DIFFERENTIAL EXPRESSION OF MHC CLASS I ANTIGENS ON THE PLACENTA OF THE RAT

A Mechanism for the Survival of the Fetal Allograft

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The acceptance of the fetal allograft in the face of a completely competent maternal immune system remains a perplexing and critical problem in transplantation immunology. Varieties of potential mechanisms by which the fetus could avoid rejection have been proposed by Medawar (1) and by Billingham (2), and these proposals set the stage for much of the work in this field. The mechanisms proposed to prevent immune destruction of the fetus by the mother have postulated roles for blocking antibodies, immunosuppressive antibody-antigen complexes, nonimmunoglobulin immunosuppressive molecules, suppressor cells, and selective maternal immunoincompetence (reviewed in references 3–8). Many of these arguments turn on the question of what type of transplantation antigens encoded by the MHC are present in the placenta. Class I antigens have been demonstrated in the placenta of the mouse (9–13), human (14–21), and rat (22–24) using alloantisera and mAbs. Other studies in the human showed that the placenta also carries unique class I, or class I–like, antigens, the TLX antigens (25, 26), and class I molecules that carry only broadly crossreactive public antigenic determinants (27). Class II antigens are absent from the placenta in both the mouse (9, 28) and the human (14–19, 29).

Studies in the rat demonstrated that during pregnancy the maternal strain in certain mating combinations made an antibody response to the paternal component of the fetal histocompatibility antigens without any prior sensitization (22, 30, 31). The most potent response occurred when the $u \times a$ (female × male) haplotypes were mated. The alloantibody (22) and mAb (30) responses were directed against a placental class I molecule, the pregnancy-associated (Pa)[1] antigen, that has a broadly shared public antigenic determinant but not the allele-specific determinant of a classical class I transplantation antigen. The Pa antigen isolated from lymphocytes has a heavy chain of 46 kD and is associated with the β_2-microglobulin molecule. It appears to be controlled by a diallelic system: the $a, d, f, b,$ and m haplotypes carry the Pa antigen, whereas the $n, c, l, u, g, k,$ and h haplotypes do not. Using alloantisera, both the Pa antigen and the allele-specific RT1.A^a antigen were shown to be in the basal, but not in the

labyrinthine, trophoblast, but only the Pa antigen elicited an antibody response in the pregnant female (23, 32). This differential antibody response suggests that the Pa antigen is exposed on the surface of the basal trophoblast but that the A^a antigen is not.

The present study examines this hypothesis by exploring the expression of the Pa and A^a antigens in the placenta using mAbs that are specific to each antigen and both light and electron microscropy. The rat is a uniquely suitable animal with which to explore this question because the very low level of its MHC polymorphism allows one to study a serologically simple system and thereby to make a clear distinction between the allele-specific class I transplantation antigens and the unique Pa antigen (33).

Materials and Methods

Serology. All of the animals used in this study were from our colony at the University of Pittsburgh School of Medicine. The pregnancy combination in which the strongest antipaternal antibody response was elicited ($u \times a$) (22, 31) was used in all of the experiments. The allele-specific mAbs were made as previously described (34), and the anti-Pa mAb (mAb 124) was elicited in WF (u) females immunized only by pregnancy after mating with DA (a) males. The anti-A^a mAb (mAb 211) was made in the (MR $\times$ WKA)F$_1$ anti-DA strain combination, and the anti–class II mAb (mAb 348) that reacted with the a and k haplotypes was made in the BN anti-BN.1I (BI) strain combination. The OX3 mAb was purchased from Accurate Chemical & Scientific Co., Westbury, NY. The anti-A^n mAb (mAb 42), which was used as a negative control, was obtained from the [BN.1U(WF) $\times$ BN.1A(DA)]F$_1$ anti-BI strain combination (34). The RT1 specificities of the strains used to raise the mAbs are shown in Table I, and the reactivities of the monoclonal antibodies are shown in Table II. The haplotypes and strains of the rats used to test the reactivity of the mAbs are: a, DA, BN.1A(DA); b, BUF, BN.1B(BUF); c, AUG, PVG, BN.1C(AUG); d, DA.1D(BDV); f, DA.1F(AS2); g, BN.1G(KGH); h, DA.1H(HW); k, BN.1K(WKA); l, BN.1L(LEW); lvl, BN.1LV1(F344); m, DA.1M(MNR); n, BN,LEW.1N(BN); o, DA.1O(MR); and u, WF, YO, BN.1U(WF), BN.1U(YO).

Immunohistochemistry: Light Microscopy. These techniques have been described in detail (32). Briefly, the placentas at different days of gestation were removed from animals anesthetized by fluothane and perfused with phosphate-buffered 0.15 M NaCl, pH 7.2, at 37° through a cannula inserted into the ascending aorta and using a peristaltic pump (model 1203; Harvard Apparatus Co., Natick, MA) at a flow rate of 114 ml/min. The placentas were drained through the uterine veins, and perfusion was continued until the venous arcade was devoid of blood. Then 2.0-mm full-thickness slices of the placenta were placed in phosphate-buffered 0.15 M NaCl, pH 7.2, at 4°C for 1 min and quick-frozen in Tissue Tek O.C.T. embedding medium (Miles Laboratories Inc., Naperville, IL) in a container of 2-methylbutane suspended in liquid nitrogen at −70°C.

TABLE I

Strains Used for Raising Antisera and mAbs

Strain	Haplotype	RT1 specificities				
		A	Pa	B	D	E
MR	*o*	d	a	a	a	—
WKA	*k*	k	—	k	k	—
DA, BN.1A(DA)	*a*	a	a	a	a	—
BN	*n*	n	—	n	n	—
BN.1I(BI)	*i*	n	—	a	a	u
BN.1U(WF)	*u*	u	—	u	u	u

Table II

Characterization of mAbs

Haplotype of test cells	Reactivity of mAbs				
	Class I			Class II	
	124(Pa)	211(A^a)	42(A^n)	348(B/D^a)	OX3
a	256	2048	0	256	0
d	256	0	0	0	0
f	256	0	0	0	0
b	16	0	0	0	0
m	8	0	0	0	0
n	0	0	128	0	0
k	0	0	0	256	0
l	0	0	0	0	64
u	0	0	0	0	64
c, g, h	0	0	0	0	0
Antibody isotype:	IgG1	IgG2b	IgG2a	IgG2b	IgG (mouse)
Hemagglutination:	+	+	+	0	0
Cytotoxicity:	0	0	0	0	0
Binding assay*:	+	+	+	+	+

* Radioimmunoassay and cytofluorimetry.

The affinity-purified mAbs were biotinylated using biotinyl-N-hydroxysuccinimide ester dissolved in DMSO (1 mg/ml) and tested by hemagglutination or by binding assay to demonstrate that they retained their reactivity. The tissues were stained by the avidin-biotin complex immunoperoxidase method to localize the Pa and A^a antigens.

Blocking studies were performed to demonstrate that the mAbs to the Pa and A^a antigens detected separate molecules. The unlabeled blocking antibody was first applied to the slide, and then the biotinylated test antibody was added after washing off the excess blocking antibody. The various antibody combinations were: (a) unlabeled anti-Pa antibody followed by labeled anti-A^a antibody; (b) unlabeled anti-A^a antibody followed by labeled anti-A^a antibody; (c) unlabeled anti-A^a antibody followed by labeled anti-Pa antibody; and (d) unlabeled anti-Pa antibody followed by labeled anti-Pa antibody. The intensity of the immunoperoxidase staining was graded visually by light microscopy on a scale of 0 to +++. The original magnifications are given in the figures.

To examine the potential presence of class II antigens on the placenta, the immunoperoxidase technique was applied using the anti-B/D^a mAb (mAb 348) and the anti-B/D^u mAb (OX3). In this study, T and B lymphocytes prepared by nylon-wool chromatography were used as negative and positive controls, respectively.

