Cervical Antibodies to Herpes Simplex Virus Proteins in Pregnancy and Puerperium: A Pilot Study

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ABSTRACT

Objective: This study was undertaken to evaluate the changes in total and anti-herpes simplex virus (HSV)-specific cervical IgA and IgG antibody profiles during and after pregnancy.

Methods: Serum and cervical secretions were obtained from pregnant patients before 20 weeks gestation, at 34-36 weeks gestation, and at 6 weeks postpartum and tested for total IgA and IgG antibody and for IgA and IgG to HSV proteins by Western blot.

Results: Seven women were HSV seronegative, 14 HSV-1 seropositive, and 14 HSV-2 + HSV-1 seropositive. Minimal changes in the serum anti-HSV profiles were seen over the 3 visits. The total cervical IgA, IgG, and protein levels did not change between the 2 pregnancy visits but tended to increase at the postpartum visit. No consistent change in cervical HSV-specific IgA and IgG was seen during pregnancy, but the levels increased markedly at the postpartum visit.

Conclusions: Lower cervical anti-HSV antibody levels may be related to the previously reported increased frequency of a reactivation of HSV during late pregnancy. Further evaluation is necessary to confirm and quantify the changes in genital immunity during pregnancy and to evaluate whether the increased levels at the postpartum visit are sustained.

KEY WORDS
Local immunity, IgA, IgG, genital infection, Western blot

Genital herpes simplex virus (HSV) infections are common among pregnant women, with up to 40% having antibody to HSV-2.1-4 The risk factors for the perinatal transmission of HSV include recent primary genital infection, cervical rather than only labial HSV shedding, use of fetal-scalp electrodes, and lack of maternal antibody, particularly antibody to an HSV-2 type-specific glycoprotein, gG.1,5-9 Among some women with recurrent genital HSV infections, reactivation increases in the third trimester,10 which may result in a greater risk of perinatal transmission. The factors responsible for the increased rate of reactivation in late pregnancy have not been elucidated, but may be associated with the immunologic changes characteristic of late pregnancy.11,12

One possible contributor to the increased genital HSV reactivation in late pregnancy is a change in local antibody levels. In addition to serum antibody, a genital HSV infection induces mucosal antibodies,13-19 which are directed against a number of viral proteins, that are demonstrable months after the infection and neutralize HSV in vitro. Although systemic levels of immunoglobulins do not appear to

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Clinical Study

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改变期间妊娠，妊娠对粘膜抗体的影响尚未评估。为了评估妊娠对循环和局部宫颈总和抗-HSV 特异性免疫球蛋白的影响，我们使用定量法检测宫颈总 IgA 和 IgG 水平和半定量方法分析 HSV 特异性 IgA 和 IgG 在妊娠初的 serum 和 cervical 秘密分泌物中收集并分析了妊娠第一部分妊娠、34-36 周妊娠和产后 6 周。

SUBJECTS AND METHODS

Study Population and Protocol

妊娠妇女被纳入研究，妊娠期间，妊娠妇女被列为妊娠前的 clinic 海湾医疗中心和 Harbormedical Center 之间的 January 1991 和 March 1992。所有受试者均以同意书的形式得到同意，该同意书由华盛顿大学 Institutional Review Board 批准。患者在妊娠前 20 周被纳入研究，然后在妊娠 34-36 周妊娠和 6 周后妊娠。在 55 名患者中，37 名患者至少完成了 2 次访问。在 18 名（33%）没有完成研究的患者中，13 名患者由于医学原因而被排除（自发性流产 3 名和 placenta previa 1 名）。所有患者在妊娠期间的 HS 经验病史和症状调查问卷均被完成。病毒培养按先前的描述进行。血清标本用于检测 HSV-1 和 HSV-2 的 IgA 和 IgG 由 Western blot 法分析。

Cervical Secretions

宫颈分泌物

宫颈分泌物被分为先前的描述。简要地，带入阴道镜，2 片眼药水 tear-flow 测试条（"Sno-strips," Akorn, Inc., Abita Springs, LA) 被放在阴道上，直到给到无缝的 10 秒。这个技术提供了每种分泌物的恒定体积（平均体积 = 357 ± 77 µl/sample)。18 名患者被单独采样，饱和的条被密封在无菌的标本中，并冷冻在 -20°C。

