Ist. Lomb. Sci. Lett. B **97**, 291 (1963); V. H. Denenberg, G. A. Hudgens, M. X. Zarrow, *Science* **143**, 380 (1964); D. Mainardi, M. Marsan, A. Pasquali, *Atti. Soc. Ital. Sci. Nat. Mus. Civ. Stor. Nat. Milano* **104**, 325 (1965); J. N. Marr and L. E. Gardner, *J. Genet. Psychol.* **107**, 167 (1965); D. M. Quandango and E. M. Banks, *Anim. Behav.* **18**, 379 (1970); D. Müller-Schwarze and C. Müller-Schwarze, *Nature (London)* **229**, 55 (1971); G. K. Beauchamp and E. H. Hess, *J. Comp. Physiol. Psychol.* **85**, 383 (1973); U. W. Huck and E. M. Banks, *Anim. Behav.* **28**, 1053 (1980).

3. J. R. Alberts, in *Mammalian Olfaction, Reproductive Processes and Behavior*, R. L. Doty, Ed. (Academic Press, New York, 1976), pp. 67–91; P. J. Singh, A. M. Tucker, M. A. Hofer, *Physiol. Behav.* **17**, 373 (1976).
4. M. A. Hofer, H. Shair, P. J. Singh, *Physiol. Behav.* **17**, 131 (1976); M. H. Teicher and E. M. Blass, *Science* **193**, 422 (1976).
5. M. Wilhelmsson and K. Larsson, *Physiol. Behav.* **11**, 227 (1973); K. Larsson, *ibid.* **14**, 195 (1975); D. H. Thor and K. J. Flannelly, *ibid.* **19**, 411 (1977).
6. A. M. Gawienowski and M. Stacewicz-Sapuntzaki, *Behav. Biol.* **23**, 267 (1978); P. E. Pedersen and E. M. Blass, in *Development of Perception: Psychobiological Perspectives*, R. N. Aslin, J. R. Alberts, M. R. Petersen, Eds. (Academic Press, New York, 1981), vol. 1, pp. 359–381.
7. P. E. Pedersen and E. M. Blass, *Dev. Psychobiol.* **15**, 349 (1982); P. E. Pedersen, C. L. Williams, E. M. Blass, *J. Exp. Psychol. Anim. Behav. Process.* **8**, 329 (1982).
8. B. A. MacFarlane, P. E. Pedersen, C. E. Cornell, E. M. Blass, *Anim. Behav.* **31**, 462 (1983).
9. T. J. Fillion and E. M. Blass, *ibid.*, in press.
10. S. A. Barnett, *The Rat: A Study in Behavior* (Univ. of Chicago Press, Chicago, ed. 2, 1975).
11. T. J. Fillion and E. M. Blass, *J. Comp. Psychol.* **99**, 328 (1985).
12. The difference in ejaculation latency between the two groups of C males was not due to their mating with females that were differentially receptive. On a four-point scale that evaluated the intensity of female sexual behavior during the first 5 minutes of the mating test, citral-scented females scored 1.7 ± 0.2 (mean ± standard error) whereas untreated females scored 2.0 ± 0.2.
13. While mount and intromission latencies reflect essentially the same pattern shown by the ejaculation latencies, these differences are not statistically significant. Frequency and interval measures did not reflect developmental treatments. The only nonejaculatory measure that attained statistical significance was duration of anogenital sniffing. Males mating with citral-scented females sniffed less, regardless of rearing condition [$F(65) = 6.704$, $P < 0.05$]. The biological significance, if any, of this difference is not known. Possibly the fresh citral odor was slightly irritating to the males.
14. Supported by NIH Grant in Aid of Research AM 18560 and Research Scientist Award MH 00524 to E.M.B. This study represents a portion of T.J.F.'s dissertation submitted to Johns Hopkins University in partial fulfillment of requirements for the Ph.D. We thank M. Book and P. DiMuzio for valuable technical assistance.

