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Prevalence of Antibodies Against Virus-Like Particles of *Epidermodysplasia Verruciformis*-Associated HPV8 in Patients at Risk of Skin Cancer

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There is increasing evidence for widespread occurrences of infection with Epidermodysplasia verruciformis-related human papillomaviruses, both in the general population and in immunosuppressed patients. In order to test for the prevalence of antibodies directed against the native L1 epitopes exposed on the surface of the virions, we have established an IgG-specific enzyme-linked immunosorbent assay with L1 virus-like particles of the Epidermodysplasia verruciformis-specific human papillomavirus 8 as antigen to screen 567 representative serum samples from the general population and immunosuppressed/dermatologic patients. Among healthy European donors (n = 210), 7.6% were found to be seropositive. In a group of renal transplant recipients (n = 185) the antibody prevalence was elevated to 21.1%, irrespective of the presence or absence of skin cancer. High positivity rates could be detected among (i) immunocompetent patients with nonmelanoma skin tumors (45.6%, n = 79) and (ii) Psoralene/UVA treated psoriasis patients (42.9%, n = 42). In contrast, anti-human papillomavirus 8-virus-like particle antibodies were found in only 6.8% of Hodgkin lymphoma patients (n = 44). ***Key words: psoriasis/renal transplant recipients/seroepidemiology/UV light. J Invest Dermatol 111:696–701, 1998***

Clinically apparent infections of the skin by human papillomaviruses (HPV) result in a variety of hyperplastic, papillomatous, or verrucous tumors of the squamous epithelium (Shah and Howley, 1996; Wieland and Pfister, 1997). Almost all lesions induced by the cutaneous HPV types in immunocompetent individuals are benign and eventually regress due to a complex, poorly understood response of the immune system (Tindle and Frazer, 1994).

A striking exception to this rule is the rare skin disease Epidermodysplasia verruciformis (EV). EV patients suffer from multiple, life-long persisting flat warts and macular skin lesions, frequently disseminated all over the body. EV tumors develop after infection by several HPV types that are specific for induction of EV lesions (Orth, 1987; Fuchs and Pfister, 1996). Within 20–30 y from onset of the disease, the primarily benign skin lesions undergo malignant conversion in 30%–60% of the patients, mostly on sun-exposed areas of the body (Tanigaki *et al*, 1986; Orth, 1987). Interestingly, out of over 20 EV-associated HPV types, HPV5 and HPV8 can be found in more than 90% of the EV cancers (Pfister, 1992). There are data arguing for some undefined genetic defect of the cell-mediated immunity, probably responsible for the susceptibility of EV patients to infections by specific HPV types (Jablonska and Majewski, 1994).

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Abbreviations: EV, Epidermodysplasia verruciformis; PBST, PBS/0.05%Tween20; RTR, renal transplant recipient; VLP, virus-like particle.

The EV-HPV were originally believed to infect the EV patients only; however, recent studies clearly demonstrated that DNA of EV viruses may also occur in normal human skin, as well as in premalignant and malignant skin tumors in patients without EV (Pfister and ter Schegget, 1997). Using a nested polymerase chain reaction technique, sequences of EV-specific HPV could be detected in up to 80% of squamous cell carcinomas of immunosuppressed renal transplant recipients (RTR) (de Jong-Tieben *et al*, 1995). Prevalences of 62% and 41% have also been reported for squamous cell carcinomas and basal cell carcinomas of immunocompetent patients, respectively (Pfister and ter Schegget, 1997). An intriguing hypothesis concerning a source of the widespread skin infections by EV-specific HPV types has recently been proposed by Boxman *et al* (1997), who detected subclinically persisting EV-HPV-DNA in plucked hairs of 45% of healthy donors and of all RTR examined.

A high prevalence of EV-HPV infections was also indicated by a seroepidemiologic study using western blots with bacterially expressed major capsid protein L1 of HPV8 (Steger *et al*, 1990), which detected specific anti-L1 IgG antibodies in 20% of the general population and in 40%, 73%, and 48% of patients with basaliomas, squamous cell carcinomas, and Hodgkin's disease, respectively. The antibody prevalence in RTR was 50% (Bouwes Bavinck *et al*, 1993). Antigens in the form of denatured proteins consist predominantly of linear epitopes and may therefore fail to detect antibodies specific for conformational epitopes on the surface of the virions. On the other hand, linear L1 proteins contain conserved cross-reactive regions that may be recognized by a broad range of type-common antibodies.

