JOURNAL OF VIROLOGY, Sept. 1990, p. 4399–4406
0022-538X/90/094399-08$02.00/0

Vol. 64, No. 9

Prevalence of Antibodies to Human Papillomavirus Type 8 in Human Sera

GERTRUD STEGER,[1] MARELIES OLSZEWSKY,[2] EGGERT STOCKFLETH,[3] AND HERBERT PFISTER[1]*

Institut für Klinische und Molekulare Virologie[1] and Dermatologische Klinik,[2] Friedrich-Alexander Universität, D-8520 Erlangen, and Universitätshautklinik Eppendorf, D-2000 Hamburg,[3] Federal Republic of Germany

Received 19 March 1990/Accepted 13 June 1990

The epidermodysplasia verruciformis-associated human papillomavirus type 8 (HPV-8) poses a high risk for malignant conversion of skin lesions in patients with epidermodysplasia verruciformis. For seroepidemiological studies, the HPV-8 open reading frames for E1, E2, E4, E6, E7, and L1 were bacterially expressed as β-galactosidase fusion proteins, which were purified by preparative gel electrophoresis. Cleavage with the protease FX_a at the engineered recognition site separated the β-galactosidase polypeptide part from the viral polypeptide. Western blot analysis of 445 serum samples from a randomly selected population with the entire L1 as antigen revealed HPV-8-specific immunoglobulin G antibodies in 20% of the samples. The percentage of positive sera did not significantly differ in different age groups. In some sera, we could also detect immunoglobulin M antibodies. The use of two shortened L1 polypeptides as antigen indicated that there are at least two reactive epitopes in the case of HPV-8 L1. Several sera contained antibodies to the early proteins E1, E2, E4, and E7. E1 and E7 were predominantly detected by sera which were negative for L1. In one case, we found antibodies to E6. Two of four sera of patients with epidermodysplasia verruciformis reacted with HPV-8 L1. The prevalence of anti-HPV-8-L1 antibodies in patients with malignant melanomas was comparable to that in the normal population (27.8%) but was significantly higher in patients with cervical cancer (37.5%), basaliomas (40%), and squamous cell skin carcinomas (72.7%) and in immunocompromised patients with Hodgkin's disease (47.7%).

A large group of human papillomaviruses (HPVs) was found to be associated with flat warts and macular skin lesions of patients with epidermodysplasia verruciformis (EV). These viruses deserve special interest because some representatives pose a high risk of developing into skin cancer (26). The EV-associated HPVs are occasionally detected in warts and squamous cell carcinomas of transplant recipients and patients with Hodgkin's disease (8, 10, 24, 34), for whom infection is assumed to be facilitated by immunosuppression analogous to that found with EV patients, who consistently show defects of cell-mediated immunity (15). In the normal population, these viruses apparently do not induce the typical lesions of EV but are occasionally encountered in various skin tumors. HPV-9- and HPV-25-related viruses have been demonstrated in three keratoacanthomas (30, 36), and HPV-20 and HPV-36 have been found in a basal cell carcinoma (26) and a solar keratosis (20), respectively. These reports indicate that EV-specific HPVs may occur in the immunocompetent normal population, which will thus form the reservoir for these viruses. They may furthermore be involved in cutaneous oncogenesis, although the DNAs of EV-associated HPVs are usually not detectable in skin tumors of the general population.

A preliminary seroepidemiologic study by immune electron microscopy revealed HPV-8 particle-agglutinating antibodies in 7 of 72 sera from healthy donors (32). A further analysis was not possible due to the lack of specific antigen. This problem can now be overcome by procaryotic expression of viral antigens, as in the case of HPV-6 and HPV-16 (17–19, 23), to demonstrate antibodies reactive with early and late proteins. An immune response was observed against HPV-6 L1, L2, and E2 and against HPV-16 L2, E4, and E7 proteins. It is of interest that anti-HPV-16 E7 antibodies were significantly more prevalent in sera from cervical cancer patients (20.5%) than from controls (1.4%), which suggests some correlation with HPV-related oncogenesis (19).

To analyze the immune response against the EV-specific HPV-8, we expressed early and late proteins in bacteria after cloning the appropriate open reading frames (ORFs) into pROS (4). The viral moiety could be separated from the β-galactosidase (β-Gal) fusion protein by cleavage with the protease factor X_a (FX_a) at the engineered recognition site. It was thus possible to exclude reactions of human antibodies with the procaryotic protein. The genomic organization of HPV-8 is comparable to that of other HPVs (6). The L1 ORF encodes the major structural protein (37), and the E2 ORF encodes the virus-specific transactivator of transcription (14). In contrast to genital HPVs, the E6 and not E7 ORF showed transforming activity in in vitro tests with mouse fibroblasts, whereas E7 of HPV-8 seems to be involved in the regulation of DNA replication, as described for bovine papillomavirus type 1 (BPV-1) (13).

