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Early embryonic histamine-releasing factor: a new model for human implantation

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In this paper are presented preliminary results on the identification of a histamine-releasing factor. This factor was partially purified by means of affinity heparin–Sepharose chromatography from media in which 2-cell human embryos were cultured. The culture was performed in preparation for an in-vitro fertilization (IVF) programme. The mean value of histamine release evoked by the embryo-derived histamine-releasing factor (EHRF) was 56.7%. The release was not due to cytotoxicity since no histamine release was obtained with unsensitized cells and when the assay was performed at 4°C. As a control, no histamine release was obtained using medium from unfertilized oocytes or medium alone. The EHRF could be one of the first signals from the embryo to the uterus. The immunosuppressive activity of histamine is well known, and we suggest that the local secretion of histamine *in vivo* by uterine mast cells, alone or in cooperation with other factors and/or mechanisms could play a role in preventing maternal immuno-rejection at the implantation stage.

Introduction

The way in which the maternal immune system is regulated during early pregnancy and how an embryo escapes the expected maternal immuno-rejection is a fundamental, yet largely unsolved, question in biology. Several hypotheses have been suggested to explain successful embryo–maternal balance, and reports on the embryo as actively interfering with normal maternal graft rejection mechanism have recently gained much attention (Rolfe *et al.*, 1983; Muchmore and Decker, 1985; Mayumi *et al.*, 1985; Cocchiara and Geraci, 1985; Cocchiara *et al.*, 1986a, 1986b).

Systemic immuno-suppressive factors such as hormones, steroids and pregnancy-related proteins have been reported to interfere with implantation, however, they are not present in concentrations great enough to modulate the action of primed maternal lymphocytes. Therefore the mechanism primarily responsible for fetal maintenance is likely to act locally at the level of the uterus inhibiting the activity of sensitized maternal lymphocytes since at implantation the maternal immune system is not totally impaired (Edwards, 1980; Billington, 1985).

An induction stage must, however, precede the regulatory process to initiate activation of suppressor cells and we suggest here that EHRF could have such a role leading to the release of histamine. Histamine is a biogenic amine present in almost all mammalian species and it has been well demonstrated that suppressor cells can be activated *in vitro* by various stimuli, thereby modulating the immune system (Beer *et al.*, 1984).

Materials and methods

The embryo culture medium was Menezo's medium (Menezo, 1976) containing 10% (v:v) heat-inactivated maternal serum. The human oocyte, usually collected by laparoscopy, was incubated at 37°C in 1 ml of medium containing ~50 000 spermatozoa. After 12 h the medium was changed and the embryo was transferred into 1 ml of fresh medium containing 10% serum and cultivated for ~24 h until the 2–4-cell stage and then transferred to the maternal uterus.

The 24-h medium was centrifuged to remove debris and stored at −20°C until used. Ten such media were pooled and chromatographed through an affinity heparin–Sepharose column previously equilibrated with 0.01 M sodium phosphate buffer at pH 7.3 containing 0.15 M NaCl. The bound material eluted in 1 ml was concentrated 10 times by increasing the salt concentration of the starting buffer to 1.5 M NaCl and no activity was lost in the column. The bound fraction contained all the histamine-releasing activity and this fraction was therefore called EHRF. Histamine release was measured by means of an enzymatic–isotopic microassay (Taylor *et al.*, 1980) using sensitized human leukocytes obtained from atopic donors (Geraci *et al.*, 1985). The same amount of culture medium, from unfertilized oocytes also containing spermatozoa was collected and used as a control in addition to the medium alone.

All the supernatants from culture media with or without embryos or oocytes were partially purified (manuscript in preparation) through an affinity chromatography column of heparin–Sepharose. Each EHRF preparation was derived from 10 supernatants, and was used for ~100 histamine-release tests. To date we have obtained four different preparations sharing a similar histamine-releasing activity. The protein concentration of each EHRF preparation was ~0.7 mg/ml, and 1–10 μg of protein were used in each histamine-release assay.

