JOURNAL OF VIROLOGY, May 1996, p. 3298–3301 Vol. 70, No. 5
0022-538X/96/$04.00+0

Assessment of the Serological Relatedness of Genital Human Papillomaviruses by Hemagglutination Inhibition

RICHARD B. S. RODEN,[1] NANCY L. HUBBERT,[1] REINHARD KIRNBAUER,[2] NEIL D. CHRISTENSEN,[3] DOUGLAS R. LOWY,[1] AND JOHN T. SCHILLER[1]*

Laboratory of Cellular Oncology, National Cancer Institute, Bethesda, Maryland 20892[1]; Division of Immunology, Allergy and Infectious Diseases, Department of Dermatology, University of Vienna Medical School, A-1090 Vienna, Austria[2]; and Department of Pathology, The Milton S. Hershey Center, Hershey, Pennsylvania 17033[3]

Received 21 December 1995/Accepted 9 February 1996

To assess the potential for cross-protection among genital human papillomavirus (HPV) types in virus-like particle (VLP)-based vaccinations, inhibition of HPV VLP-mediated hemagglutination by rabbit antisera raised against HPV type 6b (HPV-6b), HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, and HPV-45 was analyzed. Only highly homologous types (HPV-6b and HPV-11, and HPV-18 and HPV-45) exhibited detectable serological cross-reaction for the class of antibodies that inhibit virion-to-cell surface binding. However, analysis of neutralizing monoclonal antibodies to several animal and human papillomaviruses indicated that over half of these antibodies do not prevent cell surface binding, but these latter antibodies do not appear to be more cross-reactive in enzyme-linked immunosorbent assays than those that mediate inhibition of hemagglutination. The data strongly suggest that while there may be limited cross-protection between highly (>85% L1 amino acid identity) homologous types, protection by HPV VLP-based vaccines will be predominantly type specific.

Experimental and epidemiologic studies support the concept that human papillomaviruses (HPVs) are important etiologic agents in cervical carcinogenesis (19, 31). Certain high-risk genital HPV types such as HPV type 16 (HPV-16), HPV-18, HPV-31, HPV-33, and HPV-45, but not low-risk HPV-6 and HPV-11, are associated with cervical and other anogenital cancers (1).

When expressed from a strong heterologous promoter in eukaryotic cells, the major papillomavirus capsid protein, L1, self-assembles into virus-like particles (VLPs) (13, 16, 25, 26). Immunization with L1 VLPs, but not unassembled L1, stimulates production of high titers of neutralizing antibodies (5, 16). This property and the absence of viral genome suggest that VLPs could be used as a safe prophylactic vaccine to prevent papillomavirus infection (27). Studies of the efficacy of prophylactic VLP-based vaccines, which have been conducted with animal papillomaviruses, such as cottontail rabbit papillomavirus (CRPV), canine oral papillomavirus (COPV), and bovine papillomavirus type 4 (BPV-4), indicate that VLPs effectively prevent experimental papillomavirus infection (2, 17, 29). Protection can also be obtained by passive transfer of serum antibodies from vaccinated to naive animals, suggesting that the protection is mediated by neutralizing antibodies (2, 29).

Although in vivo and in vitro data suggest that the neutralizing antibody response to papillomaviruses (or VLPs) is type specific when distantly related papillomaviruses have been examined (7, 15), the serological relatedness of more closely related HPVs is less clear. In particular, the L1 sequences of certain common genital HPVs share strong homology (Table 1) (3), but the degree to which antivirion antibodies cross-neutralize is unknown. Such information is important for understanding the evolution of papillomaviruses and for determining how many HPV types might be needed in a VLP-based vaccine that could protect against most genital HPV infections.

