

Immune Activation in Cervical Neoplasia: Cross-Sectional Association between Plasma Soluble Interleukin 2 Receptor Levels and Disease

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Abstract

In a previous study (Tsukui *et al.*, *Cancer Res.*, 56: 3967–3974, 1996), we observed an inverse association between degree of cervical neoplasia and interleukin (IL) 2 production by peripheral blood mononuclear cells in response to human papillomavirus (HPV) 16 E6 and E7 peptides *in vitro*. This suggested that a Th1-mediated cellular immune response might be important in host immunological control of HPV infection and that a lack of such a response might predispose to progression of cervical disease. To follow up on these findings, we have conducted a cross-sectional study of women with various degrees of cervical neoplasia to investigate the association between overall immune activation and cervical disease. A total of 235 women were recruited into our study; 120 of these women were participants in our previous study in which IL-2 production in response to HPV-16-specific peptides was measured. The study population included 34 women with invasive cancer, 62 women with high-grade squamous intraepithelial lesions (HSILs), and 105 women with low-grade squamous intraepithelial lesions (LSILs). In addition, 34 cytologically normal women with no past history of squamous intraepithelial lesions despite confirmed

HPV-16 infection in the 5 years preceding the study were selected as controls. As our measure of overall immune activation, serum samples obtained from study participants were tested for soluble IL-2 receptor (sIL-2R) level using an ELISA method. The mean sIL-2R levels were found to increase with increasing disease severity ($P_{\text{trend}} = 0.0002$). Among cytologically normal, HPV-exposed women, the mean receptor level in serum was 465.8 units/ml compared to 467.6 units/ml among LSIL subjects, 514.9 units/ml among HSIL subjects, and 695.5 units/ml among women with invasive cervical cancer. Similarly, the proportion of women with elevated sIL-2R levels (defined as ≥ 450 units/ml) increased with increasing disease severity from 35.2% among normal study subjects to 70.6% among cancer patients ($P_{\text{trend}} = 0.003$). Among the subgroup of subjects for whom *in vitro* IL-2 production in response to HPV-16-specific peptides was measured, we examined the association between *in vitro* IL-2 production and serum levels of sIL-2R. sIL-2R levels were higher, on average, among those women who were positive in our IL-2 production assay compared to those who were negative, but the differences did not reach statistical significance ($P > 0.05$). We also observed a trend of increasing sIL-2R level with increasing disease severity both in women who were positive and in women who were negative for our IL-2 production assay, but the trend was only significant among those who were negative for IL-2 production ($P_{\text{trend}} = 0.01$). Results from our studies suggest that although the immune system of women with cervical neoplasia is nonspecifically activated as disease severity increases, the ability of those women with HSILs or cancer to mount a Th1-mediated immune response to HPV peptides appears to decrease compared to women with LSILs or normal women infected with HPV. Increased overall activation along with decreased Th1 immune response among women with increasing cervical disease severity might be explained by an increased Th2-mediated immune response, a response that we hypothesize is ineffective in controlling the viral infection and its early cytological manifestations. Future studies should directly assess Th2-mediated responses to confirm this hypothesis. Also, future efforts should be aimed at determining whether the associations observed are causally related to disease progression or an effect of the disease.

Introduction

Cervical HPV³ infection and the related LSILs of the cervix are precursors to the more clinically important HSILs of the cervix

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³ The abbreviations used are: HPV, human papillomavirus; PBMC, peripheral blood mononuclear cell; IL, interleukin; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; IL-2R, IL-2 receptor; sIL-2R, soluble IL-2R; Th1, T-helper cell type 1; LSM, lymphocyte separation medium; STM, Specimen Transport Medium.

