LOCALIZATION OF H-2 ANTIGENS ON MOUSE TROPHOBLAST CELLS*

By SASWATI CHATTERJEE-HASROUNI AND PEEYUSH K. LALA

From the Department of Anatomy, McGill University, Montreal, PQ, Canada H3A 2B2

The survival and growth of the fetoplacental units resulting from allogeneic matings, and thus potentially alien to the mother, constitute an example of natural allografts defying all the known laws of tissue transplantation. The immunobiology of fetomaternal relationship has been the focal point of extensive investigation over a long time, but the precise mechanisms that allow allogeneic pregnancies to proceed to term have still remained unclear.

The various hypotheses that have been forwarded to explain this phenomenon include the following (a) the uterus is an immunologically privileged site (1), (b) there is a general depression of the maternal immune functions possibly resulting from pregnancy-associated humoral factors and hormones (2–5), (c) a neutral barrier existing at the fetomaternal tissue interface prevents immune interactions between the maternal and fetally-derived cells (6–8), (d) histocompatibility antigens are absent from the surface of placental trophoblast cells that remain in direct contact with maternal blood (9–11), (e) there is a blocking of the effector arm of the maternal immune response by noncytotoxic antibodies or antigen-antibody complexes (12, 13).

The immunologically privileged site hypothesis is untenable because the uterus shows normal immunoreactivity against artificially-introduced allogeneic cells (14). Similarly, the occurrence of a neutral barrier owing to a sialomucin-rich substance called fibrinoid is not universal. It has been shown to be absent from certain species (15). Furthermore, despite some general depression of maternal immune responses during pregnancy, maternal lymphocytes have been shown to be fully immunocompetent in vitro (16, 17).

The question of the presence or absence of histocompatibility antigens on trophoblast-cell surface still remains a highly controversial issue, as indicated by apparently conflicting data from different laboratories (10, 11, 18–23). Variation in technical sensitivity, as well as a lack of precise identification of the antigen-bearing placental cells in question may account for some of the discrepancies. The present study was therefore undertaken to evaluate the existence of H-2 antigens of both the paternal and maternal haplotypes on the surface of mouse trophoblast cells at different stages of pregnancy. High levels of sensitivity, specificity, and resolution of antigen detection at the cellular level were the prime criteria in the experimental designs. Trophoblast cells were identified in sections and then reidentified in smears of single-cell suspensions to be utilized for this purpose. H-2 antigens were examined with the aid of a

sensitive radioimmunolabeling method employing monospecific antisera, followed by quantitative radioautography.

Results revealed that significant levels of H-2 antigens of both parental haplotypes are present on the surface of trophoblast cells and the antigen density increases rapidly near term.

Materials and Methods

Mice. Allogeneic pregnancies were set up by mating young adult CBA/J females with C57BL/6J males (The Jackson Laboratory, Bar Harbor, Maine). The day of appearance of a vaginal plug was taken as day 0 of pregnancy.

Preparation of Single-Cell Suspensions

PLACENTA. Placentae from four to five mice were removed, rinsed, and minced into small pieces (≈ 1 mm^3) with iris scissors. They were incubated in 80 ml of 0.3% collagenase (Sigma Chemical Co., St. Louis, Mo.) made up in Ca^{++}, Mg^{++}-free phosphate-buffered saline (PBS, pH 7.4)[1] containing 0.02% disodium ethylene diamine tetracetate for 30 min at room temperature, at the end of which the mixture was passed through a stainless steel screen (80 mesh/sq. inch) and centrifuged at 400 g for 7 min. The pellet was resuspended in Hanks[1] balanced salt solution (HBSS, Grand Island Biological Co., Grand Island, N. Y.) and washed. Erythrocytes were lysed by treatment with a buffered NH$_4$Cl (0.168 M) solution and clumps were removed by layering the cell suspension on fetal calf serum (FCS), as reported from this laboratory (24). Cells were then resuspended in minimal essential medium (MEM, Grand Island Biological Co.) containing 10% FCS (10% MEM-FCS).

THYMUS. Thymuses were removed, rinsed, and minced in ice-cold 10% MEM-FCS. The cell suspension was subjected to a clump removal procedure, resuspended in 10% MEM-FCS, and adjusted to a concentration of 20 $\times$ 10^6 cells/ml.

SERA. Monospecific anti-H-2 sera produced at The Jackson Laboratory under a contract to the National Institutes of Health were obtained through the courtesy of Dr. J. G. Ray of the National Institutes of Allergy and Infectious Diseases, Bethesda, Md. Details pertaining to the production and cytotoxicity of the sera are presented in Table I. Normal mouse sera (NMS) were obtained by bleeding C57BL/6J mice (at least 12 wk old). Goat-anti-mouse IgG (GAMG) was an IgG-rich fraction (Meloy Laboratories, Inc., Springfield, Va.). This antibody appears to have some significant anti-light chain activity. All sera were heat-inactivated at 56°C for 30 min before storage at −20°C.

RADIOIODINATIONS. Radioiodination of GAMG was carried out by a modification of the chloramine T oxidation method of Greenwood et al. (25), as reported by us earlier (24).

DETECTION OF SURFACE H-2 ANTIGENS. An effective sandwich-labeling technique was designed in this laboratory (P. K. Lala and K. S. Rahil unpublished observations) to detect and quantitate H-2 antigens on the cell surface. This involved an exposure of cells to anti-H-2 sera, followed by ^{125}I-labeled GAMG. But such a protocol was applicable only to cells (eg. thymocytes) that did not bear any native Ig molecules on their surface. Because trophoblast cells were found to show substantial direct labeling with ^{125}I-GAMG, a three-step sandwich-labeling technique was devised, including an initial masking step.

