Antibodies to human papillomavirus type 11 virus-like particles in sera of patients with genital warts and in control groups

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We analysed by ELISA a total of 478 human sera for the presence of antibodies to HPV-11 virus-like particles. The sera were obtained from patients with current genital warts (group CO), from males attending the hospital for fertility disorders (group MA), from blood donors (group BD) and from patients hospitalized for reasons unrelated to HPV infections (group HO). Antibody prevalence was higher in male patients of group CO (23.0%) as compared to males of groups MA (3.2%; P < 0.0001), HO (5.3%; P = 0.01) and BD (16.7%; NS). In addition, there was a significant difference in antibody titre between the males of group CO compared to group MA. Within the whole sample the absorbance of sera from females was higher than in specimens from males (P < 0.0001). A small subset of the sera was also tested by radioimmunoprecipitation assay (RIPA). There was good agreement between the data obtained by ELISA and RIPA.

Since papillomaviruses cannot be replicated in conventional experimental systems, studies on the host immune response following papillomavirus infections in man were mostly initiated when recombinant virus proteins became available. Information about the presence of antibodies to virus structural proteins in individuals with and without papillomavirus-associated disease is of particular interest as it may indicate whether prior exposure to the virus induces immunity against reinfection and/or recurrent disease. Human papillomavirus (HPV) type 11 infects mostly mucosal epithelia and is (besides HPV-6) the major causative agent of genital warts (condylomata acuminata) and of laryngeal papillomas. HPV-11 virions obtained from experimentally induced condylomata (Kreider et al., 1985) were used to analyse sera from patients with laryngeal papillomas or genital warts (Bonnez et al., 1991, 1992, 1993; Christensen et al., 1992). Antibodies were detected by ELISA in 9.5–50% of cases and in 0–3% of controls. Similar results were obtained when HPV-11 virus-like particles (VLPs) were used, which were generated by expression of the L1 protein in baculovirus (Rose et al., 1993; Heim et al., 1995). In previous studies the controls were either individuals without sexual contacts or without clinical evidence of HPV infection.

Human serum samples derived from 174 patients with newly diagnosed genital warts (condylomata acuminata; group CO; mean age 31.7 years, range 21–87) were tested for the presence of IgG antibodies to HPV-11 VLPs consisting of the L1 protein. This group consisted of 126 males and 48 females. For comparison we analysed sera obtained from the following populations: group MA, 124 male patients with fertility disorders attending the same clinic as the condylomata patients (mean age 35.6 years, range 17–73); group BD, 88 blood donors, 66 males and 22 females without information about a present or past genital HPV infection (mean age 41.5 years, range 22–65; Müller et al., 1995a); and group HO, 92 patients hospitalized for various reasons but without known records of genital HPV infections, 39 males and 53 females (mean age 38.5 years, range 22–60; Jochmus-Kudielka et al., 1989).

Generation of HPV-11 L1 VLPs by recombinant baculovirus has been described before (Müller et al., 1995b). VLPs were examined by electron microscopy (Müller et al., 1995b). ELISA was performed as described previously (Müller et al., 1995a). Fifty µl of purified VLPs (diluted 1:100 in PBS) were used for coating of microtitre wells. As a control, sera were tested in buffer wells without VLPs but otherwise treated identically. Serum specimens were tested in duplicate. For each serum, the mean reactivity of buffer wells was subtracted from the mean reactivity of wells coated with VLPs to obtain the net A405 value. The cut-off for the HPV-11 VLP L1 ELISA was calculated as 0.635 following the procedure described before (Müller et al., 1995a) using data from sera of the 124 males of group MA.
Male infertility patients (MA)

Condyloma patients (CO)

Blood donors (BD)

Hospital group (HO)

Fig. 1. Distribution of frequency of sera from different groups (MA, CO, BD, HO) reacting at different levels as measured by extinction at 405 nm ($A_{405}$). The cut-off of 0.63 is indicated by arrows.

Table 1. IgG immune response to HPV-11 L1 VLPs in sera of different donors

Significantly different values are shown in bold.

<table>
<thead>
<tr>
<th>Donors</th>
<th>Positive sera (%)</th>
<th>Median $A_{405}$</th>
<th>$A_{405}$ 10th percentile</th>
<th>$A_{405}$ 90th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>All</td>
<td>Male</td>
</tr>
<tr>
<td>MA (m = 124)</td>
<td>3.2*</td>
<td>-</td>
<td>3.2</td>
<td>0.15*</td>
</tr>
<tr>
<td>BD (m = 66; f = 22)</td>
<td>16.7†</td>
<td>22.7‡</td>
<td>18.2</td>
<td>0.31†</td>
</tr>
<tr>
<td>CO (m = 126; f = 48)</td>
<td>23.0</td>
<td>25.0†</td>
<td>23.6</td>
<td>0.25</td>
</tr>
<tr>
<td>HO (m = 39; f = 53)</td>
<td>5.3§</td>
<td>2.13§</td>
<td>16.0</td>
<td>0.33†</td>
</tr>
<tr>
<td>All (m = 355; f = 123)</td>
<td>13.2‖</td>
<td>22.0‖</td>
<td>15.1</td>
<td>0.23**</td>
</tr>
</tbody>
</table>