In the meantime, artificially synthesized, empty papillomavirus capsids [virus-like particles (VLP)] became available and were

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successfully employed as antigen in serologic studies (Schiller and Roden, 1995). VLP can be generated by self-assembly of L1 or L1 and L2 proteins overexpressed in vaccinia virus-, baculovirus-, semliki forest virus-, or yeast-systems, and display structural features very closely resembling those of the native virions (Hagensee *et al*, 1994).

Serum antibodies against HPV-VLP turned out to be a useful marker of HPV infection with 58%–89% of individuals with clinical lesions or HPV DNA being seropositive compared with 6%–19% of individuals without HPV DNA or disease history (Carter *et al*, 1994, 1995; Kirnbauer *et al*, 1994; Heim *et al*, 1995). Prolonged exposure to viral antigen turned out to be a primary determinant of a detectable antibody response (Wideroff *et al*, 1995). Unlike DNA-based assays, VLP-enzyme-linked immunosorbent assay (ELISA) are able to detect past infections. Antibodies were shown to persist after loss of HPV DNA (Wikstrom *et al*, 1995). Seroreactivity to HPV16 VLP in currently HPV DNA negative women strongly correlated with their number of sexual partners and proved to be superior to HPV DNA prevalence as a marker for cervical cancer risk in high-risk populations (Nonnenmacher *et al*, 1996; Wideroff *et al*, 1996). In contrast to HPV DNA detection, serologic analysis does not depend on a precise sampling of the tissue, which obviously is a particular problem in epidemiologic studies of skin infections.

Because HPV8 is one of the candidates to play an important role in cutaneous carcinogenesis and represents one of the best studied EV-HPV, we were interested in the actual seroprevalence as defined by a VLP-ELISA for this specific type. We report here on the establishment of an ELISA with HPV8-VLP as antigen and present the results of screening for specific IgG antibodies in the general population and in immunocompetent patients with skin tumors. Patients with Hodgkin lymphoma served as a control group with another type of malignant disease, and PUVA-treated psoriasis patients represented UV-exposed individuals with a benign hyperproliferative skin disorder. Immunosuppressed RTR were included due to an increased risk of skin cancer (Hoxtell *et al*, 1977; Bouwes Bavinck and Berkhout, 1997).

MATERIALS AND METHODS

Construction of the recombinant baculovirus expressing HPV8 L1 protein To construct recombinant baculoviruses expressing the L1 protein of HPV8, the L1 open reading frame was amplified using the primers 5′-TAGTAGGGATCCGC-CACCATGGCAGTGTGGCAA-3′ and 5′-ATGA-TGAAGCTTCCTTGAATACTGTGT-3′, which correspond to HPV8 sequences between nt. 5851–5865 and 7445–7431 (Fuchs *et al*, 1986) and contain recognition sites for BamHI and HindIII engineered into their 5′ parts, respectively. The cloned genomic DNA of HPV8 was used as a polymerase chain reaction template. The amplified L1 gene was cloned into baculovirus transfer vector (pBlueBacIII, Invitrogen, Leek, The Netherlands) via BamHI and HindIII sites followed by recombination with linearized wild-type AcMNPV baculovirus DNA. Resulting recombinant baculoviruses were plaque purified in the semi-solid agarose medium containing 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal). Expression of the capsid proteins by recombinant baculoviruses was determined by immunofluorescence, coomassie blue staining, and immunoblot as described in detail by Ghim *et al* (1996).