22 May 1985; accepted 18 October 1985

Detection of Papillomavirus DNA in Human Semen

RONALD S. OSTROW, KAREN R. ZACHOW, MICHIHITO NIIMURA, TAKASHI OKAGAKI, SIGFRID MULLER, MITCHELL BENDER, ANTHONY J. FARAS

Human papillomavirus DNA has been detected in the semen of three patients, two of whom have severe chronic wart disease. These data support the contention that sexual transmission of human papillomavirus DNA could occur via semen, a possibility suggested by epidemiological data on the sexual transmission of human papillomavirus.

SEVERAL HUMAN PAPILLOMAVIRUS (HPV) DNA genomes have been shown to be physically associated with various types of benign, premalignant, and malignant lesions of the anogenital tract (*1–8*). Epidemiological evidence has suggested a correlation between some male penile cancers and female partner cervical carcinomas (*9*) and has indicated that male sexual partners of women with various benign or premalignant cervical lesions were at high risk for having penile lesions (*10*). Because the incidence of HPV in the male is far lower than in the female (*9, 10*), there are questions about the possible modes of sexual transmission of HPV. In an attempt to determine whether semen can serve as a reservoir for HPV DNA or HPV virus, we have used the Southern blot hybridization procedure to examine semen from several patients exhibiting severe chronic HPV infections. We have found HPV-5 or HPV-2 DNA in semen from three of the patients, an indication that sexual transmission of HPV may occur directly through the delivery of HPV-containing semen.

In our ongoing studies on the nature of severe chronic and recurring papillomavirus-associated diseases, we have concentrated on two groups of patients. (i) Patients with epidermodysplasia verruciformis (EV) disease have a chronic and familial condition characterized by flat warts and pigmented papules which in one out of three patients will progress to squamous cell carcinoma in sun-exposed areas of the skin (*11, 12*). (ii) Veterinarians and meat-handlers have a high incidence of wart disease and frequent recurrence after treatment (*13, 14*). We extracted the total DNA from semen samples of three patients from each of these groups and analyzed it by the Southern blot hybridization technique, using nick-translated probes of molecularly cloned HPV DNA's (*15–17*). The sensitivity of these analyses as determined by reconstruction experiments indicated that less than 0.06 genome copy per diploid cell equivalent could be detected for homologous HPV types and about 0.6 copy per cell for heterologous HPV types detected under conditions of reduced stringency.

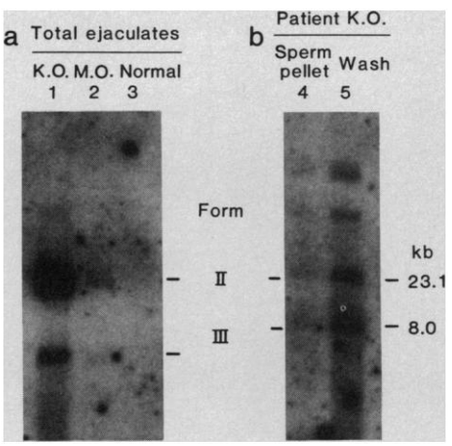

Fig. 1. Detection of HPV-5 in the semen of two patients. (a) Fresh or frozen semen from an EV patient (K.O.), lane 1, his son (M.O.), lane 2, and a normal donor, lane 3, were treated with a mixture of 1 percent sodium dodecyl sulfate and Pronase (500 mg/ml) overnight at 37°C. Donors wore gloves to prevent possible contamination of samples. After extraction with phenol, phenol:chloroform (1:1 by volume), and chloroform, the ethanol-concentrated nucleic acids were treated with ribonuclease A and extracted as above. Total cellular DNA (about 15 μg) was analyzed by electrophoresis in agarose gels, transferred to nitrocellulose, and hybridized under stringent conditions with a ^{32}P-labeled HPV-5 probe. Form II and form III represent the positions of nicked, double-stranded or linear, double-stranded native HPV DNA, respectively. (b) The semen of patient K.O., obtained about 5 months after the sample analyzed in (a), was pelleted and washed three times with isotonic saline. Both the sperm pellet, lane 4, and pooled supernatant washes, lane 5, were extracted for DNA as above. Size of linear HPV DNA and markers are given in kilobases (kb).

