Type 1 Diabetes Mellitus:

Insights into Pathogenesis and Clinical Implications

Daniel W. Foster, M.D.

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I. INTRODUCTION

Diabetes mellitus is currently classified into three large categories: (1) insulin-dependent diabetes (Type 1); (2) noninsulin-dependent diabetes (Type 2) and (3) secondary diabetes. In the United States the overall prevalence of the disease is probably around 1% of the population despite estimates of 5% by the National Diabetes Advisory Board and other diabetes-related groups (1). Obviously certain subsets have much higher prevalence; e.g., 50-70% of Pima Indians above the age of 65 have diabetes. The prevalence of insulin-dependent diabetes has been estimated to be 0.25% in the United States and western Europe, a figure based on symptomatic disease identified by the age of 20. As will be noted below, a significant number of patients initially characterized as having noninsulin-dependent disease with onset in the middle years probably have a late-appearing variant of insulin-dependent diabetes. It is possible, therefore, that the general rule of one Type 1 patient for every three to four Type 2 patients may have to be revised upward.

Today's subject has been reviewed in these rounds in 1982 and 1984 but sufficient progress has been made to warrant an update. Advances in Type 2 disease, though steady, have been less illuminating. I plan to cover current understanding of the pathophysiology of Type 1 diabetes and then draw some clinical implications. The findings are of intrinsic scientific interest and may be applicable to a broad array of other autoimmune diseases. In addition, because diabetes is associated with a devastating array of complications that continue unabated despite the availability of insulin (2), there is great pressure to prevent or cure the disease.

1. Foster DW. Diabetes mellitus. In The Metabolic Basis of Inherited Disease, 5th edition, JB Stanbury, JB Wyngaarden, DS Fredrickson, JL Goldstein, MS Brown (eds). New York, McGraw-Hill, 1983, pp 99-117.
2. Unger RH, Foster DW. Diabetes mellitus. In Williams Textbook of Endocrinology, 7th edition, JD Wilson, DW Foster (eds). Philadelphia, W. B. Saunders, 1985, pp 1018-1080.

II. Pathogenesis

1. Overview

A current overview of the pathogenesis of Type 1 diabetes is shown in Table 1. According to this view susceptibility is genetically determined with the primary clinical markers being the presence of HLA DR3, DR4 or the DR3/DR4 heterozygous state. It is presumed that in most cases some environmental event initiates the destructive process, with primary attention focused on viruses as the trigger. The need for an environmental "trigger" primarily devolves from studies of concordance in identical twins which indicate that if one twin has diabetes the other develops the disease less than 50% of the time.

Table 1

The pathogenesis of Type 1 diabetes mellitus

Genetic susceptibility	HLA DR3, DR4 (other genes?)
↓	
Environmental event	Virus (?)
↓	
Activation of autoimmunity	Self → nonself transition
+	
Insulitis	Infiltration of activated T lymphocytes
↓	
Immune attack on beta cells	Islet cell antibodies, cell mediated immunity
↓	
Diabetes	> 90% beta cells destroyed (alpha cells unopposed)

The critical issue appears to be activation of the immune system whereby both humoral and cytotoxic attack on the beta cells is initiated. Presumably some sort of self → nonself transition is involved. A common (invariant?) accompaniment of the process is the development of insulitis, an infiltration of activated T lymphocytes in the islets. With time the beta cell population is essentially completely destroyed (> 90%) with the consequence that alpha cells are derepressed, overproducing glucagon (see Table 2). The elevated glucagon/insulin ratio then results in hyperglycemia and/or ketoacidosis.

Table 2

Table COMPARISON OF PANCREATIC WEIGHT AND MASS OF ENDOCRINE CELLS AT AUTOPSY

	Total Pancreatic Weight (Mean and Range, g)	Weight of Pancreatic Endocrine Component (mg)	Total Mass of Endocrine Cells (mg)				
			β	α	δ	PP	α/β Ratio
Normals	82 (67–110)	1395	850	225	125	190	0.26
Type 1 IDDM	40 (26–51)	413	0	150	90	185	∞
Type 2 NIDDM	73 (55–100)	1449	825	375	100	180	0.45

Data from Rahier J, et al.: Cellular composition of the human diabetic pancreas. Diabetologia 1983; 24:366–371. Mass of endocrine cells was estimated from Figure 3 of the cited reference and should be considered approximate.

Several recent reviews cover the above points (3-5).

3. Lernmark A. Molecular biology of Type 1 (insulin-dependent) diabetes mellitus. Diabetologia 1985; 28:195-203.
4. Eisenbarth GS. Type 1 diabetes mellitus. A chronic autoimmune disease. N Engl J Med 1986; 314:1360-1368.
5. Bottazzo GF. Death of a beta cell: homicide or suicide? Diabetic Med 1986; 3:119-130.

2. Review of the immune system and terminology

A skeletal overview of the immune system and the HLA D region will be presented first. A minimum understanding of both systems is required for what follows.

a. The immune system. A schematic view of the immune system is shown in Figure 1.

Figure 1

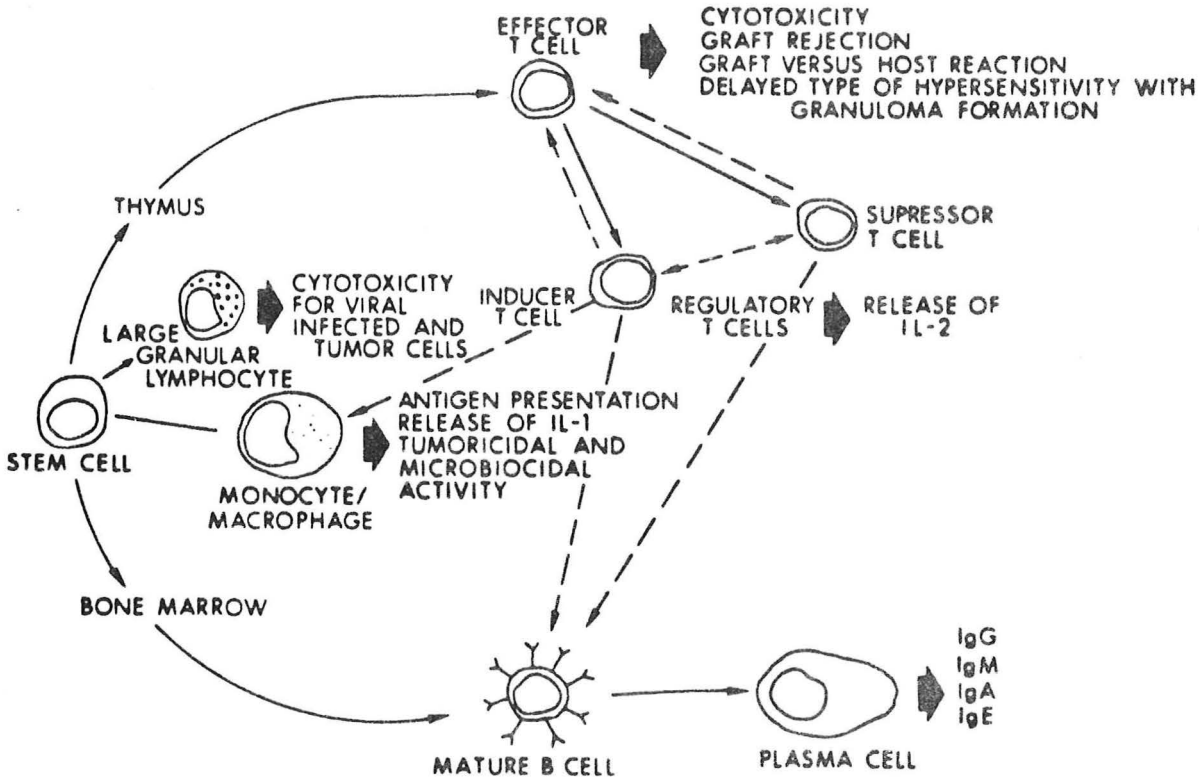


FIGURE *Schematic representation of cellular interactions involved in the generation of cell-mediated and humoral immunity.*

(From: BF Haynes, AS Fauci. Introduction to clinical immunology. In Harrison's Principles of Internal Medicine, 11th edition, E Braunwald, KJ Isselbacher, RG Petersdorf, JD Wilson, JB Martin, AS Fauci (eds). New York, McGraw-Hill, 1987, pp 328-337.)

Two functional arms develop from a primordial stem cell: the T lymphocyte system (responsible for cell-mediated immunity and immunoregulation) and the B lymphocyte system (responsible for humoral immunity) (6). The effector T cell mediates graft rejection, graft versus host reaction, delayed hypersensitivity and granuloma formation. The B cell matures to a plasma cell and produces antibodies. The other important cells are:

Macrophages. Macrophages are present in both blood and tissues. In tissues they derive from circulating macrocytes. They are the monocytes primary antigen presenting cells that interact with the helper T cell to activate an immune response. They also produce interleukin 1 (IL-1), a regulatory lymphokine, and carry out bacteriocidal, tumoricidal and (occasionally) cytotoxic functions. Macrophages express class II HLA molecules constitutively.

Helper/Inducer T cells. These regulatory T lymphocytes normally enhance the immune response. They are usually defined by the presence of CD4 (T4, OKT4) antigen on the surface of the cell as determined by monoclonal antibodies. However, T lymphocytes bearing CD4 can have cytolytic (killer) function against virally infected cells (7,8). It is now known that helper T lymphocytes not only "turn up" antibody response in the B cell system but also have the capacity to induce suppressor T cell formation. They produce interleukin 2 (IL-2).

Suppressor T cells. These cells "turn down" both humoral and cell mediated immune responses. They are ordinarily identified by the presence of the CD8 (T8, OKT8) antigen on the cell surface. It was originally thought that suppressor and cytolytic function were carried out by the same cell, but this is now known not to be true (7).

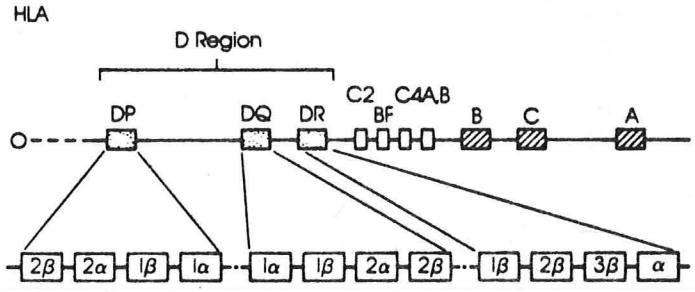
Cytotoxic (killer) T cells. These cells carry the CD8 antigen but are distinct from suppressor cells. They are thought to be primarily involved in the defense against viruses.

Large granular lymphocytes (natural killer or NK cells, null cells). These cells do not bear surface markers of the T cell line. They are cytolytic against antibody-coated cells (binding to the Fc portion of bound antibody) as well as non-antibody coated cells, especially malignant cells. The former activity is called antibody-dependent cellular toxicity (ADCC) while the latter is designated natural killer function.

When the body is exposed to a foreign antigen the response normally occurs as follows: the foreign antigen (e.g., bacteria, virus) is engulfed by the monocyte/macrophage with the constituent protein being broken down to short peptides. These peptides are then inserted into the plasma membrane where they bind to a class II HLA molecule on the surface of the cell which is constitutively present and of a type that is genetically determined. A major function of the class II molecule is to hold the peptide in favorable position for recognition by the T cell receptor on the helper T cell (9). In the processing, interleukin 1 (IL) is produced. A clonally derived helper T cell then binds to the antigen producing cell. Ordinarily "docking" of the helper T cell on the antigen presenting cell requires the presence of a self class II molecule on the latter (called MHC restriction). The T cell receptor, a highly complex and variable molecular structure, was discussed in these rounds on December 18, 1986 by J. D. Capra (10). It appears to have as its functional unit the juxtaposed Ti and T3 antigens. It is best thought of as having an essentially unlimited repertoire, like immunoglobulins. Once activated the helper/inducer cell carries out its multiple functions. How these functions are directed is only now being worked out.

b. The HLA D region. The human leukocyte antigens (HLA) are encoded in the major histocompatibility gene complex located on the short arm of the 6th chromosome in humans. A schematic representation is shown in Figure 2.