Purification of virus-like particles High Five-insect cells (Trichoplusia ni 5B1–4, Invitrogen) were cultivated in Express Five-serum-free medium (Gibco, Karlsruhe, Germany) at 27°C. Confluent cultures were collected and infected with baculo-HPV8 L1-virus stock. Seventy-two hour post-infection cells were harvested, the pellet was resuspended in a total volume of 5 ml phosphate-buffered saline (PBS), 1 mM phenylmethylsulfonyl fluoride, 10 μg Aprotinin per ml, and sonicated on ice for 3×10 s at 40 W. The crude extract was loaded on top of a 40% (wt/vol) sucrose/PBS-cushion in SW28 tubes and centrifuged at $113{,}000 \times g$ for 2.5 h at 4°C. The pellet was resuspended in 29% (wt/vol) CsCl/PBS and centrifuged to equilibrium at $183{,}000 \times g$ in a 50TI rotor for 60 h. Fractions were collected from the bottom of the tubes and dialyzed against PBS three times for 1 h at 4°C. Fractions were tested for immunoreactivity in the ELISA with an HPV8 L1-positive and a negative serum. Protein concentration of VLP preparations was determined by BCA Protein-Assay (Pierce, Oud Beijerland, The Netherlands).

Electron microscopy Diluted VLP preparations were immobilized on 300 mesh Formvar/carbon-coated grids and negatively stained with 1% uranyl acetate. VLP were examined with a Zeiss EM9 S2 transmission electron microscope at the magnification of ×30,000.

Establishment of the VLP-ELISA The ELISA procedure was established by using the following antigens: a bacterially expressed denatured HPV8 L1 protein (Steger *et al*, 1990), denatured and assembled VLP. A guinea pig anti-serum directed against denatured L1 protein and sera of two EV patients known to be HPV8 DNA positive served for antigen detection. Five sera from 0.5 to 1.5 y old children were initially used as supposedly negative controls.

PBS/0.05% Tween 20 (PBST) was chosen as a blocking solution because of the best signal-to-background ratio. Sera dilutions, the washing solution, and the washing procedure were optimized. By these means the saturating amount of antigen for each well was determined.

VLP-ELISA Nunc Maxisorp-ELISA plates were coated with 100 ng of the VLP preparations in 50 μl PBS for 1 h at 37°C. After three washings with PBS the wells were blocked by incubation with 200 μl PBST for 1 h at 37°C. Patients' sera were diluted 1:50 in PBST and incubated in duplicate wells for 1 h at room temperature. After five washes with PBST the peroxidase-conjugated goat anti-human IgG (Fcγ fragment-specific, Affipure, Dianova, Hamburg, Germany), diluted 1:20,000 in PBST was added to the wells for 1 h at room temperature. Following five washes with PBST, 100 μl of the peroxidase substrate diammonium 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonate) (ABTS, Boehringer, Mannheim, Germany) was added and incubated for 45 min at room temperature. The optical densities were determined in the ELISA reader at 405 nm.

For each serum sample the OD value of an antigen-free well was subtracted from the mean value of duplicate wells coated with VLP. A positive serum with an OD of 1.0 was included on each plate. The definition of the cut-off value for HPV8 and HPV16 antigen was based on the distribution of the OD values of 210 or 149 sera from the general population, excluding the outliers (Heim *et al*, 1995). The individual values exceeding the mean + 3 SD were excluded, and the remaining readings were averaged again until no further values exceeded the recalculated mean + 3 SD.

Western blot analysis Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out under reducing conditions in 10% polyacrylamid slab gels using the discontinuous buffer system of Laemmli (1970). VLP were denatured by heating at 95°C for 10 min in sodium dodecyl sulfate-sample buffer. Following electrophoresis, proteins were stained with Coomassie brilliant blue R250. For the western blot, proteins were transferred to nitrocellulose membrane for 2 h by the semi-dry blotting procedure (Kyhse-Andersen, 1984). The nitrocellulose membrane was stained for protein with Ponceau S and destained completely in PBST. Unspecific binding sites on nitrocellulose membrane were saturated with 1% bovine serum albumin and 1% dry milk in PBST for 1 h at room temperature. Guinea pig anti-HPV8 L1 anti-serum (Steger *et al*, 1988) was diluted 1:800 in blocking solution. Specific antigen-antibody complexes were detected by rabbit anti-guinea pig IgG peroxidase conjugate (1:3000) using the ECL-system (Amersham, Freiburg, Germany) for detection.

