the presence of the viral genome in these cases suggests that sexual transmission by this route could indeed occur. Unequivocal evidence implicating semen-mediated transmission of HPV would require a much larger sample of male sexual partners of women with benign, premalignant, and malignant HPV-associated lesions. The results of such a study would help to clarify whether the presence of HPV nucleotide sequences in semen represents a general phenomenon in males at risk for sexually transmitted diseases or rather is an unusual event found only in patients with rare chronic and debilitating wart diseases.

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The NOD Mouse: Recessive Diabetogenic Gene in the Major Histocompatibility Complex

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Examination of the histocompatibility region of the nonobese diabetic (NOD) mouse with antibodies against class II glycoproteins (products of immune response genes of the major histocompatibility complex I-A and I-E), hybrid T-cell clones, and mixed-lymphocyte cultures and analysis of restriction fragment length polymorphisms indicate that the NOD mouse has a unique class II major histocompatibility complex with no expression of surface I-E, no messenger RNA for I-E_α, and an I-A not recognized by any monoclonal antibodies or hybrid T-cell clones studied. In crosses of NOD mice with control C3H mice, the development of diabetes was dependent on homozygosity for the NOD mouse's unique major histocompatibility region.

THE NOD (NONOBESE DIABETIC) mouse develops type I diabetes mellitus characterized by pancreatic beta cell destruction in association with a massive lymphocytic infiltration of islets (*1–4*). Development of diabetes mellitus is dependent on intact T-cell function and is presumed to be autoimmune in etiology (*5*).

In both man and BB rat, type I diabetes is in part determined by an unknown gene or genes within the major histocompatibility complex (MHC). Two categories of genes within the MHC are immune response genes coding for dimeric polypeptides (I-A and I-E) and genes coding for single-chain glycoprotein transplantation antigens (class I). We used several techniques to analyze the MHC of the NOD mouse. Serologic analysis of class I histocompatibility alleles indicates that the NOD mouse is H-2K^d and H-2D^b (*6*). We also studied the reactivity of monoclonal antibodies to the I-A and I-E glycoprotein molecules and the interaction of a conventional antiserum to Ia with NOD splenocytes (Table 1) (*7–12*). As a control for the percentage of B lymphocytes in the spleen of each strain, we analyzed the reactivity of antimouse immunoglobulin.

Conventional antiserum designated FIa1,2,3,7, made by immunizing A.TH mice with A.TL cells, reacted with splenocytes of NOD, BALB/c, and C57BL/6 mice. None of the monoclonal antibodies to class II antigens studied, however, reacted with NOD splenocytes, although all were reactive with either or both splenocytes from the control strains [BALB/c (H-2^d) and C57BL/6 (H-2^b)]. Five of the specific monoclonal antibodies reacted with I-A^b and four with I-A^d, in addition to exhibiting their other H-2 specificities. Monoclonal antibody 14-4-4 reacts with all H-2 haplotypes expressing surface I-E molecules (*9*). It

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Table 1. Percentage of splenocytes reacting with specific antibodies to class II antigens. Fluorescein isothiocyanate–conjugated goat antibody to mouse immunoglobulin was used as a control for the percentage of B lymphocytes. Specificities of FIa1,2,3,7 (A.TH anti-A.TL) and monoclonals are as follows: FIa1,2,3,7, H-2$^{b,d,f,h,i,k,m,o,p,q,tb,td,te,tl,y\text{-}kly,y\text{-}sg}$ (*7*); 3JP, I-A^{b,i,k,p,q,t,s,u,v} but not I-A^d (*8*); Af6-120, I-A^b but not I-A^d; 17-227, I-A^{b,k} but not I-A^{f,g,r,s} (*9*); 14-4-4, I-E^{k,d,p,r} but not strains lacking surface I-E such as H-2^{b,s,f,q} (*10*); MKD6, I-A^d but not I-A^{a,b,f} (*11*); 25-9-17, I-A^{b,d}; and M5/114, I-A^{b,d} and I-E^{d,k} (*12*). Splenocytes isolated with Ficoll-400 (lymphocyte-M; Cederlane Laboratories) were incubated with monoclonal antibodies on ice for 45 minutes and washed three times. Then the cells were stained with fluorescein-conjugated F(ab')$_2$ fragment goat antibody to mouse immunoglobulin G (gamma-chain specific; Cappel). The percentage of fluorescence-positive cells was determined by Coulter Epics fluorescence cytometer analysis. Normal mouse serum and nonspecific monoclonal antibody (P3X63) fluorescence were subtracted for FIa1,2,3,7 and the monoclonals, respectively.