MATERIALS AND METHODS

Bacterial strains and plasmids. The procaryotic vector pROS used for expression cloning contains the first 375 codons of the β-Gal gene under control of the *lacZ* promotor. These sequences are followed by a synthetic oligonucleotide which encodes the cleavage site of FX_a. Cleavage sites for restriction enzymes producing blunt ends in all three reading frames are joined, followed by stop codons in all

* Corresponding author.

three reading frames (4). *Escherichia coli* BMH7881 (21) and W3110 (35) were used for propagation of the expression vector and protein production. HPV-8 was originally cloned in pBR322 as 1,965- and 5,689-base-pair *Bam*HI-*Eco*RI fragments (32). Both HPV-8 inserts were isolated and ligated to reconstitute the L1 ORF. After recutting the DNA with *Bam*HI, it was ligated into the *Bam*HI site of pUC9. All HPV-8 DNA fragments used for expression cloning were isolated from this clone.

Construction of plasmids and synthesis of fusion proteins. Expression plasmids were constructed by insertion of HPV DNA fragments with blunt ends. After cutting the HPV DNA with the appropriate restriction enzymes, the fragments were isolated with Gene Clean (Bio 101, Inc.) and ligated with T4 DNA ligase into the *Sma*I, *Eco*RV, or *Stu*I site of pROS to obtain continuous β-Gal-virus open reading frames. The cleaved vector DNA was treated with alkaline phosphatase. The DNA preparations were used to transform competent bacteria. The transformants were selected in Luria broth supplemented with 100 μg of ampicillin per ml. HPV-containing clones were identified by colony hybridization as described previously (11), and plasmids were prepared from recombinants (1) and characterized by restriction enzyme digestion.

BMH7881 or W3110 harboring HPV expression plasmids was precultured at 37°C. The overnight culture was diluted 1:50 and incubated for 2 h and subsequently for another 3 h in the presence of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). The cells were pelleted, suspended, and boiled in Laemmli sample buffer and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (22). For preparative purification, a 500-ml culture containing an expression clone was grown to the mid-log phase, followed by 4 h of incubation in the presence of 1 mM IPTG.

Purification of fusion proteins and cleavage with protease FX_a. The cell pellets were suspended in lysis buffer (50 mM Tris hydrochloride [pH 8.0], 25% sucrose, 1 mM EDTA) and then incubated for 30 min on ice in the presence of lysozyme (0.2%), followed by a 30-min digestion with DNase I. After adding the detergent buffer (0.2 M NaCl, 1% deoxycholic acid, 1% Nonidet P-40, 20 mM Tris hydrochloride [pH 7.5], 2 mM EDTA), the insoluble proteins were contrifuged at 45,000 × *g* for 10 min. The pellet was washed two or three times with 0.5% Triton X-100–1 mM EDTA and once in between with 0.1 M citrate buffer, pH 3.0. The remaining pellet, containing the fusion protein and some membrane proteins of *E. coli*, was suspended and boiled in 2 ml of Laemmli sample buffer and separated by SDS–10% PAGE. After a lane of the electrophoretic gel was stained with Coomassie brilliant blue to obtain a marker, the unfixed fusion protein was cut out, electroeluted, and dialyzed three times for 2 h against 10 mM Tris hydrochloride, pH 8.3. The cleavage of the β-Gal-virus fusion protein with the protease FX_a was carried out for 24 h at room temperature in the presence of 50 mM Tris hydrochloride (pH 8.3)–100 mM NaCl, in some cases with 0.1% SDS or 1% Triton X-100. The cleavage conditions for the protease digestion had to be tested for each fusion protein separately. The protease FX_a was isolated from bovine blood as described previously (7).

Western blot analysis. For Western immunoblot analysis, about 1 μg of the cleaved fusion protein per lane was subjected to SDS-PAGE (12.5 or 15% polyacrylamide) and transferred onto nitrocellulose filters (2). The filters were stained with Ponceau S (Sigma Chemical Co.), cut in strips, destained, and coated by incubation in NET buffer (2% bovine serum albumin, 150 mM NaCl, 5 mM EDTA, Tris hydrochloride [pH 7.4])–0.2% Tween 20 for 1 h. The incubation with human sera or antisera, which were diluted in NET–0.2% Tween 20, was carried out overnight. After washing the strips three times for 10 min each in phosphate-buffered saline–0.2% Tween 20, the incubation with the second conjugate followed for 2 h. Protein A-peroxidase (Sigma) and anti-human immunoglobulin G (IgG)-peroxidase (Dako, Hamburg, Federal Republic of Germany) were diluted 1:1,000, and anti-human IgM-peroxidase (Sigma) was diluted 1:2,000 in NET–0.2% Tween 20. After three 10-min washings with phosphate-buffered saline–0.2% Tween 20, the antibody binding was detected by adding 3 mM 4-chloro-1-naphthol and 0.025% H_2O_2. Sera were preadsorbed with bacterial lysates, as described previously (37).

