

DISTRIBUTION OF MATERNAL IMMUNOGLOBULINS IN THE MOUSE UTERUS AND EMBRYO IN THE DAYS AFTER IMPLANTATION*

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Enhancing maternal antibodies are among the various factors currently thought to play a role in protecting the mammalian embryo from rejection by the mother (1). Immunoglobulins having enhancing or immunosuppressive properties can actually be eluted from late placentae, in mice or in humans (2-4). While it is now well established that immunoglobulins are transferred from the mother to the fetus in the last third of pregnancy (for review see reference 5), little is known concerning the distribution of maternal immunoglobulins in the uterus and the embryo in the days after implantation. In this paper we report an immunoperoxidase study of such distribution in the mouse uterus and embryo from day 5 to 10 after fertilization. It is shown that the embryo is surrounded by maternal immunoglobulins at the time of implantation and continues to be invested by immunoglobulins throughout the period we studied. Trophoblast giant cells heavily concentrate immunoglobulins, adding support to their possible role as an immunological filter.

Materials and Methods

Matings between inbred mice, either from the A strain or from the BTBRTF/Nev strain (wild type or heterozygous for the *T* gene at the *T/t* locus) were used with similar results. The day a vaginal plug was found was considered day 0 of gestation. Primiparous females were killed by cervical dislocation at 5, 6, 7, 8, 9, and 10 days. Capsules were dissected out of the uterus in cold 4% formaldehyde (freshly prepared from paraformaldehyde) in 0.05 M phosphate buffer, pH 7.4. Intact capsules were fixed for 24 h at 4°C with slight shaking, then washed for another 24 h in several changes of cold 0.1 M phosphate buffer pH 7.4. They were then embedded in polyethylene glycol 1,000 according to Mazurkiewicz and Nakane (6) and cut into 5 μ m sections. Polyethylene glycol was removed in a 5% glycerol solution, and sections were incubated for 1 h at room temperature in sheep Fab anti-mouse immunoglobulins labeled with peroxidase (Institut Pasteur, Paris) at a concentration of 65 μ g/ml in phosphate-buffered saline pH 7.4 (PBS).¹ Sections were then washed in PBS and stained at pH 7.2 in diaminobenzidine and hydrogen peroxide according to Mazurkiewicz and Nakane (6) except for a final H₂O₂ concentration of 0.01%.

Three types of controls were used: (a) incubation with PBS alone instead of peroxidase conjugates. Under the conditions we used, no endogenous peroxidase activity could be detected, either in the erythrocytes or in the uterine epithelium (7); (b) incubation with sheep Fab anti-

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¹ Abbreviations used in this paper: PBS, phosphate-buffered saline, pH 7.4.

human immunoglobulins labeled with peroxidase (Institut Pasteur, Paris): a faint staining was observed only where mouse immunoglobulin concentration was highest. Cross-reactivity between mouse and human immunoglobulins has been described (8); and (c) preincubation with unlabeled sheep Fab anti-mouse immunoglobulins (Institut Pasteur, Paris) completely blocked further staining with peroxidase-labeled sheep Fab anti-mouse immunoglobulins. No inhibition could be observed when unlabeled normal sheep serum was used instead of unlabeled sheep Fab anti-mouse immunoglobulins.

Photographs were taken in the light microscope with and without phase contrast to locate the immunoglobulins revealed by a brown deposit. Hematoxylin counterstaining was sometimes used.