Immunocytochemistry: Electron Microscopy. Both single- and double-label immunogold techniques were used to obtain electron photomicrographs (35–37). In addition, the number of particles per μm of membrane and per μm^2 of cytoplasm was counted on single-labeled photomicrographs. The test placentas were obtained from WF or DA females pregnant by DA males, and the negative control placentas were obtained from WF females pregnant by WF males.

The placentas were removed, and 1-mm full-thickness slices were immediately fixed in 2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.3, for 1 h. After fixation they were rinsed twice in 0.1 M phosphate buffer, pH 7.3, and postfixed in 2% osmium tetroxide buffered in 0.1 M phosphate, pH 7.3, for 2 h. The tissues were then dehydrated in increasing concentrations of ethanol, cleared in propylene oxide, and embedded in epoxy resin.

Semithin (1 μm), plastic-embedded sections were cut and stained with toluidine blue. These sections were examined by light microscopy, and the blocks that were morphologically well preserved and showed both basal and labyrinthine zones were selected. Ultrathin

sections (70 nm) from the selected blocks were mounted on nickel grids (300 mesh; Electron Microscopy Sciences, Fort Washington, PA), processed for immunocytochemical staining, counterstained with uranyl acetate and lead citrate, and examined with a Philips EM 300 electron microscope. The original magnifications are given in the figures.

For the ultrastructural localization of the Pa and A^a antigens, the postembedding staphylococcal protein A–gold (PAG) technique using ultrathin sections mounted on nickel grids was used (38–40). The sections on nickel grids were etched by floating them on a drop of freshly prepared 0.5% sodium metaperiodate for 1 h, following a modification of the method by Bendayan et al. (40, 41). They were washed several times in 0.025 M Tris buffer, pH 8.2, and then were placed on drops of 1% BSA in 0.025 M Tris buffer for 10 min. Without further washing, the grids were transferred to a drop of antibody–protein A–gold complex and incubated for 2 h at 37°. They were washed several times in 0.025 M Tris buffer, blotted with filter paper, and dried. Finally, the grids were fixed in 1% glutaraldehyde for 15 min, washed several times with distilled water, dried, and counterstained with uranyl acetate and lead citrate.

The antibody–protein A–gold complexes were prepared by incubating different dilutions of the mAbs with 1:50 dilutions of protein A–gold EM grade (Janssen Life Sciences, Westchester, PA) for 2 h at 37°C. The best dilution for staining with anti-Pa–PAG (20 nm) and with anti-A^a–PAG (10 nm) complexes was 1:100. Double-labeling a single section (40, 41) was performed by the sequential addition of anti-A^a–PAG 10 and anti-Pa–PAG 20 to opposite faces of the tissue, respectively. To demonstrate the specificity of the staining, two controls were used. First, incubation of sections from the WF × DA and WF × WF placentas was done with the unrelated anti-A^n antibody–protein A–gold complex (PAG 10 and PAG 20) for 2 h at 37°C; both types of placentas should not stain. Second, incubation of the WF × WF placental sections with the anti-Pa–PAG 20 and with the anti-A^a–PAG 10 for 2 h at 37°C should not show any staining.

To validate the experimental approach further, the ultrastructural localization of the Pa and A^a antigens on splenic lymphocytes was examined by the double-label technique. Lymphocytes from the DA and WF strains were incubated sequentially with anti-A^a–PAG 10 and anti-Pa–PAG 20 antibodies or with anti-A^n antibody labeled with PAG 10 and with PAG 20, as described above. The results should correlate with the serological findings: DA lymphocytes should stain with both the anti-A^a and anti-Pa antibodies; WF lymphocytes should not stain with either antibody; and neither the DA nor the WF lymphocytes should stain with the anti-A^n antibody.

The number of the anti-Pa, anti-A^a, and anti-A^n mAbs bound was counted per μm length of the cell membrane and μm^2 of cytoplasm in the basophilic trophoblast cells of the basal zone of the WF × DA, DA × DA and WF × WF placentas and in the labyrinthine zone cells of the same placentas using single-label electron photomicrographs at different magnifications. The area of the cell and the length of the cell membrane were measured using computerized morphometry (Bioquant II with Apple II microcomputer; R & M Biometrics, Inc., Nashville, TN). Each high magnification electron photomicrograph was counted as one field, and each low magnification photomicrograph was divided into quarters, with each quarter being counted as one field. The number of gold particles counted was then divided by the area of the cytoplasm or by the length of the cell membrane to obtain the appropriate parameter. The counts obtained from each field were used to calculate the average and the standard deviation.

Immunochemistry of Pa and A^a Antigens on Trophoblast Cells and on Lymphocytes. Trophoblast cells were purified from carefully separated basal zones from the placentas of WF rats pregnant by DA males at 14–18 d of gestation following essentially the procedure of Zuckermann and Head (42) with some modifications (42a). Using a discontinuous density gradient of Hypaque-Ficoll, a cell fraction from the 1.04–1.06 density interface that contained >70% trophoblast cells was obtained. These cells were positive by immunocytochemical staining with the anti-Pa and anti-A^a mAbs and with rabbit anti–human SP1 antibody (43). They had the morphological features of trophoblast cells: 20–60 μm in diameter, one or more nuclei, usually more than one nucleolus, and basophilic staining. Trophoblast cells prepared from the placentas of WF × WF pregnancies stained with the

anti–human SP1 antibody but not with the anti-Pa and anti-A^a antibodies. The contaminant cells in the WF × DA preparation were lymphocytes that were negative for all three antigens, showing that they were of maternal origin and that the cell preparations were not contaminated with fetal blood. Only 5% of the cells in the size range of the trophoblast cells were negative for the A^a and Pa antigens; they were probably glycogen cells.

The trophoblast cells isolated from 36 placentas (8 × 10^5 cells) and splenic lymphocytes (2 × 10^8) obtained from DA rats were surface-labeled with ^{125}I and extracted with 1% NP-40, as described (34). The preparations were reacted with 2 ml Sepharose 4B coupled with goat anti–rat Ig (affinity-purified Ig, 2 mg/ml gel) to remove the radiolabeled Igs. Aliquots of the samples without further purification or their glycoprotein fractions purified by lentil-lectin chromatography (34) were reacted overnight with the affinity-purified mAb 124 or 211. The immune complexes were precipitated overnight with equivalent amounts of affinity-purified goat anti–rat IgG. The precipitates were washed, counted for radioactivity, and analyzed by SDS-PAGE, as described (34). The relative amounts of the class I heavy chains were estimated by densitometric measurements of the autoradiographs using an Ultroscan XL Densitometer (LKB Instruments, Inc., Gaithersburg, MD).

Results

Both the anti-Pa and anti-A^a mAbs reacted with the endovascular and interstitial trophoblast of the decidua, the basal trophoblast, Reichert's membrane, and the yolk sac epithelium of the WF × DA placenta (Fig. 1). Although the staining pattern of these antibodies was the same, they reacted with different molecules, as shown by the blocking studies (Fig. 2). Prior reactivity with the anti-Pa antibody did not affect the reactivity with the anti-A^a antibody, and vice versa. Each antibody did, however, block the homologous reaction. The reactivities of these antibodies with the different components of the placenta and of the decidua at successive gestational ages are presented in Table III.

The question of whether class II MHC antigens are present in the placenta was examined immunocytochemically using anti–class II mAbs to the two antigens that could potentially be found: B/D^a (mAb 348) and B/D^u (OX3) (Table II). Both antibodies stained B (Fig. 3, B and F), but not T (Fig. 3, A and E) lymphocytes of the appropriate specificity. By contrast, neither antibody stained the semiallogeneic WF × DA placenta (Figure 3, C and G); the anti-B/D^a antibody did not stain the syngeneic DA × DA placenta (Fig. 3D); and the anti-B/D^u antibody did not stain the syngeneic WF × WF placenta (Fig. 3H). Hence, the placenta does not have any class II antigens on the cell membrane or in the cell cytoplasm.

