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IMMUNOGLOBULIN LEVELS AND ANTIBODY TO *CANDIDA ALBICANS* IN HUMAN CERVICOVAGINAL SECRETIONS

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SUMMARY

Human cervicovaginal secretions were examined for immunoglobulin content and for antibody to *Candida albicans*. The predominant immunoglobulin class of the secretions was IgA, accounting for approximately 65% of the total immunoglobulin. Most of the IgA was eluted from Sephadex G-200 in the excluded peak and was associated with secretory component; it therefore had the characteristics of 'secretory IgA'. With increasing age, the IgA content fell and the IgG content rose in cervicovaginal secretions. No IgE could be detected in the secretions. Antibody to *C. albicans* was found to be predominantly of the IgA class.

INTRODUCTION

It has been repeatedly shown that the immunoglobulin composition of the mucous secretions of the lacrimal, respiratory and gastrointestinal tracts is different from that of blood-serum (Tomasi & Bienenstock, 1968). The major immunoglobulin class in external secretions is IgA, and in addition, secretory IgA differs in several of its physicochemical characteristics from serum IgA (Tomasi & Bienenstock; 1968). Whether the female genital tract can be considered a part of the 'secretory immunologic system', as it has been called (Symposium of the Secretory Immunologic System, 1969) has been questioned. In a recent study of the proteins of cervical mucus, the IgA : IgG ratio was noted to be 1 : 5, i.e. approximately equal to that of serum (Masson, Heremans & Ferin, 1969). In another study on sixteen 'vaginal fluid' specimens similar results were obtained (Chordirker & Tomasi, 1963). However, several studies have indicated that antibody in cervical or vaginal mucus was of local origin (Straus, 1961, Straus, 1965, Parish *et al.*, 1967 and Kerr, 1955). Thus we undertook to re-examine the immunoglobulins and to investigate the immunoglobulin classes of antibody to *Candida albicans* in cervicovaginal secretions of normal adults.

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MATERIALS AND METHODS

Volunteers

131 normal adults were obtained from a university hospital gynaecology clinic. Routine history and physical examination were obtained, with special notice taken of the time in the menstrual cycle, problems of sterility and history of vaginal infection.

Specimens

A cervicovaginal washing was obtained from each of the volunteers by gently instilling 10 ml physiological saline into the cervical os, and aspirating the mucus-buffer mixture from the os and from the fornix of the vagina. The specimen was then mixed thoroughly using a vortex stirrer, one drop of 0·1% sodium azide was added, and the specimen was concentrated five times by negative pressure dialysis. The specimens were kept at −20°C until studied. They were centrifuged at 3000 rev/min for 30 min to remove debris prior to immunoglobulin or antibody determinations.

Immunoglobulin concentrations were determined by radioactive single radial immunoprecipitation in agar (Rowe, 1969). Specific antisera to serum IgA, IgG, IgM, and secretory component* were used. The antisera to IgA and IgM were obtained from sheep immunized with purified myeloma proteins, and the antisera were absorbed with cord serum. The antiserum to IgG was obtained from sheep immunized with Cohn's fraction II of normal serum further purified by DEAE-cellulose chromatography and the antiserum was absorbed with Fab fragment prepared by papain digestion of IgG. The antisera were shown to be monospecific by immunoelectrophoresis against whole serum. The antiserum to secretory component has been described elsewhere (Waldman *et al.*, 1970). Pooled serum from healthy adult males, batch 67/97, was used as a quantitative reference preparation for serum proteins.† The quantitation standard for secretory IgA has been described elsewhere (Waldman *et al.*, 1970). IgE levels were determined by the radioactive single radial diffusion method achieving a sensitivity of approximately 100 ng/ml (Rowe, 1969). The non-immunoglobulin proteins albumin and transferrin were also determined by the single radial immunoprecipitation method, using anti-albumin obtained commercially (Inst. of Sera and Vaccines, Prague), and anti-transferrin prepared by immunizing rabbits with transferrin isolated from serum, using the serum 67/97 as standard.

Antibody to *Candida albicans* was measured by three methods: (a) Co-precipitation of ^{125}I-labelled antigen and antibody to it by specific anti-immunoglobulin antisera: the antigen mixture was prepared by lyophilizing a dialysed culture filtrate of a 7-week culture.‡ The antigen was labelled with ^{125}I using the chloramine-T method (McConahey &

* Kindly provided by Dr J. P. Mack, Institut de Biochemie, University of Lausanne.

