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Antibody-Mediated Neutralization In Vivo of Infectious Papillomaviruses

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Specific antibody-mediated neutralization of infectious human papillomavirus type 11 (HPV-11) was achieved in the athymic mouse xenograft system, in which HPV-11 induced morphological transformation of human foreskin. Virus-specific neutralization was demonstrated by the ability of an HPV-11-specific polyclonal antiserum to neutralize HPV-11 infectivity and not bovine papillomavirus type 1 (BPV-1) or cottontail rabbit papillomavirus (CRPV) infectivity. In all three virus infectivity systems, neutralization was detected by the failure of the virus suspension to induce morphological transformation of the appropriate skin xenografts placed under the renal capsule of athymic mice. Rabbit polyclonal antisera were also generated against intact virions of both BPV-1 and CRPV, and neutralizing activity was tested in the xenograft system with BPV-1 and fetal bovine skin and with CRPV and rabbit ear skin. The three polyclonal antisera contained virus-specific neutralizing antibodies, demonstrating that neutralizing epitopes existed on all three papillomaviruses and that these epitopes were antigenically non-cross-reactive. The athymic mouse xenograft system was a useful model for detecting papillomavirus-specific neutralizing antibodies and offers the only opportunity for the analysis of neutralizing antibodies to human papillomaviruses.

Human papillomaviruses (HPVs) are double-stranded DNA viruses which induce hyperproliferative lesions in epithelial tissues. Over 50 different HPV types have been identified by liquid hybridization assays in which less than 50% DNA homology is used as a criterion for defining different types (3). HPV types associated with laryngeal and anogenital lesions have been implicated as causative agents in the carcinomatous transformation of the larynx and cervix (5, 9). Low viral particle yields from natural lesions (1) and the lack of suitable animal and tissue culture models for assaying residual viral infectivity have hampered the study of the immunological responses to these HPV types. We recently developed an animal model system which allows HPV type 11 (HPV-11)-induced transformation of human tissue as well as replication of infectious virions (12, 13). This animal model system, which uses virus-infected xenografts transplanted beneath the renal capsule of athymic mice, provides, for the first time, an animal model for the assay of antibody-mediated neutralization of an infectious HPV type.

The only models that are currently available for testing the neutralization of papillomaviruses (PVs) are for two non-HPVs, bovine PV and cottontail rabbit PV (CRPV). The models include neutralization of bovine PV type 1 (BPV-1)-induced transformation of mouse C127 cells in tissue culture (6, 16), protection in cattle following BPV-1 challenge (15), and neutralization of CRPV-induced papillomatous transformation of domestic rabbit skin (10).

The purpose of the current study was to describe an animal model for antibody neutralization of infectious PVs. Rabbit polyclonal antisera generated against intact virions of HPV-11, BPV-1, and CRPV were used as a source of neutralizing antibodies. These three polyclonal antisera specifically neutralized PV infections by preventing morphological transformation of the appropriate PV-infected skin when transplanted beneath the renal capsule of athymic mice. The results demonstrated that the neutralizing epitopes located on the surfaces of these three PVs were antigenically non-cross-reactive.

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MATERIALS AND METHODS

Animals. Female athymic *nu/nu* mice were purchased from Harlan Sprague Dawley, Inc., Madison, Wis., and used when 6 to 8 weeks old. Male BALB/c mice were purchased from the National Cancer Institute, Bethesda, Md., and used when 12 weeks old. New Zealand White (NZW) rabbits were purchased from Hazelton Research Products, Inc., Denver, Pa., and used when 3 months old.

Preparation and purification of virus stocks. HPV-11 and CRPV were prepared from cysts developed in the athymic *nu/nu* mouse xenograft system as previously described (2, 12, 13). CRPV was prepared from two separate sources (2). CRPV-KA was obtained from naturally occurring papillomas of Kansas wild cottontail rabbits (11), and CRPV-PA was prepared from cysts grown in athymic mice by incubating Pennsylvania wild cottontail rabbit skin chips with CRPV-KA suspensions and grafting the chips under the renal capsule. BPV-1 was prepared from bovine warts kindly provided by Carl Olson (University of Wisconsin, Madison). PV particles were purified on CsCl gradients and stored in 10 mM Tris–1 mM EDTA buffer (pH 7.5) at −70°C (2).