Placental cells in suspension were adjusted to a concentration of 20 $\times$ 10^6 cells per ml in 10% MEM-FCS. An 0.1-ml vol of this suspension was incubated with 0.1 ml unlabeled goat anti-mouse IgG (2.6 mg/ml) for 30 min at 4°C. This step was designed to mask any native IgG molecules bound to the cell surface and thus prevent their direct labeling with radioiodinated anti-IgG subsequently. Preliminary experiments showed that this concentration of anti-IgG provided effective masking. The cells were then washed twice through discontinuous FCS gradients (50% MEM-FCS, 75% MEM-FCS, and 100% FCS). Next, the cells were incubated

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[1] *Abbreviations used in this paper:* FCS, fetal calf serum; GAMG, goat-anti-mouse IgG; HBSS, Hanks' balanced salt solution; MEM, minimal essential medium; NMS, normal mouse serum; PAS, periodic acid Schiff; PBS, phosphate-buffered saline.

TABLE I

Details of Monospecific H-2-Antisera Used

Antiserum		Strains used for raising antisera		Cytotoxicity (50%) titre
Code No.	Haplotype specificity	Donor strain	Recipient strain	
D-23b	Anti-H-2K^k	B10.A	(B10.D2 × SJL)	700
D-5b AF	Anti-H-2D^k	C3H-H-2°	(C3H.JK × HTG)	4,000
D-2	Anti-H-2D^b	B10	(B10.A(5R) × LP.R111)	200

with various anti-H-2 sera (1/40 final dilution) for 30 min at 4°C. Controls were always included in which cells were incubated with NMS rather than anti-H-2 serum. After two further washes through FCS gradients, the cells were incubated with radioiodinated GAMG at a final concentration of 10 μg/ml. The choice of concentrations of anti-H-2 sera and radiolabeled GAMG was based on pilot studies, providing optimal specific labeling for thymocytes with countable number of silver grains. After two further washes through FCS gradients cells were resuspended in 10% MEM-FCS and spun down in small pellets. They were suspended in a minute volume of FCS and smeared on slides subbed in gelatin-chrome alum. The slides were fixed in methanol for 40 min before they were processed for radioautography (26).

ABSORPTION. Anti-H-2 sera (at a dilution of 1/10) were absorbed once with H-2-matched thymocytes at a concentration of 300 × 10^6 cells/ml for 30 min at room temperature, followed by another 30-min incubation at 4°C. Absorbed antisera were then used in the three-step sandwich-labeling technique as described before to evaluate the extent of removal of specific anti-H-2 activity.

Identification of Cells in Sections and Smears.

PLACENTA. (a) Sections. The histological appearance of mouse placentae of various gestational ages taken from CBA females mated with C57BL/6 males was examined in conventional sections of paraffin-embedded material (5 μm thick, stained with periodic acid Schiff (PAS)), as well as in semithin sections of Epon-embedded materials (0.5 μm thick, stained with toluidine blue).

(b) Smears. Smears of single cell suspensions from placenta were stained with Giemsa as well as PAS-hematoxylin. Trophoblast cells were distinguished from decidual cells by PAS staining as well as a correlation of morphological characteristics observed in semithin sections, as described in Results. Criteria established on these grounds were utilized to identify trophoblast cells in radioautographs. The area of each trophoblast cell in smears was measured by using an eyepiece area grid in which each square measured 8 μm × 8 μm.

THYMUS. Small lymphocytes were identified by their morphology. They were small cells (8 μm or less in diameter) with round, dark nuclei, some of which were slightly indented, and a very thin rim of cytoplasm.

Evaluation of Radioautographs

The background number of silver grains was estimated according to the method of Lala and Patt (27). Areas comparable to the size of cells being counted were scored in cell-free regions of the slide. 100 such areas were counted and the background count for the each experiment was determined on the basis of a grain count distribution over such areas. Based on these findings, the threshold for positive labeling was set at 11 grains per unit area (64 μm^2) of trophoblast cells and 6 grains for a small lymphocyte from the thymus. 100 consecutive trophoblast cells in debris-free areas of each sample were scored for plotting silver grain count distributions. In the case of thymus, 500 small lymphocytes were scored.

Results

Anatomy of the Fetomaternal Junction. The typical constituent cell types of the mouse placenta as observed in 0.5-μm thick sections are indicated in Fig. 1. Within a
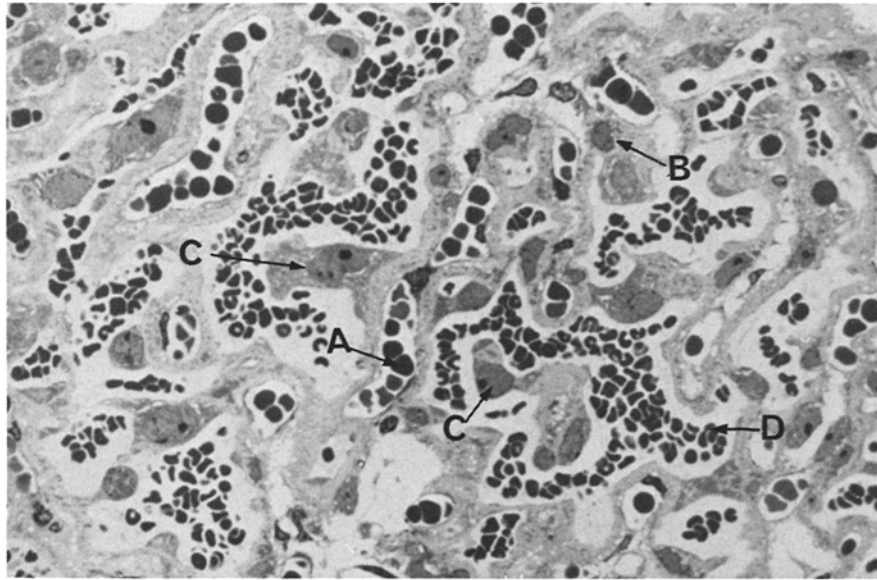
Fig. 1. 0.5 μm thick section of the mouse placenta, showing A—erythrocytes within a fetal capillary; B—monocytoid cell with a convoluted nucleus; C—trophoblast cell lining a maternal sinusoid; D—erythrocytes within a maternal sinusoid. Toluidine blue, × 1,200