† The immunoglobulin content of a similar reference standard (67/86) has been reported (Rowe, Anderson & Grab, 1970). Because the estimates of immunoglobulins performed in different laboratories showed significant variability, the average activity of 67/86 has been set at 100 units/ml for IgG, IgA and IgM. It is the recommendation of the World Health Organization that immunoglobulin concentrations be expressed in International Units. The concentration of secretory IgA cannot be expressed in IU because a serum standard is inaccurate. Therefore, we have found it necessary to express the immunoglobulin concentrations in secretions in mg/ml rather than the preferred IU. For the purpose of this work it was considered that the following protein concentrations were present in one ampoule of 67/97 reconstituted with 1 ml of distilled water: albumin 40, IgG 12, IgA 2, IgM 1, transferrin 2, all values in mg/ml.

‡ Kindly provided by Dr H. Scholer, F. Hoffmann—La Roche & Co., Basel.

Dixon, 1966). This was then dialysed against frequent changes of PBS for 48–72 hr to get rid of the unbound ^{125}I. The labelled antigen was then diluted to contain 0·1 mg/ml (this gave $0{\cdot}5–1{\cdot}0 \times 10^6$ cpm in a gamma-well scintillation counter). 0·1 ml of the antigen was then mixed with varying amounts of specimen to be tested, incubated at 37°C for 30 min, then overnight at 4°C. To this mixture was added 0·1 ml of a specific anti-immunoglobulin antiserum (anti-IgA, anti-IgG or anti-IgM), and the mixture placed at 4°C overnight once more. The mixture was then centrifuged at 3,000 rev/min for 30 min at 4°C. The supernatant was removed and counted. The sediment was washed twice with cold PBS and then counted in a gamma-well scintillation counter. The amounts of the three reagents (antigen, specimen and antiserum) used in the test were adjusted to ensure that the antigen and the antiserum were greatly in excess. (b) Radio-immunoelectrophoresis was carried out by the method of Yagi *et al.* (1963) using the ^{125}I-labelled *C. albicans* antigen. (c) Passive haemagglutination was carried out by the method of Johnson, Brenner & Hall (1966).

RESULTS

Immunoglobulin concentration: in cervicovaginal secretions from 131 normal women, IgA was detected in 117, with a mean level of 0·22 mg/ml; IgG in 124, with a mean level of 0·12 mg/ml; and IgM in 41, with a mean of 0·01 mg/ml (Table 1). In 74 samples, IgA was the predominant immunoglobulin class, in 48 IgG was predominant and in 9 the 2 were equal. The percentage of the total immunoglobulin that was IgA averaged 64% for all the samples. In older age groups the percentage of total immunoglobulin that was IgA decreased, and the IgG increased. There was a significantly greater percentage of IgG in the cervicovaginal secretions of women over 50 as compared to women under 30 ($P<0{\cdot}05$). In a variety of conditions such as pregnancy, sterility, vaginitis, etc. the IgA:IgG ratio in cervicovaginal secretions remained unchanged (Table 1). The time in the menstrual cycle also caused no significant changes in the immunoglobulin composition of the cervicovaginal secretions. No IgE could be detected in any of the samples.

Three women with absent cervices were available for study, and the immunoglobulin distribution was no different from normal. Cervical secretions, free of contamination with vaginal secretions, were obtained by first washing out the vagina, and then instilling saline into the cervical os and aspirating the mucus-saline mixture. The immunoglobulin content of these secretions was no different from that of the whole cervicovaginal secretions.

Albumin and transferrin levels are also shown in Table 1, with a mean level of 0·012 mg/ml for transferrin, and 0·069 for albumin.

Correlation between various proteins in cervicovaginal secretions: there was a statistically significant correlation between all of the proteins measured in the cervicovaginal secretions, the strongest correlation being between transferrin and IgM. The poorest correlation was between IgA and transferrin levels.