Electron microscopic localization of the Pa and A^a antigens was done by the immunogold technique using both the single- and double-labeling approaches. The control study for this approach was the demonstration that both the Pa and A^a antigens are on the surface and in the cytoplasm of DA lymphocytes (Fig. 4A), with which they react serologically, but not on WF lymphocytes (Fig. 4B), with which they do not react; neither type of lymphocyte stained with the anti-A^n antibody (Fig. 4C).

Staining the WF × DA placenta by the double-label immunogold technique (Fig. 5) showed that the basal trophoblast had both the Pa and A^a antigens in the cytoplasm but that only the Pa antigen was expressed on the placental surface (Fig. 5A). The labyrinth did not contain either the Pa or A^a antigen (Fig. 5B), and the placenta did not stain with the unrelated anti-A^n mAb (Fig. 5, E and F).
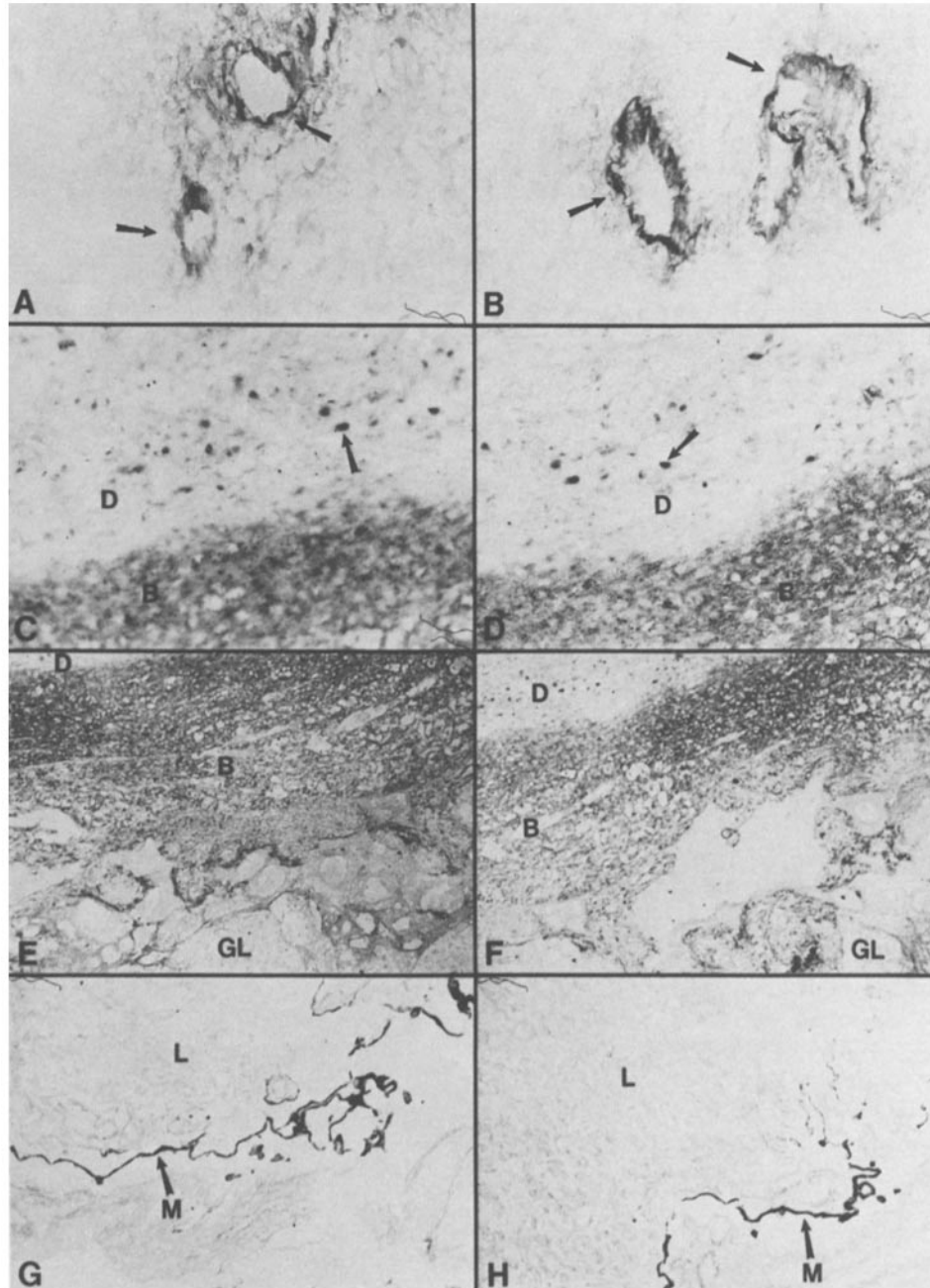
FIGURE 1. Staining of the WF × DA placenta (day 16 of gestation) by the avidin-biotin complex method with the anti-A^a mAb (mAb 211) (A, C, E, G) and the anti-Pa mAb (mAb 124) (B, D, F, H). (A and B) Metrial gland with staining of the endovascular trophoblast lining the lumina of maternal vascular spaces in the decidua (arrows). × 340. (C and D) Decidual tissue (D) with staining of the interstitial trophoblast (arrow) and basal zone trophoblast (B). × 340. (E and F) Basal zone trophoblast (B) is stained, but decidua (D) and glycogen cells (GL) are not. × 140. (G and H) Reichert's membrane (M) is stained, but the labyrinthine trophoblast (L) is not. × 140.
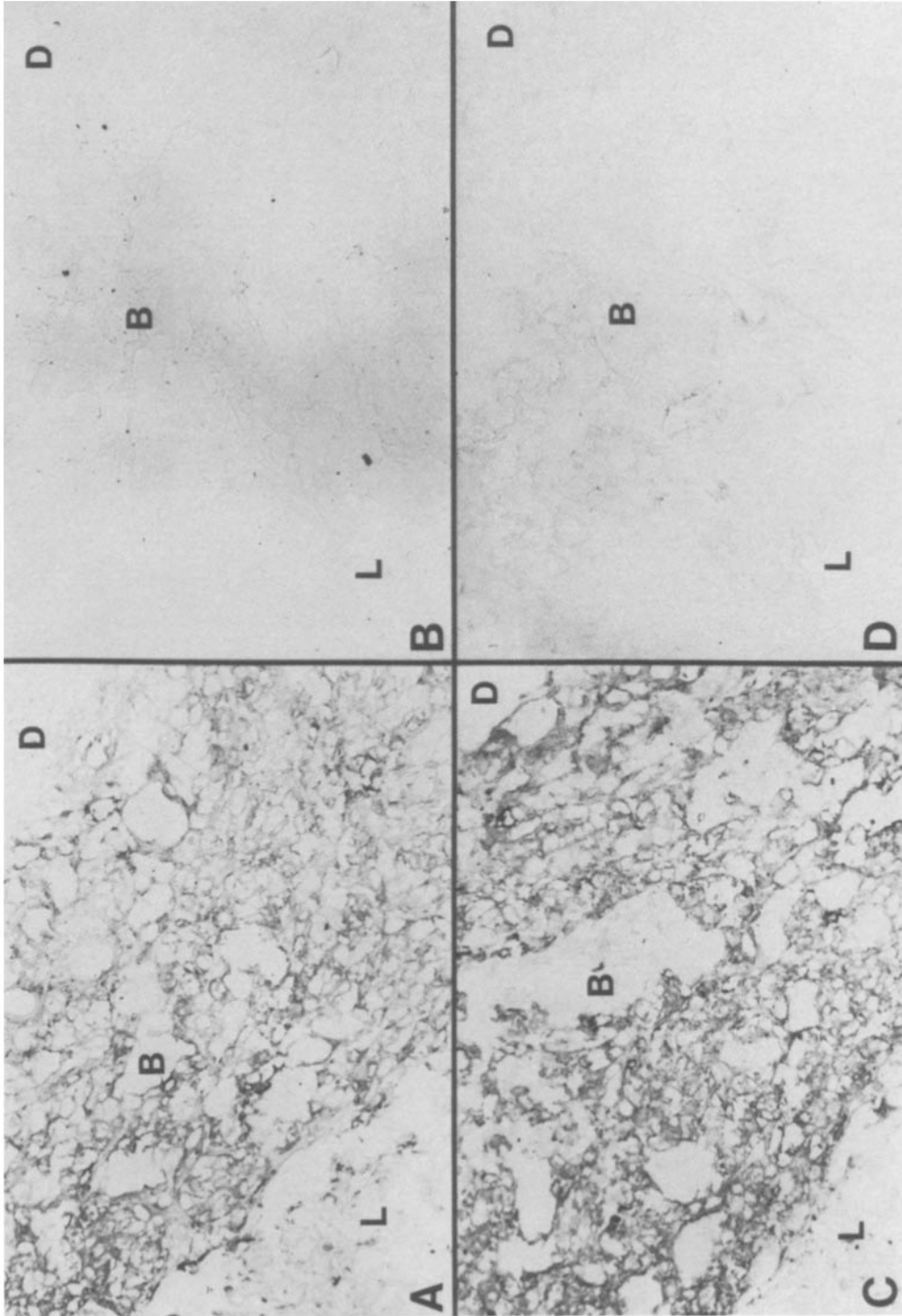
FIGURE 2. Blocking studies on the WF × DA placenta (day 16 of gestation) using the anti-Pa and anti-A^a mAbs and the avidin-biotin complex method. (A) Unlabeled anti-Pa mAb (blocking antibody) followed by biotinylated anti-A^a mAb (test antibody): the basal trophoblast (B) is stained, but the labyrinthine trophoblast (L) and the decidua (D) are not. (B) Unlabeled anti-A^a followed by biotinylated anti-A^a: no staining. (C) Unlabeled anti-A^a followed by biotinylated anti-Pa: the basal trophoblast (B) is stained, but the labyrinthine trophoblast (L) and the decidua (D) are not. (D) Unlabeled anti-Pa followed by biotinylated anti-Pa: no staining. × 140.
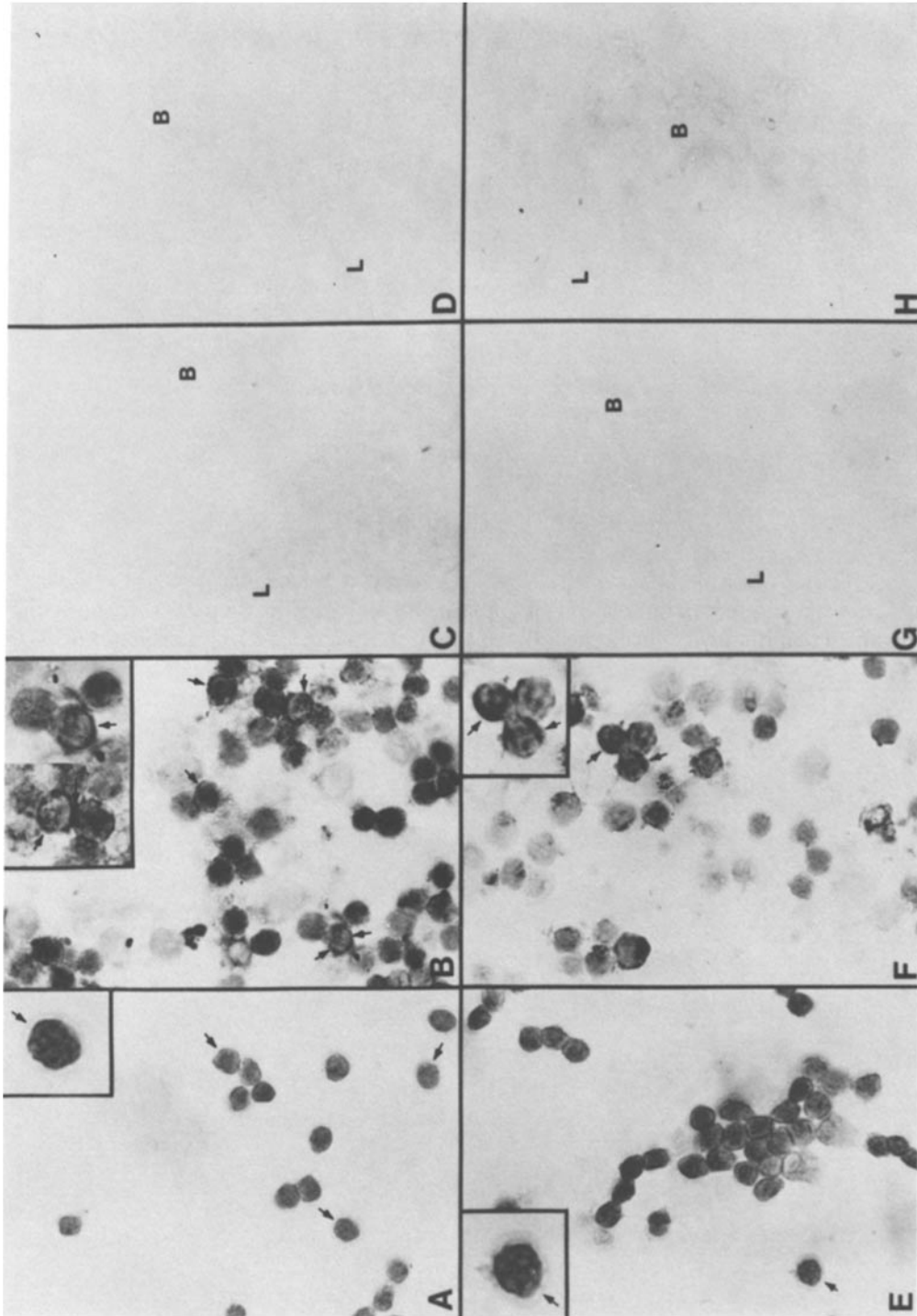
TABLE III

