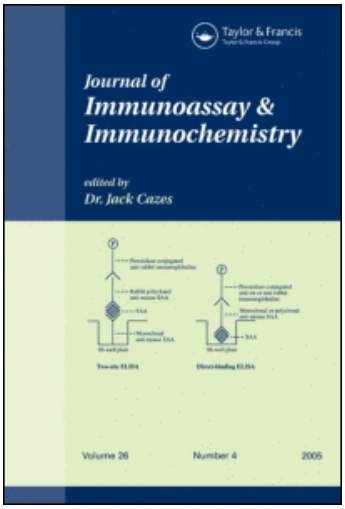

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VALIDATION OF A HIGH SENSITIVE IMMUNOENZYMATIC ASSAY TO ESTABLISH THE ORIGIN OF IMMUNOGLOBULINS IN FEMALE GENITAL SECRETIONS

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VALIDATION OF A HIGH SENSITIVE IMMUNOENZYMATIC ASSAY TO ESTABLISH THE ORIGIN OF IMMUNOGLOBULINS IN FEMALE GENITAL SECRETIONS

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ABSTRACT

Several studies were carried out to characterize the humoral immune response on mucosal genital surfaces. However, the results obtained so far were particularly conflicting due to the absence of validation methods. The aim of this study was to develop and validate a quantitative ELISA method, which is sensitive and reproducible, to measure immunoglobulin and secretory immunoglobulin concentrations in

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various biological fluids. This quantitative, sensitive (detection limit = 1 μg/L) and reproducible (coefficient of variation <15%) method could be of interest to study the effects of viral infections on mucosal non-specific immune response in genital tract. To explore the humoral response, serum, saliva, vaginal secretions, and cervicovaginal secretions from 18 women, 20–45 years old, were evaluated for total-IgA, secretory IgA, IgM, and IgG. Albumin level was also evaluated by immuno-nephelometry. The secretion rates of immunoglobulins were measured by calculating their relative coefficients of excretion by reference to albumin. Despite large individual variations, median immunoglobulin levels were higher in the endocervical secretions than in the cervicovaginal secretions. When we compared the rates of immunoglobulins in genital fluids, IgG prevalence was higher (80%) in cervicovaginal and endocervical secretions than IgA prevalence (12%). In contrast, digestive mucosal secretions, such as saliva, contained mostly IgA (80%). In cervicovaginal and endocervical secretions, IgG and IgM originated mainly from serum, whereas a local synthesis provided total-IgA and secretory IgA. These results allowed us to raise a possible hypothesis for the origin of immunoglobulins in the genital tract. They illustrated the peculiar feature of the female reproductive tract and the difficulty for this tissue to contribute in the mucosal associated lymphoid tissue. The low secretory-IgA and total-IgA levels could explain the particular sensitivity of the vagina and the cervix to infections.

INTRODUCTION

Local protection of the mucous membranes is provided mainly by a local immune defense system, comprised of secretory immunoglobulins (Ig). This system acts on the mucosal surface where it deactivates viruses, exerts anti-microbial properties, and links with exogenous antigens.(1–3) In most studies, the quantification of Ig in female genital secretions was performed without validated methods, especially without taking into consideration the matrix effect on the reaction. Moreover, the determination of all molecular forms of Ig is rarely done in the same assay.(4–6)

The secretory immune system has two functionally independent compartments: a systemic compartment which includes bone marrow, spleen, lymph nodes, and mucosal compartment which involves lymphoid tissues in