placental villus, a fetal capillary possessing a very thin endothelium is seen, surrounded by an acellular matrix, possibly representing extraembryonic mesoderm. Within this matrix, cells containing convoluted nuclei with distinct nuclear membranes are seen, whose morphology is suggestive of a monocyte-macrophage type of cell. Whether these cells are of fetal or maternal origin is undetermined. The outermost cells which are in direct contact with the blood in the maternal sinusoids are the trophoblast cells. These are large cells containing abundant cytoplasm, often showing cytoplasmic processes, possessing single or multiple nuclei that exhibit a stippled chromatin pattern and contain two or more nucleoli.

A careful comparison of sections with smears of cell suspensions allowed an identification of trophoblast cells in the latter preparations (Figs. 2 and 3). They are large pale staining cells, ranging in area from 300 μm^2 to 1,600 μm^2, containing a few cytoplasmic vacuoles. The plasma membrane is irregular and often ruffled around the edges. Most (approximately two-thirds) of the cells were uninucleate. The rest were bi- or multinucleate. These cells were further distinguished from decidual cells by virtue of their negative staining with PAS. Decidual cells are smaller in size and are always PAS positive because of their high intracellular glycogen content. The incidence of the latter cells was very low in placental-cell preparations. Among other cell types encountered in smears were large macrophage-type cells that contained highly vacuolated cytoplasm, often containing inclusions, and relatively small, dark-staining eccentric nuclei. They showed mild to moderate PAS reactions in the cytoplasm. Besides the above cells, blood cells of various types (lymphocytes, monocytes, erythroid cells, and occasional granulocytes) were also encountered, and easily identified by their morphology (Fig. 3).

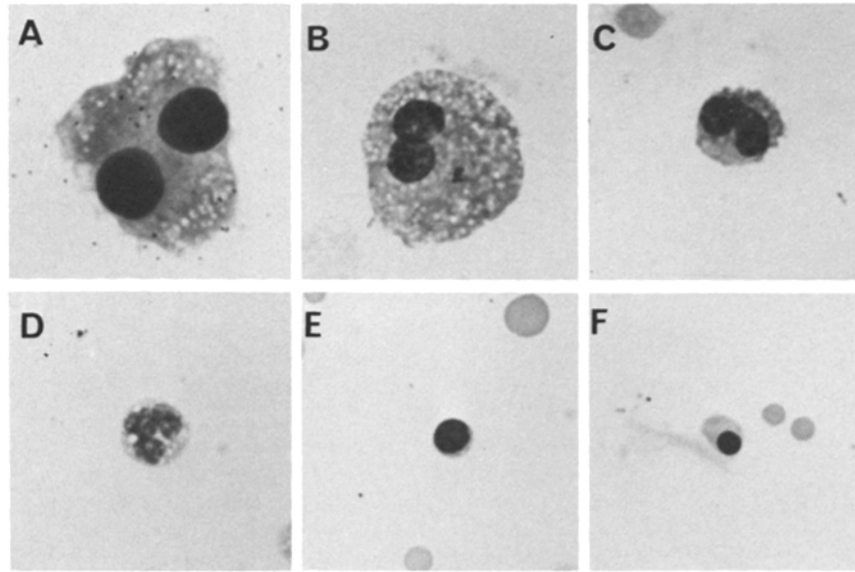*Examination of H-2 Antigens on Trophoblast Cells.* The presence of paternal and
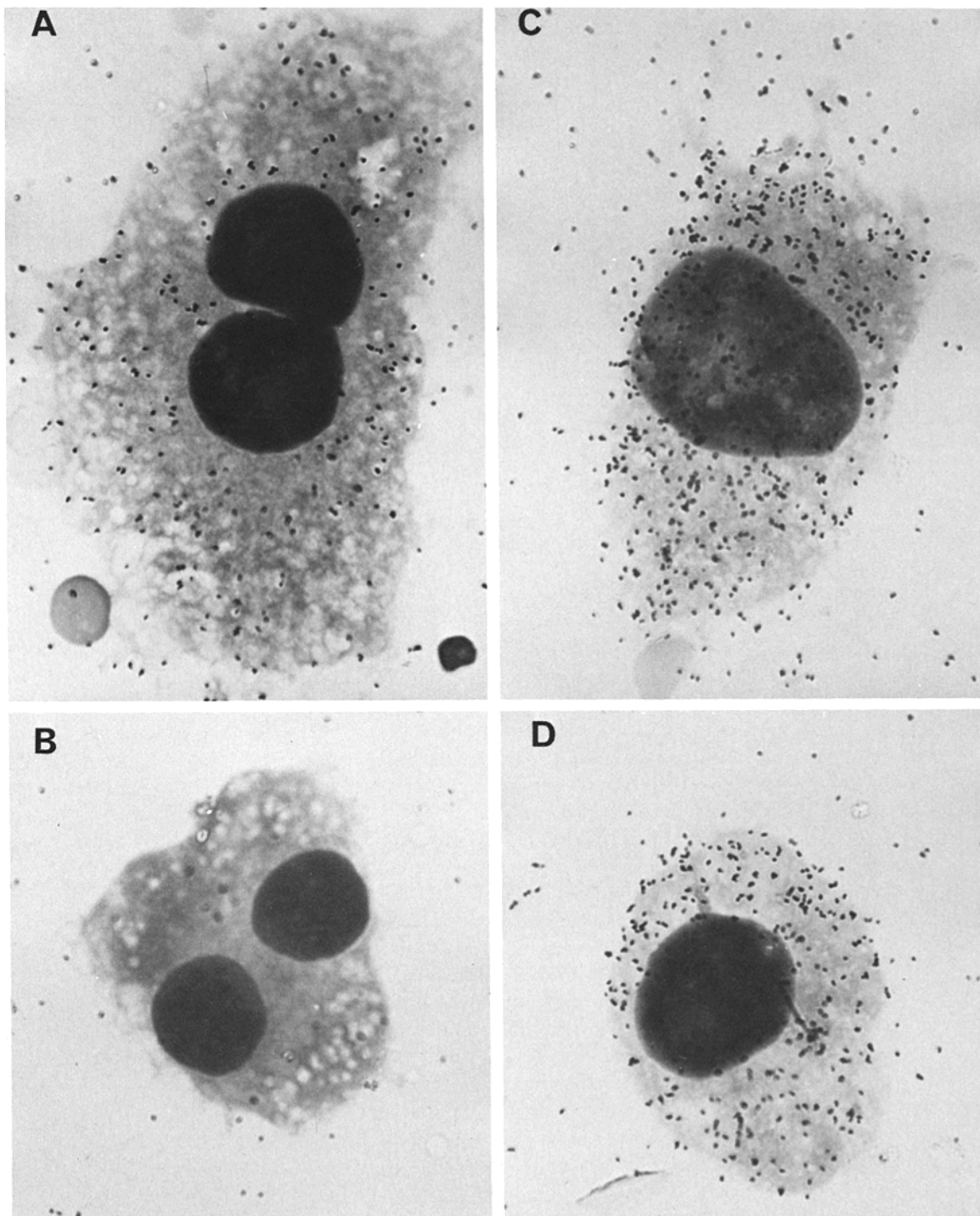
Fig. 3. Morphology of various cells encountered in smears of placental cell suspensions. A—trophoblast cell; B—large macrophage; C—monocyte; D—granulocyte; E—small lymphocyte; F—nucleated erythroid cell (late normoblast). Giemsa, × 1,200