Human sera, hyperimmune sera, and wart extract. Randomly selected sera from 445 patients who had diseases unrelated to HPV infection were taken from the diagnostic department of our institute. Sera from patients with human immunodeficiency virus infections were excluded. Sera from 160 patients with various skin lesions were obtained from the Department of Dermatology, Friedrich-Alexander Universität, Erlangen, Federal Republic of Germany. Most of these patients suffered from malignant melanomas and basaliomas. Sera from 44 patients with Hodgkin's disease were collected at the Department of Dermatology, Universitätshautklinik Eppendorf, Hamburg, Federal Republic of Germany. Sera from cervical cancer patients were kindly provided by I. Jochmus Kudielka and L. Gissmann (19). Sera from EV patients were obtained from G. Albrecht, G. Fierlbeck, A. Gassenmaier, and F. Nürnberger. Skin biopsies were available in each case, and the persisting HPV types were determined in our laboratory by standard protocols (31). HPV-8 E7- and L1-specific antisera were prepared by immunization of a rabbit and a guinea pig with β-Gal fusion proteins (35, 37), and the antiserum against SDS-disrupted BPV-1 virions was from Dako. Rabbit antisera induced by pooled plantar warts and skin lesions from an EV patient (28) were obtained from F. Pass (Department of Dermatology, University of Minnesota, Minneapolis, Minn.). The antisera were diluted 1:100 when used in Western blots. Protein extracts were prepared from typed HPV-1 and HPV-5 warts and from a skin wart pool, as previously described (37), and used in Western blots.

RESULTS

Expression of ORFs of HPV-8 and purification of the proteins. For the expression of the major capsid protein L1 and the early proteins of HPV-8 blunt-ended fragments starting shortly behind the 5′ ends of the ORFs and ending beyond the stop codons (Fig. 1) were inserted into pROS. Two smaller fragments of ORF L1 were also expressed. The *Rsa*I fragment L1R (positions 7115 to 7330) is homologous to the HPV-6 L1 sequence, which encodes the epitope recognized by naturally occurring human antibodies (18). The apparent molecular masses of the β-Gal-HPV-8 fusion proteins synthesized in bacteria and analyzed by SDS-PAGE closely correlated with the predicted values from the sequence data (Fig. 2). Only the E7-β-Gal fusion protein migrated slower than expected. All fusion proteins were stable, except for the E4-β-Gal fusion protein, which regularly degraded to some extent.

Prior to electrophoretic purification in a preparative scale, the insoluble β-Gal fusion proteins were enriched from bacterial extracts by removing soluble material with several

