Localization of type I interferon in murine trophoblast and decidua during decidual formation

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Summary. Mouse trophoblast and decidua were examined by means of immunohistochemistry to define the localization of type I interferon. The decidua were stained for type I interferon at the time of implantation. The strong reaction was first observed in the primary decidual zone on day 5 and subsequently in the secondary decidual zone on day 6. After day 10, the decidua basalis and decidua capsularis showed a strong reaction.

At the one-cell stage, embryos were weakly labelled, but a positive reaction was recognized in compacted morulae. Blastocysts on days 3 and 4 were positive in trophoblast and inner cell mass and a strong reaction was observed in the primitive endoderm on day 4. The visceral endoderm on day 5 and the trophoblast on day 6 were positive. After day 10, the trophoblast giant cells, labyrinth, visceral yolk sac and fetal blood cells gave a positive reaction.

This study is the first demonstration of type I interferon localization *in situ* in mouse trophoblast and decidua during decidual formation.

Keywords: type I interferon; implantation; decidualization; trophoblast; mouse

Introduction

Implantation and placentation are essential processes of reproduction in all mammals except the monotremes. These events have immunological implications, as mammalian embryos have different genotypes from their mothers. Some hypotheses have been proposed to explain why embryos are not rejected by their mothers: for example, embryos lack major histocompatibility complex (MHC) class I antigens at the time of implantation (Sellens, 1977; Desoye *et al.*, 1988); murine trophoblast resists lysis by allospecific, cytotoxic T lymphocytes (Zuckermann & Head, 1987), and natural killer cells that may injure murine embryos are inactivated by prostaglandin derived from murine decidua (Scodras *et al.*, 1990). Because these hypotheses are incomplete, the strategies by which embryos escape from maternal rejection have still to be defined.

Interferons are powerful, inducible, effector molecules with a wide spectrum of regulatory functions (Romeo *et al.*, 1989). The existence of type I interferon in human and murine placental tissues has suggested that it may play a role in regulating feto–maternal relationships (Fowler *et al.*, 1980; Bocci *et al.*, 1985; Duc-Goiron *et al.*, 1985; Chard *et al.*, 1986; Howatson *et al.*, 1988). Although the presence of type I interferon was reported in the syncytiotrophoblast and the maternal decidua of human placenta, its origin and function are unknown.

In this study, the localization of type I interferon in murine trophoblast and decidua during decidual formation was examined by immunohistochemistry. Its possible role in preventing maternal rejection of the embryo is discussed.

Materials and Methods

Animals

Virgin female mice, 8–12 weeks old, from the inbred strain C57BL/10ScSc (Japan SLC, Hamamatsu, Japan) were mated with males of a similar age of the inbred strain C57BL/10BRSSgSn (Institute of Medical Science, University of Tokyo, Japan). All animals were maintained on a standard programme of 14 h light:10 h dark. The day on which vaginal plugs were found was designated as day 0. Female mice at each stage were anaesthetized with pentobarbital and 0.9% NaCl solution was perfused through the heart followed by 50 ml of Carnoy's fixative at 4°C. Uteri were surgically excised from mice during the normal oestrous cycle and on days 0, 1, 2, 3, 3.75, 4, 5, 6, 8, 10 and 16, and oviducts were excised from mice on days 0, 1 and 2. The tissues were fixed in the same fixative for 1.5 h and dehydrated in absolute ethanol at 4°C. They were immersed in polyesterwax:ethanol, 1:1 (P:E = 1:1) at 37°C for 1.5 h and in P:E = 9:1 at 37°C for 1.5 h and finally embedded in P:E = 9:1. Sections were cut at 4 µm.

Immunohistochemical procedures

Immunohistochemistry was carried out at room temperature using rabbit antimouse α and β -interferon antiserum (Paesel, Frankfurt, Germany) as the first antibody. The activity of the antiserum is 1.0×10^4 NIH Neutralizing Reference Units ml⁻¹. The sections were immersed in absolute ethanol for 5 min to remove polyesterwax. They were then rehydrated through a series of ethanols, washed with distilled water and rinsed with 0.01 mol phosphate-buffered saline l⁻¹ (PBS, pH 7.2). The sections were incubated in the following reagents: 1% bovine serum albumin (BSA) in PBS for 15 min; rabbit antimouse α and β -interferon antiserum (1:500 dilution) for 2 h; PBS wash; 1% BSA in PBS for 15 min; biotinylated goat antirabbit immunoglobulin G (Organon Teknika NV-Cappel Products, USA) for 45 min; PBS wash; 1% BSA in PBS for 15 min; avidin–biotin complex (Vector Laboratories Inc., Burlingame, CA, USA) for 45 min; and PBS wash. Afterwards, they were stained with 0.5 mg diaminobenzidine ml⁻¹ in PBS containing 0.1% hydrogen peroxide and counterstained with haematoxylin. For negative controls, (i) normal rabbit serum and (ii) rabbit antimouse α and β -interferon antiserum that had been incubated with 1300-fold unit excess of mouse α -interferon (Lee Biomolecular Research Lab., Inc., San Diego, USA) for 45 min at 4°C were used instead of the antiserum.