maternal type H-2 antigens was examined on trophoblast cells obtained from placentae in CBA females (H-2K^k, D^k) mated with C57BL/6 males (H-2K^b, D^b). The H-2 haplotype of the resulting fetus should therefore be H-2K^{kb}, D^{kb}. In this study, H-2K^k, D^k, and D^b antigens were examined; H-2K^b could not be examined because of the unavailability of the antiserum at the time.

Tests of Specificity of Alloantisera Binding to Thymocytes. Binding of several anti-H-2 sera was examined on target cells of known H-2 haplotypes (CBA, C57BL/6 and CBA × C57BL/6 F$_1$ thymocytes). This was done with a two-step sandwich-labeling technique in which cells were first exposed to the individual alloantisera (or normal mouse serum in controls), and then to radioiodinated GAMG. Results are presented in Fig. 4. A total of 95.8% of CBA thymocytes were labeled (ie., showed six or more grains) with anti-H-2K^k, 58% with anti-H-2D^k and 0% with anti-H-2D^b serum. Although 47.2% of C57BL/6 thymocytes labeled with anti-H-2D^b, no labeling occurred with anti-H-2K^k, and only negligible (9.5%) levels with anti-H-2D^k serum. Almost all the F$_1$ thymocytes were labeled (98.8%) with anti-H-2K^k serum. No labeling of thymocytes of any strain was seen in the normal serum controls. Grain count distributions of labeled cells in Fig. 4 provides an indication of the intensity of

Fig. 2. The morphology of (CBA × C57B1/6)F$_1$ trophoblast cells in smears after radioautography in H-2-labeling experiments. The incubation steps during the labeling protocol were as follows:

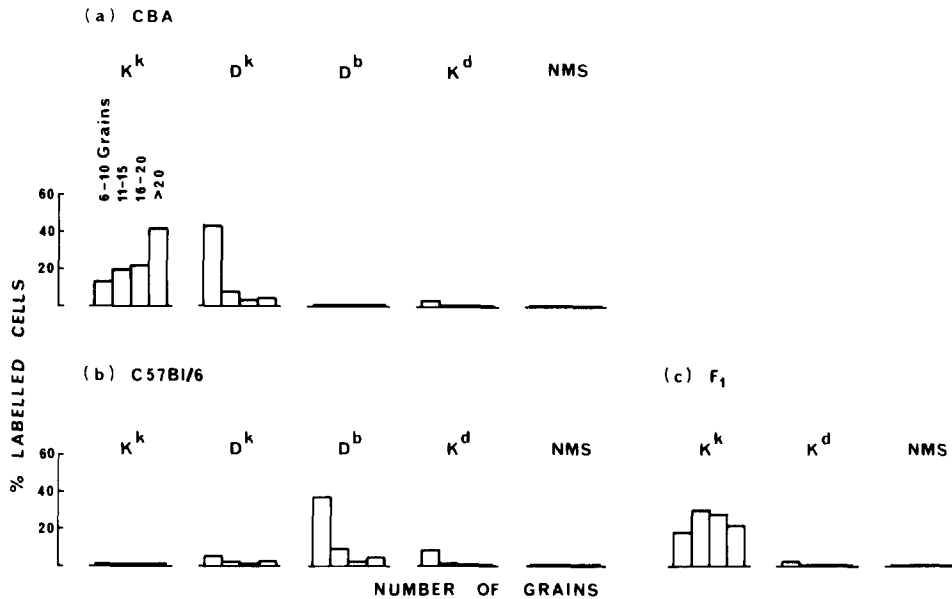| Step I	Step II	Step III
A—Nil	Nil	^{125}I-anti-IgG
B—Anti-IgG	NMS	^{125}I-anti-IgG
C—Anti-IgG	Anti-H-2K^k	^{125}I-anti-IgG
D—Anti-IgG	Anti-H-2D^b	^{125}I-anti-IgG

Giemsa, × 3,000

Fig. 4. The labeling patterns of (a) CBA, (b) C57Bl/6, and (c) (CBA × C57Bl/6)F₁ thymocytes with various anti-H-2 sera.

binding of each antiserum. Thus, the various antisera showed only specific binding with the appropriate target thymocytes.