Specificities	Antibody to immunoglobulin (B lymphocytes)	FIa1,2,3,7 (I-A,E)	3JP (I-A)	AF6-120 (I-A)	17-227 (I-A)	14-4-4 (I-E)	MDK6 (I-A)	25-9-17 (I-A)	M5/114 (I-A,-E)
NOD	44	26	6	0	0	0	0	0	0
BALB/c (H-2^d)	52	35	5	0	1	31	49	39	36
C57BL/6 (H-2^b)	54	34	47	50	34	1	0	50	40

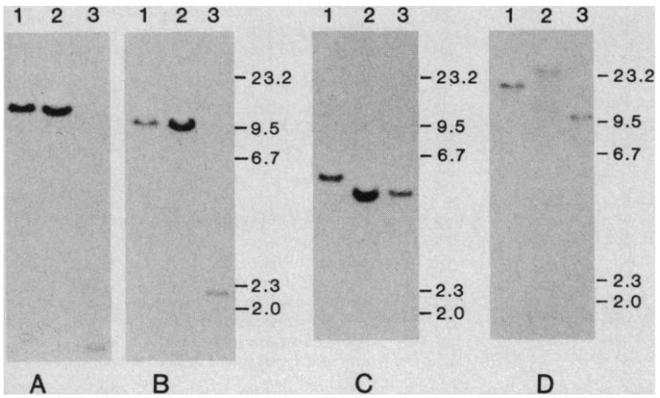

Fig. 1. Restriction enzyme length polymorphism analysis of BALB/c (H-2^d, lane 1), NOD (lane 2), and C57BL/6 (H-2^b, lane 3) class II MHC genes with Hind III and A_α probe (A), Bam HI and A_β probe (B), Pvu II and E_α probe (C), and Kpn I and E_β probe (D). The A_α probe was a 1.3-kb Hind III genomic fragment of C57BL/10 encoding part of the α_1 and all of the α_2, transmembrane, and intracytoplasmic domains (*22*). The A_β probe was a 2.1-kb genomic fragment of H-2^k encoding the β_2, transmembrane, and intracytoplasmic domains (*23*). The E_α probe was a 1.4-kb Bgl II fragment of a Pst I subclone of a cosmid clone, cosI^d-α-1, encoding the α_1 domains of E^d (*24*). The E_β probe was a 7.2-kb Hind III genomic fragment encoding the β_1 and β_2 domains of E^d (*25*). DNA was digested with the restriction enzymes and DNA fragments were subjected to electrophoresis on 0.8 percent agarose gels. Hybridizations with ^{32}P-labeled DNA probes were done at 42°C for 20 hours with washes for 1 hour at 50°C in 50 m*M* NaCl, 3 m*M* trisodium citrate, and 0.1 percent sodium dodecyl sulfate. Autoradiograms were exposed for 14 hours at −70°C with an intensifying screen. Sizes of DNA marker fragments are shown in kilobases.

did not react with NOD splenocytes or C57BL/6 mice (H-2^b). C57BL/6 mice have a deletion of their I-E gene (*13*). Thus the H-2 haplotype of the NOD mouse differs at I-A from the many known H-2 haplotypes recognized by the monoclonal antibodies tested (although reactivity with antiserum FIa1,2,3,7 suggests that the I-A gene is expressed), and NOD mice probably do not express surface I-E molecules. To determine if NOD mice, like H-2^b mice (for example, C57BL) (*13*), lack I-E_α messenger RNA (mRNA), we isolated RNA from NOD mice, subjected it to electrophoresis and analyzed it with A_β, A_α, E_β, and E_α gene probes. The three strains expressed RNA for A_β, A_α, and E_β genes. NOD mice had no detectable E_α RNA bands; BALB/c mice expressed E_α mRNA, but the C57BL/6 mice, which have a deletion in the E_α gene, did not.

We tested the ability of NOD splenocytes to induce interleukin-2 production by using a series of I-A^d and I-A^b restricted autoreactive, alloreactive, and antigen-specific T-cell hybrid clones (*14*). By analogy to the monoclonal antibody serology, none of the I-A^d or I-A^b restricted T-cell clones were activated by NOD mouse splenocytes. In mixed-lymphocyte culture assays (Table 2), NOD splenocytes induced proliferation or proliferated when cocultured with splenocytes of H-2^b, H-2^k, H-2^g, and H-$2^{b/d}$ (F_1 heterozygote) mice. Thus, on the basis of monoclonal serology T-cell clone response and mixed-lymphocyte culture, the class II genes of NOD mice differed from those of the known mice strains tested.

