Anti-fertility effect of passive immunization against progesterone is influenced by genotype

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ABSTRACT

The anti-fertility effect of a monoclonal progesterone antibody injected i.p. 32 h after mating is influenced by genotype since a single dose of 5.7 nmol immunoglobulin G successfully blocked the establishment of pregnancy in BALB/c but not in F1 hybrid mice (CBA male × BALB/c female). Progesterone concentrations in circulation were significantly higher at days 3 and 4 after mating in F1 females compared with those of the BALB/c stock. Moreover, the pattern of mitotic activity in the endometrium after passive immunization differed between the two genotypes. In treated BALB/c mice there was no increase in mitotic activity in stromal cells at days 3, 4 and 5 after mating (in contrast to BALB/c control females in which the number of stromal mitoses increased sharply). In F1 females there was a transient effect of antibody at day 3 (no increase in stromal mitoses but enhanced mitotic activity in the glandular epithelium compared with F1 control females), and subsequently a normal increase in mitotic divisions in stromal cells. The hypothesis is proposed that passive immunization against progesterone at 32 h after mating will only block the establishment of pregnancy in genotypes in which there is a gradual, rather than a steep rise in circulating progesterone concentrations during the preimplantation period. In F1 mice, high concentrations of circulating progesterone at days 3 and 4 of pregnancy apparently over-ride the effect of antibody and facilitate the normal development of stromal mitotic activity associated with the onset of implantation.


INTRODUCTION

We have previously reported that a single injection of monoclonal progesterone antibody 32 h after mating blocks pregnancy in BALB/c mice (Wright, Feinstein, Heap et al. 1982). The minimum effective dose in this strain is about 1 nmol, and the effect can be reversed by the administration of progesterone (Rider, McRae, Heap & Feinstein, 1985). Passive immunization at this stage also blocks pregnancy in another inbred strain, CBA (Wang, Rider, Heap & Feinstein, 1984), though we now report that it is ineffective in an F1 hybrid (CBA, sub-line Carter, male × BALB/c, sub-line Jackson, female). An explanation of the contrasting action of passive immunization in BALB/c and F1 mice may reside in different circulating concentrations of progesterone during early pregnancy, or in a differential sensitivity of endometrial cells to progesterone.

Two techniques were used to examine which of these possibilities is correct. The concentrations of progesterone in plasma at various times of gestation, and in particular during the preimplantation period, was measured by radioimmunoassay to determine whether it was significantly higher in F1 compared with BALB/c untreated females, and therefore more difficult to neutralize by passive immunization. After antibody injection at 32 h after mating, plasma progesterone concentrations measured by radioimmunoassay rise substantially due to the increase in antibody-bound steroid (Wang et al. 1984), but in BALB/c mice there is a lack of endometrial sensitization at the normal time even though circulating concentrations remain high (Rider et al. 1985). The radioimmuno-
assay values obtained represent the sum of steroid bound to antibody, albumin and corticosteroid-binding globulin in addition to the small quantity of unbound steroid. To examine whether the different response to antibody in the two genotypes was due to differences in the amount of biologically available steroid in circulation after antibody treatment, we have examined the progesterone-dependent transition from epithelial to stromal cell division in the preimplantation endometrium. During early pregnancy in the mouse, cells of the luminal and glandular epithelium of the uterus undergo a precise sequential pattern of mitotic activity. Maximum activity occurs in the cells of the luminal epithelium at the time of mating, in the glandular epithelium on day 3 of pregnancy, and in the stroma on days 4 and 5. Cell division in the epithelia is stimulated by oestrogen secreted at pro-oestrus, and the abrupt change to stromal cell division is caused by lutelal secretion of progesterone enhanced by low concentrations of oestrogen (Finn & Martin, 1967; Martin & Finn, 1968). The transition from epithelial to stromal mitotic activity is therefore a sensitive cellular indicator of the availability of progesterone in the circulation for interaction with target cell receptors.

MATERIALS AND METHODS

Animals

Mature virgin mice (BALB/cJ and F1 females from the mating, CBA/Ca male × BALB/cJ female) were housed in a light- (14 h light: 10 h darkness; lights off 20.00 h) and temperature-controlled (22 °C) room and fed a mouse and rat diet (Labsure; Christopher Hill Group Ltd, Poole, Dorset). Female mice were caged with males of the same genotype and pregnancy was dated from the morning on which a vaginal plug was detected (day 1). Mating was presumed to have taken place at 02.00 h (time 0) (Bronson, Dagg & Snell, 1966).