R. S. Ostrow, K. R. Zachow, A. J. Faras, Institute of Human Genetics and Department of Microbiology, University of Minnesota Medical School, Minneapolis 55455.
M. Niimura, Department of Dermatology, Jikei University School of Medicine, 19-18 Nishishimbashi 3-Chome, Minato-Ku, Tokyo, Japan.
T. Okagaki, Departments of Obstetrics and Gynecology, Laboratory Medicine, and Pathology, University of Minnesota Medical School, Minneapolis 55455.
Sigfrid Muller, Department of Dermatology, Mayo Clinic, Rochester, MI 55905.
M. Bender, Department of Dermatology, University of Minnesota Medical School, Minneapolis 55455.

We initially examined semen from an HPV-5–infected EV patient (K.O.) (*18*) and a former meat-handler (G.M.) with a debilitating wart disease of the hands and chronic lymphatic leukemia. The semen from both patients contained HPV DNA (Figs. 1 and 2). Several warts from the hands of G.M. were found by stringent hybridization to contain both HPV-2 and HPV-3 DNA. We confirmed this by molecularly cloning and characterizing each of these HPV DNA species. Only HPV-2 DNA was found in the semen of this patient.

In an attempt to determine whether semen from the progeny of the EV patient also contained HPV DNA, we analyzed a DNA extract from his son's (M.O.) semen and found HPV-related DNA present (Fig. 1). This subject exhibited no clinical evidence of any wart disease. Since hybridization was conducted under stringent conditions with an HPV-5 probe, the DNA detected in semen from this patient represented HPV-5–related nucleotide sequences. In this case, as with the others, the small amount of HPV DNA detected did not permit extensive restriction endonuclease analyses. Therefore, it cannot be ruled out that the viral DNA found in donor M.O. might be one of the many HPV DNA types found in EV patients that cross hybridize with HPV-5 DNA under these conditions.

In an attempt to determine whether the HPV DNA present in these semen samples was associated with sperm, we centrifuged semen samples of two patients (K.O. and G.M.) and gently washed them with isotonic saline. Approximately 95 percent of the HPV DNA was found associated with extracts of the washes and not with the sperm pellet (Figs. 1 and 2). This suggests that the HPV DNA was not associated with sperm in the semen samples but rather was present as either free viral particles or HPV DNA. Although the presence of free viral particles could not be detected in the semen by electron microscopy (suggesting that the viral DNA present may not be associated with viral particles in these patients), this issue could not be unequivocally resolved in these studies because of an inadequate supply of semen from these patients for more rigorous density gradient hybridization analyses.

Ten additional semen specimens from normal donors were examined under conditions of low stringency with an HPV-6 probe in order to determine the prevalence of HPV DNA in a sampling of the general population. There was no evidence for the presence of HPV nucleotide sequences in these samples when compared to a negative foreskin DNA control (Fig. 3). Similar negative results were obtained with HPV-16 and HPV-18 DNA probes.

Papillomavirus-associated lesions of the male (*19*) and female (*20*) urethra have recently been reported. Although there was no clinical evidence for urinary papillomas in our patients, we sought to ascertain whether exfoliated HPV-infected cells or virions being shed by an undetected urethral lesion in these patients might be responsible for our initial results. The first and second 5-ml fractions of urine samples from these patients were extracted for DNA analysis. We were unable to detect HPV DNA in these samples by the Southern blot hybridization procedure. This finding suggests that the positive hybridization results on the semen samples do not reflect HPV shedding from urinary or bladder lesions. No clinical evidence of genital neoplasia was observed in the spouses of these patients, although subclinical infections could not be ruled out at this time.