Results

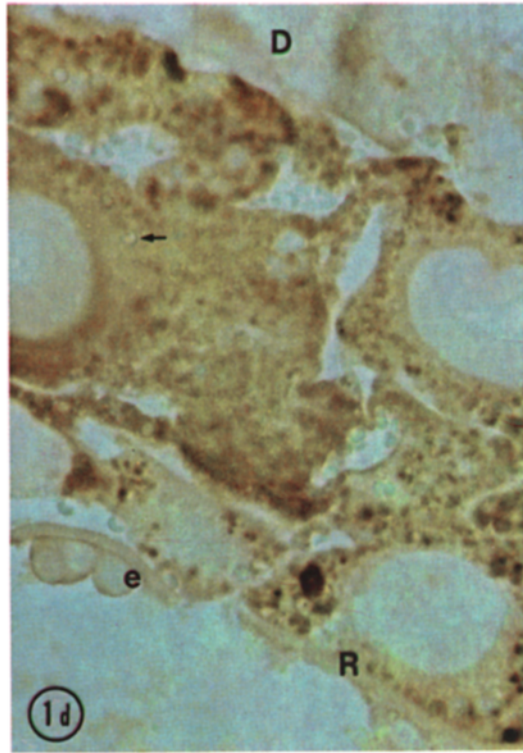
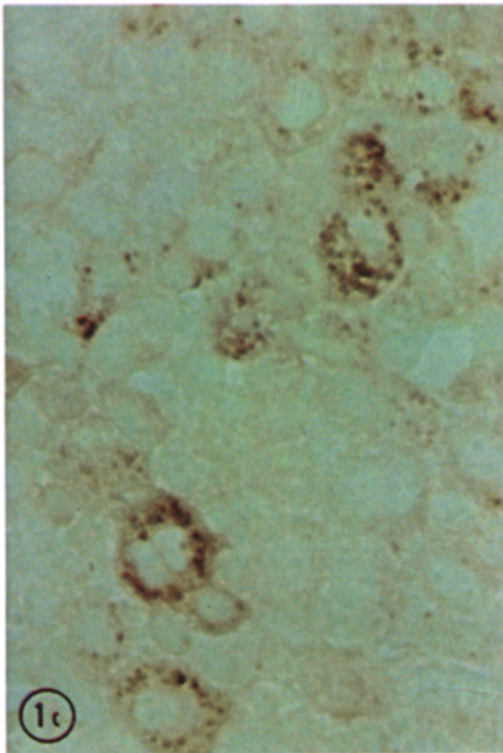
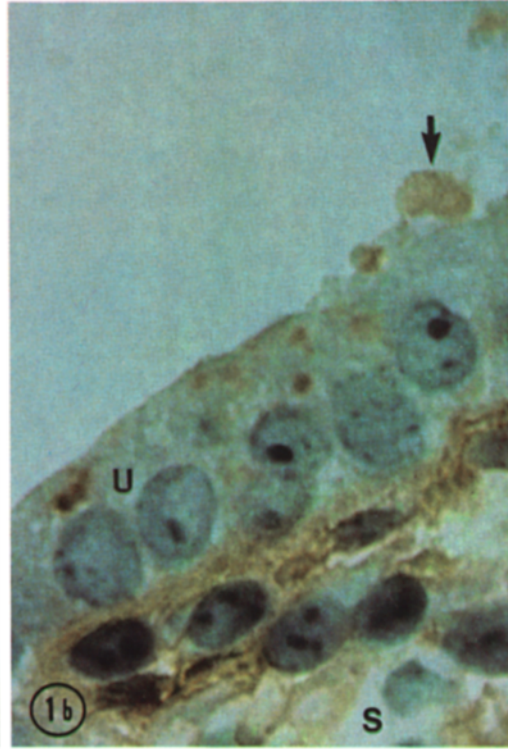
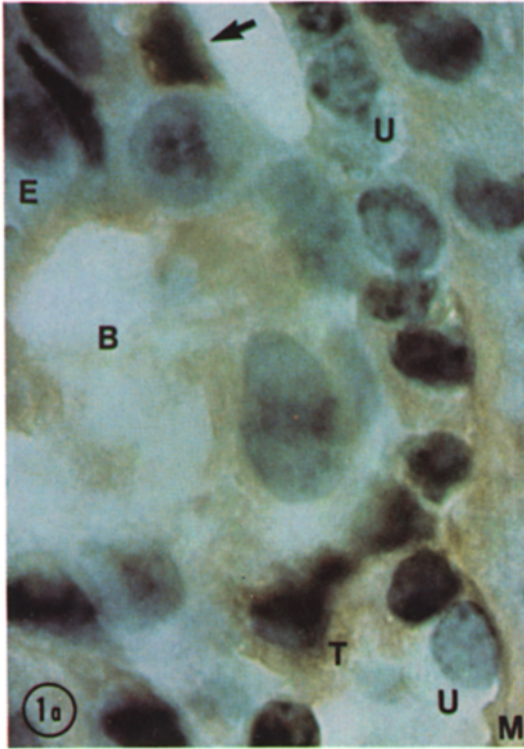
5-Day Stage. On day 5 the embryo is implanted. It often keeps a blastocyst-like appearance with the abembryonic part of the trophoctoderm closely attached to the uterine epithelium. It is surrounded by the uterine stroma undergoing decidualization. Uterine glands are still present at the margin of the capsule.