The somewhat lower binding of the anti-D sera can possibly be attributed to the relatively low immunogenicity of the H-2D antigens as well as to batch differences, as this batch of anti-H-2D^k serum gave consistently lower labeling than previous batches of other series. In addition to these three alloantisera that were employed for the studies on trophoblasts (results to be presented later), another alloantisera (anti-H-2K^d), unrelated to all the above mouse strains, was also tested for any nonspecific binding by the thymocytes; little or no labeling was detectable (Fig. 4) in these cases.

The results of prior absorption of the antisera with H-2-matched thymocytes are presented later.

Masking of Previously Bound Immunoglobulin on Trophoblast Cells. Direct exposure of placental cell suspensions to radiolabeled GAMG resulted in heavy labeling of most of the trophoblast cells, as seen in Figs. 2 a and 5 a. This labeling was attributed to the presence of immunoglobulin molecules that were previously bound to the cell surface. Such binding could theoretically result from (*a*) antibodies directed against paternally derived antigens (H-2 or non H-2 in nature), (*b*) antibodies directed against embryonic antigens, (*c*) immunoglobulin bound by Fc receptors and (*d*) immunoglobulins which were in the process of transfer from the mother to the fetus. Because of this labeling of pre-existing Ig molecules it was impossible to determine, in a two-step sandwhich-labeling assay for H-2 antigens (Fig. 5 d) how much of the labeling was attributable to the presence of H-2 antigens. An effective masking of the native Ig molecules by treatment with unlabeled GAMG (Figs. 2 b, 5 b, and 5 c) allowed an evaluation of specific labeling due to surface H-2 antigens by using the three-step procedure (Figs. 2 c, 2 d, and 5 e).

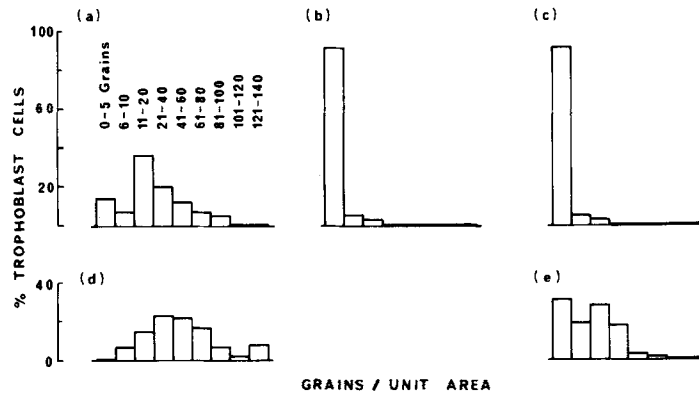*Evaluation of Paternal and Maternal Type H-2 Antigens on Trophoblast Cells.* Experiments

FIG. 5. The effects of the prior exposure of trophoblast cells to nonradioactive goat anti-mouse IgG on their labeling patterns with the sandwich technique. Grain counts are expressed per unit area of 64 μm^2. Steps in the various labeling protocols were as follows:

	Step I	Step II	Step III
a)	Nil	Nil	^{125}I-anti-IgG
b)	Anti-IgG	10% MEM-FCS	^{125}I-anti-IgG
c)	Anti-IgG	NMS	^{125}I-anti-IgG
d)	Nil	Anti-H-2K^k	^{125}I-anti-IgG
e)	Anti-IgG	Anti-H-2K^k	^{125}I-anti-IgG

were carried out on days 14, 16, and 18 of gestation. Figs. 6–8 represent the labeling patterns of trophoblast cells for the various H-2 antigens on different days of pregnancy, as indicated by the grain count distribution. On day 14, 75% of the trophoblast cells labeled for H-2K^k and 58% for D^k (the maternal haplotypes) and 75% of the cells labeled for H-2D^b (the paternal haplotype). On day 16, the labeling indices were 80, 74, and 70% for H-2K^k, D^k, and D^b, respectively. On day 18, 81% labeled for H-2K^k, 85% for D^k, and 93% for D^b.

It may be concluded from these results that both maternal and paternal type H-2 antigens are expressed on the surface of mouse trophoblast cells as early as day 14. Furthermore, a slight increase in the incidence of cells labeling for H-2 is seen in the later time points (Fig. 9).

To examine the relative density of H-2 antigens on trophoblast cells on different days of gestation, the median grain counts of labeled cells were calculated and plotted (Fig. 10). Between days 14 and 16, no change in the labeling intensity was observed. However, a rapid increase in the median grain count of labeled cells was noted between day 16 (16–18 grains) and day 18 (26–28 grains). A similar rise was also seen when the median grain count of all cells rather than labeled cells was examined (from 14 to 15 grains on day 16 and from 20 to 25 grains on day 18). This implies a notable increase in the density of H-2 antigens on the cell surface. The observed increment in the incidence of cells with detectable H-2 antigens (Fig. 9) may indeed be a result of this increase in the H-2 density rather than an acquisition of H-2 antigens by more cells.

