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Could interleukin-33 and its suppressor of tumorigenicity 2 (ST2) receptor have a role in cervical human papillomavirus (HPV) infections?

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ABSTRACT

Why most women can clear human papillomavirus (HPV) infections while others can develop permanent infections. The stimulation of immunotolerance of the immune system of the host by the persistent HPV infection may be the answer to this question. Interleukin-33 (IL-33) may play a role in the pathogenesis of HPV infection, this hypothesis was thought to be due to the rapid release of IL-33 from damaged cells following tissue damage, necrosis, and activation of the inflammasome. Thus, in this study, the role of IL-33/suppressor of tumorigenicity 2 (ST2) was emphasized in HPV positive and HPV negative cervical tissues. A total of 80 were assessed. The reduced levels of IL-33 and ST2 are associated with cervical HPV infections. There was a statistically significant 42% positive correlation between IL-33 and ST2 in the HPV-positive group. Surprisingly, our data showed no significant difference between the expression levels of IL-33 or ST2 and working status, type of delivery, pre- and post-operative pathology, cigarette, educational status, locality, birth control method, gynecological, and colposcopic findings. We found that as a result of our study; low IL-33 and ST2 levels were associated with HPV infections

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Introduction

Why most women can clear human papillomavirus (HPV) infections while others can develop permanent infections that may progress to cervical cancer? The answer lies in the interplay between HPV and the immune system determines. It is seen that the immune system plays an important role in the regression or progression of cervical dysplasia. An effective immune response increases the spontaneous clearance of the virus, whereas a weak immune response often initiates a pathological process [1,2]. Mechanisms underlying the pathogenesis of HPV-related diseases are complicated and remain unclear. To illustrate the role of the immune system in HPV, scientists have examined immunocompromised women due to human immunodeficiency virus (HIV). In a study by Duerr et al., HIV-infected women had an increased risk of HPV infection and increased incidence of squamous intraepithelial lesions due to depression in their immune status [3]. Measurement of proteins secreted from immune cells, such as cytokines, chemokines, and soluble receptors, may help to detect early changes in the immune response predicting whether an HPV infection will pass into cervical cancer. Interleukin-33 (IL-33) may play a role in the pathogenesis of HPV infection, this hypothesis was thought to be due to the rapid release of IL-33 from damaged cells following tissue damage, necrosis, and activation of the inflammasome. In addition, IL-33 is involved in a variety of diseases with proinflammatory or protective roles depending on the cellular and cytokine context, including infection-related diseases. IL-33 induces synthesis of various chemokines and cytokines by binding to its heterodimeric receptor suppressor of tumorigenicity 2 (ST2) and interleukin-1 receptor (IL-1R) accessory protein [4]. İnterestingly, HPV-related diseases and IL-33/ST2 relationship have not been investigated so far. The markers measured in intralesional cervical tissues may better reflect the cervical environment in localized infections such as HPV and affect the development of cervical cancer. Thus, in this study, the role of IL-33/ST2 in terms of cervical HPV infection is emphasized. To the best of our knowledge, our study is the first to demonstrate that IL-33 and/or ST2 may be potential markers of prognosis for patients with cervical HPV infections.

Materials and methods

Study population

A total of 80 Turkish women attending a gynecological outpatient clinic of Erzincan University and expressing a desire for access to cervical cancer screening were assessed between June 2015 and April 2017. The inclusion criteria were as follows: the person (1) with a history of current or past sexual activity, (2) who were not pregnant at the time of enrollment, (3) with no history of total uterus or cervical resection, (4) who provided agreement to undergo an HPV test and participate in this study, and (5) Patients infected with a single type HPV. The study was approved by the Ethics Committee of Faculty of Medicine, Erzincan University, in accordance with the Declaration of Helsinki. Written informed consent was obtained directly from all patients and controls for the collection of samples. All

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participants were asked to complete a short questionnaire to determine their socio-demographic and reproductive characteristics.

Cervical specimen collection and pap smear

Cervicovaginal specimens were collected from all participants by a gynecologist according to standard operating procedure. Slides were stained according to standard protocols, reviewed by a trained local cytopathologist, and results were graded according to the 2004 Bethesda Classification system. The cytological classifications were the following: (1) within normal limits or reactive cellular changes (normal) and (2) atypical squamous cells (a) atypical squamous cells of undetermined significance (ASC-US), (b) low-grade squamous intraepithelial lesion (LSIL), (c) high-grade squamous intraepithelial lesion (HSIL).