Monoclonal antibody

Monoclonal progesterone antibody was prepared and characterized as previously described (Wright et al. 1982; Wang et al. 1984). Ascites fluid from cloned (DB3) cells was pooled and its immunoglobulin G (IgG) concentration (38.0 nmol/ml) was measured in a Beckman Model E analytical ultracentrifuge equipped with Schlieren optics by comparison with a known concentration of IgG. At 32 h post coitum (p.c.) mice were randomized and given a single i.p. injection of ascites fluid containing 5-7 nmol IgG (minimum effective dose which blocks pregnancy in BALB/c mice, 1 nmol; Heap, Rider & Feinstein, 1984); control females received either an equivalent volume of ascites fluid from a mouse myeloma (McPc 1748) or physiological saline since pregnancy rates were the same after either treatment.

Plasma progesterone levels

Blood samples were collected at autopsy by cardiac puncture under ether anesthesia. Blood was placed in heparinized tubes and the plasma, separated by centrifugation at 4 °C, was stored at −20 °C before assay. Plasma samples were thawed to room temperature, diluted 10- to 200-fold with sodium phosphate buffer (0.05 mol/l, pH 7.4) and 100 μl portions were extracted in duplicate with 5.0 ml diethyl ether. After shaking for 10 min the ether phase was decanted and evaporated to dryness at 40 °C under a stream of nitrogen. The residue was subjected to progesterone radioimmunoassay according to the method described by Ricketts, Galil, Ackland et al. (1980) and validated for the mouse in our laboratory (Wang et al. 1984). A polyclonal antiserum (18/3, kindly supplied by Dr A. P. F. Flint of this Institute) was raised in sheep against 11α-hemisuccinyl-bovine serum albumin and used at a dilution of 1:6000. Its specificity has been reported by Sheldrick, Mitchell & Flint (1980).

The recovery of [3H]progesterone added to plasma was 96.5 ± 1.6% (mean ± s.e.m., six observations), and the sensitivity of the assay was 0.09 ± 0.02 pmol/assay tube (calculated from 2 × s.d. below the value at zero concentration). Adding 3-18 and 6-37 pmol progesterone to 1 ml plasma from male mice gave values of 3-07 ± 0.2 and 6-27 ± 0.5 pmol/ml respectively (six observations). The intra- and interassay coefficients of variation were 18.2 and 12.8% respectively.

Mitotic activity

BALB/c and F1 nulliparous females were injected i.p. with antibody 32 h after mating as previously described. Two hours before autopsy at 14:00–16:00 h on days 3, 4 and 5 of pregnancy, 0.1 mg colchicine (Sigma Chemical Co. Ltd, Poole, Dorset) in glass-distilled water was administered s.c. and mitotic figures in the uterus were counted as described previously (Finn & Martin, 1967). Briefly, the uteri were removed and fixed in Bouin's fluid and transverse sections (5 μm) were prepared from a piece taken from the middle region of one uterine horn. Counts of all cells in mitosis in the luminal epithelium, the glandular epithelium, and connective tissue stroma were made on one section taken at random from one uterine horn.

Statistical analysis

The experimental results are expressed as means ± s.e.m. Mean differences between groups were considered to be significant if the appropriate test gave a P
value of <0.05. Differences between means were determined by Student's $t$-test or Bayesian methods on a difference between two proportions (Walters, 1985).

RESULTS

Anti-fertility effect in BALB/c and F₁ mice

Table 1 shows that monoclonal progesterone antibody blocked pregnancy in BALB/c, but was ineffective in F₁ females. At autopsy on day 10 p.c., one of ten BALB/c treated females remained pregnant compared with nine of thirteen F₁ treated females. There was no significant difference between the two stocks in the number of corpora lutea, though the number in antibody-treated F₁ mice was greater than that in control F₁ mice at day 10 p.c. The numbers in antibody-treated and control BALB/c mice were similar at day 10 p.c.

Plasma progesterone

Plasma concentrations of progesterone in normal untreated mice (BALB/c and F₁) were 10–15 nmol/l at oestrus (Fig. 1). After fertilization the concentration in BALB/c females increased slowly to a peak of about 90 nmol/l at day 6 p.c., declined to a value of about 60 nmol/l at day 10 p.c., and then increased up to a value of about 190 nmol/l shortly before parturition. In F₁ females, the concentration increased more sharply after fertilization so that by days 3 and 4 p.c. it was significantly ($P < 0.001$) higher than that in BALB/c females at a corresponding time. No statistically significant difference was observed at any other stage of pregnancy. The mean concentration at the time of antibody injection was 19.3±4.0 nmol/l in BALB/c females and 28.1±6.2 nmol/l in F₁ females.