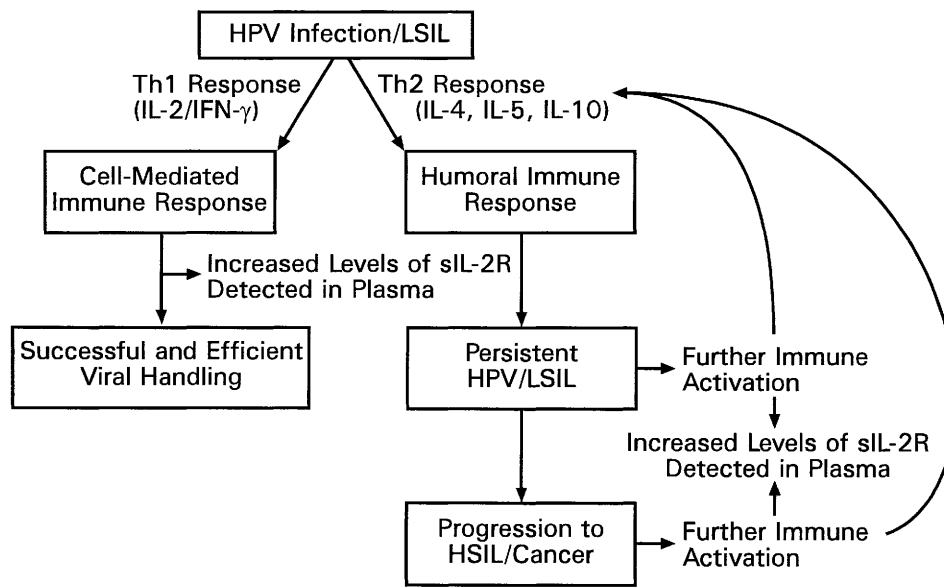


Fig. 1. Working hypothesis: role of overall levels of immune activation and Th1/Th2-type immune responses and cervical disease.

and cervical cancer (1, 2). Whereas genital HPV infection and its early cytological manifestation, LSILs, are relatively common among sexually active women, HSILs and cancer are rare. This is due, in part, to the early detection and control of LSILs by Pap smear screening programs, but is believed to also reflect the ability of the host to mount an immune response capable of controlling the infection and its low-grade cellular manifestations. In fact, HPV infection and LSILs have been shown to commonly regress without any intervention (3, 4). In one study, spontaneous regression of HPV infection among cytologically normal women was observed to be 59% over an average of 15 months among women initially found to be HPV DNA positive by PCR (3). Similarly, studies of LSILs diagnosed by cytology have noted high rates of spontaneous regression. In a study of 555 women with mild cervical dysplasia, a 62% regression rate was observed over an average of 39 months, with progression to high-grade lesions observed in only 16% of women followed (4).

Studies suggest that host immune response to HPV (cell-mediated response in particular) is critical for effective control of HPV infection (5–15). Animal studies have demonstrated that immunized animals are protected from papillomavirus infection and the development of neoplasia and that immunization facilitates regression of existing lesions (6, 7, 11). In addition, the incidence of HPV-related diseases is increased among transplant recipients and HIV patients, both of whom are known to have reduced cell-mediated immunity with maintenance of normal humoral immune function (5, 8). Furthermore, investigators have observed the infiltration of CD4+ (T-helper cells) and CD8+ (T-cytotoxic cells) T cells in spontaneously regressing warts, depletion of antigen-presenting cells in the cervix of women with cervical cancer, and an association between HLA haplotypes and cervical disease risk, all of which suggest an important role for the cellular immune system in the host response to HPV infection and low-grade cervical lesions (9, 10, 12–15).