Preparation of polyclonal antisera. Polyclonal antisera reactive to whole PV virions were prepared in NZW rabbits by monthly intramuscular injections of CsCl-banded HPV-11, CRPV-PA, and BPV-1 at 20 μg per injection in phosphate-buffered saline (PBS) (2). Three days after the third or fourth (HPV-11) injection, blood was removed from the ear artery, and heat-inactivated (56°C for 30 min) sera were prepared and stored at −70°C. Absorptions of the polyclonal antisera against human foreskin tissue and BALB/c mouse kidney tissue were carried out by incubation of the polyclonal antisera for 30 min on ice with minced fragments of the respective tissue, followed by centrifugation and collection of the sera as supernatants.

ELISA. Polyclonal antisera were tested for reactivity to both intact and disrupted HPV-11, BPV-1, and CRPV-PA by an enzyme-linked immunosorbent assay (ELISA) as previously described (2). In brief, intact virions at 300 ng of protein per well in PBS (pH 7.0) were attached to ELISA plate wells by incubation for 1 h at 37°C (4). All subsequent incubations were done at 22°C. The plates were washed three times with PBS, unattached sites were blocked by incubation for 1 h in blocking buffer (5% milk protein powder in PBS [pH 7.0]), and polyclonal antisera at a 1:200 dilution in blocking buffer were added and incubated for 1 h. The plates were washed with PBS, and alkaline phosphatase-conjugated swine anti-rabbit antisera (DAKO Corp., Via Real, Calif.) at a 1:1,000 dilution in blocking buffer were added and incubated for 1 h, followed by four washes with PBS and the addition of substrate. Disrupted virus was prepared by incubation of intact virions in carbonate buffer (0.2 mM Na_2CO_3, 0.01 mM dithiothreitol [pH 10.6]) on ice for 2 h (7), dried onto ELISA plate wells by overnight incubation at 37°C, and processed as described above for intact virions.

Neutralization assays. Two methods for detecting antibody-mediated neutralization of infectious PV virions were examined. The xenograft system (12, 13) was used to assay the neutralization of HPV-11-induced condylomatous transformation of human foreskin, BPV-1-induced morphological transformation of fetal bovine skin, and CRPV-induced (CRPV-KA) morphological transformation of NZW domestic rabbit ear skin, all transplanted beneath the renal capsule of athymic mice. The second method utilized NZW domestic rabbits only for the assay of antibody-mediated neutralization of infectious CRPV (CRPV-KA) (10).

For the neutralization of HPV-11 infectivity, serial fourfold dilutions of polyclonal antisera (nonabsorbed) in PBS (100 μl) were added to aliquots of infectious HPV-11 in PBS (100 μl) and incubated for 1 h at 37°C. HPV-11 in PBS alone was included as a positive control for infectivity. Human foreskin chips (5 to 10 2- by 2-mm pieces) were added to each dilution series and incubated for 1 h at 37°C. The chips were transplanted under the renal capsule of athymic mice, and condylomatous transformation and cyst size (in millimeters) were determined after 100 days (13).

Neutralization of BPV-1 infectivity was assayed similarly to that of HPV-11 infectivity but with BPV-1 and fetal bovine skin chips instead of HPV-11 and human foreskin chips. Cyst size (in millimeters) was determined after 60 days.

