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NOTES

Evaluation of Temperature Sensitivity of Human Papillomavirus Type 11 by Using the Human Xenograft Severe Combined Immunodeficiency Mouse Model

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The temperature sensitivity of human papillomavirus type 11 was evaluated by using a human xenograft severe combined immunodeficiency mouse model. Incubation of the virus for 1 h at a temperature higher than 56°C but lower than 72°C was sufficient to inhibit the virally induced growth of infected human tissue. However, 100°C was necessary to completely inactivate HPV type 11 genome expression.

The conditions for heat inactivation of human papillomaviruses (HPVs) have not been rigorously established. Investigations of human autogenous wart vaccines prepared from condylomata acuminata suggested that incubation at 56°C for 1 to 2 h is sufficient for inactivation, but the infectivity of the preparation was not determined prior to inoculation in those studies (1, 7). More recently, Smith et al. (9) described an in vitro model of HPV type 11 (HPV-11) infection and evaluated the ability of a single temperature, 60°C for 1 h, to abrogate viral expression, as measured by the presence of E1^E4 mRNA. In the present study, we used the severe combined immunodeficiency mouse model of productive HPV-11 infection (2) to evaluate the susceptibility of HPV-11 to a broad range of temperatures. HPV-11 is, with HPV-6, the main causative agent of condyloma acuminatum.

Five-hundred-microliter aliquots of a standardized HPV-$11_{Hershey}$ suspension (batch 4/90; 1:10 dilution) were incubated for 1 h at one of the following temperatures: 37, 56, 72, 88, or 100°C. Incubations at 37, 56, 72, and 88°C were carried out in water baths; for those at 100°C, a heat block was used. All tubes were tightly sealed to prevent evaporation. A human neonatal foreskin specimen obtained on the same day was prepared as described previously (2), and the 1-by-1-mm fragments were divided into the five heat-treated viral suspensions. Additional foreskin fragments were snap-frozen in liquid nitrogen and were saved at −80°C to serve as controls. After a 1-h incubation at 37°C, one foreskin fragment corresponding to each of the heat treatment groups was grafted under the renal capsule of each kidney of three 6-week-old mice with severe combined immunodeficiency (C.B-17/*Icr Tac-scid DF*; Taconic Farms, Germantown, N.Y.). The experiment was duplicated with a different foreskin specimen on a different day. The mice were sacrificed 12 weeks postimplantation, and the kidneys were recovered. Graft size was expressed as the geometric mean diameter [GMD; GMD = $3\sqrt{(\text{length} \times \text{width} \times \text{height})}$]. Portions of the grafts were snap-frozen in liquid nitrogen and were stored at −80°C until analysis for viral expression. The protocols described here were approved by the University of Rochester Committee on Animal Research and Highland Hospital of Rochester Human Investigation Committee.

To demonstrate HPV-11 viral expression, we chose to detect the E1^E4 mRNA species because it is the most abundant of the mRNAs originating from the early region of the genome. Extraction of total RNA and reverse transcriptase (RT)-PCR of HPV-11 have been described elsewhere (3). In the present study, we used a new forward primer (p827; 5′-CAA GGA TGG CGG ACG ATT CAG-3′; nucleotides 827 to 847) and a new reverse primer (p3584R; 5′-GCA CTA TAG GCG TAG CTG CAC-3′; nucleotides 3563 to 3584), which amplify a 281-bp fragment derived from the E1^E4 mRNA. To provide a positive control for the RT-PCR, we assayed the samples for the human β-actin mRNA transcript. The primer-binding sites for β-actin PCR amplification were selected from the second (forward primer, 5′-CGCCGCGCTCGTCGTCG-3′) and fourth (reverse primer, 5′-GTAGCCGCGCTCGGTG-3′) exons of the β-actin transcript. Amplification with these primers produces a 582-bp product from the spliced β-actin transcript, as opposed to a 1,152-bp product from the unspliced transcript or the genomic β-actin locus. Corresponding uninfected foreskin fragments were used as negative controls.

Statistical analysis was done by using CSS:STATISTICA/w version 4.3 software (Statsoft, Tulsa, Okla.). The effect of temperature on graft size was evaluated by the Kruskal-Wallis test by using the Bonferroni multiple comparison procedure for pairwise comparisons (5). The effect of temperature on viral expression was assessed by logistic regression, adjusting for foreskin effect. Size comparison between the RT-PCR-positive and -negative samples was done by Mann-Whitney's U test. Two-tailed P values of less than or equal to 0.05 were considered significant.