After antibody treatment plasma concentrations of progesterone at day 3 p.c. had increased to values at

![Plasma progesterone concentrations in normal untreated BALB/c (solid line) and F₁ hybrid (CBA male × BALB/c female) (broken line) mice. Oestrus was detected by the vaginal smear method and females were placed with males of the same genotype (plug = day 1 of pregnancy). Cardiac blood was collected under ether anaesthesia and the concentration of progesterone in the plasma was determined by radioimmunoassay as described in the text. Data are means ± s.e.m. of measurements from at least five females at each day of pregnancy. ***$P < 0.001$ comparison between groups (Student's $t$-test).](image_url)

**TABLE 1.** Effect of monoclonal progesterone antibody on pregnancy in BALB/c and F₁ mice. F₁ females from the mating CBA male × BALB/c female were crossed with F₁ males. Antibody was given as a single i.p. injection (5.7 nmol in 200 µl saline) 32 h after mating. Control females received an equivalent volume of ascites fluid from mouse myeloma McPc 1748 in saline

<table>
<thead>
<tr>
<th>Stock</th>
<th>Day of autopsy (p.c.)</th>
<th>No. of pregnant/ no. mated</th>
<th>No. of corpora lutea (mean ± S.E.M.)</th>
<th>Total no. of eggs or implantation (av. number/pregnant female)</th>
<th>Plasma progesterone (nmol/l)</th>
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</thead>
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<tr>
<td>Antibody-treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BALB/c</td>
<td>3</td>
<td>7/8</td>
<td>11.5±0.6</td>
<td>54(7.7)</td>
<td>533±55(7)</td>
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<td></td>
<td>5</td>
<td>2/12a</td>
<td>12.8±1.0</td>
<td>9(4.5)</td>
<td>321; 541(2)</td>
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<tr>
<td></td>
<td>10</td>
<td>1/10b,c</td>
<td>11.9±1.7</td>
<td>5(5-0)d</td>
<td>111(1)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>118±24(9)f</td>
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<tr>
<td></td>
<td>3</td>
<td>10/15</td>
<td>12.8±0.5</td>
<td>73(7-3)</td>
<td>588±77(10)</td>
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<tr>
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<td>8/10a</td>
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<tr>
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<td>12.2±0.8</td>
<td>112(12-4)</td>
<td>415±78(9)f</td>
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<td>Controls</td>
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<td></td>
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<tr>
<td>BALB/c</td>
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<td>9.3±0.9</td>
<td>79(9-9)d</td>
<td>99±7(5)</td>
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<tr>
<td></td>
<td>10</td>
<td>9/11</td>
<td>9.3±0.2</td>
<td>98(10-9)</td>
<td>106±18(5)f</td>
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</tbody>
</table>

* Embryos were flushed from the oviducts at day 3 post coitum (p.c.); dye sites were counted after injecting 1% (w/v) Pontamine blue in saline into the tail vein 15 min before autopsy at day 5 p.c.; and implantation sites were counted at autopsy at day 10 p.c. Values with same superscript are significantly different when tested by Bayesian methods on differences between two proportions, ($P < 0.01$), a–d, (Walters, 1985); or by Student's $t$-test ($P < 0.05$), e and f; ($P < 0.01$), g.
least eightfold higher than those in untreated females (Fig. 1). After day 3 p.c., the values in BALB/c females declined, whereas those in F₁ females were only slightly reduced by day 10 p.c.

Mitotic activity

The pattern of cell division in untreated females was similar in BALB/c and F₁ mice, but differed from previous findings in respect of the transition from epithelial to stromal mitosis which had started by day 3 p.c., at least 12 h earlier than reported by Finn & Martin (1967). At days 4 and 5 all animals in the control groups showed the expected high incidence of mitotic activity in stromal cells and the low incidence in epithelial cells (Fig. 2).

After antibody treatment of BALB/c females, mitotic activity at day 3 p.c. was significantly (P < 0.001) greater in luminal and glandular epithelia than that observed in control females. At day 4 p.c. the wave of activity was greatly reduced not only in epithelial cells, as in control females, but also in stromal cells, unlike control females. At day 5 p.c. a more complex pattern emerged with a modest increase in stromal activity and a shift back to mitotic divisions in the luminal epithelium, both events differing significantly from those observed in control females.

In antibody-treated F₁ females only the glandular epithelium remained significantly stimulated at day 3 p.c., and there was no increase in stromal mitoses compared with F₁ control females. At day 4 and 5 p.c. mitotic divisions in the stroma increased and those in the luminal and glandular epithelia decreased in a manner similar to the findings in control females.