Effects of Prior Absorption of Anti-H-2 Activity from Sera on Trophoblast Labeling. To ensure that the observed H-2 antisera binding by trophoblast cells was not due to non-H-2-related (eg. anti-viral) antibodies in the sera, sera absorbed with H-2-matched thymocytes from young mice were also tested on trophoblast cells. Removal
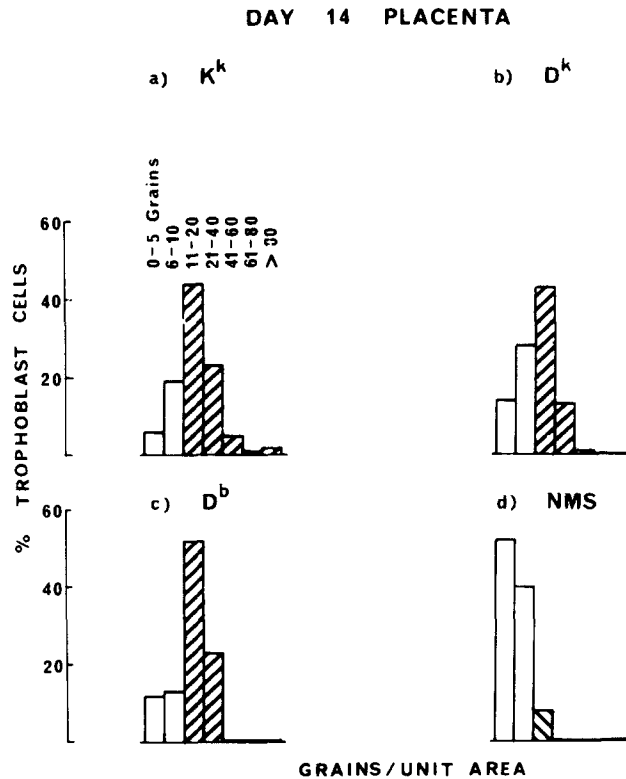
Fig. 6. The labeling patterns of 14-d old trophoblast cells (per unit area of 64 μm²) with various anti-H-2 sera (a, b, c). (d) Represents normal mouse serum control. Shaded areas represent positive labeling above threshold.

of specific H-2-related activity from the sera, as judged by a decline or elimination of thymocyte labeling, also resulted in a similar decline or elimination of trophoblast labeling with the three-step sandwich technique in all cases. For example, a single absorption of anti-H-2K^k serum with CBA thymocytes resulted in a decline of CBA thymocyte labeling from 96 to 19% (6$^+$ grains), and a decline in the median grain count of all thymocytes from 18 to 4. For late gestational F$_1$ trophoblast cells, the labeling index (11$^+$ grains) declined from 88 to 12% and the median grain count per unit area (8 μm²) dropped from 22 to 4.

Examination of Anti-Paternal Antibodies in Maternal Serum. Because a substantial proportion of the trophoblast-cell population was seen to exhibit detectable H-2 antigens, including those of the paternal haplotype, the possible presence of antibodies directed against the paternal type antigens in the maternal circulation during pregnancy was investigated. Paternal thymocytes were incubated with heat-inactivated serum collected from primiparous mice on days 13, 15, 16, and 18 of gestation, after which they were incubated with radioiodinated goat anti-mouse IgG. As shown in Fig. 11a, no labeling above controls (incubated with NMS) was seen. When paternal thymocytes were incubated with heat-inactivated sera from multiparous mice on the 18th day of their third pregnancy (Fig. 11b), no positive labeling was observed earlier. Hence, it was concluded that no anti-paternal antibodies in the
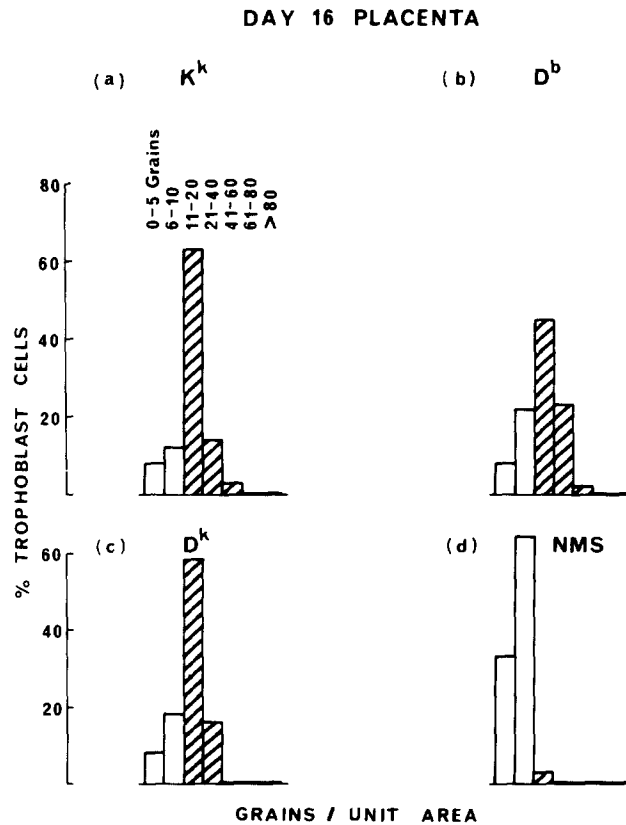
DAY 16 PLACENTA

Fig. 7. The labeling patterns of 16-d old trophoblast cells (per unit area of 64 μm^2) with various anti-H-2 sera (a, b, c). (d) represents normal mouse serum control. Shaded areas represent positive labeling above threshold.

circulation of pregnant primiparous or multiparous mice were detectable by our techniques.

Discussion

The present study was designed to re-examine the unresolved question of the existence of major histocompatibility antigens on the surface of mouse trophoblast cells.

The three-step sandwich technique employed in the present experiments was designed to provide a high degree of sensitivity without the loss of specificity, as well as a good resolution at the cellular level.

The high sensitivity of the present technique was achieved by a sandwich rather than a direct assay, because of an amplification of labeling introduced by the second antibody. Furthermore, radioautography permitted an increased detection of the label as opposed to other techniques such as immunofluorescence (28). This also allowed a quantitation of the relative intensity of binding which, under constant labeling conditions, reflected the relative antigen density on the cell surface.