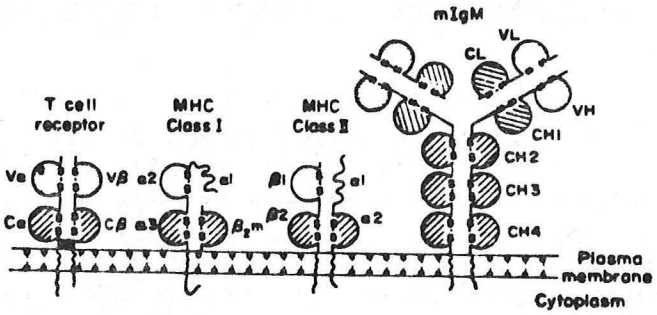
Figure 2



(From: CB Carpenter. The major histocompatibility gene complex. In Harrison's Principles of Internal Medicine, 11th edition, E Braunwald, KJ Isselbacher, RG Petersdorf, JD Wilson, JB Martin, AS Fauci (eds). New York, McGraw-Hill, 1987, pp 337-342.)

Each site is designated by letter with identifiable alleles given numbers (e.g., DR3). Genes coded by the A, B and C sites are designated "class I" molecules while those in the D region are called "class II." Complement-related antigens are classified as "class III" molecules. All of these antigens are present in the plasma membrane with a short cytoplasmic anchor (Figure 3). Quantitatively the DR antigens are most prominent. Their overall 2 chain anatomy is similar.

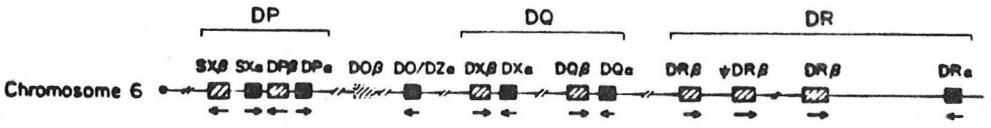
Figure 3 (ref 8)



Class I molecules are present on all nucleated cells while class II molecules are normally present only on cells of the immune system: monocyte/macrophages, B lymphocytes and activated T lymphocytes. It is of interest that class I but not class II molecules are closely associated with the insulin receptor (11,12).

Each of the D region sites has several genes. Terminology varies. In Figure 2 there are 2 alpha and 2 beta genes listed for the DP and DQ regions and a single invariant alpha gene and 3 beta genes at DR. An alternative terminology is shown in Figure 4.

Figure 4 (ref 8)



Here the second set of DP genes is called SX_α and SX_β while the second set at DQ is designated DX_α and DX_β. DZ_α or DO/DZ_α is known but not localized. How these genes function is not yet understood but it is presumed that only one is active in a given individual (13). The second (middle) DR_β gene is a pseudogene, missing the first domain found in $DR_{\beta I}$ and $DR_{\beta III}$.

Functionally class I molecules are thought to have a primary role in defending against viruses via cell mediated immunity. Class I directed cytotoxic T lymphocytes are $CD3^+8^+4^-$. Class II directed T lymphocytes function primarily as restriction agents in activation and regulation of the immune response to foreign or self antigens. Their surface makeup is $CD3^+4^+8^-$. A subset of the $T4^+8^-$ cells can function in cytotoxic reactions analogous to the $T8^+T4^-$ cells (8).

As noted above the primary role of the HLA D region molecules is to bind short peptides derived from foreign antigens (9). Each class II molecule has only a single binding site which interacts with a number of peptides (14). Competitive inhibition can be demonstrated between the immunogenic peptides (Fig 5). Cross-linking experiments show that both α and β chains are involved in binding.

Figure 5 (ref 14)

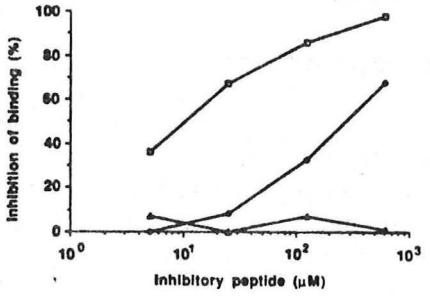


Fig. Inhibition of binding of ^{125}I-labeled HEL-(46–61) to I-A^k. Purified I-A^k (at 40 μM) was incubated with labeled peptide and a dose range of inhibitory peptide for 2 days at room temperature. The degree of binding of labeled peptide to Ia was determined by gel filtration and the percent inhibition of binding was plotted against the concentration of inhibitory peptide used. The inhibitory peptides used were: Ha(130–142) (□), HEL(74–86) (◆), Myo(132–153) (△).

There appears to be a degree of homology between the amino acid sequence of the foreign peptide bound and an internal "ligand" in the histocompatibility protein. The internal ligand is presumed, therefore, to be complementary to the foreign antigen binding site. In one fascinating experiment a peptide (P12-26) derived from bacteriophage lambda repressor (cI) was found to bind to I-A^d, I-E^d and I-E^k (purified class II molecules from the mouse, also called Ia molecules) as shown in Table 3.

Table 3 (ref 9)

Class II antigen	Percent of peptide bound
$I\text{-}A^d$	1.6 ± 0.8 (n = 5)
$I\text{-}E^d$	8.9 ± 2.2 (n = 7)*
$I\text{-}A^k$	0.3 ± 0.5 (n = 5)
$I\text{-}E^k$	2.3 ± 1.7 (n = 5)

*Level of binding is significantly different from the other three class II molecules at the >99 percent confidence level.

Table Binding of ^{125}I-labeled cI P12-26 analog to class II molecules. The peptide P12-26 was modified by the addition of tyrosine residue to the NH_2-terminus to serve as an acceptor for the ^{125}I. Ia molecules were purified from Nonidet P-40 (NP-40) lysates of A20 ($H2^d$) or AKTB-1b ($H2^k$) cells by affinity chromatography using the monoclonal antibodies. MK-D6 ($I\text{-}A^d$–specific), 10-3-6 ($I\text{-}A^k$–specific) or 14-4-4 ($I\text{-}E^{d/k}$–specific) were coupled to Sepharose 4B beads (Pharmacia Fine Chemicals, Sweden) (*5*). The gel filtration assay for determining the degree of association between immunogenic peptides and Ia has been described (*38*). Briefly, 40 μM of purified Ia in 1 percent solution of NP-40 and phosphate-buffered saline was mixed with 0.2 μM of ^{125}I-labeled peptide (approximately 200,000 count/min for each experiment) and incubated for 48 hours at room temperature to allow for formation of the Ia-peptide complex. The Ia-peptide complexes were separated from free peptide by gel filtration, and the percentage of peptide bound to Ia was calculated as the ratio of the ^{125}I-labeled peptide in the void volume to the total ^{125}I-labeled peptide recovered.

When bound to $1\text{-}A^d$ in vitro, the complex stimulated T cell hybridomas but despite being more tightly bound to $1\text{-}E^d$, the $1\text{-}E^d$ complex could not activate T cells. Further, after immunization of BALB/c mice with cI, 300 T cell hybridomas were obtained, none of which could be stimulated by P12-26 in the presence of $I\text{-}E^d$ although they could be activated with the $I\text{-}A^d$ bound peptide. In unfractionated T cells isolated from the blood of 26 immunized mice the stimulating activity of P12-26 was blocked by a monoclonal antibody against $1\text{-}A^d$ but not by an anti $I\text{-}E^d$. It was noted that there was marked amino acid homology between the phage repressor peptide and a region of the class II molecule:

λ repressor cI: Leu Glu Asp Ala Arg Arg Leu Lys Ala Ile Tyr Glu Lys

$I\text{-}E_\beta^d$: Leu Glu Asp Ala Arg Ala Ser Val Asp Thr Tyr Cys Arg

The authors speculate that intense homology may produce "invisibility" to the helper T cell which interprets the foreign peptide as self because it mimics the "internal ligand" and is tightly bound to the class II molecule (9). Thus one might have two sequences:

Self class II + "mimicking" foreign epitope → no activation
Self class II + less homologous epitope → activation

A further implication, which will not be explored here, is that alloreactivity may be due to T cell recognition of the "internal ligand" of a foreign class II molecule; i.e., a given T cell could react in an identical way to a foreign ligand bound to a self class II molecule or foreign class II molecule which had "bound" its own internal ligand.

Obviously these interpretations are highly tentative, but they could be informative for autoimmune diabetes.

c. Assays. The D region is analyzed in several ways. DR and DQ alleles can be identified by serologic testing (alloantisera) radioimmunoassays, monoclonal antibodies and T cell responses. DP products are primarily identified by T cell responses but are also identified by monoclonal antibodies (13). The two main cellular tests are:

The homozygous typing cell (HTC) test. This test utilizes proliferative responses of T lymphocytes (usually assessed by tritiated thymidine uptake) to stimulating cells that are putatively homozygous for DR and DQ products (such cells may not be homozygous for DP). The

determinants are labeled Dw (little w means "workshop") and indicates that antigens are ill-defined or provisional. In this test if the stimulating cell bears antigens different from the responder (test) cell, ^{3}H thymidine uptake is stimulated. If there is little or no stimulation it is presumed that a shared D determinant is present.

The primed lymphocyte typing (PLT) test. A determinant recognized by T lymphocytes in this test is designated LD. These determinants are epitopic and associated with a single molecular product in contrast to Dw which measures a summation of LD. Stimulating and responding cells are incubated for 10 days, allowing the responders to revert to a primed but non-dividing status. When they are exposed to 3rd party cells they quickly respond, allowing identification of differences that might not be picked up in the HTC.

Serologically defined and T lymphocyte determined antigens are not identical. Overlapping determinants are called supertypic, meaning that they are found associated with two or more other antigens which are designated subtypic. For example DQw1 is supertypic to DR1, DR2 and DRw6; i.e., individuals can be DQw1/DR1, DQw1/DR2, etc. Conversely all DR1, 2, and w6 cells will likely bear DQw1. DR determinants may also be supertypic for Dw (Figure 6).

Figure 6 (ref 13)

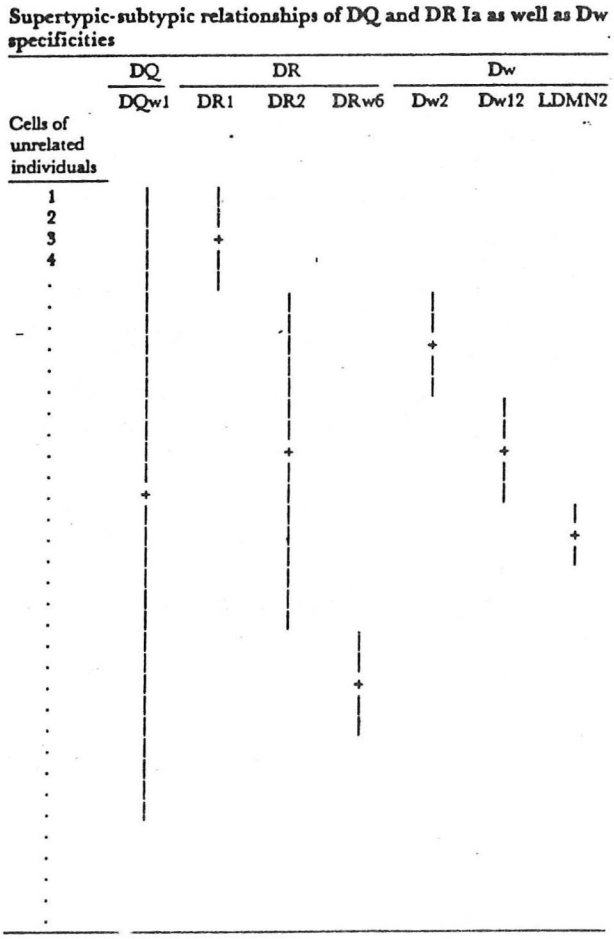


Fig. A schematic representation of the supertypic–subtypic relationship of DR-DQ Ia and Dw specificities illustrated for DQw1.

DR1, 2 and w6 are also seen associated with DQ specificities of non-DQw1 individuals; Dw subtypes are shown only for DR2. The length of the vertical lines (which indicate cells that are positive for the specificity under which the lines appear) is not intended to indicate antigen frequencies.