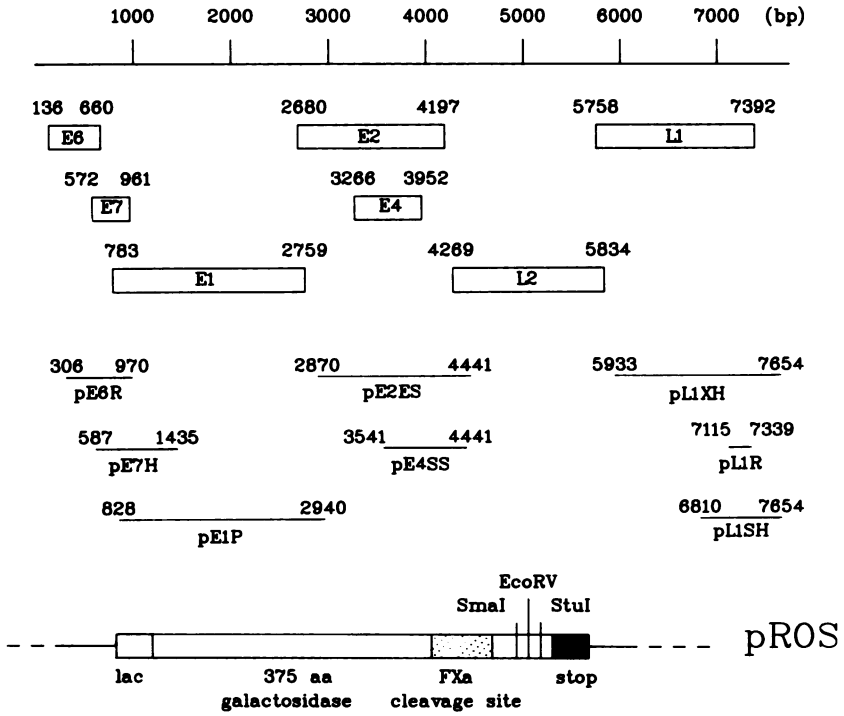


FIG. 1. Construction of expression plasmids containing HPV-8 DNA fragments. The genome organization of HPV-8, with the ORFs and their respective nucleotide positions, is shown. The segments, which were inserted into the *Sma*I, *Eco*RV, or *Stu*I sites of pROS, with their nucleotide positions and the names of the resulting clones are indicated. At the bottom is a diagram of the procaryotic expression vector pROS, with the *lac* promotor, 375 amino acids (aa) of β-Gal, the engineered FX_a cleavage site, the multiple cloning site, and translation stop codons.

washes, as described in Materials and Methods. In the case of E4, the bacteria were lysed only in SDS-containing sample buffer and directly subjected to preparative gel electrophoresis. The fusion proteins were recovered by electroelution and dialysis and cleaved with the protease

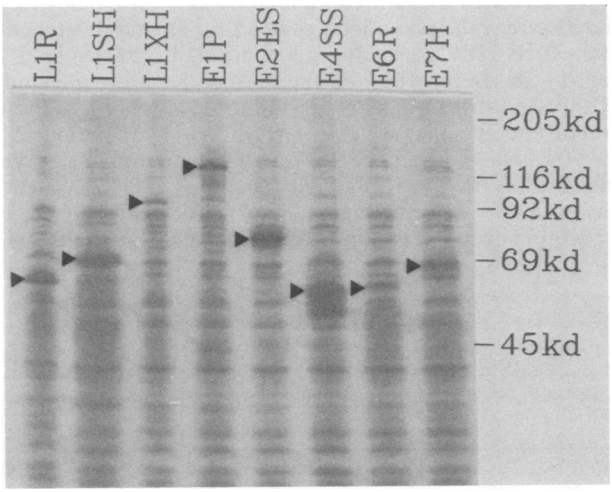


FIG. 2. SDS-PAGE of HPV-8 β-Gal fusion proteins (▶). Total protein extracts of induced *E. coli* BMH7118 and W3110 containing HPV-8 expression plasmids (for designations, see Fig. 1) were separated by SDS-PAGE on 10% polyacrylamide gels and stained with Coomassie brilliant blue. The positions of the molecular mass standards are shown on the right.

FX_a into the β-Gal (50 kilodaltons) and viral parts and used as antigen for Western blot analysis.

Prevalence of HPV-8-specific antibodies. To test for the prevalence of antibodies to L1 of HPV-8 in the human population, we used the purified L1XH protein encoded by the *Xmn*I-*Hin*cII-fragment (positions 5,839 to 7,654) as antigen in Western blots. It has a molecular mass of 54 kilodaltons (Fig. 3, lane L1) and represents the entire L1 ORF except for the 59 amino-terminal amino acids. The protein is recognized by an antiserum, which was generated against the bacterially expressed middle third of the HPV-8 L1 and is specific for EV-associated HPVs (37). The L1XH protein is also recognized by an antiserum induced by native virus particles from an EV patient (28). A Western blot with the group-specific antiserum against SDS-denatured BPV particles also showed a positive reaction, indicating that the L1XH of HPV-8 harbors genus-specific epitopes too. An antiserum, however, raised against virus particles from a plantar wart pool and reacting with the L1 in protein extracts from an HPV-1-induced wart and from pooled skin warts showed no reaction with the bacterially expressed L1 of HPV-8 (Fig. 3). This confirms that immunization with intact particles usually does not induce group-specific antibodies and that HPV-1-specific antibodies do not react with HPV-8 L1XH.

Randomly selected human sera from donors of various age groups were used at a dilution of 1:20 for an initial screening. Different sera reacted selectively with L1XH or β-Gal, with both proteins, or with none of them (Fig. 4). Two L1XH-positive human sera also recognized proteins in an HPV-5-induced lesion but did not react with the L1 protein

