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An Antigen Chimera of Poliovirus Induces Antibodies against Human Papillomavirus Type 16

OWEN JENKINS,[1] JOHN CASON,[2] KAREN L. BURKE,[1] DECLAN LUNNEY,[3] ALISON GILLEN,[1] DAKSHA PATEL,[3] DENNIS J. McCANCE,[2,3] AND JEFFREY W. ALMOND[1]*

United Medical and Dental Schools Department of Microbiology, University of Reading, London Road, Reading RG1 5AQ,[1] and Department of Microbiology, Guy's Campus, London SE1 9RT,[3] and Dimbleby Laboratory of Cancer Virology, United Medical and Dental Schools St. Thomas's Campus, London SE1 7EH,[2] United Kingdom

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It has been established that the surface of poliovirus type 1 can be extensively modified to incorporate antigenic domains from other poliovirus serotypes and from unrelated viruses. The fact that the modified (chimeric) viruses exhibit dual antigenicity and immunogenicity led us to explore the possibility of using the Sabin vaccine strain of poliovirus type 1 as a vector for the presentation of antigenic domains from human papillomavirus type 16 (HPV-16), a virus associated with the development of cervical carcinoma. We report here the construction and characterization of a chimeric poliovirus containing a 16-residue sequence derived from the major capsid protein (L1) of HPV-16. This virus chimera stimulated the production in rabbits of antibodies which recognized the HPV-16-derived peptide and an L1 fusion protein synthesized in *Escherichia coli* and detected HPV-16 in human biopsy material by immunoperoxidase staining. The possibility that poliovirus-HPV chimeras could be used as vaccines against HPV-16 is discussed.

The live attenuated poliovirus vaccine of Sabin (OPV) has been used widely for more than 25 years (18). Of the three serotypes constituting OPV, the type 1 strain has an excellent safety record and is probably the safest live vaccine virus ever used in humans (1). We reported recently the construction of a poliovirus type 1-type 3 chimera which exhibits dual antigenicity and immunogenicity (4). This virus was constructed by the replacement of a major antigenic site (site 1 [19]) of the poliovirus type 1 vaccine strain with the corresponding region of a type 3 strain. Similar recombinants based on the neurovirulent poliovirus type 1 strain P1/Mahoney have also been reported (16, 21, 22). These experiments suggested that it would be possible to incorporate antigenic domains from unrelated viruses into the poliovirus particle without destroying viability and that viruses modified in this way may have vaccine potential. To this end, a poliovirus cDNA cassette designated pCAS1 (5) was constructed to facilitate the rapid incorporation of foreign sequences into the region encoding antigenic site 1 of poliovirus. With this cassette system, a number of viable chimeras have been constructed. One such chimera incorporating an epitope from the transmembrane glycoprotein (gp41) of human immunodeficiency virus type 1 was recently described (11). Rabbit antisera raised to this chimera were shown to be specific for human immunodeficiency virus type 1 gp41 in peptide binding assays and by Western blot (immunoblot) analysis. Moreover, the antisera also neutralized a wide range of human immunodeficiency virus isolates and inhibited virus-induced cell fusion. These results confirmed the potential of poliovirus as a vector for the presentation of foreign antigens and its use in the development of novel vaccines. We describe here the construction and characterization of a poliovirus chimera containing an epitope from human papillomavirus type 16 (HPV-16).

Certain HPVs are associated with premalignant and malignant disease of the genitourinary tract, in particular carcinoma of the cervix (3, 9). HPV-16 is one of the commonest viruses associated with all grades (I to III) of cervical intraepithelial neoplasia, a premalignant lesion localized in the transformation zone of the cervix, and with malignant carcinoma of the cervix (17). In addition, HPV-16 is associated with progression of mild cervical intraepithelial neoplasia (grade I) to severe cervical intraepithelial neoplasia (grade III) (6). This association of HPV with cervical disease has been confirmed by a number of groups using DNA-DNA hybridization to detect viral genomes in abnormal cells. Type-specific antibodies to HPVs have not been produced because these viruses do not replicate in cell cultures and intact virus particles in lesions are present only in very small quantities. Furthermore, the major capsid proteins (L1s) of different papillomaviruses are highly conserved and therefore a polyclonal antiserum raised against bacterial fusion proteins incorporating the L1 of one HPV type invariably cross-reacts with the L1s of several other types (24). Recently, however, Patel et al. (24) raised monoclonal antibodies (MAbs) against a β-galactosidase fusion protein containing part of the L1 of HPV-16 (residues 172 to 375). These MAbs are HPV-16 type specific and react with L1 fusion protein in Western blots and with human biopsy sections judged HPV-16 positive by in situ DNA-DNA hybridization. Furthermore, the MAbs were able to detect HPV-16 antigen in routine sections of cervical intraepithelial neoplasia tissue. Subsequent mapping of the MAb-binding sites with a series of 17 overlapping synthetic peptides corresponding in sequence to residues 172 to 375 of L1-HPV-16 revealed that four MAbs (8C4, ID6, 3DI, and 5A4) recognize peptide 269-284 (sequence, Glu-Asn-Val-Pro-Asp-Asp-Leu-Tyr-Ile-Lys-Gly-Ser-Gly-Ser-Thr-Ala), whereas MAb IC6 recognizes peptide 299-313 (7). We therefore chose to incorporate the 16 amino acids of L1-HPV-16 peptide 269-284 into antigenic site 1 of the Sabin 1 vaccine strain of poliovirus to create a virus with the potential to grow readily in tissue culture and which may stimulate an anti-HPV-16 response in humans after replication in the gut.