At this stage the embryo already appears to be surrounded by immunoglobulins. At the implantation site (Fig. 1a) the trophoblast cells in contact with the maternal epithelium contain immunoglobulins in their cytoplasm. Immunoglobulins can also accumulate at this point, and they can sometimes be seen crossing the degenerating epithelial cells from the stroma. Immunoglobulins are also present in some other mural or polar trophoctoderm cells, either as a lining on the surface or within the cytoplasm. The blastocoel contains immunoglobulins as well as the early endoderm. Although immunoglobulins are not visible in the remnant of the uterine lumen, they can be seen lining the apical edges of some of the intact epithelial cells. When the trophoblast cells have invaded the maternal tissue a little more, some of them contain one or two granules of immunoglobulins in their cytoplasm.

In the decidua surrounding the implantation site, few immunoglobulins are seen except on the basement membrane of the uterine epithelium itself. This contrasts with the outer part of the stroma where immunoglobulins are abundant between the cells, chiefly around and in the uterine glands. Plasma cells can often be seen next to the glands (Fig. 2).

Above the implantation site, the closed uterine lumen often contains highly labeled free immunoglobulins and immunoglobulin-containing cells which can be seen crossing the epithelium. Some of these cells look like plasma cells; others are round and mononuclear. Most epithelial cells are unstained. However, some epithelial cells of the closing uterine lumen contain immunoglobulins, either diffusely in the cytoplasm, sometimes in continuity with the stained basement membrane, or as granules in the apices. Some of these look as if they were budding out of the cells. In the area of the uterus between the embryonic capsules, there are also some deposits of immunoglobulins in the lumen (Fig. 1b). These are fairly close to the apices of the cells, and it is difficult to tell whether they are actual parts of the cytoplasm or part of the uterine lumen fluid. The stroma under the epithelium is here heavily labeled. Most of the glands are filled with immunoglobulins, and plasma cells are widely present along the glands throughout the mucosa. In some instances, immunoglobulins can be seen crossing the epithelium of a gland.

6-Day Stage. The 6-day embryo is a typical egg cylinder containing embry-



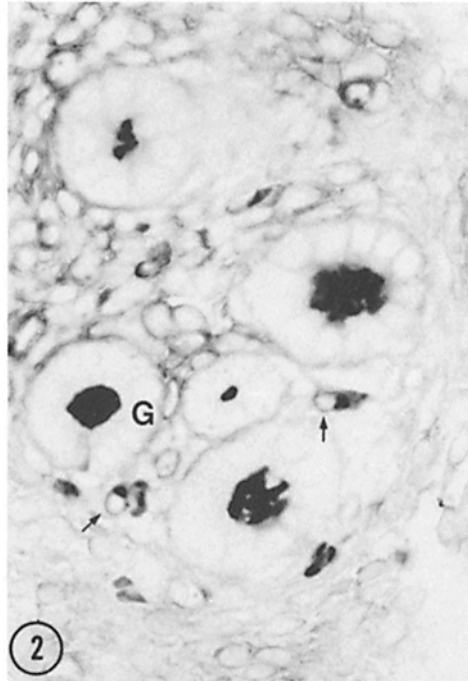


FIG. 2. Uterine glands (G) and stroma at the margin of the capsule in a 5-day-old pregnant uterus. The lumens of the glands contain large amounts of immunoglobulins (dark deposits). Plasma cells (arrows) are next to the glands; the stroma is infiltrated with immunoglobulins. ($\times 430$; no counterstaining).

onic and extraembryonic components. The ectoplacental cone is well differentiated, some trophoblastic giant cells can be seen, and the decidual reaction is increasing. The main feature at this stage is the presence of immunoglobulins in the vacuolated visceral endoderm (Fig. 3). All vacuolated endodermal cells contain immunoglobulins as granules in their apical cytoplasm, and sometimes more diffusely in the rest of the cell with occasionally a condensation around the nucleus. Depending upon the developmental stage reached in each individual