It has been hypothesized that a Th1 response to HPV infection and LSILs is important for successful handling of this virus and that a shift to a Th2 response results in an ineffective response to HPV infection (16). It is thought that Th1-type

responses (mediated through the production of various cytokines, including IL-2 and IFN- γ) augment cell-mediated immunity, whereas Th2 responses (mediated by a different set of cytokines, including IL-4, IL-5, and IL-10) preferentially augment humoral immunity (see also Fig. 1). This Th1/Th2 hypothesis is congruent with data demonstrating that Th1-type responses preferentially protect against various chronic parasitic, bacterial, and viral diseases (17–22). Also, results from a previous study conducted by us demonstrated an inverse association between a Th1-type response and cervical neoplasia (16). In this previous study, levels of IL-2 produced *in vitro* by PBMCs in response to stimulation with HPV-specific peptides were measured as a marker of a Th1-type response, and the fraction of responders were found to correlate inversely with severity of cervical disease; *i.e.*, the fraction of women making IL-2 in response to HPV-16 E6 and E7 peptides decreased as disease severity increased. These results could not be attributed to a generalized state of immune dysfunction because responses to a control antigen, influenza virus, occurred in a comparable proportion of women in each disease group examined. Thus, results from this previous study supported our *a priori* hypothesis that the ability of a woman to respond to HPV-specific peptides and to produce cytokines (IL-2 in this case) that preferentially activate cell-mediated responses would be a useful biomarker of adequate immune response to HPV.

In the present study, we examined an index of overall cellular immune activation among women with various degrees of HPV infection and cervical neoplasia and correlated these findings with those from our previous study, which examined IL-2 levels produced *in vitro* in response to HPV-specific peptides. As a marker of overall cellular immune activation, we measured plasma levels of sIL-2R (23, 24). sIL-2R is one of several markers of immune activation and was chosen for this study for the following reasons: (a) IL-2R is expressed in mononuclear cells, such as T cells, B cells, and monocytes (23–26); (b) the α -chain (also known as Tac) of the IL-2R is expressed only in activated mononuclear cells (23, 24); (c) the IL-2R α chain has been shown to be released by mononuclear cells in fully soluble form and to be detectable in plasma (27–30); and (d) previous studies have demonstrated elevated

serum levels of sIL-2R in various autoimmune conditions (including rheumatoid arthritis and systemic lupus erythematosus), among transplant recipients who subsequently reject their graft, and in individuals with a variety of viral infections (including HIV and hepatitis) or advanced hematological tumors (23, 31–37).

The present cross-sectional study examined levels of sIL-2R in plasma collected from 235 women with cervical cancer, HSILs, LSILs, or normal cytology despite exposure to HPV. One hundred twenty women previously examined in our study of *in vitro* response to HPV-specific peptides were included in the present study (16). Thus, we were able to correlate sIL-2R levels with disease and with our previous measure of Th1 response to HPV in a subset of women.

Materials and Methods

Study Subjects. The present study of 235 women was designed as a cross-sectional study to examine the association between plasma levels of sIL-2R and cervical neoplasia. Cases were women with histologically confirmed invasive cervical cancer ($n = 34$) or evidence of HSILs ($n = 62$) or LSILs ($n = 105$; 72 confirmed, 33 equivocal) confirmed by expert pathology review (D. R. S., M. E. S., R. J. K.). Subjects were seen either at one of the Kaiser Permanente clinics in Portland, Oregon (HSIL and LSIL cases) or at the Simmons Cancer Center of the University of Texas Southwestern Medical Center in Dallas, Texas (invasive cancers). Controls were a group of 34 women who were participants in a large cohort study conducted at the same Kaiser Permanente clinics from which the LSIL and HSIL cases were recruited (38). These 34 control subjects were women who were originally selected as controls for our study examining *in vitro* response of PBMCs to HPV-16 peptides. They were all cytologically normal with no history of squamous intraepithelial lesions at the time of enrollment into the cohort (1989–1990) but were also known to have been HPV-16 DNA positive an average of 3.5 years prior to the time of their participation in the present study (1994–1995). The 235 women examined in this study represent 78.9% of 298 women determined to be eligible for study in the participating clinics and represent a convenience sample of women with various degrees of cervical disease. Among the 63 women who did not participate in the study, 44 (14.8%) refused blood collection. The remaining 19 women agreed to participate, but their samples were not tested due to sample hemolysis ($n = 11$; 3.7%) or loss ($n = 8$; 2.7%).