Specimen Preparation

样本准备

样本被解冻。在 Sno-strips 被移除后，标本被孵育，然后在 13,960 g 15 min。超折射体积被记录，并且 50-µl 部分从每个标本用于蛋白分析（Bio-Rad, Richmond, CA)。羽状的标本用于检测来自 Hemoccult (Smith-Kline Diagnostics, San Jose, CA)，但正向的标本未被分析。

Total Cervical IgG, IgA Determinations

总宫颈 IgG, IgA 定量

纯化的人 IgA（Sigma, St. Louis, MO) 和人的 IgG 标准品（Pierce, Rockford, IL) 分别用 2 倍稀释至 1:10,000 和 1:80,000。在碳酸缓冲液（pH 9.6）中分散入 96-well plates。分泌物被 2 倍稀释至 1:40（IgA）和 1:200（IgG）。结合抗体被用 Peroxidase-conjugated goat anti-human chain (Tago, Burlingame, CA) 和 3,3',5,5'-tetramethylbenzidine (TMB; Kirkegard & Perry, Gaithersburg, MD) 检测。在 450 nm 测定吸光度值。2 点选择在标准曲线的直线部分，并被计算出回归线。两台标本连续在标本曲线的范围内的线性部分被选择，并 IgA 和 IgG 浓度在宫颈标本中被外推。
human IgG (y chain diluted 1:10,000 in PBS; Boehringer Mannheim Biochemicals, Indianapolis, IN).

After being thoroughly washed with PBS/Tween, the blots were incubated for 1 min with a commercial Western blotting detection system based on chemiluminescence as directed by the manufacturer (Amersham, Arlington Heights, IL). The blots were then covered with plastic wrap and exposed to Hyperfilm-ECL (Amersham) for a range of times: 10 sec to 3 min. The film was then developed in a Kodak X-Omat processor.

All specimens from each patient were tested against the same lot of ECL-Western blot strips and in the same immunoblot run. For an accurate comparison of antibody profiles across time points, each set of blots for HSV-specific IgA or IgG was developed for the same length of time. The changes in relative levels of IgA or IgG to HSV were scored if at least 3 major bands showed a marked difference in intensity or if the bands were present in 1 specimen and absent in the other specimens being compared. The reactive bands were identified by migration characteristics as described previously.22,23

Statistical Analysis

The median levels of protein and total IgG and IgA from the cervix at each visit were compared using the 2-tailed Wilcoxon's signed-rank test.24 The changes scored as increased or decreased levels of HSV-specific cervical IgA and IgG by ECL-Western blot between visits were evaluated using a 2-tailed sign test.24

RESULTS

HSV Serostatus

Of the 37 patients who completed the study, 35 subjects had at least 2 cervical samples taken for local antibody measurements that were Hemoccult negative. Of these, 7 (20%) were HSV seronegative, 14 (40%) had antibodies to HSV-1, 10 (29%) had antibodies to HSV-2, and 4 (11%) had antibodies to both HSV-1 and HSV-2. In the following analyses, the 4 patients with antibodies to both HSV-1 and HSV-2 were grouped with the 10 who had antibodies to HSV-2 only.

Prenatal Cervical Protein, IgA, and IgG Levels

Thirty-five women had cervical specimens taken during the third trimester for comparison with enrollment samples for total cervical protein concentrations, total IgA, and total IgG. The results according to HSV serostatus are shown in Table 1. No significant differences in median protein, total IgA, or total IgG values were found between the enrollment and third-trimester samples in seronegative women or in HSV-1 or HSV-2 seropositive women compared with HSV seronegative women. Moreover, all values were similar between the 2 time points.

Postpartum Changes in Total Protein, Total IgA, and IgG

Nineteen of 35 (54%) women had evaluable cervical specimen pairs from third-trimester and postpartum visits (Table 1). Overall, the median values of total protein, IgA, and IgG rose 3-fold to 5-fold in the postpartum samples. This rise was especially apparent in local IgA which rose from a median of 1.32 to 6.3 meg/ml (P < 0.005). Of interest, this rise was seen mainly in the HSV seronegative and HSV-1 seropositive subsets. The median IgA values rose approximately 10-fold in seronegative women (P = 0.08) and HSV-1 seropositive women (P = 0.03) and by 2-fold in HSV-2 seropositive women (P = 0.35) (Table 1).