Results

Little reaction for type I interferon was observed in the uterus during the normal oestrous cycle or on days 0, 1, 2 and 3, except for a weak reaction in the cell membrane and cytoplasm of luminal and glandular epithelia (Fig. 1a). However, in the uterus at the time of implantation on day 4, most of the decidualized cells showed diffuse labelling of type I interferon in the cytoplasm (Fig. 1b). There were also a few stromal cells, distant from the attached blastocyst, which were morphologically decidualized, but showed weak labelling. Endometrial stromal cells that had not been decidualized showed little reaction. The labelling in luminal and glandular epithelia became slightly stronger and these tissues continued to react for type I interferon during pregnancy. Six hours before the stage of day 4, a small proportion of uterine endometrial stromal cells was decidualized and type I interferon was detected in the cytoplasm of these cells. In uteri on day 5, the primary decidual zone (PDZ) and secondary decidual zone (SDZ) showed positive staining in the cytoplasm. The staining of the PDZ was more intense than that of the SDZ (Fig. 1c). In uteri on days 6 and 8, there was increased staining over a wide area of the SDZ. In uteri after day 10, the decidua basalis and decidua capsularis were intensely positive in the cytoplasm, and decidual tissue neighbouring trophoblast giant cells showed an especially strong reaction (Fig. 1e). The reaction of the decidua was stronger than that of the fetal trophoblast. No reaction was observed in controls using normal rabbit serum and rabbit antimouse α and β -interferon antiserum absorbed with mouse α -interferon (Fig. 1d).

One-cell embryos on day 0 showed little reaction for type I interferon (Fig. 2a), but 2-cell embryos on day 1 were weakly positive. The labelling of type I interferon gradually increased in the cytoplasm of compacted morulae (Fig. 2b). Blastocysts on day 3 were strongly positive in the cytoplasm of the trophoblast and inner cell mass (Fig. 2c). In hatched blastocysts on day 4, the trophoblast and inner cell mass was weakly labelled, and the primitive endoderm reacted

Fig. 1. Localization of type I interferon in mouse uteri during the normal oestrous cycle and pregnancy (bar = 100 µm): (a) weak labelling was observed only in luminal and glandular epithelia in a proestrous uterus; L: luminal epithelium; G: glandular epithelium; (b) positive staining was observed in most of the decidualized cells (large arrow) and blastocyst on day 4. There were also stromal cells that were morphologically decidualized, but showed little labelling (small arrow). In blastocyst, the reaction of primitive endoderm was evident; D: decidua; T: trophoblast; I: inner cell mass; PE: primitive endoderm; (c) positive reaction was observed in primary and secondary decidual zones and visceral endoderm of the embryo on day 5. The reaction of the primary decidual zone was strong (arrow); (d) negative control at the stage of day 5 using normal rabbit serum; P: primary decidual zone; S: secondary decidual zone; T: trophoblast; VE: visceral endoderm; Em: embryonic ectoderm; Ex: extra-embryonic ectoderm; (e) decidua basalis, trophoblast giant cells and labyrinth on day 10 were positive. Decidua basalis neighbouring trophoblast giant cells showed a strong reaction (arrow). Spongiotrophoblast was negative; DB: decidua basalis; TG: trophoblast giant cell; SP: spongiotrophoblast; LA: labyrinth.

prominently (Fig. 1b). In embryos on day 5, little labelling was observed in the trophoblast and embryonic and extra-embryonic ectoderm, while a clear reaction was detected in the cytoplasm and blastocoelic cell membrane of the visceral endoderm (Fig. 1c). In embryos on day 6, although the reaction in the cytoplasm of the trophoblast increased, the labelling in the cytoplasm of the visceral endoderm appeared to decrease. The ectoplacental cone and other embryonic tissues showed little labelling. On day 8, a strong reaction was exhibited not only in the cytoplasm of the trophoblast, but also in the cytoplasm and cell membrane of the visceral yolk sac. After day 10, there were reactions in the cytoplasm of the trophoblast giant cells and the cytoplasm and cell membrane of the labyrinth. The cytoplasm and cell membrane of the visceral yolk sac and the cytoplasm of fetal

Fig. 2. Reaction of type I interferon in mouse preimplantation embryos (bar = 100 μ m): (a) little reaction was observed in one-cell embryos on day 0; (b) positive reaction was observed in a morula on day 2; (c) strong reaction was observed in trophoblast and inner cell mass of an early blastocyst on day 3; (d) negative control at the stage of day 3 using normal rabbit serum; T: trophoblast; I: inner cell mass.

blood cells were also positive. The reaction in the labyrinth increased as the pregnancy proceeded. The spongiotrophoblast, which has been reported to react for paternal MHC class I antigens (Redline & Lu, 1989), showed little reaction (Fig. 1e). These results are summarized in Tables 1 and 2.