Studied collectives Sera from five different patient groups were enrolled in this study. As a control group representing the general population, 210 sera of healthy blood donors, collected in the University Hospital of Cologne, were tested. Sera of five EV patients were provided by the Department of Dermatology, Warsaw; two were obtained from G. Fierlbeck, Dermatologic University Hospital in Tübingen.

The group of RTR consisted of 127 patients from the Transplantation Center Leiden, the Netherlands (Bouwes Bavinck *et al*, 1991, 1993). All patients were immunosuppressed for about 13 y. Another 58 sera from RTR were provided by the Department of Dermatology, University of Edinburgh, Scotland. Sera of 44 Morbus Hodgkin patients and 42 psoriasis patients subjected to treatment with psoralene and UVA light (PUVA therapy) originated from the University Hospital Hamburg-Eppendorf.

Sera of 79 patients with skin tumors (basaliomas, spinaliomas, and/or M. Bowen-lesions) were collected at the Dermatologic University Hospital in Kiel.

RESULTS

Characterization of HPV8 VLP The dialyzed fractions from the CsCl gradients were initially analyzed by reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis to identify those

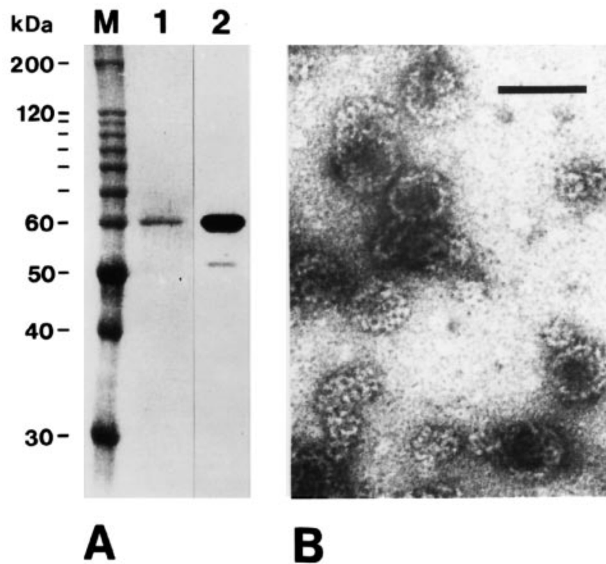

Figure 1. Analysis of HPV8-LI VLP. (*A*) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of purified HPV8 L1 VLP. M, molecular weight marker 10 kDa ladder; 1, Coomassie blue stained gel; 2, western blot with guinea pig anti-HPV8 L1 anti-serum. (*B*) Electron micrograph of negatively stained HPV8 L1 VLP. *Scale bar:* 100 nm.

containing the L1 protein. The central fractions of the gradients showed a prominent 60 kDa protein band, corresponding to the approximate molecular weight expected for the HPV8 L1 polypeptide. The identity of this protein as L1 was verified by immunoblot with anti-HPV8 L1 guinea pig anti-serum (**Fig 1*A***). Besides the 60 kDa L1 protein, a weak band of about 52 kDa was detectable in western blots. As previously reported (Pfister *et al*, 1977), this fraction most likely represents a degradation product of the full-length L1 protein.

Analysis by transmission electron microscopy revealed spherical particles with a diameter of about 60 nm that seemed to be composed of capsomers; however, it also contained smaller, irregular structures (**Fig 1*B***). The CsCl fractions with an average buoyant density of 1.3 g per ml contained the highest concentrations of L1 protein and virus-like particles and corresponded to visible bands in the gradients.

VLP-containing fractions were also strongly positive in the ELISA test using an EV patient's serum (Fierlbeck *et al*, 1989). The hyperimmune guinea pig anti-serum to bacterially expressed HPV8-L1 protein (Steger *et al*, 1988) reacted both with assembled and with disrupted VLP (5% sodium dodecyl sulfate, 20 mM dithiothreitol, 10 min 95°C); however, the denatured capsids were not detected by the EV serum (data not shown).

