known to carry a susceptibility to islet autoimmunity, is sufficient to cause the slow development of a syndrome of islet autoimmunity progressing to mild hyperglycemia. Thus, the low MHC class I expression in the NOD mouse may contribute to the cause of the spontaneous form of murine autoimmune diabetes.

Decreased MHC class I expression and function in human type I diabetes. To determine whether the correlation between low MHC class I expression and hyperglycemia is also true of human type I diabetes, we examined MHC class I expression on cells and cell lines from various groups of controls, prediabetics, and long-term diabetics. Prediabetics can be identified years before the onset of hyperglycemia by the presence of circulating antibodies to insulin or islet cells (*24–29*). Prediabetics who produce antibodies to both insulin (Ins^+) and islet cell antigens (ICA^+) are at the highest risk; most Ins^+ICA^+ individuals progress to hyperglycemia within 5 years of detection. Less than 10 percent of prediabetics that produce autoantibodies to only insulin (Ins^+ICA^-) become hyperglycemic in five years. Individuals with the Ins^-ICA^+ phenotype are more heterogenous in rate of progression (approximately 42 percent become diabetic in 5 years).

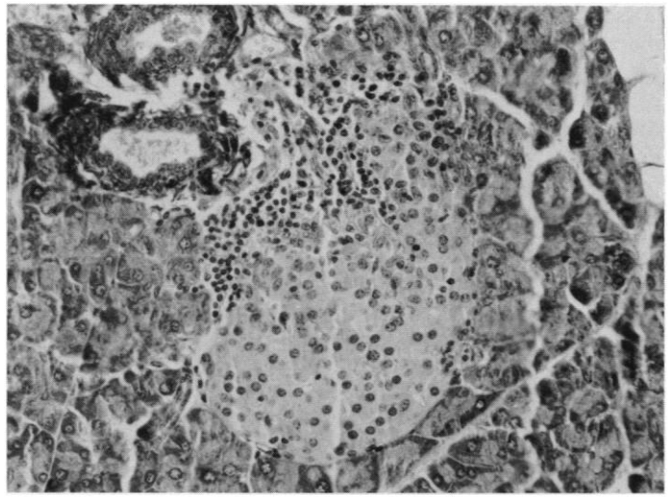

Fig. 3. Insulinitis in a transgenic MHC class I–deficient mouse. Hematoxylin and eosin stained section of pancreas from an 18-month-old mouse, homozygous for β_2-microglobulin deficiency and thus lacking in MHC class I expression. The field shows an islet of Langerhans surrounded by lymphocytes. When killed, this mouse had a blood glucose of 345 mg/dl and serum antibodies reactive with islets, as measured by indirect immunofluorescence on frozen sections of mouse pancreas.

We measured with flow cytometry the amount of MHC class I on the surface of PBL's from well-characterized individuals from these various groups, as well as long-term diabetics, sets of identical (monozygotic) twins discordant for diabetes, first degree relatives of type I diabetics, and nondiabetic controls (Fig. 4). MHC class I expression and density, as assessed by the binding of MAb W6/32, was significantly reduced in all ten high-risk prediabetics (Ins^+ICA^+, normoglycemic individuals), and 19 of 20 long-term diabetics as compared to age-matched controls (Fig. 4). All four diabetic twins had less MHC class I than their nondiabetic monozygotic twin siblings. MHC class I expression was also diminished in all ten Epstein-Barr virus (EBV) transformed B cell lines newly established from long-term diabetics when compared to EBV transformed B cell lines from ten nondiabetic controls. In contrast, normal MHC class I antigen expression was observed on cells from all five low-risk prediabetics (Ins^+ICA^-), ten normoglycemic, autoantibody negative first-degree relatives, and 39 control individuals. The MCF of MHC class I epitopes was also decreased in the same four groups (Fig. 4). Significantly, MHC class II expression, as assessed with MAb I-2 FITC showed no difference between type I diabetic and nondiabetic cells, whether assessed on freshly isolated PBL's or EBV-transformed B cells.