In vitro assays of cross-neutralization by antisera to heterologous HPV types represent the most relevant assays for evaluating the potential for cross-protection (20). However, for high-risk genital HPVs, the requisite assays are not yet available because of the lack of a source of infectious virus and/or of an appropriate infectivity assay (12). Therefore, several attempts have been made to develop surrogate assays for neutralization to determine the serological relatedness of the HPVs. Binding by antisera raised against VLPs of one HPV type to VLPs derived from another type in an enzyme-linked immunosorbent assay (ELISA) can demonstrate the presence (or absence) of cross-reactive epitopes (24). However, it is unclear what proportion of such cross-reactive antibodies is neutralizing, especially because VLP preparations usually contain significant quantities of improperly folded L1 that elicits and binds to nonneutralizing cross-reactive antibodies. In addition, contaminants common to VLP preparations used for immunization and antigen on the ELISA plate may give false-positive results.

Alternatively, the ability of antibodies to inhibit virion interaction with cell surfaces can be measured by inhibition of VLP-mediated agglutination of mouse erythrocytes (21). An advantage of this assay is that antibodies must bind to correctly assembled capsids to prevent cell surface binding. Therefore, it does not measure antibodies to internal or denatured epitopes. The induction of sera with high hemagglutination inhibition (HAI) titers to the homologous papillomavirus has been found to correlate with both in vitro neutralization of BPV-1 virions and protection of rabbits from experimental infection with CRPV (21). However, a preliminary analysis of monoclonal antibodies raised against BPV-1 virions suggested that cell surface binding inhibition assays may be more stringent than neutralization, in that a subset of the monoclonal neutralizing antibodies did not inhibit viral binding to the cell surface (21, 23).

In this study, we have examined the ability of polyclonal rabbit sera to VLPs derived from a number of common high-

* Corresponding author. Mailing address: Laboratory of Cellular Oncology, Building 36, Room 1D-28, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892. Phone: (301) 496-6539. Fax: (301) 480-5322. Electronic mail address: schillej@dc37a.nci.nih.gov.

TABLE 1. L1 amino acid sequence identity comparison between common genital HPV types and a variant[a]

HPV type	L1 amino acid sequence identity (%) between HPV types							
	6b	11	16K	18	31	33	45	16Z
6b	100	92.4	69.6	64.3	69.8	69.6	65.5	69.4
11	92.4	100	69.1	62.7	70.3	68.8	64.0	68.9
16K	69.6	69.1	100	66.0	83.5	80.9	66.5	98.6
18	64.3	62.7	66.0	100	65.1	65.8	87.6	65.6
31	69.8	70.3	83.5	65.1	100	78.4	64.2	83.1
33	69.6	68.8	80.9	65.8	78.4	100	65.9	80.3
45	65.5	64.0	66.5	87.6	64.2	65.9	100	65.8
16Z	69.4	68.9	98.6	65.6	83.1	80.3	65.8	100

[a] Sequence comparison was performed by using the Genetics Computer Group (University of Wisconsin) computer program Bestfit with a gap creation penalty of 3.00 and a gap extension penalty of 0.10 and is presented as maximum percent sequence identity.

and low-risk genital HPV types to inhibit hemagglutination mediated by VLPs derived from each of these HPV types. The ability of a number of neutralizing monoclonal antibodies to inhibit VLP-mediated hemagglutination was also explored to assess what proportion of neutralizing antibodies is measured in the assay.

Cross-inhibition of HPV VLP-mediated hemagglutination by antisera to different HPV types. Recombinant baculovirus expressed VLPs derived from seven of the most common genital HPV types (HPV-6b, -11, -16, -18, -31, -33, and -45), as well as VLPs of the L1 from a variant of HPV-16 isolated in Zaire (4, 14) and the assembly-deficient L1 of the reference strain of HPV-16 L1 (18, 28), were purified by sucrose gradient and cesium chloride gradient centrifugation as previously described (21). Polyclonal antisera to purified VLPs were generated by three immunizations of New Zealand White rabbits with 300 μg of L1 protein, the first immunization with Freund's complete adjuvant and subsequent booster immunizations with Freund's incomplete adjuvant at 2-week intervals. Sera were precleared of nonspecific hemagglutinating activity and heated for 30 min at 56°C (11) and threefold serially diluted in phosphate-buffered saline (PBS) containing 1 mg of bovine serum albumin per ml (21). The minimum concentration of VLPs necessary for complete agglutination of 50 μl of a 1% suspension of mouse erythrocytes was determined empirically for use in these assays. Different amounts of L1 protein were used for different types, because the efficiency of hemagglutination varied among types and preparations, presumably a result of differences in the percentage of L1 that was properly assembled.