Histamine release was expressed as a percentage of the total cellular content of the amine; all values were corrected for the spontaneous release occurring in the absence of any stimulus. The values reported are the mean of at least triplicate tests. Standard deviation (SD) usually did not exceed 10%. The statistical evaluation of the results was effected by Pearson's χ^2 test or by Student's *t*-test.

Results

Supernatants of several human embryo culture media and unfertilized oocyte culture media were obtained during an IVF programme (Cocchiara *et al.*, 1984), and stored at −20°C until used. Whole human blood of atopic patients (Geraci *et al.*, 1985) was used as a source of sensitized basophils for the histamine-release assay. Table I shows representative results obtained using EHRF and basophils from different atopic donors.

A mean value of 56.7% of histamine release was obtained in respect of the total histamine blood content, when supernatant from embryo cultures was used. The histamine release values ranged between 38 and 71% of the total histamine, depending

Table I. Histamine release from leukocytes of atopic patients using EHRF

Patient	Histamine release induced by (c.p.m. mean ± SD)		
	None	EHRF[a]	Percentage[b]
I.D.L.	609 ± 0.04	2406 ± 0.07	49
A.C.	680 ± 0.10	2500 ± 0.10	71
R.S.	410 ± 0.21	3498 ± 0.12	69
F.D.	590 ± 0.12	1837 ± 0.08	38
Mean percent release			56.7

[a]EHRF (10 μg/ml) represents the bound fraction of the heparin–Sepharose chromatography derived from a pool of 10 embryo culture media.
[b]Percentage of histamine release calculated with respect to the total histamine content corrected for spontaneous release.

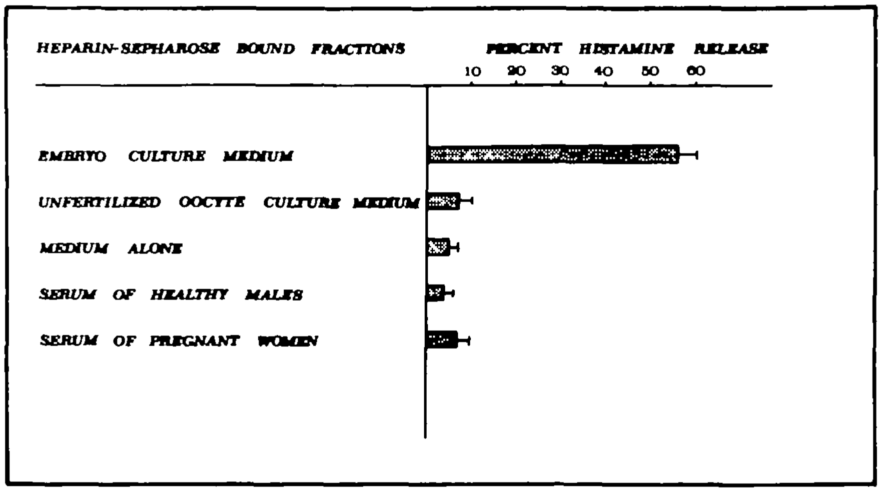

Fig. 1. Histamine release of different media and sera after chromatography on heparin – Sepharose column

on the variability among the different basophil donors. Very low values of histamine release were obtained in absence of any stimulus (Table I) and they represent the spontaneous release observed throughout the assay conditions.

To test if EHRF was secreted at fertilization, the histamine-release assay was performed with a medium of unfertilized oocytes. As shown in Figure 1, no histamine release was obtained by using such a medium. To exclude the possibility that the histamine release detected in the embryo medium was due to some aspecific secretagogue present in the mother's serum used to prepare the growth medium, medium alone was used. The results reported in Figure 1, once again suggest the growing embryo as being responsible for the EHRF production. Additional controls such as healthy males' serum or pregnant serum gave negative results.