* Corresponding author.

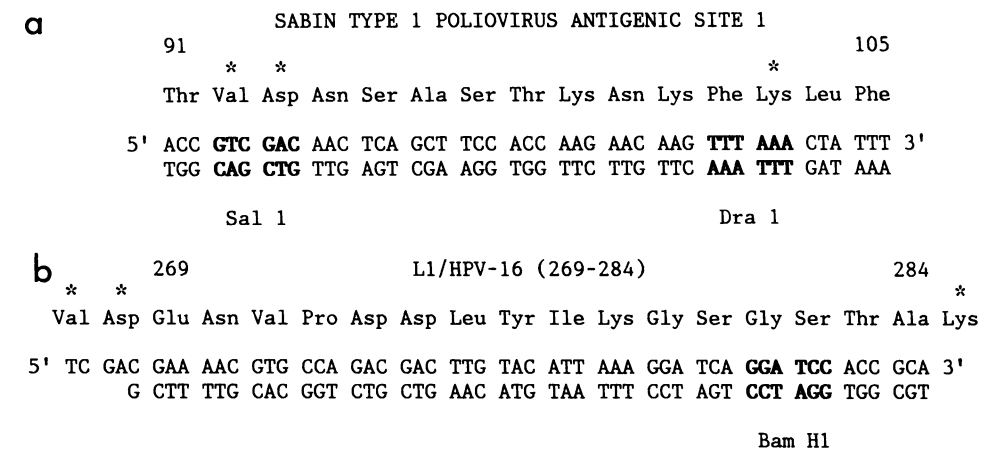

FIG. 1. Construction of S1/HPV-16/L1. (For details of construction, see Materials and Methods). (a) Nucleotide and amino acid sequence of antigenic site 1 of poliovirus Sabin type 1 in expression vector pCAS1 (5). Locations of the introduced unique restriction sites are shown, together with amino acid numbers of capsid protein VP1. (b) Nucleotide and amino acid sequence of complementary synthetic oligonucleotides encoding residues 269 to 284 of L1-HPV-16 and terminating in *Sal*I- and *Dra*I-compatible ends. Residues marked with an asterisk (*) correspond to those in panel a.

MATERIALS AND METHODS

Construction of chimeric poliovirus (S1/HPV-16/L1). The vector cassette pCAS1 (5) was used. (Briefly, this pBR322-based plasmid contains a full-length infectious cDNA of Sabin type 1 under the control of a T7 promoter. Unique *Sal*I and *Dra*I restriction sites facilitate the removal of the region encoding antigenic site 1 of the virus and its replacement with complementary synthetic oligonucleotides encoding desired changes. Infectious chimeric virus is recovered after transfection of HEp-2c monolayers with transcripts produced in vitro by T7 RNA polymerase.) The two synthetic oligonucleotides (Fig. 1b) were synthesized on a Miligen/Biosearch 8600 DNA synthesizer by using phosphoamidite chemistry and purified by polyacrylamide gel electrophoresis. Each oligonucleotide (600 ng) was annealed by boiling for 3 min and cooling slowly to room temperature. Approximately 100 ng of annealed DNA was ligated to pCAS1 previously digested with *Dra*I and *Sal*I. The resulting DNA was used to transform competent *Escherichia coli* MC1061. Plasmid DNA from transformant colonies selected on tetracycline (10 μg/ml) was screened by *Bam*HI restriction enzyme digestion (a *Bam*HI site was introduced in the synthetic oligonucleotides to assist screening). After *Nae*I digestion of correct plasmids, T7 runoff transcripts were prepared and transfected onto subconfluent HEp-2c cells in 75-cm^2 tissue culture flasks (28). After total cytopathic effect (6 days), the resultant chimeric virus was purified and the sequence of the modified antigenic site 1 was verified by the methods described by Rico-Hesse et al. (26).