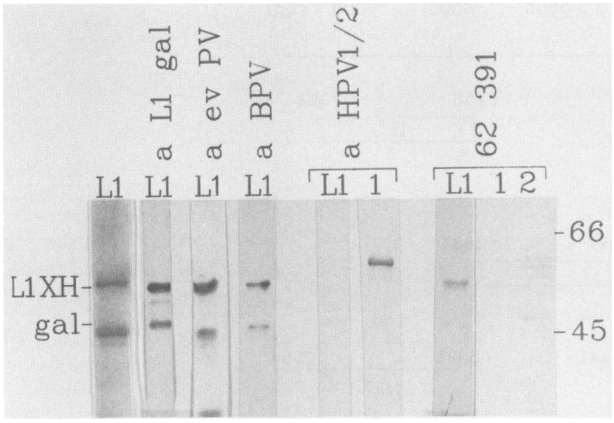


FIG. 3. Characterization of antigenic properties of the bacterially derived HPV-8 L1 protein. The FX_a-cleaved L1-β-Gal fusion protein was stained with Coomassie brilliant blue (left lane L1) or used for Western blots (lanes L1). Protein extracts of a plantar wart (1) and of an HPV-5-induced wart (2) were used as control antigens. The blots were incubated with antisera against engineered β-Gal–HPV-8 L1 (lane a L1 gal), native particles from EV lesions (lane a ev PV), papillomavirus genus-specific antigens (lane a BPV), native particles from pooled plantar warts (lane a HPV1/2), and a human serum sample (lane 62 391). Positions of the molecular mass markers (kilodaltons) are shown on the right.

in an HPV-1-induced wart (Fig. 3). To narrow down the immunoreactive epitope of L1 of HPV-8, 40 sera with high titers against L1XH were tested with L1SH and L1R proteins. Only 23 of the sera recognized the carboxy-terminal third of L1, and none of the sera reacted with the protein encoded by the *Rsa*I fragment (Fig. 5). Preadsorption of sera with L1XH and L1SH fusion proteins confirmed that there are at least two reactive epitopes in the case of HPV-8 L1 (Fig. 5C).

A total of 88 of 445 sera tested contained IgG antibodies to L1XH (19.7%). The majority showed titers below 1:100, but nine sera exceeded 1:150 and one serum sample exceeded 1:300. The percentage of positive sera did not significantly differ between different age groups (Table 1). Already, 17.6% of the children up to 6 years old were positive. Of 49 sera from children, 7 revealed HPV-8-specific IgM antibodies. All IgM-positive sera also contained IgG antibodies. Among 44 sera from adults older than 37 years, we found 9 sera to be anti-HPV-8 IgM positive. Three of these sera were negative for IgG antibodies.

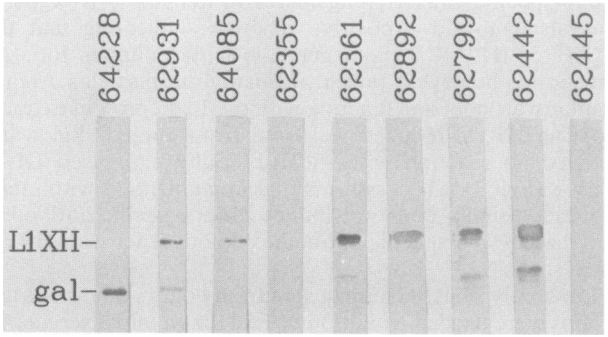
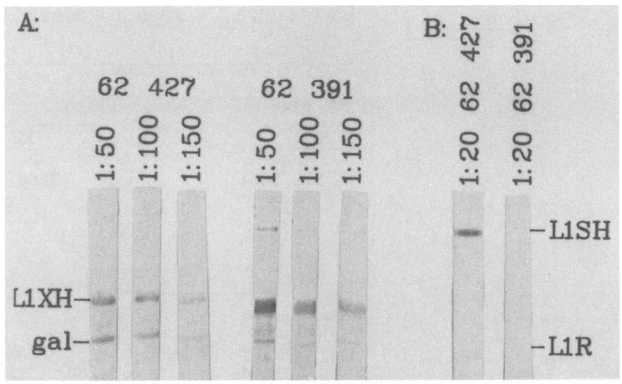


FIG. 4. Detection of antibodies to the bacterially derived, cleaved L1XH of HPV-8 in different human sera (numbered lanes) at a dilution of 1:20. The positions of L1XH and β-Gal are indicated on the left.



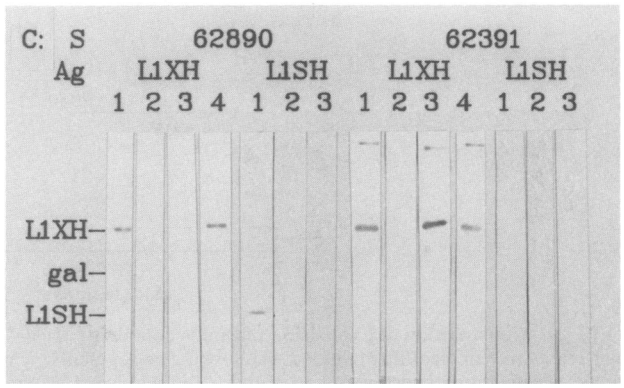


FIG. 5. Identification of different antigenic determinants of L1. (A) Two human sera were used at three dilutions in Western blots with L1XH as antigen. (B) The same sera were used at a dilution of 1:20 in Western blots with the shortened L1 proteins, L1SH, and L1R as antigens. (C) Two sera (S) which reacted with L1XH and L1SH or L1XH only were tested with L1XH and L1SH antigens (Ag) either untreated (lanes 1) or preadsorbed with lysates of L1XH (lanes 2), L1SH (lanes 3), or β-Gal (lanes 4) expressing bacteria. The positions of the proteins are indicated at the sides of the strips.

