Short Communication

Determinants and Correlation of Systemic and Cervical Concentrations of Total IgA and IgG

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Abstract

We compared systemic and cervical total IgA and IgG during the menstrual cycle among 154 women who attended clinic visits at follicular/early, periovulatory/mid, and luteal/late phases of menstrual cycle. Paired serum and cervical secretions were tested at each visit for total IgA and IgG using ELISA. Geometric mean titers for systemic IgA and IgG were 1.92 and 8.25 mg/mL, respectively. There were no differences in titers by menstrual cycle phase, neither were they correlated to cervical titers (ρ = 0.17 and 0.16, respectively). The lack of correlation between systemic and cervical total IgA and IgG suggests that systemic concentrations are not reflective of cervical levels. (Cancer Epidemiol Biomarkers Prev 2009;18(10):2672–6)

Introduction

Vaccines that protect against infection with oncogenic human papillomaviruses (HPV) types 16 and 18 have recently been licensed for use. These vaccines have been shown to be highly effective at preventing infection with homologous HPV types, and initial findings suggest that partial protection against some heterologous HPV types phylogenetically related to HPV-16/18 might also be conferred through vaccination (1, 2). Because vaccination is administered systemically whereas protection occurs locally at the mucosal sites, there is an interest in better understanding the patterns of both systemic and local immune responses and their interrelationship.

Recently, we and others reported that cervical total IgA and IgG levels and antigen-specific antibodies fluctuate over the menstrual cycle, being lowest around ovulation and highest in the periovulatory and luteal phases of the cycle (3-5). It is not clear, however, whether systemic levels of total IgA and IgG levels also fluctuate over the menstrual cycle, whether other correlates of systemic levels exist, and to what extent systemic IgA and IgG levels are predictive of levels at the cervix. In this analysis, we sought to investigate the patterns and determinants of systemic IgA and IgG levels overall and during the menstrual cycle, and to compare the correlation between systemic and cervical total IgA and IgG levels among unvaccinated women.

Materials and Methods

Study Population. Data to address the aims of this study are from the National Cancer Institute–sponsored Proyecto Epidemiológico Guanacaste Study, described in detail elsewhere (6). Briefly, the primary aim of this population-based cohort was to study the natural history of HPV infection and cervical intraepithelial neoplasia. Between 1993 and 1994, 10,049 women from the Guanacaste province in Costa Rica enrolled and were followed for up to 7 y.

For the present evaluation, a subset of women who were 25 to 35 y old, with an intact uterus, not pregnant, and without evidence of cervical high-grade disease were contacted to participate in a substudy to investigate mucosal (cervical and oral) and systemic IgA and IgG over the menstrual cycle. For this substudy (described in detail in ref. 5), women were additionally required to have regular menstrual cycles (cycle lengths of 25-35 d), and to be willing to come for three clinic visits coinciding with follicular/early (days 5-8 in cycle), periovulatory/mid, (days 14-16 in cycle), and luteal/late (days 19-22 in cycle) phases. At each visit, plasma was collected for measuring IgA and IgG, and an interview administered inquiring about health and behaviors. Additionally, a pelvic exam was done, at which time cervical secretions were collected using a cellulose-based Ultracell sponge for local IgA and IgG determination. All women signed an informed consent. The study was approved by the institutional review

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boards of the National Cancer Institute and INCIENSA, Costa Rica. A total of 202 women were invited to participate, of whom 199 met the eligibility criteria, and 196 provided specimens required for this analysis. We further excluded 17 women who were breast-feeding at study entry and 28 whose menstrual cycles were either too short (<25 d; $n = 9$), too long (>36 d; $n = 15$), or missing ($n = 4$), leaving 154 women in the analytic sample.

Determination of Immunoglobulin Levels. Cervical and systemic IgA and IgG levels were determined by using an ELISA in duplicate according to the instructions of the manufacturer (Bethyl Corporation; ref. 7). Cervical IgA and IgG were standardized to account for differences in the volume of secretion collected. To do so, 5 to 10 unused sponges randomly picked from each lot were weighed; the mean weights of these sponges was assumed to be the dry weight of all sponges from that lot. We standardized each IgA and IgG level using the following formula:

[Specimen weight – average dry sponge weight + 0.6 (i.e., weight of 600 μL extraction buffer)]/ (specimen weight – average dry sponge weight)

Statistical Methods. Cervical and systemic IgA and IgG levels were checked for distributional properties and log-transformed to account for skewed properties. Geometric mean (GM) concentrations are presented. To determine the factors independently associated with systemic IgA and IgG levels, we applied multivariate models for correlated data. Generalized estimating equations models with an unstructured correlation structure were used to estimate SEs and coefficients adjusted for multiple observations for each woman. Spearman coefficients (ρ) were calculated for the correlation between systemic and cervical IgA and IgG levels. We also calculated correlations for subgroups defined by oral contraceptive use, time in menstrual cycle, smoking status, cervical hemoglobin contamination, indication of genital infection or inflammation, HPV status, or cytologic abnormality. All analyses were done using STATA 9.2.