A high level of specificity of labeling was achieved (a) by the use of monospecific antisera directed against the K or D end antigens, and (b) by introducing a masking
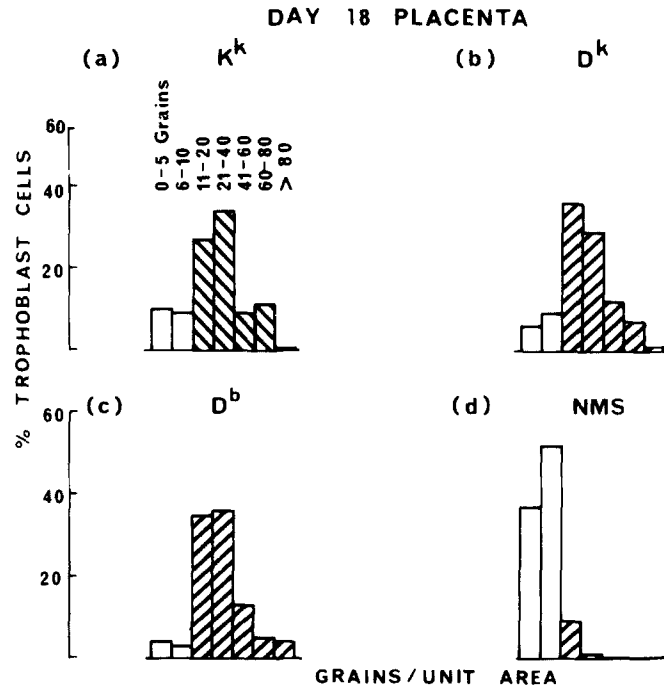
FIG. 8.   The labeling patterns of 18-d old trophoblast cells (per unit area of 64 μm²) with various anti-H-2 sera (a, b, c). (d) represents normal mouse serum control. Shaded areas represent positive labeling above threshold.

step in the present technique to eliminate the binding of ¹²⁵I-goat anti-mouse IgG to pre-existing Ig molecules on the surface of trophoblast cells. The specificity of the antisera was further established by testing their binding to H-2-matched and un-matched thymocytes. Finally, absorption experiments ensured that the antiserum binding by the trophoblast cells did not result from non-H-2-related antibodies in the sera.

A high resolution of labeling at the cellular level was achieved by the use of single-cell suspensions rather than sections. A choice of the method of cell dispersion used in this study was based on the criteria of high cell recovery, high cell viability, good morphological integrity of the trophoblast cells, and finally a retention of surface antigenic characteristics. The latter criterion prohibited the inclusion of proteolytic enzymes in the dispersion medium. Identity of trophoblast cells in smears was unequivocally established by morphological criteria based on a comparison with 0.5 μm thick sections as well as the cytochemical property of PAS-negative reaction. Because viable single cells were used at 4°C during the labeling procedure, any positive labeling resulted from antigenic sites on the cell surface rather than within the cytoplasm.

By using the three-step sandwich-labeling technique, H-2 antigens of both maternal and paternal haplotypes were identified on trophoblast cells between days 14 and 18 of gestation. Thus, no preferential suppression of the paternal haplotype was observed. The relative-labeling intensities (as given by the median grain count of labeled cells per unit area of 64 μm²) of individual H-2 antigens were very similar for 14- and 16-

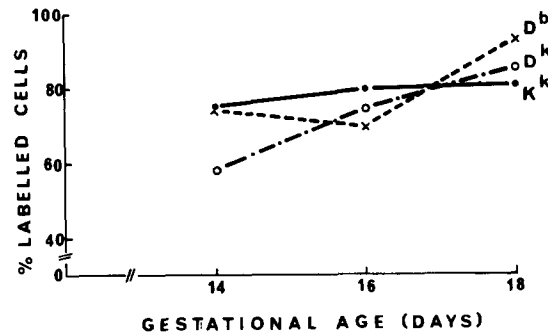SASWATI CHATTERJEE-HASROUNI AND PEEYUSH K. LALA

Fig. 9. The labeling incidence of trophoblast cells for H-2K^k, D^k, and D^b antigens at different days of gestation.

d-old trophoblasts, which in turn were comparable to that for F_1 thymocytes. For example, the respective median grain counts resulting from a labeling of H-2K^k antigens were 18.5, 16.3, and 16 for these cells. Therefore, it may be suggested that the extent of H-2 antigen expression on 14- to 16-d trophoblast cells is as high as that on a normal thymocyte. On day 18, there was a further rise (50%) in the density of all H-2 antigens on the trophoblasts.

Nondetection of histocompatibility antigens on mouse trophoblast in some investigations may have stemmed from a lesser sensitivity of the techniques employed. For example, Billington et al. (20) had negative results using an immunoperoxidase method, but obtained positive results by using mixed hemadsorption method. With the latter technique, some investigators (19, 21) also reported positive results at various gestational ages. However, in all of the above mentioned studies, the alloantisera used were not monospecific and thus may have included antibodies to alloantigens other than H-2. Recent findings of Wegmann et al. (29) of rapid and specific accumulation of injected anti-paternal type alloantibodies at the mouse placental site is consistent with our findings of the presence of H-2 antigens on trophoblast cells.

In the case of human trophoblasts most studies have been unable to detect HLA (10, 11, 18, 23). Whether they are due to technical limitations or represent a real phenomenon is an open question. Present results in the mouse indicate that further work is also needed in the human. Recently, Faulk et al. (30) reported a group of antigens shared between human trophoblast cells and blood mononuclear cells, which they called TA$_2$, as opposed to TA$_1$, which are shared with human tissue culture cell lines. Although the latter antigens may be oncofetal in nature, the former may include HLA among various other antigenic moieties.

The present findings on H-2 antigens on trophoblasts indicate that mechanisms other than the absence of H-2 antigens must be operative in the protection of the fetoplacental unit. Although the present results cannot completely exclude a preferential distribution of H-2 antigens on the fetal side of trophoblast cell surface, such a possibility is highly unlikely; on no occasion did labeled trophoblast cells in smears show a polarization of silver grains. Furthermore, a possible role of antigens determined by loci other than K or D in the major histocompatibility complex for allograft rejection in vivo and its implications for fetomaternal protection remains unknown at present.