mucosa and external secretory glands (intestinal, bronchial, nasal, mammary, salivary, and lachrymal glands).(7,8) All are linked by mucosa-associated lymphoid tissue (MALT) with inductive and effector sites.(9) Recent studies have shown, however, that the genital tract mucosa seems to function differently; it has been demonstrated that there was a poor induction of specific antibodies in the mucosa genital surface when other compartments were strongly activated by antigens.(5,10) Human IgA occur in several molecular forms: monomeric (mIgA), polymeric (pIgA), and secretory IgA (S-IgA), with two subclasses (IgA_1 and IgA_2) showing differential distributions between the mucosal and circulatory compartments of the immune system.(11) The receptor for polymeric Igs is a glycoprotein synthesized by the epithelial cells of mucosal and exocrine glands. Its extracellular region is released by proteolytic cleavage as a secretory component (SC). Polymeric Ig receptor (pIgR) plays a major role in the transepithelial transport of polymeric IgA and IgM.(7) It is mainly found in external secretions bound to S-IgA and secretory IgM (S-IgM), and also in its free form.(7) Analyses of cervicovaginal lavage samples have shown the presence of both IgG and IgA in female genital secretions. Detailed studies have revealed that the number of Ig isotypes present in secretions varies at different sites in the female genital tract (12) and varies over the course of the menstrual cycle.(5) There are local variations in Ig-producing cells, epithelial receptor-mediated IgA secretions, and transudation of serum Ig. Nevertheless, there are many contradictory results concerning the origin and the relative ratio of Ig at different levels of the female reproductive tract.

To obtain information about the concentrations of different Igs present in secretions associated with mucosa surfaces, as in the cervix and vagina, our laboratory has developed, first, a procedure to collect genital fluids (cervicovaginal and endocervical fluids) without causing epithelial damage, and second, a quantitative analysis by enzyme linked immuno sorbent assay (ELISA).

Endocervical secretions were obtained by application of adsorbent filter strips originally designed for collection of human ophthalmic secretions.(13) Cervicovaginal secretions were obtained by vaginal lavage. Serum and saliva samples were, respectively, used as systemic and upper digestive compartment controls. The salivary, cervicovaginal, or endocervical relative coefficient of excretion (RCE), by reference to albumin, as well as the comparison of specific activities, permitted to determine the immunoglobulin's origin (secretion or transudation).(11)

The purpose of this study was to find a reliable method for evaluating S-IgA, total-IgA, IgM, and IgG in various biological fluids in order to obtain quantitative results. Using the French Society of Clinical Biology test for validation, we used the method on healthy women to observe the

specific nature of the female reproductive tract with Ig imbalance.(14) This study permitted us to raise a hypothesis on the origin of Ig in genital fluids, and in the future, it will be used to explore non-specific genital immune response in infectious diseases.

EXPERIMENTAL

Subjects

Eighteen human papillomavirus (HPV) and human immunodeficiency virus (HIV) negative women (aged 17–42 years) attended the Department of Obstetrics and Gynecology (CHU Besançon, France). Written informed consent was obtained from each woman. At the beginning, patients filled in a form with general questions. This form gave information about the last menses, date of the first and the last sexual intercourse, previous gynecological problems, and use or not of the pill. Exclusion criteria were vaginal douching 48 h before, alcoholism, and liver diseases, leucorrhees, menses, and cervical bleeding. From this population, 10 women were chosen at random and samples were pooled for validation of an ELISA.

Biologic Fluid Collection

To explore all compartments of the female genital tract and their relationships with the MALT, different types of samples were chosen.

Saliva Samples

Saliva samples were obtained within a period of five minutes in each side of the mouth, with a "Salivette"(TM) (Sarstedt, Orsay, France) which was placed between gum and cheek in the axe of the ostium Stenon canal. The "Salivette"(TM) was left in contact for 10 min and then centrifuged at 2500 rpm during 10 min to separate saliva from "Salivette"(TM).

Endocervical Secretion Samples

Endocervical secretion samples were taken before lavages by application of an adsorbent filter strip (Sno Strip(TM), Akorm Inc., Abita Springs, LA, USA) originally designed for collection of human ophthalmic secretions

used for Schirmer test.(13) After visualization of the cervix with a speculum without lubrication, a filter strip was applied on the external orifice of the cervix. The strip was then immersed into 0.5 mL of phosphate buffer salt (PBS) at pH 7.4 (Sigma Chemical, Bourgoin-Jallieu, France) with 0.1% of sodium azide and 0.0025% of aprotinine. After low-speed mechanical agitation, the strip was removed. There was no blood contamination, as confirmed by the Hem Check-1$^{(TM)}$ test.