Results

Plasma IgA and IgG GMs were 1.92 mg/mL [95% confidence interval (CI), 0.89-4.11; range, 0.44-4.88] and 8.25 mg/mL (CI, 5.57-12.21; range, 4.91-15.86), respectively. These compared with cervical IgA and IgG GMs of 0.28 mg/mL (CI, 0.04-2.27; range, 0.007-22.70) and 0.64 mg/mL (CI, 0.05-8.00; range, 0.008-38.84), respectively.

We evaluated factors associated with systemic IgA and IgG levels between women. Neither IgA nor IgG in the circulation varied significantly over the menstrual cycle (Table 1). When we restricted the evaluation to naturally cycling women, circulating IgA levels were 2.07, 2.00, and 2.03 mg/mL at the follicular, periovulatory, and luteal phases, respectively. Similarly, IgG levels were 8.46, 8.15, and 8.00 mg/mL at the follicular, periovulatory, and luteal phases, respectively. No other covariates evaluated (Table 1) were found to strongly predict systemic IgA or IgG levels. In final multivariate models, after adjusting for multiple visits per person, oral contraceptive use was found to be the only factor to be significantly associated with systemic IgA levels (data not shown); women using oral contraceptives had lower IgA levels than naturally cycling women

Table 1. Systemic and cervical IgA and IgG concentrations (GM) and correlations by covariates

	N (%)	IgA (mg/mL)			IgG (mg/mL)		
		Systemic	Cervix	ρ	Systemic	Cervix	ρ
Overall: GM	426 (100.0)	1.92	0.28	**0.17**	8.25	0.64	**0.16**
Visit							
Follicular	139 (32.6)	1.80	0.47	**0.25**	8.67	1.16	0.14
Periovulatory	146 (34.3)	1.86	0.20	0.04	8.22	0.41	0.12
Luteal	141 (33.1)	1.87	0.29	**0.17**	8.08	0.70	0.12
Recent oral contraceptive use							
Yes	188 (44.1)	1.71	0.35	**0.24**	8.41	1.03	**0.22**
No	238 (55.9)	2.01	0.27	**0.21**	8.25	0.48	0.09
Age (y)							
26-29	121 (28.4)	1.94	0.27	**0.22**	8.06	0.65	0.12
30-32	136 (31.9)	1.86	0.33	**0.25**	8.72	0.87	0.05
33-35	169 (39.7)	1.99	0.33	0.06	8.24	0.81	**0.25**
Smoking							
No	388 (91.1)	1.92	0.30	**0.16**	8.37	0.81	**0.15**
Yes	38 (8.9)	2.08	0.44	0.22	7.21	0.52	0.31
Recent illness							
No	293 (68.8)	1.95	0.33	**0.14**	8.25	0.83	**0.23**
Yes	133 (31.2)	1.91	0.28	**0.24**	8.55	0.76	0.05
Any sexually transmitted disease							
No	307 (73.4)	1.97	0.31	**0.12**	8.42	0.67	**0.22**
Yes	111 (26.6)	1.75	0.33	**0.32**	8.05	0.96	–0.005
HPV infection							
No	348 (83.9)	1.98	0.30	**0.15**	8.40	0.73	**0.22**
Yes	67 (16.1)	1.70	0.33	**0.36**	7.87	0.99	–0.09
Blood in cervical sample							
No	77 (18.1)	2.04	0.15	**0.27**	8.38	0.28	0.02
Trace	220 (51.6)	1.85	0.29	**0.19**	8.23	0.86	**0.19**
Visible	129 (30.3)	1.97	0.43	0.15	8.58	1.14	0.13

NOTE: Values in boldface indicates $P < 0.05$.