To further define the class II genes of the NOD mouse, we digested DNA from NOD, BALB/c, and C57BL/6 mice with restriction enzymes, subjected it to electrophoresis and analyzed it by Southern blotting with murine class II genomic probes. Using an A_α genomic probe with restriction enzyme Hind III (Fig. 1A) or an A_β genomic probe with Bam HI (Fig. 1B) or Hind III, we found that the NOD mouse is identical to the BALB/c (H-2^d) mouse and differs from the C57BL/6 (H-2^b) mouse (Fig. 1). Using an E_α genomic probe with Pvu II (Fig. 1C) or Bam HI and an E_β genomic probe with Kpn I (Fig. 1D) or Bam HI, we found that the NOD DNA differs from both H-2^d and H-2^b DNA.

After having defined restriction fragment length polymorphisms of the NOD class II MHC, we crossed NOD mice with C3H mice (H-2^k) and determined the linkage of diabetes to inheritance of A_β restriction fragment length polymorphisms. The A_β hybridizing fragment resulting from Bam HI digestion of NOD mice is a 9.5-kilobase (kb) band and is readily distinguished from a 2-kb band of C3H mice (Fig. 2). In our inbred NOD strain, diabetes occurred in approximately 80 percent of females and 20 percent of males by 7 months of age (*1*). In our breeding study, diabetes occurred in 0 of 81 female F_1 animals (C3H × NOD). Among backcross animals [female F_1(C3H × NOD) × male NOD] 12 of 77 female and 1 of 58 male offspring developed overt diabetes. Among F_2 mice, 3 of 117 intercrosses developed diabetes. All diabetic ani-

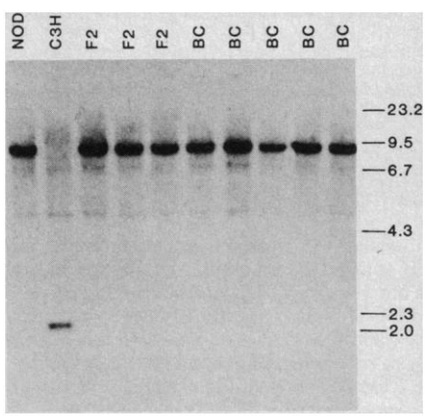

Fig. 2. Results of an analysis of the restriction fragment length polymorphisms of NOD and C3H (parental strains) and diabetic F_2 and backcross (BC) animals with Bam HI and an A_β probe. All diabetic animals were homozygous for the NOD 9.5-kb fragment and lacked the C3H 2.1-kb fragment. Sizes of DNA marker fragments are shown in kilobases.

Table 2. Results for the mixed culture of NOD, B6D2F1 (B/D F1), BIO.GD, BIO.HTG, and BIO.BR lymphocytes. Lymph nodes were pooled from three mice of each strain and dissociated by gentle passage through a wire screen. The resulting cell suspension was washed three times with medium and utilized as responders or stimulators. Responder cells (5×10^5) were cultured with irradiated stimulator cells (5×10^5) at 1000 rads in RPMI 1640 medium containing 2 percent normal human serum and 2-mercaptoethanol (5×10^{-5} *M*) for 3 days. All cultures were exposed to 0.5 μCi of [^{3}H]thymidine for 1 day. [^{3}H]thymidine incorporation is expressed as mean counts per minute for triplicate cultures ± standard deviation.

Responder	Stimulator	H-2 Haplotype				[^{3}H]Thymidine uptake
		K	A	E	D	
B6D2F1	B6D2F1	b/d	b/d	b/d	b/d	2,945 ± 163
	NOD	d			b	45,588 ± 1,453
	BIO.GD	d	d	b	b	3,088 ± 492
	BIO.HTG	d	d	d	b	2,926 ± 454
	BIO.BR	k	k	k	k	126,440 ± 5,844
BIO.GD	BIO.GD	d	d	b	b	3,324 ± 354
	NOD	d			b	48,445 ± 3,346
BIO.HTG	BIO.HTG	d	d	d	b	2,024 ± 321
	NOD	d			b	36,462 ± 3,759
NOD	NOD	d			b	2,143 ± 216
	BIO.GD	d	d	b	b	82,029 ± 7,538
	BIO.HTG	d	d	d	b	83,268 ± 2,543
	C57BL/6	b	b	b	b	44,614 ± 4,026

mals analyzed [seven of seven backcrosses, three of three intercrosses (typing of eight animals is shown in Fig. 2)] were homozygous for the NOD 9.5-kb band ($P < 0.0001$, binominal distribution), suggesting a recessive contribution to diabetes susceptibility. Because the incidence of diabetes in backcross females was only 15.6 percent, we hypothesize that there is at least one and probably two or more susceptibility genes in addition to the MHC-linked diabetogenic gene.