Cervicovaginal Secretions

Cervicovaginal secretions were obtained using a lavage technique described by Bélec et al.(15) Three milliliters of sterile PBS were instilled into the vagina with a pipette. After flux and reflux cycles lasting 60 s, 2 to 3 mL of fluid were collected. This sample was centrifuged for 10 min at 2500 rpm. The supernatant was used to measure Igs, while the pellet-containing cells and other mucus was frozen for later studies.

Blood Samples

Blood samples were taken on a 10 mL dry tube. All sera were stored at -80°C.

Sandwich Enzyme Linked Immunosorbent Method

S-IgA, total-IgA, IgM, and IgG were assayed by ELISA in all biological fluids. Immulon Immuno Plates$^{(TM)}$ (Dynex Microtiter, Issy-les-moulineaux, France) were coated for 1 h at 37°C and incubated overnight at 4°C with purified antibody to human IgA (rabbit anti-α chain polyclonal IgG purified, Dako: Dakopatts, Copenhagen, Denmark, A262, 5 μg/mL), to human SC (rabbit anti-Human SC, Dako A187, 1 μg/mL), to human IgM (rabbit anti-μ chain polyclonal IgG purified, Dako, A425, 1 μg/mL), or to human IgG (rabbit anti-γ chain polyclonal IgG purified, Dako, A423, 1 μg/mL) in veronal buffer (25 mmol/L, pH 8.6). Non-specific protein binding sites were blocked by incubation of plates with blocking solution (PBS BSA 1%, Sigma P3813, Bourgoin Jallieu, France). Serial two-fold dilutions of tested samples and standard purified human antibody (Human S-IgA, 55905 Cappel: ICN, Aurora, USA; Human IgA, Dako, X594; Human IgM, Dako, X595; Human IgG X593) were added to the wells. The plates were incubated for 1 h at 37°C under agitation. They were washed three times

with an automatic plate washer (Wallac; Turku, Finland) and then incubated for 1 h, under agitation, with peroxidase conjugated antibodies (rabbit anti-human IgA/HRP, Dako P0216, 0.25 μg/mL; rabbit anti-human IgM/HRP, Dako P0215, 0.25 μg/mL; rabbit anti-human IgG/HRP, Dako P0214, 0.125 μg/mL).

The reaction was developed by adding *o*-phenyl diamine (Sigma P1526, 0.8 mg/mL of citrate tampon and 1 μL of H_2O_2/mL of buffer). After 10 min in the dark, it was stopped by adding $2N\ H_2SO_4$ and optical densities were read at 492 nm with a Titter multiscanplus (MK II$^{(TM)}$, UK). Data were analyzed by software (Titersoft$^{(TM)}$, Flow Laboratory, Herts, UK). Quantitative results were determined by reference to the standard curves. Concentrations were corrected by dilution factors taken into account, Sno trip$^{(TM)}$ absorption, and dilutions for ELISA test.

Immunonephelometry

Immunonephelometry was used to measure human serum albumin in all biologic fluids (Immage$^{(TM)}$, Beckman, Fullerton, CA, USA). IgA, IgM, and IgG were quantified by the same technique as the control. S-IgA was not measured by this method because no validation exists for this isotype.

Criteria of Reliability

If ELISA is not a novative technique, described for the first time in 1971 for Ig dosages with IgG labeled with peroxydase, it presents, however, many advantages: it is simple, rapid, sensitive, and applicable to series. To find reliable data about female genital tract immunity, we validated an ELISA for all Igs (S-IgA, total-IgA, IgM, and IgG) in biological fluids (serum, saliva, cervicovaginal secretions, and endocervical secretions). Several parameters were necessary to efficiently validate this test. Linearity was measured for each Ig with two standard tests; the limits were determined on the basis of measured dilutions in biological fluids on an adapted matrix or from original solutions.(14) Detection limit (DL) was measured with reagent blanks and their standard deviations (SD) were calculated by the formula: $DL = \text{Mean (reagent blank)} + 3\,SD$. Reproducibility was measured in three different tests and was repeated 15 times. The within-series precision was measured with the same tests and repeated during three days.