Prevalence of anti-HPV8 VLP antibodies in different collectives The results of the serologic assays are summarized in **Fig 2** and **Table I**. As a control group representing the general population, 210 sera of healthy blood donors were tested. The cut-off value for HPV8-VLP ELISA was determined to be 0.304. The OD values of serologic reactions in the general population revealed a normal distribution. In the control group 16 of 210 sera reacted with VLP. In contrast, four of seven sera from patients with EV tested positive. Three of these patients were known to be infected with HPV8 by DNA analysis and two of them were seropositive (Fierlbeck *et al*, 1989; S. Jablonska, Department of Dermatology, Warsaw, Poland, personal communication).

Apart from EV, patients with nonmelanoma skin tumors had the highest prevalence of antibodies (45.6%) among the different groups. Ten of 14 spinalioma patients and eight of 12 Morbus Bowen patients had IgG antibodies reactive with HPV8 VLP, whereas only 19 of 54 patients with basal cell carcinomas were seropositive.

Of 44 sera of Hodgkin-lymphoma patients, three were positive for anti-HPV8 VLP antibodies, which corresponds to the seroprevalence in the general population.

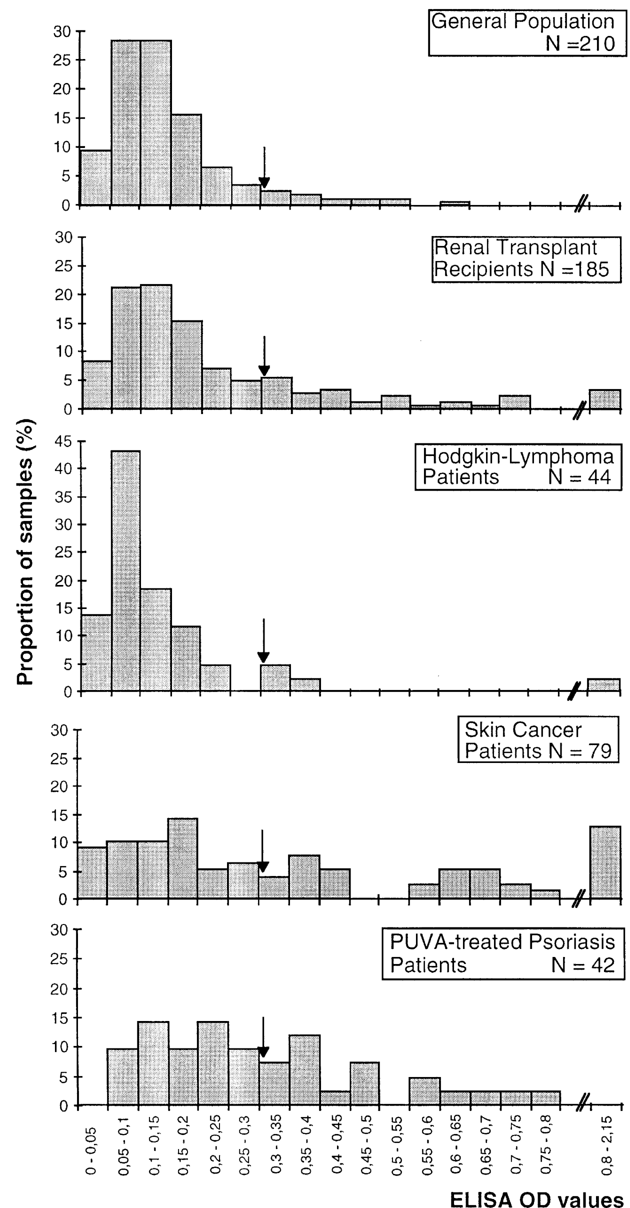

Figure 2. Distribution of frequency of sera from different groups reacting with HPV8 L1 VLP at different levels as measured by extinction at 405 nm. The cut-off of 0.304 is indicated by *arrows*.

Psoriasis patients treated with Psoralene/UVA showed a surprisingly high seropositivity; 43 per cent of the sera contained antibodies to the HPV8 major capsid protein.

In the immunosuppressed RTR, 39 of 185 sera contained IgG antibodies to HPV8 VLP (21.1%). This prevalence appears to be significantly elevated when compared with the general population ($p < 0.001$). A similar seropositivity was observed for patients with spinaliomas (26.7%) and basal cell carcinomas (22.2%), respectively.