Table 1. Reaction of type I interferon during the preimplantation period in mice

Stage	Endometrial stroma	Embryo
Day 0	—	—
Day 1	—	+ —
Day 2	—	+
Day 3	—	+
Day 4	+	+

Discussion

In the present study, type I interferon appeared in murine decidua at the time of implantation and in murine trophoblast at the early blastocyst stage on day 3. Judging by the intensity of immuno-staining, the quantity of type I interferon seemed to be regulated during pregnancy. That is, the

Table 2. Decidual, embryonic or fetal tissue stained for type I interferon during decidual formation in mice

Stage	Decidual tissue	Embryonic or fetal tissue
Day 4	Decidua	Primitive endoderm
Day 5	PDZ* SDZ†	Visceral endoderm
Day 6	PDZ SDZ	Trophoblast
Day 8	SDZ	Trophoblast Visceral yolk sac
Day 10	Decidua basalis Decidua capsularis	Trophoblast giant cell Labyrinth Visceral yolk sac Fetal blood cells
Day 16	Decidua basalis Decidua capsularis	Trophoblast giant cell Labyrinth Visceral yolk sac Fetal blood cells

*PDZ, primary decidual zone.

†SDZ, secondary decidual zone.

reaction for type I interferon in the primary decidual zone on day 5 was stronger than that in the early decidua on day 4, and the reaction in the secondary decidual zone increased as the pregnancy proceeded. The labelling in the blastocyst decreased at the time of implantation. The labelling in the trophoblast increased again after day 6 and the reaction in the labyrinth increased after day 10. However, it appeared that type I interferon was uniformly present at the embryo–maternal and feto–maternal interfaces throughout pregnancy.

It has been suggested that type I interferon may play an important role in regulating feto–maternal relationships (Chard *et al.*, 1986). Further, it is well known that interferons prolong the survival of allografts (Mobraaten *et al.*, 1973; Hirsch *et al.*, 1974). These results suggest that type I interferon in murine decidua and trophoblast may be essential for maternal acceptance of embryos and fetuses.

Type I interferon in the decidua appeared at the time of implantation. This interferon does not seem to be a trigger in the decidualization of stromal cells, since the molecule became detectable after day 4, when these cells had already become decidualized in their morphology. It has been reported that α -interferon does not induce decidualization in suitably sensitized uteri in mice (Baker & Nieder, 1990).

Luminal and glandular epithelia reacted for type I interferon during the normal oestrous cycle and pregnancy. Type I interferon in these tissues may be involved in the normal physiological status of uteri.

Type I interferon was also detected in murine preimplantation embryos. The strongest reaction was observed at the early blastocyst stage. The trophoblast of ungulate blastocysts has been reported to synthesize and release α -interferon during the peri-implantation period, and the molecule has been shown to have an antiluteolytic function (Imakawa *et al.*, 1987; Cross & Roberts, 1989; Gnatek, 1989; Lifsey *et al.*, 1989; Roberts, 1989; Farin *et al.*, 1990). In mouse embryos, however, it is unclear whether the type I interferon has antiluteolytic activity or not, since luteolysis occurs in different ways in mice and ungulates.

Although the function of type I interferon in murine preimplantation embryos is unknown, interferons are reported to affect cell proliferation and differentiation (Romeo *et al.*, 1989).

As embryos undergo cell proliferation and differentiation at the preimplantation stage, type I interferon may exert an important controlling influence on cell proliferation and differentiation in embryos. Baker & Nieder (1990) did not detect interferon activity in the conditioned-media-containing proteins secreted from murine peri-implantation blastocysts and in the lysed blastocysts, but some proteins with molecular masses in the range of murine α -interferon were demonstrated in the conditioned media. The quantity of type I interferon may have been too small to be detected in their bioassay system, despite its presence in murine embryos as demonstrated by the present study.

Nonpolymorphic MHC class I antigens have been detected in human cytotrophoblast and rat basal trophoblast (Ellis, 1990; Ellis *et al.*, 1990; Kanbour-Shakir, 1990). These antigens are thought to have important roles at the feto-maternal interface. In mice, however, polymorphic classical MHC class I antigens are reported to be detected on the cell surface of spongiotrophoblast (Redline & Lu, 1989). Zuckermann & Head (1986) found that MHC class I antigens on the cell surface of murine trophoblast were induced by α/β -interferon (type I interferon) or γ -interferon (type II interferon) *in vitro*. There is a strong possibility that these MHC antigens may be induced *in vivo* by type I interferon produced by the trophoblast giant cells, labyrinth and decidua. More detailed research is necessary to ascertain the significance of type I interferon at the embryo-maternal and feto-maternal interfaces.

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