Physicochemical characteristics of IgA in cervicovaginal secretions: the elution pattern obtained when pooled cervicovaginal secretions were chromatographed on Sephadex G-200 is shown in Fig. 1. Two such pools were made and the results were similar. An approximation of the amount of IgA that was associated with secretory component was made by expressing the amount of secretory component in terms of the secretory IgA standard. Then the total IgA, as determined by the serum IgA standard, minus the amount of IgA associated with secretory component (corrected for the approximately four-fold

TABLE 1. Concentration of various proteins in cervicovaginal secretions

Patients (Numbers)	IgA	IgG	IgM	Transferrin	Albumin
By age					
20–29 (66)	66%* (0·23 ± 0·05)	31% (0·11 ± 0·02)	3% (0·01 ± 0·003)	0·020† (± 0·0003)	0·069 (± 0·03)
30–39 (35)	62% (0·15 ± 0·01)	33% (0·08 ± 0·02)	5% (0·01 ± 0·0006)	0·007 (± 0·002)	0·053 (± 0·02)
40–49 (18)	63% (0·28 ± 0·02)	36% (0·16 ± 0·02)	1% (0·01 ± 0·0008)	0·007 (± 0·003)	0·027 (± 0·01)
50 or greater (12)	57% (0·30 ± 0·01)	40% (0·21 ± 0·01)	3% (0·02 ± 0·001)	0·003 (± 0·001)	0·030 (± 0·01)
Day of menstrual cycle					
1–10 (13)	61% (0·25 ± 0·05)	37% (0·15 ± 0·03)	2% (0·01 ± 0·002)	—	—
11–20 (19)	64% (0·18 ± 0·04)	32% (0·09 ± 0·01)	4% (0·01 ± 0·001)	—	—
21–30 (12)	60% (0·15 ± 0·03)	36% (0·09 ± 0·02)	4% (0·01 ± 0·001)	—	—
Pregnant					
<12 weeks (25)	68% (0·25 ± 0·05)	30% (0·11 ± 0·02)	2% (0·01 ± 0·001)	—	—
12 weeks or greater (26)	57% (0·13 ± 0·04)	39% (0·09 ± 0·02)	4% (0·01 ± 0·001)	—	—
6 weeks post-partum (12)	68% (0·23 ± 0·03)	29% (0·10 ± 0·02)	3% (0·01 ± 0·001)	0·027 (± 0·01)	0·100 (± 0·05)
Sterility (8)	76% (0·13 ± 0·02)	24% (0·04 ± 0·01)	<1% (0)	0·005 (± 0·002)	0·010 (± 0·006)
Oral contraceptive (4)	62% (0·08 ± 0·01)	38% (0·05 ± 0·01)	<1% (0)	—	—
Vaginitis (42)	67% (0·28 ± 0·04)	31% (0·13 ± 0·03)	2% (0·01 ± 0·002)	0·014 (± 0·005)	0·106 (± 0·04)
Absent cervix (3)	65% (0·15 ± 0·04)	30% (0·07 ± 0·02)	5% (0·01 ± 0·003)	0·005 (± 0·002)	0·015 (± 0·01)
Cervical secretions (5)	77% (0·08 ± 0·02)	19% (0·02 ± 0·01)	4% (0·01 ± 0·0008)	—	—

* Percent of total immunoglobulin (mg/ml ± standard error of the mean).
† mg/ml (± standard error of the mean).

difference between IgA determined by a serum IgA standard and a secretory IgA standard), was called 'IgA without secretory component'. Thus about 70% of the IgA was secretory IgA and eluted mainly in the descending limb of the first peak. A small amount of free secretory component was present in the cervicovaginal secretions and eluted between the 'IgG' and 'albumin' peaks. There was no evidence of significant degradation of any immunoglobulins to smaller than their 'normal' size.

Antibody to Candida albicans: (a) Co-precipitation of labelled antigen with its antibody by specific anti-immunoglobulin antiserum, was detected in nine of ten cervicovaginal

