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An Autoradiographic Study of the Implantation of Transferred Mouse Blastocysts

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Abstract

Mouse blastocysts collected on day 4 were cultured in [^{3}H]thymidine (0·01 μCi/ml) for 24 h and transferred to the uteri of pseudopregnant recipients. Autoradiography revealed that when such blastocysts were allowed to develop for 48 h *in utero*, label was apparent in the nuclei of decidual cells. The experimental conditions were physiological since blastocysts developed into normal offspring when gestation was allowed to proceed in pseudopregnant recipient animals. The transfer of foetal DNA into maternal decidual cells may be of important immunological significance.

Introduction

The success of the foetal allograft remains an immunological paradox (Beer and Billingham 1971; Edwards *et al.* 1975). Placentation has appeared many times during the evolution of the animal kingdom, and its presence in invertebrates exhibiting cellular immunity but no humoral immune responses supposes that the mechanisms involved in the avoidance of maternal immune rejection are cellular (Kaye *et al.* 1972).

It appears from various lines of evidence that the developing mouse blastocyst expresses paternal antigens prior to implantation. Wudl and Chapman (1976), using an enzyme marker assay, demonstrated the expression of the paternal genome in the blastocyst, and Carter (1976), using a mixed immune haemadsorption technique, showed the presence of maternal and paternal antigens on mouse blastocyst trophoblast.

The H-Y (male) antigen has been detected on pre-implantation mouse blastocysts by cell lysis in the presence of H-Y antibody and complement (Krco and Goldberg 1976). The presence of the major histocompatibility antigens (H-2) on experimentally delayed blastocysts and their disappearance when implantation was eventually induced has been demonstrated using immuno-peroxidase labelling (Searle *et al.* 1976). Similar results were obtained for the paternal H-2 antigens by Hakanssen *et al.* (1975) using an isotope antiglobulin technique. Antigenicity of the human blastocyst lacks direct proof but may be inferred from the aggregation of maternal lymphocytes at the site of implantation on day 12 of development (Hamilton 1970).

The means by which the developing blastocyst avoids the maternal response remains uncertain. However, previous workers have noted the uptake of labelled maternal nuclear material by the developing trophoblast which might then alter the antigenic nature of the trophoblast and thus separate the maternal and foetal tissues immunologically (Galassi 1967; Avery and Hunt 1969).

The present study aimed to extend these observations by labelling the DNA of mouse blastocysts with [^{3}H]thymidine *in vitro* and to ascertain the fate of labelled foetal DNA following implantation.

Materials and Methods

Animals

Randomly bred Swiss albino mice of the Quackenbush special (QS) strain were used. They were housed at 21°C and received artificial fluorescent lighting between 0600 and 1800 h. The animals were divided into two experimental groups.

Group I: 30 females were administered 5 i.u. pregnant mare's serum gonadotrophin (PMSG, Folligon, Organon) by intraperitoneal injection followed 48 h later by 5 i.u. human chorionic gonadotrophin (HCG, Pregnyl, Organon). They were then mated (two females per male) overnight and on the following morning were examined for the presence of a vaginal plug indicating day 1 of gestation.

Group II: 30 females were mated with vasectomized males (two per male) and examined daily for the presence of a vaginal plug. Animals which showed plugs on the same day as pregnant animals were used as the pseudopregnant recipients.

Embryo Culture

On day 4, pregnant females of group I were killed by cervical dislocation, and the blastocysts flushed from the excised uterine horns. The blastocysts were collected under a dissecting microscope, washed twice in culture medium (Brinster 1965), then cultured in 20-μl droplets in plastic tissue culture dishes (Falcon Plastics USA, 60 by 15 mm). Half of the blastocysts were cultured in [^{3}H]thymidine at a concentration of 0·01 μCi/ml of culture medium (Radiochemical Centre Amersham, specific activity 2 Ci/mmol) and the remainder were cultured in non-radioactive medium. The blastocysts were cultured for 24 h at 37°C in humidified 5% CO_2 in air under light-weight paraffin oil.