The column of heparin – Sepharose used allows a partial purification of the EHRF since all of the histamine-release activity was found in the bound fraction. The heparin – Sepharose unbound material from all the media and sera tested gave negative results. The results shown in Figure 1 are the mean ± SD of $n = 20$ experiments performed in triplicate. The percentage of histamine release calculated for 20 experiments was significantly higher in the bound embryo medium fraction ($P < 0.05$).

Discussion

Several modifications have been reported to occur in the uterus during the early stages of pregnancy (Edwards, 1980). The permeability of endometrial capillaries adjacent to the blastocyst increases at implantation, and such a permeability can be modified by the local action of histamine. An increased permeability can support embryo implantation by increasing the adhesion of the embryo to the endometrium. Histamine, in addition, has been well demonstrated (Beer *et al.*, 1984) to modulate the immune response, and it could be an important candidate for a leading role in implantation.

We propose here a new model to explain embryo implantation. The human embryo at an early stage secretes a factor capable of inducing release of histamine from uterine mast cells and the histamine acts locally to stimulate suppressor cells to produce histamine-derived suppressor factors. These factors alone or in cooperation with other factors or mechanisms suppress the local maternal immune response allowing the embryo to survive and implant. Such a model suggests that the embryo does not need to produce a huge amount of factor since the message is then amplified by the several molecules of histamine released.

To explain why the histamine produced does not affect the mother's immune system as a whole but acts only at the uterine level, we suggest that histaminase known to appear in plasma during pregnancy a day or two after implantation (Bell, 1986) can destroy the excess histamine produced. EHRF is secreted into the culture medium by the embryo, after the fertilization step, since neither the oocyte nor the sperm present in the unfertilized oocyte medium release such a factor.

The histamine assay used in this study gave negative results with serum from either healthy males or pregnant women's serum. Although the histamine assay is very sensitive, trace amounts of EHRF in serum will be better tested as soon as a specific antibody against EHRF can be obtained. To test for the presence of histamine-releasing activity, whole human blood from atopic donors was used because these are the most useful cells for the purpose. However, this in-vitro result could probably reflect the in-vivo situation since experiments carried out with human endometrium obtained by hysterectomy gave preliminary results (assessed by the release of histamine by uterine mast cells on challenge with EHRF) supporting the results obtained with basophils (manuscript in preparation). The different histamine-release values obtained with different atopic donors is due to variations in sensitivity among the donors. This would suggest an interaction of EHRF with IgE molecules on the basophil or mast cells, but additional and more specific data are needed.

Since EHRF binds to the heparin – Sepharose column, the factor could contain some lipid group, and the affinity step concentrated and partially purified the EHRF.

In conclusion, the data presented here suggest that the human embryo at a very early stage of development produces a histamine-releasing factor capable of inducing histamine release by basophils that could represent the first message for implantation. In addition a new model suggesting histamine as the immunosuppressive molecule capable of allowing the survival of the embryo allograft during the implantation stage is presented.

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Announcement

New Aspects in Female Contraception — Update and Trends: 2nd Heidelberg International Symposium under the patronage of the 8th World Congress of Sexology 13–16 June 1987, Heidelberg, FRG

The 2nd Heidelberg International Symposium on 'New Aspects in Female Contraception: Update and Trends' will be held at the congress centre from 13–16 June 1987 in Heidelberg, FRG.

President: Professor Benno Runnebaum, M.D., Department of Obstetrics and Gynaecology, Division of Gynaecological Endocrinology, University of Heidelberg.

The programme will include plenary lectures, free oral and poster communications. Headlines of the Symposium are: oral contraception, epidemiology, cardiovascular risks, carbohydrate and lipid metabolism, blood coagulation, CNS effects, low dose pills, new progestins, risks and benefits, new IUD technology, GnRH – analogues, temperature computers, implantables, barriers and spermicides, immunological contraception, injectables, politics in contraception.

The deadline for submission of abstracts to be considered for presentation is 1 February 1987. Registration fees: DM 120 (US$60) before 1 February 1987; DM 200 (US$100) after 1 February 1987.

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