Both in the control group and in RTR a significant fluctuation in seroprevalence for different age groups of patients was not observed (**Table II**). There was no significant correlation between seropositivity and sex seen in the control group, RTR, and skin tumor patients (data not shown).

Positive cases from the general population showed moderate seroreactivity, with OD values not exceeding 0.65. In contrast 23% of the skin tumor patients revealed stronger ELISA reactions with OD values in the range of 0.65–2.15.

A total of 391 sera was tested in parallel for ELISA reactivity against HPV8 and HPV16 VLP (**Fig 3**). Both anti-HPV8 and anti-

Table I. Serum ELISA reactivity to HPV8 L1-VLP of control and patient groups

Collective	n	HPV8 pos.	Pos. (%)	95% conf. interval
Skin tumor patients	79	36	45.6	34.6; 56.6
SCC[a]	14	10	71.4	47.8; 95.1
M. Bowen	21	8	66.7	40.0; 93.3
BCC[a]	54	19	35.2	22.4; 47.9
General population	210	16	7.6	4.0; 11.2
Controls				
Hodgkin lymphoma patients	44	3	6.8	0.0; 14.3
Psoriasis patients (PUVA-treated)	42	18	42.9	27.9; 57.8
Immunosuppressed population	185	39	21.1	15.2; 27.0
RTR from Edinburgh	58	10	17.2	7.5; 27.0
RTR from Leiden	127	29	22.8	15.5; 30.1
without skin cancer	89	20	22.5	13.8; 31.1
with skin cancer	28	9	23.7	10.2; 37.2
SCC[a]	30	8	26.7	10.8; 42.5
BCC[a]	18	4	22.2	3.0; 41.4

[a]SCC, squamous cell carcinoma; BCC, basal cell carcinoma. Values of patients with SCC *and* BCC were counted to both groups.

Table II. Age distribution of patients and seroprevalences to HPV8-VLP in three different collectives

	General population		RTR		Skin tumor patients	
Age group (y)	n	% pos.	n	% pos.	n	% pos.
0–10	21	9.5	0	–	0	–
10–30	76	5.3	8	25.0	0	–
30–50	66	10.6	73	20.5	5	0
>50	47	6.4	46	26.1	74	48.6
Total	210	7.6	127	22.8	79	45.6

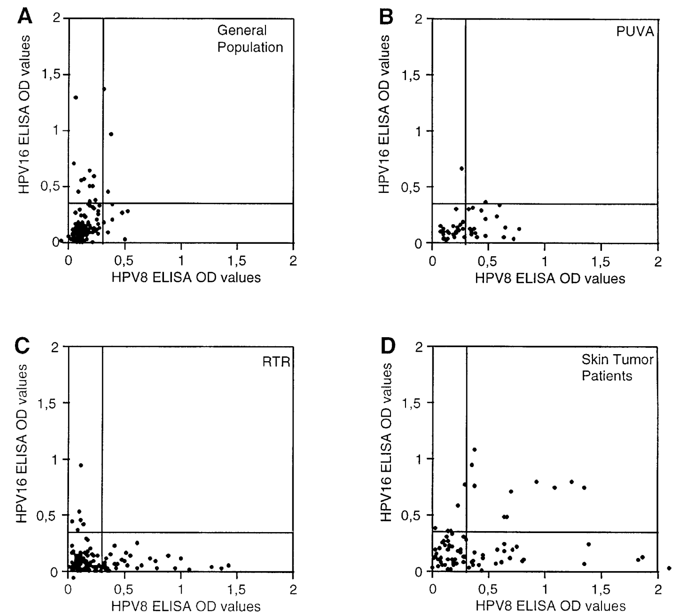

Figure 3. Correlation of serum ELISA IgG reactivity to HPV8 and HPV16 L1 VLP. Sera from (*A*) general population (n = 145); (*B*) psoralene/UVA-treated psoriasis patients (n = 42); (*C*) RTR (n = 125); (*D*) skin tumor patients (n = 79). The cut-off for the HPV8 VLP-ELISA (0.304) is indicated by vertical lines, and the cut-off for the HPV16 VLP-ELISA (0.348) by horizontal lines.