Both the xenograft system and CRPV-induced papillomatous transformation of epithelial tissue of NZW rabbits were used to assay for the neutralization of CRPV infectivity. Identical dilutions of CRPV and polyclonal antisera were used in both animal models to compare the relative sensitivity of each system for CRPV-neutralizing antibodies. The xenograft system for assaying CRPV neutralization was similar to the HPV-11 and BPV-1 assays but utilized CRPV and NZW rabbit ear skin chips. Nonimmune heat-inactivated rabbit serum (50%) was added to all dilutions of test neutralizing polyclonal antisera containing CRPV and NZW rabbit ear skin chips for both of the 37°C, 1-h incubation steps. Cyst size (in millimeters) was determined after 60 days.

Neutralization of CRPV-induced papilloma formation on adult NZW rabbits was assayed as previously described (10). Serial fourfold dilutions of test nonabsorbed polyclonal antisera in PBS were incubated with aliquots of infectious CRPV for 1 h at 37°C. The mixtures (50 μl) were spread onto separate 1- by 1-cm scarified sites on the shaved backs of NZW rabbits, and papilloma size was monitored weekly starting at day 21 after infection. Papillomas were measured in three dimensions (length, width, and height in millimeters), and geometric mean diameters were calculated for each site. The means ± standard errors of the mean of geometric mean diameters of papillomas for each antiserum dilution point were calculated.

Statistical analyses. Geometric mean diameters of cysts and papillomas for each antiserum dilution point were compared with those for untreated controls by using Student's *t* test.

RESULTS

Polyclonal antisera used in neutralization assays were generated against CsCl-banded intact HPV-11, CRPV (CRPV-PA), and BPV-1 particles (polyclonal antisera 639, 7380, and 3866, respectively). These sera were examined initially by an ELISA for reactivity to intact and disrupted virions in two separate experiments (Table 1). Preliminary experiments (2) demonstrated that polyclonal antisera generated against HPV-11 (polyclonal antiserum 639) and CRPV (polyclonal antiserum 7380) obtained from cysts generated in athymic mice were cross-reactive to both intact HPV-11 and intact CRPV but not to intact BPV-1. This cross-reactivity may have been due to antibodies generated against a common nonviral tissue contaminant present in the viral preparations; therefore, these sera were preabsorbed against BALB/c mouse kidney tissue and human foreskin tissue and reexamined by the ELISA (Table 1). Absorption of these sera against mouse kidney tissue fragments removed most of the cross-reactivity, and the absorbed sera thus reacted specifically to the appropriate intact virus with minimal cross-reactivity. Polyclonal antiserum 3866 raised to BPV-1 particles purified from bovine warts reacted strongly to intact BPV-1 but not to intact HPV-11 or CRPV. When the polyclonal antisera were examined for reactivity to disrupted virus, only serum 3866 (anti-BPV-1) showed strong reactivity to disrupted BPV-1 antigen.

Titration of polyclonal antisera 639, 3866, and 7380 against intact HPV-11, BPV-1, and CRPV, respectively, demonstrated that the titers of virus-specific antibodies present in each polyclonal antiserum were similar (data not shown).

Neutralization assay. (i) Xenograft system. The three polyclonal antisera (nonabsorbed) as well as normal rabbit serum were tested for the neutralization of PV infectivity with the appropriate combinations of PV and host tissue transplanted beneath the renal capsule of athymic mice. Effective neutralization was determined by comparing cyst size and microscopic morphology in hematoxylin-eosin-stained sections.

(a) HPV-11. Human foreskin chips were incubated with HPV-11 which had been preincubated for 1 h with dilutions of polyclonal antisera 639 (anti-HPV-11), 3866 (anti-BPV-1), 7380 (anti-CRPV), and 7341 (normal rabbit serum). The chips were grafted subrenally in athymic mice, and average geometric mean diameters of cyst sizes were determined 100 days later (Fig. 1A). A large and significant reduction in cyst size was obtained for all dilutions (up to 1:128) of polyclonal antiserum 639 (anti-HPV-11). Neither polyclonal antiserum 3866 (anti-BPV-1) nor polyclonal antiserum 7341 (normal rabbit serum) had an effect on cyst size at all dilutions tested, whereas polyclonal antiserum 7380 (anti-CRPV) at the lowest dilution (1:2) resulted in a large (but not significant) cyst size reduction.