Hybrid capture 2 (HC2) high-risk HPV deoxyribonucleic acid (DNA) test

All cervical specimens were analyzed for the presence or absence of 13 hrHPV genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) with the digene® HC2 high-risk HPV DNA test (Qiagen, Gaithersburg, MD) according to the manufacturer's instructions.

Enzyme-linked immunosorbent assay (ELISA)

Tissue homogenate

Tissues were washed in ice-cold PBS (pH = 7.2–7.4), weighed before homogenization. Tissues were chopped into small pieces and homogenized with ice on a glass homogenizer PBS (pH = 7.2–7.4) was added. After that, subjected it to freeze-thaw cycles. The homogenates were centrifuged for 5 min at 5000×*g*. Remove the supernatant and aliquot and store at −20 °C until used for the ELISA assays.

Tissue levels of IL-33 and ST2 were quantified using enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's directions (Elabscience, Biotechnology Co., Ltd, Wuhan, China). The sensitivity of these assays for IL-33 and ST2 was 9.38 and 18.75 pg/mL, respectively. Each sample was measured in duplicate. Absorptions of standards and samples were obtained at 450 nm using the Epoch spectrophotometer (BioTek Instruments, Inc., Winooski, VT). The standard curve was plotted with standard concentration on the x-axis and absorbance on the y-axis to determine IL-33 and ST2 levels.

Statistical analysis

Statistical package program SPSS version 20.0 (IBM Corp. Released 2011, IBM SPSS Statistics for Windows, Armonk, NY; IBM firm) was wont to appraise the information. Variables mean ± standard deviation and median (maximum–minimum) percentage and frequency values are used. In addition, the homogeneity of the variances from the preconditions of the parametric tests was checked by the 'Levene' test. The assumption of normality was checked by the 'Shapiro-Wilk' test. 'Student's t Test' was used once constant quantity check stipulations were provided for variations between the 2 groups; 'Mann–Whitney-U check' was used once constant quantity test stipulations were not provided. One-way ANOVA for three or more group comparisons and the Bonferroni-Dunn test from Kruskal–Wallis and multiple comparison tests were used when not provided by the Tukey HSD test from multiple comparison tests. McNemar Bowker's Test, Fisher's Exact Test, Chi-Square Test, Sensitivity and selectivity calculations, positive predictive value, negative predictive value were calculated when categorical data were analyzed. When the expected eyewear is less than 20%, the values are determined by 'Monte Carlo Simulation Method' to include these views in the analysis. The relationship between two variables was evaluated by the Kendall rank correlation coefficient when the parametric test prerequisites were not met. Statistical significance was accepted as $p<.05$ and $p<.01$.

Results

The study was carried out on 80 female patients aged between 22 and 68 years. The mean age of the patients was 36.32 ± 10.90 and 31.23 ± 12.39 years in HPV positive and negative women, respectively. The mean age of the patients with HPV (+) was higher than the HPV (−) but not statistically significant ($p>.05$). The characteristics of the 80 women included in this study and HPV genotypes detected in our study are reported in Table 1.

Current tobacco use in HPV positive and negative women was reported as 32.5 and 10%, respectively, and a statistically significant difference was found between smoking status ($p=.014$). In both groups less than half of the women initially used oral contraceptives. There is a statistically significant difference between cases related to contraceptive use ($p=.001$). The rates of no cellular changes in cervical cytology were 87.5 and 100% in HPV infected and non-infected patients, respectively. Two cases of low-grade squamous intraepithelial lesion and two cases of atypical squamous cells of undetermined significance (ASCUS) were reported among the HPV infected women included in analysis, but it was not statistically significant ($p=.149$).

The properties of the samples selected for analysis are shown in Table 2.

The reduced levels of IL-33 and ST2 are associated with cervical HPV infections. There was a statistically significant 42% positive correlation between IL-33 and ST2 in the HPV-positive group (Table 2).

We also analyzed whether there is a difference between the expression levels of IL-33 and ST2 and the clinicopathological features of HPV (Table 3).