The autologous mixed lymphocyte reaction (AMLR) is an assay that models immunologic self recognition in vitro. T cells are mixed with irradiated antigen-presenting cells (APC's) from the same

individual. Normal T cells proliferate slightly and show specificity and immunologic memory; it is characterized by T cell activation controlled by the MHC antigens on the APC. This assay has been used to study individuals with various chronic autoimmune diseases (including established type I diabetes) and shows the uniform and paradoxical finding that T cells from such patients have decreased activation and proliferation in response to antigens presented by autologous cells in vitro, when compared to the response seen in AMLR's of cells from control individuals.

Because MHC class I antigen expression was depressed in some prediabetics, we performed AMLR's with cells from the various subsets of prediabetics that had been characterized by autoantibody pattern and rate of progression to hyperglycemia (Fig. 5). In addition to the measurement of [^{3}H]thymidine incorporation, cell counts were commonly performed, which in each case confirmed the proliferative response reported by [^{3}H]thymidine incorporation. Ins$^+$ICA$^+$ prediabetics had depressed T cell proliferation in the AMLR in nine of eleven subjects, when compared to simultaneously studied age-matched controls. When the Ins$^+$ICA$^+$ individuals were restudied approximately 1 to 2 years later (when they randomly returned to clinic), each of the nine retested subjects still had an abnormally depressed proliferation to self. During the first 2 years of observation, all of the individuals with a depressed proliferative response in the AMLR became hyperglycemic. Of the two Ins$^+$ICA$^+$ individuals who initially had augmented proliferation to self, one converted to depressed proliferation during the first two years of observation and then became diabetic in the fourth year; in contrast, the second Ins$^+$ICA$^+$ individual with augmented proliferation in the AMLR continued to show normal blood sugar throughout 5 years of observation.

The group of nine Ins$^+$ICA$^-$ individuals in 16 of 16 AMLR assays had augmented T cell proliferation relative to age-matched controls or to first-degree relatives who had no autoantibodies and whose response in the AMLR was indistinguishable from that of age-matched controls. During five years of observation only one of these nine individuals became hyperglycemic.

The correlation of depressed T cell proliferative response in the AMLR with progression to clinical diabetes and its relative independence from the autoantibody profile is most clearly illustrated by the patterns seen in the prediabetic group who are Ins$^-$ICA$^+$. Four of these eight individuals consistently had depressed proliferative responses in the AMLR; three of the four became diabetic in the first two years of observation and the fourth has decreased insulin secretion during intravenous glucose stimulation, an indicator of impending hyperglycemia. In contrast, three of the four Ins$^-$ICA$^+$ prediabetics with an augmented AMLR response continued to show normal blood sugar throughout 5 years of observation and have a normal insulin response to intravenous glucose. Thus, depressed reactivity to autologous antigen in vitro is associated with rapid and complete disease expression, whereas augmented autoproliferation appears protective, even with an "unfavorable" pattern of autoantibody expression.