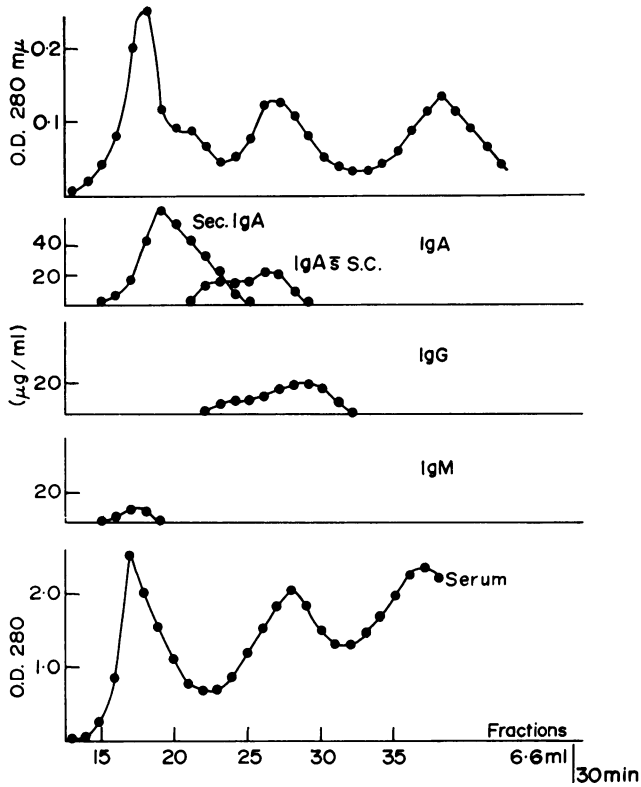

FIG. 1. Elution from Sephadex G-200 of a pool of cervicovaginal secretions. The dimensions of the column were 90×3 cm. The optical density and the concentrations of IgA, IgG, IgM and secretory component were measured on each unconcentrated fraction. The total IgA minus the IgA accounted for by the secretory component was assumed to be IgA without secretory component (IgA s̄ S.C.). For reference purposes, the elution pattern of a serum specimen from one of the patients is also shown.

TABLE 2. Antibody to *C. albicans* in cervicovaginal secretions

Patients	Co-precipitation method			RIEP			Passive haemagglutination
	IgA	IgG	IgM	IgA	IgG	IgM	
Total	49 (8/10)*	4 (3/10)	0 (0/10)	3/6†	1/6	0/6	1:8 (7/13)‡
With monilia infection	10 (2/5)	6 (1/5)	0 (0/5)	0/2	0/2	0/2	1:2 (2/5)

* μg of antigen bound/100 ml of specimen (number positive/number tested).
† Number positive/number tested.
‡ Mean titre (number positive/number tested).

secretion samples tested (Table 2). Eight of ten specimens had IgA antibody, three of ten IgG antibody, and none had IgM antibody. Only one specimen had more IgG than IgA antibody; and 88% of the detectable activity in all samples was in the IgA class.

The antibody activity in the IgA of the cervicovaginal secretions was not related to the amount of IgA present in the secretions, which indicates that the test was not measuring some non-specific co-precipitation of labelled material. Also confirming this were the findings that when an excess of unlabelled antigen was added to the secretion, there was nearly 100% inhibition of the precipitation of labelled material, and that when a myeloma IgA was used in the test, no labelled material was precipitated. (b) Radio-immunoelectrophoresis, using ^{125}I-labelled mucopolysaccharide antigen, showed the presence of IgA antibody in three of six specimens tested, and IgG antibody in one of six specimens tested. (c) Passive haemagglutination was positive in seven of thirteen specimens tested.

C. albicans organism was cultured from the secretions of five of the patients. In this group, antibody titres by the various methods used were much lower (Table 2).

When antibody to *C. albicans* was measured on pooled fractions following Sephadex G-200 chromatography, most of the antibody activity, as measured by passive haemagglutination, was found in the excluded peak.

DISCUSSION

These results indicate that cervicovaginal secretions, like those of the lacrimal, respiratory, and gastrointestinal tracts, have the characteristics which have led to the term 'secretory immunologic system' (Tomasi & Bienenstock, 1968; Small & Waldman, 1969). These characteristics are (a) immunoglobulin to albumin ratio higher than serum, indicating that plasma proteins are not present simply as a result of transudation: in the present study the immunoglobulin to albumin ratio was 5:1, as opposed to 1:3 for serum; (b) the predominance of an immunoglobulin class (IgA) which is a minor component in serum: in the present study IgA constituted about 65% of the immunoglobulin present; (c) unique physicochemical structure of the IgA in secretions: the IgA of cervicovaginal secretions has been shown to be predominantly polymeric and to contain secretory component; (d) antibody in external secretions is predominantly of the IgA class: antibody to *C. albicans* was shown to be mainly IgA.