* $P < 0.0001$ in comparison to males in group CO.
† Not significant in comparison to males in group CO.
‡ Not significant in comparison to males in group BD.
§ $P = 0.01$ compared to males in group CO.
¶ $P = 0.02$ compared to males in group HO.
‖ $P = 0.21$ in comparison to males in group CO.
** $P < 0.0001$.

The statistical analysis compared proportions of positive sera using the $\chi^2$ test of independence and, in cases of small samples sizes, the Fisher Exact test. For continuous measurements, differences between groups were examined using the Wilcoxon rank-sum test. Two-sided alpha levels of less than 0.05 were considered statistically significant.

The distribution of the reactions in different sera is shown in Fig. 1. All results are summarized in Table 1. When the calculated cut-off value ($A_{405} = 0.635$) was applied, four of the donors of group MA (3.2%) were shown to be positive. In none of these males was there any clinical evidence of condylomata acuminata and none was aware of having had genital warts prior to the visit. For comparison with the donors of group MA we first analysed the data obtained from the male donors of the groups BD, CO and HO. Among the 126 male patients with proven condylomata acuminata attending the same dermatology clinic at Hamburg University as the donors of group MA, 29 (23.0%) had serum antibodies directed against HPV-11 L1 VLPs. The difference in antibody response between these two populations was statistically highly significant ($P < 0.0001$; $\chi^2$ test). There was also a significantly lower positivity rate among the males of group HO (9.3%) as compared to the condylomata patients (group CO; $P = 0.01$). The rate of positivity among the male donors in group BD was also lower (16.7%) but not statistically significant ($P = 0.3$).

In order to exclude bias introduced by the calculated cut-off
value, statistical analysis was performed by comparing the amount of antibody response (given by the $A_{405}$ value) rather than the proportion of positives in the different populations. Among male condylomata acuminata patients (group CO) the median (10th and 90th percentile) was 0.25 (0.15, 0.39). Within male donors of groups MA, BD and HO the values were 0.15 (0, 0.43), 0.31 (0.13, 0.80) and 0.33 (0.12, 0.58), respectively. Compared to the parameters obtained for the male condylomata acuminata patients the differences in seroreactivities were statistically significant for donors of group MA ($P < 0.0001$) but not for the males of groups BD ($P = 0.4$) or HO ($P = 0.4$).

To validate the results obtained by the HPV-11-specific VLP L1 ELISA, we performed radioimmunoprecipitation assays (RIPA) with some of the samples. HPV-16 E7-specific RIPA was used previously and proved to detect antibodies in HPV-16-positive cervical cancer patients with higher sensitivity than the HPV-16 E7 protein ELISA (Nindl et al., 1994). For the HPV-11 L1-specific RIPA the protein was produced by in vitro transcription and translation. The HPV-11 L1 open reading frame used for baculovirus expression (Müller et al., 1995 b; see above) was cloned into Bluescript SK (Stratagene). One μg was used in a coupled transcription–translation reticulocyte system (Promega) and radiolabelled HPV-11 L1 protein was obtained in the presence of $[^{35}S]$methionine. The protein was detected by autoradiography using a mouse monoclonal antibody raised against HPV-11 VLPs (21B2; M. Müller, unpublished results). This antibody was included as positive control in each experiment (Fig. 2a). In contrast, three monoclonal antibodies prepared against HPV-11 virions (H11A3, B2, H3; kindly provided by N. D. Christensen, The Milton S. Hershey Medical Center, Hershey, Penn., USA) failed to react with the HPV-11 L1 protein (data not shown). When tested by ELISA or RIPA these antibodies were shown to react with intact but not with disrupted virions (Christensen et al., 1990; N. D. Christensen, personal communication). Hence we concluded that the HPV-11 protein produced in vitro does not take the authentic three-dimensional structure. This assumption was supported by the failure of this protein to form VLPs when analysed by CsCl gradient centrifugation (data not shown).