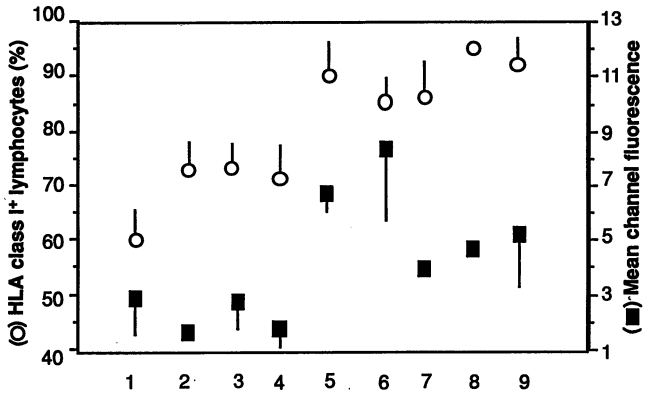

Fig. 4. MHC class I expression is deficient on diabetic human lymphocytes. Flow cytometry analysis (mean ± SEM) of human peripheral blood lymphocytes showing binding of MAb W6/32 (anti-HLA,A-B-C; American Type Tissue Culture, Rockville, MD). The human lymphocytes were prepared for immunofluorescence as described (*33*), with the use of a Ficoll gradient. Flow cytometry gates excluded the remaining red blood cells and debris and included T cells, B cells, and macrophages. A "wide gate" with exclusion of debris was used for the EBV cell lines. Prediabetics in this study are defined as normoglycemic first-degree relatives of type I diabetics who consistently have high titer cytoplasmic islet cell autoantibodies [>40 Juvenile Diabetes Foundation (JDF) units], insulin autoantibodies (>80 nanounits per milliliter) or both. Serum was tested for islet cell autoantibodies (ICA) on frozen sections of Wistar-Furth rat pancreas and human pancreas with fluorescein isothiocyanate– or peroxidase-conjugated protein A and quantified as JDF units per International Diabetes Workshop standards (*34–37*). Serum was assayed for insulin binding by competitive radioimmunoassay as described (*38*). Both assays were consistent with international standards. Lymphocytes from 1, Isn$^+$ICA$^+$ prediabetics (18 to 24 years old, mean = 16.7 years, n = 10); 2, the diabetic twin (n = 4); 3, long-term type I diabetics (n = 19); 4, newly produced EBV-transformed B cell lines from long-term diabetics (n = 10); 5, normal controls (n = 27); 6, Ins$^+$ICA$^-$ prediabetics (10 to 34 years old, mean = 19.7 years, n = 5); 7, the nondiabetic discordant twin (n = 5); 8, antibody negative, normoglycemic first-degree relatives (n = 10); and 9, EBV-transformed B cell lines from normal controls (n = 10) were studied.

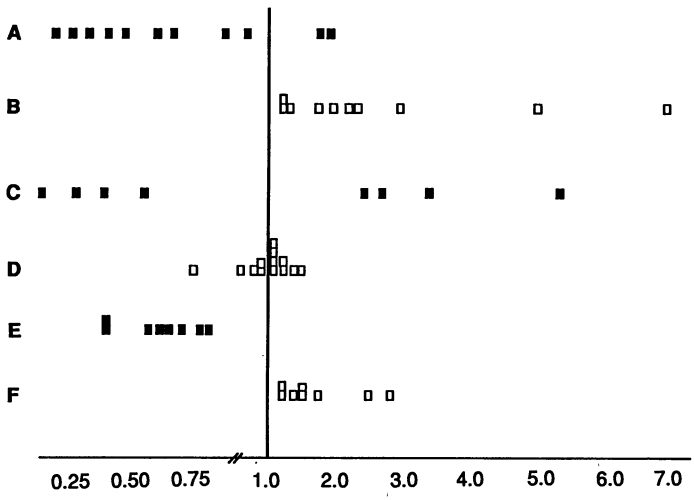

Fig. 5. Autologous antigen stimulation of T cells from prediabetics, twins discordant for type I diabetes, and controls. A, High risk Ins$^+$ICA$^+$ prediabetics; B, low risk Ins$^+$ICA$^-$ prediabetics; C, intermediate risk Ins$^-$ICA$^+$ prediabetics; D, first-degree relatives; E, the diabetic twin of twin sets discordant for type I diabetes; and F, the nondiabetic twin from the discordant twin sets. Human PBL's were freshly prepared from a Ficoll-Hypaque density gradient (Pharmacia Chemicals, Piscataway, NJ). The PBL's were removed from the top layer and T cells were prepared by rosetting with 5 percent sheep erythrocytes (more than 95 percent were positive with a MAb to CD3). The non-T cell population was primarily B cells and macrophages. Autologous mixed lymphocyte reactions were performed in RPMI 1640 with 10 percent fetal calf serum, glutamine, and 1 percent penicillin-streptomycin. For [^{3}H]thymidine measurements in the AMLR or MLR, 1×10^5 responder T cells were cultured with 1×10^5 irradiated (3000 to 5000 rads) autologous non-T cells in a total volume of 200 μl in 96-well, round bottomed plates for 6 or 8 days, as triplicate cultures. [^{3}H]Thymidine was added 18 hours before harvesting, and incorporation was measured by liquid scintillation counting. For each experimental subject, an age-matched (±18 months) control subject was analyzed simultaneously. Stimulation index is obtained by dividing the counts of [^{3}H]thymine incorporation observed in the experimental conditions by that observed in the concurrent AMLR carried out with cells from the age-matched control subject. In this figure, the twins of any particular twin set were not necessarily studied on the same day.