Biological Specimens. A total of 30–70 ml of peripheral blood were collected into heparinized Vacutainer tubes, kept at room temperature, and sent to the processing laboratory in Bethesda, MD, via overnight air carrier. At the processing laboratory, plasma was recovered by centrifugation, aliquoted, and stored at -70°C until ready for sIL-2R testing. PBMCs were separated on lymphocyte separation medium (LSM, Organon Teknika Corp., Durham, NC) and used fresh for *in vitro* stimulation with HPV-specific antigens. Ten-ml cervicovaginal lavage samples were collected from all Portland subjects with the exception of 39 women for whom a Dacron swab sample stored in STM (Digene Corporation, Silver Spring, MD) was used as the source of cervicovaginal cells. Tumor blocks were obtained from the invasive cancer cases recruited from Dallas, TX. Lavages, STM samples, or tissue blocks were used for HPV DNA testing.

Measurement of sIL-2R. Plasma samples were tested for sIL-2R (10,000 smaller than the M_r 55,000–60,000 membrane-bound IL-2R protein) using CellFree IL-2R test kits (Endogen,

Cambridge, MA). In brief, frozen samples were allowed to thaw at room temperature prior to preparation of sIL-2R analysis by the procedure provided in the test kit. The 96-well test plate provided space for 1 blank, 6 standards (0, 384, 823, 1658, 3424, and 6487 units/ml), 2 controls, and 39 test samples run in duplicate. The plates received 50 μl /well of designated standard, control, or test sample and, except for the blanks, 50 μl of horseradish peroxidase-conjugated murine monoclonal antibody to human IL-2R. The plates were sealed and incubated on a stationary tabletop at room temperature (24°C) for 3 h. Following incubation, the plates were unsealed, the solutions were aspirated from all wells, and the plates were washed three times with approximately 250 μl /well each of wash buffer (buffered solution of detergent). After removal of the final wash aspirate, 100 μl of chromogen solution (containing *O*-phenylenediamine and fillers) were added to each well, including the blanks, and the plates were incubated uncovered for 30 min at room temperature (24°C) in a stationary position. Upon completion of incubation, 50 μl of stop solution (5.8 ml of concentrated sulfuric acid to 80 ml of deionized water) were added to all wells, including the blanks. The plates were read on a MAXline microplate reader (Molecular Devices Corp., Menlo Park, CA) at an absorbance of 490 nm and analyzed by SOFTmax software, Version 2.01 (Molecular Devices Corp.). Samples from 30 study subjects (12.6%) were selected at random and tested in duplicate. Duplicate testing was performed in a blind fashion. Comparison of results obtained from these blind replicates revealed good agreement; the Pearson correlation coefficient between the two measurements was 0.95. The mean sIL-2R level for these 30 samples was 465.7 units/ml at first measurement (median, 413.5; range, 264–894) and 477.5 units/ml at second measurement (median, 431.5; range, 214–843). The coefficient of variation, calculated as the SD of the sIL-2R levels divided by the mean level, was 7.63%.

***In Vitro* Stimulation and Measurement of IL-2.** *In vitro* stimulation assays and measurement of IL-2 from supernatants of proliferated cultures were performed as described previously (16). In brief, cultures containing 3×10^5 PBMCs were either unstimulated or stimulated with overlapping sets of soluble 10–16-mer HPV-16 E6 and E7 peptides (six peptide mixtures were used, three containing E6 peptides and three containing E7 peptides), influenza virus, or phytohemagglutinin, each in triplicate. The soluble peptides used in the mixtures covered 88% of the E6 sequence and the entire E7 sequence. On day 6, supernatants were harvested from each well and assayed for IL-2. IL-2 in the culture supernatant was measured by culturing CTLL-2 cells with the supernatant described above at three dilutions (1:2, 1:4, and 1:8). After 24 h, 1 μCi of [^3H]thymidine was added for the final 18 h, cells were harvested, and uptake of tritium into DNA was determined using a β -plate scintillation counter (LKB, Uppsala, Sweden) as a measure of cell proliferation. Cell proliferation levels are reflective of IL-2 levels in the supernatant, because CTLL-2 cells are dependent on IL-2 for growth. A sample was considered positive for IL-2 production upon stimulation only if it met three separate criteria, each for at least two of the three dilutions at which testing was performed: (a) the mean [^3H]thymidine incorporation (cpm) of each triplicate set had to be at least twice that of the medium control background; (b) the difference between the mean cpm of each triplicate set and the mean cpm of the medium control background had to be greater than 500; and (c) the experimental triplicate sets had to be statistically significantly greater than the medium control triplicate sets by Student's *t* test ($P < 0.05$).

