

Expression of Interleukin-5 and Tumor Necrosis Factor Alpha in Cervical Carcinoma[▽]

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Interleukin-5 (IL-5) levels were significantly higher in vaginal washing fluids from patients with cervical carcinoma than in those from patients with carcinoma in situ and controls. Tumor necrosis factor alpha levels did not differ among the three groups. Detection of IL-5 in cervical secretions may be a useful marker for evaluating aggressive local immune response in cervical carcinoma.

Cellular immune response mediated by cytokines is the main defense against tumors related to cervical carcinogenesis. Previous studies have suggested that decreased T helper 1 (Th1) and increased Th2 responses are associated with cervical carcinogenesis (2, 3, 5, 6, 8). However, Th1 and Th2 cytokines, such as interleukin-1 β (IL-1 β), IL-10, IL-12, tumor necrosis factor alpha (TNF- α), and transforming growth factor beta, were elevated in cervicovaginal washings from patients with cervical carcinoma (12). Another study reported that the concentrations of TNF- α and IL-10 were increased in cases of cervical intraepithelial neoplasia (1). Although each study was different in terms of increased cytokine profiles, the results reveal that both Th1 and Th2 cytokines are increased in cervical carcinoma, which differs from previous reports of a shift toward a Th2 cytokine pattern during cervical carcinogenesis.

To date, most studies have focused on the cytokine profiles of the systemic immune response by analysis of peripheral blood. This study was designed to evaluate the cytokine secretion profiles for the Th2 cytokine IL-5 and the Th1 cytokine TNF- α in cervicovaginal secretions. In addition, we aimed to verify whether the levels of cytokines were related to eosinophil counts and human papillomavirus (HPV) DNA titers.

Women with abnormal cervical cytology who had been referred to the Women's Cancer Clinics of Severance Hospital between May 2006 and November 2006 were included. For all women, a cervical biopsy and HPV sampling were performed. Informed consent was obtained from each patient prior to enrollment. We recruited women who were diagnosed histologically with cervical carcinoma ($n = 20$) or carcinoma in situ (CIS) of the uterine cervix ($n = 6$). Women histologically diagnosed with chronic nonspecific inflammation were recruited for the control group ($n = 10$) (Table 1).

For cervicovaginal sample collection, all individuals lay in the supine position in a gynecological examination chair. Vaginal wash samples were collected by instilling 5 ml of phos-

phate-buffered saline, and approximately 3 ml was recovered by aspiration. Due to the presence of microbiota, a protease inhibitor cocktail was added (10 mM EGTA, 150 mM NaN₃, 0.01% [wt/vol] leupeptin [Sigma, St. Louis, MO], 0.02 M Pefabloc [Boehringer Mannheim, Indianapolis, IN]). IL-5 and TNF- α levels were measured using a commercially available human enzyme-linked immunosorbent assay kit (Biosource International, Inc., Camarillo, CA), according to the manufacturer's instructions. Cervical samples for HPV detection and typing were taken by a cervical sampler (Digene Corporation), and HPV DNA titers were measured by the Hybrid Capture 2 HPV DNA test (Digene Corporation, Gaithersburg, MD). HPV genotyping was performed with HPV DNAChip, a PCR-based DNA microarray system provided by Microarray Center, Biomedlab Co. (Seoul, South Korea). Peripheral venous blood samples were collected from patients with cervical carcinoma. A differential leukocyte count was performed with a Sysmex XE-2100 analyzer.

The cytokine data were presented as medians and interquartile ranges. The nonparametric Kruskal-Wallis test was used to assess the difference in cytokine levels between groups. Inter-group comparisons were evaluated by Dunn multiple-comparison tests. Correlations between the levels of cytokine in each group and eosinophil counts and HPV DNA titers were determined by the Spearman correlation coefficient. The statistical tests and graphing were performed using Prism 4 Windows software (GraphPad, Inc., San Diego, CA). P values of <0.05 were considered to be statistically significant.

The median IL-5 concentrations in the cervical carcinoma, CIS, and control groups were 25.50 pg/ml (interquartile range, 14.25 to 54.25 pg/ml), 12.50 pg/ml (10.50 to 19.00 pg/ml), and

TABLE 1. Demographic and clinical characteristics of patients in the control, CIS, and cervical carcinoma groups

Group (no. of patients)	Median age (yr) (range)	% with HPV infection	
		Total	Types 16 and 18
Control (10)	40.0 (28–58)	70	0
CIS (6)	44.5 (36–52)	100	66
Carcinoma (20)	55.0 (33–87)	100	50

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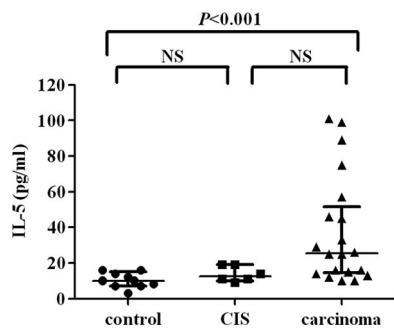


FIG. 1. Distribution of IL-5 (pg/ml) concentrations in cervicovaginal washings from the control ($n = 10$), CIS ($n = 6$), and cervical carcinoma ($n = 20$) groups. Estimated concentrations are plotted for each sample. Median levels and interquartile ranges are indicated. In women with cervical carcinoma, those with CIS, and the controls, the median IL-5 concentrations were 22.50 pg/ml (interquartile range, 14.25 to 54.25 pg/ml), 12.50 pg/ml (10.50 to 19.00 pg/ml), and 10.00 pg/ml (7.00 to 14.50 pg/ml), respectively. Those in women with cervical carcinoma were significantly higher than those in controls ($P < 0.001$; Kruskal-Wallis test followed by Dunn's multiple-comparison test). *, $P < 0.001$; NS, no significance.