Growth characteristics of S1/HPV-16/L1. Confluent HEp-2c monolayers in 35-mm tissue culture dishes (Sterilin) were washed twice with phosphate-buffered saline (PBS) and infected at a multiplicity of infection of 10 PFU per cell. Virus was adsorbed for 30 min at room temperature, and then prewarmed medium was added and the plates were incubated at 34°C. At the time points indicated in Fig. 2, medium was aspirated and the cells were washed twice in PBS before being scraped from the dish into a 1.5-ml tube. Cells were pelleted and then lysed by being resuspended in 0.1 ml of buffer containing 0.1 M Tris (pH 7.5), 0.1 M NaCl, 1.5 mM $MgCl_2$, and 0.25% (vol/vol) Nonidet P-40 (Sigma Chemical Co., St. Louis, Mo.). Virus titers in the lysate were determined by plaque assay on HEp-2c cells grown in six-well dishes (Corning Glass Works, Corning, N.Y.).

Rabbit sera. Three rabbits were inoculated subcutaneously with approximately 250 μg of sucrose cushion-purified (26) S1/HPV-16/L1 in complete Freund adjuvant. Animals were bled and boosted with 250 μg of purified chimera (subcutaneously) in incomplete Freund adjuvant at 14 days (bleed 1), 28 days (bleed 2), and 42 days (bleed 3), and a final bleed was taken at 56 days (bleed 4).

ELISA. Rabbit sera were assayed for antibodies to S1/

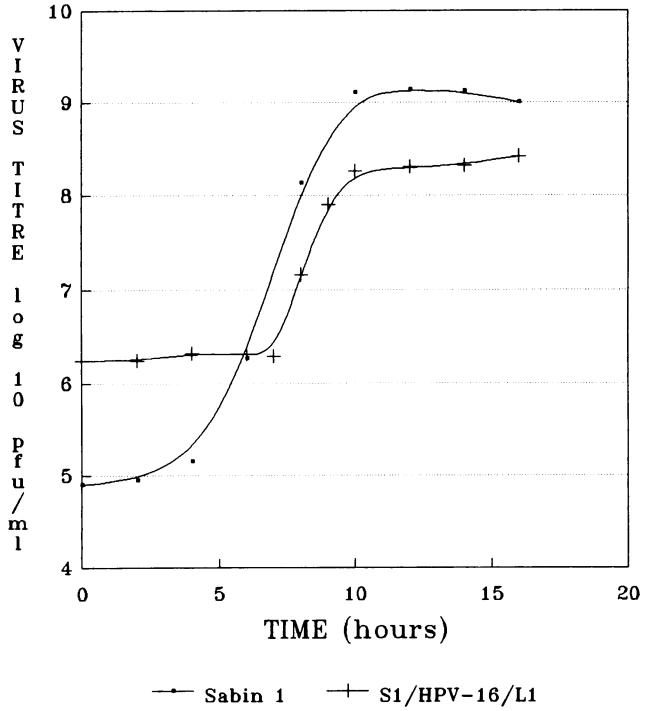

FIG. 2. One-step growth curve comparing the replication of S1/HPV-16/L1 (+) with that of the Sabin strain of poliovirus type 1 (■).

TABLE 1. Reactivity of S1/HPV-16/L1 and Sabin type 1 with MAbs and polyclonal antibodies in a neutralization assay[a]

Virus	MAb and polyclonal antisera	Against site:	Titer[b]	
			S1/HPV-16/L1	Sabin 1
HPV-16	MAbs			
	8C4	L1-HPV-16 (269-284)	1.3	<
	ID6	L1-HPV-16 (269-284)	1.0	<
	3D1	L1-HPV-16 (269-284)	<	<
	5A4	L1-HPV-16 (269-284)	1.9	<
	IC6	L1-HPV-16 (299-313)	<	<
Sabin 1	955	1	<	>3.1
	271	2	>3.1	>3.1
	423	3	>3.1	>3.1
HPV-16	Polyclonal sera			
	DI (prebleed)	L1-HPV-16 (172-375)/β-galactosidase fusion protein	<	<
	DI	L1-HPV-16 (172-375)/β-galactosidase fusion protein	1.5	<
Sabin 1	Sheep 16	Sabin 1	>3.1	>3.1
Chimera	Rabbit 1 (prebleed)	S1/HPV-16/L1	<	<
	Rabbit 1 (fourth bleed)	S1/HPV-16/L1	>3.1	3.1
	Rabbit 2 (prebleed)	S1/HPV-16/L1	<	<
	Rabbit 2 (fourth bleed)	S1/HPV-16/L1	2.8	2.8
	Rabbit 3 (prebleed)	S1/HPV-16/L1	<	<
	Rabbit 3 (fourth bleed)	S1/HPV-16/L1	2.8	2.8