Each serum was tested against every HPV VLP type and against rhesus papillomavirus (RhPV) type 1 VLPs (which exhibits 77.4% amino acid identity with HPV-16 L1) in an HAI assay to assess serological relatedness (Table 2). Certain HPV types consistently generated higher HAI titers against their homologous VLPs than did other HPV types, i.e., HAI titers from HPV-16 and HPV-18 were greater than those generated by HPV-6b, -11, -31, -33, and -45. The differences appeared to correspond to the efficiency of VLP assembly, since HAI titer was inversely correlated with the quantity of L1 used in the assays.

None of the HPV types showed consistent and reciprocal cross-inhibition of hemagglutination, except for the two most closely related pairs: HPV-6b with HPV-11 and HPV-18 with HPV-45 (Table 2). Even with these pairs, whose L1 proteins show 92.4 and 87.6% sequence identity, respectively (Table 1), cross-inhibition was relatively weak. Antiserum to HPV-6b demonstrated 2- or 3-$\log_3$-unit-greater reactivity with HPV-6b VLPs than HPV-11 VLPs and vice versa. This cross-reaction correlates with the partial neutralization of HPV-11 infectivity in the xenograft model exhibited by polyclonal antisera to HPV-6 VLPs and the significant reactivity of HPV-6 VLP antiserum with HPV-11 virions in ELISAs (6). Reactions between HPV-18 and HPV-45 showed 3- or 4-$\log_3$-unit difference between the homologous and heterologous VLPs, consistent with their weaker sequence homology compared with HPV-6b and HPV-11. Weak cross-reactivity between HPV-16 and HPV-33 was observed in some assays but was not detected consistently, suggesting that any potential cross-reactivity is

TABLE 2. Type specificity of HAI

VLP	Titer of rabbit antiserum to VLP[a]										
	HPV-6b L1 + L2	HPV-11 L1 + L2	HPV-16K L1 + L2	HPV-18 L1 + L2	HPV-31 L1	HPV-33 L1 + L2	HPV-45 L1 + L2	HPV-16K L1	HPV-16Z L1	HPV-16P L1	HPV-6b, -11, -16, and -18
HPV-6b L1 + L2	24,300	900	*	*	*	*	*	*	*	*	8,100
HPV-11 L1 + L2	900	8,100	*	*	*	*	*	*	*	*	8,100
HPV-16K L1 + L2	*	*	24,300	*	*	300	*	24,300	8,100	*	8,100
HPV-18 L1 + L2	*	*	*	24,300	*	*	300	*	*	*	8,100
HPV-31 L1	*	*	*	*	2,700	*	*	*	*	*	*
HPV-33 L1 + L2	*	*	300	*	300	8,100	*	*	*	*	*
HPV-45 L1 + L2	*	*	*	300	*	*	8,100	*	*	*	300
HPV-16K L1	*	*	72,900	*	*	300	*	218,700	24,300	*	24,300
HPV-16Z L1	*	*	24,300	*	*	*	*	8,100	8,100	*	8,100
RhPV L1 + L2	*	*	*	*	*	*	*	*	*	*	*

[a] Titers are expressed as the median maximum antiserum dilution for HAI in three independent assays. Titers equivalent to those of the preimmune sera are indicated by asterisks. HPV-16K, HPV-16Z, and HPV-16P correspond to the German 114/K, Zairian Z-1194, and nonassembling reference Asp-202–to–His mutant HPV-16 L1 isolates, respectively. HPV-6b L1+L2, HPV-11 L1+L2, HPV-16K L1+L2, HPV-18 L1+L2, HPV-31 L1, HPV-33 L1+L2, HPV-45 L1+L2, HPV-16K L1, HPV-16Z L1, and RhPV L1+L2 VLP preparations were diluted 256, 512, 2,000, 1,000, 256, 512, 512, 4,000, 256, and 256 times, respectively.