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7. Acuto O, Reinherz EL. The human T-cell receptor. Structure and function. N Engl J Med 1985; 312:1100-1111.
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9. Guillet JG, Lai MZ, Briner TJ, Buus S, Sette A, Grey HM, Smith JA, Gefter ML. Immunological self, nonself discrimination. Science 1987; 235:865-870.
10. Capra JD. The T cell receptor and human disease. Internal Medicine Grand Rounds, December 18, 1986.
11. Phillips ML, Moule ML, Delovitch TL, Yip CC. Class I histocompatibility antigens and insulin receptors: evidence for interactions. Proc Natl Acad Sci USA 1986; 83:3474-3478.
12. Due C, Simonsen M, Olsson L. The major histocompatibility complex class I heavy chain as a structural subunit of the human cell membrane insulin receptor: implications for the range of biological functions of histocompatibility antigens. Proc Natl Acad Sci USA 1986; 83:6007-6011.
13. Bach FH. The HLA class II genes and products: the HLA-D region. Immunol Today 1985; 6:89-94.
14. Buus S, Sette A, Colon SM, Miles C, Grey HM. The relation between major histocompatibility complex (MHC) restriction and the capacity of Ia to bind immunogenic peptides. Science 1987; 235:1353-1358.

3. Genetics

The genetics of diabetes in humans is not well understood. Phenotypic expression (overt disease) does not allow conclusions to be drawn in classic Mendelian terms. It is likewise not known whether there is one or more than one gene for diabetes. It is essentially certain that at least one gene is located in the major histocompatibility region of the 6th chromosome as noted. More than 90%, probably 95%, of patients with Type 1 diabetes carry HLA DR3 or DR4 with a significant percentage bearing the heterozygous DR3/4 combination (2-5, 15). Representative figures are shown in Table 4 (where X represents any allele other than DR3 or 4).

Table 4 (ref 15)

TABLE
HLA-DR status and race

	DR3x	DR4x	DR3/4	DRx/x
Whites	23%	36%	30%	11%
(N = 172)	(39)	(62)	(52)	(19)
Blacks	53%	12%	29%	6%
(N = 17)	(9)	(2)	(5)	(1)

The gene frequency of DR3 and 4 varies from 20-40% in nondiabetic populations, but averages 25% in the United States. Whether there is a second gene on the 6th chromosome or on another chromosome remains uncertain. Candidate sites have been the 5' upstream region of the insulin gene on chromosome 11, the region coding for the heavy chain of immunoglobulins (Gm allotype) on chromosome 14, the gene for the kappa light chain (Km allotype) on chromosome 2 and the Kidd marker on chromosome 2. By and large these associations have proven to be weak or non-existent (5,16-21). Polymorphisms at the haptoglobin site have also been discussed (22) but not confirmed. Against this background several additional points can be made. For purposes of discussion I will divide them into 2 sections, Family and Population Studies and Molecular Genetics.

a. Family and population studies

(1) The risk of Type 1 diabetes for first degree relatives of a proband with insulin-dependent disease varies from 2-8% (23-25). Table 5 shows the adjusted risk to age 80 for white families with 1 child or parent having Type 1 diabetes. The percent risk for blacks (26) and hispanics (27) will be slightly less but has not been precisely worked out. The HLA distribution in American blacks reflects that found in whites and presumably represents racial admixtures since it does not mirror the makeup found in African blacks (28,29). The number of families having two parents with Type 1 diabetes is too small to have valid studies of risk.

Table 5 (data from ref 25)

Empirical risk for Type 1 diabetes to age 80

Proband's age at onset	Parents (n=1083)	Siblings (n=982)	Children (n=649)
		% ± SEM	
< 25 yr	2.2 ± 0.6	6.9 ± 1.3	5.6 ± 2.8
≥ 25 yr	4.9 ± 1.4	5.8 ± 1.8	4.3 ± 2.2
Total	2.9 ± 0.6	6.6 ± 1.1	4.9 ± 1.7

(2) Diabetic fathers are more likely to transmit disease to children than diabetic mothers. It is quite remarkable that children of fathers with Type 1 diabetes are approximately 5 times more likely to inherit the disease than children of diabetic mothers (30). This is illustrated in Figure 7. The reason for this is not clear. Warram et al (30) speculated along 2 lines: diminished recombination between linked loci during gametogenesis in men and selective spontaneous loss of the diabetic fetus in utero. The former would be important only if recombinations resulted in lesser risk of diabetes (breaking of diabetogenic combinations). There is no firm evidence supporting either possibility.

Figure 7 (ref 30)

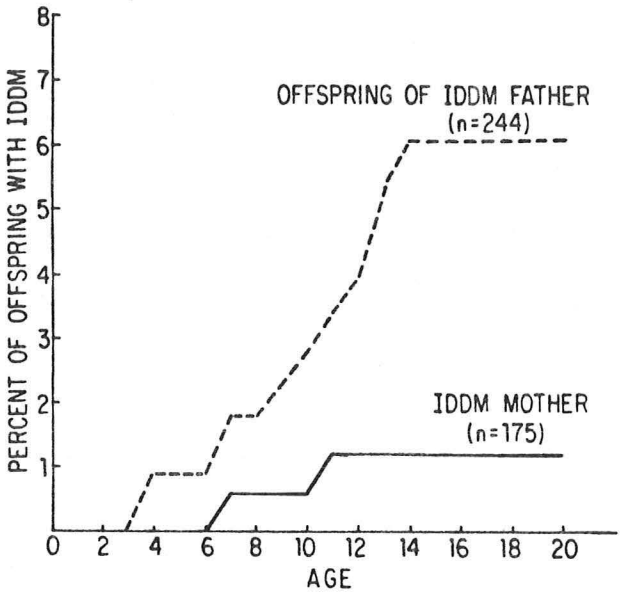

Figure Cumulative Risk of Insulin-Dependent Diabetes Mellitus (IDDM) Up to Age 20 in Offspring of a Parent with Juvenile-Onset IDDM, According to the Sex of the Diabetic Parent.

(3) There is preferential (nonrandom) transfer of diabetes-linked HLA genes from nondiabetic parents to offspring, especially diabetic offspring. Two studies indicate that there is preferential paternal transmission (transfer distortion) of diabetic alleles. Kay, Wilton and Dawkins (31) examined 102 offspring in 26 families where 15 mothers and 14 fathers were heterozygous for HLA B18, BfF, DR3. The diabetogenic allele (DR3 containing haplotype) was passed to 50% of the children when the mother was the carrier but 68% of the children when the father was the carrier. Slightly different results were found by Vadheim et al (32) who showed that DR3 was transferred to children substantially more than 50% of the time by both mothers and fathers. DR4 was passed to diabetic children in excess of 50% by both parents but paternal transmission was greater than maternal transmission in both diabetic and nondiabetic offspring. Mothers did not appear to pass DR3 to nondiabetic children in excess (see Table 6).

Table 6 (data from ref 32)

Transmission of DR3 and DR4 from parent to offspring

	Father		Mother	
	HLA DR3	HLA DR4	HLA DR3	HLA DR4
IDDM child	75.4%	83.1%	71.4%	64.5%
no IDDM child	61.2%	59.2%	53.7%	45.5%
total	68.0%	72.1%	65.1%	55.6%

Parents homozygous for DR3 or DR4 were eliminated so that results were unambiguous. Preferential passage of DR4 from the father relative to the mother was not seen in a smaller study (33). Transmission distortion may account for the fact that the diabetogenic gene(s) are not decreasing in the population despite lethal complications that shorten life expectancy (32).

(4) It is possible that the putative HLA DR4-linked gene is transmitted in dominant fashion while the putative DR3-linked gene is recessive. The discussion of inheritance of susceptibility to diabetes continues to be the subject of lively debate (34-36). It is known that either DR3 and DR4 alone increase the risk of diabetes and that the DR3/DR4 heterozygous state induces the highest risk. It is further known that risk of disease amongst siblings is haplotype dependent; i.e., if a nondiabetic sibling shares both haplotypes with the diabetic sibling the chance of getting diabetes is at least 20% and perhaps higher (see Fig. 8). If one haplotype is shared the risk is 5% and if neither are shared it is 1% (4).

Figure 8 (ref 22)

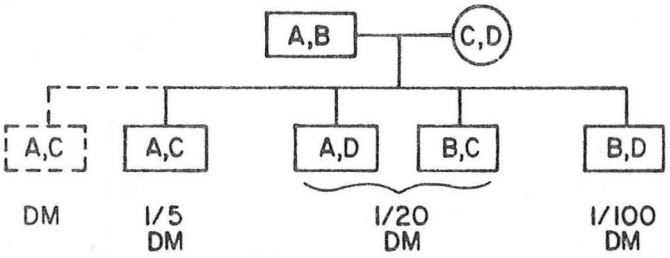

FIG. Risk of type I diabetes mellitus among siblings of proband, relative to HAL type (A, C). *Broken line* denotes proband with diabetes mellitus (DM), fractions indicate approximate risk of diabetes in siblings, and letters denote HLA identity. From Eisenbarth (*Triangle, SANDOZ J Med Sci*, copyright SANDOZ LTD, Basel, Switzerland).

For this reason the most popular model for inheritance is "mixed" in Mendelian terms meaning that one gene acts in recessive fashion and the other in the dominant mode (36). Rubinstein and coworkers (35) have argued against this view on the grounds that the DR3/DR4 excess in Type 1 diabetes is seen in the first child with the disease but not the second (Table 7). They thus believe that excess DR3/DR4 cannot be directly related to susceptibility but only indirectly through interaction with environmental triggers. They suppose that when the environmental risk is low DR3/DR4 bearing subjects are peculiarly vulnerable. Thus the first child to get diabetes would have a higher chance of being DR3/DR4 while second children would have a higher environmental risk for some unspecified reason and thus get diabetes from a less "genetic" HLA. One possibility, I suppose, would be increased virulence of the virus after passage through a pancreas (37).

Table 7 (ref 35)

TABLE
Frequency of DR3/4 heterozygotes among index cases and affected sibs in families with conducive parents

	Simplex (N = 158)	Multiplex (N = 43)	All (N = 201)
Conductive parents*	43 (27%)	17 (40%)	60 (30%)
DR3/4 indices	33 (77%)	13 (76%)†	46 (77%)‡
DR3/4 later-affected sibs		10/25 (40%)	10/25 (40%)

*One parent DR3/X and the other DR4/X. However, one may be DR3/4.
†χ^2 = 5.43; $P \sim .02$.
‡χ^2 = 10.56, $P \sim .001$.

Regardless of the reason, they use the first sibling/second sibling findings to remove the necessity for a "dominant" element and argue for a purely recessive model. This paper, although interesting, is not very persuasive to me. More impressive is the study of MacDonald et al (33) who looked at inheritance of DR3 and DR4 in 37 families where a parent and child both had Type 1 diabetes. The diabetic parent contributed DR4 to the diabetic child in 78% of the cases (versus a gene frequency for DR4 in the general diabetic population of 43%) (Table 8).

Table 8 (ref 33)

Table *HLA-DR* alleles transmitted to diabetic offspring by their parents with IDDM

Subject	*DR* type, sex, and age at diagnosis: Offspring	Parent	*DR* allele transmitted to offspring
1	*3,4*; M (3)	*4,4*; M (17)	*4*
2a	*3,4*; F (5)	*4,4*; M (12)	*4*
2b*	*3,4*; F (2)		*4*
3	*3,4*; M (1)	*1,3*; M (1)	*3*
4	*1,4*; F (7)	*1,4*; F (12)	*4*
5	*3,4*; M (7)	*4,5*; F (10)	*4*
6	*1,4*; M (2)	*4,5*; M (21)	*4*
7a	*3,4*; M (4)	*4,4*; M (29)	*4*
7b*	*3,4*; M (8)		*4*
8	*4,4*; F (10)	*4,5*; M (11)	*4*
9	*3,4*; M (3)	*2,4*; M (1)	*4*
10	*4,4*; M (10)	*4,4*; M (10)	*4*
11	*3,7*; M (10)	*3,4*; M (13)	*3*
12	*1,4*; M (1)	*1,4*; M (7)	*4*
13	*1,4*; F (19)	*3,4*; M (39)	*4*
14	*4,5*; M (14)	*1,4*; M (15)	*4*
15	*3,4*; M (13)	*3,4*; F (32)	*4*
16a	*4,4*; M (6)	*4,4*; M (19)	*4*
16b	*4,4*; M (6)		*4*
16c	*4,4*; M (10)		*4*
16d	*4,w6*; M (11)		*4*
16e*	*4,4*; F (14)		*4*
17	*4,w6*; F (6)	*4,4*; M (39)	*4*
18	*1,9*; F (9)	*1,9*; F (27)	*9*
19	*3,4*; M (13)	*3,4*; F (9)	*4*
20	*1,3*; M (6)	*1,3*; M (14)	*1*
21	*1,4*; M (18)	*4,6*; M (23)	*4*
22	*4,5*; M (10)	*3,5*; M (14)	*5*
23	*3,4*; M (3)	*4,8*; M (7)	*4*
24	*4,9*; M (5)	*3,4*; M (4)	*4*
25	*3,4*; F (12)	*1,3*; M (29)	*3*
26	*2,2*; F (6 mo)	*2,7*; F (7 mo)	*2*
27	*3,w6*; F (1)	*3,w6*; M (20)	*w6*
28	*4,7*; F (7)	*4,5*; M (25)	*4*
29	*3,4*; M (3)	*2,4*; M (29)	*4*
30	*4,4*; M (7)	*3,4*; M (13)	*4*
31	*4,4*; F (3)	*4,7*; F (31)	*4*
32	*2,4*; F (7)	*4,4*; M (9)	*4*
33	*4,5*; M (5)	*4,7*; M (46)	*4*
34	*4,7*; F (2)	*3,4*; M (35)	*4*
35	*3,4*; M (12)	*1,4*; F (31)	*4*
36a	*4,w6*; M (11)	*3,4*; M (4)	*4*
36b	*1,4*; F (17)		*4*
37	*3,4*; M (11)	*1,4*; M (36)	*4*

Numbers in parentheses indicate age in yr unless specified otherwise.
*These offspring received a haplotype containing *DR4* from the diabetic parent that is different from the one received by the other sibling(s).