Table 1 Characteristics of the study population

Characteristics	Normal controls (n = 34) ^a	LSIL (n = 105)	HSIL (n = 62)	Cancer (n = 34)
Age (yr) ^b				
Median	28.5	30	31.5	41.1
Mean	31.3	32.4	32.3	45.7
Range	(22–51)	(17–79)	(19–49)	(30–68)
Current HPV ^c				
% positive	20.6%	57.1%	68.9%	92.9%
% HPV-16	17.7%	25.7%	44.8%	78.6%
% HPV-other	2.9%	31.4%	24.1%	14.3%

^a All normal controls are known to have been HPV-16 DNA positive at some time during the 5 years preceding this study.

^b Two invasive cancer cases with unknown age at diagnosis were excluded.

^c HPV DNA data are available on 34 normal controls, 105 LSIL cases, 58 HSIL cases, and 14 cancer cases.

HPV DNA Testing. HPV testing was conducted on lavage and STM samples using the Hybrid Capture tube test (Digene Corp.) formatted to detect 16 HPV types (types 6, 11, 16, 18, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 56, and 58), as described previously (39). Samples were screened for overall positivity, and those found to be positive were further tested using an HPV-16-specific Hybrid Capture probe to determine which HPV positive samples were positive for HPV-16. For cervical tumor specimens, DNA was processed from formalin-fixed, paraffin-embedded sections (50 μ m) as described (40). HPV DNA was detected using the HPV general primer-mediated GP5+/6+ amplification system as described (41). A cellular 268-bp β -globin fragment was amplified to verify that each sample contained DNA of sufficient quality (42).

Statistical Methods. Median and mean levels of sIL-2R by disease status and IL-2 positivity were computed and compared by using Student's *t* test, ANOVA, and test for linear trend (43). Contingency table analyses were used to examine the association of categorical sIL-2R levels with disease status and with IL-2 production. The Pearson χ^2 test and the Mantel-Haenszel χ^2 test for trend were used to determine statistical significance of the findings (43). Analysis was also performed following log transformation of sIL-2R values to normalize the data. Results were similar to those obtained using untransformed data. Thus, results presented herein are based on analysis of untransformed data.

Results

Two-hundred thirty-five women were included in the present analysis. The average age of women in different disease categories is presented in Table 1. Normal control women were, on average, the youngest group studied (median age, 28.5 years), whereas women with invasive disease were the oldest (median age, 41.1 years; $P < 0.001$). HPV DNA prevalence by disease status is also presented in Table 1. HPV status was known for 211 (89.8%) subjects. Nearly all women with invasive disease for whom HPV data were available were HPV DNA positive (92.9%), and the majority of these were positive for HPV-16. Positivity rates among women with HSILs and LSILs were 68.9% and 57.1%, respectively. HPV-16 DNA positivity accounted for nearly two-thirds of positive HSILs and one-half of positive LSILs. Although all women selected as normal controls were known to be positive for HPV-16 DNA at some time in the 5 years immediately preceding recruitment into the present study, only seven (20.6%) were positive for HPV DNA

