Effect of Local Infection and Oral Contraception on Immunoglobulin Levels in Cervical Mucus

ELISABETH J. CHIPPERFIELD and B. A. EVANS

Department of Venereology, Middlesex Hospital, London, W1N 8AA, England

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The concentrations of immunoglobulin A (IgA) and immunoglobulin G (IgG) were estimated in cervical mucus from 115 patients attending a clinic for sexually transmitted diseases. The patients were divided into two groups; those using combined estrogen/progestogen oral contraceptives, and those with presumed normal ovulatory cycles. Gonorrhea, trichomoniasis, candidosis, and herpes genitalis were diagnosed by conventional smear and culture techniques, and the two groups were subdivided according to these diagnoses. Gonorrhea, trichomoniasis, herpetic, and nonspecific cervicitis all caused marked increases in the mean concentrations when compared with a control group of uninfected patients with natural cycles (P < 0.01). Patients with candidosis and contacts of men with nonspecific urethritis showed a lesser rise. IgG/IgA ratios lower than that of serum suggested a considerable locally produced contribution of IgA. Oral contraception with the combined pill also caused a significant increase in mean IgA and IgG levels even in the absence of local infection (P < 0.01). An increase in the IgG/IgA ratio of this group may indicate that the hormonal effect was manifest through increased serum transudation. IgM was also detected more commonly in patients taking the pill. The marked effect of local disease on immunoglobulin levels in cervical mucus which occurs even in asymptomatic patients emphasizes the importance of screening for infection when studying these secretions.

Increasing interest is being shown in the role of cervical mucus in fertility and fertility regulation. However, cervical mucus may also have an important role to play in the protection of the uterus, which is sterile, from invasion by microorganisms present in the vagina. Although it is likely that the physical properties of mucus are factors in protection, it is probable that immunological mechanisms are of even greater importance.

Antibodies against normal vaginal flora including Candida albicans have been reported in cervicovaginal secretions (6, 13, 16), but there have been no reports of antibody or of immunoglobulin levels in the cervical mucus of patients with local genital infection caused by virulent pathogens such as Neisseria gonorrhoeae, Trichomonas vaginalis, and Herpesvirus hominis type 2. These pathogens rarely invade the uterus. Previously we demonstrated an increase in the numbers of immunoglobulin A (IgA)-, immunoglobulin G (IgG)-, and immunoglobulin M (IgM)-producing plasma cells in endocervical biopsies taken from infected patients and postulated that this local response prevents invasion of the uterus and oviducts (3). To see whether this increase in plasma cells resulted in an increase in immunoglobulin concentration in cervical mucus, we decided to measure IgA, IgG, and IgM levels in mucus from infected patients and to compare these with the concentrations in mucus from uninfected patients.

Although cervical mucus, unlike cervicovaginal secretions, will not be contaminated with vaginal fluid, it probably derives immunoglobulins both from local immunocytes and by diffusion from the systemic circulation, as well as from secretions of the endometrium and oviducts. Hormonal changes have been found to influence immunoglobulin levels in this composite secretion (5). Accordingly our patients were not only grouped by diagnosis, but were divided into those with presumed normal ovulatory cycles and those taking the combined estrogen/progestogen contraceptive pill in order to ascertain the independent effect of these hormones on immunoglobulin concentration in cervical mucus.

1 Present address: Department of Genito-Urinary Medicine, West London Hospital, London W6 7DQ, England.
MATERIALS AND METHODS

Collection of mucus. Cervical mucus was collected from patients attending the clinic for sexually transmitted diseases at the Middlesex Hospital, London. A 1-ml syringe, inserted into the cervical canal up to the hilt of the Luer fitting, was used to aspirate the mucus, and any blood-stained specimens were discarded. The volume of mucus obtained was measured and the specimens were stored at -20°C until required. Before use, the mucus was diluted five-fold in phosphate-buffered saline (pH 7.2), mixed well, and incubated at 37°C for 1 h (10).

Measurement of IgA and IgG. The concentration of IgA and IgG in the supernatant was measured by radial immunodiffusion in gel. Sheep antiserum to human IgA and IgG were obtained from Wellcome Reagents Limited, Beckenham, Kent. The majority of samples (81%) were measured in triplicate (12), 5 µl of the diluted mucus being placed in each well. In 15% of the samples, duplicates were measured and in 4% there was sufficient for only one estimation to be made. There was good agreement between triplicate measurements, all being within 10% of each other. The standard for comparison was pooled serum from 200 patients attending the clinic, which had been calibrated against the British Working Standard for Human Serum Immunoglobulins; IgG, IgA, and IgM (67/99) were supplied by the Medical Research Council, Mill Hill, London. The pooled serum contained 132 IU of IgG per ml and 129 IU of IgA per ml. Portions of this were stored at -20°C and a fresh sample was used for each batch of immunoglobulin determinations. Concentrations in cervical mucus were thus obtained as IU per milliliter but were converted to micromilligrams per 100 ml by using the data of Hobbs (7); the standard 67/99 is equivalent to 70% of the mean normal adult serum containing IgG (950 mg/100 ml) and IgA (248 mg/100 ml).