The specimen volume, our measure of adequacy and uniformity of the sampling technique, showed no significant change between the pre- and postpartum samples (P = 0.50, P = 0.51, P = 0.35, and P = 0.63 for HSV seronegative, HSV-1 seropositive, HSV-2 seropositive, and all patients, respectively) or between the HSV seronegative and HSV-1 vs. HSV-2 seropositive specimens.

Enrollment and Third-Trimester Cervical HSV-1 IgA Profiles

Fourteen women who were seropositive for HSV-1 had samples taken both at the enrollment visit and during the third trimester for comparison of HSV cervical antibody profiles. Of these sample pairs, 3 had no detectable IgA to HSV-1 in either sample. Of the 11 with detectable antibodies, 5 had unchanging profiles of HSV-1 cervical IgA and 3 had increases and 3 had decreases in the number and intensity of reactive bands on ECL-Western blot (Table 2). An example of the low prenatal levels of cervical IgA to HSV is shown in Figure 1A (lanes 1 and 2). Note that detectable cervical IgG to HSV was present at all sampling points (Fig. 1B). The serum IgA (Fig. 1C) and IgG antibody
TABLE 1. Cervical protein, IgA, and IgG levels at each visit

| HSV status     | No. of patients | Visit                  | Median protein (range), µg/µl | Median IgA (range), µg/µl | Median IgG (range), µg/µl | P value | P value | P value |
|----------------|-----------------|------------------------|--------------------------------|---------------------------|---------------------------|---------|---------|---------|
| Negative       | 7               | Enrollment            | 19 (5-53)                      | 2.23 (0.12-5.54)          | 2.78 (0-23.31)            | 0.35    | 0.18    | 0.50    |
|                |                 | 3rd trimester         | 40 (10-51)                     | 1.32 (0-4.05)             | 2.41 (0-5.76)             |         |         |         |
|                | 5               | 3rd trimester         | 24 (10-51)                     | 1.87 (0-4.05)             | 3.41 (0-3.11)             |         |         |         |
|                |                 | Postpartum            | 250 (30-308)                   | 12.16 (0.05-45.15)        | 14.33 (0.03-21.34)        | P = 0.07| P = 0.08| P = 0.08|
| HSV-1 only     | 14              | Enrollment            | 22.5 (4-230)                   | 1.87 (0.01-19.61)         | 3.31 (0.1-36.49)          | 0.92    | 0.73    | 0.69    |
|                |                 | 3rd trimester         | 29 (10-134)                    | 1.44 (0.01-7.26)          | 3.42 (0.16-18.42)         |         |         |         |
|                |                 | Postpartum            | 29 (10-134)                    | 1.59 (0.32-7.26)          | 3.35 (0.83-18.42)         |         |         |         |
| HSV-2          | 14              | Enrollment            | 25 (0-63)                      | 1.83 (0.03-12.71)         | 4.14 (0.16-41.09)         | 0.68    | 0.22    | 0.70    |
|                |                 | 3rd trimester         | 22.5 (0-484)                   | 1.51 (0.03-10.12)         | 2.82 (0.252-33.79)        |         |         |         |
|                |                 | Postpartum            | 24 (15-90)                     | 0.97 (0.22-7.56)          | 8.47 (1.17-53.79)         | P = 0.69| P = 0.35| P = 0.89|
| Total          | 35              | Enrollment            | 21 (0-230)                     | 1.93 (0.01-19.61)         | 3.49 (0.4-41.09)          |         |         |         |
|                |                 | 3rd trimester         | 29 (0-484)                     | 1.41 (0-10.12)            | 3.11 (0-33.79)            |         |         |         |
|                |                 | Postpartum            | 29 (10-134)                    | 1.32 (0.7-5.76)           | 2.72 (0.3-33.79)          | P = 0.05| P < 0.005| P = 0.08|