When DR3 was transmitted it usually came from the nondiabetic parent. The percentage of diabetic offspring carrying DR3 was higher than the control population suggesting that it incurred susceptibility to diabetes, but the effect was relatively weak. These findings are in accord with a dominant activity of DR4 and a recessive activity for DR3.

(5) There is evidence that the clinical course of diabetes may be modified by the HLA D region genes. Two studies have suggested that Type 1 patients bearing DR3 have a milder illness with less ketoacidosis than subjects bearing DR4 (15,38). Moreover, Dw3/Dw4 heterozygotes have more rapid destruction of β cells and shorter remission ("honeymoon") periods than non DR3/DR4 subjects (39). T lymphocyte response to antigen is lower when HLA restriction is DR3 linked and higher when DR4 linked (40).

b. Molecular genetics

The critical question at the molecular level is whether the putative diabetes susceptibility gene(s) on the 6th chromosome are in the D region itself or only in linkage disequilibrium with genes in the D region. (Linkage disequilibrium means that the genes associate non-randomly; i.e., a diabetes gene segregates with DR4 or DR3 but not DR2). The concept of linkage is based on the fact that diabetes may occur in the absence of the high risk DR3/DR4 genes (35,41) and that "extended" haplotypes often confer greater degree of risk than DR3 or DR4 alone (42-45). Examples of such extended haplotypes are HLA-B8, C4-B1, Bf-S, HLA-DR3, GLO-2 and HLA-B15, C4-A3B3, Bf-S, HLA-DR4 both of which predict development of diabetes better than DR3 and DR4 assayed as single alleles, suggesting the possibility of gene location outside the D region.

On the other hand it is now known that serologic assessment of the DR alleles is too broad and that particular subtypes to a given DR may confer much higher risk than that ascertained by the serologic agent (46-48). Detailed discussion is beyond the scope of this presentation but analysis by restriction endonucleases has allowed subclassification of D region grouping based on restriction fragment length polymorphisms (RFLP). Although the putative diabetic gene could be at any D site the most informative probes for both susceptibility (49-55) and resistance (54) derive from DQ (both α (49,51) and β (50, 52-54) clones).

An illustration follows. It is known that persons with Type 1 diabetes rarely express HLA DR2, which has been designated a "protective" or "resistance" allele. Occasionally, however, diabetes occurs with DR2. When 11 such patients were studied it was found that 9 carried a DQ_{β} allele designated "DQR1" (52). Only 2 healthy controls expressed this allele (Fig 9). The interesting point is that DQR1 is normally associated with HLA DR1, a known allele increasing risk to diabetes. Thus one could conclude that recombinations had shifted the genetic makeup from the normal HLA DR1/DQR1 to HLA DR2/DQR1, thereby converting HLA DR2 from resistant to susceptible. The fact that susceptibility to diabetes is much narrower than indicated from serologic tests has been confirmed at the cellular level (Table 9).

Figure 9 (ref 52)

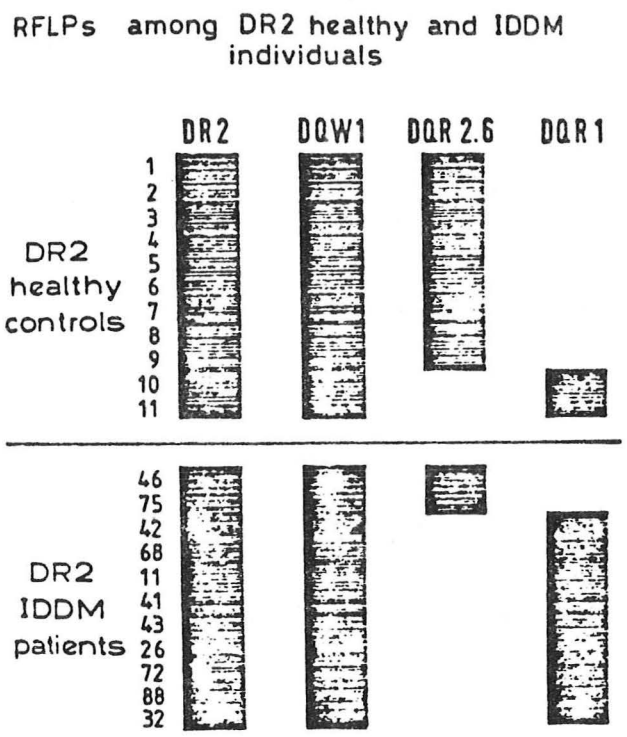

Fig. The healthy controls are DR-matched with the IDDM patients and belong to the same ethnic group: the French population

Table 9 (ref 52)

Table Subdivision of HLA-DR2

Serology		Cellular		2-D gel electrophoresis			RFLP			Disease association
DR	DQ	MLC	PLT	DR			Eco RI (DQ β)	Eco RV (DQ β)	DQR subset	
				β1	β2	β3				
DR2	DQw1	Dw2			β2 (1)		2.75 kb 12 kb	5.2 kb	DQR2.6	Multiple sclerosis Narcolepsy
DR2	DQw1	Dw12			β2 (2)		24 kb	14 kb	DQR12	?
DR2 short	DQw1	Dw"AZH"	MN2		β2 (3)		16 kb	2.6 kb	DQR1	IDDM

Here it can be seen that subjects bearing HLA DR2 and the supertypic DQw1 have vastly differing disease susceptibilities depending on DQ subsets. If one has DQR2.6 and Dw2 by mixed lymphocyte culture (MLC) the vulnerability is to multiple sclerosis. If one has Dw"AZH" by MLC, "MN2" by primed lymphocyte testing and DQR by restrictive fragment length polymorphism, then the susceptibility is to Type 1 diabetes.

Similar narrowing of specificities between disease susceptibility was reported by Nepom et al (53). Both Type 1 diabetes and juvenile rheumatoid arthritis (JRA) are associated with HLA DR4. However, the

two can be clearly distinguished by a DQ_{β} probe. Thus:

IDDM = $DR4^{+}_{+}$, DQ3.2
JRA = $DR4^{+}_{+}$, DQ3.1

An interesting approach to informative RFLP analysis is to use pooled DNA samples from a disease group and matched controls, rather than testing individual patients (56).

It is likely that further investigation will narrow the abnormalities still further since sequencing of D region genes has already shown "hot spots" of gene conversion (57) and distinct nucleotide abnormalities that correlate with Type 1 diabetes (58). An example is shown in Figure 10 where a consensus sequence of a DRβ chain first domain is compared to sequences in DR3, DRw6a, DRw6b and DR5. In a, the β1 locus, a "hot" spot is seen between 190 and 210.

Figure 10 (ref 57)

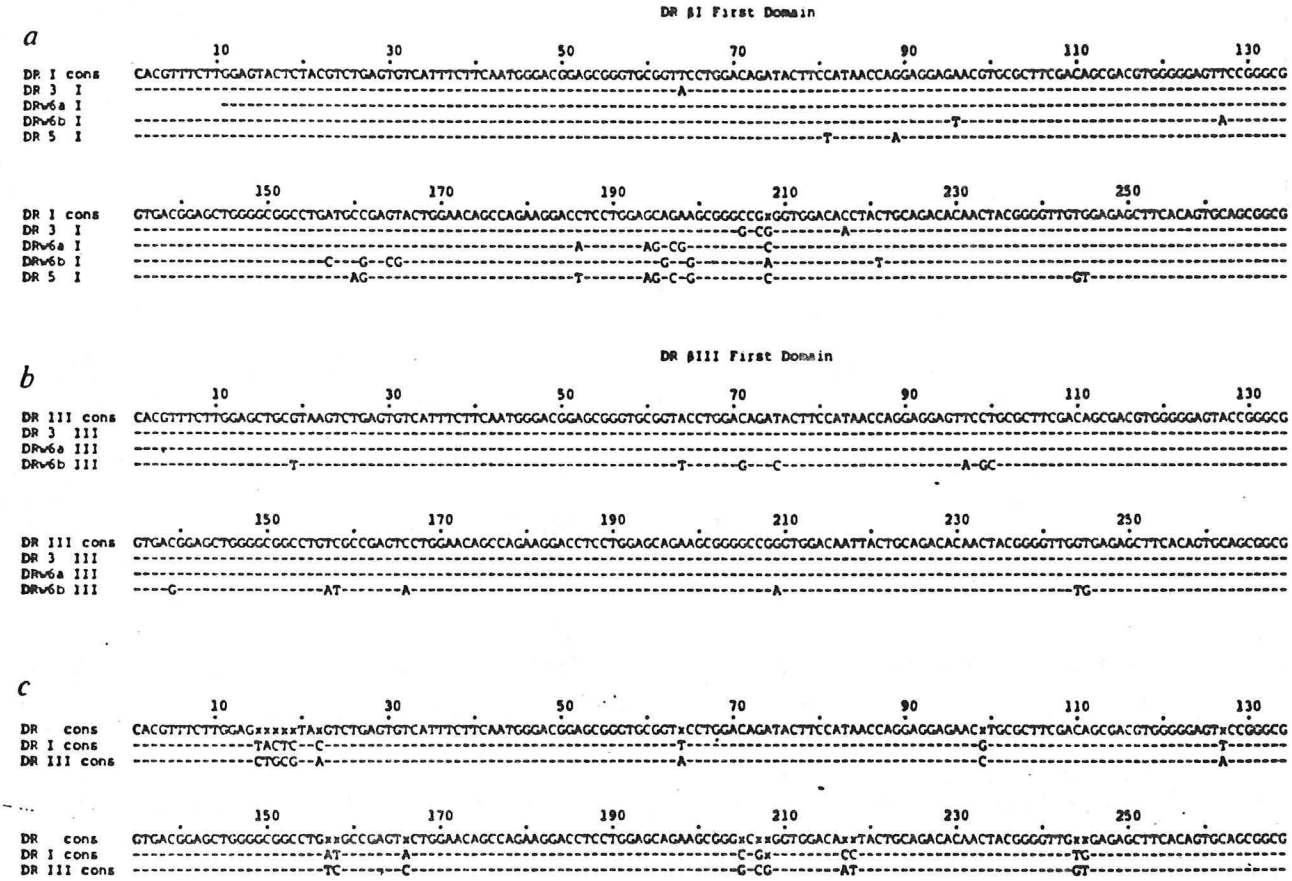

Fig. Sequence of the DR β-chain first domain of the *DR3* haplotype, two *DRw6* haplotypes and a published *DR5* haplotype[15]. *a*, *βI* locus; *b*, *βIII* locus. The consensus sequence (cons) is the base found at that position in at least two of the sequences. *c*, Comparison of locus *I* and locus *III* sequence differences by aligning the consensus sequences for each locus and deriving a *DRβ* consensus. Regions where *βI* and *βIII* differ from each other and which may influence locus-specific conformation of the protein product are easily visualized.
Methods. Sequences were obtained from subclones of the first domain exon of previously described cosmid and phage genomic clones[6,11], by both the chemical[23] and dideoxy[24] methods, as well as from cDNA clones[5]. *DR3* sequences are from a cosmid library of the consanguineous homozygous typing cell line AVL; *DRw6a* sequences are derived from a cosmid library and cDNA library of the consanguinous homozygous typing cell line HHK; *DRw6b* sequences are from a Charon 30 phage library of a *DR4*, *DRw6* line. The *DRw6* genes were identified by Southern blot comparisons with *DRw6* and *DR4* genomic DNAs (see ref. 25 for an example). The cDNA-derived sequence of *DRw6b βIII*[5] as well as a phage-derived sequence[25] have been published previously.