The Expression of Pa and A^a Antigens at Different Gestational Ages in the WF × DA Rat Placenta and in Adjacent Tissues

Localization	Intensity of staining at different gestational ages (d)*									
	12		14		16		18		20	
	Pa	A^a	Pa	A^a	Pa	A^a	Pa	A^a	Pa	A^a
Maternal uterine wall										
Endovascular trophoblast lining of blood vessels in metrial gland	++	++	++	++	++	++	++	++	++	++
Individual cells in decidua (interstitial trophoblast)	++	++	++	++	++	++	++	++	++	++
Placenta										
Basal zone trophoblast										
Basophilic and giant cells‡	++	++	++	++	++	++	++	++	++	++
Basophilic cells in intimate contact with maternal sinus	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Glycogen cells	-	-	-	-	-	-	-	-	-	-
Labyrinthine zone trophoblast	-	-	-	-	-	-	-	-	-	-
Reichert's membrane	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Yolk sac epithelium	++	++	++	++	++	++	++	++	++	++

* −, negative; +, weak; ++, moderate; +++, strong. The mAb 124 was used to stain the Pa antigen, and the mAb 211, the A^a antigen.
‡ The staining of the giant cells was variable.

Tissues from the WF × WF placenta did not stain at all (Fig. 5, C and D). Fig. 6 shows a higher magnification of the WF × DA placenta stained with both the anti-Pa and anti-A^a antibodies. By contrast, the placenta from the syngeneic DA × DA pregnancy had both the Pa and the A^a antigens expressed on its surface as well as in its cytoplasm (Fig. 7), but not in the labyrinth (Table IV).