FIG. 1. (a) Junction of the trophoblast (T) and the uterine epithelium (U) in a 5-day-old, just implanted, mouse embryo. Immunoglobulins (brown deposit) are present in the trophoblast cells at the junction as well as around the inner cell mass (arrow). They are also present in the blastocoel (B), in the apices of the early endoderm cells (E) and on the uterine epithelium basement membrane (M) ($\times 1360$; hematoxylin counterstaining). (b) Uterine lumen epithelium (U) between the implantation sites of a 5-day-old pregnant uterus. Granules of immunoglobulins are present in the epithelial cells and in the lumen, either free or within cytoplasmic processes (arrow). Immunoglobulins outline the basement membrane and are abundant in the stroma (S) ($\times 540$; hematoxylin counterstaining). (c) 7-day-old decidua. Some of the cells contain granules of immunoglobulins in their cytoplasm ($\times 540$; no counterstaining). (d) 9-day-old trophoblast giant cells. They contain large amounts of immunoglobulins, either as granular brown deposits or diffusely in the cytoplasm. Erythrocytes are present in the adjacent capillaries as well as within the giant cells themselves (arrow). Reichert's membrane (R) and distal endoderm cells (e) are moderately stained. The decidua (D) is very faintly stained ($\times 1360$; no counterstaining).

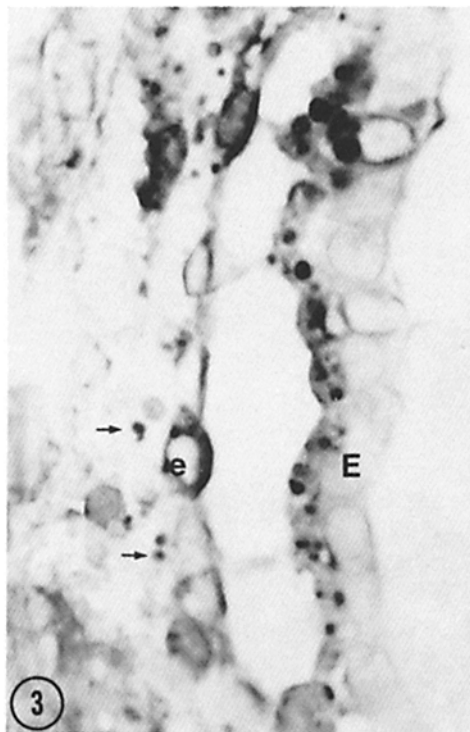


FIG. 3. Longitudinal section of the extra-embryonic area of a 6-day-old embryo. All proximal endoderm cells (E) contain granules of immunoglobulins in their apices; in some cells immunoglobulins are also present in the rest of the cytoplasm and around the nucleus. Distal endoderm cells (e) are outlined by immunoglobulins. Immunoglobulin granules (arrow) are also present in the capillaries and/or in the early trophoblast cells next to Reichert's membrane. ($\times 430$; no counterstaining).

embryo, immunoglobulins are present in endodermal cells all around the embryo, or, in later stages, are restricted to an area covering the extra-embryonic endoderm and the upper third of the embryonic endoderm. When endoderm cells become flat around the tip of the embryonic ectoderm, they no longer contain any immunoglobulins. Some very faint staining can occasionally be observed between the cells deep in the ectoderm, but as a rule the ectoderm and the ectoplacental cone are absolutely devoid of any immunoglobulins. Reichert's membrane is slightly stained, but the luminal surface of some distal endoderm cells is often lined by a thick layer of immunoglobulins (Fig. 3).

Some trophoblast cells already contain large amounts of immunoglobulins. They appear as granules in the cytoplasm and sometimes concentrate in the perinuclear region. However, most of the invasive trophoblast giant cells are negative. The positive ones are sometimes more numerous on the edges of the ectoplacental cone. The capillaries next to the trophoblast and the embryo sometimes contain granules of positive material (Fig. 3). This is different from the usual staining of immunoglobulins in the blood vessels, where they appear as large patches uniformly stained, or as a thick lining on the endothelium. Whether the granules sometimes observed here are already in the cytoplasmic

processes of the developing trophoblastic cells or free in the capillaries, they may correspond to what are suggested to be antigen-antibody complexes in the immunofluorescence studies of Voisin and Chaouat (2) in mouse placentae and of Tung (9) in kidneys of pregnant mice and guinea pigs. These granules can still be found at 7 days.

