Soluble Interleukin 2 Receptor Levels and Cervical Neoplasia: Results from a Population-based Case-Control Study in Costa Rica

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Abstract

Progression from infection with human papillomavirus (HPV) to cervical cancer in some women is thought to involve a permissive host environment, one in which immune response is mobilized in an inappropriate manner. In a previous study (A. Hildesheim *et al.*, Cancer Epidemiol. Biomark. Prev., *6:* 807–813, 1997), increasing levels of soluble interleukin 2 receptor (sIL-2R), a known proxy for general immune activation, was found to be positively associated with increasing levels of cervical neoplasia. We attempted to confirm this finding by conducting a nested case-control study of 478 women within a 10,000-woman population-based cohort in Costa Rica. We selected for the study all of the women diagnosed (at enrollment into the cohort) with: (*a*) low-grade squamous intraepithelial lesions (LSIL, $n = 191$); (*b*) high-grade squamous intraepithelial lesions (HSIL, $n = 130$); or (*c*) cancer ($n = 37$). Controls were 120 cytologically normal, HPV-negative women selected from a random sample of the entire cohort. A questionnaire was administered to participants to elicit information on cervical cancer risk factors. All of the women received a pelvic examination during which cervical cells were collected and used for HPV DNA testing by PCR. Blood samples were also collected. Plasma obtained from the blood samples was tested for sIL-2R levels by ELISA. Results indicated that sIL-2R levels increased with age. Among controls, we observed that 44.3% of women over the age of 50 had high levels of sIL-2R (defined as >735 units/ml) compared with 15.8% of women <30 years of age ($P = 0.008$). When women with cervical disease (LSIL+) were compared with controls, women in the upper quartile of the sIL-2R distribution had an age-adjusted odds ratio (OR) of 2.1 [95% confidence interval (CI), 1.1–4.1]. Comparing each advancing state of neoplasia with its precursor, we found that women with LSIL had higher sIL-2R levels than controls (OR for upper quartile of sIL-2R, 2.3; 95% CI, 1.1–5.2; comparing LSIL cases with controls); women diagnosed with HSIL were similar to the LSIL group (OR for upper quartile of sIL-2R, 1.1; 95% CI, 0.5–2.4; comparing HSIL cases with LSIL cases); and those with cancer had higher sIL-2R levels than subjects with an HSIL diagnosis (OR for upper quartile of sIL-2R = 1.8; 95% CI, 0.5–7.1; comparing cancer cases with HSIL cases). These data suggest that among our study subjects, sIL-2R levels most likely rise as a response to the events of infection and cancerous invasion, but that sIL-2R levels are unlikely to be predictive of disease progression among women with LSIL.

Introduction

At a global incidence of about 471,000 cases per year, cervical cancer is the second most common cancer in women worldwide, with the highest rates seen in developing countries (1). The earliest etiological step identified is infection by HPV.[2] The virus causes LSIL, which can progress to HSIL and then cancer. However, HPV is unlikely to be a sufficient cause for the development of HSIL or cancer because LSIL is relatively very common (2). Infection is usually transient, but persistence of the infection is believed to be associated with progression to HSIL, the immediate precursor to cancer. The specific types of HPV most strongly associated with HSIL and cancer are also those found to be associated with persistence (3).

Persistence is also likely a function of the host immune system, especially the absence of a positive lymphoproliferative response to infection (4). Although the host immune response to HPV infection is poorly understood, it has been postulated that the cellular, or Th1 branch of the immune response, as opposed to the Th2 or humoral response, plays a crucial role in eliminating HPV. Transplant recipients and HIV patients, both

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[2] The abbreviations used are: HPV, human papillomavirus; LSIL, low-grade squamous intraepithelial lesions; HSIL, high-grade squamous intraepithelial lesions; Th1, T-helper cell type 1; Th2, T-helper cell type 2; IL-2R, interleukin 2 receptor; sIL-2R, soluble IL-2R; STD, sexually transmitted disease; OR, odds ratio; CI, confidence interval.

of whom have impaired cell-mediated immunity but normal humoral immunity, have an increased prevalence of HPV-related diseases (5, 6). Tsukui *et al.* reported that a greater proportion of peripheral lymphocytes taken from women with cervical lesions showed impaired *in vitro* Th1 cytokine production compared with their normal counterparts, and that this proportion grew with increasing severity of disease (7). Furthermore, Clerici *et al.* showed that while cases with more extensive cervical disease were more likely to have a lower Th1 cytokine response than controls, they were also more likely to have a higher Th2 response, which suggests that the activation of the humoral rather than the cellular branch may be an inappropriate response to HPV (8).