[a] Rabbit antibodies were prepared as described in the Materials and Methods. Other antibodies were prepared as described previously (12, 24).
[b] Neutralization titers were determined by challenge with 100 tissue culture infective doses in a standard microdilution assay and are expressed as $\log_{10}$ of the dilution giving 50% inhibition of cytopathic effect. <, Less than 1 $\log_{10}$.

HPV-16/L1 by the following procedure. Wells of enzyme-linked immunosorbent assay (ELISA) plates were coated with 90 μl per well of L1-HPV-16 fusion protein (at 0.25 mg/liter of PBS) or peptide at 2 mg/liter of PBS (peptides were conjugated via carbodiimide to bovine serum albumin) for 1 h at room temperature. Plates were then washed three times with PBS containing 0.02% (vol/vol) Tween-20 (BDH Ltd., Poole, England), and nonspecific sites were blocked by the addition of 90 μl per well of a solution containing 1 g of bovine serum albumin per liter in PBS. After further washing, the test sera (50 μl) were added at various dilutions (in PBS) and incubated for 2 h. Plates were then washed, and 50 μl per well of horseradish peroxidase-labeled goat anti-rabbit antisera (1/500 dilution in PBS; Dako Ltd.) was added for 1 h. Plates were developed with *ortho*-phenylenediamine (Sigma Chemical Co. Ltd., Poole, England) in citric acid buffer for about 15 min. The color reaction was stopped by the addition of 25 μl of 2 M H_2SO_4 per well, and the A_{490} was determined on a Dynatech MR-70 plate reader.

Immunoperoxidase staining of HPV-16-positive tissue sections. HPV-16 positivity was assessed by in situ DNA-DNA hybridization with HPV-16 DNA (8) and immunohistochemistry with HPV-16 MAb (24). Formyl mercury-fixed tissue embedded in paraffin wax was sectioned and then dewaxed in xylene, washed in alcohol, and hydrated. These sections were incubated in 0.5% (vol/vol) sodium thiosulfate to remove the mercury. Immunoperoxidase staining with the rabbit antisera raised to S1/HPV-16/L1 was done by the method described by Jenkins et al. (14).

RESULTS

Recovery of chimeric poliovirus. Six days after transfection of HEp-2c monolayers with RNA transcripts of the modified poliovirus cDNA, a total cytopathic effect was observed. A chimeric virus designated S1/HPV-16/L1 was recovered from tissue culture fluid, and nucleotide sequence determination from purified viral RNA of the region encoding antigenic site 1 confirmed the presence of HPV-16-derived sequences. A second chimera containing residues 299 to 313 of L1-HPV-16 (recognized by MAb IC6) failed to produce viable virus after transfection for reasons which are unclear.

Growth of S1/HPV-16/L1. A one-step growth curve comparing the replication of S1/HPV-16/L1 with that of the Sabin strain of poliovirus type 1 is shown in Fig. 2. The chimera replicated slightly less well than the unmodified Sabin 1 in HEp-2c cells, producing a titer of $10^{8.4}$ compared with 10^9 for Sabin 1. The plaques formed by S1/HPV-16/L1 were indistinguishable in size from those of Sabin 1 (data not shown).

Antigenicity of S1/HPV-16/L1. The antigenic character of S1/HPV-16/L1 was examined in a standard neutralization assay (Table 1). The poliovirus chimera was neutralized by the anti-HPV-16 MAbs 8C4, ID6, and 5A4, which are specific for peptide 269-284 of L1-HPV-16, and by a polyclonal serum raised against an L1-HPV-16/β-galactosidase fusion protein. However, the virus was not neutralized by MAbs which recognized other regions of L1-HPV-16. In a radioimmunoprecipitation assay, a 1/400 dilution of MAb 5A4 recognized S1/HPV-16/L1, whereas MAb IC6 failed to react at a dilution of 1/10. Neither antibody recognized Sabin 1 (data not shown). This suggests that the antigenicity of the HPV-16 sequence expressed on the surface of poliovirus closely resembles that of the papillomavirus capsid antigen found in infected cells, although it was of interest that one MAb specific for peptide 269-284 (3D1) did not neutralize and failed to recognize the chimera in a radioimmunoprecipitation assay. The chimera was also neutralized by polyclonal Sabin 1 antiserum and by MAbs raised against Sabin 1 antigenic sites 2 and 3. As expected, the chimera was not neutralized by a site 1-specific MAb (955; Table 1).