The decidua surrounding the embryo is generally less stained than the outer part of the capsule; it contains polygonal decidual cells that are closely packed and surrounded by a layer of immunoglobulins between each cell; some immunoglobulins are also present in the peripheral cytoplasm. In outer areas of the capsule, in what may still be part of the swollen edematous uterine stroma undergoing decidual transformation, round cells are often seen with highly labeled cytoplasm either with granules or with a diffuse staining. Some of these cells are binucleate or multinucleate. Oval cells with a densely stained cytoplasm are often present on the edges of the capsule. Some areas of the boundary between trophoblast and decidua are heavily labeled with thick, apparently acellular deposits of immunoglobulins, although this does not form a continuous sheath. This feature remains detectable at 7 and 8 days.

7-Day Stage. On the 7th day, when the primitive streak becomes apparent, the overall appearance of the distribution of immunoglobulins is similar to the 6-day stage. Vacuolated visceral endoderm cells take up immunoglobulins, but the rest of the embryo, the ectoplacental cone in particular, is unstained. Traces of immunoglobulins can occasionally be seen in the proamniotic cavity. Reichert's membrane is usually stained. More trophoblast giant cells are stained, as a rule, around the embryo. Some contain immunoglobulins in limited amount, while others may contain large quantities. Immunoglobulins appear as granules or diffusely in the cytoplasm, and sometimes sharply outline the nucleus. In the decidua the cell surfaces are coated with immunoglobulins; these can also be seen in the cytoplasm (Fig. 4). Some of them also contain granules of immunoglobulins in their cytoplasm (Fig. 1c); such cells can be found throughout the decidua but chiefly in the outer part of the capsule.

8-Day Stage. At 8 days the embryo is now separated into various cavities: amniotic cavity, exocoel, and ectoplacental cavity. The yolk sac begins to differentiate out of the visceral endoderm, and the allantois from the primitive streak. The embryo is now surrounded by numerous obvious trophoblastic giant cells.

The fluids of the various cavities of the embryo appear to contain immunoglobulins: the edges of the embryonic and extraembryonic cells facing the cavities are outlined by immunoglobulins and, in some sections, the actual stained fluid can be seen, especially in the yolk sac cavity between the stained Reichert's membrane and the embryo (Fig. 5). All the visceral yolk sac cells contain granules of immunoglobulins as observed earlier in the extraembryonic endoderm. Numerous trophoblastic giant cells now contain significant amounts of immunoglobulins. The decidua and the cells on the edges of the capsule keep the same appearance as on day 7.

9- and 10-Day Stages. On day 9 and 10, the embryo is surrounded by the amnion and the yolk sac membrane. Most of the trophoblast giant cells are heavily concentrating immunoglobulins, which appear as granules but also as a

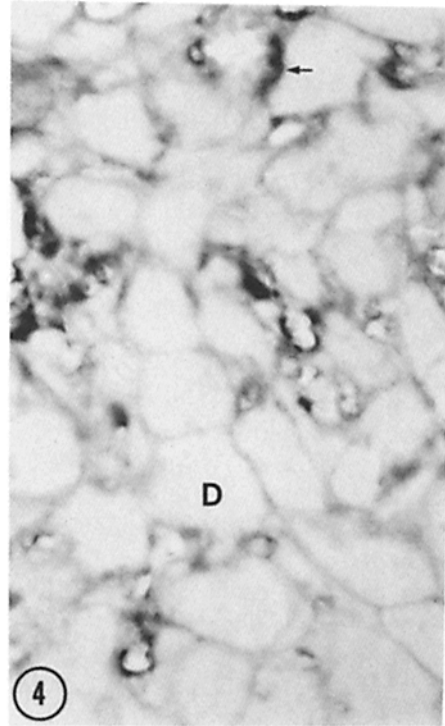


FIG. 4. 7-day-old decidua. Each decidual cell (D) is surrounded by a layer of immunoglobulins. Heavier deposits are present on the edges of the capillaries (arrow). ($\times 430$; no counterstaining).

diffuse slight staining of the cytoplasm (Fig. 1*d*). As a whole the trophoblast giant cells constitute a layer filled with immunoglobulins, although a few giant cells appear less stained than their immediate neighbors. Most of the giant cells also phagocytize erythrocytes. Reichert's membrane is usually stained, often very heavily, as well as the fluid in the yolk cavity. Visceral yolk sac cells contain immunoglobulin granules in their apices as well as immunoglobulins diffusely present throughout the cell (Fig. 6). Immunoglobulins are also sometimes present in the vitelline vessels and on the surface of some of the hemocytoblasts in the yolk sac blood islands.