Increased levels of sIL-2R are known to a be a proxy for general immune activation. It is a nonspecific marker expressed by various cell populations, including both activated Th1 and activated Th2 cells (9). Unlike the more complicated functional immune assays used to measure Th1 and Th2 specific immune responses, sIL-2R can be measured with relative ease because a portion of it is proteolytically cleaved and is found in serum. In a previous cross-sectional study in Portland, Oregon, we observed increasing sIL-2R levels associated with worsening disease status (10). Taken in conjunction with parallel findings in which increasing Th1-specific cytokine production was associated with reduced risk of high-grade disease (7), this finding supports the hypothesis that a Th1-type response may be protective against HPV-related cervical disease, whereas activation that preferentially triggers a Th2 type of response is not.

The present study attempted to confirm the positive association of sIL-2R with disease observed in our previous study in the context of a larger population-based case-control study, taking into account detailed information on cofactors such as HPV type assessed by PCR and other cervical cancer risk factors.

Materials and Methods

Overall Study Design. The 478 women selected for the present nested case-control study are participants in a 10,000-woman population-based natural history study of cervical neoplasia in Costa Rica. The study is being conducted in Guanacaste, a rural province of Costa Rica with consistently high rates of invasive cervical cancer. Details of the cohort design and implementation have been described elsewhere (11).

In brief, the Costa Rica cohort is a random sample comprising approximately one-sixth of the total adult female population in Guanacaste Province. There were 10,738 women eligible for the study, of whom 10,049 (93.6%) were successfully enrolled between June 1993 and December 1994. Participants were interviewed with a brief standardized questionnaire on the major cervical cancer risk factors, including socioeconomic, demographic, sexual, reproductive, medical, and smoking history. Pelvic examinations were performed on all of the women reporting previous sexual activity ($n = 9175$; 96.9% of those eligible for pelvic examination). The examination included visual inspection, conventional Pap smear, ThinPrep slide, collection of cervical cells for cytological/virological studies, and two photographic images of the cervix (Cervigram). In addition, blood samples from 9969 women (99.2% of those interviewed) were taken. Those with abnormal cytological or Cervigram results were referred for colposcopy ($n = 2199$), as were a 2% random sample of the sexually active cohort consisting of 176 women. At the colposcopy visit, biopsies were taken if lesions were observed. Disease status at enrollment was determined by a review of cytological and histological material by expert pathologists (M. E. S, M. A., M. L. H.) as previously described in detail (11). To supplement the number of invasive cervical cancer cases available for study, medical records at all of the main tertiary care centers in Costa Rica were reviewed on a weekly basis, and all of the women from Guanacaste diagnosed with cancer of the cervix during the enrollment phase of our natural history project were asked to participate in our study. In all, 31 women were so identified, and from them an additional 25 (77%) women with cervical cancer were recruited.

Study Subjects. For the present study, we selected as cases all of the women diagnosed at enrollment into our cohort with LSIL or more severe disease. This comprised 191 women diagnosed with LSIL, 130 women diagnosed with HSIL, and 37 women diagnosed with invasive cervical cancer (12 from screening, supplemented by an additional 25). In addition, from among the random sample of 176 women described above, we selected as controls 120 cytologically normal women who tested negative for HPV DNA (as described below). Cytologically normal women who tested positive for HPV DNA at the cervix were excluded from the control group because HPV infection and LSIL are now understood to be varying manifestations of the same disease complex; inclusion of HPV-positive individuals as controls would, therefore, have been akin to including cases in the control group.

Measurement of sIL-2R. Blood samples were transported daily to field stations where aliquots of plasma were frozen at −30°C, shipped weekly on ice packs to San Jose, stored at −70°C, and sent periodically on dry ice to the National Cancer Institute repository. Samples were tested for sIL-2R using Cell-Free IL2R test kits (Endogen, Inc. Cambridge, MA). In brief, frozen plasma was allowed to thaw at room temperature and sealed onto test plates to be incubated with murine monoclonal antibody to human IL-2R for 3 h at room temp. After incubation, the plates were unsealed, and the solutions were aspirated from all of the wells; the plates were then washed three times with approximately 250 μl of wash buffer per each well. After the final wash aspirate was removed and 100 μl of chromogen solution was added, the plates were incubated uncovered for 30 min at room temperature, and 50 μl of Stop Solution was added. 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 (version 2.01) software (Molecular Devices Corp.) Methods were identical to those used in our previous study (10). Reproducibility of our testing method was demonstrated in the previous study, in which a random sample of subjects were selected for duplicate testing in a blind fashion. Comparison of the results obtained from those blind replicates revealed good reliability; the Pearson correlation coefficient between the two measurements was 0.95, and the coefficient of variation was 7.63%.