Discussion

Risk factors that could prevent the natural clearance of HPV persistent infection in some populations have been an important source of interest. Some studies have found that genetic and lifestyle factors can significantly improve the likelihood of persistent infection development [5,6]. For example, many studies have found that both smoking and alcohol use are important risk factors for persistent oral and genital HPV infection [5,7]. Carcinogens in cigarette smoke have been suggested to increase viral load as well as the possibility of cancer transformation of HPV-infected epithelial cells [8]. In our study, although there was no history of alcohol use in both groups, current tobacco use in HPV positive and negative women was reported as 32.5 and 10%, respectively.

A number of genetic risk factors have been identified which make the individual susceptible to persistent HPV infection, but the association is not particularly strong. Human leukocyte antigen (HLA) is a genetic marker in which some alleles appear to

Table 1. Demographic and reproductive characteristics of the patients.

		Group			
		HPV positive (%)	HPV negative	Total	*p*
Occupation					
Housewife	*n*	36	35	71	.723
	%	90.0%	87.5%	88.8%	
Worker	*n*	4	5	9	
	%	10.0%	12.5%	11.3%	
Operation					
TAH-BSO	*n*	4	32	36	.001**
	%	10.0%	80.0%	45.0%	
LEEP	*n*	6	1	7	
	%	15.0%	2.5%	8.8%	
Cervical biopsy	*n*	30	7	37	
	%	75.0%	17.5%	46.3%	
HPV Type					
HPV 16	*n*	18	0	18	.001**
	%	45.0%	0.0%	22.5%	
HPV 18	*n*	2	0	2	
	%	5.0%	0.0%	2.5%	
HPV 33	*n*	2	0	2	
	%	5.0%	0.0%	2.5%	
HPV 39	*n*	1	0	1	
	%	2.5%	0.0%	1.3%	
HPV 45	*n*	1	0	1	
	%	2.5%	0.0%	1.3%	
HPV 51	*n*	3	0	3	
	%	7.5%	0.0%	3.8%	
HPV 52	*n*	1	0	1	
	%	2.5%	0.0%	1.3%	
HPV 61	*n*	1	0	1	
	%	2.5%	0.0%	1.3%	
HPV 66	*n*	2	0	2	
	%	5.0%	0.0%	2.5%	
HPV other types	*n*	9	0	9	
	%	22.5%	0.0%	11.3%	
HPV negative	*n*	0	40	40	
	%	0.0%	100.0%	50.0%	
Preoperative pathology					
Normal	*n*	35	40	75	.149
	%	87.5%	100.0%	93.8%	
ASCUS	*n*	2	0	2	
	%	5.0%	0.0%	2.5%	
LGSIL	*n*	2	0	2	
	%	5.0%	0.0%	2.5%	
Infection	*n*	1	0	1	
	%	2.5%	0.0%	1.3%	
Postoperative pathology					
CIN1	*n*	2	0	2	.027*
	%	5.0%	0.0%	2.5%	
CIN2	*n*	2	0	2	
	%	5.0%	0.0%	2.5%	
CIN3	*n*	1	0	1	
	%	2.5%	0.0%	1.3%	
Normal	*n*	35	40	75	
	%	87.5%	100.0%	93.8%	
Smoking					
No	*n*	27	36	63	.014*
	%	67.5%	90.0%	78.8%	
Yes	*n*	13	4	17	
	%	32.5%	10.0%	21.3%	
Education					
Illiterate	*n*	3	6	9	.043*
	%	7.5%	15.0%	11.3%	
Primary education	*n*	17	25	42	
	%	42.5%	62.5%	52.5%	
High school	*n*	14	4	18	
	%	35.0%	10.0%	22.5%	
University	*n*	6	5	11	
	%	15.0%	12.5%	13.8%	
Residence					
Rural	*n*	6	34	40	.001**
	%	15.0%	85.0%	50.0%	
Urban	*n*	34	6	40	
	%	85.0%	15.0%	50.0%	
Contraception					
None	*n*	6	22	28	.001**
	%	15.0%	55.0%	35.0%	
IUCDs	*n*	18	5	23	
	%	45.0%	12.5%	28.8%	
Injectables	*n*	4	2	6	
	%	10.0%	5.0%	7.5%	
POPs	*n*	5	1	6	
	%	12.5%	2.5%	7.5%	
Condoms	*n*	4	8	12	
	%	10.0%	20.0%	15.0%	
COCs	*n*	3	2	5	
	%	7.5%	5.0%	6.3%	
Gynecological findings					
Normal	*n*	26	39	65	.003**
	%	65.0%	97.5%	81.3%	
Genital warts	*n*	10	1	11	
	%	25.0%	2.5%	13.8%	
Intermens. bleeding	*n*	2	0	2	
	%	5.0%	0.0%	2.5%	
Postcoital bleeding	*n*	2	0	2	
	%	5.0%	0.0%	2.5%	
Colposcopy					
Normal	*n*	14	33	47	.001**
	%	35.0%	82.5%	58.8%	
Acetowhite epithel	*n*	13	2	15	
	%	32.5%	5.0%	18.8%	
leukoplakia	*n*	3	1	4	
	%	7.5%	2.5%	5.0%	
Atypical vascularization	*n*	4	3	7	
	%	10.0%	7.5%	8.8%	
Mosaicism	*n*	3	1	4	
	%	7.5%	2.5%	5.0%	
Punctuation	*n*	3	0	3	
	%	7.5%	0.0%	3.8%	
Total	*n*	40	40	80	
	%	100.0%	100.0%	100.0%	