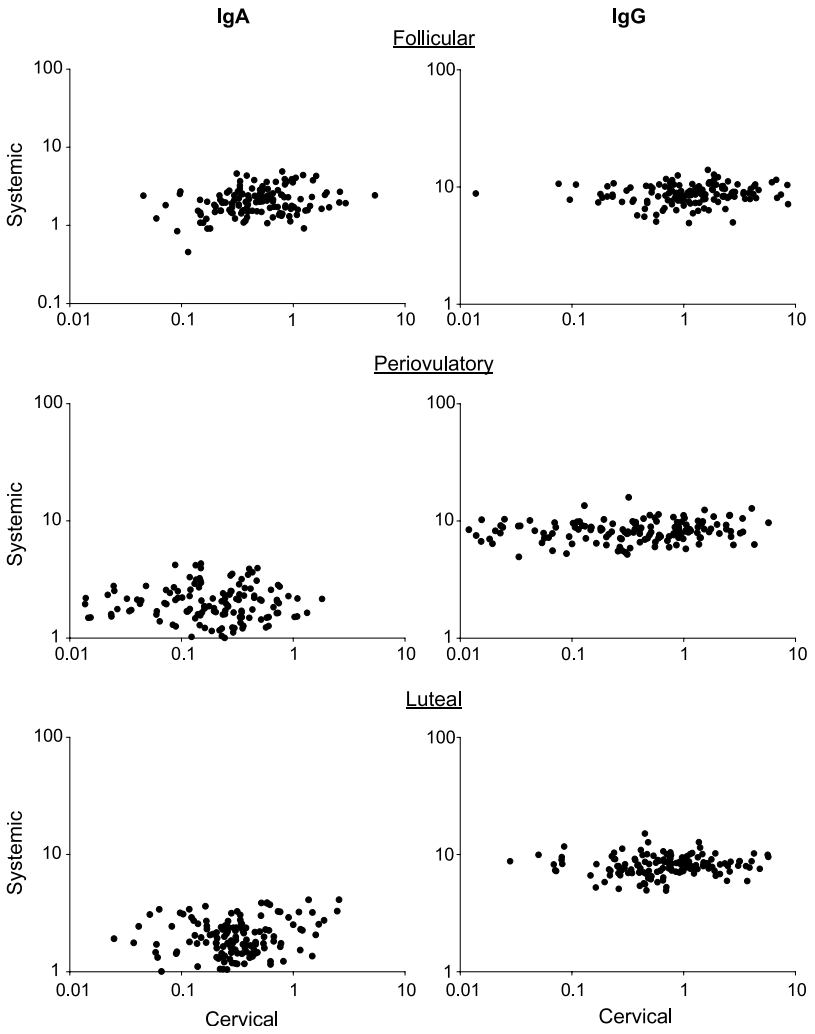

Figure 1. IgA and IgG concentrations (mg/mL) in cervix and plasma by menstrual cycle. In order to keep the same scale for all graphs, we removed two individuals from two of the graphs. One was in the IgG periovulatory graph and had cervical IgG levels of 0.008 and systemic levels of 7.81. Similarly, one was in the IgA luteal graph, and had cervical IgA levels of 0.008 and systemic IgA levels of 2.70.

(percent change, 0.85; 95% CI, 0.76-0.95). We did not identify independent factors associated with systemic IgG (data not shown).

Next, we evaluated the degree of correlation between systemic and cervical IgA and IgG. Overall, systemic and cervical IgA or IgG were not highly correlated (ρ = 0.17, and 0.16, respectively); correlations remained low when we stratified by time in menstrual cycle (Table 1). We further investigated systemic and cervical IgA and IgG correlation in subgroups such as oral contraceptive use, age, smoking status, recent illness, evidence of sexually transmitted infection, and presence of blood in the cervical samples, and did not find meaningful correlation between plasma and cervical IgA and IgG levels within any of these subgroups (Table 1). Figure 1 graphically depicts the low correlation observed between systemic and cervical immunoglobulins according to phase of the menstrual cycle. It is interesting to note the small degree of variability observed across women in systemic IgA (range, 0.44-4.88; CI, 0.89-4.11) and IgG (range, 4.91-15.86; CI, 5.57-12.21) levels relative to the much larger variability observed in mucosal levels across women [range and CI, 0.007-22.70 (0.04-2.27) and 0.008-38.84 (0.05-8.00) for IgA and IgG, respectively].