Table 1. AMLR regulation between discordant type I diabetic twins. Age matched controls were analyzed with each twin set. MHC typing was performed on each twin pair to confirm the identical twin status except for set G. This represents an analysis performed on some of the same patients in Fig. 4, but, at separate times. The AMLR reactions were performed at a 1:1 ratio of stimulators to responders. The stimulators were irradiated to prevent proliferation. A paired T test between rows 1 and 2, rows 1 and 5, and rows 2 and 5 in seven of the seven twin sets revealed a P value < 0.01. A paired T test between rows 3 and 4, rows 3 and 5, rows 2 and 4, and rows 1 and 3 in seven of seven twin sets revealed a P value of < 0.001.

Responder T cells	Stimulatory APC's	[^{3}H]Thymidine incorporation (cpm) of responder cells from twin sets:						
		A	B	C	D	E	F	G
1. Diabetic	Diabetic	3,279 ± 129	3,266 ± 354	3,218 ± 167	2,557 ± 57	7,115 ± 252	3,171 ± 152	4,271 ± 161
2. Diabetic	Nondiabetic	7,093 ± 112	6,881 ± 191	6,411 ± 143	5,905 ± 85	14,153 ± 986	9,756 ± 149	18,271 ± 592
3. Nondiabetic	Diabetic	3,159 ± 223	2,211 ± 141	3,020 ± 166	2,416 ± 141	4,332 ± 235	2,755 ± 112	2,593 ± 161
4. Nondiabetic	Nondiabetic	6,994 ± 889	5,849 ± 213	6,234 ± 188	5,277 ± 134	12,150 ± 138	8,164 ± 163	12,341 ± 297
5. Control	Control	4,771 ± 239	4,222 ± 625	5,002 ± 201	3,319 ± 195	10,452 ± 332	7,111 ± 223	6,899 ± 128

The abnormal T cell proliferative response of prediabetic individuals was confined to the response to autologous or self antigens; parallel allogeneic mixed lymphocyte reactions, in which prediabetic T cells or APCs (antigen presenting cells) were cocultured with APCs or T cells from a nondiabetic, allogeneic donor, gave normal proliferative responses (*30*).

Correlation of low T cell proliferation to self with defective antigen presentation. Inasmuch as the twin sets, although discordant for type I diabetes, are genetically identical, it is feasible to mix the T cells and APC's in various combinations to determine whether the hypoproliferative response in the AMLR of the diabetic is the result of defective APC's, T cells, or both. We performed AMLR's with cells from seven twin sets discordant for type I diabetes (Table 1). The depressed proliferation of these syngeneic mixed lymphocyte reactions appeared to be primarily due to defective stimulation by antigen-presenting cells from the diabetic donor. The T cells of the nondiabetic and diabetic twin responded equally poorly to the APC's from the diabetic twin. In contrast, the T cells from the diabetic twin, when incubated with the syngeneic antigen-presenting cells from the nondiabetic twin, proliferated vigorously, to an extent that exceeds significantly (by 1.65 times) that of a simultaneously incubated AMLR conducted with cells from control individuals and is even slightly greater (by about 15 percent) than that seen in the autologous reaction from the nondiabetic twin. Thus, the depressed T cell proliferation in response to autologous antigens in vitro, observed in these diabetic subjects, is predominantly ascribable to defective presentation of endogenous antigens.

We evaluated the generation of cytotoxic T cells in the syngeneic-MLR's of the discordant twins by incubating the four possible combinations of irradiated APC's with T cells from the diabetic and nondiabetic twin. After 7 days T cells were purified from each of these reactions and assayed for cytotoxic activity against ^{51}Cr-labeled target lymphocytes prepared from both the diabetic and nondiabetic twin (Table 2). The generation of cytotoxic T cells was low in three of the incubations, as expected for an AMLR. However, in the reaction with APC's from the nondiabetic twin and T cells from the diabetic twin, cytotoxic T cells reactive to the nondiabetic twin target were generated almost as efficiently as in a parallel allogeneic-MLR. In contrast to the conventional allogeneic mixed lymphocyte reaction, the T cell receptors of the cytotoxic cells generated in the twin incubation recognized MHC class I molecules on the nondiabetic target cells; incubations of the nondiabetic twin targets with antibodies to MHC class I selectively prevented target cell lysis by activated diabetic T cells. The diabetic cytotoxic T cells extensively lysed syngeneic nondiabetic twin targets, yet failed to