We screened 21 sera containing no IgG antibodies to L1 and 33 sera with IgG antibodies to L1 for an immune reaction to the early HPV-8 proteins at a dilution of 1:20 (Table 2). Of the 54 sera, 14 reacted with the purified E4 protein encoded by the *Sma*I-*Stu*I fragment (positions 3541 to 4441). The E2 protein encoded by the *Eco*RV-*Stu*I fragment (positions 2870 to 4441) was recognized in 7 cases, and the E1 protein encoded by the *Pvu*I fragment (positions 828 to 2940) was recognized by 12 sera. Eight sera revealed antibodies to the E7 protein encoded by the *Hae*III fragment (positions 557 to 1436) (Fig. 6), and antibodies could be detected once with

TABLE 1. Prevalence of HPV-8 L1 antibodies in human sera

Age (yr)	L1 antibody prevalence in sera		
	No. tested	No. positive	% Positive
0 to 6	108	19	17.6
7 to 16	46	10	21.7
17 to 26	69	13	18.8
27 to 36	71	15	21.1
37 to 46	59	12	20.3
47 to 56	42	6	14.3
>57	50	13	26.0

TABLE 2. Reactivities of 54 sera with HPV-8 antigens

No. of sera	Reactivity with:					
	L1	E7	E6	E4	E2	E1
17	+	−	−	−	−	−
4	+	−	−	−	+	−
2	+	+	−	+	−	−
6	+	−	−	+	−	−
1	+	−	−	+	+	−
1	+	−	−	−	−	+
1	+	−	−	−	+	+
1	+	−	−	+	−	+
1	+	+	+	+	−	+
2	−	+	−	−	−	−
1	−	−	−	+	−	−
1	−	−	−	+	+	+
5	−	−	−	−	−	+
2	−	+	−	−	−	+
1	−	+	−	+	−	−
9	−	−	−	−	−	−

the E6 protein encoded by the *Rsa*I fragment (positions 306 to 970). The majority of the anti-E2- and anti-E4-positive sera were also positive for anti-L1, in contrast to anti-E7- and anti-E1-positive sera, which were predominantly anti-L1 negative. The specificity of the reactions was confirmed in selected cases by demonstrating that reactivity was lost following preadsorption with lysates of bacteria expressing the respective viral protein but was retained following preadsorption with β-Gal-containing lysates (Fig. 7).

Two of four patients with EV revealed anti-HPV-8 L1 antibodies. One was infected with HPV-5, -8, -19, -20, and -25, and the other (patient 1) was infected with HPV-5 and -12. The negative sera were from patients infected with HPV-5, -8, and -20 (patient 2) or HPV-5 only. The sera of patients 1 and 2 reacted with either HPV-8 E1 or E4.

The prevalence of HPV-8 L1 antibodies in 201 patients with various skin lesions was tested with L1XH antigen at a serum dilution of 1:20 (Table 3). Of 54 sera from patients with malignant melanomas, 15 were L1 positive (27.8%), which corresponds to the values observed in the normal population. Of 60 sera from patients who suffered from basaliomas and 11 sera from patients with squamous cell skin carcinomas, 24 (40%) and 8, respectively, had antibodies to L1, which was significantly more than in the unselected population (P = 0.0004 and 0.0009, respectively). Among 32 sera from cervical cancer patients, 12 reacted with HPV-8 L1 (37.5%).

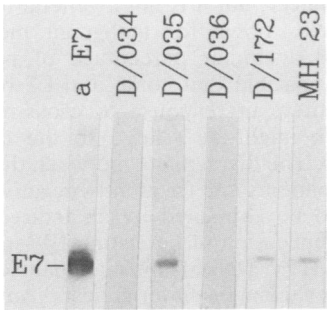


FIG. 6. Detection of antibodies to the bacterially derived E7 protein of HPV-8. Shown is a Western blot with an antiserum generated against the E7-β-Gal fusion protein (lane a E7) and blots with different human sera used at a dilution of 1:20 (remaining lanes). The position of the cleaved E7 protein is indicated.