The extraembryonic coelom between yolk sac membrane and amnion is sometimes faintly stained, as well as the edges of the amnion cells and the early epidermis of the embryo proper. This suggests again that these structures are bathed in immunoglobulin-containing fluids. In the embryo the gut is the only organ containing notable amounts of immunoglobulins. In cross-sections the gut lumen is filled with immunoglobulins (Fig. 7). These are also present on the luminal part of the gut epithelial cells, very rarely as granules, usually as a diffuse staining; some immunoglobulins are occasionally present in the entire epithelial cell and in the underlying tissue. In some sections the junctions between yolk sac membrane and gut can be seen: immunoglobulin-containing yolk sac fluid seems to be "swallowed" in the gut (Fig. 8). Sagittal sections of the embryo show immunoglobulins to be present on the whole length of the gut. By

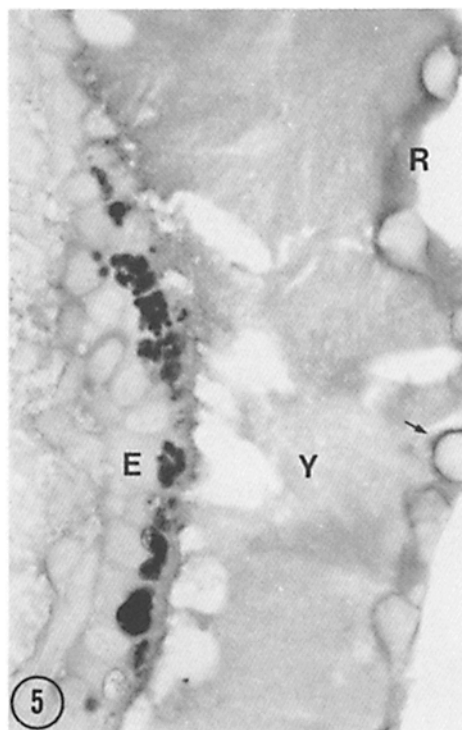


FIG. 5. Longitudinal section of the yolk cavity of an 8-day-old embryo. The yolk cavity (Y) is filled with immunoglobulins. Visceral yolk sac cells (E) contain immunoglobulins in their apices. Reichert's membrane (R) and the edges of the distal endoderm cells (arrow) are stained as well. ($\times 430$; no counterstaining).

contrast the lung buds do not contain any immunoglobulin. The edges of the lumens of embryonic blood vessels are sometimes faintly outlined.

The decidua cells show much less staining compared to earlier stages, although most are still coated with immunoglobulins. In the forming chorio-allantoic placenta little staining is found as compared with the giant cells and the yolk sac around the embryo. A layer of trophoblast giant cells remains stained through the placenta, but the area where allantois, ectoplacental cone, and chorion meet is almost devoid of immunoglobulins. When chorionic villi can be seen, they are stained only on the maternal side, the embryonic vessels filled with primary blood cells being unstained. The maternal decidual cells above the forming placenta usually keep their intercellular coating of immunoglobulins, and the lumens of the maternal vessels in the placenta are positive. The new uterine lumen is being formed at this stage; when it can be seen on the section it is filled with immunoglobulins.

Discussion

Transmission of maternal immunoglobulins to the mouse embryo seems to take place at the time of implantation and during the several days that follow. Immediately before or at the time of implantation, the trophectoderm of the blastocyst becomes coated with immunoglobulins and apparently transports



FIG. 6. 9-day-old visceral yolk sac membrane. Endoderm-derived cells (E) contain granules of immunoglobulins in their apices and also as a diffuse staining of some cells. ($\times 430$; no counterstaining).

them to the blastocoel. As the implanted blastocyst grows, immunoglobulins can be found in the vacuolated endoderm surrounding the egg cylinder, and then, in growing amounts in the trophoblastic giant cells and in the various fluids surrounding the embryo. Endoderm-derived yolk sac cells actively take up immunoglobulins from the yolk cavity, and trophoblast giant cells soon constitute a "barrier" filled with immunoglobulins around the embryo.

It is very likely that most, if not all, of the immunoglobulins observed in this study are of maternal origin: in the mouse the first immunoglobulin-containing cells have been detected in the fetal liver at 12 days (10). It is, however, not possible to indicate what classes the observed immunoglobulins belong to: the reagent used in this study is prepared by immunoadsorption on polymers of mouse IgG and can thus react with mouse IgG and mouse immunoglobulin light chains (J. de Saint Martin, personal communication).