Table 2. Generation of cytotoxicity directed toward self MHC class I on antigen-presenting cells. Two additional twin sets were assayed, with comparable results. Diabetic (D), nondiabetic (N), twin T cells or control (C-1 or C-2) T cells were stimulated for seven days at a 1:1 ratio with syngeneic irradiated non-T cells from self or identical twin in Table 1. At day 7 the responding T cells of the AMLR were harvested over Ficoll and the CTL assay performed. Target lymphocytes represent frozen lymphocytes from the donors thawed 24 hours before the assay. Targets were labeled with Na_2CrO_4 at 50 to 150 mCi of ^{51}Cr for 1 × 10^6 cells at 37°C for 1 hour on a slow shaking platform, washed twice, and cultured with syngeneic stimulated T cells for 10 hours at 37°C without and with (*) MAb W6/32. Culture supernatant (100 μl) was harvested and counts per minute measured in a gamma counter. The above experiment was performed in triplicate. Target cells were treated with a 1:100 dilution of sterile polyclonal mouse antibody to human MHC class I for 30 minutes at room temperature, washed, and added to the culture plate for the cytotoxicity assay. Three of the four monozygotic twin sets were verified by MHC typing.

T cells	APC's	Target cells	Effector to target cell ratio [^{51}Cr release (%)]		
			5:1	10:1	40:1
			Twin set 1		
D	D	D	0.9 ± 0.4	4.5 ± 0.3	5.4 ± 0.2
D	D	N	3.6 ± 0.2	2.8 ± 3.2	6.3 ± 1.4
D	N	D	2.0 ± 0.5	2.3 ± 0.5	2.7 ± 0.5
D	N	N	20.1 ± 1.5	40.0 ± 2.0	59.0 ± 6.2
D	N	N*	2.9 ± 0.6	4.2 ± 1.7	17.1 ± 1.2
N	D	D	0.9 ± 0.1	2.5 ± 0.1	0.2 ± 0.6
N	D	N	2.0 ± 1.8	3.1 ± 3.6	0.7 ± 0.35
N	N	D	2.6 ± 2.2	6.3 ± 4.0	6.4 ± 5.5
N	N	N	2.1 ± 0.4	2.5 ± 3.1	4.8 ± 0.2
C-1	C-2	C-2	22.0 ± 8.5	72.3 ± 9.1	81.0 ± 17.0
			Twin set 2		
D	D	D	1.2 ± 0.7	1.8 ± 0.3	2.4 ± 0.3
D	D	N	1.5 ± 0.4	1.6 ± 0.8	1.6 ± 0.2
D	N	D	0.5 ± 0.4	2.0 ± 0.7	1.9 ± 1.6
D	N	N	30.0 ± 9.7	51.0 ± 7.9	63.0 ± 15.0
D	N	N*	2.3 ± 0.3	4.7 ± 5.0	4.8 ± 2.9
N	D	D	0.9 ± 0.3	0.9 ± 0.6	0.83 ± 0.2
N	D	N	0.7 ± 0.4	1.5 ± 0.3	1.4 ± 0.2
N	N	D	0.4 ± 0.2	1.5 ± 0.3	2.4 ± 0.2
N	N	N	1.2 ± 1.2	1.9 ± 0.3	1.3 ± 0.3
C-1	C-2	C-2	25.0 ± 0.3	40.0 ± 9.0	55.0 ± 5.7

lyse autologous diabetic twin targets. Thus, a subset of diabetic T cells is activated by peptide antigens presented by the MHC class I molecules of the nondiabetic twin APC's; the cytotoxic T cells thereby generated are capable of reengaging MHC class I molecules on the targets derived from the nondiabetic twin, but not the syngeneic MHC class I molecules on the targets of the diabetic twin.