Tissue sections were prepared from all grafts and exam-

TABLE 1. ELISA of polyclonal antiserum reactivity to HPV-11, CRPV, and BPV-1

Expt and viral antigen	Mean ELISA optical density readings for the following polyserum:									
	Normal rabbit serum (7341)	Anti-HPV-11				Anti-CRPV			Anti-BPV-1 (3866)	Group-specific antigen (DAKO)
		639	639-M1[a]	639-M4[b]	639-H1[c]	7380	7380-M2[d]	7380-H1[c]		
1										
HPV-11										
Intact	0.001	0.568	0.492	0.353	0.407	0.095	0.011	0.065	0.007	0.066
Disrupted	0.010	0.136	0.090	0.056	0.040	0.098	0.012	0.075	0.044	0.577
CRPV										
Intact	0.008	0.643	0.028	0.021	0.529	1.167	0.269	1.085	0.009	0.007
Disrupted	0.009	0.565	0.040	0.008	0.428	0.731	0.068	0.830	0.036	0.096
BPV-1										
Intact	0.003	0.005	0.004	0.004	0.000	0.006	0.004	0.008	0.815	0.295
Disrupted	0.047	0.010	0.009	0.006	0.007	0.016	0.011	0.018	0.552	2.000
2										
HPV-11 (intact)	0.025	1.327	0.891	0.543	0.972	0.708	0.045	0.643	0.143	NT[e]
CRPV (intact)	0.002	1.237	0.139	0.014	1.057	1.514	0.570	1.341	0.016	NT
BPV-1 (intact)	0.003	0.026	0.020	0.008	0.001	0.010	0.006	0.004	0.612	NT

[a] Absorbed against BALB/c mouse kidney tissue (one time).
[b] Absorbed against BALB/c mouse kidney tissue (four times).
[c] Absorbed against human foreskin tissue (one time).
[d] Absorbed against BALB/c mouse kidney tissue (two times).
[e] NT, Not tested.

ined microscopically for koilocytosis as an indicator of HPV-11-induced morphological transformation. The numbers of grafts transformed, grafts surviving, and grafts attempted for each dilution point are shown in Table 2. For all dilutions of polyclonal antiserum 639 (anti-HPV-11), the surviving grafts were small normal epidermal cysts without foci of koilocytosis. For the lowest dilution (1:2) of polyclonal antiserum 7380 (anti-CRPV), two of four grafts were dead, and of the two surviving grafts, one contained koilocytotic foci, indicating morphological transformation. Most of the cysts that developed for all dilutions of polyclonal antisera 3866 (anti-BPV-1) and 7341 (normal rabbit serum) and PBS alone showed a transformed phenotype (29 of 33, or 88%; Table 2).

(b) BPV-1. BPV-1-induced morphological transformation of fetal calf skin transplanted beneath the renal capsule of athymic mice was used to test antibody-mediated neutralization of BPV-1 infectivity. Cyst size was determined after 60 days, and sections of the grafts were examined microscopically (Fig. 1B and Table 2). Polyclonal antiserum 3866 (anti-BPV-1) strongly reduced cyst size up to a dilution of 1:32. Polyclonal antisera 639 (anti-HPV-11) and 7380 (anti-CRPV) had no effect on cyst size over all the dilutions tested, but polyclonal antiserum 7341 (normal rabbit serum) at the lowest dilution (1:2) resulted in a significant reduction in cyst size. Microscopic examination of the cysts showed that for serum 3866 at the lowest dilution (1:2), the grafts were all untransformed, and at a dilution of 1:8, two of four grafts were untransformed. For all other dilutions points, the cysts were morphologically transformed.

