

Study of precipitation reactions to *Actinomyces israelii* antigens in uterine secretions

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SUMMARY Uterine secretions were obtained from 110 women and analysed by counterimmunoelectrophoresis for the occurrence of precipitation reactions against *Actinomyces israelii* antigens. Precipitation reactions were found in secretions from seven women and a correlation was found between these reactions and long term use of plastic intrauterine devices. The precipitating components could not be proved to be immunoglobulins; neither could identity be shown with IgG precipitins in reference serum. The nature and the importance of the precipitating components are discussed.

Actinomyces israelii, the predominant pathogen of human actinomycosis, has recently been identified as a part of the indigenous genital flora.¹⁻³ Actinomycotic involvement of the female pelvis is often associated with long term use of intrauterine contraceptive devices (IUCDs).⁴ Prevalence studies have, however, failed to show an increase in colonisation rate with duration of IUCD use.⁵ An association with IUCD use and the occurrence of *Actinomyces* like organisms in Papanicolaou stained cervicovaginal smears was first documented by Gupta *et al* in 1976.⁶ The importance of the identification of *Actinomyces* like organisms in cervicovaginal smears is not clear; nor are the conditions for their formation. Factors other than the mere existence of *Actinomyces* in the genital tracts of women with IUCDs must be important for disease development. Specific antibodies against *A israelii* have been detected in serum from patients with genital actinomycosis.^{7,8} In an attempt to study the possibility of detecting a local antibody response to *A israelii* in uterine secretions the following study was performed.

Material and methods

SUBJECTS

A group of women participating in a study of the effects of long term use of IUCDs, which will be presented elsewhere (Persson *et al*, in preparation),

were examined. As reference, a number of women without IUCDs and with IUCDs for less than three years, including women with *Actinomyces* like organisms detected in routine cervical smears, were examined. The women were subdivided into groups as follows.

Group 1: seven asymptomatic women using barrier methods of contraception.

Group 2: 78 women using copper IUCDs. Eleven women had used the IUCDs for two to three years and four of them were included because they had *Actinomyces* like organisms detected in routine cervical smears. The remaining 67 women had been fitted with the same copper IUCD for six to eight years. Results of analysis of cervical smears were not available from these women.

Group 3: 25 women who had had plastic IUCDs for more than six years (Table).

None of the IUCD users had symptoms or signs of genital infections. From two women repeated samples were obtained after two and 12 months.

SAMPLING PROCEDURES

Uterine secretions

Uterine secretions were obtained for analysis from all women examined. The cervix was wiped with a chlorhexidine solution and a sterile plastic catheter attached to a syringe was introduced into the uterine cavity. A slight suction was applied with the syringe to fill the catheter with uterine secretions. After extraction, the mucus filled part of the catheter was cut off and put into a box with a damp cotton swab to avoid drying of the sample.

Different contraceptive methods used by women examined related to results of analyses of uterine and cervical secretions by counterimmunoelectrophoresis

Group	No of women	Contraceptive method used	Precipitation reactions detected in	
			Uterine secretions	Cervical secretions
1	7	Barrier methods	0	0
2	78	Copper IUCDs	2	1*
3	25	Plastic IUCDs	5	ND
Total	110		7	1

*Cervical secretions obtained from 11 women.
ND = not done.

Cervical secretions

Cervical secretions were obtained from the women in group 1 and from the 11 women in group 2 who had had copper devices for two to three years. As much as possible of the mucus visible in the cervical os was suctioned into a catheter before obtaining uterine samples. The catheters were then handled as described above.

Serum samples

Ten millilitres of blood was drawn from each woman for serological analysis.

All sampling was performed at the time of ovulation or in the secretory phase of the menstrual cycle.

LABORATORY METHODS

Counterimmunoelectrophoresis

Counterimmunoelectrophoresis was performed in a 1% (wt/vol) agarose solution (Litex, Glostrup, Denmark) cast as a 1 mm thick layer on plates made of polyester (Gel Bond).⁹ A 0.075 M Tris/Barbital buffer, at pH 8.6, was used for the gels and the electrode vessels. In the primary analysis, wells holding 10 μ l were cut in parallel rows 1 cm apart. Aliquots of 10 μ l of *A israelii* antigen, produced as described elsewhere^{8,10} and containing 0.005 μ g protein/ml, were placed in the wells nearest to the cathode in each row. Arbitrary amounts of secretions were cut by means of scissors, in portions suitable to fill the wells nearest to the anode. Electrophoresis was then carried out at 10 V per cm at 10–12°C for 30 min in an apparatus suitable for the purpose (Medelco, Bromma, Sweden). After a wash in physiological saline the plates were stained in a solution of 0.5% (wt/vol) Coomassie brilliant blue R-250 in ethanol, acetic acid, and water (40/10/50) and destained in the same solution without Coomassie brilliant blue. They were then examined for precipitin lines.

A second analysis, according to Moody,¹¹ was performed to compare the identities of precipitates with those obtained when using a defined reference

serum. The sample of secretions was put in a well close to one with reference antiserum, both close to the anode. The antigen was placed in a well opposite these and closer to the cathode. Electrophoresis was then performed as described above.

Serum analyses for the detection of precipitating antibodies were performed using counterimmunoelectrophoresis as described above but with *A israelii* antigen containing 0.2 μ g protein/ml.