Immunoglobulins in Pregnant Uteri. Few morphological studies have been reported on the distribution of immunoglobulins in the uterus and embryo during pregnancy. Immunofluorescence studies of human placenta (4, 11) have shown IgG to be present on the trophoblastic basement membranes both at term and at 10–18 wk gestation. In younger human trophoblast, however, IgG can also be detected as globules in the cytoplasm (12). In the 15-day mouse placenta, immunofluorescence studies show the presence of IgG on various structures such as giant cells, Reichert's membrane, and the spongiotrophoblast (2); in this study, the amount of deposited immunoglobulins seemed to increase with multi-

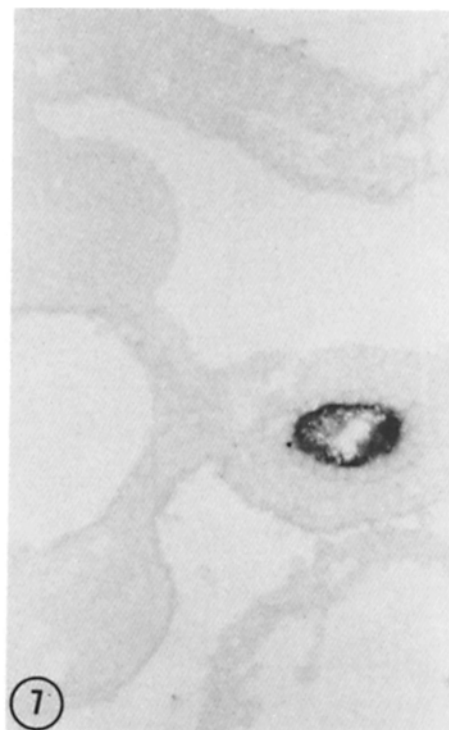


FIG. 7. Transverse section of a 10-day-old embryonic gut. The lumen contains large amounts of immunoglobulins. ($\times 170$; no counterstaining).

parity in hybrid matings. In our study, primiparous females in inbred matings seem to produce an already detectable and early transfer of immunoglobulins although mostly outside of the chorioallantoic placenta. Relatively early stages have been studied in the rat by Anderson (13): injected ^{131}I -labeled homologous gamma globulins are detected in locations similar to those reported here for 9-10 day mouse uteri.

Additional information on the presence of immunoglobulins in early embryonic development can be gleaned from purely histochemical studies. Immunoglobulins are glycoproteins and thus may give a PAS-positive amylase-resistant staining (14). A similarity in the distribution of PAS-positive enzyme-resistant material and immunoglobulins in early postimplantation mouse embryos was indeed suggested by Chiquoine (15). His findings and those of other investigators in mice and rats (16-19) correlate well with the data presented in this paper. Furthermore, in the 16-day-old mouse placenta, a PAS-positive enzyme-resistant fibrinoid layer is present at the junction of trophoblast cells and decidua (20), a location where heavy deposits of immunoglobulins were sometimes observed in our study at earlier stages.

Possible Mechanisms of Distribution and Transmission. In the uterus the main features reported in this study are the large amounts of immunoglobulins and plasma cells present in the stroma and the presence of immunoglobulins between and in the decidua cells in the days after implantation.