This discrimination between syngeneic MHC class I molecules could occur if the nondiabetic twin presented unique endogenous peptide antigens. However, we favor the hypothesis that presentation of endogenous peptide antigens common to both twins is impaired in the diabetic twin; therefore self tolerance is defective, which results in an in vivo autoimmune response. The potentially autoreactive T cells that persist in the diabetic can be activated in vitro because the syngeneic MHC class I molecules on the APC of the nondiabetic twin present the self antigen in an effective fashion. Thus, the presentation of endogenous cytoplasmic antigens on MHC class I may be a crucial pathway for the establishment of self tolerance.

MHC class I expression and autoimmunity. We observed defective expression of MHC class I molecules in the prediabetic and hyperglycemic phases of the NOD mouse, β_2-microglobulin–deficient mice, and humans. Low MHC class I expression in diabetics also correlated with faulty antigen presentation and generation of CTL's. The defective expression of MHC class I correlated better with the risk of progression to hyperglycemia than did the presence of active islet autoimmunity or genetic susceptibility per se. Because low MHC class I expression precedes hyperglycemia and is present in the prediabetic phase, the abnormality in MHC class I expression is probably not secondary to the hyperglycemia or to some other metabolic concomitant of insulin deficiency.

Low MHC class I expression is probably not a consequence of active β cell autoimmunity. The Ins^+ICA^- subset of human prediabetics has autoimmunity to many islet constituents. Nevertheless, the expression of MHC class I on the PBL's of the Ins^+ICA^- prediabetics is normal, and their rate of progression to hyperglycemia is slow and incomplete. Thus, the presence of low MHC class I expression is correlated to, and probably predictive of, the risk of progression to hyperglycemia in both man and the NOD mouse.

The presence of an autoimmune diabetic syndrome in the mice that have both alleles for β_2-microglobulin inactivated shows that defective MHC class I expression and function by itself can be causative of type I diabetes. The mechanism by which β cell autoimmunity develops in these transgenic mice is unknown although participation of a virus is always possible. However, these mice lack $CD8^+$ T cells; therefore, $CD8^+$ T cells are not required for autoimmune β cell destruction. The autoimmunity may instead be mediated by $CD4^+$ T cells or less likely NK cells, which preferentially attack targets that are low in MHC class I (*31*).

The demonstration that deficient MHC class I expression and function can by itself cause autoimmune diabetes suggests that the defective function and expression of MHC class I in the spontaneous syndromes of autoimmune diabetes in man and the NOD mouse could contribute to the development of autoimmune diabetes. The existence of a mutation in the putative peptide transporter (*HAM* genes) in the NOD mouse supports this hypothesis.

The transporter genes act in trans to allow cell surface expression of MHC class I molecules, presumably because both antigenic peptides and β_2-microglobulin are required for the assembly and stability of mature MHC class I molecules and to ensure their transport to the cell surface. Thus, defective expression and mutation of the transporter gene in NOD splenocytes provides a mechanism for low MHC class I expression. In addition, the location of the transporter gene within the MHC class II locus establishes the transporter gene as a candidate for the elusive MHC class II related diabetes susceptibility gene of the NOD mouse. Conclusive evidence requires the demonstration that this mutation causes the decrease in mRNA, that the low mRNA be accompanied by a decrease in transporter polypeptides, and that inactivation of the transporter gene produce the anticipated phenotypes.

We hypothesize that MHC class I expression and function may be involved in the pathogenesis of human type I diabetes. Because of the similarities between class I expression and autoimmune diabetes in humans and in the NOD mouse model, we suggest that deficiencies in transporter locus in humans may yet be found. The correlation between MHC class I expression and progression to hyperglycemia, together with the observation that defective MHC class I expression (and hyperglycemia) is not an inevitable consequence of a diabetes-susceptible genotype, suggests that interventions will be found that functionally complement the gene defects underlying low MHC class I expression, and that these interventions will slow or halt the progression of β cell autoimmunity to true diabetes mellitus.