It is interesting that the diabetogenic histocompatibility gene functions in a "recessive" manner. The recessive nature of the MHC contribution to the development of diabetes is analogous to the results obtained from breeding studies in the BB rat, which also develops type I diabetes (*15*). Among these animals the incidence of diabetes in individuals homozygous for the MHC (RT1-u) is ten times greater than that in heterozygous individuals in crosses with Lewis, Brown Norway, and BBN RT1-A rats (*15, 16*). In human type I diabetes there is a higher incidence of concordance in diabetic siblings sharing both histocompatibility regions (20 percent) than in those sharing one histocompatibility antigen haplotype (5 percent) (*17*).

Class I and class II MHC-linked gene products are expressed in a codominant fashion on the cell surface. Responsiveness to foreign antigens controlled by class II genes is inherited as a dominant trait (*18*). The recessiveness of the diabetogenic influence of the NOD MHC genes contrasts with the "dominant" inheritance of class II–determined responsiveness to foreign antigens and can be explained by at least three mechanisms. First, if the MHC-linked diabetogenic gene is a class II immune-response gene, a lack of diabetes in heterozygotes may be due to decreased cell-surface expression of a specific α:β class II heterodimer on antigen-presenting cells, lymphocytes, or possibly the target tissue. Since class II molecules are α:β heterodimers, the α and β chains can associate in mice heterozygous at I-A loci, which would reduce the expression of the parental-type heterodimers (*19*). Such decreased parental heterodimer expression can cause a proportional decrease in the responsiveness of MHC-restricted T-cell clones (*14*). Second, the anti-islet immune response involved in type I diabetes of NOD mice may be controlled by a class II immune "suppressor" gene. In such a system, low responsiveness is inherited as a dominant trait and high responsiveness is inherited as a recessive trait (*20*). Third, the MHC-linked gene involved in the pathogenesis of type I diabetes mellitus may involve a deletion (for example, lack of an I-E chain gene). Congenital adrenal hyperplasia results from a deficiency in a 21-hydroxylase enzyme and is inherited as an MHC-linked autosomal recessive trait (*21*). Further breeding studies and analyses of the unique MHC of the NOD mouse should allow direct testing of the three hypotheses.

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Pinealocyte Projections into the Mammalian Brain Revealed with S-Antigen Antiserum

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Neural processes from mammalian pinealocytes have been discovered in several brain areas. These processes were visualized immunocytochemically in the Djungarian hamster, *Phodopus sungorus*, with an antiserum against bovine retinal S-antigen and traced as far as the region of the posterior commissure and habenular nuclei. This result indicates that pineal-to-brain connections exist in the mammal, and that the mammalian pineal gland, currently thought of only as a neuroendocrine organ, may communicate directly with select brain regions by way of these projections. The existence of mammalian pinealocyte projections is consistent with the view that these cells are not of glial origin but are derivatives of photoreceptor cells of the pineal complex of lower vertebrates that transmit signals to the brain by neural projections.

THE PINEAL ORGAN HAS UNDERgone remarkable structural and functional modification during evolution (*1*). In poikilotherms it has a prominent photosensory apparatus and sends neural projections to the brain (*2*). In contrast, the mammalian pineal gland is not directly photosensitive, but is regulated by light acting on the lateral eyes and a circuit of central and peripheral neural structures (*3*); in addition, it is generally thought that the mammalian pineal gland acts as a neuroendocrine organ which controls target tissue exclusively through the circadian release of melatonin and perhaps other hormones into the circulation (*4*). However, we now present evidence that mammalian pinealocytes send projections into the brain, pointing to the possibility that some mammalian pinealocytes might function as neurons.

A group of 13 Djungarian hamsters were studied (*5*). In this species, a superficial pineal gland is connected to a deep pineal gland by a pineal stalk (Fig. 1). The superficial pineal gland was surgically removed from three animals 1 week before fixation

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