The shift of small nucleotide segments from one gene to another could result in the appearance of epitopes in the protein structure of the gene product that would cause immune activation, say but that would not be reflected in serologic tests. Thus an epitope conferring susceptibility to diabetes might appear in the context of a DR2/DR2 individual or an epitope causing celiac disease might appear as a $DR4_{\beta}$ allele rather than the usual $DQ2_{\beta}$ allele. The role of gene conversion has been extensively explored in the murine histocompatibility complex and is well established (59). An example of a human gene conversion is shown in Figure 11 where the βIII gene of DR6 was donor to βI of DR3.

Figure 11 (ref 57)

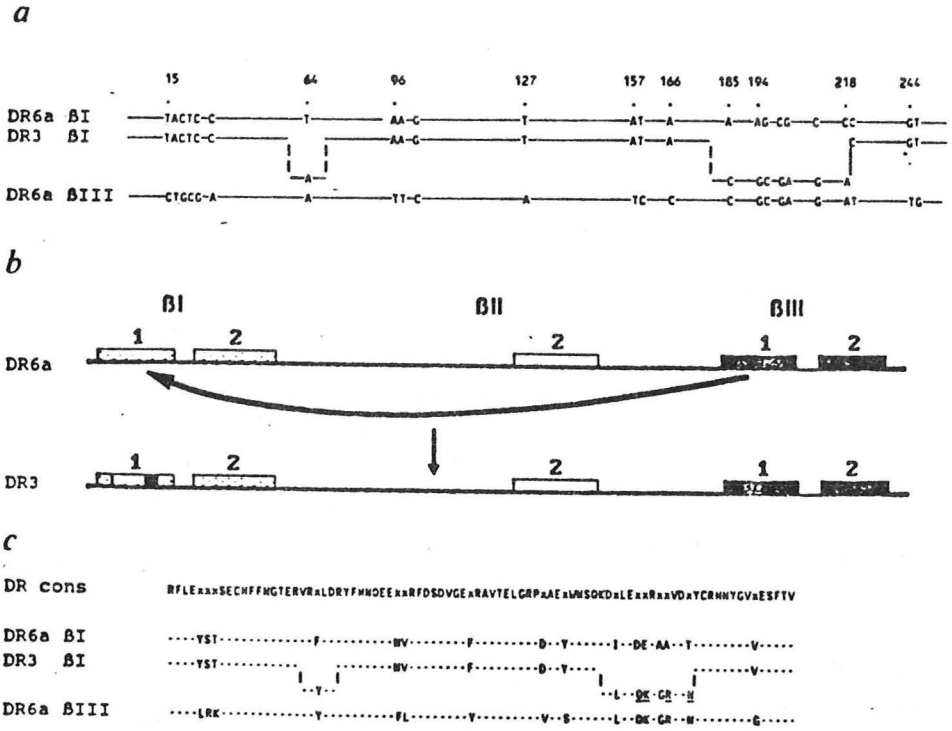

Fig. Intrachromosomal gene conversion between two *DRw6 β*-chain loci genes. *a*, The first-domain sequences of the two *DR6a* loci are aligned with the sequence of the *DR3 βI* locus. A solid line indicates identical sequences. Relevant nucleotide positions are shown to facilitate comparison with the sequence in Fig. 1. Because of the uncertainty in the limits of the conversion event, dotted lines are used to indicate cross-over points. *b*, Schematic representation of genes participating in the conversion event; *c*, amino-acid sequence of the first domains of the DR3 *βI* and DRw6a *βI* and *βIII* chains showing the block of amino acids transferred by the conversion event. Non-conservative amino-acid changes in the DR3 *βI* chain are underlined. The consensus approach is used to aid visualization of amino-acid differences between the *βI* and *βIII* loci. The block of amino acids transferred must interact with these differences in the generation of the novel epitope.

Finally, the system becomes even more complicated because of the possibility of transcomplementation. Transcomplementation refers to the construction of hybrid molecules between chains of opposite haplotypes on the cell surface; i.e., a DQ_{α} chain from one haplotype (say a DR3) could mix with a DQ_{β} chain of its paired haplotype (say a DR4) to form a $DQw3_{\alpha}$ $DQw2_{\beta}$ hybrid. This phenomenon, well-known in mice, has now been demonstrated directly in Type 1 diabetes in humans (60). The initial experiment is shown in Figure 12. In this figure F1D is a transformed (EB virus) B lymphocyte line from a Type 1 diabetic subject who serologically was an HLA DR3/DR4 heterozygote. HA and F1F are the parental cell lines. Monoclonal antibody 17.15 is DQw3 specific while antibody 17.1 is DQw2 specific. In the experiment cells were labeled with [^{3}H] leucine and the class II HLA molecules were precipitated with one or the other of the antibodies followed by electrophoresis of the α and β chains. Panel B shows the proband's pattern using the DQw3 specific antibody. Three spots are seen: the DQw3 α and β chains equivalent to parent HA and the DQw2 α chain of parent F1F.

Figure 12 (ref 60)

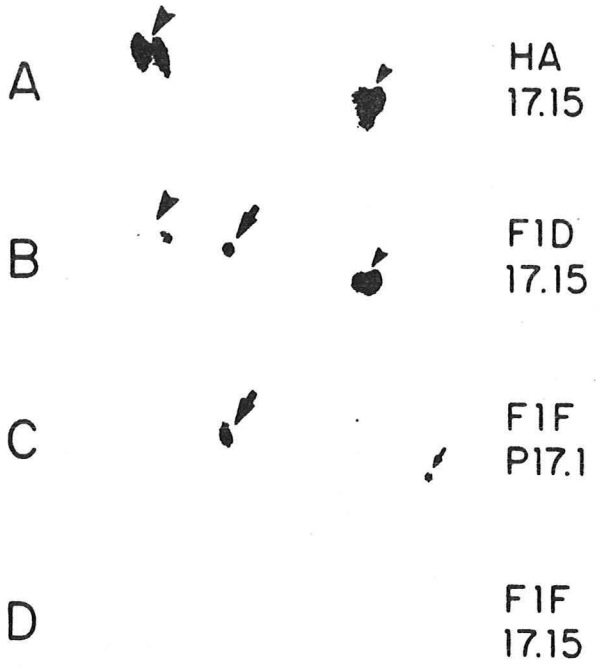

FIG. Electrophoretic analysis of HLA-DQ α- and β-chains. Radiolabeled HLA class II molecules were immunoprecipitated with DQ-reactive monoclonal antibodies and analyzed by 2-dimensional polyacrylamide electrophoresis. Acidic end of gel is on *left*, and basic end is on *right*, with higher molecular wieght at *top* of each autoradiograph. *A*: parental DQw3-associated α- (*large arrowhead*) and β- (*small arrowhead*) chains immunoprecipitated from cell line HA with monoclonal antibody 17.15. *B*: heterozygous diabetic cell line F1D (DR3'4, DQw2 w3) was immunoprecipitated with monoclonal antibody 17.15; only the DQw3 β-chain is seen, although both DQw2 and DQw3 α-chains coprecipitate. C: parental DQw2-associated α- (*large arrow*) and β- (*small arrow*) chains from cell F1F precipitated with monoclonal antibody P17.1. Antibody 17.15 does not react with DQw2 parental cell F1F (*D*).

This indicates the DQw2α DQw3β hybrid since DQw2 could not be visualized by the antibody in any other configuration.

* (DQw3α) (DQw3β) *
* (DQw3α) DQw2β
DQw2α DQw2β
* DQw2α (DQw3β) *

(○) recognizable by mAB 17.15

* found

Since the DQw2β chain was not visible the reverse hybrid DQw3α/DQw2β was not constructed. Analysis of the spots by HPLC after tryptic digestion confirmed the hybrid molecule (Fig 13).

Figure 13 (ref 60)

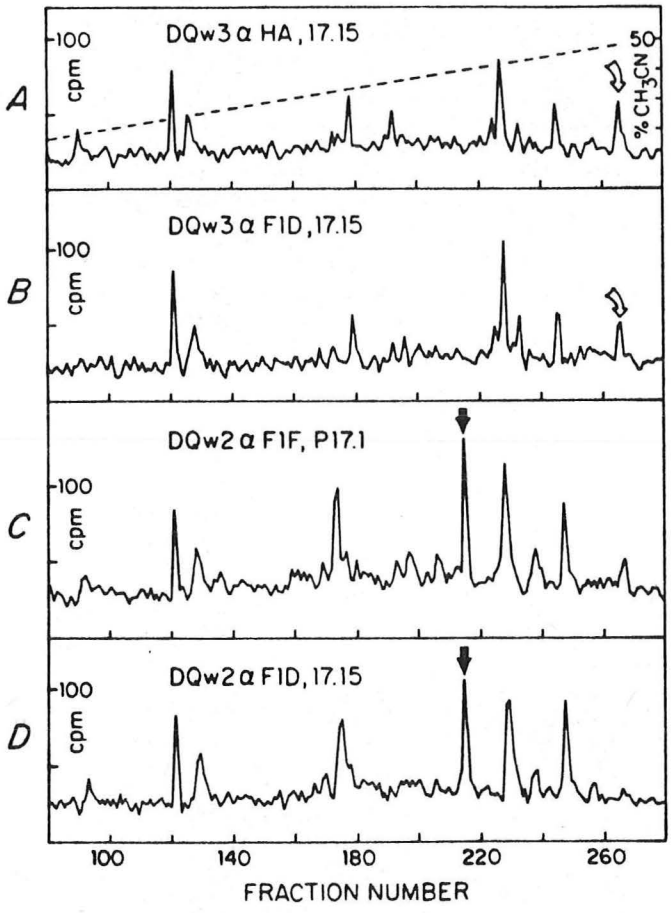

FIG. Peptide map analysis of DQ α-chains purified by 2-dimensional gel electrophoresis. DQ α-chain spots were purified by 2-dimensional gel electrophoresis. DQ α-chain spots were recovered by elution from polyacrylamide gels (Fig. 1) and analyzed by reverse-phase HPLC. Tryptic peptides from purified α-chains were eluted from octadecyl silica column with ascending acetonitrile gradient (*dashed line* in *A*). Peptide maps from parental DQw3 and DQw2 α-chains are shown in *A* and *C*, respectively. Peptide maps from DQw3 and DQw2 α-chains precipitated from heterozygous diabetic cell line F1D with monoclonal antibody 17.15 are shown in *B* and *D*. *Open arrow*, peak at fraction 266 characteristic of DQw3 α-chains; *closed arrow*, peak at fraction 215 unique to DQw2 α-chains.

Conclusion: On the basis of new data I think it is likely that the susceptibility genes for Type 1 diabetes are D genes themselves and not just a linked gene.

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4. Environmental event

As noted above the suggested requirement for a non-genetic trigger from the environment for the initiation of diabetes is based primarily on the observation that monozygotic twins are concordant for Type 1

diabetes less than 50% of the time and that siblings who are HLA haplotype identical are concordant 20-30% of the time (4). Although chemicals such as the rat poison Vacor can induce insulin-dependent diabetes (61) most attention has focused on viruses. The evidence for a viral cause is really quite soft as has been reviewed (62). On one occasion Coxsackie B4 was isolated from the pancreas of a boy dying after recent onset diabetes with ketoacidosis (63). When injected into rodents diabetes developed. A Coxsackie B5 virus isolated from the stool of a 16 month old child who developed diabetes caused glucose intolerance in mice (64). Perhaps the best documented relationship is with rubella infection in infancy. Twelve to twenty percent of children with the congenital rubella syndrome develop Type 1 diabetes (65). Affected subjects carry the typical HLA predisposing genes and develop islet cell antibodies. Rubella virus causes diabetes in the golden Syrian hamster. Certain differences between rubella induced diabetes and ordinary Type 1 diabetes have been reported: the rubella associated patients develop thyroiditis, have abnormal circulating T lymphocytes and demonstrate disturbed immune responses (22).

