LEVELS OF IMMUNOGLOBULINS IN THE SERUM AND UTERINE FLUID OF WOMEN USING AN INTRAUTERINE CONTRACEPTIVE DEVICE

R. K. CHANDRA, P. K. MALKANI AND K. BHASIN

Department of Paediatrics (Immunology Division) and Obstetrics and Gynaecology, All India Institute of Medical Sciences, New Delhi 16, India

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Summary. The serum concentration of IgM was significantly higher in women using an IUD. Uterine fluid aspirates from such women contained large molecular weight proteins more frequently than control samples. Of twenty uterine luminal fluid collections, thirteen showed locally produced IgM, sixteen IgG and twenty IgA. It is suggested that secretory immunoglobulins may play a significant rôle in physiopathological mechanisms associated with the contraceptive action of IUDs.

INTRODUCTION

The mechanism of IUD action is still unknown (Eckstein, 1970; Edwards, 1970). Attention has recently been focused on the changes in the endometrial environment. Sagiroglu & Sagiroglu (1970) demonstrated a large number of leucocytes, fibroblasts and macrophages in the IUD smears. Gupta, Malkani & Bhasin (1971) observed that the so-called 'inert' IUD material provoked an acute inflammatory response which was the forerunner of macrophage accumulation, the severity of the reaction being proportionate to the duration of usage.

Holub, Reyner & Forman (1971) studied the levels of serum immunoglobulins G and M in women using an IUD and found them to be significantly elevated. They postulated that both these immunoglobulins may be involved in the foreign body reaction to an IUD, possibly through a lymphocytotoxic or leucocyte chemotactic effect. Serum and secretory IgA were not studied. If the action of an IUD is immunologically mediated, a significant alteration in the immunoglobulin content of uterine luminal fluid might be expected. Our observations show that such changes do indeed occur.

MATERIALS AND METHODS

The study was carried out on thirty women attending the Family Planning Research Clinic of the All India Institute of Medical Sciences, New Delhi. Twenty of the women were using an IUD and ten controls were healthy women not using a device. The uterine fluid was aspirated by a syringe with a polythene cannula into 1 ml sterile normal saline. At the same time, 5 ml blood
was collected. The samples were collected around the middle of the menstrual cycle. Each woman was tested once only.

*Estimation of protein concentrations*

Serum levels of albumin, IgG, IgA, IgM and $\alpha_2$-macroglobulin were estimated by the single radial diffusion in agar gel method (Mancini, Carbonara & Heremans, 1965), using monospecific antisera raised in rabbits. The uterine fluid aspirates were concentrated ten times in Visking dialysis tubing using negative suction, and the presence of various proteins was detected by the method of Ouchterlony (1962). In samples positive for a particular protein, the concentration of the protein was estimated either by single radial diffusion (Mancini *et al.*, 1965) or by the modified double diffusion method (Soothill, 1962).

The Reference Standard for serum immunoglobulins was obtained from Dr D. S. Rowe of the World Health Organization, Lausanne. Pooled plasma from ten healthy adults served as the reference standard for $\alpha_2$-macroglobulin.

*Locally produced immunoglobulins*

The quantity of immunoglobulins produced locally in the female genital tract was calculated by the following formulae derived from the studies of Donovan, Johansson, Bennich & Soothill (1970) on nasal polyp fluid:

For $\text{IgM} = F_M - \left(\frac{F_x}{S_x} \times S_M\right)$;

For $\text{IgG} = F_G - \left(\frac{F_x + F_{lb}}{S_x + S_{lb}} \times S_G\right)$;

For $\text{IgA} = as for IgG above, where F stands for uterine fluid and S for serum. The IgM concentration in uterine fluid ($F_M$), when corrected for the amount possibly filtered from the vascular compartment by reference to the ratio F/S for $\alpha_2$-macroglobulin which has a closely similar molecular weight but is immunologically inert, gives the amount of IgM which must be derived from local production and release into the uterine luminal fluid. The log molecular weight of IgG and of IgA is approximately half that of the sum of albumin and $\alpha_2$-macroglobulin, and the above formulae for these two immunoglobulins takes this into account.

**RESULTS**

The values of serum concentrations of various immunoglobulins in healthy women and in those using an IUD are shown in Table 1. There was a slight but statistically significant increase in IgM in the experimental group (0.02 $< P < 0.05$). The levels of IgA and IgG were comparable in the two groups. All the uterine fluid aspirates from women using an IUD contained albumin and IgA. The other proteins were not present in all the collections. The detection rate of the large molecular weight proteins was, however, much higher in the women using an IUD than in the controls (Table 2).
Immunoglobulins in body fluids of women with IUDs

Table 1. Serum levels of immunoglobulins in controls and in women using an IUD

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of women</th>
<th>Serum concentration (mg/100 ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgG</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>1645 ± 371</td>
</tr>
<tr>
<td>IUD</td>
<td>20</td>
<td>1730 ± 495</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.02 &lt; P &lt; 0.05</td>
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</table>

Immunoglobulin values are expressed in terms of the geometric means and standard deviations.

