Published in final edited form as:

Cancer Epidemiol Biomarkers Prev. 2009 October ; 18(10): 2672–2676. doi:
10.1158/1055-9965.EPI-09-0348.

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

Mahboobeh Safaeian^{1,*}, Troy Kemp², Roni T Falk¹, Ana Cecilia Rodriguez³, Allan Hildesheim¹, Marcus Williams², Carolina Porras³, Rolando Herrero³, and Ligia A. Pinto²

¹Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD ²HPV Immunology Laboratory, SAIC-Frederick, Inc., NCI-Frederick, Frederick, Maryland ³Proyecto Epidemiológico Guanacaste, Fundación INCIENSA, Costa Rica

Abstract

We compared systemic and cervical total immunoglobulins A (IgA) and G (IgG) during the menstrual cycle among 154 women, who attended clinic visits at follicular/early, peri-ovulatory/mid, and luteal/late phases of menstrual cycle. Paired serum and cervical secretions were tested at each visit for total IgA and IgG by ELISA.

Geometric mean titers for systemic IgA and IgG were 1.92 and 8.25mg/ml, respectively. There were no differences by menstrual cycle phase, neither were they correlated to cervical titers ($p=0.17$ 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.

Keywords

cervical; systemic; IgA; IgG; menstrual cycle

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 ^{5;13}. Since vaccination is administered systemically while protection occurs locally at the mucosal sites, there is an interest in better understanding patterns of both systemic and local immune responses and their interrelationship.

Recently, we and others reported that cervical total immunoglobulin A (IgA) and G (IgG) levels and antigen-specific antibodies fluctuate over the menstrual cycle, being lowest around ovulation and highest in the peri-ovulatory and luteal phases of the cycle ^{3;11;14}. 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

*Corresponding author: Division of Cancer Epidemiology and Genetics, Infections and Immunoepidemiology Branch, National Cancer Institute, 6120 Executive Boulevard, Suite 550, Rockville, MD 20852, Phone: (301) 594-2934, Fax: (301) 402-0916, safaeianm@mail.nih.gov.

Conflict of interest: All authors declare no conflict of interest.

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 & Methods

Study population

Data to address the aims of this study are from the NCI sponsored Proyecto Epidemiológico Guanacaste (PEG) study, described in detail elsewhere⁶. Briefly, the primary aim of this population-based cohort was to study the natural history of HPV infection and cervical intraepithelial neoplasia. Between 1993-1994, 10,049 women from the Guanacaste province in Costa Rica enrolled and were followed for up to seven years.

For the present evaluation, a subset of women who were 25–35 years old, with intact uterus, not pregnant and without evidence of cervical high grade disease were contacted to participate in a sub-study to investigate mucosal (cervical and oral) and systemic IgA and IgG over the menstrual cycle. For this sub-study (described in detail elsewhere)¹⁴ women were further required to have regular menstrual cycles (cycle length of 25–35 days), and be willing to come for 3 clinic visits coinciding with follicular/early (days 5-8 in cycle), peri-ovulatory/mid, (days 14-16 in cycle), and luteal/late (days 19-22 in cycle) phase. 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 performed, 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 IRBs of NCI 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 cycle was either too short (<25 days; n=9), too long (>36 days; n=15), or missing (n=4) leaving 154 women in the analytic sample.

Determination of IG Levels

Cervical and systemic IgA and IgG levels were determined by using an enzyme-linked immunoassay (Bethyl Corporation, Montgomery, TX) in duplicate, per manufacturer's instruction⁴. Cervical IgA and IgG were standardized to account for differences in volume of secretion collected. To do so, five to ten unused sponges randomly picked from each lot were weighed; mean weight 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:

$$\frac{(\text{Specimen weight} - \text{Average dry sponge weight} + 0.6 \text{ (i.e. weight of 600uL extraction buffer)})}{(\text{Specimen weight} - \text{Average dry sponge weight})}$$

Statistical Methods

Cervical and systemic IgA and IgG levels were checked for distributional properties and log-transformed to account for skewed properties. Geometric mean (GMT) concentrations are presented. To determine factors independently associated with systemic IgA and IgG levels, we applied multivariate models for correlated data. Generalized estimating equations (GEE) models with an unstructured correlation structure were used to estimate standard errors and coefficients adjusted for multiple observations for each woman. Spearman coefficients (ρ) were calculated for the correlation between systemic and cervical IgA and IgG. We also calculated correlations for subgroups defined by oral contraceptive (OC) use, time in menstrual cycle,

smoking status, cervical hemoglobin contamination, indication of genital infection or inflammation, HPV status or cytologic abnormality.