It is usually assumed that diabetogenic viruses act by inducing autoimmunity. However, under certain circumstances they appear to act directly via inflammatory destruction and not through immune mechanisms (66-68). As will be noted below, an early lesion in all forms of diabetes is loss of glucose induced insulin release. Infection of golden Syrian hamsters with Venezuelan encephalitis virus results in a similar defect even though permanent diabetes or altered structure of the islets does not result (69).

Indirect evidence for a possible role of viruses continues to accumulate. For example between 1982 and 1984 the incidence of IDDM doubled in Poland, strongly suggesting a major alteration in environmental risk factors (70). Two-thirds of children with new onset diabetes in Sweden had IgM antibodies against Coxsackie B while only 12% of control children had evidence of recent infection (71).

Eisenbarth (22) has posed the two critical questions regarding genetics and environment:

(1) Is an environmental trigger necessary to develop Type 1 diabetes?

(2) Can the environment alter the probability of developing Type 1 diabetes?

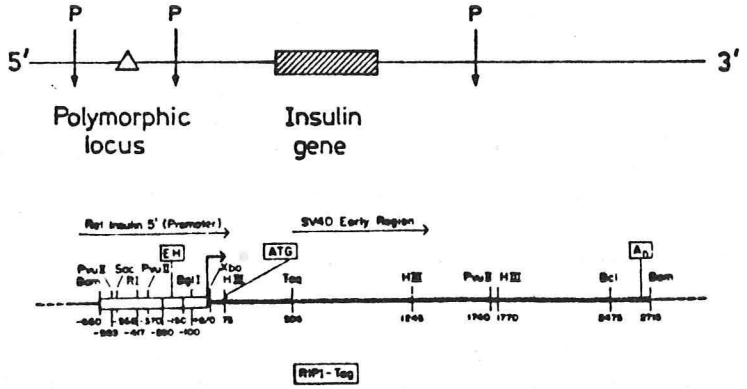
He answers the first question "perhaps not" and the second "yes." Supporting evidence against the necessity of an environmental factor includes the following:

- The non-obese diabetic (NOD) mouse and the Biobreeding (BB) rat, models of Type 1 diabetes, appear to develop diabetes independent of an environmental factor (the latter even in a germ free environment). Diet can prevent diabetes in the BB animal but does so by modulating the immune system (72).

- Identical twins may not be identical shortly after birth because of diversity introduced in immunoglobulin molecules and the T cell receptor (7). This removes the power of the concordance argument from twin studies.

- The same genetic lesion may produce apparently different clinical responses. Thus diabetes-prone BB animals who remain non-diabetic have diminished insulin response to glucose although the defect is not as marked as in the frankly diabetic BB rats (Fig 14) (73). Diabetes never develops after 140 days in diabetes-prone animals. In other words the same genotype produced differing phenotypes even when animals were kept in identical environments.

- Beta cell genes may express themselves at differing times and whether they induce autoimmunity may depend on the time of expression. This is illustrated by work from Hanahan and colleagues (74,75). A fusion gene was prepared between the insulin promoter and SV40 virus T antigen. A schematic diagram of the insulin gene is shown above and the actual fusion gene below.



When the fusion gene was placed into fertilized eggs it was expressed exclusively in the pancreatic β cells. In certain strains antibodies to T developed. Early expression of T seemed to induce tolerance while later expression seemed to be associated with autoimmunity.

Figure 14 (ref 73)

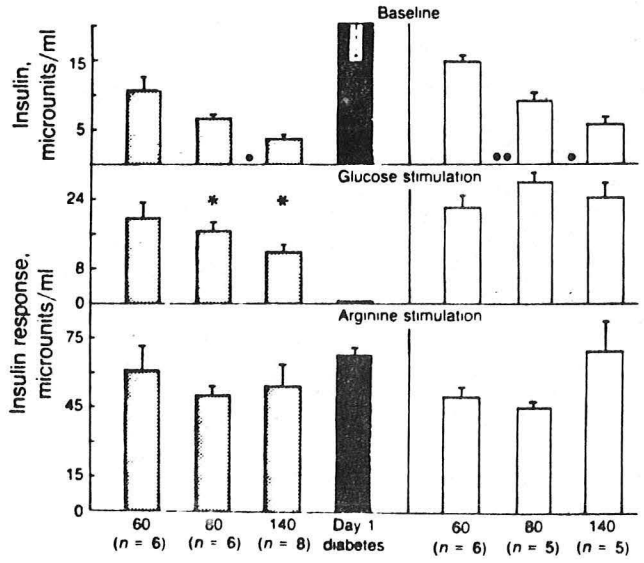


FIG. Baseline levels of insulin and the insulin response to glucose (10 mM) and to arginine (10 mM) in perfused pancreata of nondiabetic diabetes-prone BB/W rats (stippled bars) at 60, 80, and 140 days of age; age-matched diabetes-resistant BB/W controls (open bars); and diabetic BB/W rats (solid bars) on the first day of diabetes (●, $P < 0.05$ vs. preceding age; ●●, $P < 0.01$ vs. preceding age; *, $P < 0.05$ vs. age-matched diabetes resistant group; ⊂, not statistically significant). There was no response to glucose or to arginine on day 14 of diabetes (data not shown); baseline insulin secretion on that day averaged 2.1 ± 0.1 microunits/ml.

Conclusion: I believe it possible, even likely, that some patients with Type 1 diabetes develop the disease in the absence of an environmental trigger. In most cases the process is probably induced or speeded by an environmental factor, acting in the self → nonself transition described below.

5. The autoimmune process

There seems to be little doubt that Type 1 diabetes is an autoimmune disease (3-5,22,76). Islet cell antibodies of a variety of types (cytoplasmic, surface, complement fixing and immunoprecipitating) are ubiquitous in newly diagnosed diabetic subjects and are likewise present prior to development of symptomatic disease (3). Anti-insulin antibodies are common (77-80). They probably represent simply another marker of the autoimmune process, although some workers continue to think they serve to differentiate a particular subset of the disease (81). Common viruses have the capacity to induce insulin autoantibodies but only rarely cause the appearance of islet cell antibodies (82). Chicken pox had the highest incidence (81%), but positive responses were also seen with mumps, rubella and measles infections. Persuasive evidence that autoimmunity is cause rather than consequence of beta cell destruction comes from the observation that a variety of immune interventions prevents diabetes in the NOD and BB models (76) and under certain circumstances in humans (22).

The critical question is what causes the autoimmune syndrome (self → nonself transition)? The answers are not known but interest has focused on two areas: (1) an abnormality in suppressor T cell function and (2) the appearance of class II HLA molecules on the surface of beta cells in the pancreas where they are not normally found.

a. Is there a defect in suppressor T cell function in Type 1 diabetes (and other autoimmune diseases)? The answer to this question cannot be given with certainty. Direct evidence for a functional defect in immune suppression is essentially nonexistent. Peripheral blood monocytes from normal individuals reportedly inhibit cytotoxic attack against isolated islets by activated T lymphocytes from patients with Type 1 diabetes while similar cells from diabetic subjects do not, but the experiments are very unimpressive (83).

It is known that lymphopenia is necessary for development of diabetes in the BB rat and that a specific subset of T cells (RT6) are involved (84). This subset contains both helper and suppressor T cells. Infusion of helper T cells prevents the appearance of diabetes in these animals. For this reason helper/suppressor T cell ratios have been extensively examined in human Type 1 diabetes. Early reports suggested disproportionate deficiencies of suppressor T cells (which would leave helper cells unbalanced, favoring exuberant immune response) but better recent studies have failed to confirm (85-87). Modest lymphopenia is generally found but surprisingly the T4 subset of cells (ordinarily considered to be primarily helper) has generally been found to be more depressed than the T8 suppressor fraction (85-88) (Table 10).

Table 10 (ref 85)

TABLE
White blood cells, total lymphocytes, and lymphocyte subset counts in the adult controls and in the diabetic patients (per mm^3)

	Control adult subjects	Recently diagnosed patients	Long-standing diabetes	Extrapancreatic autoimmune manifestations
Total WBC	6950 ± 370	7500 ± 1300	6600 ± 550	8300 ± 1300
Total lymphocytes	2200 ± 140	2200 ± 340	2200 ± 130	2300 ± 210
OKT3	1340 ± 90	1260 ± 240	920 ± 50	1160 ± 160
OKT4	900 ± 60	630 ± 145	440 ± 50	800 ± 150
OKT8	460 ± 30	420 ± 120	370 ± 30	380 ± 40

Results are presented as mean values ± SEM. Number of subjects studied was 10–20 in each group.

This results in low T4/T8 ratios in a number of patients, which should, in theory, suppress humoral immunity (Table 11).

Table 11* (ref 85)

TABLE
Anti-islet humoral immunity (^{51}Cr release test), IRI secretory index, and OKT4/OKT8 ratio in diabetic patients

Case no	OKT4/OKT8 ratio	^{51}Cr release test (%)	IRI secretory index (%)
1	4.80*	63*	0*
2	3.10*	68*	0*
3	1.60	31	10*
4	3.70*	39	0*
5	1.80	43*	0*
6	1.27	20	20*
7	2.90*	55*	0*
8	1.33	46*	98
9	1.40	50*	0*
10	1.78	48*	90
11	2.92*	13	35*
12	5.50*	10	0*
13	2.26	23	0*
14	1.32	19	0*
15	2.26	58*	30*
16	3.92*	25	0*
17	0.94	61*	40
18	3.19*	15	0*
19	0.70	54*	0*
21	1.00	59*	30*
22	1.81	21	0*
23	2.60*	58*	125
24	1.50	38	0*
25	3.25*	19	0*
26	2.20	51*	59
27	1.20	69*	0*
31	2.30	14	102
32	1.30	60*	0*
33	2.02	13	
34	1.70	93*	130
35	0.75	88*	30*
36	1.50	49*	170
37	1.00	57*	157
38	0.50	88*	95
39	1.10	64*	240
40	2.00	12	0*
41	2.40	17	75
42	5.40*	30	0*
43	0.90	88*	7*
44	0.65	57*	86
45	1.10	56*	0*
46	3.30*	36	0*
47	2.50	29	10*
48	2.10	15	0*
49	0.60	81*	20*
50	1.71	55*	0*
51	2.40	32	0*
52	1.90	55*	0*
53	0.40	81*	0*
54	3.40*	27	0*
56	3.75*	48*	0*

*Asterisks denote values out of normal range

*^{51}Cr release test is a measure of complement fixing islet cell antibody induced β cell leakage; IRI secretory index is a measure of insulin release in response to glucose + theophylline.

This may be important, since in lupus erythematosus (89), multiple sclerosis (90) and probably rheumatoid arthritis (91) T4+ cells (T4+2H4+) that induce suppressor clones are deficient, accounting for decreased suppressor activity in mixed lymphocyte culture together with increased IgG production in vitro. In other words, deficiency of a T4 helper population results in diminished suppressor function, presumably due to absence of a small but important fraction of $T8^+$ cells. A corollary is that T4/T8 ratios or even absolute numbers of T4 or T8 bearing cells may be insufficient to understand regulatory dysfunction in the immune system. An entirely plausible scenario might be:

↓ T4+ (inducer) subset → ↓ T8 suppressor subset → activated immune systerm

Conclusion: Decreased suppressor function may be important in the autoimmunity of Type 1 diabetes.

b. Is the self → nonself transition mediated by the appearance of class II HLA molecules on the surface of the beta cell? This is the most popular theory for endocrine autoimmunity following the hypothesis published by Bottazzo, Pujol-Borrell and Hanafusa in 1983 (92). The basic idea was that the appearance of the class II molecule would allow presentation of autoantigens (presumably already on the cell surface) in such a way that the immune system would see them as foreign. It is clear that class II molecules are found on the beta cells of humans dying with Type 1 diabetes (93-94). They are not expressed on alpha cells. Class II antigens have also been found in human thyroid epithelial cells (92,95).