**Wilcoxon’s signed-rank test, 2-tailed P value comparing paired visits as indicated.

TABLE 2. Changes in ECL-Western blot cervical antibody profiles against HSV proteins between visits

| HSV serologic status | No change | Increase | Decrease | Total | No change | Increase | Decrease | Total |
|----------------------|-----------|----------|----------|-------|-----------|----------|----------|-------|
| Enrollment and 3rd trimester |           |          |          |       |           |          |          |       |
| HSV-1                | 5 (45%)   | 3 (27%)  | 3 (27%)  | 11    | 7 (50%)   | 3 (21%)  | 4 (29%)  | 14    |
| HSV-2                | 2 (20%)   | 2 (20%)  | 6 (60%)  | 10    | 4 (29%)   | 6 (42%)  | 4 (29%)  | 14    |
| Total                | 7 (33%)   | 5 (24%)  | 9 (43%)  | 21    | 11 (39%)  | 9 (32%)  | 8 (29%)  | 28    |
| 3rd trimester and postpartum |       |          |          |       |           |          |          |       |
| HSV-1                | 0         | 9 (90%)  | 1 (10%)  | 10    | 1 (10%)   | 8 (80%)  | 1 (10%)  | 10    |
| HSV-2                | 0         | 5 (83%)  | 1 (17%)  | 6     | 0         | 4 (67%)  | 2 (33%)  | 6     |
| Total                | 0         | 14 (88%) | 2 (12%)  | 16    | 1 (6%)    | 12 (75%) | 3 (19%)  | 16**  |

*ECL-Western blot profiles of cervical IgA and cervical IgG were compared between enrollment and 3rd-trimester blots and between 3rd-trimester and postpartum blots, as illustrated in Figures 1 and 2. Numbers in parentheses are the percentages of subjects with a given result within the population of pairs scored (“Total”). Increase = at least 3 major bands on ECL-Western blot were markedly increased in intensity or were present in the second but not the first blot of the pair. Decrease = at least 3 major bands on ECL-Western blot were markedly decreased in intensity or were present on the first but not the second blot of the pair. All blots for the same subject were run at the same time under the same conditions for each antibody type (IgA or IgG).

*Wilcoxon’s signed-rank test, 2-tailed P value comparing paired visits as indicated.

profiles to HSV-1 (Fig. 1D) did not change over time. No cervical IgA or IgG antibodies against HSV-1 or HSV-2 proteins were detected at any of the 3 visits in seronegative patients.

Five of the 6 women who had apparent changes in cervical IgA profiles had concordant changes (either increases or decreases) in their total protein levels as well. The volumes of the paired specimens in 5 of
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| Cervical | Serum |
|---------|-------|
| IgA | IgG | IgA | IgG |

Fig. 1. Cervical and serum antibody profiles from samples obtained over time from an HSV-1 seropositive patient. ECL-Western blots were performed to detect cervical antibodies to HSV-1 (A, B) and serum antibodies to HSV-1 (C, D). The left blot in each panel is from specimens taken at enrollment, the middle blot from specimens taken during the third trimester, and the right blot from specimens taken 6 weeks postpartum. Note the dramatic increase in band number and intensity in cervical IgA and IgG to HSV-1 between the third-trimester and postpartum samples. In contrast, the serum profiles show no change in the IgA profile to HSV-1 over the 3 time points. The serum IgG profiles, while difficult to read at this exposure because of the high IgG, show evidence of a decrease in intensity in the third-trimester serum and recovery of intensity in the postpartum serum.

the 6 pairs with changes were either similar between the pairs or showed discordant change, suggesting that sampling differences alone were not sufficient to explain the changes. The sixth pair had a lower volume in the third-trimester sample along with decreasing apparent levels of IgA to HSV-1; however, her IgG levels increased between the 2 specimens. Of note was that 1 subject had labial blisters that were culture positive for HSV-1 at the time of her third-trimester sampling; her HSV-1-specific IgA in genital secretions decreased to undetectable levels at this sampling. None of the other genital cultures obtained were positive for HSV during the study.

Postpartum Cervical HSV-1 IgA
Fourteen women with HSV-1 antibody had specimens taken in the third trimester and at 6 weeks postpartum. The postpartum samples from 4 women contained blood and could not be evaluated for cervical antibody. Of the 10 available pairs, all had demonstrable HSV-IgA in the postpartum cervical secretions and 9 (90%) had clear increases in cervical HSV-IgA profile complexity and intensity, while 1 had a decrease (Table 2). Figure 1A (lane 3) gives an example of the increased intensity and complexity of the IgA profile postpartum.