Discussion

We were interested in investigating the patterns and correlates of systemic IgA and IgG, and the relation between systemic and cervical IgA and IgG during the menstrual cycle. We found very low correlation between plasma and cervical IgA or IgG concentrations overall, or by phase of menstrual cycle. Furthermore, we observed that whereas cervical IgA and IgG levels vary considerably between women, systemic levels seem to be at a much more constant level between individuals than cervical levels. Taken together, these data suggest that systemic humoral immune responses, as measured by total immunoglobulin levels do not represent the local humoral response. Our findings are consistent with previous, smaller studies that also observed a lack of correlation between systemic and local levels of both immunoglobulins and cytokines (8-10).

Interestingly, we found that the ratio of cervical IgA to serum IgA (0.14) was higher than the ratio of cervical IgG to serum IgG (0.08, $P > 0.001$; data not shown), which may be in agreement with the local production of IgA, in addition to transudating from the serum.

In a previous study of the correlates of cervical IgA and IgG, we showed a sharp decline in total cervical

IgA and IgG levels around ovulation among naturally cycling women (5), consistent with smaller reports from other groups (3, 4). Furthermore, the levels of cervical IgA and IgG were markedly lower in women using oral contraceptives, suggesting that the immune response in the cervix may be mitigated by steroid sex hormones (5). In contrast, in the present report, based on the same study participants, we did not find variations in systemic IgA or IgG levels during the menstrual cycle, suggesting a lack of effect of menstrual cycle hormones on total levels of systemic IgA and IgG. Consistent with this, we also observed no association between oral contraceptive use and systemic IgG levels. We did, however, observe that oral contraceptive users have lower systemic IgA levels than naturally cycling women. Whether this latter observation is real or spurious is not known and requires independent replication in future studies.

Our finding of a lack of correlation between systemic and cervical total IgA and IgG contrasts with previous reports of a modest to high correlation between circulating and cervical levels of HPV-specific antibodies among women who received HPV vaccination (11, 12). A recent publication from our group (11), reported correlations ranging from 0.73 to 0.75 between systemic and cervical levels of antibodies against HPV-specific antigens (HPV-16 and HPV-18, respectively) among HPV-vaccinated women. Due to this apparent discrepancy, we evaluated the correlation between systemic and cervical levels of total IgG (IgA was not tested) in vaccinated women from our previous study. Consistent with findings from the present report, there was also a lack of correlation between systemic and cervical levels of total IgG in the vaccinated population (Spearman correlation, −0.25). The discrepant findings could therefore be reconciled as follows: (*a*) although the exact source of immunoglobulins in the cervix is unknown, and in the absence of HPV exposure via vaccination, the lack of correlation between systemic and cervical levels of total immunoglobulins suggests that passive transfer of antibodies from blood to the cervix might not explain local immunoglobulin levels in the cervix. Other mechanisms such as active or selective transport mechanisms, and local production of immunoglobulins are likely to contribute to the total pool of immunoglobulins at the cervix (13, 14); and (*b*) the modest to high correlation observed between systemic and cervical levels of HPV-specific antibodies among vaccinated women suggests that anti-HPV cervical antibodies induced by i.m. vaccination likely derive from transudation from blood leading to the high correlation observed between systemic and cervical levels among vaccinated women. However, induction of local immune responses is also possible, as shown in other models of parenteral immunization (13, 14). Finally, these findings indicate that the correlations between systemic and local immunoglobulins for a particular antigen-specific antibody do not reflect the correlations of total immunoglobulins or other antibodies induced by different antigens and via various potential routes of exposure.

Surprisingly, we also did not find evidence for correlation between systemic and cervical levels in analysis that stratified by blood contamination in the cervical secretions, despite the fact that in our earlier work, we observed significantly higher levels of cervical IgA and IgG if blood had been detected in the cervical secretions (5). We believe the lack of correlation between systemic and local levels in the presence of blood may be due to the much lower variability in systemic but higher variability (at least two order of magnitudes difference) between the lowest and highest values of the cervical IgA and IgG levels.

A unique feature of the present study was the large number of women evaluated for both systemic and cervical IgA and IgG levels. Interestingly, the mean systemic IgA and IgG levels (2.1 and 8.4 mg/mL, respectively), and cervical IgA and IgG levels (0.50 and 1.27 mg/mL, respectively) were comparable to mean IgA and IgG levels in serum in other published studies (15).

In summary, in contrast with mucosal humoral responses, we observed that systemic levels of IgA and IgG were not affected by menstrual cycle and hormonal changes. In addition, systemic levels of total immunoglobulins do not mirror cervical levels, indicating that the mechanisms of expression and regulation of these molecules locally is complex and deserves further study to better understand the determinants of effective humoral immune defenses against infection in the female genital tract.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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