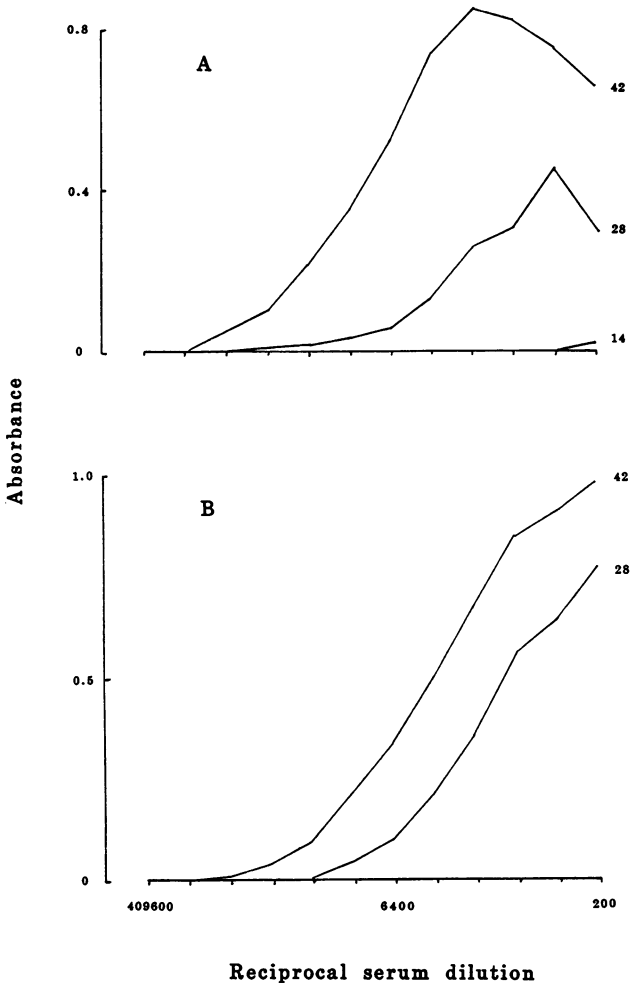

FIG. 3. Reactivity of rabbit antisera raised to S1/HPV-16/L1. Antisera taken 14, 28, and 42 days postinoculation were tested for reactivity to β-galactosidase/L1-HPV-16 (amino acids 172 to 375) fusion protein (A) and L1-HPV-16 peptide 269-284 (B) in ELISA. One representative set of antisera is shown. Preimmune and immune rabbit antisera to Sabin 1 (no HPV-16 insert) did not recognize any of these antigens in ELISAs.

Receptor specificity. Infection of HEp-2c monolayers by S1/HPV-16/L1 was blocked by an MAb specific for the cellular receptor for polioviruses (20), demonstrating that the virus retained its capacity to bind to and enter cells via the normal route used by polioviruses. This observation also suggests that the extensive modification to antigenic site 1 did not confer novel receptor-binding properties on the virus.

Immunogenicity of S1/HPV-16/L1. The immunogenicity of S1/HPV-16/L1 was assessed in rabbits by subcutaneous inoculation in adjuvant. Sequential bleeds from each of three animals showed an increase in antibodies which neutralized the chimera (Table 1). The sera also showed an increase in reactivity in ELISAs against the β-galactosidase/L1-HPV-16 fusion protein (amino acids 172 to 375) (Fig. 3A) and the L1-HPV-16 peptide 269-284 (Fig. 3B). Similar results were obtained when a different fusion protein of tryptophan E synthetase and L1-HPV-16 residues 1 to 505 (a gift from D. Galloway, Seattle, Wash.) were used as a solid-phase target. No reactivity against L1-HPV-16 peptides 299-313 and 329-343 was observed (data not shown).

Immunoperoxidase staining of HPV-16-positive tissue sections. Since HPV-16 cannot as yet be cultured in vitro, large amounts of purified virions are unavailable for immunological assay. The ability of the rabbit antisera raised against S1/HPV-16/L1 to recognize HPV-16 virions was therefore tested by immunoperoxidase staining of HPV-16-positive human biopsy material. Representative rabbit antisera raised against S1/HPV-16/L1 (third bleed serum from rabbit 1) contained antibodies which detected HPV-16 antigen in human tissue (Fig. 4B), whereas preimmune rabbit antiserum did not contain such antibody (Fig. 4A). Immune rabbit antisera to Sabin type 1 did not react with any tissue sections (data not shown).