HPV DNA Testing. As described previously (12), cervical cells collected using a Dacron swab and stored in 1 ml of specimen transport medium (STM, Digene Corporation, Silver Spring, MD) were analyzed for the presence of HPV DNA by the L1 consensus primer PCR technique. In brief, cellular material was incubated with Proteinase K and then amplified with the MY09/MY11 L1 consensus primers using the PCR. The PCR solution (10 μl) was gel-electrophoresed, transferred by Southern blot to nylon filters, and hybridized to generic HPV probes and β-globin. Samples hybridizing to the β-globin probe but negative for the generic probe were considered HPV-negative. PCR products generically positive for HPV were then replica-probed using 42 different type-specific HPV probes.

Table 1 Distribution of age, HPV, and sIL-2R by study group

Characteristic	*P*	Controls	LSIL	HSIL	Cancer
Age					
N		12	191	130	37
Mean (SD)	<0.0001	41.6 ± 13	31.5 ± 11	39.2 ± 15	50.3 ± 17
Median		40	29	34	46
Range		18–73	18–85	18–85	20–83
HPV					
N			190	130	35
HPV +	0.001		134 (70.5%)	113 (86.9%)	30 (85.7%)
HPV 16 +	0.001		23 (12.1%)	56 (43.1%)	16 (45.7%)
16 +: HPV+			17.2%	49.6%	53.3%
sIL-2R (units/ml)					
N		112	176	121	36
Mean (SD)	0.08	611 ± 216	679 ± 636	689 ± 390	874 ± 870
Mean[a] (SE)	0.11	588 ± 50	713 ± 41	680 ± 47	802 ± 89
Median		586	572	580	665
Range		254–1198	259–8100	254–2882	15–5577

[a] Age adjusted.

Samples that were positive by the generic probe mix but negative by all of the type-specific probes were considered to represent "uncharacterized" HPV types.

Statistical Methods. The difference between means was analyzed using one-way ANOVA and analysis of covariance to adjust for age. The association between sIL-2r and cofactors among controls was analyzed using standard contingency table methods and a Mantel-Haenzel χ^2 test for linear trend. We used unconditional logisitic regression to estimate ORs, with adjustment for age, smoking, parity, and hormonal contraceptive use. When examining the different stages in the natural history of cervical neoplasia (*i.e.,* LSIL, HSIL, and cancer) we compared each advancing stage of disease against the stage that preceded it (*i.e.,* LSIL *versus* cytologically normal/HPV negative controls; HSIL *versus* LSIL; cancer *versus* HSIL). This was done to allow for a direct assessment of the association between sIL-2r levels and each advancing stage of disease.

Results

Four hundred and seventy-eight women were included in the analysis for this study. The average age, HPV status, and mean sIL-2R levels of the women in each disease category are presented in Table 1. The women with LSIL were, on average, the youngest group studied (median age = 29 years), whereas women with invasive disease were the oldest (median age = 46 years; standard χ^2 $P < 0.0001$). HPV testing was available on 475 (99.4%) of the 478 women studied. Women with LSIL had lower detection of HPV (70.5%) than those with HSIL or invasive disease (86.9 and 85.7%, respectively). About one-half of the HPV-positive HSIL (49.6%) and cervical cancer cases (53.3%) were specifically positive for HPV 16, and 17.2% of the HPV-positive LSIL cases tested positive for HPV 16.

Four hundred and forty-five (93.1%) women were tested for sIL-2R. Of the four study groups, sIL-2R levels were on average the lowest among the controls (age adjusted mean = 588 units/ml). The low-grade and high-grade groups had similar levels of sIL-2R (age adjusted means were, respectively, 713 and 680 units/ml), whereas the women with invasive cancer had higher average levels, although not significantly so (age adjusted mean = 802 units/ml; for difference in means, $P = 0.102$; $P_{trend} = 0.051$).