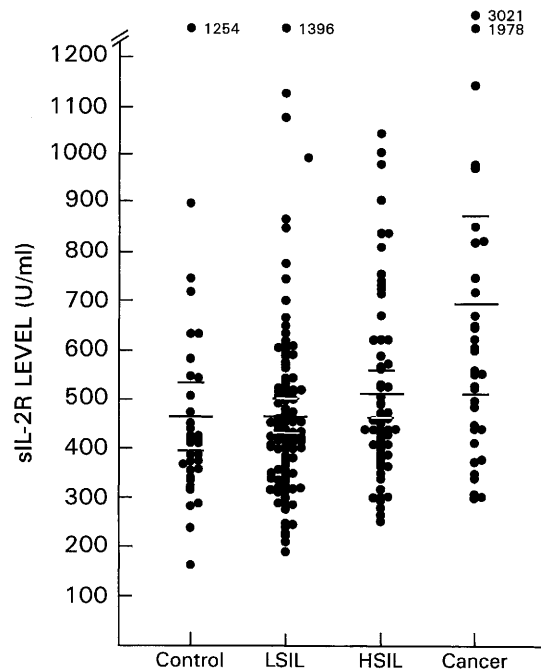


Fig. 2. Mean and 95% confidence interval of sIL-2R level among women with LSILs, HSILs, and cancer and controls.

Table 2 Distribution (%) and mean level of sIL-2R by disease status

	Normal controls (n = 34)	LSIL (n = 105)	HSIL (n = 62)	Cancer (n = 34)
sIL-2R ^a				
<370 units/ml	11 (32.4%)	30 (28.6%)	12 (19.4%)	5 (14.7%)
370–449 units/ml	11 (32.4%)	28 (26.7%)	18 (29.0%)	5 (14.7%)
≥450 units/ml	12 (35.2%)	47 (44.7%)	32 (51.6%)	24 (70.6%)
Mean level, units/ml (SD)	465.8 (205.2)	467.6 (183.2)	514.9 (191.3)	695.5 (518.8)
Median level, units/ml	413.5	431.0	452.5	557.5
sIL-2R range	164–1254	190–1396	251–1043	298–3021

^a Mantel-Haenszel χ^2 test for trend, 8.7; $P = 0.003$.

at the time of our study, and all but one woman was positive for HPV-16 DNA.

sIL-2R levels obtained from individual study subjects are plotted by disease category in Fig. 2. Women in the normal control group had the lowest overall sIL-2R levels (mean, 465.8 units/ml), whereas women diagnosed with invasive cervical cancer had the highest overall levels (mean, 695.5 units/ml; P comparing two means = 0.02). Women in the HSIL group had levels that were intermediate between normal women and those with invasive disease (mean, 514.9 units/ml), whereas those in the LSIL group had overall levels that were very similar to those observed for the normal control group (mean, 467.6 units/ml; $P_{\text{trend}} = 0.0002$).

Soluble receptor levels were categorized approximately into tertiles based on the distribution among controls (<370 units/ml, 370–449 units/ml, and ≥450 units/ml) and compared among the four study groups (Table 2). The proportion of women with elevated levels of sIL-2R (≥450 units/ml) was highest for women in the invasive cancer group (70.6%),

Table 3 Mean sIL-2R level by *in vitro* IL-2 response,^a and disease status

	IL-2 negative (n = 96) ^b	IL-2 positive (n = 24) ^a	P ^c
Disease category			
Normal (n = 23)	384.7 (406)	483.9 (474)	0.10
LSIL (n = 50)	474.5 (426)	511.4 (461)	0.56
HSIL/cancer (n = 47)	579.0 (459)	576.7 (528)	0.98
P ^d	0.01	0.29	

^a IL-2 production *in vitro* in response to HPV-specific peptides.

^b Mean (median) sIL-2R level, units/ml.

^c Comparing mean sIL-2R levels between women positive and negative for IL-2 production.

^d Test for linear trend, comparing mean sIL-2R levels between women in different disease categories, within strata of IL-2 production.

intermediate for women in the HSIL (51.6%) and LSIL groups (44.7%) and lowest among normal women (35.2%; $P_{\text{trend}} = 0.003$).