These observations indicated that the antisera to the S1/HPV-16/L1 chimera can not only recognize the parent peptide but can also recognize this sequence when it is expressed on a large fusion protein or when it is expressed naturally in infected tissue sections.

DISCUSSION

We have previously shown that the Sabin vaccine strain of poliovirus type 1 can accommodate and express foreign epitopes on its surface and that the resulting chimeric viruses exhibit both dual antigenicity and immunogenicity (4, 11). The poliovirus system provides a novel method for presenting HPV epitopes to the immune system. We chose to insert a well-characterized epitope from the major capsid protein, L1, of HPV-16 since such sites may potentially induce HPV-neutralizing antibodies and hence immunity to de novo HPV-16 infections. Whether poliovirus chimeras would be able to induce protective immunity to HPV-16 is unknown and as yet difficult to assess since in vitro neutralization tests and animal models are not available for HPV-induced disease. Animal models do exist, however, for diseases caused by cottontail rabbit papillomavirus and by bovine papillomavirus (BPV). Mice and rabbits inoculated with L1-β-galactosidase fusion proteins from BPV type 1 (BPV-1) produced antibodies which neutralized infectious BPV particles in an in vitro assay (25). The amino acid homologies between the L1 proteins of HPV-16 and BPV-1 are such that epitopes in BPV may be predicted from the knowledge of those identified in HPV-16. The use of bovine enterovirus as an expression vector is presently being investigated (10), and this could form a parallel system to investigate protective immunity to BPV-1 in cattle. Malignant cells from cervical carcinomas frequently contain HPV-16 DNA and contain low levels of E6 and E7, the major transforming proteins of HPV (2, 27), but they do not express L1 proteins. Alternative chimeras based on these early proteins designed to stimulate cellular responses may offer possible protection against both de novo and established infection. It is encouraging that vaccinia recombinants containing the middle and large T antigens of polyomavirus have been shown to protect rats against tumors induced with the full-length genome. In addition, rejection of tumors was observed in rats 11 and 15 days after inoculation with tumor cells when they were immunized with both recombinants (15).

Evidence presented in Fig. 2 shows that poliovirus-HPV chimeras grow readily in tissue culture and can be used to produce large amounts of precisely defined HPV epitopes. These chimeras may also have applications as antigenic targets for the detection of antibodies to individual HPV types and thereby be useful for the diagnosis of particular HPV-induced diseases.