*$p<.05$.
**$p<.01$.

Table 2. IL-33 and ST2 expression in HPV negative and positive cervical tissues. Relationship between continuous variables according to HPV positive and negative.

Group	N	Mean	Std. deviation	Std. error mean	*p*
IL-33 pg/mL					
HPV positive	40	251.92	382.58	60.49	.039*
HPV negative	40	504.96	507.00	80.16	
ST2 pg/mL					
HPV positive	40	1538.22	416.13	65.80	.045*
HPV negative	40	1650.31	431.69	68.26	

Group	ST2 pg/mL	Age	Gravida	Parity
HPV positive				
IL-33 pg/mL				
r	.420**	−.238	−.049	.033
p	.007	.140	.765	.841
n	40	40	40	40
ST2 pg/mL				
r	–	−.090	−.191	−.190
p	–	.579	.238	.241
n	–	40	40	40
HPV negative				
IL-33 pg/mL				
r	.354*	.360*	.379*	.343*
p	.025	.022	.016	.030
n	40	40	40	40
ST2 pg/mL				
r	–	−.113	−.063	−.081
p	–	.488	.700	.618
n	–	40	40	40

*$p<.05$.

Table 3. Differences between IL-33 and ST2 expression with clinicopathological features of HPV negative and positive cervical tissue.