Morphometric analysis of the electron photomicrographs using single-labeled tissues quantified these observations. Specific staining for the Pa and A^a antigens was found in the basophilic trophoblast cells of the WF × DA placenta, whereas the labyrinthine cells the WF × DA placenta and the entire WF × WF placenta showed only nonspecific background staining for both antigens (Table IV). In the basophilic cells of the WF × DA placenta, the Pa antigen was found both on the membrane and in the cytoplasm, whereas the A^a antigen was only in the cytoplasm (Table V). By contrast, both antigens were expressed on the membrane and were present in the cytoplasm of the syngeneic DA × DA placenta (Table V).

Both mAb 124 and 211 precipitated molecules consisting of a heavy chain of

FIGURE 3. Staining of the placenta for class II MHC antigens and demonstration of the specificity of the reagents using T and B lymphocytes. The lymphocytes were counterstained with hematoxylin, but the placenta was not counterstained. DA lymphocytes (A and B), and the WF × DA (C) and DA × DA (D) placentas (day 16 of gestation) were stained with the anti-B/D^a mAb 348. (A) T cell preparation showing no staining of the cell membrane (arrows). × 1,000 and inset × 2,200. (B) B cell preparation showing staining of the cell membrane (arrows). × 1,000 and insets × 1,400 and × 1,600. Neither the WF × DA (C) nor the DA × DA (D) placenta showed any staining of the basal (B) or labyrinthine (L) trophoblast. × 100. WF lymphocytes (E and F), and the WF × DA (G) and WF × WF (H) placentas (day 16 of gestation) were stained with the anti-B/D^u mAb OX3. (E) T cell preparation showing no staining of the cell membrane (arrows). × 1,000 and inset × 2,200. (F) B cell preparation showing staining of the cell membrane (arrows). × 1,000 and insert × 1,400. Neither the WF × DA (G) nor the WF × WF (H) placenta showed any staining of the basal (B) or labyrinthine (L) trophoblast. × 100.
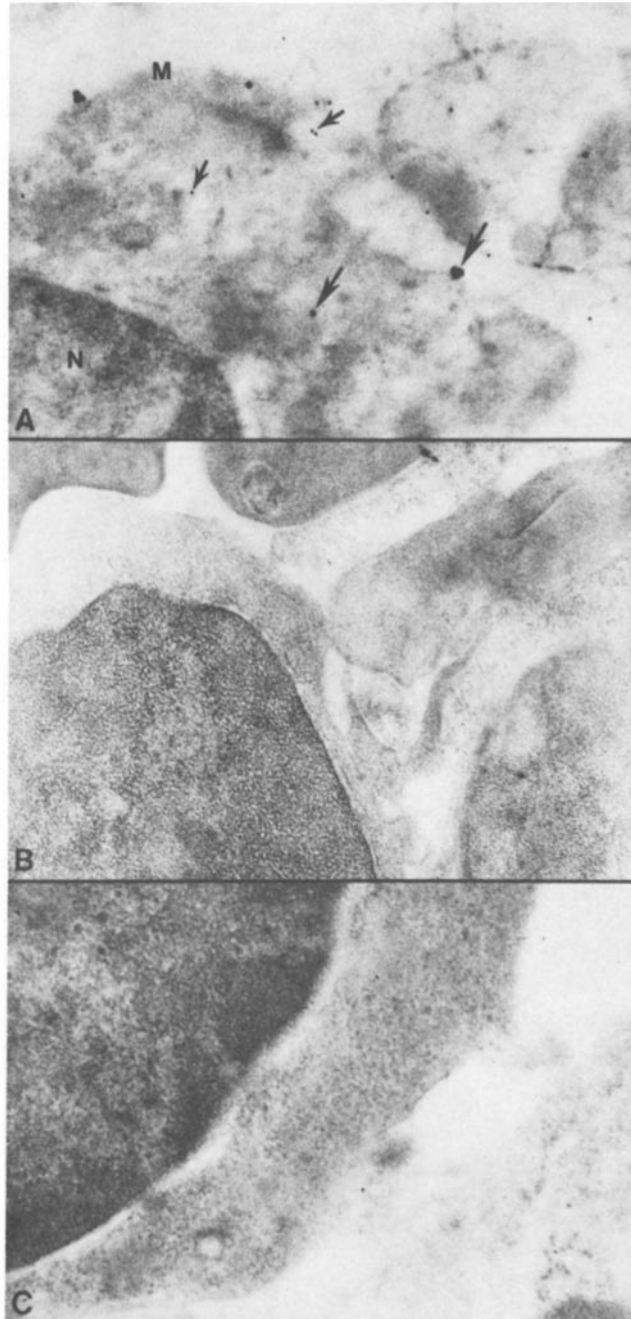
FIGURE 4. Staining of splenic lymphocytes using the double-label protein A–gold technique with 20-nm gold particles on the anti-Pa mAb (mAb 124) and 10-nm gold particles on the anti-A^a mAb (mAb 211). The lymphocytes were fixed in paraformaldehyde and osmium tetraoxide, embedded in epoxy resin, and stained with the antibodies. (N) nucleus, (M) cell membrane. (A) DA lymphocyte stained with both the anti-Pa (large arrows) and anti-A^a (small arrows) antibodies. Both antigens are present on the cell membrane and in the cytoplasm. (B) WF lymphocyte did not react with either antibody (unrelated cell control). (C) DA lymphocyte did not react with the immunogold-labeled anti-A^n mAb (mAb 42) (unrelated antibody control). × 58,000.

46 kD and a light chain of 12 kD from DA lymphocytes (Fig. 8). These molecules could be precipitated from lentil-lectin–purified glycoproteins, and SDS-PAGE analysis of their precipitates from equivalent amounts of ^{125}I-labeled glycoproteins reacted with excess amounts of the mAb showed more radioactivity associated with the A^a antigen than with the Pa antigen (A^a/Pa = 2.2) (Fig. 8). These
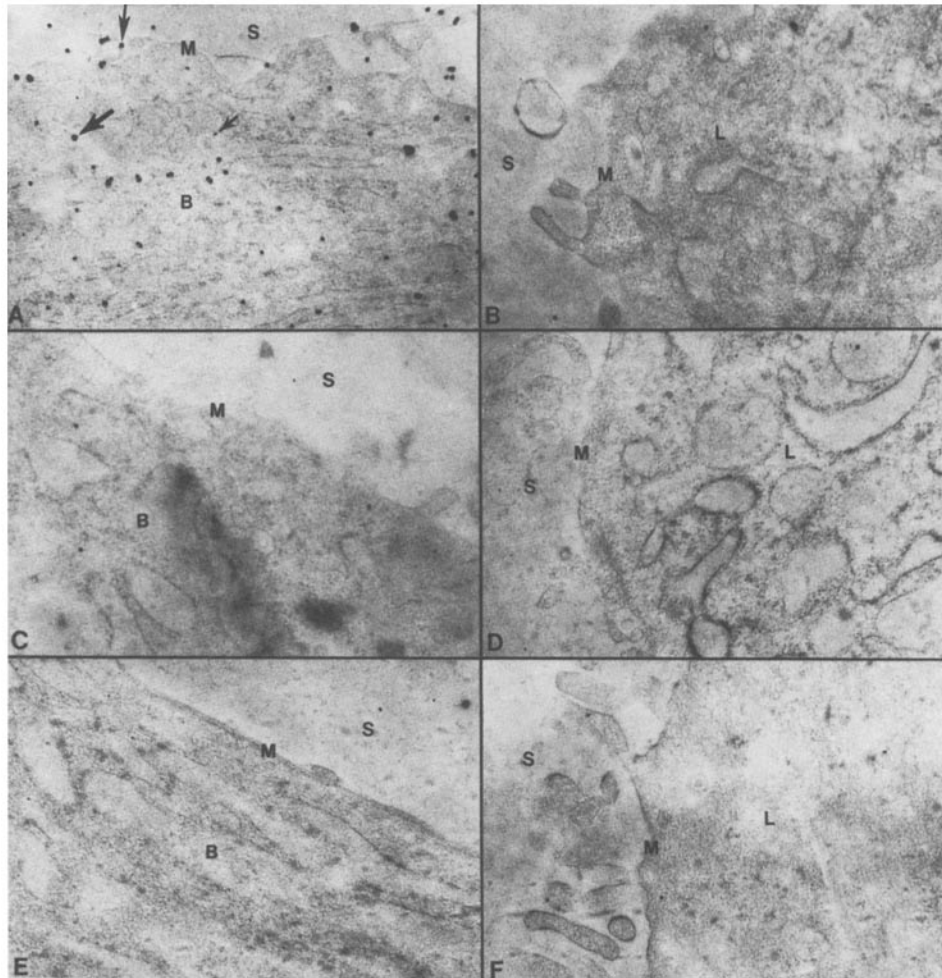
FIGURE 5. Staining of the rat placenta (day 16 of gestation) using the double-label protein A–gold technique, as described in Fig. 4, with anti-Pa (*large arrows*) and anti-A^a (*small arrow*) mAbs. (*B*) basal trophoblast, (*M*) cell membrane, (*S*) maternal sinus, and (*L*) labyrinthine trophoblast. (*A*) Basal trophoblast of the WF × DA placenta showing specific staining for only the Pa antigen on the cell membrane and for both the Pa and A^a antigens in the cell cytoplasm. (*B–F*) The much lower levels of nonspecific background staining. (*B*) Labyrinthine trophoblast of the WF × DA placenta did not stain with either antibody. Basal (*C*) and labyrinthine (*D*) trophoblast of the WF × WF placenta did not stain with either antibody (unrelated tissue control). Neither the basal (*E*) nor the labyrinthine (*F*) trophoblast of the WF × DA placenta stained with the anti-A^n antibody (unrelated antibody control). × 58,000.