TABLE 3. Frequency of HPV-8 L1 antibodies in sera from patients and controls

Condition (no. of patients)	Prevalence of L1 antibodies in sera		P
	L1 positive	% Positive	
Controls (445)	88	19.7	
Malignant melanomas (54)	15	27.8	0.25
Basaliomas (60)	24	40.0	0.0004
Hodgkin's disease (44)	21	47.7	0.0001
Squamous cell skin carcinomas (11)	8	72.7	0.0009
Cervical cancers (32)	12	37.5	0.035

Additionally, we found HPV-8 L1 antibodies in 21 of 44 sera from patients with Hodgkin's disease. The prevalence of HPV-8 L1 antibodies in these immunocompromised patients was significantly higher than in controls ($P < 0.0001$).

The sera from tumor patients were screened for antibodies against the early proteins E6 and E7, which might be particularly involved in oncogenic transformation. We found antibodies to E6 in only 1 of 34 sera from patients with basaliomas and also in 1 of 34 sera from patients with Hodgkin's disease. Antibodies to E7 could be detected in 6 of 187 sera from tumor patients and in 4 of 34 sera from patients with Hodgkin's disease, which corresponds to the prevalence of HPV-8 E7 antibodies in the control group.

DISCUSSION

This study revealed antibodies against bacterially expressed early and late proteins of the EV-associated HPV-8 in a large number of sera from donors without apparent skin disease. The viral antigens were synthesized as fusion proteins but were separated from the β-Gal protein by proteolytic cleavage before using them in Western blots. We can thus exclude false-positive results due to antibody reactions with the bacterial protein, which are frequently observed at low serum dilutions. This precaution was particularly important because the anti-HPV-8 titers were low, as is quite common in the papillomavirus field (29).

Antibodies against the major capsid protein of HPV-8 were detected in 20% of the sera, which is even more than anticipated from a previous immune electron microscopy study (32) and probably due to the higher sensitivity of Western blotting. The prevalence of anti-HPV-8 L1 antibodies was on the same order of magnitude as that of anti-HPV-1 virion antibodies, which range from 30 to 50% when determined by radioimmunoassay (33). Taking into account positive reactions with early antigens (in 57% of the anti-L1-negative sera [Table 2]), the overall prevalence of anti-HPV-8 antibodies was about 60%. The anti-HPV-8 L1 antibody prevalence did not significantly change according to age, in contrast to the results with HPV-1 for which a clear maximum was observed with teenagers, coinciding with the highest incidence of HPV-1-induced plantar warts. Our data indicate that HPV-8 infection occurs very early in life. An apparent constant prevalence may be partially maintained by the disappearance of detectable antibodies and a new immune response due to primary infections, which is supported by evidence of the presence of IgM antibodies also in older age groups. Finding anti-HPV-8 antibodies even in young children is in line with data on genital HPV-6 and -16 (19, 23). Whereas congenital transmission was discussed as a possible route of early infection in these cases, HPV-8 could easily be transmitted in addition just by skin contact.

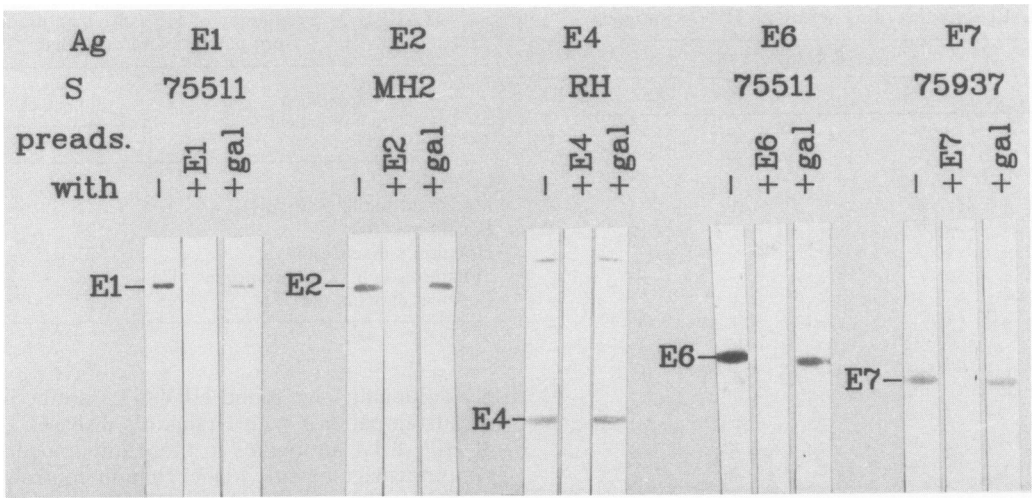


FIG. 7. Specificity of the reactivity of human sera (S) with early HPV-8 antigens (Ag). The sera were tested with respective Western blots either untreated (lanes −) or following preadsorption with lysates of HPV-8 protein or β-Gal-expressing bacteria (lanes +). The positions of the proteins are indicated.