Table 2. The presence of various proteins in the uterine fluid aspirates from controls and from women using an IUD

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of women</th>
<th>No. of women with positive specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Albumin</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>IUD</td>
<td>20</td>
<td>20</td>
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</tbody>
</table>

Text-fig. 1. The uterine fluid aspirate/serum ratio for a series of proteins divided by a similar ratio for albumin, plotted against the molecular weight of the protein on a log scale.
The uterine fluid/serum ratio for various proteins divided by a similar ratio for albumin is shown in Text-fig. 1. There was a significantly lower concentration of α₂-macroglobulin in the uterine fluid compared with the amount of albumin filtered, reflecting some degree of selectivity based on molecular size. The amount of locally produced immunoglobulins in women with an IUD is shown in Text-fig. 2. All the samples showed IgA in excess of the amount to be expected by passive filtration from vascular permeability. This was also true for IgG in sixteen instances and for IgM in thirteen instances.

**DISCUSSION**

Interest in secretory immunoglobulins is relatively recent. It stemmed from the observation that factors other than the level of serum antibody were involved in recovery from and resistance to infections. Mucoantibodies are a better parameter to measure than serum levels as an index of effectiveness of many immunization procedures. The chief immunoglobulin in extravascular secretions is IgA, which is different in molecular size, chemical structure and antigenic function from serum IgA (Tomasi & Bienenstock, 1968). It is produced locally by plasma cells located beneath the mucosal surface of many organs, e.g. the lungs and gastro-intestinal tract. Two molecules of IgA of 7S class combine together and this dimer then unites with the secretory component elaborated by the epithelial cells before being released over the mucosal surface.

There are several technical problems encountered in the analysis of secretory
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immunoglobulins. In many fluids, the total protein concentration, and presumably that of immunoglobulins, varies significantly in the same individual. The rate of fluid secreted is one important influencing factor. Since immunoglobulin concentration in most extravascular fluids is small, methods of concentration or steps to increase the sensitivity of quantitative techniques have to be adopted. Since secretory IgA is different from serum IgA, and may contain 7S, 11S and polymeric 18S to 20S types, the use of the same reference serum standard is, at best, an arbitrary and relative choice. Degradation of immunoglobulins by proteolytic enzymes found in many secretions poses another problem. The specificity of antisera directed against 11S secretory IgA is debatable.

There is very little information concerning the immunoglobulin composition of uterine luminal secretion. That such antibodies are important in local defence is suggested by studies in veterinary medicine on the three major infections causing infertility in cattle—brucellosis, trichomoniasis and vibriosis. There is a dissociation between serum antibody level and vaginal–uterine fluid antibody level (Tomasi & Bienenstock, 1968). Quantitative estimates of immunoglobulins in secretions of the human female genital tract are not available. Qualitative immunoelectrophoresis suggests the presence of IgA, IgG and IgM in this order of frequency (Anzai, Ibayashi, Aldrich & Carpenter, 1963). Herve, Robey & Sergent (1965) found the protein composition of follicular fluid to be similar to that of the normal serum. It is possible that hormone-induced changes in immunoglobulins, seen in the serum of women using oral contraceptives (Chandra, 1972) and during pregnancy (Chandra, Malkani & Bhasin, 1973), may also be reflected in the uterine fluid and this may vary at different times of the ovulatory cycle.

There was a slight but significant increase in serum IgM but not in IgG and IgA. This was also observed in women who had been using an IUD for more than 1 year. It is possible that factors other than the mere presence of an IUD may have been responsible for the profound rise in IgM and IgG noticed by Holub et al. (1971). Since the morphology of the cellular exudate elicited by an IUD does not show any significant change after the device has been in situ for a month or more, the immunoglobulin changes resulting from the presence of the IUD as a foreign body should occur fairly early. The time of sampling, in relation to the menstrual cycle, the presence or absence of coincidental infection, hormone-induced changes in the blood vascular compartment volume and other factors may be important.

The method of analysis for the estimation of locally produced immunoglobulins employed here takes into account the effect of non-specific inflammatory extravasation of immunoglobulins from the serum. The use of immunologically inert protein concentrations—albumin and α2-macroglobulin—permits the separate calculation of immunoglobulin amounts derived from enhanced vascular permeability and that produced locally. We found that IgA, and to a lesser extent IgG, was present in quantities larger than can be explained by vascular permeability. This local immunoglobulin may be significant in the physiopathological mechanisms associated with the contraceptive action of an IUD. Other biochemical changes induced by an IUD (reviewed
by Eckstein, 1970) do not seem to have a causal rôle to play. It is not known if plasma cells are present in the normal uterus. Even if they are not, this does not exclude their appearance in pathological states, as occurs in the liver in the event of cirrhosis. The loose cellular matrix around an IUD consists principally of polymorphs followed in 2 to 3 days by mononuclear cells and macrophages. Plasma cells were not seen (Gupta et al., 1971). Their probable submucosal location, as occurs in other mucosal surfaces, may prevent them from participating actively in the cellular reaction to an IUD.

REFERENCES