TABLE 3. HAI by neutralizing monoclonal antibodies to papillomavirus capsids

Monoclonal antibody	Result[a] of HAI assay using:						In vitro neutralization[c]
	BPV virions	CRPV L1 + L2	HPV-6b L1 + L2	HPV-11 L1 + L2	HPV-16K L1	HPV-16K L1 + L2	
B1.A1	+	−	−	−	−	−	Yes
5B6	−	−	−	−	−	−	Yes
MAb 3	+	−	−	−	−	−	Yes
MAb 6	+	−	−	−	−	−	Yes
MAb 9	+	−	−	−	−	−	Yes
CRPV-1A	−	−	−	−	−	−	Yes
CRPV-2C	−	−	−	−	−	−	Yes
CRPV-4B	−	−	−	−	−	−	Yes
CRPV-5A	−	−	−	−	−	−	Yes
H11.B2	−	−	−	−	−	−	Yes
H11.H3	−	−	−	−	−	−	Yes
H11.G5	−	−	−	−	−	−	Yes
H11.F1	−	−	−	−	−	−	Yes
H16.E70	−	−	−	−	+	+	?
H16.S1[b]	−	−	−	−	−	−	?
H16.U4	−	−	−	−	−	−	?
H16.V5	−	−	−	−	+	+	?

[a] Symbols: +, inhibited hemagglutination; −, did not inhibit hemagglutination.
[b] Monoclonal antibody H16.S1 recognizes a linear surface epitope that is also present on HPV-18 and -31 (4a).
[c] Of virions of the type against which monoclonal antibodies were generated (6, 8, 9, 23).

below the sensitivity of the HAI assay. None of the sera inhibited hemagglutination by RhPV VLPs. The lack of cross-reactivity between most genital HPV types suggests that HPV genotypes whose L1 amino acid sequence has less than 85% identity with that of other types probably represent distinct serotypes. The absence of serological cross-reactivity presumably helps an infecting HPV to evade a prior host humoral immune response directed against other HPV types, a feature that may account in part for the multiplicity of HPV genotypes.

Superinfection with a new variant of HPV-16 is rare despite a probability in promiscuous individuals, suggesting that the immune response generated by infected individuals offers some degree of protection from variants (30). To investigate the possibility that this protection is mediated by neutralizing antibodies, antisera to two relatively divergent HPV-16 variants from Germany and Zaire (114/K and Z-1194, respectively), which differ in seven residues in L1, were tested by the HAI assay. Sera to these variants did not preferentially inhibit hemagglutination by the homologous variant over the heterologous variant (Table 2). These data are consistent with previous results using human sera in ELISAs (4).

Immunization with either L1 or L1+L2 HPV-16 VLPs induced similarly high HAI titers. This result is in agreement with the analysis of BPV neutralization in vitro using antisera to BPV VLPs containing L1 alone versus L1 and L2 (19). Antiserum to an assembly-deficient mutant of HPV-16 L1 encoded by the reference strain of HPV-16 did not generate significant HAI titers, as would be expected from the inability of this antiserum to inhibit the binding of radioiodinated HPV-16 VLPs to human foreskin keratinocytes (18, 22).

We also measured the HAI activity of serum obtained from a single rabbit that had been immunized simultaneously with VLP preparations derived from four different HPV types (HPV-6b, -11, -16, and -18). This rabbit developed HAI titers equivalent to, or a single $\log_3$ unit dilution less than, those seen in rabbits vaccinated with only one of the types. This result indicates that the humoral immune response to one type does not unduly influence the response to other types. If humans respond similarly, use of a polyvalent HPV VLP vaccine would therefore not be expected to diminish significantly the humoral immune response to individual types.