Table 1 summarizes the results of the study. Animal deaths were perioperative and were not related to the grafts themselves. Among the grafts available for analysis, the overall size differences among the five groups were highly significant (P = 0.0001). The median size of the grafts decreased abruptly with a rise in temperature from 56 to 72°C (Fig. 1). Choosing the

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TABLE 1. Results of the study

Virus incubation temp (°C)	No. of grafts				Median (range) GMD (mm)	No. (%) of grafts HPV-11 positive by RT-PCR
	Implanted	Lost because of:		Available for analysis		
		Animal death	Dislodgement			
37	12			12	4.19 (1.00–7.39)	12 (100)
56	12	4	1	7	4.59 (1.00–6.54)	5 (71.4)
72	12		1	11	1.82 (1.26–3.63)	6 (54.5)
88	12	4		8	1.59 (1.00–1.59)	3 (37.5)
100	12			12[a]	1.35 (1.00–3.00)	0 (0)

[a] Because 1 graft was negative for β-actin mRNA, only 11 grafts were evaluable by RT-PCR.

group in which the virus was treated at 100°C as the control, pairwise comparisons were statistically significant between the groups in which the virus was treated at 100 and 37°C and the groups in which the virus was treated at 100 and 56°C. There were no significant differences between the groups in which the virus was treated at 100 and 88°C or between the groups in which the virus was treated at 100 and 72°C. This suggests that the temperature sufficient to inhibit the growth of HPV-11-infected tissue is higher than 56°C but lower than 72°C.

Table 1 shows that there was a progressive decrease in the number of grafts exhibiting viral expression as the virus incubation temperature increased. All samples were positive for the presence of HPV-11 mRNA in the group in which the virus was treated at 37°C, and all samples were negative in the group in which the virus was treated at 100°C. The effect of temperature was significant ($P = 0.001$; 48% of variance explained by goodness of fit), with no foreskin effect ($P = 0.90$). These results suggest that a 1-h incubation at 100°C is sufficient to abrogate all infectivity of the virus, since 0 of 11 (0%) of the samples were positive for the presence of HPV-11 mRNA. RT-PCR was predictive of graft size, because there was a clear difference in the median (range) size of grafts that contained HPV-11 mRNA, 3.63 (1.0 to 7.37) mm, and those that did not, 1.44 (1.0 to 2.62) mm ($P = 10^{-6}$) (Fig. 2).

The results of the present study demonstrate that incubation at 100°C for 1 h completely inactivates HPV-11 infectivity, as measured by expression of E1^E4 mRNA (the sensitivity of our RT-PCR assay was approximately 10 to 100 copies of E1^E4 mRNA). However, lower temperatures (e.g., 72°C) are sufficient to significantly reduce infectivity, as measured by graft size. The reasons for these differential effects are not known. Because serial dilution of the virus results in grafts that are smaller in size (1a), we speculate that, to occur, graft growth requires a high viral copy number per cell and that temperatures significantly below 100°C suffice to inactivate a substantial proportion of infectious viral particles, effectively decreasing the viral copy number below a critical threshold. Heat inactivation has been studied for animal papillomaviruses. Bryan and Beard (4) reported that a 15-min treatment at 57°C is sufficient to inactivate the cottontail rabbit papillomavirus (4). In contrast, Shope (8) found that this virus requires a 30-min exposure at 70°C for inactivation. Working with the virus of rabbit oral papillomatosis, Parsons and Kidd (6) determined that 30 min at 75°C is necessary for inactivation. Our findings on the growth of grafts infected with HPV-11 are more consistent with the latter two observations. Smith et al. (9) found that a 1-h treatment at 60°C is sufficient to abrogate HPV-11 expression, in comparison with the 100°C that we found abrogated HPV-11 expression in our study. Possible explanations for this dissimilarity include a lower titer of their viral inoculum, a lower sensitivity of their in vitro model in comparison with that of our in vivo model, and a lower degree of sensitivity of their RT-PCR assay.

In conclusion, the results of the present study indicate that HPV-11 is a hardy virus and that standard steam sterilization procedures may be necessary to prevent nosocomial transmission of HPV by instruments used for the diagnosis and treatment of HPV diseases.

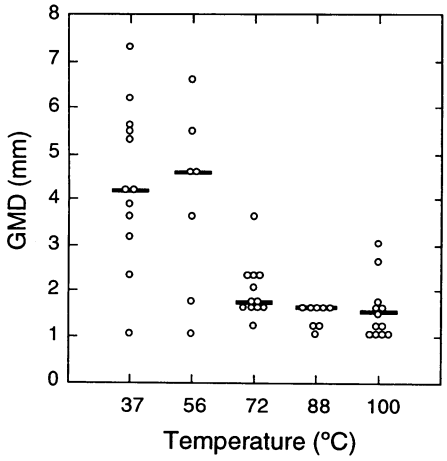

FIG. 1. Graft sizes by HPV-11 incubation temperature. For each group, the horizontal bar represents the median.

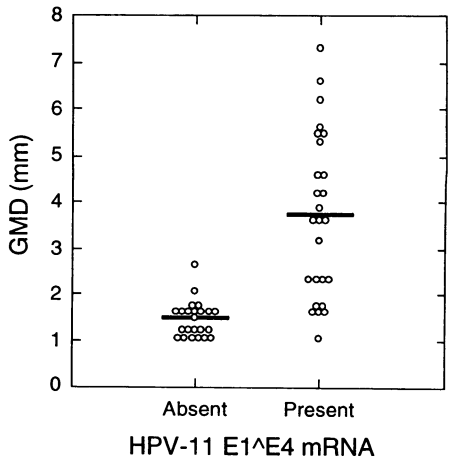

FIG. 2. Relationship between graft size and the presence or absence of viral expression in the graft. For each group, the horizontal bar represents the median.

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