A human papillomavirus (HPV) vaccine consisting of virus-like particles (VLPs) was recently approved for human use. It is generally assumed that VLP vaccines protect by inducing type-specific neutralizing antibodies. Preclinical animal models cannot be used to test for protection against HPV infections due to species restriction. We developed a model using chimeric HPV capsid/cottontail rabbit papillomavirus (CRPV) genome particles to permit the direct testing of HPV VLP vaccines in rabbits. Animals vaccinated with CRPV, HPV type 16 (HPV-16), or HPV-11 VLPs were challenged with both homologous (CRPV capsid) and chimeric (HPV-16 capsid) particles. Strong type-specific protection was observed, demonstrating the potential application of this approach.
into 293TT cells (Fig. 2) (9). These experiments yielded results consistent with the VLP-binding assays in that pseudovirion neutralization was exclusively and consistently seen with sera from vaccine-matched animals. Neutralization titers ranged from approximately 1/10^5 to 1/10^6, demonstrating the production of high titers of neutralizing antibodies in vaccinated animals.

At 4 weeks after the final immunization with VLPs, animals were challenged with both homologous (CRPV capsid/CRPV genome) and chimeric (HPV-16 capsid/CRPV genome) infectious particles. All rabbits were inoculated with both particle types at five sites each on the dorsal skin. The ability of the VLP vaccinations to protect against type-specific infection was evaluated in vivo by observation of the appearance and growth of papillomas at the sites of challenge (Fig. 3). Consistent with the in vitro data, animals vaccinated with CRPV and HPV-16 VLPs showed evidence of significant protection against the vaccine-matched virus but not against the antigenically unre-
lated infectious particles. Since all animals were challenged with both homologous (CRPV/CRPV) and chimeric (HPV-16/CRPV) particles, the significantly faster appearance and growth rate of papillomas at sites inoculated with the vaccine-mismatched particles is strong evidence for both capsid-dependent infection and type-specific protection (Fig. 4). Both the homologous (CRPV/CRPV) and the chimeric (HPV-16/CRPV) infectious particles induced papillomas at equivalent growth rates on each of the rabbits immunized with HPV-11 VLPs, demonstrating that viral challenge was similar between the two types of infectious particles. These results provide no evidence of a cross-protective response in vivo between HPV-11 and HPV-16. The delayed appearance of small numbers of papillomas at sites inoculated with the
antigenically matched particles (beginning at week 4) may be due to (i) residual escape from antibody-mediated neutralization of the high titer of input infectious particles, (ii) capsid-independent infection by CRPV DNA, and/or (iii) the lack of an assisting L1-specific cell-mediated response (in the animals vaccinated with HPV-16 VLPs). The model as described provides opportunities to examine each of these possible outcomes in more detailed future experiments. The growth rates, morphologies and histologies of papillomas produced by each type of infectious particle were indistinguishable and comparable to what we have previously seen with papillomas caused by native CRPV virions (data not shown).

FIG. 3. Papilloma occurrence and growth at sites inoculated with papillomavirus particles. New Zealand White rabbits were vaccinated with CRPV (circles), HPV-16 (triangles), or HPV-11 (squares) L1 VLPs as described in the text. At 4 weeks after the third immunization, scarified sites on the dorsal skin of each rabbit were inoculated with 10 μl of a stock of homologous (CRPV capsid/CRPV genome [open symbols]) or chimeric (HPV-16 capsid/CRPV genome [solid symbols]) particles purified from DNase-treated 293TT cell lysates using an Optiprep density gradient (3, 3a). (a) The mean papilloma size (± the SD) is calculated by using the geometric mean diameters (GMD) for all sites receiving the inoculum. (b) The frequencies of papillomas induced at all sites inoculated with the homologous or chimeric infectious particles are shown separately for the three vaccine groups.

FIG. 4. Papillomas on selected rabbits 4 weeks after challenge with infectious particles. Rabbits vaccinated with CRPV (a), HPV-16 (b), or HPV-11 (c) VLPs were inoculated at five sites each with homologous (CRPV capsid/CRPV genome) particles on the left side (L) and with chimeric (HPV-16 capsids/CRPV genomes) particles on the right side (R).
Our experiments demonstrate that chimeric HPV particles can be used in an animal model to test the efficacy of HPV L1 VLP and HPV L2-based vaccines to prevent lesions induced by papillomavirus infections. This in vivo approach provides a rigorous test of immunity since vaccinated animals can be challenged with low- or high-titer infectious particles under controlled conditions and be evaluated for the appearance and growth rates of papillomavirus-induced lesions. This model also provides opportunities to test for protection against a number of different HPV particle types on the same animal to address questions regarding potential cross-protection of related and unrelated HPV types and to determine what contributory role a cell-mediated immune response to HPV capsid proteins may play in the protection generated by these capsid vaccines.

REFERENCES