Because both age (Table 1) and sIL-2R levels (Table 2) were positively associated with disease status, we examined the possible confounding effect of age on the sIL-2R-disease association noted in Table 2. We observed, within each of the case and control groups, that age was inversely associated with sIL-2R levels in our population (*i.e.*, soluble receptor levels decreased with increasing age), suggesting that the association between sIL-2R levels and disease that we observed in this study cannot be explained by age confounding (data not shown). In fact, because age is positively associated with disease and inversely related to sIL-2R levels, the expectation is that control for age would strengthen the association between sIL-2R level and disease.

Because selection of our normal controls was conditional on having a history of HPV-16 infection, we also restricted our analysis to those women known to be HPV-16 DNA positive (*i.e.*, we excluded from analysis those women with LSILs, HSILs, or cancer not known to be HPV-16 positive). A non-significant pattern similar to that reported in Fig. 2 was observed. In our HPV-16-restricted analysis, the mean (and median) sIL-2R levels observed among women in each disease group were as follows: 465.8 units/ml (413.5 units/ml) for the normal control group; 487.7 units/ml (417 units/ml) for the LSIL group; 494.6 units/ml (452.5 units/ml) for the HSIL group; and 566.4 units/ml (441 units/ml) for the group of women diagnosed with invasive cancer ($P_{\text{trend}} = 0.20$).

Among the control and LSIL study groups, we examined sIL-2R levels separately by HPV DNA status at the time of our study. Among controls who tested negative for HPV DNA at the time of our study ($n = 27$), the mean sIL-2R level was 464.1 units/ml (median, 408 units/ml). Among HPV DNA-positive controls ($n = 7$), the comparable figure was 472.4 units/ml (median, 434 units/ml). The mean sIL-2R level for the two groups was not found to be significantly different ($P = 0.93$). Similarly, among the LSIL group, the mean sIL-2R level was 457.6 units/ml (median, 426 units/ml) among those who were HPV DNA negative ($n = 45$) and 475.2 units/ml (median, 434.5 units/ml) among those who were HPV DNA positive ($n = 60$). Again, the mean sIL-2R level for these two groups was not significantly different ($P = 0.63$).

Among the 120 women for whom IL-2 results were available from our *in vitro* stimulation assays, sIL-2R and IL-2 results were compared (Table 3). Comparisons were made within study groups to avoid confounding by disease. In this analysis, women with HSILs and cancer were combined be-

cause only five women with HSILs and two women with cancer tested positive for IL-2 production. sIL-2R levels were nonsignificantly elevated among women with positive IL-2 production compared to those classified as IL-2 negative in the normal and LSIL study groups. Among women with HSILs/invasive cancer, the median sIL-2R level was found to be nonsignificantly higher among women testing positive for IL-2 production, but the mean sIL-2R levels among women testing negative and positive for IL-2 production were found to be nearly identical. When categorical analyses were performed, the proportion of women positive on the IL-2 assay was higher among women with elevated sIL-2R levels (≥ 450 units/ml) compared to women with low levels of the receptor (< 370 units/ml) in each of the study groups (data not shown). Although we were hampered by small sample numbers, we also compared the mean and median level of sIL-2R by disease status separately for women who tested positive and negative for IL-2 production (Table 3). The mean and median sIL-2R level was found to increase with disease severity among both groups of women. However, a statistically significant trend of increasing sIL-2R level with increasing disease severity was only observed among the more sizable group of women who tested negative for IL-2 production ($P_{\text{trend}} = 0.01$).

Discussion

Results from the present analysis suggest a positive association between levels of sIL-2R in plasma and cervical neoplasia among women exposed to HPV infection. Given that the α -chain of IL-2R measured in our study is known to be expressed in activated but not resting mononuclear cells (including T cells, B cells, and monocytes; Refs. 23–26), our results can be interpreted as indicating that the level of overall immune activation increases in women as severity of cervical disease increases.