Embryo Transfer

The blastocysts were washed twice in culture medium and healthy embryos transferred to the pseudopregnant recipients on day 5. The recipients were anaesthetized with Avertin and each uterine horn exteriorized through a dorsal incision; five blastocysts were introduced into the uterine horn at the utero–tubal junction with a Pasteur pipette. [^{3}H]Thymidine-cultured blastocysts were placed in the left uterine horn and control blastocysts in the right horn. Recipient females were killed on days 7 and 8 by cervical dislocation 15 min after injection of 0·2 ml of 0·5% Pontamine sky blue 6BX in saline into the tail vein to facilitate identification of the implanting blastocysts (Orsini and McLaren 1967).

Fixation and Autoradiography of Embryos

Following cervical dislocation the whole animal was infused via the left ventricle with saline followed by 2·5% glutaraldehyde in phosphate buffer (pH 7·4). The dissected uterus with the embryos *in situ* was transferred to 2·5% glutaraldehyde in phosphate buffer at pH 7·4 overnight, post-fixed in 2% osmium tetroxide, dehydrated, and embedded in Araldite. Transverse sections 0·5 μm thick were cut on a Sorvall JB4 microtome, heat fixed on acid-cleaned slides, and dipped in Ilford K5 nuclear research emulsion (Ilford, Essex). The slides were exposed for 35 days and then processed through Kodak D 19 developer at 20°C for 4 min and stained with 2% toluidine blue. Stained sections were examined and photographed using a Zeiss photomicroscope.

Results

Blastocyst Culture and Transfer

Group I

Approximately one-half of the PMS/HCG-treated females had a vaginal plug on day 1 and some 100 blastocysts were collected on day 4. Following 24-h culture

approximately 40 well-developed blastocysts were harvested from each of the [^{3}H]thymidine and control cultures.

Group II

Approximately one-third of the females had a vaginal plug on day 1 and pseudopregnancy was confirmed at operation on day 5 by the presence of corpora lutea.

Of the five blastocysts transferred on day 5 to the right and left uterine horns one or two normal embryos were found in each of the six recipients at Caesarean section on day 18. A similar number of developing embryos was found in each of the six test animals killed on days 7 and 8.

Autoradiography

Day-7 embryos derived from [^{3}H]thymidine-cultured blastocysts exhibited nuclear labelling of both embryonic and trophoblast cells. Nuclear grain counts of adjacent decidual cells were 50% of those of the embryo. Nuclear grains, still above background levels, were seen in the intermediate zone decidual cells and peripheral uterine cells. Day-7 embryos from control cultures exhibited uniformly low grain counts throughout (Table 1).

Table 1. Mean grain counts per nucleus of labelled cells on day 7 in the uterus of pseudopregnant recipients following the implantation of blastocysts

Blastocysts were previously cultured for 24 h in control medium and in [^{3}H]thymidine. Each of the embryos was selected from separate recipients

Embryo	Inner cell mass	Trophoblast	Adjacent decidual cells	Int. zone decidual cells	Peripheral uterine cells	Uterine muscle cells
			(*a*) Blastocysts from control medium			
1	0·8	0·9	1·6	1·4	0·6	0·5
2	0·4	1·0	1·5	1·5	0·7	0·5
3	0·9	1·1	1·9	1·8	1·0	0·7
Mean±s.e.	0·7±0·2	1·0±0·1	1·7±0·1	1·6±0·1	0·8±0·1	0·6±0·1
			(*b*) Blastocysts from [^{3}H]thymidine medium			
1	9·4	9·0	4·3	3·3	3·4	0·3
2	12·0	12·0	6·0	4·0	3·0	0·3
3	8·6	8·2	4·2	3·2	2·8	0·4
Mean±s.e.	10·0±1·0	9·7±1·2	4·8±0·6	3·5±0·3	3·1±0·2	0·3±0·0
P^A	<0·001	<0·01	<0·01	<0·01	<0·001	<0·05

[A] Derived from Student's *t*-test.

