Intermediate Size Papovavirus Particles in Pregnancy Urine

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SUMMARY

By electron microscopy of negatively stained urinary sediments, papovavirus particles of size intermediate between papillomavirus and polyomavirus have been detected in the urine of a pregnant woman. The excretion of size variants of these viruses and the absence of detectable papillomas in any of the women who were found to excrete only papillomavirus represent new findings.

Since the discovery of the association of human polyomaviruses with cases of progressive multifocal leucoencephalopathy (Padgett et al., 1971) and following renal transplantation (Gardner et al., 1971; Lecatsas et al., 1973), these agents have been detected in a variety of immunodeficiency states (Lecatsas et al., 1976a, b; 1977) and recently JC polyomavirus has been detected in the urine of pregnant women (Coleman et al., 1977; Lecatsas & Boes, 1980). By exploiting the size difference which exists between the papovavirus genera, we have since detected papillomavirus in the urine of pregnant women (Lecatsas & Boes, 1979) and in this communication we report the finding in the urine of one such woman of virus particles which are intermediate in size between polyomavirus and papillomavirus, suggesting that in vivo variants of these two genera may arise spontaneously.

During routine investigations for the presence of virus particles in urine during pregnancy, employing the technique of negative-contrast electron microscopy, 10 ml of midstream urine from a healthy 23-year-old prima gravida (EMS) was centrifuged at 100000 g for 1 h at 4 °C using a Damon IEC B-60 ultracentrifuge. After discarding the supernatant the resultant pellet was dissolved and re-centrifuged in deionized water under the same conditions. This pellet was then dissolved in 1 ml deionized water and used for negative-staining electron microscopy with 3% phosphotungstic acid employing formvar–carbon-coated grids and a Philips EM 300 electron microscope operated at 60 kV.

Comparative size measurements of virus particles were carried out using polyomavirus obtained from the urine of a male renal transplant recipient (SJN), papillomavirus obtained from four pregnancy patients (WJS, MET, SMN, KLL) and virus obtained from patient EMS which varied in size but which possessed a typical papovavirus morphology. One-hundred of the latter particles, 50 polyomavirus particles from patient SJN who excreted the BK form of the virus and a pooled sample of 33 papillomavirus particles from the four pregnant women were measured with respect to particle diameter. All particle measurements were carried out directly on electron microscope negatives taken at an instrumental magnification of 42000 using a Nikon model 6C comparator measuring to 1 μm.

Fig. 1 (A) shows papillomavirus particles obtained from patient KLL and Fig. 1 (B to D) show papovavirus particles from patient EMS with diam. varying from 52 to 35 nm. Size distribution of the three virus forms is shown in Fig. 2. BK polyomavirus particles varied from 37 to 43 nm in diam., while papillomavirus particles varied from 55 to 63 nm. Particles from patient EMS varied from 33 to 59 nm, spanning both polyomavirus and papillomavirus size ranges, with approx. 50% (44) of the particles being intermediate in size between the two genera. The peaks of 41 and 52 nm within the EMS particle range suggest that the size range of polyomavirus and papillomavirus are represented in this sample. Efforts to grow the virus from EMS have so far proved unsuccessful and the small quantity of virus precluded DNA extraction and determination of mol. wt. distribution. Urine specimens from EMS were
Fig. 1. Urine-derived virus particles negatively stained with 3% phosphotungstic acid at pH 8. (A) Papillomavirus (a) from patient KLL. (B, C, D) Particle size variation measured in nm in patient EMS: (B) b, 52 nm; c, 51 nm. (C) d, 35 nm; e, 45 nm. (D) f, 44.5 nm; g, 46 nm; h, 47 nm; i, 49 nm.

Fig. 2. Size distribution of virus particles obtained from urine. Polyomavirus from renal transplant patient SJN (50 particles); papillomavirus from pregnancy patients WJS, MET, SMN and KLL (33 particles); virus particles from pregnancy patient EMS (100 particles).
negative for virus by electron microscopy 2 days after initial detection of the virus and have remained negative to date.