Rocket immunoelectrophoresis

Rocket immunoelectrophoresis was performed as described elsewhere.¹² A 1% (wt/vol) agarose solution (Litex, Glostrup, Denmark) dissolved in Tris/Barbital buffer, pH 8.6, was mixed with rabbit antihuman immunoglobulins (A 107, Dakopatts, Copenhagen, Denmark) and cast to form a 1 mm thick gel on polyester plates (Gel Bond) measuring 10 \times 10 cm. The antiserum was added in amounts of 4 μ l/cm². Wells holding 5 μ l were cut in a row nearest to the cathode. Standards of immunoglobulins with protein contents of 16 mg/ml, 8 mg/ml, and 4 mg/ml were used. These and the test samples were carbamylated before testing by incubation for 30 min at 45°C after mixing with a KOCN solution in borate buffer.¹³ The samples were then applied and electrophoresis was performed at 1–2 V per cm at 10–12°C overnight. The gels were washed and stained as described above. The immunoglobulin concentration of the test samples was determined by interpolation on the standard curve obtained.

Crossed immunoelectrophoresis

Crossed immunoelectrophoresis with an intermediate gel was performed as described by Axelsen.¹⁴

Direct microscopy and cultures

Smears for direct microscopy were prepared from the secretions and inoculations for anaerobic culture were made on supplemented brain heart infusion and on gelatin metronidazole cadmium sulphate agar media. The smears were examined by Gram

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staining and by the use of immunofluorescent techniques. Further proceedings were the same as described elsewhere.^{2 15 16}

Assay design

All specimens were taken to the laboratory within 24 h. Specimens for cultures and direct microscopy were prepared immediately after the arrival of the samples in the laboratory. The secretions were then transferred into tubes, frozen, and stored at -20°C . All immunological analyses of the secretions were made simultaneously after thawing. All sera were analysed by counterimmunoelectrophoresis and when the precipitins persisted in more than a fourfold dilution further analysis by crossed immunoelectrophoresis was performed to determine whether or not the precipitins were specific against *A israelii*.^{7 8 10}

Results

Distinct precipitation reactions were detected by counterimmunoelectrophoresis in secretions from seven of the 110 women examined (Table). Uterine samples from three of these women were contaminated with blood, but in none of them were any precipitins detected in serum. The immunoglobulin content of six of the positive secretions was determined by rocket immunoelectrophoresis. Immunoglobulins were detected in all samples tested, with concentrations of 4 mg/ml or less. The exact concentration was not determined as the amount of sample applied was arbitrary because of the physicochemical properties of the secretions. A second counterimmunoelectrophoresis analysis was performed with the positive secretions to check the identity with a reference antiserum against *A israelii*. In none of the secretion samples was identity demonstrated with the defined precipitins in the reference serum.

Nineteen women had precipitins detected in serum by counterimmunoelectrophoresis. Eight of these persisted in a fourfold serum dilution but were defined as non-specific by crossed immunoelectrophoresis. None of these women had precipitation reactions in secretion analyses.

One woman with precipitation reactions in her secretions had had a copper IUCD for two years. The reactions were detected both in cervical and uterine secretions. She had *Actinomyces* like organisms in Papanicolaou stained cervical smears but was asymptomatic. Her IUCD was removed. Re-examination two months later showed no *Actinomyces* like organisms in cervical smears, no *A israelii*, and no precipitation reactions in her secretions. Of the remaining six women with precipita-

tions one had had a copper device for six years while five had had plastic devices for more than nine years. In one of the latter *A israelii* was identified in uterine secretions. Repeat samples were obtained from her one year later with the same results. She was still asymptomatic.

A significant difference was found ($p < 0.02$)¹⁷ in the occurrence of precipitation reactions when plastic IUCD users were compared with women with copper devices. The plastic IUCDs had been used for 9–17 years while the copper devices had been fitted for six years or less. The significance remains the same if the material is stratified for duration of IUCD use instead of type of IUCD.

Discussion

Precipitation reactions with *A israelii* antigens were found in uterine secretions from seven women. Serum precipitins against the organisms were not found in any of these women. This indicates local production of the precipitating components. The presence of antibodies in genital secretions has been documented by many authors.^{18–27} The major portion of the IgG has been attributed to transudation from serum. Secretory IgA and part of the IgG present in secretions have been shown by means of fluorescent antibody techniques to be produced by submucosal plasma cells residing locally in the endocervix and the endometrium.²⁶ When inflammation occurs in the mucous membranes both an increase in transudation of IgG from serum and an invasion of immunoglobulin producing plasma cells have been found. In the present study the precipitating components could not be shown to be immunoglobulins. In six positive cases, however, the presence of antibodies in secretions was proved but we were unable to determine the immunoglobulin class. The precipitins against *A israelii* in serum have by adsorption studies, been shown to be of the IgG class (unpublished data). Identity between the precipitating compounds in secretions and the precipitins in *A israelii* reference antiserum could not be shown. This might be explained by the precipitating components being antibodies of the secretory IgA class and thereby failing to show identity with the IgG precipitins in the reference serum. In a pilot study, attempts were made to extract antibodies from the secretions with physiological buffer followed by centrifugation. No precipitation reactions were shown with the extracts. Reactions were, however, found when assaying the whole body of the same secretions, as described above. A number of components in the mucus—for example, lysozyme—might cause precipitations in the assays used and the precipitation reactions detected might

be due to non-specific non-antigen-antibody reactions.^{24 28}

Precipitation reactions in secretions were detected significantly more often in women with plastic IUCDs than in women with copper devices. All plastic IUCDs of the women with precipitation reactions had been used for long periods of time. This is concordant with the disease of genital actinomycosis occurring mainly in women after long term IUCD use.⁴ Duguid *et al*²⁹ found *Actinomyces* like organisms more often in women with plastic IUCDs than in women with copper devices. We can only speculate as to whether the precipitation reactions found in these assays are important for the formation of *Actinomyces* like organisms. The exact nature of the reacting components and their importance for *A israelii* colonisation and in disease development needs to be further investigated.

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