Changes in uterine phosphatase levels in mice deprived of LH during early pregnancy

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Summary. Mice injected with normal rabbit serum on Day 4 of gestation showed a progressive increase in specific alkaline and acid phosphatase activities on Days 6 and 7. Mice injected with antiserum to LH on Day 4 showed a significant decrease in specific alkaline phosphatase activity estimated biochemically on Days 6, 7 and 8, but this decrease was histochemically evident only on Day 8. Similarly, acid phosphatase activity in antiserum-treated mice was significantly increased on Days 6, 7 and 8 when estimated biochemically, but only on Day 8 when studied histochemically.

Introduction

In mammals, induction of blastocyst implantation is a critically timed event dependent on the sequential interplay of two hormones, oestrogen and progesterone. Intervention at the earliest possible stage after fertilization, e.g. before implantation or soon afterwards, would be a useful and direct approach towards the control of early pregnancy.

A single injection of an antiserum to LH on Day 4 of pregnancy in mice results in the termination of pregnancy (Munshi, Purandare & Rao, 1972). Subsequent studies (Munshi & Nilsson, 1973) using glutaraldehyde-perfused tissues, however, revealed that the attachment reaction and blastocyst implantation occurred normally in antiserum-treated mice. Nevertheless, termination of pregnancy occurred on Day 8 of gestation due to massive haemorrhage in the decidua. A single injection of the antiserum given on Day 4 of pregnancy was unable to block the mitotic shift from epithelial to stromal cells, which normally occurs on Day 4 of pregnancy (Munshi *et al.*, 1973). Since no morphological change was evident in the implanting blastocyst or the immediate surrounding uterine tissue in mice deprived of LH, an attempt was made to study the enzymic changes induced in these tissues.

It is well established that alkaline and acid phosphatases are associated with the decidual cell reaction and play an important role in implantation (Finn & Hinchcliffe, 1964; Lobel, Tic & Shelesnyak, 1965a, b, c, d, e; Manning, Carter & Butler, 1969; Malinowska & Fotherby, 1975; Johnson, Calarco & Siebert, 1977). In the present study the activities of acid (EC 3.1.3.2) and alkaline (EC 3.1.3.1) phosphatase in the implantation sites of mice injected with antiserum to LH were estimated biochemically and histochemically.

Materials and Methods

Fertile female mice of the Swiss strain, weighing between 20 and 25 g, were used. The animals were maintained in air-conditioned rooms with a 14 h light/24 h schedule. The females were caged with fertile males on the afternoon of pro-oestrus and checked the next morning for the presence of a vaginal plug. The day on which a vaginal plug was observed was considered as Day 1 of pregnancy.

Antiserum to LH was prepared in rabbits by the method of Munshi & Rao (1965). At 10:00 h on Day 4 of gestation, the control animals were injected subcutaneously with 0.2 ml normal rabbit serum and the experimental animals with an equal volume of the LH antiserum. The animals were autopsied at 11:00 h on Days 6, 7 and 8. Pontamine blue dye was injected through the tail vein before autopsy to facilitate detection of implantation sites. For histochemical studies the implantation sites were immediately frozen and 8 µm sections were cut on a Pearse Slee Cryostat at -22°C . The sections were fixed in chilled acetone (-4°C) and were stained for acid and alkaline phosphatase activities by Gomori's methods as reported by Pearse (1961). The enzyme activity was graded by visual appraisal of the staining reaction from moderate (+) to intense (++++).

For biochemical estimations, the implantation sites were homogenized to 1% in 0.9% (w/v) sodium chloride. Protein was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951). Optical density was measured at 540 nm on a Gilford spectrophotometer. The activities of acid and alkaline phosphatases were estimated by the *p*-nitrophenol phosphate method of Bessey, Lowry & Brock (1946). Optical density was measured at 400 nm and the enzyme activities were expressed as units where 1 phosphatase unit is the amount of enzyme which liberates 0.05 mmol *p*-nitrophenol at 37°C for 30 min. For alkaline phosphatase activity, the values are multiplied by the correcting factor of 20 and for acid phosphatase activity they are multiplied by the correcting factor of 4.68.

The statistical significance of differences between the means was determined by Student's *t* test and *P* values of <0.05 were considered significant.

Results

The histochemical localization of phosphatase activity in the decidual tissue of the control and experimental mice is depicted in Table 1. In the control animals alkaline phosphatase activity increased between Days 6 and 7, while acid phosphatase activity remained constant. In the mice treated with LH antiserum, alkaline phosphatase decreased markedly on Day 8 but acid phosphatase activity became intense on Day 8 of pregnancy when compared to the controls.

Table 1. Histochemical localization of alkaline and acid phosphatase activity* in mouse implantation sites

Phosphatase	Control mice			Mice injected with LH antiserum		
	Day 6	Day 7	Day 8	Day 6	Day 7	Day 8
Alkaline	++	+++	+++	++	++	+
Acid	++	++	++	++	++	++++

* Estimated on a 4-point scale: +, moderate; ++, good; +++, high; +++++, intense.

Biochemical estimations of the phosphatase activities are given in Table 2. A progressive increase in the wet weight and total protein content of the uterus was observed in the control mice from Days 6 to 8. This increase was significantly inhibited in mice treated with LH antiserum. In control mice, there was a rise in specific alkaline and acid phosphatase activities from Days 6 to 7, but the activities remained unchanged on Day 8. In the antiserum-injected mice a significant decrease in alkaline phosphatase activity was observed on Days 6, 7 and 8 when compared with the respective controls. On the other hand, a significant increase in acid phosphatase activity occurred for all 3 days when compared with the respective controls.