Table III. Detection of anti-HPV8-L1 antibodies by western blot[a] and VLP-ELISA in 124 sera of RTR

Group	Western blot	VLP-ELISA	Number of sera
A	+	+	12
B	+	–	50
C	–	+	16
D	–	–	46

[a]Data from Bouwes Bavinck *et al.* (1993).

HPV16 antibodies were detected in 14 sera, 81 sera showed anti-HPV8 antibodies only, and 22 sera showed anti-HPV16 antibodies only. The prevalence of anti-HPV16 antibodies was 10.3% in the general population, 16.5% in skin cancer patients, 4.8% in RTR, and 4.8% in PUVA-treated psoriasis patients.

DISCUSSION

The development of recombinant VLP offers for the first time a reliable, generally available source of papillomaviral conformational capsid antigens. As proven by several reports on genital HPV infections, VLP may be especially useful for seroepidemiologic studies, which so far suffered from the lack of suitable reagents. The VLP-based ELISA represents a specific method for initial detection and subsequent follow-up of the humoral response to HPV virions (Schiller and Roden, 1995). In contrast to genital infections, scant attention has been focused on HPV infections of the skin. Because of emerging evidence for a high prevalence of EV-associated viruses in both normal and diseased human skin (Pfister and ter Schegget, 1997), we focused on the prevalence of antibodies against the major surface antigen of HPV8.

Due to the small number of available EV patients where HPV8 infection has been proven by detection of viral DNA, it is difficult to validate to what degree antibodies against HPV8 VLP reflect current or past HPV8 infection. Based on our limited analysis of seven EV patients, there is some indication that sensitivity (two seropositive/three DNA positive) and seropositivity of individuals at risk is similar to that observed in extensive studies with genital HPV (Kirnbauer *et al*, 1994; Carter *et al*, 1995). The significance of HPV8 DNA negative test results in random samples of EV patients is obviously limited in view of the large target organ skin.

Our results reveal a significantly higher prevalence of anti-HPV8 antibodies in individuals with skin cancer in comparison with the non-selected general population. The seroprevalence was also higher in psoriasis patients and RTR. A parallel testing of these sera in an HPV16-VLP ELISA showed no obvious cross-reactivity between HPV8 and HPV16 and demonstrated no elevated reactivity of the patients sera with HPV16 antigen in comparison with the general population. For RTR and skin cancer patients HPV8 seroprevalences generally confirm earlier data based on western blot analysis (Steger *et al*, 1990; Bouwes Bavinck *et al*, 1993); however, some of the observed prevalences differ considerably. Whereas the immunocompetent skin cancer patients showed roughly the same, high antibody prevalence in both assays, the overall positivity of immunocompetent (general population) and immunocompromised (RTR, Hodgkin patients) individuals was only about one-third of the previous immunoblot results. The difference in reactivity of sera in western blot and the VLP-ELISA became particularly obvious when comparing individual patients. With 124 RTR, we consistently found positive or negative results for 58 sera (**Table III**, groups A and D). Fifty western blot-positive sera (group B) turned out to be negative in the ELISA-test and 16 sera (group C) negative in the immunoblot reacted in the VLP-ELISA. These discrepancies can be explained best by different specificities of each method that detect different sets of antibodies.

The large number of western blot-positive/ELISA-negative sera may reflect the fact that linear epitopes presented in the immunoblot are able to react with antibodies against the subgenus of EV-

associated HPV and not against HPV8 alone (Steger *et al*, 1990; Bouwes Bavinck *et al*, 1993). In contrast, the VLP ELISA appears more type restricted. Only a partial cross-reactivity of sera was observed in a VLP-ELISA with the most closely related genital HPV types 6 and 11 (Heim *et al*, 1995); however, VLP preparations do not only contain perfectly assembled particles and it is therefore not possible to exclude limited cross-reactivity in the HPV8-ELISA with some EV-associated viruses sharing the same or similar L1 surface epitopes.