	HPV positive					HPV negative				
	N	Mean	Std. deviation	Std. error	*p*	*N*	Mean	Std. deviation	Std. error	*p*
Occupation										
IL-33										
Housewife	36	262.00	396.43	66.07	.623	35	536.53	504.01	85.19	.304
Worker	4	161.14	238.88	119.44		5	283.99	526.54	235.48	
ST2										
Housewife	36	1553.00	413.10	68.85	.508	35	1654.04	433.37	73.25	.887
Worker	4	1405.25	483.74	241.87		5	1624.21	468.67	209.60	
Birth										
IL-33										
Vaginal	33	239.3900	377.89956	65.78387	.659	34	522.1021	502.95750	86.25650	.617
C-section	7	310.9671	429.88926	162.48287		6	407.8333	567.49537	231.67901	
ST2I										
Vaginal	33	1543.7661	379.73944	66.10415	.858	34	1650.3326	438.33459	75.17376	.999
C-section	7	1512.0800	596.24857	225.36078		6	1650.1900	430.67862	175.82381	
Operation										
IL-33										
TAH-BSO	4	631.61	579.64	289.82	.013*	32	593.11	519.22	91.79	.076
LEEP	6	486.85	530.25	216.48		1	376.20			
Cervical biopsy	30	154.30	269.15	49.14		7	120.39	247.28	93.46	
ST2										
TAH-BSO	4	1952.42	217.72	108.86	.109	32	1734.54	324.33	57.33	.029*
LEEP	6	1478.19	336.12	137.22		1	1657.92			
Cervical biopsy	30	1495.00	426.79	77.92		7	1264.17	675.32	255.25	
Smoking										
IL-33										
No	27	264.514	399.993	76.979	.768	36	511.75	505.69	84.28	.803
Yes	13	225.750	357.682	99.203		4	443.84	593.78	296.89	
ST2										
No	27	1522.044	473.643	91.153	.728	36	1667.98	431.24	71.87	.445
Yes	13	1571.818	273.681	75.906		4	1491.33	464.30	232.15	
Education										
IL-33										
Illiterate	3	147.17	222.56	128.49	.651	6	780.41	581.48	237.39	.199
Primary education	17	333.33	469.95	113.98		25	519.93	496.53	99.31	
High school	14	230.33	362.02	96.75		4	477.59	601.94	300.97	
University	6	123.98	155.89	63.64		5	121.49	180.95	80.93	
ST2										
Illeterate	3	1075.44	739.97	427.22	.251	6	1639.30	430.80	175.87	.916
Primary education	17	1598.49	354.61	86.01		25	1626.37	496.54	99.31	
High school	14	1567.81	429.77	114.86		4	1651.83	311.37	155.69	
University	6	1529.80	325.00	132.68		5	1782.03	86.81	38.82	
Residence										
IL-33										
Rural	6	308.44	517.21	211.15	.7	34	528.23	514.65	88.26	.4978
Urban	34	241.94	362.99	62.25		6	373.10	481.97	196.76	
ST2										
Rural	6	1476.63	411.87	168.14	.699	34	1657.12	426.81	73.20	.816
Urban	34	1549.09	422.06	72.38		6	1611.74	499.16	203.78	
Contraception										
IL-33										
None	6	366.02	495.09	202.12	.614	22	462.83	471.77	100.58	.179
IUCDs	18	276.24	447.14	105.39		5	342.35	533.39	238.54	
Injectables	4	438.90	369.22	184.61		2	16.29	6.82	4.82	
POPs	5	132.45	169.67	75.88		1	95.16			
Condoms	4	108.57	68.90	34.45		8	758.07	563.16	199.11	
COCs	3	18.69	4.71	2.72		2	1056.08	236.53	167.26	
ST2										
None	6	1502.56	381.70	155.83	.984	22	1584.48	558.24	119.02	.896
IUCDs	18	1581.91	446.21	105.17		5	1682.30	88.51	39.58	
Injectables	4	1508.81	643.94	321.97		2	1822.08	65.57	46.37	
POPs	5	1541.74	246.34	110.17		1	1819.28			
Condoms	4	1543.45	490.81	245.41		8	1682.81	224.38	79.33	
COCs	3	1373.81	389.86	225.09		2	1908.26	78.55	55.54	
Gynecological findings										
IL-33										
Normal	26	278.26	435.32	85.37	.688	39.00	517.62	507.18	81.21	–
Genital warts	10	194.77	282.19	89.24		1.00	11.47	–	–	
Intermens. bleeding	2	431.05	166.83	117.97						
Postcoital bleeding	2	16.11	0.35	0.25						

(*continued*)

Table 3. Continued.

	HPV positive					HPV negative				
	N	Mean	Std. deviation	Std. error	*p*	*N*	Mean	Std. deviation	Std. error	*p*
ST2										
Normal	26	1569.39	448.83	88.02	.922	39.00	1647.10	436.85	69,95	–
Genital warts	10	1459.50	368.77	116.61		1.00	1775.71	–	–	
Intermens. bleeding	2	1555.47	624.22	441.39						
Postcoital bleeding	2	1509.37	118.77	83.98						
Colposcopy										
IL-33										
Normal	14	321.55	438.19	117.11	.676	33.00	500.56	500.16	87.07	.554
Acetowhite epithel	13	259.83	361.25	100.19		2.00	972.55	435.06	307.64	
leukoplakia	3	77.62	101.86	58.81		1.00	41.72			
Atypical vascularization	4	27.28	24.97	12.48		3.00	527.07	714.77	412.67	
Mosaicism	3	177.24	153.77	88.78		1.00	111.95	–	–	
Punctuation	3	441.17	738.35	426.29		–	–	–	–	
ST2										
Normal	14	1679.68	417.71	111.64	.605	33.00	1605.72	460.62	80.18	.713
Acetowhite epithel	13	1493.73	468.76	130.01		2.00	1911.33	162.92	115.21	
leukoplakia	3	1545.12	182.88	105.58		1.00	1688.29	–	–	
Atypical vascularization	4	1385.94	433.86	216.93		3.00	1900.53	132.16	76.30	
Mosaicism	3	1246.11	336.19	194.10		1.00	1811.10	–	–	
Punctuation	3	1559.11	409.00	236.14		–	–	–	–	