A total of 28 sera (11 of group MA, seven of group BD, 10 of group CO) were tested by RIPA for HPV-11 L1 antibodies (Fig. 2a). The L1 protein was precipitated by nine sera, all of which were positive in the HPV-11 VLP ELISA. Three human sera, one from each group, without detectable antibody levels against the L1 protein by RIPA revealed significant ELISA reactivities. Among the remaining 16 individuals' sera, none was found to contain HPV-11 L1-specific antibodies by RIPA or ELISA (Fig. 2b). As determined by κ statistics there was a good overall agreement of 77% between the two assays. This number did not vary significantly when the three populations were analysed separately. The only three sera with discordant results were positive by VLP ELISA but negative by RIPA. As mentioned above, the L1 protein produced in vitro did not exist in its authentic conformation; thus we speculate that the HPV-11 L1 VLP ELISA detects antibodies with slightly higher sensitivity than RIPA.

The data for the complete set of sera tested in this study were analysed for sex-specific differences in the prevalence of HPV-11-specific antibodies measured by the VLP L1 ELISA (Table 1). Sera of 47 of the 355 male donors (13.2%) and of 27 of the 123 females (22.0%) had $A_{405}$ values above the calculated cut-off. This difference was not statistically significant ($P = 0.21$). In females the distribution of absorbance

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**Fig. 2.** (a) Immunoprecipitation of radiolabelled HPV-11 L1 protein produced in vitro with eight different serum samples derived from condylomata acuminata patients (CO) and healthy male individuals with fertility disorders (MA). Samples were analysed by SDS-PAGE and autoradiographed. As a positive control a mouse monoclonal antibody (21B2) directed against HPV-11 L1 VLPs was used. A sample without added serum was included as a negative control. (b) Correlation between absorbance values obtained by HPV-11 L1 VLP ELISA (measured as $A_{405}$) and reaction by RIPA of 28 human serum samples.
values was characterized by a median (10th, 90th percentile) of 0.36 (0.13, 1.11) compared to 0.23 (0.02, 0.76) in males ($P < 0.00001$). This data indicates that in females, antibodies to the HPV-11 capsid reach higher titres than in males. When the donors within the individual groups were analysed separately, a sex-specific difference of antibody positivity was found in individuals of group HO (23.1% versus 5.3%; $P = 0.02$) and BD (22.7% versus 16.7%; difference not significant) but not in patients with proven condylomata at the time when the blood was drawn (25.0% versus 23.0%). As shown in Table 1, in all instances the titres within the females were higher than in males but none of these differences reached statistical significance.

Our data on the prevalence of HPV-11-specific antibodies in patients with genital warts are in line with other studies published previously (Bonnez et al., 1991, 1992, 1993; Christensen et al., 1992; Rose et al., 1994a, b; Heim et al., 1995). We have demonstrated that infection by HPV-11 may elicit a humoral antibody response to virus late proteins, most notably if this infection leads to clinical symptoms. Biopsies of the condylomata patients analysed in this study were not available for analysis of HPV DNA. However, based upon our previous experience with patients of the same hospital we assume that approximately one third of the genital warts contain HPV-11 and most of the remainder are positive for HPV-6 (Gissmann et al., 1984). Since there appears to be considerable cross-reactivity between HPV-6 and -11 (Heim et al., 1995), we do not expect that the rate of antibody positivity within the condylomata group would change considerably when only sera of the HPV-11-positive patients are analysed. Hence, the low proportion of antibody-positive patients (23.6%) indicates that during natural infection papillomaviruses are poor immunogens. Since, on the other hand, high titre experimental antisera can be obtained using HPV virions or VLPs (Rose et al., 1994b), the low response must be due to insufficient presentation of the virus antigen to the immune system.

Differences in antibody response to HPV VLPs between male and female patients with genital warts were reported by Carter et al. (1995) who used VLPs of HPV-6. However, our data are somewhat different as we find a sex-specific difference in donors with unknown status of genital HPV infection (groups HO and BD, although statistically not significant in the latter) but not in condylomata patients. The reason for these discordant results is not clear but it is unlikely that they are due to distinct biological properties of the HPV types used; HPV-6 and -11 are closely related in their protein composition, induce the same clinical disease and, as mentioned before, show a considerable degree of serological cross-reactivity (Dartmann et al., 1986; Heim et al., 1995). Possibly the duration of genital warts within the patients as well as the history of previous condylomata may be important factors in mounting an immune response and these parameters should be taken into account in future research. It is also feasible that, despite the structural similarities between the L1 proteins of HPV-6 and -11, they show a different degree of cross-reactivity with other HPV types which may lead to a higher positivity rate in one or the other case. According to our data, the antibody response to HPV-11 VLPs develops equally well in both sexes during an acute infection. If infection persists in a subclinical (or unrecognized) state it is conceivable that, due to the female anatomy, HPV infection may persist longer and may lead to a more pronounced immune response than in males. Since there is no evidence for a sex-specific difference in prevalence of incident or recurrent genital warts one can further speculate that IgG antibodies to HPV-11 VLPs do not confer immunity to clinical HPV infection.

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References


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