The presumption has been that D region products are induced by lymphokines with primary emphasis placed on γ interferon (92). The latter agent has been directly shown to stimulate the appearance of class II molecules on murine islets (96,97) as well as thyroid cells (98). In the BB strain only diabetes-prone animals show the effect; diabetes-resistant BB rats and normal Wistar animals do not induce with γ interferon (96). Not all findings have been consistent utilizing interferon since Wright, et al (97) found induction of class II molecules in a normal strain of mice (B10.BR $H\text{-}2^K$) and reported that both beta and non-beta cells expressed the antigens. Campbell and coworkers reported that γ interferon induced class I but not class II molecules on both human pancreatic cells obtained from brain-dead donors and a rat insulinoma cell line (99,100). On balance, however, it seems likely that γ interferon does induce the appearance of D region antigens.

Viral illness is considered the ordinary inducer of γ interferon release in humans. The overall sequence would be: virus → γ interferon production → appearance of class II molecules (92). However, it is of interest that messenger RNA for the DRα chain is readily demonstrable in normal thyroid tissue (101). The amounts of message were higher in glands from patients with autoimmune thyroid disease. This finding raises the possibility that "autonomous" (not requiring viral infection or any environmental stimulus) induction of class II molecules might occur in some patients. A possible mechanism might be decreased methylation of the structural genes for D region molecules. For example, DR expression on B lymphocytes is low in lupus erythematosus.

Examination of 12 cell lines from normal subjects showed identical methylation patterns at 5 CCGG sites in the HLA DRα locus (102). By contrast, 28 cell lines from patients with lupus erythematosus showed distinct methylation and hypermethylation patterns.

An hypothesis for self → nonself transformation based on the induction of D region antigens on the beta cell is shown in Figure 12 (103). In panel A the exogenous stimulus (virus) induces D region expression in the beta cell but it is not a diabetogenic class II molecule and this is not recognized by the helper T cell. In panel B a diabetogenic DR3 is produced and the immune system is activated with humoral and (panel C) cytotoxic attack occurring in response.

Figure 15 (ref 103)

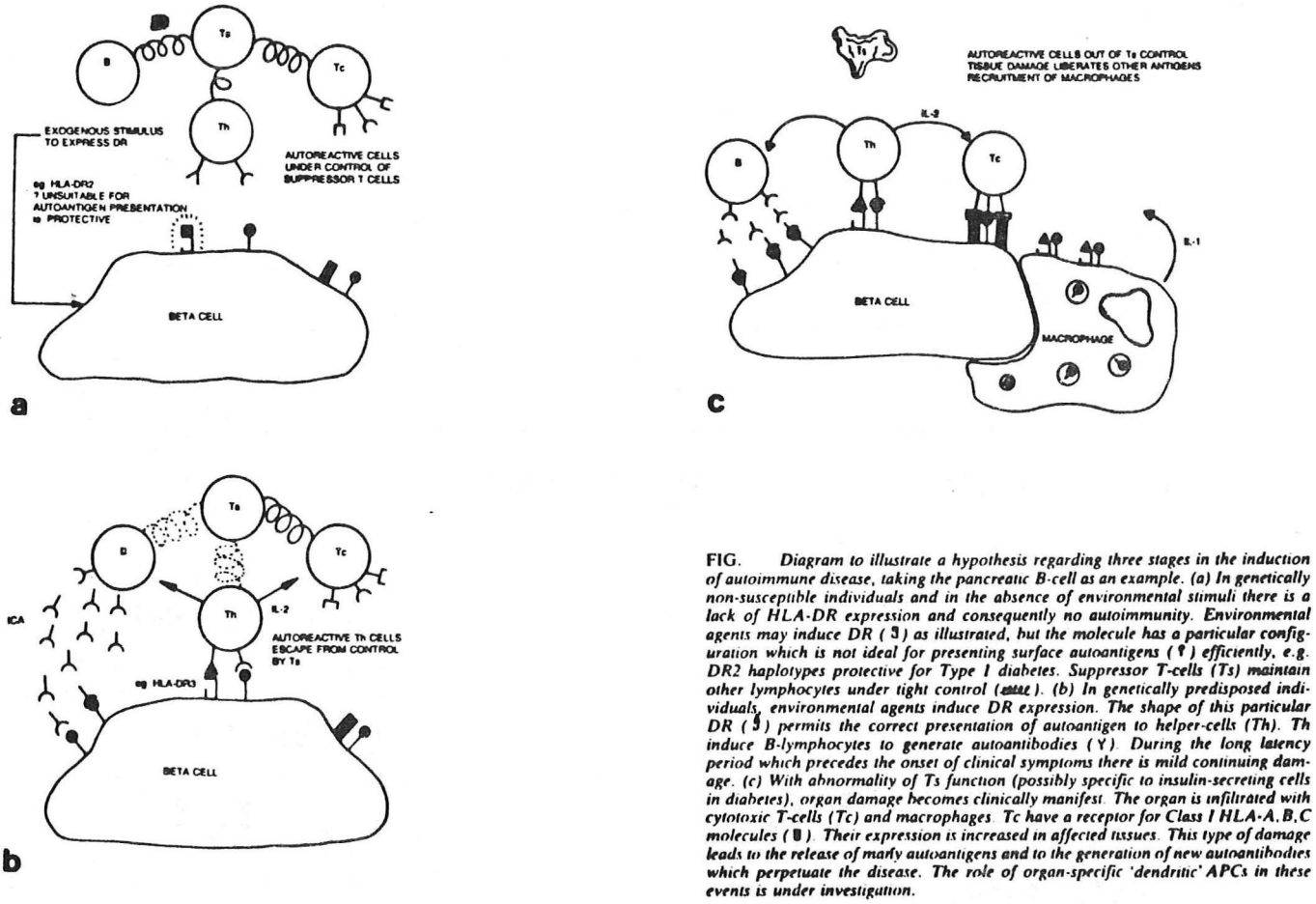


FIG. *Diagram to illustrate a hypothesis regarding three stages in the induction of autoimmune disease, taking the pancreatic B-cell as an example. (a) In genetically non-susceptible individuals and in the absence of environmental stimuli there is a lack of HLA-DR expression and consequently no autoimmunity. Environmental agents may induce DR ( ) as illustrated, but the molecule has a particular configuration which is not ideal for presenting surface autoantigens ( ) efficiently, e.g. DR2 haplotypes protective for Type I diabetes. Suppressor T-cells (Ts) maintain other lymphocytes under tight control ( ). (b) In genetically predisposed individuals, environmental agents induce DR expression. The shape of this particular DR ( ) permits the correct presentation of autoantigen to helper-cells (Th). Th induce B-lymphocytes to generate autoantibodies ( ). During the long latency period which precedes the onset of clinical symptoms there is mild continuing damage. (c) With abnormality of Ts function (possibly specific to insulin-secreting cells in diabetes), organ damage becomes clinically manifest. The organ is infiltrated with cytotoxic T-cells (Tc) and macrophages. Tc have a receptor for Class I HLA-A,B,C molecules ( ). Their expression is increased in affected tissues. This type of damage leads to the release of many autoantigens and to the generation of new autoantibodies which perpetuate the disease. The role of organ-specific 'dendritic' APCs in these events is under investigation.*

Although the scheme envisions induction of class II molecules on the beta cells, this is not a requirement. The "shared epitope" theory is based on the idea that a virus or other agent could infect any tissue (say nerve), induce class II molecules and activate the immune system against viral antigens (8). The class II restricted, virus-specific cytotoxic T lymphocytes or antibodies could then attack any target tissue bearing a surface protein with homology to the viral antigen (as few as 5-8 amino acids). Examples of such shared epitopes would be

streptococcal antigens and the heart in acute rheumatic fever (104), Escherichia coli and other gut bacteria with the acetylcholine receptor in myasthenia gravis (105) and adenovirus type 12 and A-gliadin in celiac disease (106).

Since autoimmunity is relatively rare and viral and other environmental onslaughts are common, it is clear that some sort of "narrowing" specificity is involved that prevents everyone from getting autoimmune disease following common infections. Three narrowing factors suggest themselves. First, as already discussed part of specificity resides in the likelihood that only a few genetic variants in the D region are able to present self-antigens in a way recognizable as nonself. Second, there may be only a few T lymphocytes bearing a T cell receptor capable of recognizing this configuration; e.g., one might have the right inducing D region molecule but not a recognizing T lymphocyte (7,8,10,107,108). The analogy might be the right key and the wrong lock or vice versa. Third, there may be differences in a variety of effector responses to activated immunity in Type 1 diabetes and other diseases. Candidate sites might include cellular responses (109-111), lymphokine generation (112,113) and complement activation (114).

Conclusion: At the moment I consider the appearance of D region antigens on the endocrine cells to be the most likely necessary (if not sufficient) step for activation of autoimmunity.

c. What is the target antigen? The answer to this question is not known and it may be that there are several important antigens. Initial attention was focused on a 64K protein of unknown function (3,115). Antibodies against this protein are frequent in newly diagnosed diabetic children and may precede the appearance of islet cell antibodies. They have also been seen in the diabetes-prone BB rat. A second group of candidate antigens are surface gangliosides and glycoproteins (22). One monoclonal antibody against glycoproteins has been shown to block parathyroid hormone release by raising calcium levels in the parathyroid gland; i.e., the antibody has regulatory capacities in the endocrine system. This is important because inhibition of glucose stimulation of insulin secretion appears to be the earliest functional lesion in human (116) and rodent (73) diabetes. An attractive possibility is that a primary antigen recognized by islet cell or other antibodies is the specific glucose transporter of the beta cell. In support of such a possibility, islet cell antibodies have been shown to block glucose-induced insulin release (117). Interleukin-1 can also cause loss of insulin response to glucose (118,119). It has been speculated that the newly described insulin inhibitory peptide called pancreastatin might be involved (120) but there is no evidence for this.

6. Insulitis

In the pancreas of patients with new-onset diabetes there is an infiltration of activated T lymphocytes expressing class II antigens. The cells, which surround and infiltrate islets, are mostly of the cytotoxic type but natural killer cells are also represented (121). Such infiltrations are characteristic of other forms of immune endocrinopathy. It is likely that these cells are the consequence of the autoimmune process rather than its cause.

Summary. Although conclusions must be considered tentative, the following represents my current views of the pathogenesis of Type 1 diabetes.

1. The primary genetic susceptibility gene is an altered D region molecule, sometimes DR, sometimes DQ and probably sometimes DP. Serologic and even RFLP assessments include normal, susceptible and resistant genes.

2. In most cases the autoimmune process is initiated by the appearance of class II molecules bearing a susceptibility gene and thus capable of presenting one or more surface antigens as nonself. The induction of class II antigens may be environmental (viral, chemical) but could be purely genetic. Modulating factors probably involve polymorphisms of the T cell receptor or differences in immune effector response.

3. The destruction of the beta cells is immune-mediated and involves both humoral and cellular effector areas.

4. Explanation for the age of onset (first day of life to the 8th decade) and speed of development (few weeks to a decade) remains unknown. Possibilities include an intrinsic (genetic) biologic clock, repeated viral infections (which I doubt) or a smoldering autocatalytic sequence. For the latter case the scenario might run as follows: viral infection induces class II molecules on a few beta cells → attraction of cytotoxic and regulatory T lymphocytes which destroy class II bearing cells while releasing additional lymphokines → induction of class II molecules on an additional few percent of beta cells → etc.

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III. CLINICAL IMPLICATIONS

I want to draw 4 clinical implications from the discussion on pathogenesis.

1. The classification of diabetes needs to be revised. Until recently it has been assumed that insulin-dependent diabetes mellitus develops according to a rapid course. As indicated in Figure 16 this is now known not to be true. There are both rapid and slow courses.