*$p<.05$.

have a more pronounced association with HPV infection and later failure to clear cervical cancer [9–11]. As this study was performed in a population of the same ethnic group, genetic risk factors were excluded from the study. More research is needed on these genetic markers for each gene to identify a high risk for HPV persistence and provide comprehensive preventive care accordingly.

Considering the prevalence of multiple HPV-type infections in patients, infection with more than one type of HPV was investigated as a potential determinant of subsequent persistent infection. The results suggest that previous infection with HPV increases the chance of obtaining another HPV infection [12,13]. However, it is unclear whether HPV persistence is due to a co-infection. Patients infected with more than one HPV type were excluded from this study.

Although it is not yet clear how all these co-factors interact for persistence of HPV, it has been suggested that the virus may be associated with its ability to escape from the immune system. Cytokines polarize the host immune response to the Th1 or Th2 pattern by modulating viral replication [14]. The IL-33 acts as an alarm by sending signals from the damaged cells to the local immune cells. IL-33 is involved in a variety of diseases with proinflammatory or protective roles depending on the cellular and cytokine context, including infection-related diseases (such as parasitic and viral infection). The permanence of HPV infection is a prerequisite for cervical intraepithelial neoplasia (CIN) development. Th1 type anti-HPV response has been shown to be defective in severe CIN lesions. In our study, we found that IL-33 levels in HPV infected patients were lower than those with HPV negative. These data can provide a clearer understanding of micro-environmental changes in the HPV-infected cervix that support local immune control insufficiency and the progression of lesions. IL-33 signaling is mediated via its receptor ST2L. The role of IL-33/ST2 axis has recently been implicated in infection-related diseases, but with limited data. IL-33/ST2 functions as an alarming that is released following cell necrosis to polarization to the Th2 cytokine profile to tissue damage or stress [15]. The distorted balance between Th1 cell and Th2 cell is the cellular immunity feature during HPV infection [16]. In some studies, the immune response to HPV and other viral infections has been reported to polarize the Th2 cytokine profile to facilitate neoplastic transformation and to predispose women to CIN and cervical cancer [17]. Decreased Th1 response and increased Th2 response showed suppression of cellular immunity and progression of cervical lesion [18]. In this study, the IL-33 levels were found reduced in HPV-infected cervix. Changes in IL-33 levels may provide a preliminary sign of factors that support impaired Th1 response. Further studies are needed to fully understand the mechanisms. However, the role of HPV in IL-33 expression remains unclear. Furthermore, as HPV plays a critical role in cervical cancer, we attempted to assess the possible role of IL-33/ST2 as an inducer of cancer and inflammation in response to HPV infection. Our study was the first to demonstrate that analyzes the intralesional IL-33 and ST2 levels in women presenting with and without HPV-infection.

Intraepithelial and invasive cervical lesions have a clear Th2 cytokine profile. Increased Th2 cytokine (IL-10) and reduced Thl cytokine (Interferon gamma (IFN-N), IL-12, IL-2) and tumor necrosis factor-a (TNF-α) levels were determined in cervical exudates of HPV-infected patients. In contrast to other IL-1 families, IL-33 specifically induces Th2 immune responses in various immune cell types [19]. The reduced detection of IL-33 levels in HPV-positive patients in our study would result in a decrease in Th2 cells and an increase in Th1 cells, and the balance would shift toward Th1. This finding is not consistent with findings in the literature. This difference is thought to be due to the phase-dependent effect of IL-33 on viral infections and it was revealed that interaction between IL-33 and other cytokines may be definite and further investigation is needed to elucidate the interaction details between IL-33 and other cytokines. ST2L has been found to be selectively expressed in Th2 cells but not expressed in Th1 or regulatory (Treg) T cells [20]. In a study by Rank et al., it has been shown that IL-33 can polarization of native T cells to a Th2 phenotype due to activate murine dendritic cells [21] and it may be effective on Th2 cells to increase the secretion of some Th2 cytokines, such as IL-5 and IL-13 [22]. In addition, IL-33 may be a chemoattractant for Th2 cells [23]. IL-33 may activate B1 B cells and significantly increase the production of immunoglobulin M (IgM), IL-5 and IL-13 from these cells [24]. It has been shown that IL-33 can induce degranulation, maturation,