Enrollment and Third-Trimester Cervical HSV-2 IgA Profiles
IgA to HSV-2 was compared in enrollment and third-trimester samples of 14 women who were seropositive for HSV-2. Of the 14 pairs, 4 lacked detectable IgA. Of the 10 with detectable HSV-2 antibody, 2 had no change, 2 had increasing complexity and intensity of profiles, and 6 had decreasing amounts of antibodies in their cervical secretions (Table 2). An example of a decrease in cervical IgA band number and intensity is shown in Figure 2A (lanes 1 and 2). This patient also had a decrease in cervical IgG in the third-trimester sample (Fig. 2B, lanes 1 and 2).

Postpartum Cervical HSV-2 IgA
Of the 14 HSV-2 seropositive women, 9 had specimens taken at 6 weeks postpartum. Three specimens were not used for comparison of antibody profiles because they contained blood. Five of the 6 (83%) available pairs showed clear increases in IgA complexity and intensity in the postpartum samples and the sixth showed a decrease (Table 2). The patient in Figure 2A (lane 3) exemplifies the dramatic changes in cervical IgA profiles postpartum.

Overall, in 21 evaluable subjects with detectable cervical IgA to HSV-1 or HSV-2, 43% had decreases in apparent antibody levels between enrollment and the third trimester; 24% had increases; and 33% had no apparent change. Overall, 14 of 16 (88%) HSV seropositive women had postpartum increases in their cervical IgA to HSV (P = 0.004 by 2-tailed sign test).

Enrollment and Third-Trimester Cervical HSV IgG
All 28 patients with paired specimens from enrollment and third-trimester visits had detectable cervical anti-HSV IgG. Among the 14 with HSV-1 anti-
The IgA increased while the IgG showed no change postpartum in 1 patient and the IgA increased while the IgG decreased in 1 patient. Four of 6 (67%) available pairs had pronounced increases in cervical IgG to HSV-2, while 2 (33%) had decreases. The changes in cervical HSV-2 antibodies were concordant between IgA and IgG in 5 of the 6 pairs; the sixth had an increase in IgA and a decrease in IgG postpartum. Overall, 12 patients (75%) had increased cervical IgG to HSV postpartum, while 3 (19%) had decreases and 1 (6%) had no change ($P = 0.08$ for proportion with increased postpartum IgG by 2-tailed sign test).

**Serum Antibody Profiles Pre- and Postpartum**

Sera were drawn at all 3 visits from 6 HSV-1 and 6 HSV-2 seropositive women. Serum IgA and IgG profiles were examined between enrollment and the third-trimester and postpartum visits. Among the 6 women with HSV-1 antibodies, 3 had no detectable changes, 2 had IgA (but not IgG) profiles that decreased in complexity in the third trimester and recovered to enrollment levels postpartum, and a third had IgG (but not IgA) levels that dropped at the third trimester but recovered postpartum. In this latter patient, the cervical IgG changes roughly paralleled those of serum IgG (Fig. 1B vs. Fig. 1D), while the cervical IgA changed markedly without a concomitant change in serum IgA profiles (Fig. 1A vs. Fig. 1C). Of the 6 who were HSV-2 seropositive, 3 had no detectable changes, 1 had a rise in serum IgA (but not IgG) at the third trimester, 1 had a decrease in both serum IgA and IgG at the third trimester (changes which were reflected in similar changes in cervical antibody), and 1 had a possible rise in serum IgA in the postpartum sample (Fig. 2C). Overall, of the 12 women with sequential serum samples, the changes in apparent levels of serum HSV-specific IgA and IgG over time were either not detected or were minor compared with the profile changes seen over time with cervical HSV-specific IgA and IgG.

**DISCUSSION**

This pilot study indicates that there are fluctuations in HSV-specific antibodies in cervical secretions that are not seen in serum. Although serum antibody profiles changed little between pregnancy and the postpartum visits, the total protein, total IgA, and HSV-specific antibody levels in the cervix in-
creased postpartum. The increasing intensity and complexity of cervical HSV antibody profiles, both IgA and IgG, at the postpartum visit are striking. It is tempting to assume that these relatively low levels of cervical antibodies late in pregnancy reflect broader immune suppression, which may account for the previously reported increased rate of positive genital herpes cultures during the third trimester. However, a study of more women earlier in pregnancy and for a longer period postpartum is necessary to evaluate whether the increasing levels seen postpartum are the normal levels or whether they are transient evaluations. In previous studies of non-pregnant women with either first-episode or recurrent genital HSV infections, the cultures were not positive when the cervical IgA antibody level was ≥1:2. In addition, the mean duration of genital shedding of HSV with both first-episode and recurrent outbreaks was 3 days shorter among women who developed secretory IgA compared with those without IgA. These and more recent studies are suggestive that IgA may play a role in limiting the duration of HSV shedding. However, since only 1 patient had a positive culture at any visit in our study, only concurrent, more frequent sampling for both virus and antibody in the cervix could address this question.