Measurement of IgM. IgM was measured in commercial low-level IgM immunodiffusion plates (Hyland Division, Travenol Laboratories Limited, Thetford, Norfolk, England). The sensitivity of these plates was approximately 4 mg/100 ml, but due to the five-fold dilution of the cervical mucus only those specimens with IgM concentrations above 20 mg/100 ml had detectable IgM.

Infection categories. The infections studied affect the cervix and vagina and are either transmitted by sexual intercourse or are otherwise related to sexual activity. They are either commonly asymptomatic (cervical gonorrhoea [G], nonspecific cervicitis [NSC], herpetic cervicitis) or are associated with vaginal symptoms of variable severity (trichomoniasis [TV], candidosis [CA]). Contacts of men with nonspecific urethritis (NSU) usually show no abnormality either clinically or by conventional microbiological examination.

Diagnosis of infection. Conventional slide and culture methods for the diagnosis of G, TV, and CA were performed for all patients. Additionally cultures for herpes simplex type 2 were carried out when herpetic cervicitis was suspected. The diagnosis of NSC was made on four patients with marked cervicitis from whom no pathogen was isolated. Women in whom no abnormality was detected were divided into two groups; those who attended for a check of their own accord (NAD), and another group who attended as contacts of men with NSU.

The patients were divided into two main groups; those who were using oral contraception (in all cases this was a combined pill) and those who were not. They were then further divided into subgroups according to their diagnoses. All specimens of mucus were collected at the time of diagnosis, i.e., before treatment, except for the TV group which included some specimens from patients who had been treated for trichomoniasis up to a week before. A record was kept of the last menstrual period, the interval since last sexual intercourse, and the number of sexual partners in the preceding three months. Cervical erosions were also recorded.

RESULTS

The distribution of immunoglobulin levels in cervical mucus was found as in human serum (8) to be log normal (Fig. 1). Accordingly, a log transformation of the concentration of immunoglobulin was used to calculate the mean and the standard deviation. Student's t-test was used to calculate the significance of the difference between the mean for the NAD group and the means for the other disease groups. The results are summarized in the table.

Natural cycles: IgA. The mean level for the NAD group with natural cycles was 6.71 mg/100 ml (Fig. 2). There was no increase in the CA group. Contacts of patients with NSU had a small but significant increase in IgA concentration (P < 0.05). Approximately four- and five-fold increases in mean IgA concentrations were found with G and TV, respectively. These differences were highly significant and so was that for the combined group comprising G, TV, herpes, and NSC (P < 0.0005). Twenty-one out of the 23 patients in this combined group had IgA concentrations above the mean for the NAD group.

Natural cycles: IgG. Similar results were obtained for IgG as for IgA (Fig. 3). The mean concentration in the NAD group was 13.48 mg/100 ml. There was no increase in the CA group, NSU contacts had a small increase, but G, TV, herpes, and NSC were associated with a highly significant increase in the mean concentration (P < 0.0005). Twenty-two out of 23 of this group had IgG concentrations above the mean for the NAD group.

Oral contraception: IgA. The NAD group who were taking the pill had a significantly higher mean level of IgA than the NAD group who were not taking the pill (P < 0.01) (Fig. 4). The remaining groups of patients taking the pill, who either had an infection or were contacts of patients with NSU, had increased mean...
values and at least 75% of the IgA concentrations were above the mean of the NAD group. Nevertheless, these differences were not highly significant.

**Oral contraception: IgG.** As with IgA concentrations, the effect of the pill on those in whom no abnormality was detected was to raise the mean concentration of IgG \((P = <0.05)\) (Fig. 5). There was no further increase with CA, but at least 75% of the concentrations in the other groups were above the mean for the NAD group.

**IgM.** IgM reached a detectable level \((20 \text{ mg/100 ml})\) in approximately one-fifth of the specimens, 15.3% from patients with natural cycles and 23.2% from patients using oral contraception. IgM was detected almost exclusively in specimens with higher levels of IgG and IgA, although many specimens with higher levels of IgG and IgA did not have detectable IgM.

**IgG/IgA ratios.** The distribution of IgG/IgA ratios was log normal, and statistical analysis showed no correlation with disease category or with the menstrual cycle. The arithmetic mean of the log-normal IgG/IgA ratios for all the specimens from patients with natural cycles was 2.13:1, whereas that for patients taking the pill was 2.78:1. This was significantly different \((P = <0.05)\).

Although there was a significant increase in the incidence of cervical erosions in women taking the pill (37.5%) compared with women with natural cycles (20.3%), there was no direct correlation between erosions and immunoglobulin levels. There did not appear to be any difference between the two groups in the number of sexual partners during the preceding 3 months. The mean number of partners for those taking the pill was 1.39 and for those who were not 1.33.