The labelling of day-8 embryos was not significantly above background due to the increased cell numbers of the developing embryo and the low levels of [^{3}H]thymidine in the culture medium.

Discussion

Developing mouse blastocysts are very radiosensitive; levels of [^{3}H]thymidine (specific activity 17·4 Ci/mmol) greater than 0·01 μCi/ml for 60–70 h cause significant cell damage especially in the inner cell mass (Snow 1973). Horner

and McLaren (1974) found that blastocysts cultured in [^{3}H]thymidine (specific activity 17·4 Ci/mmol) concentrations of 0·005 μCi/ml for 72 h failed to undergo normal implantation and development. This failure may possibly be ascribed to the demise of the inner cell mass, on whose integrity the trophoblast depends for subsequent development (Gardner 1975).

Studies on implantation require normal blastocysts. Previous investigations of the immunobiology of trophoblast at implantation using transferred blastocysts are difficult to interpret since damage to the inner cell may contribute to the failure of the trophoblast to divide which may not therefore be an immunological event (Johnson 1975).

The integrity of transferred blastocysts (both treated and untreated) can be assessed by their subsequent normal development to term in recipient animals. In the present study the levels of [^{3}H]thymidine present in the culture medium allowed the blastocysts to implant and develop normally. Labelling was sufficient, however, to allow the demonstration of transfer of DNA from the trophoblast to the surrounding decidual cells within 48 h of implantation in animals killed at that time.

Previous autoradiographic studies of developing embryos have involved the systemic injection of the radio label into pregnant animals. When pregnant rats were injected on day 9 with [^{3}H]thymidine intravenously or intraperitoneally the label was observed in the uterus, embryo and trophoblast but not in the decidual cells surrounding the implant (Bulmer and Peel 1974). The possible transfer of maternal nuclear material to the developing rat trophoblast on day 9 was examined by Galassi (1967) who found that when blastocysts were transferred to the uteri of pseudopregnant rats previously injected systemically with [^{3}H]thymidine, the label was found in the deciduum, embryo and trophoblast due to reutilization of [^{3}H]thymidine from the maternal circulation. However, when blastocysts were transferred to pseudopregnant uteri previously injected 'locally' with [^{3}H]thymidine, only the deciduum and trophoblast were labelled. The conclusion was that the embryo did not utilize nucleosides or nucleotides from the uterine deciduum for its DNA synthesis but that the trophoblast incorporated label in the form of macromolecular DNA. Ledoux and Charles (1967) also demonstrated the uptake by trophoblast of exogenous DNA injected into pregnant mice.

The experiments reported in this paper demonstrate that the label transferred to the uterine deciduum from blastocysts cultured in [^{3}H]thymidine prior to transfer may be DNA and not [^{3}H]thymidine. Recent studies have confirmed conclusively that cells cultured in [^{3}H]thymidine incorporate the label specifically into their newly synthesized DNA (Grieder *et al.* 1970; Eriksen and Eriksen 1972; Fakan and Hancock 1974; Shackney and Ford 1974). The present findings also draw support from the studies of Bulmer and Peel (1974) which showed that the adjacent deciduum selectively failed to incorporate systemically injected [^{3}H]thymidine.

When the cells of different species hybridize and nuclear mixing occurs, the resultant hybrid cell undergoes a marked alteration in its cell-surface antigens (Harris *et al.* 1969). Although the possibility of DNA degradation and reutilization cannot be fully excluded in these experiments, the transfer of foetal DNA to maternal cells may similarly induce an alteration in the cell-surface antigens of the decidual cells surrounding the developing embryo and thereby prevent mutual immune recognition by foetal and maternal lymphocytes. These findings may have important implications in foetal–maternal immunobiology.

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