results are consistent with our previous observations using ^{125}I-labeled red blood cells, internally labeled lymphocytes, and alloantisera to the Pa and A^a antigens (22). By contrast, no specific radioactivity could be detected in the lentil-lectin–purified glycoprotein fractions from the ^{125}I-labeled trophoblast cells precipitated with mAb 211 and 124, and SDS-PAGE analysis of the precipitates did not show any specific antigen components (results not shown). However, when the precipitates from the whole antigen extract were analyzed by SDS-PAGE, the anti-Pa antibody (mAb 124) showed a prominent specific component of 43 kD. An
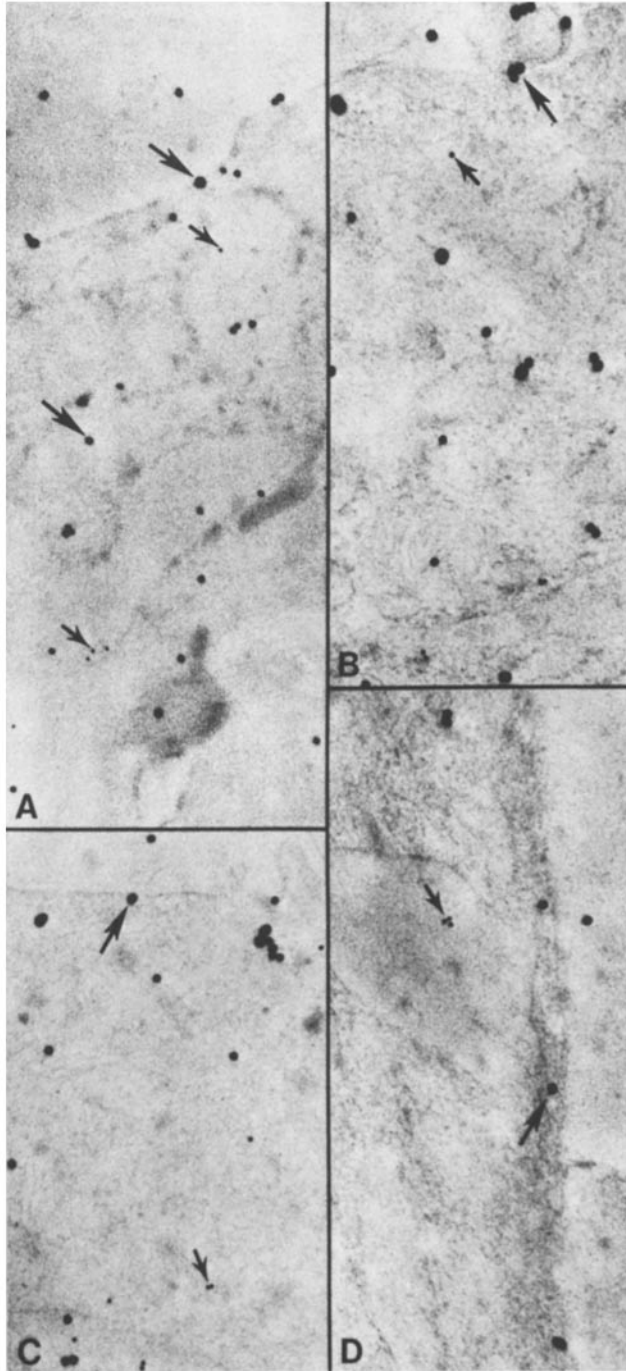
FIGURE 6. Staining of the basal trophoblast of the WF × DA placenta (day 16 of gestation) using the double-label protein A–gold technique, as described in Fig. 4. (A–D) Different fields of the same section. Only the Pa antigen (*large arrows*) is expressed on the cell membrane, whereas both the Pa and A^a (*small arrows*) antigens are present in the cell cytoplasm. × 98,000.

