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Balance of IgG Subclasses toward Human Papillomavirus Type 16 (HPV16) L1-Capsids Is a Possible Predictor for the Regression of HPV16-Positive Cervical Intraepithelial Neoplasia

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Human papillomavirus type 16 (HPV16) is known to be a major causative agent of cervical cancer. To test the hypothesis that an enhanced Th1 response favors the natural course of cervical intraepithelial neoplasia (CIN), we measured IgG subclasses toward HPV16 L1-capsids because IgG1/IgG2 balance reflects Th2 and Th1 responses, respectively. We examined IgG2/IgG1 ratios in sera from 67 anti-HPV16 L1-positive women; 18 were cytologically normal women, 29 were CIN patients, and 20 were cervical cancer patients. The IgG2 dominance (IgG2/IgG1 ratio >1) was observed in 94, 48, and 5%, respectively ($p < 0.001$). The regression rate of CIN lesions was significantly different between patients with and without IgG2 dominance: 83.3% (5/6) versus 16.7% (1/6), respectively ($p < 0.05$). These findings raise the possibility that IgG2 dominance toward HPV16 L1-capsids, i.e., Th1 dominance, may be a useful marker to predict viral clearance or the regression of HPV16-positive CIN.

Subsets of human papillomaviruses (HPVs) are believed to be primary causative agents of cervical cancer and its precursor lesions (cervical intraepithelial neoplasia [CIN]) (1, 2), with HPV16 being the highest-risk oncogenic type (3). HPV infection of the uterine cervix is common among sexually active individuals (4, 5). Most HPV infections disappear spontaneously, while only a subset of the women develop CIN lesions, a small fraction of which progress to invasive cervical cancer (6). Thus, HPV infection is not a sole causative factor for cervical cancer.

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An increased incidence of persistent HPV infection or cervical neoplasia in immunosuppressed individuals has been demonstrated (7), implicating that failure of host immunity to eradicate HPV infections may be relevant to the development of cervical neoplasia. Given this observation, we focused our research on the possibility that immunological status modulates the risk of HPV infection to develop CIN and cervical cancer. According to the recent thought, Th1 cells medicate cellular immunity while Th2 cells mediate humoral immunity (8). Thus, one may speculate that Th1/Th2 balance may determine the natural course of HPV infection of the uterine cervix.

Recently, it has been shown that Th1 and Th2 cytokines activate the production of IgG 2 and IgG1 by B cells (9-11), respectively, thus measuring the ratio of these antigen-specific IgG subclasses allowing us to evaluate Th1/Th2 balance (12, 13).

In this study, we evaluated the reactivities of IgG1 and IgG2 toward HPV16 L1-capsids (L1 virus-like particles, L1 VLPs), because HPV16 is the most common HPV type associated with cervical cancer and the L1 proteins (major capsid proteins) are highly expressed during HPV infection or in low-grade CIN lesions. Besides, the seropositive rates for HPV16 L1 capsids are higher both in cytologically normal women and in patients with cervical neoplasia compared with heretofore reported other viral antigens, such as HPV16 E4, E6 and E7 (13-18).

In the present study, IgG1/IgG2 balance of anti HPV16 L1 antibody was determined in anti-HPV16 L1 positive women with and without HPV16-positive cervical neoplasia. In addition, we analyzed the fate of CIN lesions in relation to the IgG1/IgG2 balance prospectively.

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MATERIALS AND METHODS

Study subjects. The study population consisted of subsets of 492 Japanese women including 121 women with normal cervix as judged by cytological and colposcopical diagnosis, 293 CIN and 78 cervical cancer patients. The diagnoses of CIN and cervical cancer were histologically confirmed.