Table 2. Biochemical estimation of alkaline and acid phosphatase in mouse implantation sites

	Control mice			Mice injected with LH antiserum		
	Day 6	Day 7	Day 8	Day 6	Day 7	Day 8
<i>Alkaline phosphatase</i>						
No. of animals	13	10	8	11	8	9
Uterine wt (mg)	25.92 ± 1.95 ^a	38.44 ± 2.00 ^b	54.67 ± 3.51 ^c	18.00 ± 0.90 ^c	19.81 ± 2.87 ^d	20.88 ± 2.40 ^d
Total protein (mg)	1.41 ± 0.10 ^a	1.71 ± 0.18 ^a	2.35 ± 0.21 ^b	1.06 ± 0.05 ^c	1.04 ± 0.10 ^c	1.02 ± 0.19 ^c
Total enzyme activity	76.80 ± 21.40 ^a	448.60 ± 40.6 ^b	589.00 ± 95.90 ^b	24.40 ± 4.40 ^c	52.20 ± 16.60 ^d	39.80 ± 5.80 ^c
Specific enzyme activity						
Units/mg protein	48.00 ± 10.60 ^a	278.40 ± 32.40 ^b	260.00 ± 42.00 ^b	22.00 ± 3.60 ^c	44.00 ± 9.60 ^d	44.00 ± 10.20 ^d
Units/mg tissue	2.46 ± 0.54 ^a	11.20 ± 0.54 ^b	10.60 ± 1.38 ^b	1.20 ± 0.22 ^c	2.54 ± 0.56 ^d	2.06 ± 0.32 ^d
<i>Acid phosphatase</i>						
No. of animals	6	8	7	5	8	7
Uterine wt (mg)	27.43 ± 1.52 ^a	42.20 ± 2.849 ^b	46.37 ± 4.53 ^b	16.04 ± 1.10 ^c	21.35 ± 3.63 ^c	17.31 ± 1.55 ^c
Total protein (mg)	1.35 ± 0.09 ^a	1.70 ± 0.29 ^a	1.82 ± 0.27 ^a	1.01 ± 0.07 ^b	0.71 ± 0.13 ^b	0.73 ± 0.11 ^b
Total enzyme activity	17.17 ± 1.54 ^a	27.09 ± 3.68 ^b	20.45 ± 4.21 ^c	16.28 ± 1.26 ^a	24.47 ± 3.94 ^b	18.25 ± 1.85 ^c
Specific enzyme activity						
Units/mg protein	12.60 ± 1.30 ^a	17.70 ± 2.30 ^b	11.20 ± 2.10 ^a	15.90 ± 1.10 ^c	38.80 ± 5.60 ^d	29.40 ± 7.30 ^d
Units/mg tissue	0.63 ± 0.05 ^a	0.63 ± 0.06 ^a	0.44 ± 0.09 ^a	1.01 ± 0.06 ^b	1.16 ± 0.05 ^b	1.03 ± 0.03 ^b

Values are mean ± s.e.m.

In each horizontal row, values with different superscripts are significantly different, $P < 0.05$.

Discussion

A marked increase in protein synthesis in the uterus on Day 4 of pregnancy in the mouse (Gwatkin, 1969; Aitken, 1977; Pratt, 1977), accompanied by a 10–17-fold increase in the activity of alkaline phosphatase in the decidual tissue at the site of implantation (Pritchard, 1947; Christie, 1966; Manning, Meli & Steinetz, 1966; Steinetz, Manning, Giannina & Meli, 1967; Jelinek & Jelinkova, 1975), indicates the preparation of the endometrium for implantation of the arriving blastocyst, and suggests that alkaline phosphatase is an important marker enzyme for the decidual cell reaction and could be intimately associated with implantation. Acid phosphatase, which has been shown to increase on Day 6 of pregnancy, is associated with the breakdown and phagocytosis of luminal epithelial cells at the site of implantation (Smith & Wilson, 1971; Malinowska & Fotherby, 1975; El-Shershaby & Hinchliffe, 1975).

In the present study, the increase in the wet weight and total protein content of the uterus was associated with an increase in the phosphatase activities in the implantation site of the control mice between Days 6 and 7 and thus confirms the earlier observations (Finn & Hinchliffe, 1964; El-Shershaby & Hinchliffe, 1975; Aitken, 1977). However, in the antiserum-treated mice, a decrease in total protein and uterine weight was observed. This was accompanied by a concurrent decrease in alkaline phosphatase and an increase in acid phosphatase levels when measured biochemically and compared to the respective controls on all 3 days studied, but the changes were evident histochemically only on Day 8. Lobel *et al.* (1965e) have observed a similar rise in acid phosphatase and a loss of alkaline phosphatase activities in rats treated with specific inhibitors for decidualization (MER-25, pyrathiazine or ergocornine) during pregnancy and pseudopregnancy, suggesting that changes in the two phosphatases were associated with decidual cell regression.

Alkaline phosphatase plays an important role in transferring metabolites across the cell membrane. Its high activity in the decidual tissue, associated with high protein synthesis, reflects its nutritive role for the developing blastocyst and thereby helps in the maintenance of pregnancy. Decreases in alkaline phosphatase activity, together with a decrease in total protein in the antiserum-treated animals in the present study, suggest deprivation of nutrient material to the blastocyst, inhibiting its growth and survival and resulting in the termination of pregnancy. Acid phosphatase is a lysosomal enzyme associated with the breakdown and

phagocytosis of the tissue. The significant increase in acid phosphatase activity in the antiserum-treated animals would indicate enhanced phagocytosis of the luminal epithelial cells accompanied by degeneration of the growing blastocyst and termination of pregnancy. The biochemical method of assessment of phosphatase activity, being more sensitive and quantitative, is able to demonstrate these changes well.

The Pearse Slee Cryostat and the chemicals used in the present study were obtained from a grant to the WHO CCCR, Bombay.

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Received 24 October 1978