It should be noted that HPV-2 and HPV-5 are not normally associated with lesions of the genital tract. Recent reports have indicated that primarily HPV-6 and HPV-11 are associated with the majority of benign or mildly dysplastic lesions (*6*) and that HPV-16 and HPV-18 are associated with more severe dysplastic or malignant lesions of the anogenital tract (*7*). Although we cannot unequivocally demonstrate the transmission of HPV DNA via semen to sexual partners,

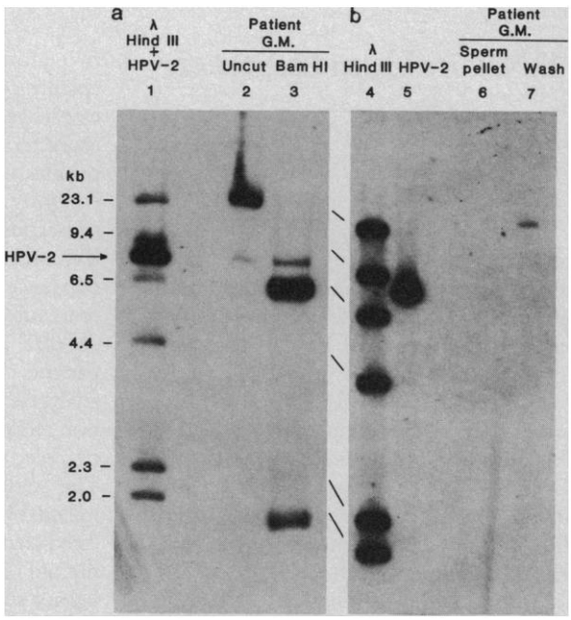

Fig. 2. Detection of HPV-2 in the semen of a former meat-handler. Semen from patient G.M. was extracted for total cellular DNA and analyzed as in Fig. 1. Characterization of the HPV DNA present with or without prior treatment with the restriction enzyme Bam HI was accomplished by stringent hybridization with ^{32}P-labeled HPV-2. (a) About 20 μg of total cellular DNA was treated in each of lanes 2 and 3. Lane 1 is a mixture of 16 pg of linearized HPV-2 DNA and radiolabeled bacteriophage λ Hind III DNA fragments. Lane 4 is radiolabeled bacteriophage λ Hind III DNA fragments, and lane 5 is 16 pg of linearized HPV-2 DNA. (b) About 4 μg total was used for both sperm pellet and wash DNA extracts in lanes 6 and 7.

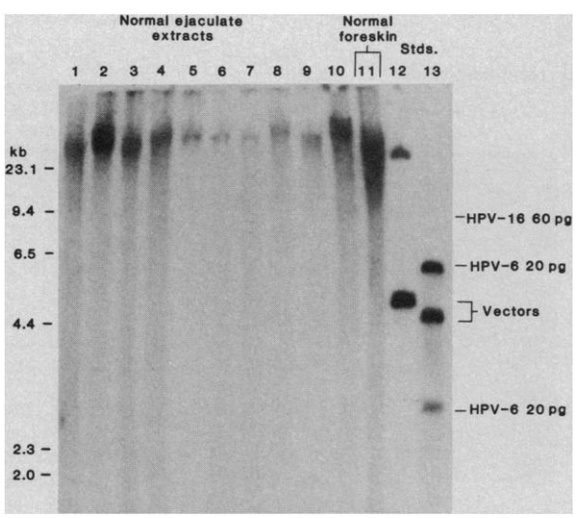

Fig. 3. Screening for HPV DNA in the semen of normal donors. Semen from healthy normal donors was extracted for total cellular DNA (lanes 1 through 10). Approximately 20 μg of each was then treated with Bam HI prior to analysis for HPV DNA. A DNA extract of a normal foreskin is also included (lane 11). ^{32}P-Labeled HPV-6–pBR322 DNA was hybridized under conditions of low stringency, which permit the detection of all HPV types. A small amount of nonspecific trapping of label in the high molecular weight region is observed under these conditions. Vector bands are pBR322 cleaved from HPV-16 at the unique Bam HI cloning site in lane 12 and the slightly smaller Eco RI-Bam HI–cleaved pBR322 excised from the two cloned fragments of HPV-6 in lane 13. The band near the top of lane 12 represents a small amount of uncleaved HPV-16–pBR322 plasmid.

