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The Physiological Interferon Response. I. Cells Attached to Intrauterine Devices Release Interferon *in Vitro* (41644)

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Abstract. Cells attached to intrauterine devices (IUDs) release interferon (IFN) in the medium during incubation *in vitro*. Most of the IFN is released during the first hour suggesting that the cells had been previously induced and were probably already producing IFN *in vivo*. Characterization of the IFN indicates that most of it is of gamma-type with a trace of alpha. Production of IFN in the uterine fluid would represent a first example of the physiological IFN response and may serve to modulate some of the mechanisms preventing implantation of blastocyst. The actual presence of IFN in the uterine secretion remains to be demonstrated.

Intrauterine devices (IUDs) are presently used as a method of birth control by about 60 million women (1). Their insertion in the uterine cavity triggers a number of reactions characterized by infiltration of neutrophils, macrophages, lymphocytes, and plasma cells into the endometrium (2), by biochemical changes in endometrial tissues (3) and in uterine secretions (4–7), by alteration of both sperm and egg transport and survival (8, 9), by hormonal responses (10–12), and finally by immunological reactions (13, 14).

It is thought that the macrophages play a major role in insuring contraception probably with more than one mechanism of action. It is interesting to note that macrophages adhere to the IUDs surface (15) and products, such as proteases and prostaglandins, are released by these cells when IUDs, removed from the uterine cavity, undergo incubation (16, 17).

Bocci (18) has reviewed the evidence that a number of agents such as mechanical stimuli, copper, proteases, and many others can act on lymphocytes and macrophages as inducers of interferon (IFN). Therefore we entertained the possibility that if cells adhered to the IUDs have been induced *in vivo*, they may release IFN which could be measured in the incubating medium. This possibility has been investigated and we are now presenting the results.

Materials and Methods. IUDs (ML Cu-250, Multilan S.A., Switzerland; Copper T-200, Schering AG, West Germany; No-Gravid, IRMED, Italy) were removed under aseptic conditions from 16 women at the fifth to sixth day of the menstrual cycle after having been *in situ* for periods of time ranging from 20 to 26 months. After removal, the tails of the devices were eliminated and the IUDs were immediately placed into tissue culture dishes (COSTAR, USA) containing 3.5 ml of RPMI 1640 medium (GIBCO, Scotland) supplemented with 10% heat-inactivated fetal calf serum (GIBCO, Scotland), penicillin (100 units/ml), and streptomycin (100 μg/ml). IUDs were then promptly incubated at 37°C in an atmosphere of 5% CO_2 in air. At predetermined times (Fig. 1), the IUDs were placed in a new dish containing 3.5 ml of fresh RPMI 1640 medium. After counting the cells detached from the IUDs, the samples were centrifuged at 4°C and supernatants stored at −80°C for assay. Moreover, two different IUDs were incubated for 30 hr without change of medium. In this case the initial volume was 6 ml and small aliquots (100 μl) were withdrawn at predetermined times (Fig. 2).

Cells detached from IUDs in the medium were counted and, at the end of incubation, IUDs were treated with 0.01% trypsin (GIBCO) for counting residual cells. After cytocentrifugation, smears were stained with May Grunwald–Giemsa for differential cell counts. Macrophages were identified after exposure to 10^6 latex beads (1.01 μm diam; Polysciences) in 1 ml of medium for 30 min at 37°C; the cells were washed to remove free

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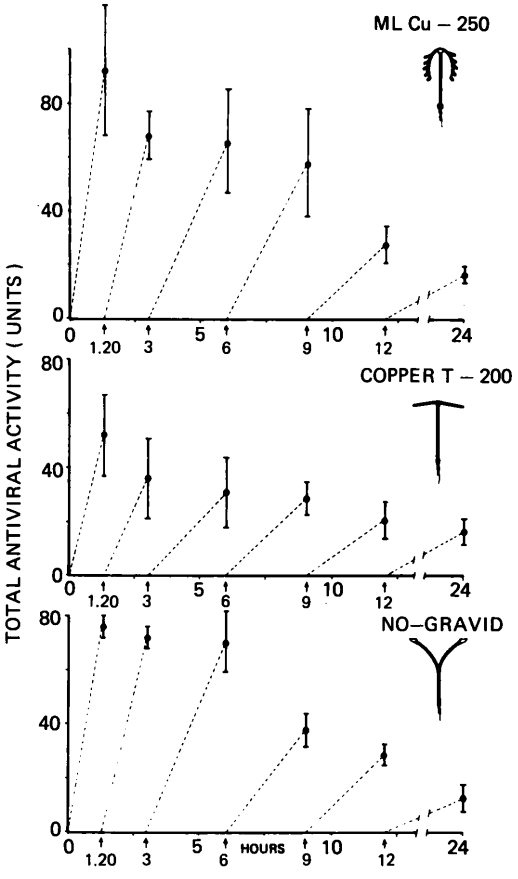

FIG. 1. Amount of interferon released from different IUDs during incubation. Devices were placed in a new dish containing 3.5 ml of fresh RPMI 1640 medium at times indicated by the arrows. Each point represents the mean ± SD of observations carried out in IUDs (ML Cu-250: $N = 4$; Copper T-200: $N = 4$; No-Gravid: $N = 6$).

beads, cytocentrifuged, and stained for nonspecific esterase activity by the method of Yam *et al.* (19).