10.00 pg/ml (7.00 to 14.50 pg/ml), respectively. IL-5 levels differed between groups ($P = 0.001$). The IL-5 concentrations in women with cervical carcinoma were significantly higher than those in the controls ($P < 0.001$) (Fig. 1). The median TNF- α concentrations in the cervical carcinoma, CIS, and control groups were 6.0 pg/ml (interquartile range, 2.25 to 8.00 pg/ml), 5.50 pg/ml (5.00 to 7.25 pg/ml), and 4.00 pg/ml (2.50 to 9.25 pg/ml), respectively. There were no significant differences in TNF- α concentration between groups ($P = 0.716$). For the cervical carcinoma, CIS, and control groups, the median ratios of IL-5/TNF- α were 5.23 (interquartile range, 2.20 to 12.88), 2.29 (1.83 to 2.74), and 2.02 (1.25 to 3.05), respectively. The ratio of IL-5/TNF- α had a tendency to increase according to the stage of the cervical lesion. However, there were no significant differences between groups ($P = 0.061$). IL-5 concentration showed no significant correlation with TNF- α concentration in the cervical carcinoma group ($r = 0.249$ and $P = 0.487$), the CIS group ($r = 0.754$ and $P = 0.086$), or the control group ($r = 0.128$ and $P = 0.590$). A positive correlation was found between eosinophil counts and IL-5 concentrations in women with cervical carcinoma ($r = 0.539$ and $P = 0.026$). In contrast, TNF- α concentrations did not correlate with eosino-

phil counts ($r = -0.011$ and $P = 0.966$) (Fig. 2). There was no correlation between HPV DNA titers and IL-5 or TNF- α concentrations in patients with cervical carcinoma or CIS ($r = 0.297$ and $P = 0.283$ or $r = 0.191$ and $P = 0.496$, respectively).

In our study, IL-5 concentrations were significantly higher in women with cervical carcinoma than in women with CIS or controls. TNF- α concentrations also tended to be higher in women with cervical carcinoma than in women with CIS or controls. Although our observation of cytokine correlations at different stages does not show a shift to the Th2 cytokine as reported previously, the ratios of IL-5/TNF- α tended to increase with the cervical lesion stages. These results indicate that the Th2 immune response in cervical carcinoma is relatively dominant.

The presence of an eosinophil infiltrate may be indicative of a less effective antitumor immune response (7, 10, 13). In addition, a dominant Th2 immune response might explain the poor clinical outcomes seen in cervical carcinoma patients with an eosinophilic tumor infiltrate (14). In this study, the higher levels of IL-5 in patients with cervical carcinoma than in those with CIS or controls suggest that the Th2 immune response is dominant in cancer tissue. The positive correlations between concentrations of IL-5 in cervicovaginal washings and eosinophil counts in peripheral blood samples suggest that IL-5 may promote eosinophil growth and activation, thereby inducing tissue infiltration in the tumor. Eosinophil counts could be elevated in peripheral blood because of this imbalance in the immune response.

The decreased Th1 cytokines, such as IL-2 and IFN- γ , and the increased Th2 cytokines, such as IL-4 and IL-10, were demonstrated to occur in the peripheral blood samples of women infected by HPV types 16 and 18 (9). Persistent high-risk HPV infection and increased viral loads have been shown to be correlated with high-grade cervical intraepithelial neoplasia and invasive cancer in previous studies (11, 15). In the present study, there were cases of high-risk HPV infection in women with cervical carcinoma and CIS. However, HPV DNA titers did not show significant correlation with cytokine levels.

The subjects of this study were classified according to their pathological diagnosis based on cervical biopsy. All controls had a chronic nonspecific inflammation. Even though histological diagnosis of chronic nonspecific inflammation is so prevalent that it should be considered the norm for parous women of reproductive age, inflammatory reaction might affect cervi-

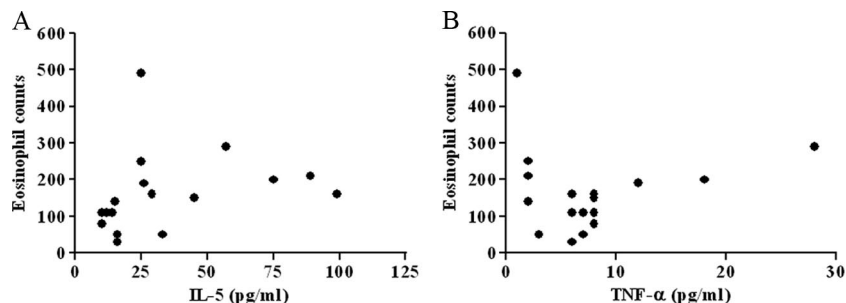


FIG. 2. Correlation between eosinophil counts and IL-5 (A) and TNF- α (B) in the cervical carcinoma group. A positive correlation was found between eosinophil counts and IL-5 in women with cervical carcinoma ($n = 17$) ($r = 0.539$ and $P = 0.026$). No correlation was observed between eosinophil counts and TNF- α ($r = -0.011$ and $P = 0.966$).

cal cytokine secretion (4). Therefore, the lack of significant differences in cytokine concentrations between patients with CIS and controls may be due to nonspecific, non-HPV-related infection.

In conclusion, our results indicate that the Th2 immune response is more active than the Th1 immune response in cervical carcinoma. In addition, cervical IL-5 concentrations in cervical carcinoma show statistically positive correlations with peripheral eosinophil counts. Therefore, detection of IL-5 in cervicovaginal secretions may be a useful marker for evaluating aggressive local and peripheral immune responses in cervical carcinoma.

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