The amounts of S-IgA, total-IgA, IgM, and IgG were detected in biological fluids. Each sample was tested in duplicate. All the results were

corrected by the dilution factor (DF) imposed by the technique for secretion collection: DF 10 for cervicovaginal lavage (16) or endocervical secretions. The last DF was calculated by difference of weight before and after secretion collection.

Salivary, Cervicovaginal, and Endocervical Relative Coefficient of Excretion (RCE)

To compare the parameters of protein excretion in the various human secretions, and with other species, we calculated the RCE for each protein. The RCE expresses a protein excretion rate relative to that of albumin which is derived entirely from plasma by passive diffusion.(11) The RCE of total S-IgA, total-IgA, IgM, and IgG in saliva, cervicovaginal secretions, and endocervical secretions were obtained according to the following formula:(17)

[(albumin in serum)/(albumin in fluid)]/[(immunoglobulins in serum)/(immunoglobulins in fluid)].

The limit between secretion and transudation is equal to 1 (RCE of albumin). Under this limit, transudation from plasmatic compartments occurs, whereas RCE greater than 1 exhibits a predominant local secretion with a small proportion of transudation.

Statistical Analysis

Results were expressed by the mean with standard deviation and also by the median value and range. Analysis of variance ($p \leq 0.05$) and Spearman's correlation test ($p \leq 0.05$) were performed using the StatView$^{(TM)}$ software for PC.

RESULTS

Linearity and Detection Limits of the Method

A graphic representation (log/log) gave the linearity that was different for all isotypes (Figure 1). Linearity for total-IgA was between 0.005 and 0.625 mg/L, it was between 0.020 and 1.250 mg/L for S-IgA, 0.010 and 1.250 mg/L for IgM, and 0.001 and 1.250 μg/L for IgG.

The higher detection limit was 4 μg/L for total-IgA and IgM, and the lower one was 1 μg/L for IgG.

