# Disappearance of paternal histocompatibility antigens from hybrid mouse blastocyst at the time of implantation

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Products of the major histocompatibility complex (H-2) are important in allograph rejection. In view of the close relationship between mother and foetus, we can consider the latter as an allograph which is however not rejected by an immunological reaction. We studied the presence of H-2 antigens on embryo membranes at the time of implantation, by immunochemical labeling using gold particles coupled with protein A. Results showed that the expression of H-2 antigens is different before and after implantation. It seems that after implantation, H-2 antigens disappear from trophoblastic membranes. This could explain the absence of immunological reaction of the mother against the foetus.

*Blastocyst outgrowth* *H-2 antigen* *Trophoblastic cell* *Protein A–gold*

## 1. INTRODUCTION

Products of the major histocompatibility complex (H-2) are part of the cell surface structures which have a number of biological functions like allograph rejection. Identification of these cell-surface products on the embryo, especially during implantation, is important in order to explain the maintenance of the close relationship existing between the mother and the foetus, since the latter can be considered as an allograph which is not submitted to immunological rejection by its host [1].

One explanation of this observation could be that the embryo tissue, the trophoblast, which is in contact with the mother's endometrium lacks histocompatibility antigens which prevent the embryo from being destroyed by the mother's immunological system [2].

Expression of major histocompatibility antigens on mouse embryos has been investigated by several approaches. It is now definite that H-2 antigens are present on mid-term and late embryos [3]. Nevertheless, it appears that on 7–9 days embryos, only the ICM cells (inner cell mass) and derivatives possess these antigens [4]. Other reports failed to show the presence of H-2 antigens on preimplantation embryos [5,6]. In 1981 however, using a sensitive cytotoxicity technique, Cozad and Warner [7] were able to detect H-2 antigens on mouse blastocyst embryos.

The purpose of the present investigation was to follow the evolution of H-2 antigens during the blastocyst stage in order to detect any change in distribution and in expression of the H-2 antigens on the trophoblastic cells before and during implantation. For the detection of these antigens, we used the very sensitive immunogoldstaining method [8].

## 2. MATERIALS AND METHODS

Two congenic mouse lines, A.AL (H-2K$^k$) and A.TL (H-2K$^s$) (Centre d'Immunopathologie INSERM, Hôpital St-Antoine, Paris) and a third inbred one, Balb/c (H-2$^d$) were used. Balb/c females strain mice were mated with A.AL and A.TL males. F1 offsprings, A.AL × Balb/c, were chosen for test experiments and A.TL × Balb/c as controls.

Blastocysts were collected at 4.5 days

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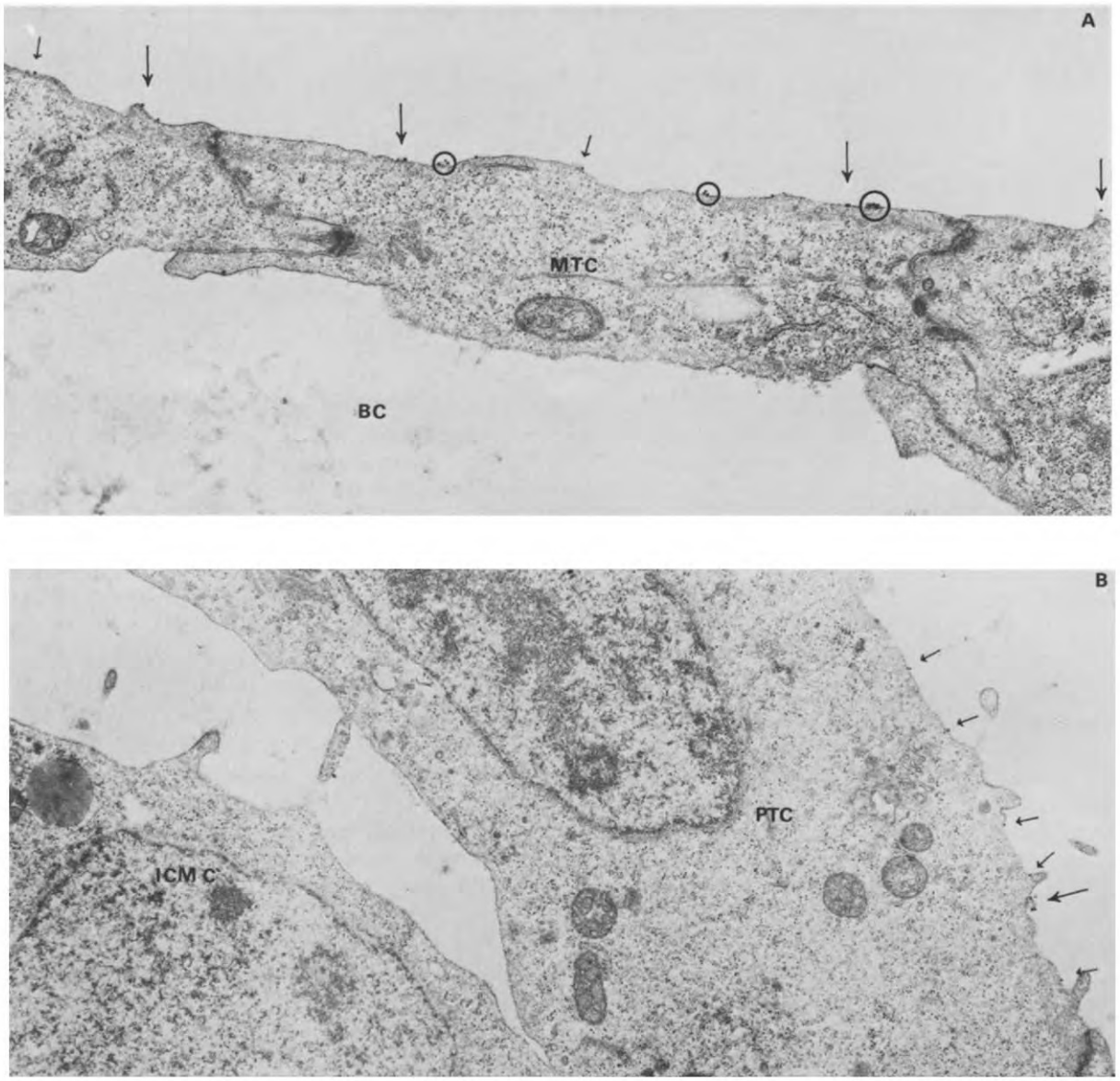