All analyses were performed using STATA 9.2.

Results

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

We evaluated factors associated with systemic IgA and IgG levels between women. Neither IgA nor IgG in 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 follicular, peri-ovulatory, and luteal phases, respectively. Similarly, IgG levels were 8.46, 8.15, and 8.00 mg/ml at follicular, peri-ovulatory, 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 (percent change 0.85, 95% confidence interval 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 plasma and cervical IgA and IgG. Overall, plasma and cervical IgA or IgG were not highly correlated ($\rho=0.17$, and 0.16 , respectively); correlations remained low when we stratified by time in menstrual cycle (Table 1). We further investigated plasma and cervical IgA and IgG correlation in subgroups such as oral contraceptive use, age, smoking status, recent illness, evidence of sexually transmitted infection, presence of blood in the cervical samples, and did not find meaningful correlation between plasma and cervical IgA and IgG within any of these sub-groups (Table 1). Figure 1 graphically depicts the low correlation observed between systemic and cervical immunoglobulins by 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. Further, we observed that while cervical IgA and IgG levels vary considerably between women, systemic levels appear 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^{1;10;12}.

Interestingly, we found 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 demonstrated a sharp decline in total cervical IgA and IgG levels around ovulation among naturally cycling women, consistent with smaller reports from other groups ^{3;11;14}. Further, 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. 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 ^{7;15}. A recent publication from our group ⁷, reported correlations ranging from 0.73 to 0.75 between systemic and cervical levels of antibodies against HPV-specific antigens (16 and 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 can therefore be reconciled as follows: 1) 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 ⁹; and 2) 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 intramuscular 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 demonstrated in other models of parenteral immunization⁸. 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 ¹⁴. We believe the lack of correlation between systemic and local levels in the presence of blood may be due to a very little 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 whom we have both systemic and cervical IgA and IgG levels. Interestingly, the mean systemic IgA and IgG (2.1 and 8.4mg/ml, respectively), and cervical IgA and IgG (0.50 and 1.27mg/ml, respectively) were comparable to mean IgA and IgG levels in serum in other published studies ².

In summary, in contrast with mucosal humoral responses, we observed that systemic levels of IgA and IgG are 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.

Acknowledgments

We would like to thank study participants for their time and effort. We would also like to acknowledge Lidiana Morera, the study nurse, and Manuel Barrantes, the field supervisor, whose efforts made this study possible. This research was supported in part by the Intramural Research Program of the National Cancer Institute, NIH. This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under contract (N01-CO-12400). The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Reference List