In our previous investigation involving 140 women (120 of whom were also participants in the present study), we observed an inverse association between degree of cervical neoplasia and IL-2 production by PBMCs upon *in vitro* stimulation with HPV-specific peptides (16). Because IL-2 is a cytokine produced by T cells undergoing a Th1-type immune response but not by T cells undergoing a Th2-type immune response or other immune cells, we concluded in that study that our findings were suggestive of a protective role of a Th1-type response against cervical disease progression. Taken together, results from these two studies suggest that whereas the immune system of women with cervical neoplasia is increasingly activated as disease severity increases, the ability of those women with HSILs or cancer to mount a Th1-mediated immune response specific for HPV peptides appears to decrease compared to women with LSILs or cytologically normal women with HPV infection. This increasing overall immune activation (as measured by serum sIL-2R levels) along with a decreasing HPV-specific Th1 immune response (as measured by IL-2 production in response to stimulation of PBMCs with HPV-16-specific peptides) among women with increasing cervical disease severity might be suggestive of an increased Th2-mediated immune response, a response previously hypothesized to be linked to ineffective response to chronic parasitic, bacterial, and viral infections (see also Fig. 1; Refs. 16–22). Alternatively, responses mediated by immune cells other than Th1 or Th2 cells or nonspecific Th1 responses might be elevated, whereas HPV E6 and E7 peptide-specific responses are decreased.

Although, to our knowledge, other studies that correlate sIL-2R levels with degree of cervical neoplasia have not been

conducted, a few previous studies have attempted to examine the association between neopterin, another serum measure of overall immune activation, and cervical neoplasia (44, 45). In concordance with our findings, these studies observed an increase in serum neopterin levels among women diagnosed with invasive cervical cancer as disease severity increased (44). However, the study that examined neopterin levels in women with preinvasive squamous intraepithelial lesions did not observe any increase in serum neopterin levels among LSIL or HSIL cases relative to population-based controls (45). It is unclear whether this discrepancy is due to differences in the populations studied or differences in the markers of immune activation used.

Potential limitations of the present study should be mentioned. First, women diagnosed with invasive cervical cancer in the present study were recruited from Texas and were not derived from the same population from which the remaining three study groups were derived. Thus, elevated sIL-2R levels among women diagnosed with cancer might theoretically reflect differences among the populations studied rather than a true disease association. This possibility is unlikely to completely explain our findings, however, given that women diagnosed with HSILs, all of whom were recruited from the same clinics from which the LSIL and normal study groups were selected, exhibited levels of plasma sIL-2R that were intermediate between those seen among cytologically normal women and cervical cancer patients. Secondly, concomitant sexually transmitted diseases were not ascertained as part of our study, and we are therefore unable to rule out the possibility that our results are due to increased concomitant infection with other agents among women with HSILs and cancer. However, we have noted in a separate analysis of women enrolled in our Portland Kaiser cohort⁴ that women diagnosed with HSILs are less likely than women diagnosed with LSILs to report multiple recent sexual partners (defined as the number of partners in the past 28 months, on average). In fact, the percentages of women diagnosed with HSILs and LSILs and cytologically normal women who reported three or more recent partners were 18, 33, and 8%, respectively. Assuming that recent sexual behavior is a good correlate of prevalence of sexually transmitted diseases, concomitant infections among diseased women is unlikely to be an explanation for our present findings.

Although the combined results of this and our previous investigation (16) are consistent with the theory that a Th1-type of immune response is protective against progression of low-grade cervical lesions, whereas a Th2-type of immune response is permissive of disease progression, additional studies are needed to directly measure humoral and Th2 types of immune response to confirm their permissive role in disease progression. Larger, more comprehensive studies are required in which both humoral and cellular immune responses are measured in parallel and in a prospective fashion to clarify the role of immune response to HPV and cervical lesions in cervical cancer pathogenesis.

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