Fig.1. Electron micrographs of trophoblastic cells from a zona-free A.AL blastocyst after immunogoldstaining of the H-2K$^k$ antigens: (A) mural trophoblastic cell; (B) polar trophoblastic cell; (○) aggregates of particles; (⟶) gold particles; (MTC) mural trophoblastic cell; (PTC) polar trophoblastic cell; (BC) blastocoelic cavity; (ICMC) 'inner cell mass' cell; × 17 750.

postcoitum in Brinster medium (BMOC-3). The culture medium used was BMOC-3 supplemented with 10% foetal calf serum (BMOC-3 + 10% FCS). Blastocysts were allowed to attach and grow out in microTest II™ tissue culture plates (Falcon 3040, Becton Dickinson Labware, Oxnard, USA) for 3 days in 5% $CO_2$, at 37°C.

Monospecific antiH-2 serum was prepared by injecting A.TL mice 10 times with $5.5 \times 10^6$ spleen cells collected from A.AL mice.

The antisera were assayed on spleen cells by immunolabeling with protein A coupled with gold particles (16 nm), using the method described by Horisberger [8]. We are indebted to Dr Ph. Coppe

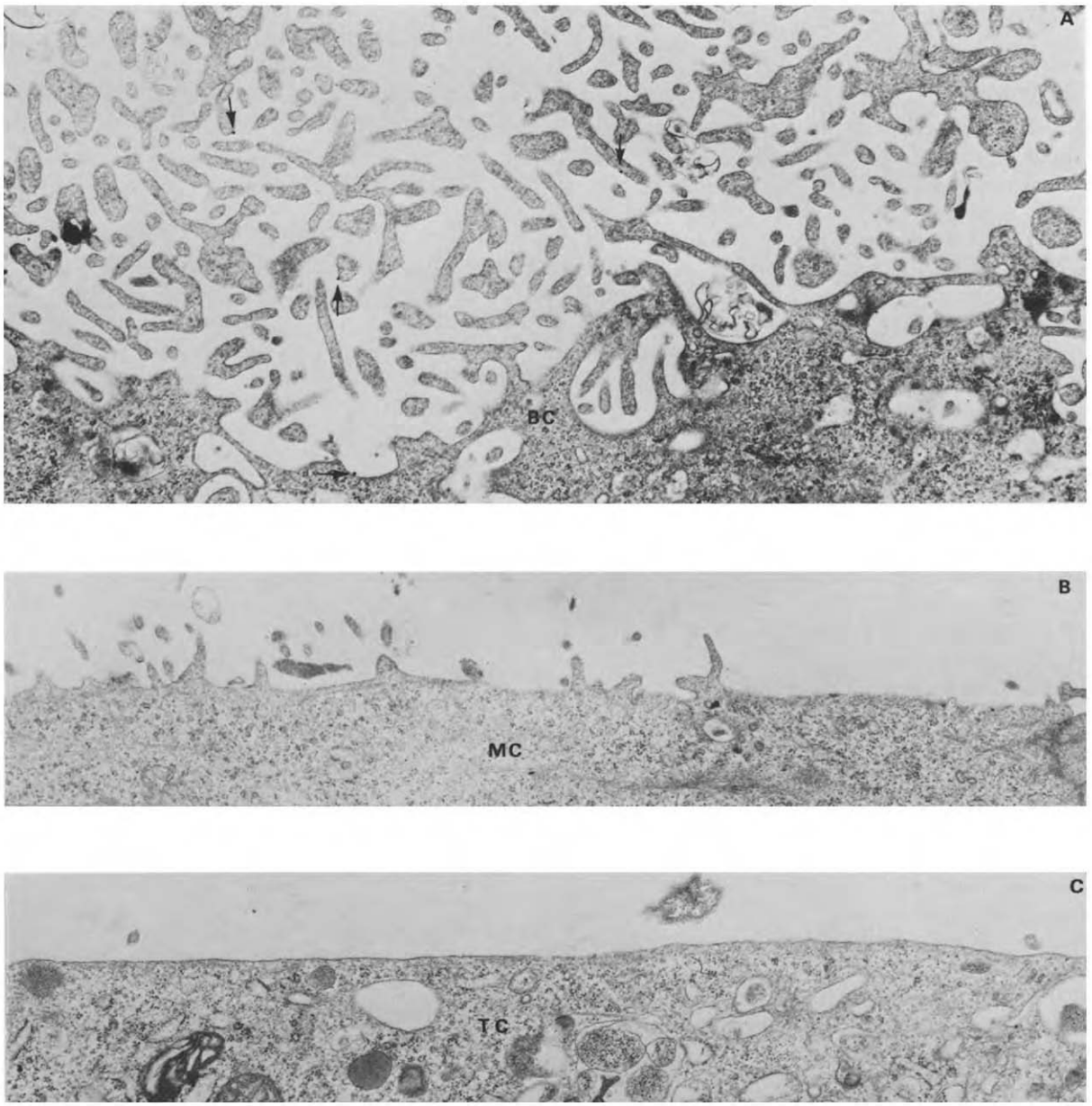


Fig.2. Electron micrographs of trophoblastic cells from an in vitro outgrowth A.AL blastocyst after immunogoldstaining of the H-2K$^k$ antigens. Cells from the lower (A), middle (B) and top (C) of the attached embryo: (⟶) single gold particles; (BC) basal cell; (MC) middle cell; (TC) top cell; × 17 750.

(Laboratoire d'Histologie) for a gift of protein A–gold complex.

For the staining experiments, embryos were incubated at 4°C with congenic antiserum diluted in cold BMOC-3 for 90 min, they were intensively washed 4 times in BMOC-3 and incubated for 60 min with rabbit anti-mouse serum diluted in BMOC-3, after intensive washing, they were incubated with protein A–gold complex for 60 min and washed until the red coloration disappeared, and then embedded in Epon.

## 3. RESULTS

### 3.1. *Zona-free blastocysts*

Visualization of surface H-2 antigens by protein A–gold complex was positive both on the mural and polar trophoblastic cells (fig.1). The pattern of labeling on the blastocyst cell surface varied from a weak staining to a discontinuous but distinct labeling on certain blastomeres. The presence of aggregates of particles (fig.1A) on the cell surface explains the variability in the labeling. However, we conclude that blastocysts after hatching express H-2 antigens on their trophoblastic membranes even if the level was low.