1. Castle PE, Hildesheim A, Bowman FP, Strickler HD, Walker JL, Pustilnik T, Edwards RP, Crowley-Nowick PA. Cervical concentrations of interleukin-10 and interleukin-12 do not correlate with plasma levels. *J Clin Immunol* 2002;22:23–27. [PubMed: 11958590]
2. Janeway, Chales A.; Travers, Paul; Walport, Mark; S, MJ. *Immunobiology*. Vol. 5. Garland Publishing; 2001. The Humoral Immune Response; p. 341-380.
3. Franklin RD, Kutteh WH. Characterization of immunoglobulins and cytokines in human cervical mucus: influence of exogenous and endogenous hormones. *J Reprod Immunol* 1999;42:93–106. [PubMed: 10221733]
4. Hamelinck D, Zhou H, Li L, Verweij C, Dillon D, Feng Z, Costa J, Haab BB. Optimized normalization for antibody microarrays and application to serum-protein profiling. *Mol Cell Proteomics* 2005;4:773–784. [PubMed: 15793073]
5. Harper DM, Franco EL, Wheeler CM, Moscicki AB, Romanowski B, Roteli-Martins CM, Jenkins D, Schuind A, Costa Clemens SA, Dubin G. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. *Lancet* 2006;367:1247–1255. [PubMed: 16631880]
6. Herrero R, Schiffman MH, Bratti C, Hildesheim A, Balmaceda I, Sherman ME, Greenberg M, Cardenas F, Gomez V, Helgesen K, Morales J, Hutchinson M, Mango L, Alfaro M, Potischman NW, Wacholder S, Swanson C, Brinton LA. Design and methods of a population-based natural history study of cervical neoplasia in a rural province of Costa Rica: the Guanacaste Project. *Rev Panam Salud Publica* 1997;1:362–375. [PubMed: 9180057]
7. Kemp TJ, Garcia-Pineres A, Falk RT, Poncellet S, Dessy F, Giannini SL, Rodriguez AC, Porras C, Herrero R, Hildesheim A, Pinto LA. Evaluation of systemic and mucosal anti-HPV16 and anti-HPV18 antibody responses from vaccinated women. *Vaccine* 2008;26:3608–3616. [PubMed: 18541349]
8. Lu FX, Abel K, Ma Z, Rourke T, Lu D, Torton J, McChesney M, Miller CJ. The strength of B cell immunity in female rhesus macaques is controlled by CD8+ T cells under the influence of ovarian steroid hormones. *Clin Exp Immunol* 2002;128:10–20. [PubMed: 11982585]
9. Lu FX, Ma Z, Moser S, Evans TG, Miller CJ. Effects of ovarian steroids on immunoglobulin-secreting cell function in healthy women. *Clin Diagn Lab Immunol* 2003;10:944–949. [PubMed: 12965931]
10. Menge AC, Naz RK. Immunoglobulin (Ig) G, IgA, and IgA subclass antibodies against fertilization antigen-1 in cervical secretions and sera of women of infertile couples. *Fertil Steril* 1993;60:658–663. [PubMed: 8405520]
11. Nardelli-Haeffliger D, Wirthner D, Schiller JT, Lowy DR, Hildesheim A, Ponci F, De GP. Specific antibody levels at the cervix during the menstrual cycle of women vaccinated with human papillomavirus 16 virus-like particles. *J Natl Cancer Inst* 2003;95:1128–1137. [PubMed: 12902442]

12. Naz RK, Butler A, Witt BR, Barad D, Menge AC. Levels of interferon-gamma and tumor necrosis factor-alpha in sera and cervical mucus of fertile and infertile women: implication in infertility. *J Reprod Immunol* 1995;29:105–117. [PubMed: 7500318]
13. Paavonen J, Jenkins D, Bosch FX, Naud P, Salmeron J, Wheeler CM, Chow SN, Apter DL, Kitchener HC, Castellsague X, De Carvalho NS, Skinner SR, Harper DM, Hedrick JA, Jaisamrarn U, Limson GA, Dionne M, Quint W, Spiessens B, Peeters P, Struyf F, Wieting SL, Lehtinen MO, Dubin G. Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial. *Lancet* 2007;369:2161–2170. [PubMed: 17602732]
14. Safaeian M, Falk RT, Rodriguez AC, Hildesheim A, Kemp T, Williams M, Morera L, Barrantes M, Herrero R, Porras C, Pinto L. Factors associated with fluctuations in IgA and IgG levels at the cervix during the menstrual cycle. *J Infect Dis* 2009;199:455–463. [PubMed: 19133811]
15. Schwarz TF, Spaczynski M, Schneider A, Wysocki J, Galaj A, Perona P, Poncelet S, Zahaf T, Hardt K, Descamps D, Dubin G. Immunogenicity and tolerability of an HPV-16/18 AS04-adjuvanted prophylactic cervical cancer vaccine in women aged 15-55 years. *Vaccine* 2009;27:581–587. [PubMed: 19022320]

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 2 individuals from 2 of the graphs. One was in the IgG peri-ovulatory 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 of 0.008 and systemic IgA was 2.70.

Table 1
Systemic and cervical IgA and IgG concentrations (GMT) and correlations by covariates:

IgA (mg/ml)					IgG (mg/ml)		
	N (%)	Systemic	Cervix	ρ	Systemic	Cervix	ρ
Overall: GMT	426 (100.0)	2.06; 1.94	0.50, 0.31	0.17	8.42, 8.30	1.27, 0.78	0.16
Visit							
Follicular	139 (32.6)	1.80	0.47	0.25	8.67	1.16	0.14
Peri-Ovulatory	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 OC 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							
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 STD							
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

		IgA (mg/ml)			IgG (mg/ml)		
	N (%)	Systemic	Cervix	p	Systemic	Cervix	p
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

Bold face indicates p-value <0.05