equivalent amount of sample precipitated by the anti-A^a antibody (mAb 211) showed a barely detectable component in this position (Pa/A^a = 23.5) (Fig. 8). These samples did not show any component in the position of the A^a and Pa antigen heavy chains precipitated from lymphocytes.
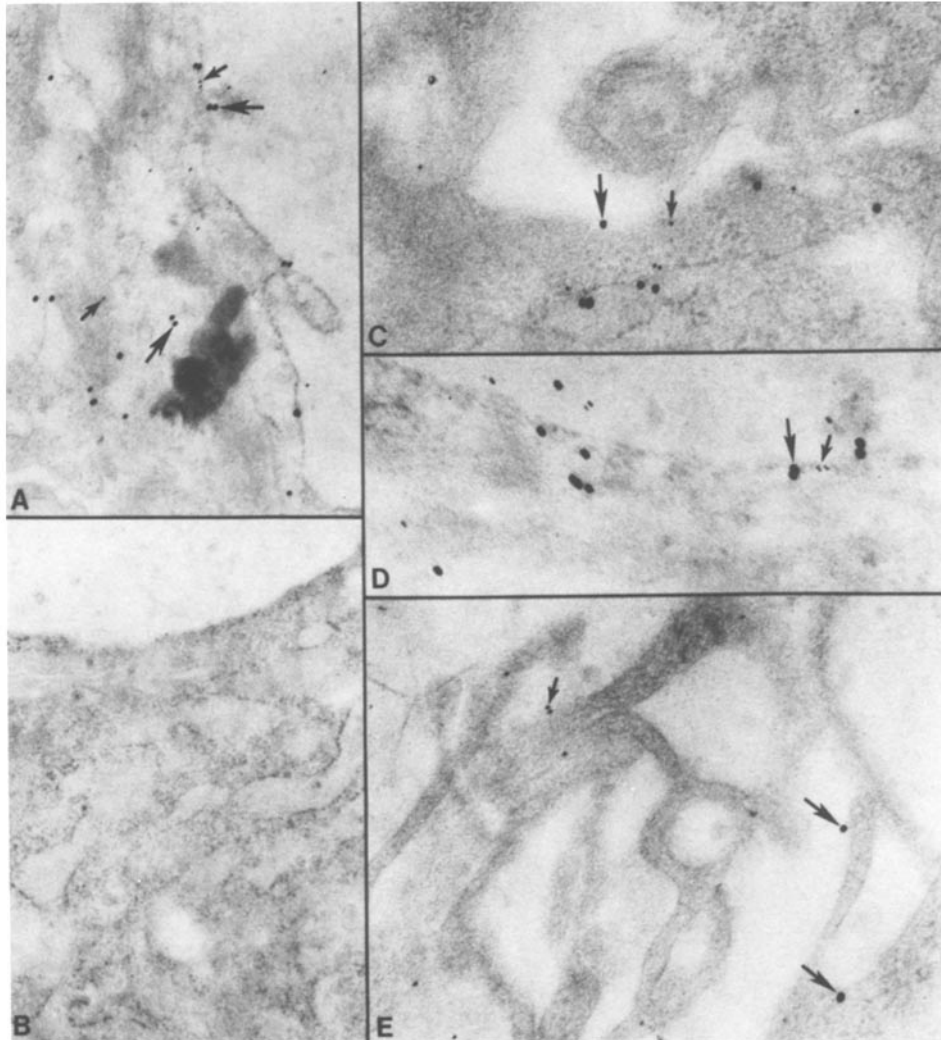
FIGURE 7. Staining of the basal trophoblast of the DA × DA placenta (day 16 of gestation) using the double-label protein A–gold technique, as described in Fig. 4. (A, C, D, E) Both the Pa antigen (*large arrows*) and the A^a antigen (*small arrows*) are expressed on the cell membrane of the DA × DA placenta, and both are present in the cell cytoplasm. (B) WF × WF placenta showing no staining with either antibody (control). × 98,000.

Discussion

There is differential expression of class I MHC antigens on the placenta in semiallogeneic pregnancies but not in syngeneic pregnancies. The Pa antigen, which elicits an antibody response during semiallogeneic pregnancies without any prior sensitization of the mother, is expressed on the membrane of the basal trophoblast cells and is present in their cytoplasm. The allele-specific class I transplantation antigen A^a is synthesized by the basal trophoblast cells but is retained in their cytoplasm in semiallogeneic pregnancies. However, it is expressed on the placental surface in the syngeneic pregnancies. The class II

TABLE IV

Staining of Placental Basophilic and Labyrinthine Cells for the Pa and Aª Antigens

Antigen	Cell	Antigen molecules/μm² cell area								
		WF × DA placenta			DA × DA placenta			WF × WF placenta (control)		
		Mean	± SD	Number of μm² counted	Mean	± SD	Number of μm² counted	Mean	± SD	Number of μm² counted
Pa	Basophilic	4.95	3.65	720	3.07	2.93	442	0.98	1.05	500
	Labyrinthine	1.19	1.19	340	0.63	1.01	203	0.76	0.85	360
Aª	Basophilic	3.90	2.52	630	3.56	1.96	702	0.91	0.99	800
	Labyrinthine	0.95	1.07	650	0.72	0.56	246	0.86	1.05	590
Aⁿ (control)	Basophilic	0.92	0.98	130	0.77	0.69	163	0.90	0.91	140
	Labyrinthine	0.98	0.98	130	0.59	0.83	120	0.88	1.01	130

TABLE V

Staining of the Membrane and the Cytoplasm of Basophilic Trophoblast Cells for the Pa and A^a Antigens

Antigen	WF × DA placenta			DA × DA placenta			WF × WF placenta (control)		
	Mean	± SD	Number of μm or μm^2 counted	Mean	± SD	Number of μm or μm^2 counted	Mean	± SD	Number of μm or μm^2 counted
Pa*	1.55	0.65	571	1.14	0.76	241	0.21	0.14	198
A^a*	0.13	0.07	625	1.13	0.63	365	0.19	0.15	286
Pa‡	3.27	2.48	1543	3.07	2.93	442	0.89	0.34	206
A^a‡	3.25	0.94	1394	3.56	1.96	702	0.76	0.28	377

* Results are antigen molecules per μm of membrane.
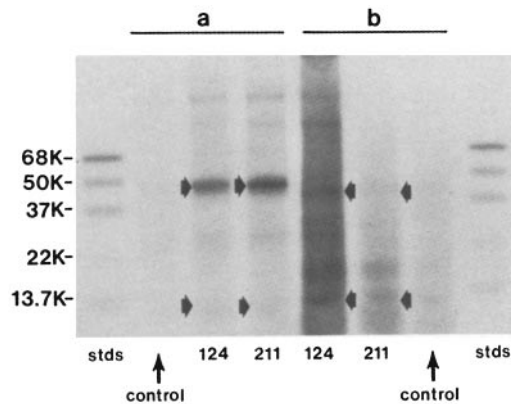‡ Results are antigen molecules per μm^2 of cytoplasm.

FIGURE 8. Autoradiogram of the SDS-PAGE analysis of the Pa and A^a antigens obtained from ^{125}I-labeled DA lymphocytes (a) or from trophoblast cells isolated from the placentas of WF females pregnant by DA males (b) using the anti-Pa mAb 124 and the anti-A^a mAb 211, respectively. The antigens were precipitated from glycoprotein extracts of the lymphocyte membranes prepared using a lentil-lectin column and from unpurified detergent extracts of trophoblast cells, since glycoproteins from these cells did not bind to lentil-lectin columns. The upper arrows indicate the antigen heavy chains, and the lower ones, the β_2-microglobulin. Based on densitometric measurements of the autoradiograms, the Pa antigen was less abundant than the A^a antigen in lymphocytes (Pa/A^a = 0.46) but considerably more abundant in trophoblast cells (Pa/A^a = 23.5).

antigens are not synthesized by the placenta in either semiallogeneic or syngeneic pregnancies. These observations indicate that two kinds of regulatory mechanisms affecting MHC antigens are operative in the placenta: one that is tissue-specific and constitutively represses the synthesis of class II antigens in both semiallogeneic and syngeneic pregnancies, and one that is inducible and represses the expression of the class I allele–specific transplantation antigens on the basal trophoblast surface in semiallogeneic pregnancies only.

Placental antigens similar to the A^a and Pa antigens in the rat have been identified in the human and in the baboon (Table VI). In the latter two species, these antigens have been demonstrated on trophoblast tissue and have been isolated from trophoblast membrane preparations using the mAb W6/32, which reacts broadly with human class I antigens. The heavy chains of these antigens isolated from lymphocytes have a molecular mass of 45 kD, whereas those isolated from trophoblast cells are smaller. We found the same difference for the A^a and Pa antigens in the rat. The two molecules obtained from lymphocytes have the characteristics of classical class I antigens with a heavy chain of 46 kD, but the

TABLE VI

Nonpolymorphic Class I Molecules on Lymphocytes and Placental Trophoblast

Species	Source of antigen	mAb	Molecular weight of heavy chain	Associated with β_2-microglobulin	Localization	Reference
Rat	Lymphocyte (DA)	124(Pa)	46,000	+	Membrane + cytoplasm	This paper
		211(A^a)	46,000	+	Membrane + cytoplasm	22, 30
	Basal trophoblast (WF × DA)	124(Pa)	43,000	+	Membrane + cytoplasm	
		211(A^a)	43,000	+	Cytoplasm	
Human	Lymphocyte	W6/32	45,000	+	Whole cell	21
	Chorionic cytotrophoblast*	W6/32	40,000	+	Whole cell	
	Choriocarcinoma	W6/32	40,000	+	Whole cell	
Baboon	Lymphocytes	W6/32	45,000	+	Whole cell	44
	Syncytial trophoblast	W6/32	41,000	+	Whole cell	

* A W6/32-reactive molecule is also present in the extravillous cytotrophoblast of the placental bed (18, 20).

trophoblast antigens have a 43-kD heavy chain. If we assume that the density of iodination is the same for the two molecules, densitometric analysis of SDS-PAGE autoradiographs shows that the A^a antigen is more abundant than the Pa antigen in lymphocytes. Assuming again a similar iodination density for the two antigens, the Pa antigen is more abundant than the A^a antigen in the trophoblast. Morphometric analyses of electron photomicrographs confirm the latter point.