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.

REFERENCES AND NOTES

1. M. Fukushima *et al.*, *Cancer Res.*, **45**, 3252 (1985).
2. T. Okagaki *et al.*, *Arch. Pathol. Lab. Med.* **108**, 567 (1984).
3. T. Okagaki *et al.*, *Int. J. Gynecol. Pathol.* **2**, 153 (1983).
4. K. Zachow *et al.*, *Nature (London)* **300**, 771 (1982).
5. M. Green *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4437 (1982).
6. L. Gissmann *et al.*, *ibid.* **80**, 560 (1983).
7. M. Durst, L. Gissmann, H. Ikenberg, H. zur Hausen, *ibid.*, p. 3812.
8. W. Lancaster, R. Kurman, L. Sanz, S. Perry, A. Jenson, *Intervirology* **20**, 202 (1983).
9. S. Graham and W. Shotz, *J. Natl. Cancer Inst.* **63**, 23 (1979).
10. R. Levine *et al.*, *Obstet. Gynecol.* **64**, 16 (1984).
11. M. Lutzner, *Bull. Cancer (Paris)* **65**, 169 (1978).
12. S. Jablonska, J. Dabrowski, K. Jakubowicz, *Cancer Res.* **32**, 583 (1972).
13. K. Bosse and E. Christophers, *Hautarzt* **15**, 80 (1964).
14. M. DePeuter, B. Declerq, A. Minnette, *Br. J. Dermatol.* **96**, 427 (1977).
15. R. Ostrow *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1634 (1982).
16. E. Southern, *J. Mol. Biol.* **98**, 503 (1975).
17. T. Maniatis, A. Jeffrey, D. Kleid, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1184 (1975).
18. R. Ostrow *et al.*, *J. Am. Acad. Dermatol.* **8**, 398 (1983).
19. W. Murphy, Y. Fu, W. Lancaster, A. Jenson, *J. Urol.* **130**, 84 (1983).
20. K. Syrjanen and S. Pyrhonen, *Scand. J. Urol. Nephrol.* **17**, 267 (1983).

21. We thank D. Weber for technical assistance in cloning the HPV's of G.M. The HPV-6 and HPV-16 were provided by L. Gissmann and H. zur Hausen. This work was supported by grants from the National Institutes of Health (CA 25462) and the Minnesota Leukemia Research Fund.

28 June 1985; accepted 20 November 1985

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

M. HATTORI, J. B. BUSE, R. A. JACKSON, L. GLIMCHER, M. E. DORF, M. MINAMI, S. MAKINO, K. MORIWAKI, H. KUZUYA, H. IMURA, W. M. STRAUSS, J. G. SEIDMAN, G. S. EISENBARTH

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

M. Hattori, J. B. Buse, R. A. Jackson, Joslin Diabetes Center, Harvard Medical School, Boston, MA 02215.
L. Glimcher, M. E. Dorf, M. Minami, Harvard Medical School, Boston, MA 02215.
S. Makino, Shionogi Aburahi Laboratories, Shiga, Japan.
K. Moriwaki, National Institute of Genetics, Shizuoka, Japan.
H. Kuzuya and H. Imura, Kyoto University School of Medicine, Kyoto, Japan.
W. M. Strauss and J. G. Seidman, Harvard Medical School, Boston, MA 02215.
G. S. Eisenbarth, Joslin Diabetes Center, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02215.

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