The results differ from those reported by Chordiker & Tomasi (1963) and Masson, Heremans & Ferin (1969). The former reported a mean IgG : IgA ratio of about six (as opposed to our 0·5) in sixteen 'vaginal secretions', but the details of how the specimens were obtained were not given. Masson *et al.*, studying cervical mucus pooled from seventy patients, found an IgG: IgA ratio of five. There is no ready explanation for the differences with our data. In a recent study reported by Hulka & Omran, the IgA: IgG ratio in cervical secretions was reported to be similar to the results in this study (Hulka & Omran, 1969). They also reported an increase in the IgA: IgG ratio in mid-cycle. They studied a small number of patients and the cyclical differences were small. We found no significant difference in immunoglobulin levels at various times during the cycle; however, small differences may have been masked since we did not take serial samples from the same patients and lumped our data into early, mid and late cycle. The cyclical changes should be studied further by obtaining serial secretions at frequent intervals from a larger group of patients.

Tourville *et al.* (1970) recently reported immunohistological localization of the immuno-

globulin containing plasma cells and epithelial cells containing secretory component (Tourville, *et al.*, 1970). They found secretory component-containing epithelial cells lining the villi of the uterine tubes and the glandular epithelium of the uterus, but none in three cervical specimens and three vaginal specimens. The paucity of immunoglobulin containing cells that they reported is not inconsistent with our results, since the immunoglobulin levels in the secretions were very low, less than 5% of the serum concentration of immunoglobulins.

There are other data in the literature which would support our finding that the female genital tract does have a local immunologic system. Straus showed that antibody titres to *Salmonella typhosa* in vaginal secretions were higher following local rather than systemic immunization (Straus, 1961). The same investigator demonstrated sperm immobilizing antibody in vaginal secretions following intravaginal immunization with killed sperm (Straus, 1965). Parish, Carron-Brown & Richards (1967) showed that cervical mucuscontained antibodies to *Escherichia coli* and *Candida albicans* at the same time that there was no antibody in blood-serum. In animals, the local production of antibody has been shown repeatedly; e.g. Kerr (1955) showed that the antibody titre to *Brucella abortus* in serum or vaginal or uterine secretions depended on the route of immunization, local instillation of vaccine giving the highest titres in the secretions. None of these workers, however, has demonstrated the immunoglobulin class of the antibody.

This study has shown that the IgA in cervicovaginal secretions contains 30% IgA that elutes from Sephadex G-200 as if it were monomeric (7S). This is in agreement with the finding that about 20% of the IgA of nasal secretions in 7S (Rossen *et al.*, 1965), and the report that the proportion of IgA in colostrum containing secretory component is 70% (Mestecky, Kraus & Voight, 1970).

We have used the term cervicovaginal secretions, but this indicates only the place the secretions were obtained, not their origins. Although histochemical studies have, in some instances, shown plasma cells containing IgA and IgG in cervical mucosa (Masson *et al.*, 1969), the origin of the proteins of the cervicovaginal secretions could be 'upstream;' i.e. from the ovary, the peritoneal cavity, the oviducts or the uterus. We were able to obtain vaginal secretions which could not have been 'contaminated' by secretions from the uterus or higher, since three of the patients had had a complete hysterectomy. IgA was the predominant immunoglobulin in those secretions, and the secretions contained secretory component; but there was not sufficient material to verify that the secretory component and IgA were on the same molecule. Since cervical secretions, relatively free of vaginal secretions, also showed a predominance of IgA, we can conclude that both the cervical and vaginal mucosae secrete IgA.

Examination of the immunoglobulin content of cervicovaginal secretions in a variety of physiologic and pathologic conditions revealed that only age appeared to alter the relative amounts of IgA, IgG and IgM. With increasing age the percentage of IgA fell and that of IgG rose. In the only other study of the influence of aging on external secretions, Alford (1968) found a decrease in nasal wash IgA concentration with increasing age.

Assuming that our *in vitro* results mirror the *in vivo* situation, the immunoglobulins present in the cervicovaginal secretions are all present in higher relative concentrations than albumin. However, transferrin, another serum protein, is also present in relatively higher concentrations than albumin. If the immunoglobulin concentrations are compared to the transferrin concentration, only IgA is present in relatively higher concentrations.

Antibody to *C. albicans* mucopolysaccharide was demonstrated in cervicovaginal

secretions, and about 90% of the antigen-binding capacity was present in the IgA class. To our knowledge, this is the first demonstration of the immunoglobulin class of antibody in secretions from the female genital tract.

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