All the 492 women were examined for HPV DNA in exfoliated cells from the cervix by PCR method and for anti-HPV16 L1 antibody in the sera using HPV16 L1-capsids ELISA. We selected study subjects by virtue of seropositivity for HPV16 L1 antibody from 120 women with HPV16 DNA-negative and cytologically normal cervix (one out of the 121 woman was positive for HPV16 DNA), 56 patients with HPV16 DNA-positive CIN I-III, and 29 patients with HPV16 DNA-positive invasive cervical cancer. The seropositive rates were 15%, 52% and 69% in the above three groups, respectively. As a result, 67 anti-HPV16 L1 positive women (18 control women with normal cervix, 29 CIN patients and 20 cervical cancer patients) were eligible for the subsequent IgG subclass analysis.

Of 29 seropositive patients with HPV16-positive CIN, 12 patients were followed up for more than 24 months, the remaining 17 patients being lost or surgically treated after the diagnosis of CIN. We analyzed the clinical outcome of CIN lesions in relation to the IgG1 and IgG2 balance.

HPV DNA detection and typing. We detected HPV DNA in exfoliated cervical cells by PCR-based method described previously (19). In brief, total cellular DNA was extracted from cervical specimens by a standard procedure. HPV DNA was amplified by PCR method using the consensus-primers for the HPV L1 region. HPV types were identified on the basis of restriction fragment length polymorphism (RFLP). This assay has been shown to identify at least 26 types of genital HPVs.

Detection of IgG reactivity toward HPV16-L1 capsids. Detection of whole IgG antibody toward HPV16 L1-capsids was performed by ELISA using HPV16 L1 VLPs as antigens as described (18). HPV16 L1 VLPs were produced using recombinant baculovirus and purified by CsCl density gradient centrifugation (18, 20). Horseradish peroxidase-conjugated goat anti-human IgG antiserum (Cappel-Organon Teknika Corp., West Chester, PA) was employed as a second antibody to detect whole IgG antibodies. Specific absorbance was calculated by subtracting the absorbance measured in mock wells without L1-capsid antigens. The pre-assigned cut-off level (0.322 optical density) for seropositivity was described elsewhere (18).

Detection of IgG subclass. IgG1 and IgG2 reactivities toward HPV16 L1-capsids were examined by modified HPV16 L1 VLP ELISA employing peroxidase-conjugated monoclonal antibodies specific to human IgG1 and IgG2, respectively (The Binding Site, Birmingham, England) as second antibodies. For each sample as well as whole IgG absorbance, IgG1- and IgG2-specific absorbance was calculated by subtracting the absorbance measured in mock wells without L1-capsid antigens.

Data analysis. When IgG2/IgG1 ratio in the sera positive for anti-HPV16 L1-capsids antibodies was greater than 1, IgG2 reactivity was considered dominant. Serological data were statistically analyzed by χ^2 test for trend, Fisher's exact probability test, and Mann-Whitney U test. P values obtained in all tests were considered to be significant when below 0.05.

RESULTS

IgG subclass reactivities toward HPV16 L1-capsids in HPV16-negative normal cervix and HPV16-positive cervical neoplasia in a cross-sectional study. All the anti-HPV16 L1 positive sera obtained at the diagnosis of the cervix from 18 control women, 29 HPV16-positive CIN patients and 20 HPV16-positive cervical cancer patients showed both IgG1 and IgG2 reactivities toward HPV16 L1-capsids. IgG2 dominance (IgG2/IgG1 ratio >1.0) was found in 17 of 18 (94%) control women, 14 of 29 (48%) CIN patients, and 1 of 20 (5%) cervical cancer patients (χ^2 test for trend $p < 0.001$) (Fig. 1). The levels of IgG2/IgG1 ratio of the control women were significantly higher compared with the CIN patients on one hand (2.0 [±0.3] versus 1.0 [±0.1] in mean [±SEM], Mann-Whitney U test $p < 0.001$), and the levels of the CIN patients were significantly higher than those of the cervical cancer patients on the other hand (1.0 [±0.1] versus 0.5 [±0.1] in mean [±SEM], Mann-Whitney U test $p < 0.01$) (Fig. 1).