Figure 16

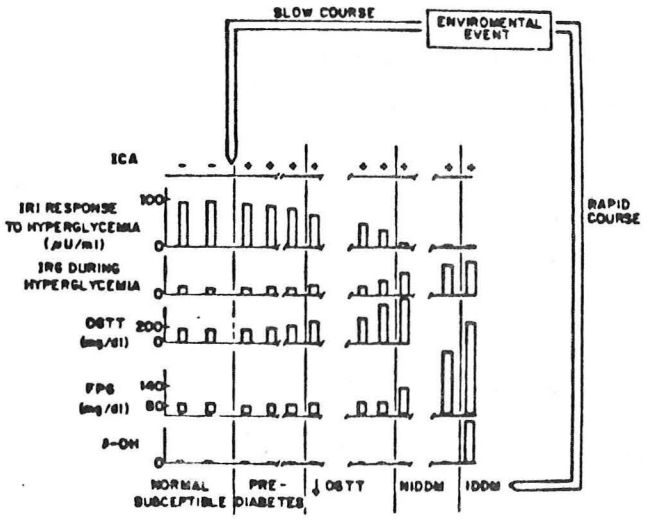

Figure Natural history of insulin-dependent diabetes mellitus. Two courses are postulated, one rapid, the other slow. In the former, symptomatic disease may appear abruptly with little evidence of a prediabetic period. In the latter, it is presumed that islet cell degeneration occurs more slowly, heralded by appearance of islet cell antibodies and gradual reduction of insulin response to glucose. Some patients pass through a non-insulin-dependent phase ordinarily considered characteristic of NIDDM on the way to IDDM. ICA = islet cell antibodies; IRI = immunoreactive insulin; IRG = immunoreactive glucagon; OGTT = oral glucose tolerance test; FPG = fasting plasma glucose; β-OH = β-hydroxybutyrate.

In the typical rapid course, the patient develops symptoms quickly, often with ketoacidosis. In the slow course, depicted schematically in the left portion of Figure 16, this is not the case. The earliest sign of abnormality is the development of islet cell antibodies. This occurs at a time where there is no elevation of the blood sugar and glucose tolerance is normal. Insulin responses to a glucose load are intact. The patient then moves into a period where the only abnormality is decreased glucose tolerance. Fasting blood sugar remains normal. In the third stage fasting hyperglycemia develops but the patient does not develop ketosis even when poorly controlled. For all the world the clinical appearance is that of noninsulin-dependent diabetes mellitus. Eisenbarth has come to the same conclusion in his stages of diabetes shown in Figure 17. His stage 5 would be equivalent to our NIDDM. With time, however, insulin dependence appears and ketoacidosis may develop, especially with stress.

Figure 17 (ref 4)

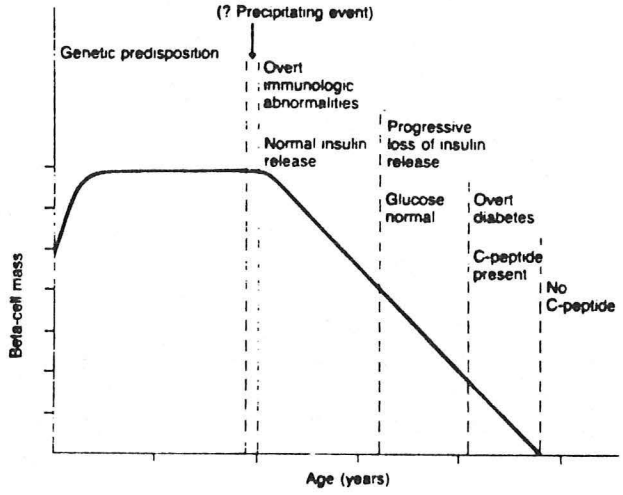

Figure Stages in the Development of Diabetes Mellitus. The stages of diabetes are listed from left to right, and hypothetical beta-cell mass is plotted against age.

It is highly likely that nonobese patients with noninsulin-dependent diabetes mellitus in fact have an autoimmune form of the disease on the slow course. For this reason, Unger and I have suggested a new classification of diabetes as shown in Figure 18. In this classification we use the terms insulin-dependent and noninsulin-dependent in the physiologic sense to indicate relative (NIDDM) and absolute (IDDM) insulin deficiency, the former being nonketoacidosis prone and the latter ketoacidosis prone. Type 1 and Type 2 are not synonyms for IDDM and NIDDM, but refer to pathogenetic mechanisms. Thus, Type 1 should only be used in association with diabetes when an autoimmune insulopathy is present. Non-destructive insulopathy should be called Type 2 disease. The characteristics of these conditions are shown in Table 12.

Figure 18

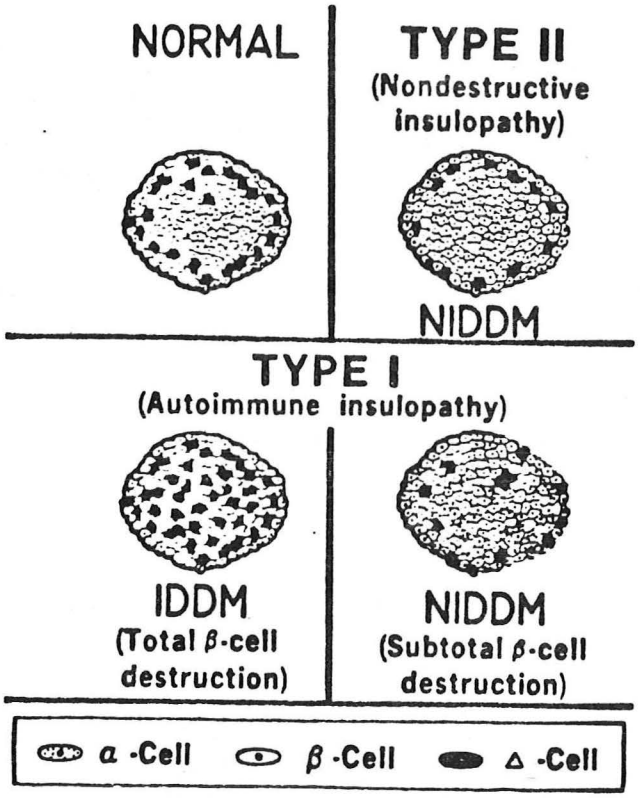

Figure Schematization of normal and diabetic islets. In Type 1 IDDM virtually no β cells are present, whereas in Type 1 NIDDM some β cells persist. In Type 2 NIDDM β cells are plentiful.

Table 12

Table POSSIBLE HETEROGENEITY IN NON-INSULIN-DEPENDENT DIABETES—FUNCTIONAL CHARACTERISTICS

Characteristic	Type 1 IDDM	Nonobese NIDDM (Type 1 Subset)	Obese NIDDM (Type 2 Subset)
Cytoplasmic islet cell antibodies	Positive	Positive	Negative
Insulin and C-peptide in plasma	Absent or very low	Low	High
Glucagon in plasma	Relative or absolute elevation	Relative or absolute elevation	Relative elevation
Effect of insulin on abnormal α-cell response to arginine	Corrected	Corrected	Not corrected

Non-insulin-dependent diabetes is considered to have two subsets, one (largely nonobese) progressing to type 1 IDDM and the other (largely obese) remaining non-insulin-dependent.

2. It should be possible to predict Type 1 diabetes in subjects at risk. By combining HLA typing, measuring islet cell antibodies and insulin (C peptide) response to intravenous glucose it is possible to identify with a moderate degree of certainty patients at risk for developing Type 1 diabetes (122-125). The identification is quite crude at present since the routine typing procedures available (HLA serologic assessment) are not precise as noted above. However, it should be

possible in the future to improve prediction rates above the current 20-40% (HLA identical siblings). Even now, however, the HLA markers and islet cell antibodies are useful in identifying patients with Type 1 NIDDM (Type 1 in evolution), especially in the middle aged or elderly (126,127).

Caveat: the presence of islet cell antibodies in a patient at risk cannot be interpreted absolutely to mean that diabetes will follow because the process may abort and the antibodies clear (128,129). This is illustrated in Figure 19 where identical twins discordant for diabetes developed islet antibodies (black bars) then cleared them (hatched bars) without ever developing the disease.

Figure 19 (ref 129)

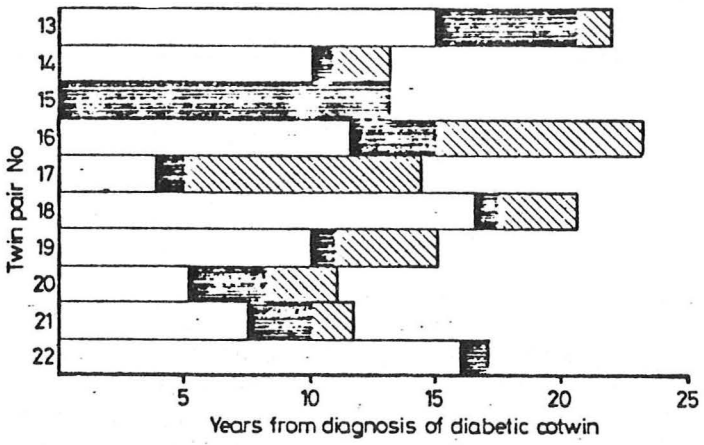

FIG —Presence of islet cell antibodies in non-diabetic cotwins in relation to time from diagnosis of diabetic twin (long term group). Black areas: tested and positive for antibodies. Hatched areas: tested and negative for antibodies. White areas: not tested.

3. Transplantation of the pancreas or isolated islets may be followed by re-enactment of the pathogenetic process. If Type 1 diabetes is an autoimmune process, then considerable concern should be expressed about the long term utility of islet cell or segmental pancreatic transplants. It would be predicted that transplantation of new tissue, particularly if from a living related donor, would reactivate the autoimmune process with subsequent destruction of the transplanted islets. Preliminary evidence from the University of Minnesota suggests that this is in fact the case as shown in Table 13. In this study four identical twins received segmental pancreatic transplants. Initial cure of diabetes occurred in all four. However, within four weeks three of the twins had a recurrence of diabetes, insulitis and the appearance of islet cell antibodies in the plasma. Biopsy of the transplanted pancreas showed no evidence of rejection. The fourth twin, who was on immunosuppression, did not redevelop diabetes and showed no evidence of either antibodies or insulitis. Although re-enactment might be less frequent with non-related donors (whose transplantation antigens would be more disparate), this is not certain.

Table 13

Table REVERSAL AND RE-ENACTMENT OF TYPE 1 DIABETES IN PANCREAS TRANSPLANTED FROM A NONDIABETIC TO A DIABETIC MONOZYGOTIC TWIN AFTER MORE THAN 15 YEARS OF DISCORDANCE

Twin Pair	Immuno-suppression	Initial Cure of Diabetes	Recurrence of Diabetes Within Four Weeks	Bx† Evidence of Rejection	Insulitis	Appearance of Islet Cell Antibodies*
1	0	+	+	0	+	+
2	0	+	+	0	+	+
3	0	+	+	0	+	+
4	+	+	0	0	no bx	–

*Appeared at approximately eight weeks. Summarized from Sutherland DER, Sibly R, Chinn P, et al. Twin to twin pancreas transplantation (TX): reversal and reenactment of the pathogenesis of type 1 diabetes. Clin Res 1984; 32:561A.
†Bx = biopsy

4. It ought to be possible to prevent Type 1 diabetes. It should be possible to modulate the immune system in such a way as to prevent diabetes. The ideal time for such a modulation would be at the appearance of islet cell antibodies in the blood because they would indicate activation of the immune system. Supportive evidence for this conclusion comes from several protocols utilizing cyclosporine as immunosuppressant. In a large study of patients with new onset diabetes mellitus (most with ketoacidosis) the administration of cyclosporine reversed diabetes in over half of cases when given within the first 6 weeks of diagnosis (130). After 6 weeks reversal did not occur. Disappearance of diabetes persisted for up to 18 months, but hyperglycemia immediately returned when cyclosporine was stopped. Other studies have not reported the same degree of remission. Since cyclosporine is a dangerous drug predisposing to the development of tumors of the lymph system and causing kidney damage when given in high doses, it would not seem desirable for use in long-term prevention (131). However, if immune intervention was begun prior to any overt beta cell damage, it is possible that milder immune modulation might prevent diabetes. One possibility would be several months treatment with cyclosporine followed by a switch to less toxic immunosuppression as is used in rheumatoid arthritis or kidney transplants. Indeed, azathioprine has been shown to induce remission in some patients (132).

For the future efforts should be directed at more specific interventions, designed to attack vulnerable points in the pathogenetic mechanism. For example, if the major inducing antigens on the beta cell can be identified it might be possible to develop a protecting peptide analogous to "peptide T" (a peptide with homology to VIP) which prevents attachment of the HIV (AIDS) virus to its target cell by blocking the T4 antigen (133). Another approach might be development of modifying peptides or antibodies for class II molecules, regulatory or effector T cells. Although such speculations have an aura of "star war" molecular biology, they may in fact prove possible.

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