The unexpected high prevalence of antibodies against genital and EV-associated HPVs raises questions on the specificity of the detection tests. In spite of extensive amino acid sequence homologies, the immune response against L1 is usually type specific in the course of natural HPV infection, as has been studied with a few HPV types (29). Our HPV-8 L1 protein did not react with an animal hyperimmune serum raised against native plantar wart particles, although it was shown to present genus-specific epitopes, and two human sera which reacted with HPV-8 L1 did not recognize HPV-1 L1 from a wart. This lack of cross-reactivity is noteworthy because, among sequenced HPVs, HPV-1 is the closest relative of HPV-8 except for the other EV-associated viruses (3). The serum of an EV patient infected by HPV-5 and HPV-12 detected the HPV-8 capsid protein. We assume that antibodies against EV-specific HPVs will generally react with HPV-8 L1 because of tremendous sequence homology (37) and therefore tentatively expect that our results are representative of the immune response against this HPV subgenus, although we certainly cannot exclude the possibility of cross-reactivities with serologically uncharacterized HPVs.

The E1 and E2 ORFs of HPVs are highly conserved, similar to the L1 ORF. In the cases of E4, E6, and E7 proteins, cross-reactivities are less likely because of considerable sequence divergence (12). Anti-HPV-8 E7-positive human sera did indeed not react with an engineered HPV-16 E7 and vice versa (data not shown). Furthermore, HPV-8 E7 expressed in eucaryotic cells could not be precipitated with antisera directed against E7 of HPV-6 and -16 (T. Iftner, G. Sagner, H. Pfister, and F. O. Wettstein, unpublished data).

Taken together, the serological data point to a high prevalence of infections with EV-specific HPVs. The infections seem to be productive (as demonstrated by anti-L1 antibodies), which guarantees virus transmission. They are obviously largely unapparent, although one has to realize that EV-specific lesions are easily overlooked as long as they occur transiently and sporadically.

Two of four EV patients showed antibodies against the capsid protein of HPV-8. One patient, who was negative in spite of being infected with HPV-8, appeared severely immunodepressed (5). The partially missing immune response is in line with previous reports of low or no titers of anti-HPV-3 antibodies in EV patients with HPV-3-induced lesions (16) and may point to specific defects of the humoral responses of EV patients.

The high percentage of anti-HPV-8 L1-positive sera among patients with Hodgkin's disease is not unexpected. Typical EV lesions induced by an HPV-20 subtype were recently described for a patient with Hodgkin's disease (10), and an impaired cell-mediated immunity of these patients may generally favor the spread of these infections.

It was a bit surprising, however, to note the high prevalence of HPV-8-specific antibodies in patients with basaliomas and squamous cell skin carcinomas, in view of the rare detection of HPV DNA in these tumors. In basaliomas, HPV-2 DNA was demonstrated twice (25) and HPV-20 DNA was found once (26). HPV-41 DNA was detected in 2 of 10 squamous cell carcinomas (9). As discussed above, an immune response against HPV-20 is likely to be detected with the HPV-8 antigen. No serological cross-reactivity was observed between HPV-2 and EV-specific HPVs (27), and nothing is known about the relationship of HPV-41 and other HPVs.

The significance of the high percentage of anti-HPV-8 L1-positive sera for cutaneous oncogenesis is difficult to assess. An increased prevalence was also noted for women with cervical cancer, but it is not a general feature of tumor patients, as was shown for malignant melanoma cases, which revealed a similar percentage of positive sera as controls. It is possible that some anti-L1 reactions reflect low antibody titers in response to cross-reactive cellular antigens, which might be related to the development of certain cancers. The discrepancy between the seropositivity of skin cancer patients and frequently negative hybridization data could also be explained by an indirect effect of the tumor on the replication of persisting EV-specific HPVs or by the loss of HPV DNA from malignant cells, i.e., by a hit-and-run mechanism of tumorigenesis. Antibodies against the transforming E6 protein (13) and against E7 were no more prevalent in patients with tumors than in controls. Thus, there is no further evidence for an increased transforming activity of the virus, in contrast to findings with cervical cancer patients, who frequently showed antibodies

against the transforming E7 protein of HPV-16 (19). This may result from a low immunogenicity of E6 proteins. If the L1 reactivity of sera from skin tumor patients was not due to the replication of EV-specific HPVs but to that of serologically more distantly related viruses, the missing E6 reactivity could be explained by the lack of cross-reactivity between these less-conserved proteins. In this case, the E6 data would not disprove a role for HPV in skin carcinogenesis, as suggested by the L1 results.

ACKNOWLEDGMENTS

We are indebted to S. Ellinger for providing pROS and purified protease FX_a.

This work was supported by the Johannes and Frieda Marohn Stiftung.

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