Although immune responses to HPVs are poorly under-

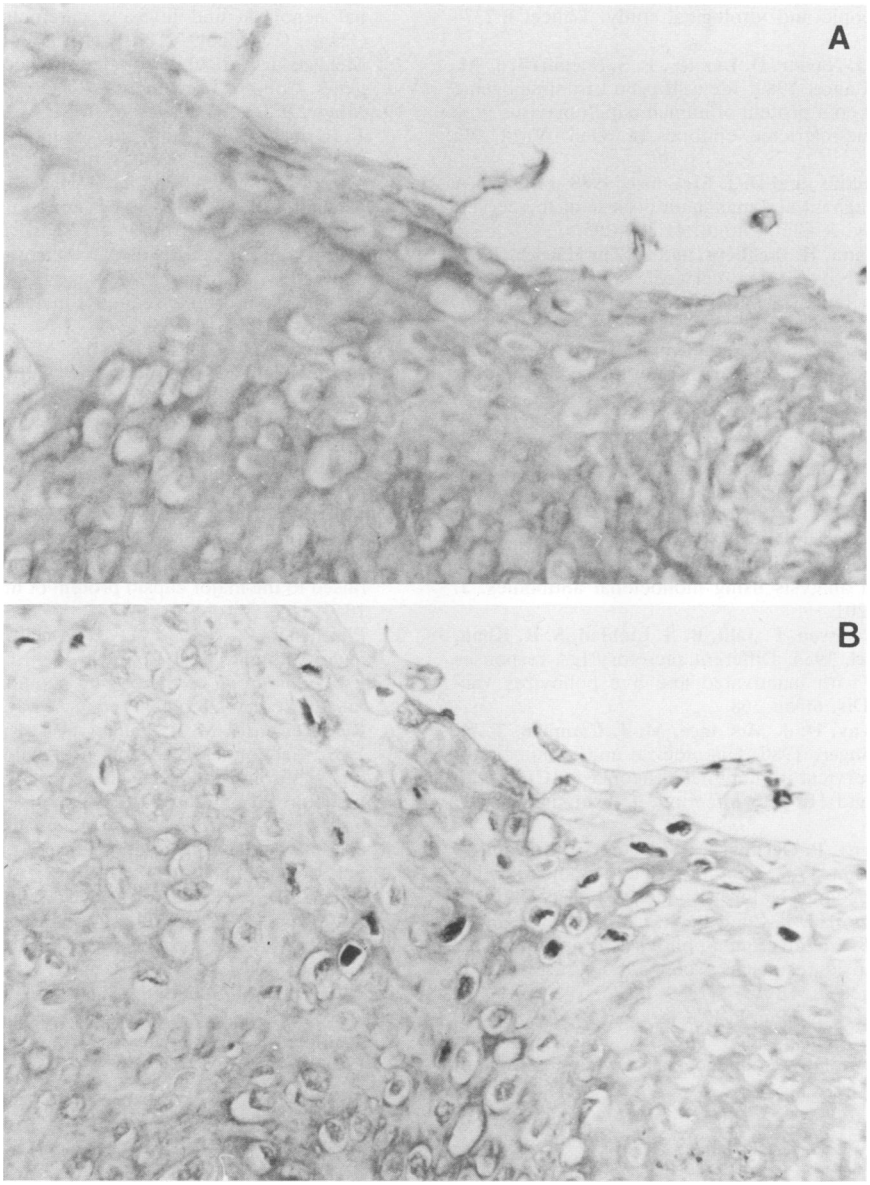

FIG. 4. Immunoperoxidase staining of HPV-16-positive tissue sections. The sections were reacted with preimmune serum (A) and third bleed serum from rabbit 1 inoculated with S1/HPV-16/L1 (B). Immune rabbit antisera to Sabin type 1 did not react with any tissue sections (data not shown).

stood, genital infections are confined to mucosal surfaces. Thus, the Sabin type 1 virus vector, which is known to stimulate good mucosal immunity (13, 23, 29), may offer the ideal vehicle to vaccinate against such pathogens.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Assaad, F., and W. C. Cockburn.** 1982. The relation between acute persisting spinal paralysis and poliomyelitis vaccine: result of a ten year enquiry. Bull. W.H.O. **60**:231–242.
2. **Banks, L., P. Spence, E. Androphy, N. Hubbert, G. Matlashewski, A. Murray, and L. Crawford.** 1987. Identification of human papillomavirus type 18 E6 polypeptide in cells derived from human cervical carcinoma. J. Gen. Virol. **68**:1351–1359.
3. **Boshart, M., L. Gissman, H. Ikenberg, A. Kleinberg, W. Scheurlen, and H. Zur Hausen.** 1984. A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. EMBO J. **3**:1151–1157.
4. **Burke, K. L., G. Dunn, M. Ferguson, P. D. Minor, and J. W. Almond.** 1988. Antigen chimaeras of poliovirus as potential new vaccines. Nature (London) **332**:81–82.
5. **Burke, K. L., D. J. Evans, O. Jenkins, J. Meredith, E. D. A. D'Souza, and J. W. Almond.** 1989. A cassette vector for the construction of antigen chimaeras of poliovirus. J. Gen. Virol. **70**:2475–2479.
6. **Campion, M. J., D. J. McCance, J. Cuzick, and A. Singer.** 1986. The progressive potential of mild cervical atypia: a prospective

cytological, colposcopic and virological study. Lancet **ii**:237–240.