The microplaque reduction assay described by Langford *et al.* (20) has been used throughout for the titration of IFN using human amnionic cells (Wish) and vesicular stomatitis virus (VSV, Indiana strain) as a challenge virus. Monolayers were infected with 50 plaque-forming units of VSV and samples were tested at least twice in duplicates. Samples were tested also on other cell lines (rat Ratec, mouse L 929 cells, and on rabbit line RL obtained from Lab-Tek, USA).

Titrations were always made employing the international reference preparation (IRP) for human IFN-α and -β (obtained from NIAID, NIH, Bethesda, Md.). Because of the unavailability of reference standard, antiviral activity of human IFN-γ was referred to the international standard for human IFN-α. The IRP of human IFN-α (G-023-901-527 with a defined potency of 4.3 Log_{10} IU/vial) and the IRP of human IFN-β (G-023-902-527 with a defined potency of 4.0 Log_{10} IU/vial) when reconstituted in 1.0 ml of sterile distilled water, had a geometric mean titer respectively, of 4.28 Log_{10} IU/ml (SD = 0.069; $N = 16$) and of 4.01 Log_{10} IU/ml (SD = 0.106; $N = 16$). All titers were reported in IU/ml and total units reported in Figs. 1 and 2 were calculated on the basis of the volumes used during incubation.

Characterization of interferon was carried out according to standard procedure using acidification of samples at pH 2 with 0.1 *M* HCl, heating, dialysis, proteolytic and other enzymic treatment, and eventual neutralization by antisera to human IFN-α, -β (Preparations G-026-502-568 and G-028-501-568 obtained through the courtesy of NIAID, NIH, Bethesda, Md.), and -γ (preparation provided by Professor J. Vilček with a neutralizing titer of about 1:2500 against 10 U/ml). For each experiment the amounts of antihuman IFN-α, -β, and -γ employed were sufficient to completely neutralize the corresponding IFNs. Following incubation the residual IFN activity in each aliquot was assayed as described above. Neutralization of antiviral activity by the specific antisera was not affected by dilution of the samples during the IFN assay.

Women using IUDs had undergone periodic control for parasitic protozoan and fungous infections and were negative.

Results. Table I indicates the type and numbers of IUDs tested and shows that there is no obvious relationship between the surface of copper wire and the total number of cells recovered from the IUDs during incubation. About 78% of the cells were macrophages, 19% were neutrophils, and 3% were lymphocytes.

The highest amount of IFN was recovered from the medium during the first 80 min of incubation (Fig. 1). This result seems more likely to depend upon the total number of cells attached to the IUD at the beginning of incubation rather than to the extent of copper wire surface. During the following periods total antiviral activity decreased and this is at-

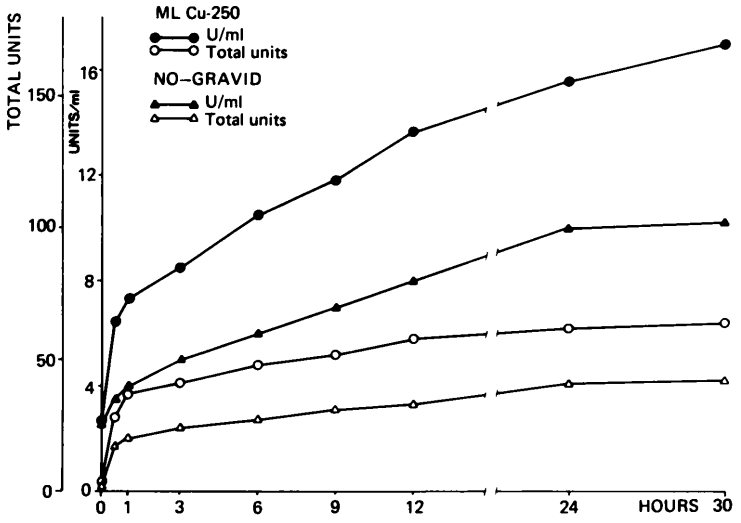

FIG. 2. Kinetic of interferon release from two different IUDs (ML Cu-250 and No-Gravid) during incubation without change of RPMI 1640 medium.

tributed to some extent to cell shedding. However, evaluation of the IFN units/cells ratio indicates its progressive decrease, implying that production of IFN per cell is actually decreasing during the incubation period. It is difficult to decide whether the decreased production of IFN is due to reduced cell viability or to an earlier shedding of IFN-producing cells.