**DISCUSSION**

This study shows that the endogenous progesterone concentrations in circulation of untreated mice were significantly greater at days 3 and 4 p.c. in F₁ compared with BALB/c females. Genetic differences in progesterone levels in pregnant mice have been reported previously by Barkley, Geschwind & Bradford (1979). Our findings suggest that the preimplantation secretory activity of the corpus luteum increases more rapidly in F₁ than in BALB/c mice. It is unlikely that the difference in plasma progesterone concentrations at days 3 and 4 p.c. is due to a change in steroid clearance rate or to the total weight of corpora lutea in the respective genotypes, since the difference is transient in nature, it is not sustained throughout the period of luteal function, and the average number of corpora lutea in the two stocks is similar. The hypothesis we propose, therefore, is that a rapidly rising rate of progesterone secretion and hence of unbound progesterone in circulation cannot be adequately neutralized by antibody given at 32 h p.c., and that this accounts for the different response observed in the two stocks.

The hypothesis is supported by examination of cellular responses in the endometrium. Epithelial mitotic divisions decline between days 1 and 3 after mating in normal untreated mice (Finn & Martin, 1967), but after antibody treatment at 32 h p.c. a very different pattern emerges in the two genotypes. In BALB/c treated mice many more mitoses were observed at day 3 in the luminal and glandular epithelia compared with control females; in F₁ mice mitotic divisions in the glandular epithelium were more numerous than in control females, but this was not so for the luminal epithelium. This indicates that the availability of progesterone in circulation to target cells is substantially reduced after antibody treatment of BALB/c mice, but less so in F₁ mice. A single injection of progesterone inhibits epithelial mitoses in oestrogen-primed ovariectomized mice (Martin, Das & Finn, 1973). Monoclonal progesterone antibody presumably has the opposite effect because it binds progesterone in circulation and allows a more prolonged action of oestrogens secreted at pro-oestrus.

**FIGURE 2.** Uterine cell division on the third, fourth and fifth days post coitum (p.c.) in BALB/c and F₁ hybrid (CBA male × BALB/c female) mice. Nulliparous females were injected 32 h p.c. with monoclonal progesterone antibody (treated groups) or ascites fluid from mouse myeloma McPc 1748 (control groups). Two hours before autopsy on days 3, 4 and 5 of pregnancy, colchicine was injected s.c. At autopsy the uterus was removed, fixed in Bouin's fluid, sectioned and the number of cells in mitosis in the luminal epithelium (L), glandular epithelium (G) and connective tissue stroma (S) were counted. Each bar represents the mean ± S.E.M. of data transformed to logarithms (to reduce heterogeneity of variance) of at least six animals. Significant differences (unpaired Student's t-test) are indicated by bars with same letter; a, c, d, f, h, P < 0.001; b, e, P < 0.02; g, P < 0.01.
leading to a sustained replenishment of oestrogen receptors in epithelial cells no longer exposed to the inhibitory action of progesterone (Hsueh, Peck & Clark, 1976). The finding that this effect is more pronounced in BALB/c than F₁ females seems to reflect a greater ability of antibody to neutralize the amount of progesterone in circulation at days 2, 3 and 4 p.c. in BALB/c than in F₁ mice. The lack of mitotic divisions in the stroma and the resumption of mitotic activity in the luminal epithelium in BALB/c mice at day 5 p.c. indicates that although circulating concentrations of progesterone are still high, insufficient hormone is available to target cell receptors in respect of the stimulation of stromal cell division. Alternatively, differences in progesterone receptor density of endometrial cells in various genotypes may result in greater uterine sensitivity to available steroid in circulation in F₁ mice after antibody treatment.

Further examination of the results highlights the relevance of regional mitotic divisions in the endometrium for successful implantation. Our findings show that implantation can occur normally even when the glandular epithelial mitotic activity at day 3 p.c. remains high after antibody treatment, as observed in BALB/c and F₁ mice. In contrast, inhibition of the onset of increased stromal mitotic divisions at day 4 p.c. by antibody is associated with a failure of implantation, as seen in BALB/c mice in the present study, and with a lack of endometrial sensitivity to a decidualizing stimulus at day 4, as observed by Rider et al. (1985).

In summary, the findings show that antibody administration at 32 h p.c. maintains a high total concentration of progesterone in circulation on account of its high affinity for the steroid, and that antibody binding diminishes steroid interaction with target cells as reflected by perturbation of the normal sequence of mitotic activity in epithelial cells and the inhibition of mitotic divisions in stromal cells. The inability of antibody to block pregnancy in F₁ mice is probably due to the steeper rise in the circulating concentration of progesterone compared with that in the inbred strain, so that the action of the hormone is more difficult to neutralize when antibody is injected at 32 h after mating.

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