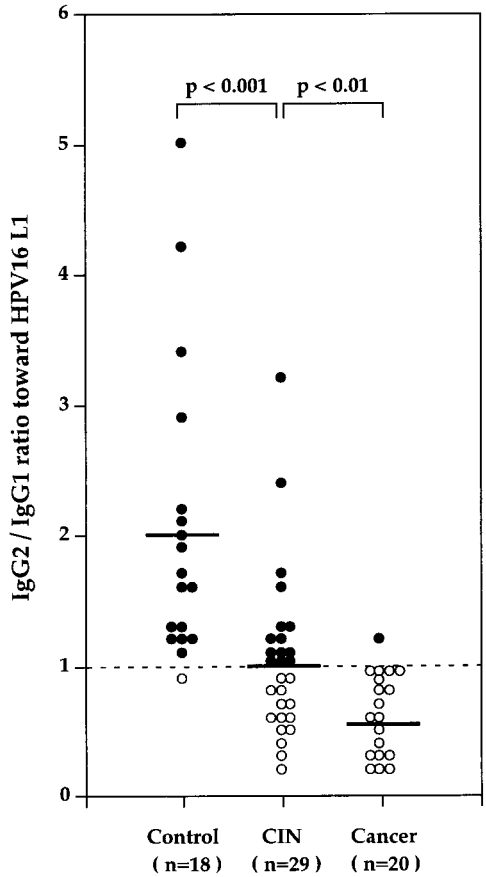

FIG. 1. IgG2/IgG1 ratios toward HPV16 L1-capsids in control women (Control), CIN patients (CIN), and cervical cancer patients (Cancer). The broken lines indicate IgG2/IgG1 ratio = 1 and solid bars show means. ●, women with IgG2 dominance. ○, women with IgG1 dominance.

The regression of CIN in relation to IgG1/IgG2 balance. Of 29 CIN patients, 12 were followed up for more than 24 months. The CIN lesion regressed to cytologically and histologically normal cervix in 5 out of 6 patients with IgG2 dominance, while the lesion regressed in only 1 out of 6 patients with IgG1 dominance (Fisher's exact probability test $p = 0.040$) (Fig.

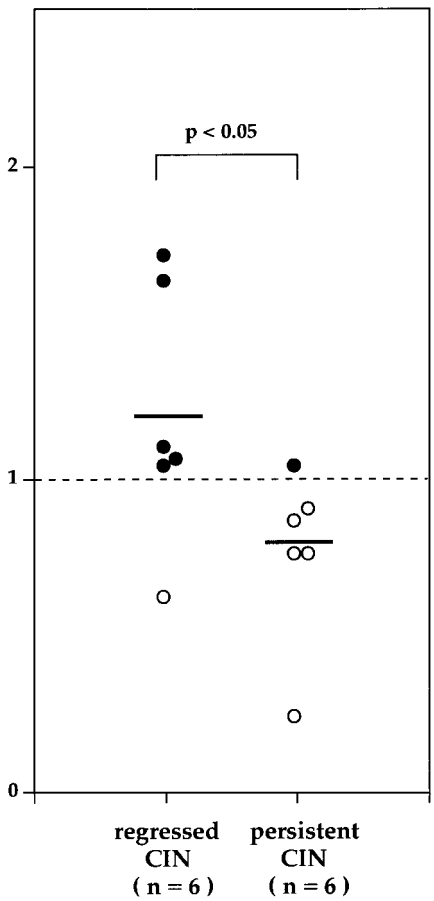

FIG. 2. IgG2/IgG1 ratios toward HPV16 L1-capsids in patients with regressed CIN and with persistent CIN. The broken lines indicate IgG2/IgG1 ratio = 1 and solid bars show means. ●, women with IgG2 dominance. ○, women with IgG1 dominance.