survival, and production of some pro-inflammatory cytokines in mast cells and basophils [25]. It is possible that the first role of IL-33 is the host defense against pathogens in terms of the biological process. In fact, IL-33/ST2 has been shown to be protective in many parasitic infections including *Leishmania* major and *Toxoplasma gondii* and is highly expressed on Th2 in animal models [26,27]. However, it is clear that IL-33 has potent activator effects on various immune cell types and may be effective on various inflammatory diseases. Miyagaki et al. reported that low IL-33 levels are associated with an increased risk of opportunistic infections in patients with HIV infection [28], providing additional evidence that low IL-33 levels reflect impaired immunity. The results of our work are consistent with this work.

Furthermore, the activity of extracellular IL-33 is controlled by binding to the soluble form of ST2 (sST2) that acts locally as an unsaturated receptor to limit the activity of the target IL-33 [29]. The absence of ST2 on cluster of differentiation 4 (CD4) T-cells impairs Th1 cell activation during viral infection and results in decreased expansion, impaired effector function, and reduced T-cell–mediated immunopathology [30]. Decreased ST2 levels that we found in our study were found to be consistent with the literature.

This study has several limitations. First of all, this is a cross-sectional study with a small sample size. Second, we analyzed the IL-33 and ST2L levels only in the intralesional cervical tissue. We could reach more accurate results if we analyzed these parameters in both cervical tissue and serum.

Conclusion

In this study, IL-33 and ST2L levels were found to be lower in HPV-infected patients than non-infected. Therefore, manipulation of the IL-33/ST2 pathway is promising for the treatment or prevention of HPV infections. Moreover, this study only finds an association between lower levels of IL-33 and HPV infection, which is not sufficient to state that this association is or even may be causal and more related work should be done.