Papovaviruses fall naturally into two groups according to their size, polyomavirus being approx. 45 nm and papillomavirus 55 nm in diam. Although particle size within the two genera will vary from one laboratory to another and also because of experimental error, the 10 nm difference in size between the two genera is generally clearly defined and is borne out in our measurements. However, the finding that almost 50% of EMS particles had diameters between the two generic sizes rules out the possibility that it was a mixture of papillomavirus and polyomavirus and indicates either the existence of a new form of papovavirus which varies in size (a remote possibility in cubic viruses) or the existence, under in vivo conditions, of genetic variants of polyomavirus and papillomavirus, a more likely explanation. Size variation in the tubular forms of human polyomavirus detected in urine has been reported (Lecatsas & Prozesky, 1975). Similar variation in spherical papovavirus particles has been limited to the occasional appearance of a few 30 nm particles in some preparations. Crawford et al. (1962) have shown that the diam. of polyomavirus 'empty' particles may vary from 47 to 57 nm, depending on the thickness of the phosphotungstate stain sprayed on the preparation. Since our particles were essentially 'full' and uniformly stained with 3% phosphotungstic acid, this factor is unlikely to have affected our results. Subsequently, Crawford & Crawford (1963) confirmed the general diam. of 45 nm and 55 nm for polyoma and papillomaviruses respectively. In addition, the largest particle in our EMS preparation measured 59 nm. Mixtures of polyoma and papillomaviruses in various clinical states, however, probably do occur. Both genera have been repeatedly detected in pregnancy urine and the possibility of the existence of size variants of both is reasonable. It is also possible that replication of these agents occurs in the same tissue. Although BK virus has been shown to replicate in the urothelium of the donor ureter following renal transplantation (Gardner et al., 1971), it is not known where these viruses replicate in pregnant women. This also holds for papillomavirus. However, replication of both genera, as well as EMS particles, in the urothelium cannot be ruled out.

Routine electron microscopy of urine sediments from renal transplant recipients in our laboratory has shown that papillomavirus is also excreted in the urine. In addition, intermediate size particles have been detected in one such patient. Attempts to grow the virus from these patients have been unsuccessful and this is probably because papillomaviruses cannot be propagated by routine culture techniques (Zur Hausen et al., 1978). Consequently, initial identification of these virus forms should be by particle size measurements followed by serological means and restriction endonuclease digestion patterns, if enough virus is available. Although no published information is yet available, our observations tend to suggest that, in pregnancy, papillomavirus excretion is at least as common as, if not more common than, polyomavirus excretion. We have recently isolated a polyomavirus from pregnancy urine which, unlike JC virus, has been successfully cultured on primary human foetal fibroblasts. To date, nine strains of BK virus have been isolated, suggesting that serotypes other than JC are likely to be found in pregnancy.

The implications of our findings raise interesting possibilities. A small percentage of human papillomas are known to become malignant and recently malignant human papillomas have been shown to be associated with the HPV-5 serotype of papillomavirus rather than with the other known serotypes (Orth et al., 1979). Although human polyomavirus has been shown to be oncogenic in experimental animals (Padgett & Walker, 1976), their implication in human tumours remains hypothetical. However, genetic variants of the two papovavirus genera may well affect oncogenic potential in papillomaviruses. In this respect the serological identity of the papillomavirus excreted during pregnancy would prove informative since no warts of any type were found in any of the patients.
Limited immunological investigation of patient EMS has shown that lymphocyte responsiveness to the mitogens concanavalin A and phytohaemagglutinin was markedly reduced. Although such findings also apply to other inherent or induced immunodeficiencies, as in organ transplantation, an immunological basis for the appearance of papovaviruses and their variants in pregnancy cannot be ruled out.

**REFERENCES**


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