Table 2 examines sIL-2R levels among controls with relation to cervical neoplasia risk factors which might confound the sIL-2R/cervical neoplasia association, including age, menopausal status, parity, oral contraceptive use, and smoking status. Age was positively related to sIL-2R levels. The oldest control women also had the highest sIL-2R levels ($P = 0.008$). As a correlated finding, a larger percentage of postmenopausal women were distributed toward the highest quartile than their premenopausal counterparts ($P = 0.001$). Women who had never used oral contraceptives tended to fall into the higher sIL-2R quartiles, whereas more of those women who had used oral contraceptives at any point fell into the lower sIL-2R levels ($P = 0.06$). Also, women who had had six or more pregnancies had a greater likelihood of being in the highest sIL-2R quartile, although the relation of parity to sIL-2R levels was not statistically significant ($P = 0.10$). There was no significant difference in the distribution of sIL-2R levels according to smoking status ($P = 0.55$), but there were very few smokers in this population.

Table 2 Distribution of sIL-2R among controls, according to selected cervical neoplasm risk factors

	N	sIL-2R levels			
		q1 (*n* = 30)	q2 (*n* = 26)	q3 (*n* = 28)	q4 (*n* = 28)
Age					
18–29	19	26.3%[a]	21.1%	36.8%	15.8%
30–39	37	35.1%	32.4%	13.5%	18.9%
40–49	26	38.5%	19.2%	23.1%	19.2%
50+	30	6.7%	16.7%	33.3%	43.3%
$P_{trend} = 0.008$					
Menopausal status					
Pre	79	32.9%	26.6%	22.8%	17.7%
Post	33	12.1%	15.2%	30.3%	42.4%
$P_{trend} = 0.001$					
Oral contraceptive use					
Never	41	14.6%	22.0%	31.7%	31.7%
Former	44	31.8%	25.0%	27.3%	15.9%
Current	27	33.3%	23.1%	10.7%	28.6%
$P_{trend} = 0.06$					
Number of pregnancies					
0–2	34	26.5%	23.5%	29.4%	20.6%
3–5	36	38.9%	27.8%	16.7%	16.7%
6+	42	16.7%	19.1%	28.6%	35.7%
$P_{trend} = 0.10$					
Smoking					
Never	104	26.0%	23.1%	26.0%	25.0%
Ever	8	37.5%	25.0%	12.5%	25.0%
$P_{trend} = 0.55$					

[a] All percentages are row percentages.

Age-adjusted ORs for sIL-2R quartiles were computed using logistic regression. When subjects with LSIL, HSIL, or cancer were combined and compared against controls, women in the second, third, and fourth quartiles of sIL-2R had age-adjusted ORs of disease of 2.2 (95% CI, 1.2–4.2), 1.9 (95% CI, 0.98–3.5), and 2.1 (95% CI, 1.1–4.1), compared with women in the lowest quartile of sIL-2R. To examine the various steps in the natural history of cervical neoplasia separately, we next compared each advancing stage of neoplasia against its precursor state (*i.e.,* LSIL *versus* controls, HSIL *versus* LSIL, and cancer *versus* HSIL). Results are presented in Table 3. When the LSIL group was compared with the control group, the highest sIL-2R quartile had a significantly higher age-adjusted OR of 2.3 (95% CI = 1.1–5.2) when compared with women in the lowest quartile of sIL-2R, but no clear dose-response trend of increasing odds of disease with increasing level of sIL-2R

Table 3 Distribution and risk associated with sIL-2R levels at different stages of cervical neoplasia

sIL-2R	(units/ml)	Controls *N*	LSIL (n = 176) *vs.* Controls (n = 112)		HSIL (n = 121) *vs.* LSIL (n = 176)		Cancer (n = 36) *vs.* HSIL (n = 121)	
			OR[a]	95% CI	OR	95% CI	OR	95% CI
Quartile 1	(0–439)	30	ref		ref		ref	
Quartile 2	(440–579)	26	2.0	(0.96–4.1)	1.4	(0.67–2.9)	0.71	(0.16–3.1)
Quartile 3	(580–734)	28	1.6	(0.75–3.4)	1.2	(0.55–2.6)	1.8	(0.45–7.1)
Quartile 4	(735+)	28	2.3	(1.1–5.2)	1.1	(0.47–2.4)	1.8	(0.47–7.1)

[a] Adjusted for age.

levels was observed. No increase in odds of disease with increased sIL-2R levels was observed when women in the HSIL group were compared with those diagnosed with LSIL, and the OR associated with the upper quartile of sIL-2R was 1.1 (95% CI, 0.47–2.4). Finally, when cancer cases were compared with those diagnosed with HSIL, a 1.8-fold increase odds of disease was observed for women in the upper *versus* lowest quartile, but the association was not statistically significant (95% CI, 0.47–7.1), and a clear trend with increasing levels of sIL-2R was not observed (P = 0.32).