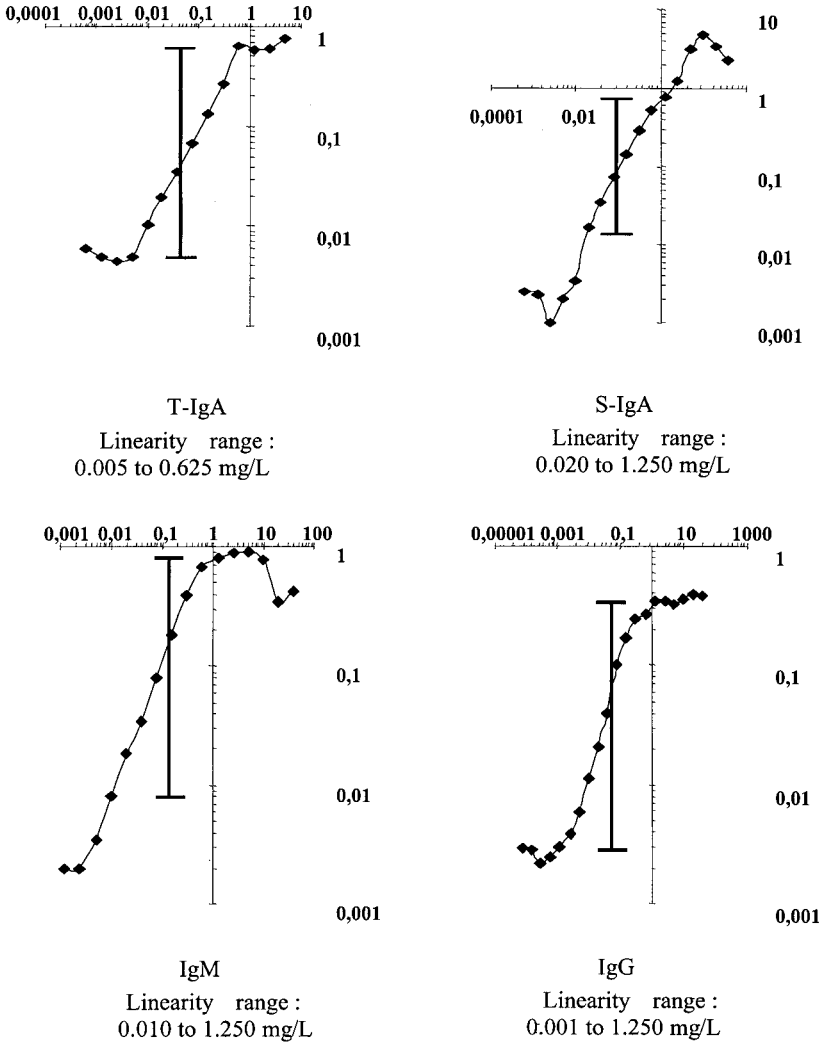

Figure 1. Linearity $(\text{Log}\, y) = f(\text{Log}\, x)$ of each protein (mg/l). Linearity limits are clearly defined by the vertical thick line. T-IgA: total-IgA; S-IgA: secretory-IgA.

Effect of the Medium on the Immunological Reaction

Figure 2 shows the optical density of the immunological reactions for all Igs measured in successive dilutions. An analytical range was chosen

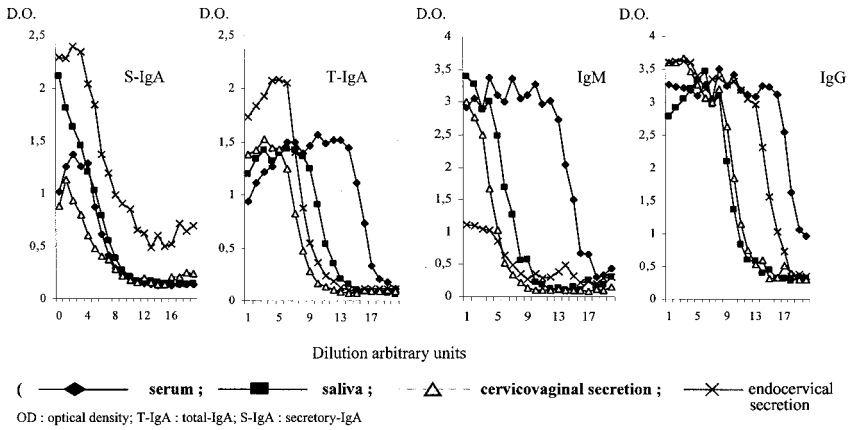

Figure 2. Effect of the medium on the immunological reaction: comparison of serial dilutions in various biological fluids.

where there were linearity and high parallelism curves. Correct dilutions were selected where the environment had no influence on the evaluation. An excellent parallelism of the dilution curves was observed in all biological fluids.

Reproducibility and Within-Series Precision

The reproducibility, expressed in coefficient of variation, was under 15% for total-IgA, 10% for S-IgA and IgM, and 11% for IgG (Table 1). Within-series precision, measured with the same tests and expressed as coefficient of variation, was between 2 and 14% for all isotypes.

Application of ELISA Method for Sera and Biological Fluids in Healthy Women

The genital and salivary S-IgA were always higher than serum S-IgA (Table 2). In all secretions, absolute Ig concentrations were lower than serum Igs, except for S-IgA. The absolute concentration of genital IgG was lower than IgG in serum (ratio 20). All isotypes in endocervical secretions were highly concentrated, compared to those found in saliva and cervicovaginal secretions. Ig concentrations were, however, in the same

Table 1. Reproducibility and Within-Series Precision of the Method

	S-IgA			Total-IgA			IgM			IgG		
	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3
Mean	0.007	0.052	0.600	0.0056	0.060	0.450	ND	0.049	0.600	0.0051	0.048	0.550
SD	0.0003	0.005	0.007	0.0008	0.007	0.007	ND	0.003	0.076	0.0005	0.005	0.040
CV (%)	4.3	9.6	1.2	14.3	11.7	1.6	ND	6.1	12.7	9.8	10.4	7.3

Mean, standard deviation (SD) and coefficient of variation (CV) of 15 repetitive samples during three consecutive days from standard solutions (concentration: C1 = 0.005 mg/L; C2 = 0.050 mg/L; C3 = 0.500 mg/L).
S-IgA: Secretory-IgA.
ND: Not done.