### 3.2. *Blastocyst outgrowths*

Blastocysts cultured for 3 days showed an outgrowth of trophoblastic cells derived from the trophoblast. In our experiments, sections were first made of cells which were attached on the plastic dish, which showed many microvilli (fig.2A). Others, going to the top of the embryo, showed cells becoming gradually smooth (fig.2B,C). None of these cells were really labeled. Very few particles could be observed on the basal cells (fig.2A, ⟶), but this very low labeling density was also present on the control with A.TL strain (not shown). On the other cells there was no labeling at all.

## 4. DISCUSSION

The presence of H-2 antigens on embryonic tissues during various stages of development has been intensively studied using a variety of methods. Nevertheless, very sensitive methods are necessary to detect these antigens on preimplantation embryos, since they are present at very low levels. Here, we chose the indirect immunolabeling method using protein A–gold particles.

Working with blastocysts which had lost their zona pellucida in utero, we first confirmed that embryos at the blastocyst stage express major paternal histocompatibility antigens from the K region [7,9,10]. However, we showed that these antigens completely disappear after in vitro implantation. This observation has to be associated with the in vivo results of Searle [4]. It appears that during the time between hatching and implantation, the expression of H-2 antigens on the trophoblast cell membrane is inhibited. The mechanism by which these antigens disappear remains unknown. However, our results suggest that their expression is intrinsically controlled and does not necessarily depend upon a maternal influence like uteroglobin [11] or hormonal factors [9]. Indeed at the time of implantation, the trophoblastic cells undergo modifications including polyploidy [12] and changes in membranes ultrastructure [13].

In view of the lack of histocompatibility antigens on the postimplantation trophoblastic cells, as compared with those of the zona-free blastocyst, it would seem that, at the time of implantation, H-2 antigenic expression is restricted. This conclusion is important with respect to the survival of the embryo when considered as an allograph. By not expressing these antigens, it escapes immunological recognition as foreign tissue and continues its development.

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