Disclosure statement

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References

[1] Araldi RP, Assaf SMR, Carvalho RF, et al. Papillomaviruses: a systematic review. Genet Mol Biol. 2017;40:1.
[2] Gadducci A, Barsotti C, Cosio S, et al. Smoking habit, immune suppression, oral contraceptive use, and hormone replacement therapy use and cervical carcinogenesis: a review of the literature. Gynecol Endocrinol. 2011;27:597–604.
[3] Duerr A, Kieke B, Warren D, et al. Human papillomavirus-associated cervical cytologic abnormalities among women with or at risk of infection with human immunodeficiency virus. Am J Obstet Gynecol. 2001;184:584–590.
[4] Nayki C, Nayki U, Kulhan M, et al. The effect of diabetes on ovaries in a rat model: the role of interleukin-33 and apoptosis. Gynecol Endocrinol. 2017;33:708–711.
[5] Haukioja A, Asunta M, Söderling E, et al. Persistent oral human papillomavirus infection is associated with smoking and elevated salivary immunoglobulin g concentration. J Clin Virol. 2014;61:101–106.
[6] Rositch AF, Koshiol J, Hudgens M, et al. Patterns of persistent genital human papillomavirus infection among women worldwide: a literature review and meta-analysis. Int J Cancer. 2013;133:1271–1285.
[7] Oh HY, Seo SS, Kim MK, et al. Synergistic effect of viral load and alcohol consumption on the risk of persistent high-risk human papillomavirus infection. PLoS One. 2014;9:e104374.
[8] Xi LF, Koutsky LA, Castle PE, et al. Relationship between cigarette smoking and human papilloma virus types 16 and 18 DNA load. Cancer Epidemiol Biomark Prev. 2009;18:3490–3496.
[9] Peng S, Trimble C, Wu L, et al. HLA-DQB1*02-restricted HPV-16 E7 peptide-specific $CD4^+$ T-cell immune responses correlate with regression of HPV-16-associated high-grade squamous intraepithelial lesions. Clin Cancer Res. 2007;13:2479–2487.
[10] Wank R, Thomssen C. High risk of squamous cell carcinoma of the cervix for women with HLA-DQw3. Nature. 1991;352:723–725.
[11] Zoodsma M, Nolte IM, Schipper M, et al. Analysis of the entire HLA region in susceptibility for cervical cancer: a comprehensive study. J Med Genet. 2005;42:e49.
[12] Liaw KL, Hildesheim A, Burk RD, et al. A prospective study of human papillomavirus (HPV) type 16 DNA detection by polymerase chain reaction and its association with acquisition and persistence of other HPV types. J Infect Dis. 2001;183:8–15.
[13] Rousseau MC, Pereira JS, Prado JC, et al. Cervical coinfection with human papillomavirus (HPV) types as a predictor of acquisition and persistence of HPV infection. J Infect Dis. 2001;184:1508–1517.
[14] Zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer. 2002;2:342–350.
[15] Sato S, Yanagawa Y, Hiraide S, et al. Cyclic AMP signaling enhances lipopolysaccharide sensitivity and interleukin-33 production in RAW264.7 macrophages. Microbiol Immunol. 2016;60:382–389.
[16] Evans MF, Adamson CS, Papillo JL, et al. Distribution of human papillomavirus types in thinprep papanicolaou tests classified according to the Bethesda 2001 terminology and correlations with patient age and biopsy outcomes. Cancer. 2006;106:1054–1064.
[17] Lamkanfi M, Dixit VM. IL-33 raises alarm. Immunity. 2009;31:5–7.
[18] Scott ME, Shvetsov YB, Thompson PJ, et al. Cervical cytokines and clearance of incident human papillomavirus infection: Hawaii HPV cohort study. Int J Cancer. 2013;133:1187–1196.
[19] Oboki K, Ohno T, Kajiwara N, et al. IL-33 is a crucial amplifier of innate rather than acquired immunity. Proc Natl Acad Sci USA. 2010;107:18581–18586.
[20] Xu D, Chan WL, Leung BP, et al. Selective expression of a stable cell surface molecule on type 2 but not type 1 helper T cells. J Exp Med. 1998;187:787–794.
[21] Rank MA, Kobayashi T, Kozaki H, et al. IL-33-activated dendritic cells induce an atypical TH2-type response. J Allergy Clin Immunol. 2009;123:1047–1054.
[22] Kurowska-Stolarska M, Kewin P, Murphy G, et al. IL-33 induces antigen-specific IL-5+ T cells and promotes allergic-induced airway inflammation independent of IL-4. J Immunol. 2008;181:4780–4790.
[23] Komai-Koma M, Xu D, Li Y, et al. IL-33 is a chemoattractant for human Th2 cells. Eur J Immunol. 2007;37:2779–2786.
[24] Komai-Koma M, Gilchrist DS, McKenzie AN, et al. IL-33 activates B1 cells and exacerbates contact sensitivity. J Immunol. 2011;186:2584–2591.
[25] Schneider E, Petit-Bertron AF, Bricard R, et al. IL-33 activates unprimed murine basophils directly in vitro and induces their in vivo expansion indirectly by promoting hematopoietic growth factor production. J Immunol. 2009;183:3591–3597.
[26] Kropf P, Herath S, Klemenz R, et al. Signaling through the T1/ST2 molecule is not necessary for Th2 differentiation but is important for the regulation of type 1 responses in nonhealing *Leishmania* major infection. Infect Immun. 2003;71:1961–1971.
[27] Jones LA, Roberts F, Nickdel MB, et al. IL-33 receptor (T1/ST2) signalling is necessary to prevent the development of encephalitis in mice infected with *Toxoplasma gondii*. Eur J Immunol. 2010;40:426–436.

[28] Miyagaki T, Sugaya M, Yokobayashi H, et al. High levels of soluble ST2 and low levels of IL-33 in sera of patients with HIV infection. J Invest Dermatol. 2011;131:794–796.
[29] Kakkar R, Lee RT. The IL-33/ST2 pathway: therapeutic target and novel biomarker. Nat Rev Drug Discov. 2008;7:827–840.
[30] Baumann C, Bonilla WV, Frohlich A, et al. T-bet- and STAT4-dependent IL-33 receptor expression directly promotes antiviral Th1 cell responses. Proc Natl Acad Sci USA. 2015;112:4056–4061.