Table 2. Concentrations (mg/L) of S-IgA, Total-IgA, IgM, and IgG in Various Biological Fluids from Healthy Women ($n = 18$)

	S-IgA	Total-IgA	IgM	IgG
Serum	12.9 ± 12.9[a] (9.1)[b]	2427.4 ± 914.3 (2311.2)	1162.5 ± 551.7 (1258.7)	18203.3 ± 8871.9 (15283.3)
Saliva	55.3 ± 35.8 (40.2)	98.9 ± 77.9 (71.4)	6.2 ± 5.1 (4.3)	25.9 ± 27.3 (12.9)
Cervicovaginal secretions	17.96 ± 10.6 (14.8)	118.3 ± 78.1 (100.9)	16.3 ± 19.2 (11.0)	466.8 ± 360.6 (459.3)
Endocervical secretions	128.9 ± 125.0 (80.8)	278.7 ± 156.4 (255.8)	31.9 ± 27.1 (24.5)	940.6 ± 687.3 (631.3)

[a]Mean ± S.D.
[b]Median.
S-IgA: Secretory-IgA.

range in the three secretory compartments, which illustrates their similar secretion characteristics.

Correlation of the Methods

Correlations between both methods (ELISA vs. Immunonephelometry) for the measurement of total-IgA, IgM, and IgG were calculated. All coefficients were markedly greater than $r^2 = 0.7029$. A correlation test gave a strong correlation ($p < 0.0001$ for all isotypes) except for S-IgA, due to the absence of immunonephelometry quantification.

Relative Coefficient of Excretions (RCE)

The RCE of Igs in the different fluids are illustrated in Figure 3. In saliva, the mean RCE of total-IgA (50.2) and IgM (5.6) was largely above 1, i.e., above the RCE of albumin, indicating a predominant local secretion of both isotypes. The mean RCE of IgG (1.2) was close to 1, demonstrating a predominant transudation of this isotype from the plasma, probably associated with a weak local secretion. In cervicovaginal and endocervical

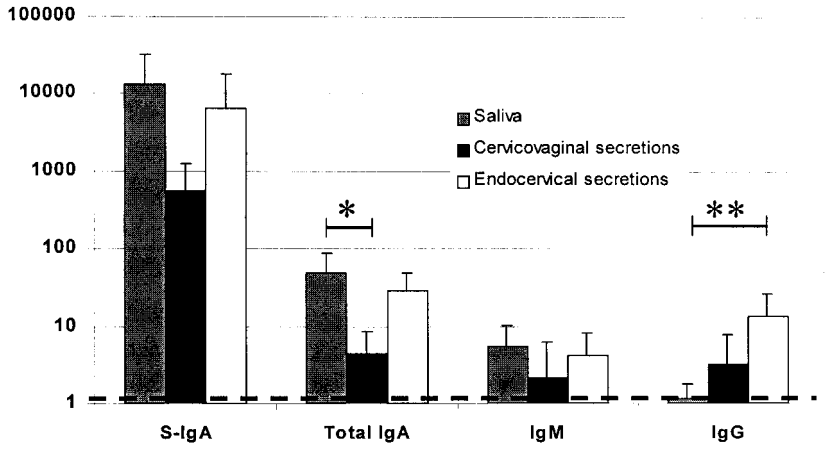

Figure 3. Results from 18 healthy women; mean of the relative coefficient of excretion (protein $= f$ [log(RCE)]) in various biological fluids for S-IgA, total-IgA, IgM, and IgG. The dashed horizontal line gives the RCE of albumin = 1; S-IgA: secretory-IgA. $*p < 0.001$; $**p < 0.0008$.

secretions, RCE of IgG were greater than 1 (3.3 and 13.6, respectively). This RCE for IgG was statistically greater in endocervical secretions compared to saliva ($p < 0.0008$). Furthermore, the mean RCE of total-IgA in cervicovaginal secretions (4.4) were lower than in saliva ($p < 0.001$), suggesting that the proportion of total-IgA originating from plasma is higher in cervicovaginal secretions than in saliva. In the three secretory compartments, the mean RCE of S-IgA was largely above 1, indicating a predominant local secretion.