(c) CRPV. Complete neutralization of CRPV-induced morphological transformation of NZW domestic rabbit ear skin was obtained at dilutions of 1:2 and 1:8 for polyclonal antiserum 7380 (anti-CRPV) only. Partial neutralization (one of three grafts untransformed) was observed for this serum at a higher dilution (1:32) (Fig. 1C and Table 2). The remaining polyclonal antisera generated against HPV-11 (639) and BPV-1 (3866) as well as normal rabbit serum (7341) had no effect on cyst size, and all grafts were morphologically transformed.

(ii) Domestic rabbits. CRPV induction of papillomas at scarified sites on the backs of NZW rabbits was used also to test for the neutralization of CRPV infectivity. Dilutions of polyclonal antisera were preincubated for 1 h at 37°C with aliquots of a suspension of unpurified CRPV prepared from Kansas wild cottontail rabbit papillomas (11) and then placed onto scarified sites as described in Materials and Methods. Growth of papillomas over time was measured to determine whether effective neutralization occurred (Fig. 2). Only polyclonal antiserum 7380 (anti-CRPV) showed neutralization of CRPV-induced papillomas, with a significant reduction in the growth of papillomas at dilutions up to 1:32. Complete protection at 1:2 and 1:8 dilutions of serum 7380 (anti-CRPV) was achieved in some rabbits, whereas in others, only a partial reduction in papilloma size was observed (Table 3 and Fig. 2). No effect on papilloma growth rates was observed for polyclonal antisera 3866 (anti-BPV-1) (Fig. 2), 639 (anti-HPV-11), and 7341 (normal rabbit serum) (Table 3), with the exception of a delay in growth at one of four challenged sites containing the lowest dilution (1:2) of serum 3866 (Fig. 2).

DISCUSSION

In this study, we present data demonstrating specific antibody-mediated neutralization of PV infectivity in the athymic mouse xenograft system. Although antibody neutralization of CRPV (10) and BPV-1 (6, 16) has been demonstrated, this is the first report of antibody neutralization of HPV infectivity. The method described here should be immediately applicable to other HPV types as infectious stocks become available. We have used this system to analyze antibody-mediated neutralization of HPV-11, BPV-1, and CRPV.