Next, we investigated the kinetic of IFN release from two types of IUDs incubated for 30 hr (Fig. 2). There is a maximum increase during the first hour followed by a slow progressive increase for the next 24 hr when it tends to level off. Unfortunately we do not know if break-down of IFN is going on at the same time so that the observed values could be the combined results of both synthesis and catabolism. This seems likely as the total amount of IFN recovered during these experiments is less than we expected from total recovery obtained in the experiments reported in Fig. 1.

To make sure that antiviral activity was due to IFN, we have characterized it according to the typical scheme reported in Table II. No antiviral protection was demonstrated when the same samples were tested on cells (rat Ratec, rabbit RL, and mouse L 929 cell lines) insensitive to human IFN.

The findings that antiviral activity was considerably (but not entirely) acid-labile, insensitive to antihuman IFN-β serum, slightly sensitive to antihuman IFN-α serum, and markedly sensitive to antihuman IFN-γ serum suggest that most of the activity can be attributed to human IFN-γ and slightly to IFN-α. Heterogeneity of cell population resident in IUDs makes plausible the release of two IFN types.

Discussion. To our knowledge this is the first report indicating that cells attached to

TABLE I. CELLS RECOVERED FROM DIFFERENT IUDS AFTER INCUBATION AND FINAL TRYPSIN TREATMENT

IUD types	Number of experiments	Total cells ($\times 10^7$) (mean ± SD)	Surface of copper wire (mm^2)
ML Cu-250	5	2.19 ± 0.33	250
Copper T-200	4	0.96 ± 0.23	200
No-Gravid	7	1.50 ± 0.61	440

TABLE II. CHARACTERIZATION OF INTERFERONS RELEASED FROM CELLS ATTACHED TO IUDS

IUD types	IFN in pooled samples (U/ml)	Treatment with: Control sheep sera (U/ml)	Anti IFN$_\alpha$ serum (U/ml)	Anti IFN$_\beta$ serum (U/ml)	Anti IFN$_\gamma$ serum (U/ml)	DNAase and RNAase (0.3%) (U/ml)	Trypsin (0.2%) (U/ml)	Heating (56°C, 1 hr) (U/ml)	Dialysis (24 hr) (U/ml)	pH 2 (U/ml)
ML Cu-250	26	26	20	24	7	25	<3	<3	28	9
Copper T-200	18	18	15	19	—	20	<3	<3	20	9
No-Gravid	20	20	16	20	<3	17	<3	<3	20	10

IUDs, besides releasing an array of other substances, can produce IFN-γ and probably a little IFN-α. This is not altogether surprising because most cells adhering to IUD are subjected in the uterine environment to a number of stimuli which can activate them. Although viruses, dsRNA, mitogens, and antigens (on sensitized lymphocytes) are among the best inducers of IFNs, the number of other compounds that can stimulate IFN production is practically endless (21).

Bocci (18) has distinguished two types of responses: the "acute" one typical of viral infections with massive production of IFN, and the "physiological" one characteristically induced in the gut and bronchial-associated lymphoid tissue or, as in this case, in the uterine cavity by the insertion of IUDs, with very limited production of IFN which act locally. We are currently working on defining the sites and the importance of the physiological IFN response and it appeared to us that the presence of IUDs in the uterus can be a good example of the response.

In this case, what could be the role of a small amount of IFN present in the uterine fluid? This is a question difficult to answer because probably alterations of the implantation and survival of blastocyst are the results of several reactions for which the presence of IFN may not be important. However, it is necessary to point out that IFN displays antiproliferative and antidifferentiation effects (22) but which IFN level will be effective in the uterus is unknown. The antiviral activity of IFN may also be useful in preventing spread of opportunistic viruses, protozoan and fungous infections in the endometrium, and it appears interesting to test if IFN is present in uterine fluids. An important question is whether leukocytes in the lumen of the uterus produce IFN in the absence of IUD. We are currently trying to find an answer by measuring antiviral activity in cervical mucus obtained from normal and IUD-bearing women.

It is known that cells adhering to IUDs release PGs *in vitro* (16) and it is noteworthy that Yaron *et al.* (23) have shown that human IFN can stimulate production of PGE in cultured fibroblasts.

On the other hand, PGs of E-type can modify the secretion of lymphokines providing a negative-feedback mechanism for the regu-

lation of the immune response (24). IFN has also immunomodulatory properties (25) and therefore its presence in the uterine fluid may help to modulate several mechanisms preventing implantation of the blastocyst.

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