DISCUSSION

In the past, several studies using ELISA methods to quantify the different molecular forms of IgA have been carried out, but none of them have explored the lower female genital tract with quantitative results.(18–20) In this study, we describe a reproducible and simple assay for Ig isotypes (S-IgA, total-IgA, IgM, and IgG) which does not require preliminary separation of the different molecular forms. All antibodies used were polyclonal and specific, with a high sensitivity for this ELISA. Some parameters needed to be taken into consideration to validate the present method. The first parameter was the determination of concentrations and, therefore, dilutions of coated and labeled antibodies. These concentrations were chosen according to quantitative and optic density criteria. Thus, we have chosen a maximum of three OD units at 492 nm. The second stage consisted of validating the technique through specific criteria described by the French Society of Clinical Biology.(14) This involved the determination of linearity, reproducibility, limit of detection, and, finally, dilutions of the samples, in order to be in a linear zone where the environment has no influence on the evaluation. Due to secretory production of S-IgA and secretory-IgM, there is undoubtedly competition between the two Igs. This could cause lower quantitative results of the secretory parts. However, Vincent and Revillard did not observe this phenomenon in the range of concentrations used.(20) Immunonephelometry served as a control test. A strong relationship existed between ELISA and immunonephelometry for each isotype, except for S-IgA which cannot be measured by nephelometry. A difference of sensitivity between the two methods was noticed: ELISA provided a better detection limit (approximately 1 μg/L) than the reference method (approximately 1 mg/L) and ELISA allowed the quantification of S-IgA. We, therefore, have chosen this method to measure Igs. The proportions in saliva and serum were identical to those published in the literature,(18,20) indicating that our trials and method were correct.

The epithelial barrier has zones of immune fragility in the female reproductive tract. IgA are the dominant Igs of mucous membranes (as digestive and bronchial tissues), with a high proportion of S-IgA. However, in the cervicovaginal secretions from healthy women, by calculating the relative distribution of Igs according to fluids, there was a very high proportion of IgG (77%) with a low percentage of total-IgA (15%), of which only 15% are S-IgA. However, in endocervical secretions, the same proportions of IgG and IgA were found with a proportion of S-IgA three times greater (46%) than that of cervicovaginal secretions, suggesting a higher production. In high digestive mucous membranes (buccal mucous membrane), the proportions of Igs are inverted. According to the literature, rather than IgG, IgA was the dominant isotype with 45% of S-IgA in cervicovaginal secretions.(19) This very high percentage is identical to that found in cervicovaginal secretions, thereby proving that the endocervical system acts as a conventional glandular epithelium for secretions. Thus, the immune profiles (cervicovaginal and endocervical secretions) resemble those of the serum (two thirds IgG, one third IgA). These results suggest that the plasmatic compartment and the female genital MALT are interdependent. In any case, IgG were the most abundant Igs in the female genital tract, according to the results obtained by Bélec et al.(16)

The presence of albumin in cervicovaginal secretions strongly suggests that passive transudation of serum borne Igs must be envisaged, especially for IgG and mIgA, which represent the principal molecular forms in the blood. We evaluated the transport of plasmatic IgG or IgA to the vaginal cavity by using the RCE calculation. This parameter, in which albumin equals 1, expresses the secretion rate of Ig independently of the concentration of Igs in serum.(4) For S-IgA, the RCE values largely exceeded the value expected from a molecular weight-affected seepage from plasma, confirming a primarily local synthesis.

The RCE of total-IgA was lower than that of secretions (but still >1). These results may be explained by the inclusion of different molecular forms of IgA (mIgA, pIgA, and S-IgA) in the calculation of the RCE. The RCE protein profiles of S-IgA and total-IgA for all fluids are symmetrical, which proves tight imbrication of molecular forms. By subtracting total-IgA and S-IgA concentrations, we can estimate the concentration of mIgA and pIgA. The pIgA and mIgA together accounted for 16 and 11% in cervicovaginal secretions and endocervical secretions, respectively. They are responsible for a significant decrease in total-IgA RCE, despite higher total-IgA concentrations. This result suggests an increased transudation from plasmatic compartment where IgA are the dominant Igs (2311 mg/L). However, a local synthesis of mIgA and pIgA, derived from secretory production, should not be ruled out: liberation in the lumen of polymeric forms without SC and/or

liberation of secretory forms with loss of SC and/or junction chain. Therefore, free SC can be found in the female genital tract.(7,21) Finally, the level of IgA found in the cervicovaginal secretions could possibly be explained by the secretion of Igs in the endocervix (ratio total-IgA endocervix/vagina = 3).