7. **Cason, J., D. Patel, J. Naylor, D. Lunney, P. S. Shepherd, J. M. Best, and D. J. McCance.** 1989. Identification of immunogenic regions of the major coat protein of human papillomavirus type 16 that contain type-restricted epitopes. J. Gen. Virol. **70**: 2973–2987.
8. **Collins, J. E., D. Jenkins, and D. J. McCance.** 1988. HPV DNA sequences in premalignant and malignant disease of the cervix: a retrospective study. J. Clin. Pathol. **41**:289–295.
9. **Durst, M., L. Gissmann, H. Ikenberg, and H. Zur Hausen.** 1983. A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from a different geographical region. Proc. Natl. Acad. Sci. USA **80**:3812–3815.
10. **Earle, J. A. P., R. A. Skuce, C. S. Fleming, E. M. Hoey, and S. J. Martin.** 1988. The complete nucleotide sequence of a bovine enterovirus. J. Gen. Virol. **69**:253–263.
11. **Evans, D. J., J. McKeating, J. M. Meredith, K. L. Burke, K. Katrak, A. John, M. Ferguson, P. D. Minor, R. A. Weiss, and J. W. Almond.** 1989. An engineered poliovirus chimaera elicits broadly reactive HIV-1 neutralizing antibodies. Nature (London) **339**:385–388.
12. **Ferguson, M., P. D. Minor, D. I. Magrath, Q. Yi-Hua, M. Spitz, and G. C. Schild.** 1984. Neutralisation epitopes on poliovirus type 3 particles: an analysis using monoclonal antibodies. J. Gen. Virol. **65**:197–201.
13. **Hanson, L. A., B. Carlsson, F. Jalil, B. S. Linblad, S. R. Khan, and A. L. van Weizel.** 1984. Different secretory IgA responses after immunization with inactivated and live poliovirus vaccines. Rev. Infect. Dis. **6**:356–368.
14. **Jenkins, D., S. K. Tay, D. J. McCance, M. J. Campion, T. K. Clarkson, and A. Singer.** 1986. Histological and immunocytochemical study of cervical intraepithelial neoplasia (CIN) with associated HPV-6 and HPV-16 infection. J. Clin. Pathol. **39**: 1177–1180.
15. **Lathe, R., M. P. Kieny, P. Gerlinger, P. Clertant, I. Guizani, F. Cuzin, and P. Chambon.** 1987. Tumour prevention and rejection with recombinant vaccinia. Nature (London) **326**:878–880.
16. **Martin, A., C. Wychowski, T. Couderc, R. Crainic, J. M. Hogle, and M. Girard.** 1988. Engineering a poliovirus type 2 antigenic site on a type 1 capsid results in a chimaeric virus which is neurovirulent for mice. EMBO J. **7**:2839–2847.
17. **McCance, D. J., M. J. Campion, P. K. Clarkson, P. M. Chesters, D. Jenkins, and A. Singer.** 1985. The prevalence of human papillomavirus type 16 DNA sequences in cervical intraepithelial neoplasia and invasive carcinoma of the cervix. Br. J. Obstet. Gynaecol. **92**:1101–1105.
18. **Melnick, J. L.** 1980. Poliomyelitis vaccines: an appraisal after 25 years. Compr. Ther. **5**:6–14.
19. **Minor, P. D., M. Ferguson, D. M. A. Evans, J. W. Almond, and J. Icenogle.** 1986. Antigenic structure of polioviruses of serotypes 1, 2 and 3. J. Gen. Virol. **67**:1283–1291.
20. **Minor, P. D., P. A. Pipkin, D. Hockley, G. C. Schild, and J. W. Almond.** 1984. Monoclonal antibodies which block cellular receptors of poliovirus. Virus Res. **1**:203–212.
21. **Murray, M. G., J. Bradley, X. Yang, E. Wimmer, E. G. Moss, and V. R. Racaniello.** 1988. Poliovirus host range is determined by a short amino acid sequence in neutralization antigenic site 1. Science **241**:213–215.
22. **Murray, M. G., R. J. Kuhn, M. Arita, N. Kawamura, A. Nomoto, and E. Wimmer.** 1988. Poliovirus type 1/type 3 antigenic hybrid virus constructed *in vitro* elicits type 1 and type 3 neutralizing antibodies in rabbits and monkeys. Proc. Natl. Acad. Sci. USA **85**:3202–3207.
23. **Ogra, P. L., and S. S. Ogra.** 1973. Local antibody response to poliovaccine in the human female genital tract. J. Immunol. **110**:1307–1311.
24. **Patel, D., P. S. Shepherd, J. A. Naylor, and D. J. McCance.** 1989. Reactivities of polyclonal and monoclonal antibodies raised to the major capsid protein of human papillomavirus type 16. J. Gen. Virol. **70**:69–77.
25. **Pilacinski, W. P., D. L. Glassman, R. A. Krzyzek, P. L. Sadowski, and A. K. Robbins.** 1984. Cloning and expression in *Escherichia coli* of the bovine papillomavirus L1 and L2 open reading frames. Biotechnology **2**:356–360.
26. **Rico-Hesse, R., M. A. Pallansch, B. K. Nottay, and O. M. Kew.** 1987. Geographic distribution of wild poliovirus type 1 genotypes. Virology **160**:311–322.
27. **Smotkin, D., and F. O. Wettstein.** 1986. Transcription of human papillomavirus type 16 early genes in a cervical cancer-derived cell line and identification of the E7 protein. Proc. Natl. Acad. Sci. USA **83**:4680–4684.
28. **van de Werf, S., J. Bradley, E. Wimmer, F. W. Studier, and J. J. Dunn.** 1986. Synthesis of infectious poliovirus RNA by purified T7 RNA polymerase. Proc. Natl. Acad. Sci. USA **83**:2330–2334.
29. **Zhaori, G., M. Sun, and P. L. Ogra.** 1988. Characteristics of the immune response to poliovirus virion polypeptides after immunization with live or inactivated polio vaccine. J. Infect. Dis. **158**:160–165.