2). In addition, the levels of IgG2/IgG1 ratio were significantly higher in patients where CIN regressed compared with patients where CIN persisted (1.2 [±0.2] versus 0.8 [±0.1] in mean [±SEM], Mann-Whitney U test $p = 0.045$) (Fig. 2).

DISCUSSION

At present, oncogenic HPV L1-capsids are the most attractive candidate for prophylactic HPV vaccine to prevent HPV infection and HPV-related cancer (21). Immunity against L1-capsids of bovine or cottontail rabbit papillomavirus was shown to protect the viral infection and regress the virus-induced tumor implanted in animals (22, 23). However, it is not yet clear whether immunity against HPV L1-capsids works in humans.

In the present study, we tested the hypothesis that Th1 response toward L1-capsids might favor elimination of HPV infection or determent of the progression of CIN to cancer. Here we demonstrated that the great majority (94%) of seropositive women with HPV16-negative normal cervix, that is, those who had been once infected with HPV16 and were cleared of it, showed IgG2-dominant reactivities (IgG2/IgG1 ratio >1). On the other hand, about half (48%) of the patients with HPV16-positive CIN and few (5%) with HPV16-positive cervical cancer showed IgG2 dominance. This cross-sectional study raises the possibility that IgG2 dominant status toward HPV16 L1-capsids, an enhanced Th1 response, may be in favor of the elimination of HPV16 infections and prevention of the progression of HPV16-positive CIN to invasive cervical cancer.

The prospective study looking at natural course of CIN patients revealed that CIN lesions of the patients with IgG2 dominance toward HPV16 L1-capsids have a tendency to regress more frequently, in keeping with the cross-sectional study. Although the number of followed up CIN patients is small, it appears that IgG2 dominance toward HPV16 L1-capsids can be a clinically useful marker which predicts the outcome of HPV16-positive CIN.

Recent cohort studies on patients with HPV16-positive CIN have demonstrated that the presence of IgG antibodies to HPV16 L1-capsids was not related to viral clearance or the regression of CIN lesions (24, 25). The observed higher incidence of HPV16 L1 IgG antibodies in HPV16-positive cancer patients (69%) seems to be consistent with this finding. These findings make sense, given that one of IgG subclass antibody toward HPV16 L1-capsids favors the regression of CIN and another IgG subclass does not, as was demonstrated here.

There are several reports indicating that Th1 cellular immunity to HPV16 E6 and E7 proteins is favorably involved in controlling HPV infection and in the regression of CIN (13, 26-28). However, it is unlikely that immunity against E6 or E7 is directly associated with viral clearance or the regression of CIN, because E6 and E7 are nuclear antigens that are overexpressed only in high-grade CIN and cervical cancer. Unlike E6 or E7, HPV capsid proteins which are expressed at an earlier stage of HPV infection may have serve as immune targets. Indeed, neutralizing IgG responses have been detected against HPV capsid proteins and, therefore, may play an important role in controlling viral spread and infection (29).

The hitherto employed methods to evaluate Th responses include lymphoproliferative response, interleukin-2 response of lymphocytes *in vitro*, cytotoxic T cell (CTL) response etc. (26-28). These seem to be unsuitable for clinical application because of low sensitivity and laboriousness. In this study, we took advantage of measuring IgG subclasses toward HPV16 L1 capsids. This method is very simple with high sensitivity compared with other methods as described. Besides, using HPV16 L1 capsids as specific antigens appeared

to be appropriate in view of the fact that the antibody against HPV16 E6 or E7 is rarely detectable in cytologically normal women and the seropositive rate is lower even in HPV16-positive CIN patients as compared with that of HPV16 L1 capsids (2-20% versus >50%) (13-17).

In summary, determination of IgG1 and IgG2 balance (IgG2/IgG1 ratio) toward HPV16 L1-capsids may serve as a useful marker for predicting elimination of HPV16 infections and the fate of HPV16-positive CIN lesions. To confirm this, a large-scale cohort study is now in progress.

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