The RCE of IgG is particular. In fact, in saliva, the RCE is very close to 1. This result was expected because buccal mucous membrane is of a glandular type which produces a very low quantity of local IgG. The IgG concentration in saliva (25 mg/L) is explained by a low transudation from the plasmatic compartment. In the case of vaginal mucous membrane, a RCE of about 5 is a sign of some local active secretion, but also of passive plasmatic transudation. This hypothesis is coherent since this epithelium is pluristratified.

Moreover, the high concentration of IgG (406 mg/L) in cervicovaginal secretions is supplied by the systemic compartment (high concentration of IgG) and a local secretion from plasma cells by diffusion through the epithelium. The small amount of plasma cells in the vagina (7.4%), described by Kutteh et al., suggests that there is high production from these same plasma cells, whereas the plasma cells producing IgA are dominant in the vagina (79%).(12) The latter, indeed, produces low amounts (17 mg/L) despite their high proportions.

The RCE of IgG for endocervical secretions is around 12. The higher concentration of IgG (940 mg/L) compared to IgA (278 mg/L) suggests that, as in the vagina, an active transudation from plasma and local excretion occurs. However, the structure of glandular epithelium does not allow the passage of IgG from the mucous membrane towards the endocervix lumen. In the case of synthesis from plasma cells, IgG must pass through the epithelium. This requires a Fcγ-receptor which links IgG and allows transcytosis.(19) A model was described in the anal glandular mucous membrane where Fcγ-receptors are situated not only on the basolateral side, but also on the apical side. A model of in vitro transcytosis on intestine cells was recently described for IgG.(21) Thus, the presence of an Fcγ-receptor allows a bidirectional passage through glandular epithelium. The other hypothesis may involve the binding of IgG to its receptor on the basolateral side, and the cell reaches the endocervix lumen by desquamation, and IgG may be released from its membrane receptors. The last hypothesis to explain the high amounts of IgG, despite an impermeable epithelium, is that mucosal damage may be counteracted by regulatory proteins produced by the epithelium.(7) In the case of plasmatic transudation, IgG must follow the same course but also pass through the capillary epithelium. Finally, to explain the preponderance of IgG, IgG plasma cells could be highly productive because their number is lower than IgA plasma cells.

The RCE of IgM, greater than 1 but less than 10, and the low concentrations found in all areas, suggest that a local secretion is predominant. This phenomenon directly reflects the intensity of a local subepithelial synthesis, as a diffusion of these large Igs across the capillary walls depends on its molecular size. Furthermore, the relatively low percentages of plasma cells in the female genital tract (12–13%) agree with previous data.(12) IgM are polymeric with potential secretory form and a local secretion (brought to light by the RCE) is carried out in the same mode as that for S-IgA.(7)

In conclusion, we have validated a reproducible and sensitive ELISA test, which is well adapted to quantify Igs in the genital tract. This study on genital secretions from healthy women points out several original facts on the physiological synthesis of Igs. Thus, the female mucosal genital tract involves two types of immune responses, a secretory type in the endocervix and in the uterus and a systemic type in the exocervix and in the vagina. These results indicate that humoral immunity in the female genital tract associates a local synthesis and a transudation from systemic Igs. IgG are, therefore, the dominant isotypes in vaginal fluids, and their production depends closely on the systemic immune response. Ig concentrations at the different levels of genital mucous membranes might be quantified to approach the problem of viral (such as HPV), bacterial, or fungal infections. In addition, this study, showing a higher IgG concentration than IgA, and the possibility to induce an S-IgA response, must be taken into account for vaccine strategy to induce a protective immune response. Because our research team is working on cervical cancer induced by HPV, this study might permit one to approach the role of the immune system in the evolution of precancerous lesions.

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