Any independent effect of the stage of the menstrual cycle on immunoglobulin levels in the NAD group and the disease groups could not
be assessed accurately because there were insufficient samples for statistical analysis. However, the time of sampling in relation to the menstrual cycle is shown in Fig. 6, and this does not show any significant differences in sampling distribution. Most samples were taken during the middle 2 weeks of the cycle, mainly because mucus is usually more plentiful at this time.

**DISCUSSION**

Two previous reports of immunoglobulin levels in female genital secretions have been based on samples obtained by saline irrigation of the vagina and endocervix, and measurements were made using all 11S IgA standard (6, 16). Despite finding similar immunoglobulin levels to ours in healthy women, Govers and Girard (6) found no rise in patients classified as having vaginal infection; neither the etiology of the vaginitis nor the use of oral contraception was stated. Waldman et al. (16) also found no immunoglobulin rise in cases of vaginitis or in four patients using oral contraception.

Schumacher (14) examined serial samples of human cervical mucus using serum IgA standards and found marked variations, particularly in IgG concentration which showed some very high pre- and post-menstrual levels. Our sampling distribution was largely determined by the availability of secretions, with the exclusion of material contaminated with either blood or plasma, which tended to occur when aspiration of the scanty mucus was attempted during the first and last weeks of the cycle. Unlike Schumacher, therefore, we were unable to obtain satisfactory serial samples from individual patients, but the relatively few samples obtained pre- and postmenstrually did not have higher levels than those obtained during the middle 2 weeks.

Our results demonstrate that local infection is associated with a rise of IgG and IgA in cervical mucus and this correlates with our previous results showing increased numbers of fluorescing plasma cells in response to infection (3). The present results also show an overall rise in IgG and IgA in patients taking an estrogen/progestogen oral contraceptive. This occurred in all

**FIG. 3.** Natural cycles: IgA concentrations (mg/100 ml) in cervical mucus according to diagnosis. The mean for each group is indicated by a bar.

**FIG. 4.** Oral contraception: IgA concentrations (mg/100 ml) in cervical mucus according to diagnosis. The mean for each group is indicated by a bar.
groups taking the pill and was not associated with evidence of increased local immunoglobulin production as shown by fluorescing plasma cells (3). Mean values of IgA and IgG in infected patients taking the pill were higher than those found in infected patients with natural cycles, and well over three-quarters of the values in the infected patients taking the pill were above the mean for uninfected patients.

Elstein (4) has shown, using disc electrophoresis of chemically solubilized cervical mucus, that the total protein content, as well as the immunoglobulins, increase in response to endogenous and exogenous progestogens. Moreover, these changes were related to reduced sperm penetrability, particularly when immunoglobulins were present. Our findings similarly show this relationship between raised cervical immunoglobulins and the use of progestogen-containing oral contraceptives, which are known to reduce sperm penetrability (11).

IgG/IgA ratios have been used to distinguish between the contributions made by transudation and local synthesis at mucous surfaces, and ratios between 1:2 (16) and 6:1 (9, 14) have been reported in cervical mucus. Calibration with a 7S serum IgA standard almost certainly resulted in a considerable underestimate of absolute IgA values in our measurements but should not have invalidated comparison between our various groups. The IgG/IgA ratio of approximately 2.5:1 suggests a considerable locally produced contribution of IgA, particularly bearing in mind that our values may be as little as one-third of the true IgA concentration (15) if the majority is 11S IgA. Firm support for local IgA synthesis has been provided by the cervical tissue culture work of Behrman and Lieberman (1) and local IgA antibody production after intravaginal immunization with C. albicans (17).

The small increase in IgG/IgA ratios found in pill-controlled cycles may suggest that the hormonal effect is manifest through increased serum transudation. Schumacher (14) described a similar effect in cycles under a sequential regimen, where estrogen is taken during the first 15 days of the cycle followed by 5 days of progestogen.

Subdivision of the infected groups according to specific pathogens shows equal rises of both IgA and IgG in response to G, herpetic infection, NSC and TV. C. albicans produced a wide range of immunoglobulin levels in the non-pill group consistent with its variable virulence. In the pill group this infection produced IgA values.

![Figure 5](http://iai.asm.org/) Oral contraception: IgG concentrations (mg/100 ml) in cervical mucus according to diagnosis. The mean for each group is indicated by a bar.

![Figure 6](http://iai.asm.org/) Frequency distribution of sampling of cervical mucus in relation to menstrual cycle.
greater than the mean for the NAD group in 90% of cases. This is consistent with the increased clinical severity of candidiasis in patients using combined oral contraception (2).

The patients known to have been in contact with men with NSU may actually fall into two groups, one with raised IgA, and one with low IgA. Those with low IgA concentrations may be unaffected by whatever agent causes NSU.

The wide scatter of IgG and IgG levels creates an initially confused impression when in point of fact it represents no more than the range of normal variability. Only serial estimations of the response in each patient, preferably measuring specific antibody production, could convey the significance of responsiveness in the individual, and this work has yet to be done.

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LITERATURE CITED