HAI by neutralizing monoclonal antibodies. As noted earlier, HAI is a stringent surrogate assay for neutralization because it measures only those antibodies that interfere with virion binding to cell surfaces. To gain some insight into the apparent frequency with which papillomaviruses induce antibodies that mediate HAI and those that do not, we examined the ability of a number of neutralizing monoclonal antibodies to inhibit hemagglutination.

Sixteen monoclonal antibodies generated against CRPV (8), BPV-1 (9, 23), or HPV-11 virions (10) or against HPV-16 L1 VLPs (4a) were determined to be directed against conformation-dependent and type-specific epitopes by ELISAs. With the exception of the four HPV-16 antibodies, all had previously been shown to neutralize the corresponding virus type (8–10, 23). These antibodies were tested for their ability to inhibit hemagglutination by homologous and heterologous particles. The presence and specific activity of each monoclonal antibody in the tissue culture supernatants was confirmed by VLP ELISAs (data not shown). The antibody concentrations in the culture supernatants were determined to be greater than 10 μg/ml (which is sufficient for HAI) using anti-mouse immunoglobulin G ELISAs and purified mouse immunoglobulin G standards (data not shown).

Only 6 of the 17 monoclonal antibodies tested inhibited hemagglutination by the homologous VLPs (Table 3). None inhibited heterologous VLP types. This finding implies that the nine neutralizing monoclonal antibodies to BPV-1, CRPV, or HPV-11 that do not inhibit hemagglutination inhibit infection at a step after virion binding to the cell surface. In addition, two of the four monoclonal antibodies to HPV-16 did not block hemagglutination. Unless there is some selection bias when generating monoclonal antibodies, these results imply that a significant fraction of virion antibodies in sera neutralize post-cell surface binding and will not be recognized in HAI assays.

The inability of the majority of neutralizing monoclonal antibodies tested to inhibit hemagglutination leaves open the

possibility that significant numbers of cross-neutralizing antibodies of this class exist for the different HPV types that were not detected by the HAI assay. However, all of the neutralizing monoclonal antibodies in this class that have been tested to date are type specific in ELISAs for even closely related types: three monoclonal antibodies to HPV-11 (H11.H3, H11.G5, and H11.F1) do not recognize HPV-6b VLPs (6), monoclonal antibody 5B6 binds BPV-1 but not BPV-4 (23), and the monoclonal antibodies to CRPV (CRPV-1A, -2C, -4B, and -5A) do not react with either BPV-1 or HPV-11 (8).

Taken together, the data suggest that the neutralizing antibodies that do not prevent virion binding to cells exhibit a type specificity that is similar to those antibodies that do inhibit hemagglutination. It therefore seems likely that most HPV genotypes represent separate serotypes. Definitive classification of serotypes and quantitation of cross-neutralization will require the ability to generate infectious virus for HPVs of any type and to develop quantitative infectivity assays for them. However, HAI is a simple assay that can monitor conformation-dependent and type-specific reactivity in individuals immunized with multiple HPV types.

We thank Carl Olson (Madison, Wis.) for the generous gift of BPV-1 papillomaviruses, Martin Sapp (Johannes Gutenberg-Universität Mainz, Mainz, Germany) for the HPV-33 L1 and L2 recombinant baculoviruses, and Brett Chaney for the multitype experiment.

R.K. was supported by the Austrian Science Foundation (FWF P-10193-MED).