The lack of change in serum IgG and IgA antibody levels over the course of pregnancy and the postpartum visit is consistent with previous studies of antibody levels in pregnant women. The serum antibody patterns do not account for the observed changes in cervical antibody levels. Although no changes were seen in the cervical levels of total protein, IgG, or IgA between the enrollment and third-trimester sampling points, the enrollment visit occurred anytime before 20 weeks gestation. The changes related to pregnancy may occur early in pregnancy, but our sampling protocol did not include first-trimester visits in all cases. One important finding of our study was the clear increase in total cervical IgA levels between the third-trimester and postpartum sampling. However, the total cervical IgG levels did not change significantly from the third-trimester to the postpartum sampling.

It is not clear why the increase in cervical total IgA and HSV-specific IgA was only 2-fold in HSV-2 positive patients compared with 6-fold in HSV-1 only or HSV antibody negative women. It did not appear to be related to the sampling technique. The volume as measured in each sample was similar at all 3 time points and in all 3 serologic groups, but only a small number of women were studied. Differences in HSV reactivation in the genital tract may be a factor influencing this observation. Our test method could not detect antibodies that were complexed with antigen. Any apparent changes in HSV-2 cervical antibodies may be affected by viral shedding. More detailed study of changes in cervical antibodies during and after pregnancy among women with genital HSV-1 infections would help to elucidate whether the specific type of HSV infecting the genital area has an effect on local antibody levels during and after pregnancy. Such studies will require more patients, more frequent sampling, and more quantitative testing methods.

The differences in local antibodies seen during pregnancy compared with postpartum values may be related to the hormone changes of pregnancy. Of interest, the serum antibody levels in pregnant women do not show significant changes during pregnancy compared with postpartum. In a study of secretory component production by uterine tissues in ovariectomized rats, progesterone was found to cause a marked decrease in the production of secretory component. This effect, which was dose dependent, occurred whether or not the animals were pretreated with estradiol. The high levels of progesterone present during pregnancy may inhibit the production of secretory component which would then limit the local IgA antibody levels. These factors could be further evaluated by more frequent sampling of cervical IgA levels throughout pregnancy and pairing them with progesterone levels since the concentration of progesterone increases markedly over the course of pregnancy. If progesterone specifically affects secretory component and not overall immunoglobulin production, it would account for differences in local antibodies without concomitant changes in serum antibody levels.

For the evaluation of the changes in local immunity suggested by this pilot study, a more comprehensive and detailed study is required. Comparing a control group of nonpregnant women sampled at similar intervals with a group of women before, during, and after pregnancy would allow an evaluation of the changes over time not related specifically to pregnancy. More frequent sampling for both local antibody levels and HSV reactivation detected by
culture and polymerase chain reaction should be included to assess the relationship between local immunity and HSV shedding. In addition, a measurement of antibody levels to an antigen not present in the genital tract such as tetanus would allow a delineation of the changes in levels related to antigenic stimulation compared with non-specific immune-system activation or changes related to pregnancy. The development of more precise methods for collection and quantitation of HSV-specific antibody levels in cervical secretions rather than just a comparison of changes in intensity and complexity of Western blot patterns is necessary to evaluate potential pregnancy-related changes in local antibody. Furthermore, since Western blots measure antibody levels to denatured proteins rather than the intact virion, a correlation of antibody levels with neutralizing activity, viral shedding, and cellular immune responses in the genital tract is necessary to evaluate the clinical significance of changes in ECL-Western blot antibody responses over time.

The methods used in our study could be adapted for a study of the mucosal immune responses to other antigens as well. An evaluation of genital immunity, including antibody response, will be crucial in evaluating the response to human immunodeficiency virus, especially to vaccines designed to protect against human immunodeficiency virus infection.

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