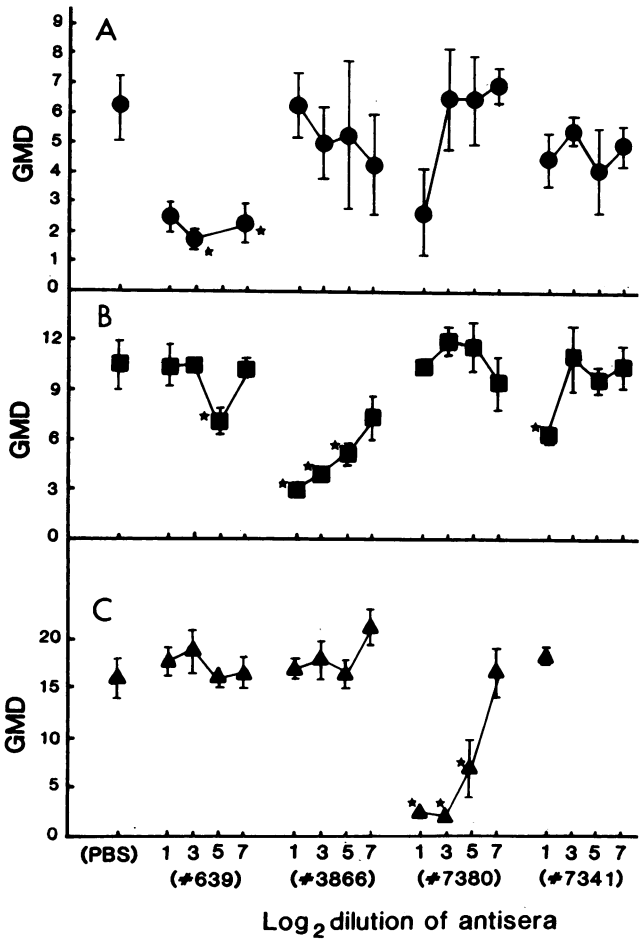

FIG. 1. Effect of titrations of polyclonal antisera 639 (anti-HPV-11), 3866 (anti-BPV-1), 7380 (anti-CRPV), and 7341 (normal rabbit serum) on the development of PV-induced morphological transformation and growth of skin chips in the xenograft system. (A) HPV-11 and human foreskin chips. (B) BPV-1 and fetal calf skin chips. (C) CRPV and NZW rabbit ear skin chips. Means ± standard errors of the means of cyst size (in millimeters) were plotted against each polyclonal antiserum dilution point for all four polyclonal antisera. (An abbreviated duplicate experiment yielded similar results). *, $P < 0.05$. GMD, Geometric mean diameter.

Polyclonal antisera generated against CsCl-banded intact PV particles were screened initially by an ELISA to confirm antibody reactivity to intact virions and to determine the specificity of each polyclonal antiserum for the three different PVs. The polyclonal antisera raised to intact virions extracted from cysts grown subrenally in athymic mice (HPV-11 and CRPV-PA) initially contained cross-reactive antibodies to both of these viruses (both intact and disrupted virion antigens) but not to BPV-1 (2; Table 1). The apparent cross-reactivity was eliminated by preabsorption of the polyclonal antisera against fresh, minced fragments of BALB/c mouse kidney but not human tissue, thereby demonstrating that the CsCl-banded PV preparations from athymic mouse cyst material probably contained mouse tissue contaminants. The level of contamination observed in the second preparation of HPV-11 (Table 1, experiment 2) was considerably higher than that present in the first experiment, as judged by the response of the nonabsorbed anti-CRPV polyclonal antiserum 7380. These contaminants have not been characterized, and it is not known whether they copurify with PV particles on CsCl density gradients or whether they are associated with the particles themselves. Polyclonal antisera preabsorbed with human foreskin tissue produced a much smaller reduction in reactivity to virion antigens than did nonabsorbed polyclonal antisera. This reactivity may represent a response to contaminating keratins because PV preparations are known also to be contaminated with these molecules (8, 14).

The reactivities of polyclonal antisera 639 (anti-HPV-11) and 7380 (anti-CRPV) were directed predominantly to external epitopes (intact virions) of the appropriate PV particles, as assessed by the ELISA following the removal of contaminating antibodies by preabsorption. In contrast, polyclonal antiserum 3866, generated against intact BPV-1, showed equally strong reactivity in the ELISA to both intact and disrupted BPV-1 virions. These results may indicate that the xenograft-derived cysts, in contrast to bovine papillomas, which were obtained from bovine lesions, contained a higher proportion of intact PV particles, possibly because the xenograft-derived cysts were internal, protected from the external environment, and not greater than 120 days old. Immunization with PV suspensions containing damaged and/or partially formed particles may generate higher levels of antibody directed to internal viral antigenic epitopes. Alternatively, BPV-1 particles may contain a higher proportion of external linear antigenic epitopes (4) (which would generate antibodies reactive to both intact and disrupted virion antigens) than do both HPV-11 and CRPV.

Data presented for antibody-induced neutralization of the infectivity of each of the three PVs in the xenograft system demonstrated effective antigen-specific neutralization. Polyclonal antiserum 639 strongly and specifically neutralized HPV-11 infectivity, with complete protection at dilutions greater than 1:128. Polyclonal antiserum 7380 specifically neutralized CRPV infectivity completely at dilutions greater than 1:8, with partial protection at a dilution of 1:32. Neutralization of BPV-1 infectivity by polyclonal antiserum 3866 was complete only at a low dilution (1:2). Partial protection at a dilution of 1:8 was observed, but complete protection at higher dilutions was not obtained despite a significant decrease in cyst size. The three polyclonal antisera, however, did not differ in antibody titer to intact virion antigens, as measured by the ELISA. Differences in effective neutralization of BPV-1 as compared with HPV-11 and CRPV may be a consequence of the increased number of potential "target cells" in the fetal calf skin chip (both epithelial and fibroblastic) for the fibropapillomavirus BPV-1. Only epithelial cells are target cells for infection by the epitheliotrophic PVs HPV-11 and CRPV. An alternative explanation is that the fibropapillomavirus BPV-1 contains more (or more antigenic) nonneutralizing external epitopes on the intact virus than do HPV-11 and CRPV. A reduction in the quantity or antigenicity of potential neutralizing epitopes would provide a mechanism for effective escape from host immune responses.