REFERENCES

1. **Bosch, F. X., M. M. Manos, N. Munoz, M. Sherman, A. M. Jansen, J. Peto, M. H. Schiffman, V. Moreno, R. Kurman, K. V. Shah, and I. B. S. C. C. Study Group.** 1995. Prevalence of human papillomavirus in cervical cancer: a worldwide prospective. J. Natl. Cancer Inst. **87:**796–802.
2. **Breitburd, F., R. Kirnbauer, N. L. Hubbert, B. Nonnenmacher, C. Trin-Dinh-Desmarquet, G. Orth, J. T. Schiller, and D. R. Lowy.** 1995. Immunization with viruslike particles from cottontail rabbit papillomavirus (CRPV) can protect against experimental CRPV infection. J. Virol. **69:**3959–3963.
3. **Chan, S. Y., H. Delius, A. L. Halpern, and H. U. Bernard.** 1995. Analysis of genomic sequences of 95 papillomavirus types: uniting typing, phylogeny, and taxonomy. J. Virol. **69:**3074–3083.
4. **Cheng, G., J. P. Icenogle, R. Kirnbauer, N. L. Hubbert, M. E. St. Louis, C. Han, E. I. Svare, S. Kruger Kjaer, D. R. Lowy, and J. T. Schiller.** 1995. Divergent human papillomavirus type 16 variants are serologically cross-reactive. J. Infect. Dis. **172:**1584–1587.

4a. **Christensen, N. D.** Unpublished data.