The existence of VLP-ELISA-positive/western blot-negative sera may be explained by a preferential immune response of these individuals against conformational surface epitopes specifically detected by the VLP-ELISA.

In spite of the fact that the immunoblot- and VLP-ELISA-tests obviously detect different repertoires of antibodies, both these methods congruently point to a widespread occurrence of EV-specific HPV and to a very high prevalence in skin cancer patients. It is remarkable that the more type-specific HPV8-VLP ELISA still detected antibodies in 7.6% of the sera from the general population. The difference in prevalence between skin cancer patients and controls appears even accentuated with the HPV8-VLP ELISA. In line with this seroepidemiologic association between HPV8 and cutaneous cancer, HPV8-DNA could be demonstrated in two of 34 squamous cell carcinomas and in one of 11 tested basal cell carcinomas of the general population (Stark *et al*, 1994; Shamanin *et al*, 1996); however, HPV8-DNA did not appear to be particularly prevalent relative to other HPV types (Pfister and ter Schegget, 1997). It is apparent that the immune response in about half of the skin cancer patients does not imply persistence of this HPV type in the malignant tumor cells, which do not support the expression of capsid proteins. The antibodies will rather reflect an increased exposure to HPV8 antigens during previous or concomitant viral replication in the nonmalignant epithelium.

Because of the low prevalence of anti-HPV8 antibodies in Hodgkin-lymphoma patients, there seems to be no association between an increased exposure to HPV8 antigens and malignancy in general. The high prevalence of HPV8-specific antibodies in skin cancer patients may be due to enhanced replication of HPV8 under conditions that promote cutaneous malignancies. Sunlight is well established as the most important risk factor for nonmelanoma skin cancer and induces local immune suppression, which can in turn facilitate viral replication (Vitaliano and Urbach, 1980; Kribke, 1994). This hypothesis is supported by the high seropositivity of PUVA-treated psoriasis patients, who develop hyperkeratotic lesions (UV keratoses) and skin cancer in a UV dose dependent manner (van Praag *et al*, 1993; Studniberg and Weller, 1993). Recently, we also observed a correlation between the presence of anti-HPV8 L1 antibodies and solar keratosis in fair skinned individuals living on Saba (Bouwes Bavinck *et al* in preparation).[1] The overall seroprevalence among 114 individuals tested on this tropical island was 46%; 37% in individuals with less than 37 solar keratoses (the median number of solar keratoses in the group studied) and 54% in those with 37 or more solar keratoses (unpublished data). The latter observation forms additional support for the hypothesis that seropositivity is promoted by UV exposure.

Another clearly recognized risk factor for skin cancer is an acquired or iatrogenic systemic immune suppression (Hoxtell *et al*, 1977). Our analysis of immunocompromised RTR revealed a 3-fold higher prevalence of HPV8-specific antibodies compared with the general population; however, there was basically no difference in the positivity rates between cancer and noncancer patients in this group, and the seroprevalence in immunocompromised skin cancer patients was considerably lower than that of the immunocompetent ones. These seemingly conflicting data may be related to the following specificities of the immunosuppressed patients: (i) the noncancer patients are at continuing high risk to finally develop skin cancer and therefore do not represent an appropriate control group in this regard; and (ii) the severe systemic immunosuppression may at least partially hamper the humoral immune response.

Taken together, the prevalence of HPV8-specific antibodies appears to be increased not only in skin cancer patients but also in populations exposed to the two major risk factors in cutaneous carcinogenesis: UV light and immunosuppression. It is not yet known if activated replication fof HPV8 is an epiphenomenon or if it plays a causal role in cancer development. It will be very interesting to see whether other HPV types detectable in cutaneous cancers follow a similar seroepidemiology. Carefully designed epidemiologic studies are necessary to assess the significance of the increased seroprevalence in HPV-related skin-specific oncogenesis. They have to include detection and typing of HPV DNA in plucked hairs and tumor specimens. It is furthermore essential to analyze all of the factors potentially influencing the immune response to the viral antigens (e.g., sex, age, skin type, sun exposure and/or UV exposure, presence of other hyperproliferative skin disorders).

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