Further adjustment for smoking, parity, oral contraceptive use, or menopause did not appreciably change the results (data not shown). A self-reported history of a diagnosis of a STD was also considered, to examine the possibility that the sIL-2R effects that we observed were due to confounding by STDs associated with HPV. Although few (4.3%) women recalled being diagnosed with a STD by a doctor, the proportion of women diagnosed with a STD was similar for each disease category (data not shown).

Discussion

The present data on serum sIL-2R represent the first to be obtained from the sampling frame of a large population-based cohort. We confirm reports that mean sIL-2R levels rise with age (9), most clearly after age 50. This is consistent with the findings that female sex and increasing age are independently associated with an increased percentage of CD4+ cells and a greater activation of T cells (13, 14). An association between sIL-2R and cervical disease was also observed. However, in contrast to our previous study of plasma sIL-2R and cervical neoplasia (10), in which the magnitude of the association increased as disease severity increased, the results from the present analysis do not suggest increasing levels of sIL-2R with increasing disease severity. Higher sIL-2R levels are most clearly associated with an increased risk of having LSIL. There is only a minimal elevation in risk for HSIL compared with LSIL and an approximately 2-fold risk for the highest sIL-2R levels among cancer cases *versus* those with HSIL.

The association of increased sIL-2R with LSIL may be due to a systemic response at the point of infection/transition to LSIL among some women. It is known from previous studies of IgG responses against HPV that the humoral branch of the immune response can be systemically activated by infection (15, 16). sIL-2R measured with concurrent LSIL may capture this initial host reaction to HPV. The association between sIL-2R and LSIL may also be due to the confounding by other sources of immune activation, including STDs or other pathogens in this population. Although the examination of STDs other than HPV in our study did not suggest residual confounding, the number of women reporting any history of non-HPV STD was very low and probably does not reflect the actual level of prevalence in the community.

We observed little evidence for an association between higher sIL-2R and the risk of having HSIL compared with having LSIL. This finding is understandable because the progression from LSIL to HSIL may be a process limited to the mucosa of the cervix and may, therefore, involve only local immune responses without systemic immune activation. A study of two other general systemic immune response markers, β-2 microglobulin and neopterin, showed an analogous lack of association with advancing cancer precursor lesions (17).

The higher sIL-2R levels observed among invasive carcinoma cases compared with HSIL cases is not surprising given that invasive disease is serious enough to induce systemic immunological changes. The levels of sIL-2R are known to rise when solid tumors metastasize (18). Here, increased sIL-2R is probably indicative of changes in response to malignancy. These data taken together suggest that among our study subjects, sIL-2R levels are most likely a marker of response to the events of infection and cancerous invasion rather than a marker of a host immune response that predicts the risk of progression from LSIL to HSIL.

This study was limited by the nature of the sIL-2R response. Our control population seemed to have a higher level of sIL-2R than in the previous study using identical methods (median sIL-2R, 413.5 units/ml *versus* 570 units/ml). Because samples from both of these studies were tested at the same laboratory using identical methods, the differences observed are unlikely to reflect laboratory differences. Also, the higher sIL-2R levels observed in the present study is not explained by the fact that our population was older (the mean ages for Portland and Costa Rica controls were 29 *versus* 40) because the age-adjusted mean levels for the two groups differ significantly (472 units/ml *versus* 600 units/ml respectively; P = 0.002). Our population may have been affected by increased background inflammation, which decreases the usefulness of an increased sIL-2R level as a marker for immune reaction to any one particular insult. sIL-2R, like β-2 microglobulin and neopterin, although a good marker of general immune response, does not—when used alone—seem to have the specificity to identify those at increased risk of developing serious cervical disease. More specific markers or functional studies would be helpful. We do not have functional data for actual Th1 cytokine production in peripheral blood monocytes from participants in this cohort and, therefore, cannot confirm the relationship suggested between the lack of Th1 activation and the increased sIL-2R response among women with cervical neoplasia. Also, our present data are only cross-sectional; studying women prospectively to see whose disease actually regresses *versus* those whose disease persists would be extremely useful. In the future, it may be beneficial to investigate the sIL-2R:IgG ratio at infection of women who later progress to HSIL or cancer, as a way of standardizing general activation against humoral re-

sponse. In ongoing work, we are currently tracking humoral and cellular responses simultaneously and prospectively.

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