Some non-antigen-specific, polyclonal antiserum-induced reductions in cyst size were observed for some of the sera tested at the lowest dilution, 1:2. For example, serum 7341 (normal rabbit serum) produced a significant reduction in the cyst size of BPV-1-infected fetal calf skin, and serum 7380 (anti-CRPV) produced a large, but not significant, reduction in the cyst size of HPV-11-infected human foreskin. These reductions in cyst size did not represent complete neutralization because surviving grafts, although smaller, contained foci of morphological transformation. The cyst size reduc-

TABLE 2. Morphological analysis of grafts following pretreatment of virus with various polyclonal antisera

Virus	Host tissue	Polyserum	No. of grafts transformed/no. of grafts surviving/no. of grafts attempted at the following antiserum dilution:				
			1:2	1:8	1:32	1:128	None (no antiserum)
HPV-11	Human foreskin	7341 (Normal rabbit serum)	3/4/4	2/2/2	3/4/4	3/4/4	
		7380 (Anti-CRPV)	1/2/4	4/4/4	2/2/2	3/3/4	
		3866 (Anti-BPV-1)	4/4/4	2/2/4	3/3/4	3/4/4	
		639 (Anti-HPV-11)	0/2/2	0/4/4	NS[a]	0/3/4	
		None (PBS)					6/6/6
BPV-1	Fetal calf skin	7341 (Normal rabbit serum)	4/4/4	3/3/3	4/4/4	4/4/4	
		7380 (Anti-CRPV)	4/4/4	4/4/4	3/3/4	4/4/4	
		3866 (Anti-BPV-1)	0/4/4	2/4/4	4/4/4	4/4/4	
		639 (Anti-HPV-11)	3/3/4	4/4/4	4/4/4	4/4/4	
		None (PBS)					6/6/6
CRPV	NZW rabbit ear skin	7341 (Normal rabbit serum)	4/4/4	NT[b]	NT	NT	
		7380 (Anti-CRPV)	0/4/4	0/4/4	2/3/4	2/2/2	
		3866 (Anti-BPV-1)	6/6/6	4/4/4	4/4/4	3/3/4	
		639 (Anti-HPV-11)	4/4/4	4/4/4	4/4/4	4/4/4	
		None (PBS)					4/4/4

[a] NS, No survivors.
[b] NT, Not tested.

tions at these low dilutions may have been due to nonspecific toxic effects of high serum concentrations (50%) on the virus and/or skin chips. No non-antigen-specific polyclonal antiserum-induced cyst size reductions were observed at a serum dilution of 1:8, whereas all antigen-specific polyclonal antisera produced significant cyst size reductions, with partial to complete neutralization of PV infectivity, at this dilution.

A comparison between the two animal model systems for the neutralization of CRPV revealed a greater sensitivity of

TABLE 3. CRPV papilloma growth at sites on NZW rabbits infected with mixtures of CRPV and dilutions of the various polyclonal antisera