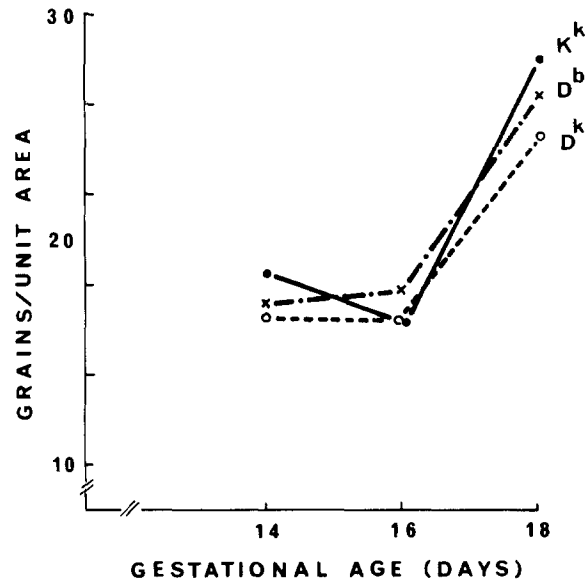Alterations in the recognition and/or effector arm of the maternal immune system,

Fig. 10. The labeling intensity of trophoblast cells (as obtained from median grain counts of labeled cells) for H-2K^k, D^k, and D^b antigens at different days of gestation.

may lead to a crippling of maternal immune responses directed towards the histoincompatible fetoplacental unit. Evidence exists to suggest the integrity of the recognition arm of the maternal cellular (12, 31) and humoral immune responses (32–34). However, a crippling of the effector arm of the maternal immune response could be accomplished by the production of noncytotoxic blocking antibodies which could combine with antigenic sites on target cells and prevent them from further sensitizing the host and from being recognized by effector lymphocytes that may have already been generated. Lysis of trophoblast cells by maternal lymphocytes, on coculture, was seen to be blocked by maternal serum (13) and the blocking function was traced to the IgG fraction.

We have however failed to detect any significant amounts of anti-paternal antibodies in the serum of either primi- or multiparous mice. Similar nondetection has also been previously reported (35). These findings do not, however, exclude the production of such antibodies in the mother; because they may be removed rapidly by the placenta which may act as an immunoabsorbent tissue (29). Furthermore, Voisin and Chaouat (36) found more Ig on the surface of the placenta in allogeneic pregnancy which bound readily to paternal thymocytes. Findings of large amounts of naturally bound Ig on trophoblast cell surface shown here and elsewhere (37–39) are consistent with this possibility. Whether such placenta-bound antibodies offer immunoprotection to the conceptus in vivo by a masking or blocking of the antigenic sites (13) remains to be determined.

Of the possible cellular mechanisms in the mother, generation of suppressor T lymphocytes has recently been reported (4, 40). As a first step to elucidate the role of the maternal lymphoid system in the maintenance of allogeneic pregnancy, we have analysed the temporal changes in the various lymphocyte subsets identified on the basis of surface markers. Such studies will be communicated separately.
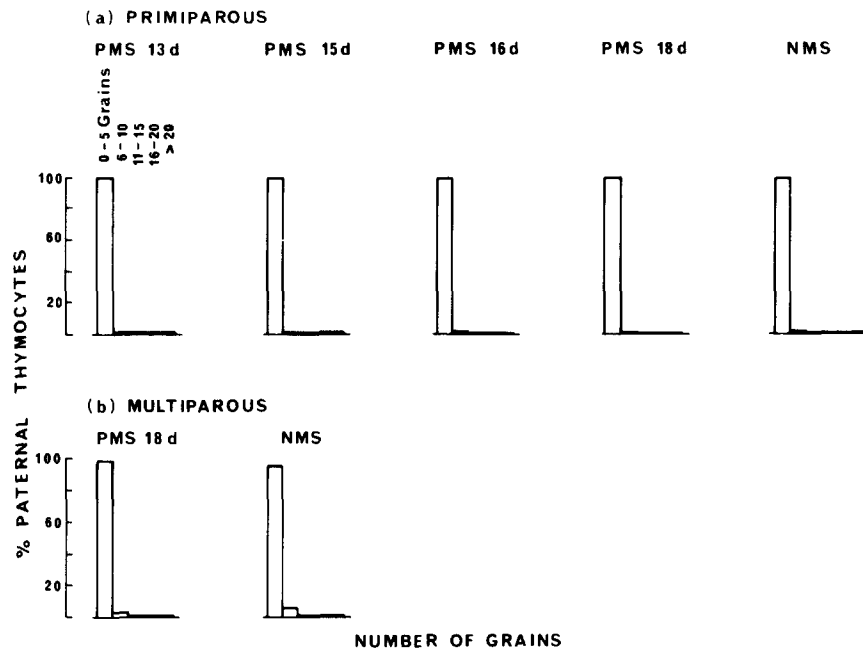
FIG. 11. Labeling patterns of paternal thymocytes after exposure to maternal sera followed by ^{125}I-labeled anti-IgG. (a) serum from primiparous mice; (b) serum from mice during their third pregnancy.

Summary

The presence of H-2 antigens of the paternal and maternal haplotypes on mouse trophoblast cells was examined at several stages of pregnancy by using a sensitive immunolabeling technique followed by quantitative radioautography. Results revealed the presence of H-2 antigens (determined by the K or D loci) of both parental haplotypes on the F_1 trophoblast cells. At 14–16 d of gestation, the antigen density was equivalent to that on adult thymocytes and there was a further 50% increase on day 18.

H-2 antigens of both parental haplotypes are also found to be expressed on 11–13 d trophoblast cells.

References

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