There can be several explanations for the abundant expression of the Pa antigen and for the absence of the A^a antigen on semiallogeneic rat trophoblast cells and for the size difference between the antigens isolated from lymphocytes and from trophoblast cells. The molecular mass difference of ~3 kD between the class I antigens in lymphocytes and in trophoblast cells could be due to a deletion of ~30 amino acid residues; the loss of a carbohydrate moiety, which has a molecular mass of 3 kD (45); or incomplete glycosylation of the carbohydrate moieties. It is unlikely that the lower molecular mass heavy chains in the trophoblast cells are Qa/Tla-like molecules for two reasons. First, the antibodies used in the studies on the rat, human, and baboon (Table VI) all precipitated 45–46 kD heavy chains from lymphocytes and smaller molecules from trophoblast cells. It seems unlikely that a classical class I locus and a Qa/Tla-like locus would encode molecules having the same antigenic specificity. Second, the locus encoding the Pa antigen in the rat maps near the RT1.A locus, which is at the opposite end of the MHC from the Qa/Tla-like region of the rat MHC (the G/C region) (33).

As a working hypothesis, we favor the interpretation that glycosylation differences account both for the differential expression of the Pa and A^a antigens and for the lower molecular weights of these antigens in the trophoblast. After their biosynthesis, the nascent heavy chains of class I antigens are inserted into the membrane of the endoplasmic reticulum, associate with β_2-microglobulin, are core-glycosylated and then terminally glycosylated, and then are expressed on the cell surface (reviewed in reference 46). The β_2-microglobulin molecule plays a critical role in posttranslational processing, intracellular transport and surface expression of class I molecules (47, 48). However, it may not be absolutely essential, because there are reports that class I heavy chains can be expressed on the cell surface without being associated with β_2-microglobulin (49–51). Other factors that can influence expression are the formation of intrachain disulfide

bridges (52), mutations or deletions in the heavy chain genes (53), *cis-* or *trans-* acting regulatory factors (54–56), and the level of glycosylation. Some investigators have found that glycosylation of HLA or H-2 class I molecules is necessary for their stable cell surface expression (57, 58), whereas others have found normal amounts of these antigens on the cell surface that are unglycosylated because of tunicamycin treatment (59, 60). Recent studies on H-2 (61) and on HLA (62) antigens that are unglycosylated because of the substitution by other amino acids of asparagine in the *N*-linked glycosylation sequence Asn-X-Ser have shown a marked reduction in the surface expression of these antigens. It has been argued by Santos-Aguado et al. (62) that it is not lack of glycosylation, but rather substitution of the Asn residue in the glycosylation sequence, that affects the surface expression of the HLA antigens, since substitution of the Ser residue in the Asn-X-Ser sequence also produces unglycosylated molecules but does not have much effect on surface expression.

We have recently shown (45) that rat MHC class I molecules contain two or three *N*-linked glycans, as in H-2 class I molecules, and that their unglycosylated molecular mass is 37.5 kD (45). Since the Pa and A^a antigens on the trophoblast do not bind to a lentil-lectin column and have heavy chains with a molecular mass intermediate between that of the glycosylated and unglycosylated class I antigens from lymphocytes, the glycosylation patterns of the trophoblast antigens may be different from those of the lymphocyte antigens. This intermediate difference in the molecular masses could be due to changes in the glycosylation patterns of these antigens, or possibly to changes in the amino acid sequence critical for glycosylation, that may selectively affect the expression of the A^a antigen on the trophoblast surface. An observation consistent with a relationship between alterations in glycosylation and the inability to bind to a lentil-lectin column is the finding that a mutant H-2 class I antigen with an altered type of glycosylation could not be retarded on a lentil-lectin column (63). The glycans of the expressed H-2 or HLA class I molecules are of the complex type (64), whereas during intracellular transport they are present as high-mannose glycans that are converted to complex glycans during the final stage of transport to the cell surface. We propose that there is a glycosylation block during intracellular conversion of the high-mannose glycans to complex ones that prevents the A^a antigen from reaching the trophoblast surface, whereas the Pa antigen is processed enough to have the sugars necessary for cell surface expression. There are recent reports that defective glycosylation, or lack of glycosylation, of some H-2 antigens decreases or stops their surface expression (65, 66).

The data presented in this paper suggest that the major mechanism by which the placenta avoids allograft rejection in semiallogeneic pregnancies is the repression of the expression of the classical class I transplantation antigens on the surface of the basal trophoblast cells and the repression of the synthesis of all class II antigens. This same mechanism may also be operative in the ability of malignant cells to avoid immune rejection.

Summary

In some mating combinations in rats, there is a maternal antibody response to the paternal antigenic components of the placenta without any previous immu-

nization of the mother. The highest response occurs in the WF (u) female mated to the DA (a) male, and it is against a unique MHC-encoded class I antigen, the Pa antigen, and not against the major allele–specific transplantation antigen of the DA strain, RT1.A^a. The development of mAbs to the Pa and A^a antigens allowed us to localize these antigens on the placenta and to explore the reason for the differential antibody response to them using immunohistochemical and biochemical techniques. Both antibodies reacted with the WF × DA placenta and stained the endovascular and interstitial trophoblast of the decidua, the basal trophoblast, Reichert's membrane, and the yolk sac epithelium, but they did not stain the labyrinthine trophoblast. Blocking studies showed that each antibody reacted with a separate molecule in the placenta. Anti–class II mAbs reactive with the a or u haplotype did not stain the WF × DA, DA × DA, or WF × WF placenta; hence, there are no class II antigens in the placenta.

Electron microscopic studies of the semiallogeneic WF × DA placenta using the immunogold technique with both single- and double-labeling showed that only the Pa antigen was expressed on the surface of the basal trophoblast, but that both the Pa and A^a antigens were in the cytoplasm of these cells; neither antigen was found in the labyrinthine trophoblast. By contrast, the placenta from the syngeneic DA × DA mating expressed both the Pa and A^a antigens on the surface of the basal trophoblast as well as in the cytoplasm; neither antigen was found in the labyrinthine trophoblast. These observations were quantified morphometrically using electron photomicrographs of single-labeled tissues.

Both the Pa and A^a antigens isolated from the plasma membrane of lymphocytes have heavy chains of 46 kD, but those antigens isolated from the plasma membrane of basal trophoblast cells have heavy chains of 43 kD. Based on densitometric measurements of autoradiographs, the Pa/A^a ratio in the basal trophoblast membrane is 23.5, whereas it is 0.46 in lymphocyte membranes. These studies show that there is differential regulation of the expression of class I antigens on basal trophoblast cells in semiallogeneic pregnancies, but not in syngeneic pregnancies, such that the major allele–specific transplantation antigen is scarcely expressed on the surface of the basal trophoblast. Instead, it is mainly the unique, pregnancy-associated (Pa) antigen that is expressed.

The observations reported here indicate that two kinds of regulatory mechanisms affecting MHC antigens are operative in the placenta: one that is tissue-specific and constitutively represses the synthesis of all class II antigens in both semiallogeneic and syngeneic pregnancies, and one that is inducible and represses the expression of the class I allele–specific transplantation antigens on the surface of the basal trophoblast cells in semiallogeneic pregnancies only.

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