Expt and polyserum	Day of measurement	No. of papillomas/no. of sites challenged at the following antiserum dilution:				
		1:2	1:8	1:32	1:128	None (no antiserum)
1						
7380 (Anti-CRPV)	31	0/3	1/3	3/3	3/3	3/3
	58	1/3	2/3	3/3	3/3	3/3
	71	1/3	2/3	3/3	3/3	3/3
3866 (Anti-BPV-1)	31	4/4	4/4	4/4	4/4	4/4
	58	4/4	4/4	4/4	4/4	4/4
	71	3/3	3/3	3/3	3/3	3/3
2						
7380 (Anti-CRPV)	34	1/2	0/2	1/2	2/2	2/2
	55	1/2	2/2	1/2	2/2	2/2
	69	1/2	2/2	1/2	2/2	2/2
639 (Anti-HPV-11)	34	2/2	2/2	2/2	2/2	2/2
	55	2/2	2/2	2/2	2/2	2/2
	69	2/2	2/2	2/2	2/2	2/2
7341 (Normal rabbit serum)	34	2/2	2/2	2/2	2/2	2/2
	55	2/2	2/2	2/2	2/2	2/2
	69	2/2	2/2	2/2	2/2	2/2

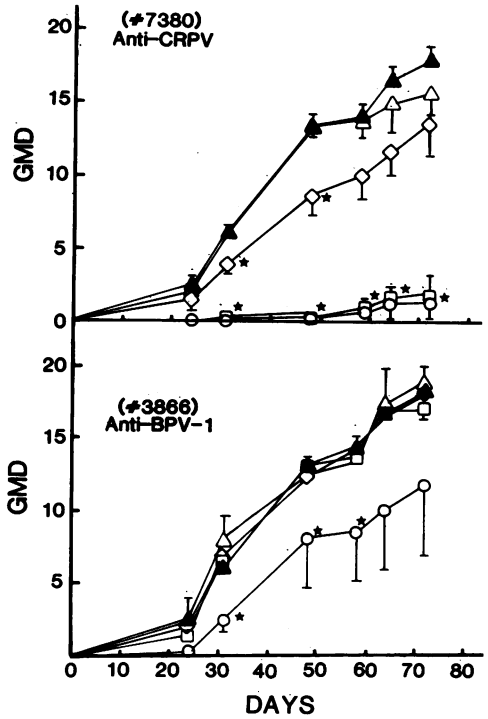

FIG. 2. Effect of polyclonal antisera 7380 (anti-CRPV) and 3866 (anti-BPV-1) on CRPV-induced growth of papillomas in NZW domestic rabbits. Dilutions of polyclonal antisera were preincubated with aliquots of infectious CRPV prior to the addition of virus to sites on the backs of the rabbits. Papilloma size (geometric mean diameter [GMD] ± standard error of the mean [in millimeters]) was plotted against time for each polyclonal antiserum dilution point (four sites per dilution point). Symbols: ▲, control (PBS alone); ○, 1:2 dilution; □, 1:8 dilution; ◇, 1:32 dilution; △, 1:128 dilution. *, $P < 0.05$.

the xenograft system than of immunocompetent NZW rabbits. Two of five rabbits tested for neutralization of CRPV infectivity by polyclonal antiserum 7380 (anti-CRPV) contained challenge sites which escaped neutralization even at the lowest dilution, 1:2. However, one of five rabbits showed complete protection at a polyclonal antiserum dilution of 1:32, the dilution at which neutralization began to break down in the xenograft system. In the NZW rabbit model, therefore, the host immune system may have contributed to the outcome such that the effectiveness of the neutralization showed substantial interrabbit variability.

In conclusion, the xenograft system is an effective model for the detection of antibody-mediated neutralization of infectious PVs. This model provides the only current system available for testing the neutralization of an infectious HPV type. The xenograft model also demonstrated marked sensitivity for the detection of neutralization of HPV-11 infectivity, with complete neutralization by an HPV-11-specific rabbit polyclonal antiserum at a dilution greater than 1:128. The greater utility of the xenograft model was demonstrated by effective neutralization of two non-HPVs, CRPV and BPV-1. Our studies clearly demonstrate the immunological specificity of neutralizing epitopes located on the three PVs (HPV-11, CRPV, and BPV-1).

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