5. **Christensen, N. D., R. Hopfl, S. L. DiAngelo, N. M. Cladel, S. D. Patrick, P. A. Welsh, L. R. Budgeon, C. A. Reed, and J. W. Kreider.** 1994. Assembled baculovirus-expressed human papillomavirus type 11 L1 capsid protein virus-like particles are recognized by neutralizing monoclonal antibodies and induce high titres of neutralizing antibodies. J. Gen. Virol. **75:**2271–2276.
6. **Christensen, N. D., R. Kirnbauer, J. T. Schiller, S. J. Ghim, R. Schlegel, A. B. Jenson, and J. W. Kreider.** 1994. Human papillomavirus types 6 and 11 have antigenically distinct strongly immunogenic conformationally dependent neutralizing epitopes. Virology **205:**329–335.
7. **Christensen, N. D., and J. W. Kreider.** 1990. Antibody-mediated neutralization in vivo of infectious papillomaviruses. J. Virol. **64:**3151–3156.
8. **Christensen, N. D., and J. W. Kreider.** 1991. Neutralization of CRPV infectivity by monoclonal antibodies that identify conformational epitopes on intact virions. Virus Res. **21:**169–179.
9. **Christensen, N. D., and J. W. Kreider.** 1993. Monoclonal antibody neutralization of BPV-1. Virus Res. **28:**195–202.
10. **Christensen, N. D., J. W. Kreider, N. M. Cladel, S. D. Patrick, and P. A. Welsh.** 1990. Monoclonal antibody-mediated neutralization of infectious human papillomavirus type 11. J. Virol. **64:**5678–5681.
11. **Favre, M., F. Breitburd, O. Croissant, and G. Orth.** 1974. Hemagglutinating activity of bovine papilloma virus. Virology **60:**572–578.
12. **Hagensee, M., and D. Galloway.** 1993. Growing human papillomaviruses and virus-like particles in the laboratory. Papillomavirus Rep. **4:**121–124.
13. **Hagensee, M. E., N. Yaegashi, and D. A. Galloway.** 1993. Self-assembly of human papillomavirus type 1 capsids by expression of the L1 protein alone or by coexpression of the L1 and L2 capsid proteins. J. Virol. **67:**315–322.
14. **Icenogle, J. P., M. Laga, D. Miller, A. T. Manoka, R. A. Tucker, and W. C. Reeves.** 1992. Genotypes and sequence variants of human papillomavirus DNAs from human immunodeficiency virus type 1-infected women with cervical intraepithelial neoplasia. J. Infect. Dis. **166:**1210–1216.
15. **Jarrett, W. F. H., B. W. O'Neill, J. M. Gaukroger, K. T. Smith, H. M. Laird, and M. S. Campo.** 1990. Studies on vaccination against papillomaviruses: the immunity after infection and vaccination with bovine papillomaviruses of different types. Vet. Rec. **126:**473–475.
16. **Kirnbauer, R., F. Booy, N. Cheng, D. R. Lowy, and J. T. Schiller.** 1992. Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. Proc. Natl. Acad. Sci. USA **89:**12180–12184.
17. **Kirnbauer, R., L. M. Chandrachud, B. W. O'Neil, E. R. Wagner, G. J. Grindlay, A. Armstrong, G. M. McGarvie, J. T. Schiller, D. R. Lowy, and M. S. Campo.** Virus-like particles of bovine papillomavirus type 4 in prophylactic and therapeutic immunization. Submitted for publication.
18. **Kirnbauer, R., J. Taub, H. Greenstone, R. Roden, M. Dürst, L. Gissmann, D. R. Lowy, and J. T. Schiller.** 1993. Efficient self-assembly of human papillomavirus type 16 L1 and L1-L2 into virus-like particles. J. Virol. **67:**6929–6936.
19. **Lowy, D. R., R. Kirnbauer, and J. T. Schiller.** 1994. Genital human papillomavirus infection. Proc. Natl. Acad. Sci. USA **91:**2436–2440.
20. **Robbins, J. B., R. Schneerson, and S. C. Szu.** 1995. Hypothesis: serum IgG antibody is sufficient to confer protection against infectious diseases by inactivating the inoculum. J. Infect. Dis. **171:**1387–1398.
21. **Roden, R. B. S., N. L. Hubbert, R. Kirnbauer, F. Breitburd, D. R. Lowy, and J. T. Schiller.** 1995. Papillomavirus L1 capsids agglutinate mouse erythrocytes through a proteinaceous receptor. J. Virol. **69:**5147–5151.
22. **Roden, R. B. S., R. Kirnbauer, A. B. Jenson, D. R. Lowy, and J. T. Schiller.** 1994. Interaction of papillomaviruses with the cell surface. J. Virol. **68:**7260–7266.
23. **Roden, R. B. S., E. M. Weissinger, D. W. Henderson, F. Booy, R. Kirnbauer, J. F. Mushinski, D. R. Lowy, and J. T. Schiller.** 1994. Neutralization of bovine papillomavirus by antibodies to L1 and L2 capsid proteins. J. Virol. **68:**7570–7574.
24. **Rose, R. C., W. Bonnez, C. Da Rin, D. J. McCance, and R. C. Reichman.** 1994. Serological differentiation of human papillomavirus types 11, 16 and 18 using recombinant virus-like particles. J. Gen. Virol. **75:**2445–2449.
25. **Rose, R. C., W. Bonnez, R. C. Reichman, and R. L. Garcea.** 1993. Expression of human papillomavirus type 11 L1 protein in insect cells: in vivo and in vitro assembly of viruslike particles. J. Virol. **67:**1936–1944.
26. **Sasagawa, T., P. Pushko, G. Steers, S. E. Gschmeissner, M. A. Hajibagheri, J. Finch, L. Crawford, and M. Tommasino.** 1995. Synthesis and assembly of virus-like particles of human papillomaviruses type 6 and type 16 in fission yeast Schizosaccharomyces pombe. Virology **206:**126–135.
27. **Schiller, J. T., and M. Okun.** 1996. Papillomavirus vaccines: current status and future prospects. Adv. Dermatol. **11:**355–380.
28. **Seedorf, K., G. Krammer, M. Durst, S. Suhai, and W. G. Rowekamp.** 1985. Human papillomavirus type 16 DNA sequence. Virology **145:**181–185.
29. **Suzich, J. A., S. Ghim, F. J. Palmer-Hill, W. I. White, J. K. Tamura, J. Bell, J. A. Newsome, A. B. Jenson, and R. Schlegel.** 1995. Systemic immunization with papillomavirus L1 protein completely prevents the development of viral mucosal papillomas. Proc. Natl. Acad. Sci. USA **92:**11553–11557.
30. **van Belkum, A., L. Juffermans, L. Schrauwen, G. van Doornum, M. Burger, and W. Quint.** 1995. Genotyping human papillomavirus type 16 isolates from persistently infected promiscuous individuals and cervical cancer patients. J. Clin. Microbiol. **33:**2957–2962.
31. **zur Hausen, H.** 1994. Molecular pathogenesis of cancer of the cervix and its causation by specific human papillomavirus types. Curr. Top. Microbiol. Immunol. **186:**131–156.