REFERENCES AND NOTES

1. H. Von Meyenburg, *Schweiz Med. Wochsch.* **21**, 554 (1940).
2. A. A. Rossini, J. P. Mordes, A. A. Like, *Annu. Rev. Immunol.* **3**, 289 (1985).
3. J. A. Todd, J. I. Bell, H. O. McDevitt, *Nature* **329**, 599 (1987).
4. H. Festenstein *et al.*, *ibid.* **322**, 64 (1986).
5. S. Makino *et al.*, *Exp. Anim.* (*Tokyo*) **34**, 425 (1985).
6. D. Serreze and E. H. Leiter, *J. Immunol.* **140**, 3801 (1988).
7. M. Hattori *et al.*, *Science* **231**, 733 (1986).
8. H. A. Erlich *et al.*, *Diabetes* **39**, 96 (1990).
9. T. Miyazaki *et al.*, *Nature* **345**, 722 (1990).
10. J. A. Todd *et al.*, *ibid.* **338**, 587 (1989).
11. J. M. Baisch *et al.*, *N. Engl. J. Med.* **322**, 1836 (1990).
12. G. Sterkers *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 6473 (1988).
13. P. A. Morel, J. S. Dorman, J. A. Todd, H. O. McDevitt, M. Trucco, *ibid.*, p. 8111.
14. A. M. Livingstone, S. J. Powis, A. G. Diamond, G. W. Butcher, J. C. Howard, *J. Exp. Med.* **170**, 777 (1989).
15. O. Rotzschke *et al.*, *Nature* **348**, 252 (1990).
16. T. Spies and R. DeMars, *ibid.* **351**, 323 (1991).
17. A. Townsend *et al.*, *ibid.* **340**, 443 (1989).
18. M. J. Bevan, *ibid.* **325**, 192 (1987).
19. J. J. Monaco, S. Cho, M. Attaya, *Science* **250**, 1723 (1990).
20. J. Trowsdale *et al.*, *Nature* **348**, 741 (1990).
21. T. Spies *et al.*, *ibid.*, p. 744.
22. M. Zijlstra *et al.*, *ibid.* **344**, 742 (1990).
23. B. H. Koller and O. Smithies, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8932 (1989).
24. A. MacCuish, W. Irvine, E. Barnes, L. Duncan, *Lancet* **ii**, 1529 (1974).
25. A. Lernmark *et al.*, *N. Engl. J. Med.* **299**, 375 (1978).
26. G. F. Bottazzo, A. Florin-Christensen, D. Doniach, *Lancet* **ii**, 1279 (1974).
27. S. Baekkeskov *et al.*, *Nature* **298**, 167 (1982).
28. J. Palmer *et al.*, *Science* **222**, 1337 (1983).
29. O. P. Ganda *et al.*, *Diabetes* **33**, 516 (1984).
30. Unpublished data. Mixed lymphocyte reactions were performed with responder T cells from prediabetics or controls mixed in various combinations with stimulator cells from prediabetics or controls. T cells from Ins^+ICA^+ or Ins^+ICA^- prediabetics proliferated normally to control APC ($P = 0.91$ and $P = 0.16$, respectively). Similarly, T cells from controls proliferated normally to allogeneic prediabetic cells ($P = 0.16$ and $P = 0.75$, respectively).
31. N. S. Liao, M. Bix, M. Zijlstra, R. Jaenisch, D. Raulet, *Science* **253**, 199 (1991).
32. J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989).
33. D. Faustman, G. Eisenbarth, J. Daley, J. Breitmeyer, *Diabetes* **38**, 1462 (1989).
34. S. Srikanta, A. Rabizadeh, M. A. K. Omar, G. S. Eisenbarth, *ibid.* **34**, 300 (1985).
35. S. Srikanta, O. P. Ganda, J. S. Soeldner, G. S. Eisenbarth, *N. Engl. J. Med.* **313**, 461 (1985).
36. H. Gleichmann and G. F. Botazzo, *Diabetes* **36**, 578 (1987).
37. P. Colman, M. Tautkus, A. Rabizadeh, C. Cahill, G. S. Eisenbarth, *Diabetes Care* **11**, 367 (1988).
38. P. Vardi, S. A. Dib, M. Tuttleman, J. E. Connelly, M. Grinbergs, A. Rabizadeg *et al.*, *Diabetes* **36**, 1286 (1987).

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