The presence of numerous plasma cells in the endometrium at 4 days of

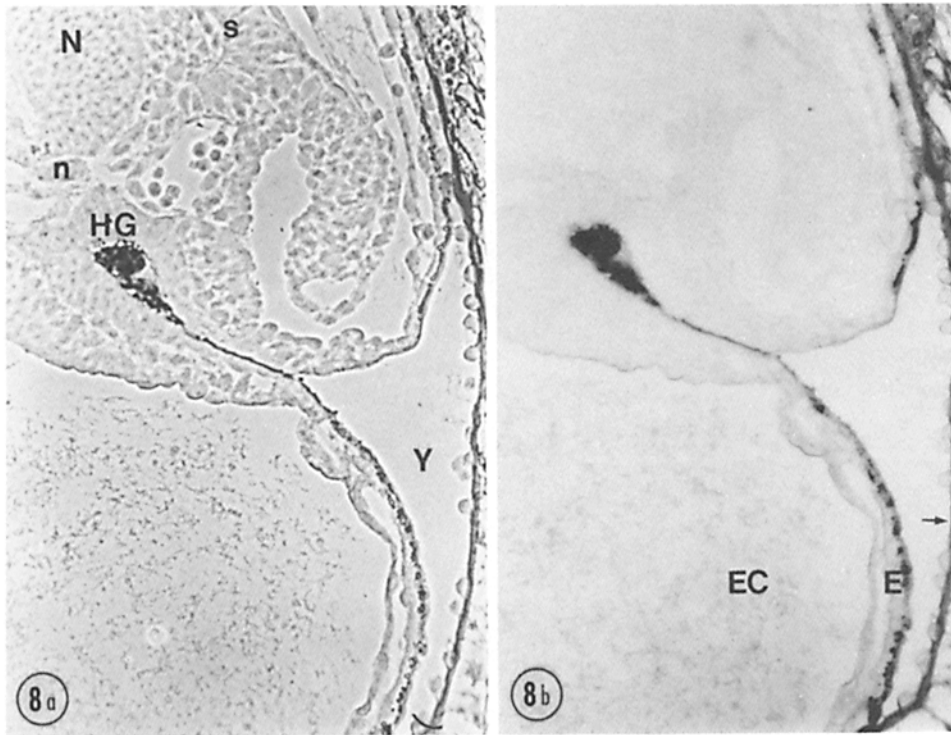


FIG. 8. Junction between the hindgut (HG) and the yolk cavity (Y) in a 10-day-old embryo. (a): under phase contrast, showing a portion of neural tube (N), notochord (n), and a somite (s). (b): without phase contrast, immunoglobulins are abundant in the gut which communicates with the yolk cavity. Visceral yolk sac cells (E) contain immunoglobulins in their apices. Reichert's membrane (arrow) is heavily stained. Some staining also occurs in the exocoelom (EC). ($\times 170$; no counterstaining).

gestation in the mouse has already been reported (16, 21). Several results suggest that there may be a hormone-dependent regulation of immune cell stimulation and migration during the estrous cycle and pregnancy (22–25). Such a programmed mechanism may be responsible for plasma cell invasion of the uterine stroma at the time of implantation. Besides the probable secretion of immunoglobulins by the plasma cells into the stroma or into the uterine glands, circulating immunoglobulins may be delivered directly to the stroma from uterine blood vessels. An increased permeability of these vessels has been indeed shown at the time of implantation in the rat (26).

In the decidua the presence of clearly detectable amounts of immunoglobulins between the cells and in their cytoplasm may reflect the use of immunoglobulins as a means of nutrition by the rapidly dividing and growing decidua cells. One cannot, however, reject the idea that such a coating may also have an immunological significance, namely to act in some way to protect maternal tissue from excessive aggression by trophoblast.

Mechanisms by which maternal immunoglobulins are transmitted to the early embryo can be suggested by previous studies dealing with free blastocysts or much later stages of development. In the 5-day-old, just-implanted blastocyst,

it is likely that the mechanisms of transfer are similar to the ones occurring in the free blastocyst. Immunoglobulins in the uterine lumen are probably coming from the uterine glands and may also cross the uterine luminal epithelium directly. Some of the immunoglobulins which can be seen in the cytoplasm of the uterine epithelium cells (Fig. 1*a*) may however be in the process of being resorbed: increased resorptive activity of the epithelium towards exogenous proteins injected into the lumen has indeed been shown in the rat before implantation (7).

At the implantation site proper, immunoglobulins are present in some trophoblast cells, even sometimes in those that are not closely associated with maternal tissue (Fig. 1*b*). Trophoblast uptake of immunoglobulins may thus occur from the uterine fluid: the mouse zona pellucida is permeable to immunoglobulins (27), and trophoblast cells before implantation possess phagocytic and pinocytotic properties (28, 29). One cannot rule out, however, that at the site of contact between trophoblast and uterine epithelium, the displacement of the epithelial cells (30) may allow the trophoblast cells to reach a capillary or the nearby stroma which contains large amounts of immunoglobulins.

Some immunoglobulins are likely to be able to cross the trophoblast, reach the blastocoel, and be picked up at the blastocoelic pole of the endoderm cells. It is known that several proteins migrating in the gamma globulin zone in electrophoresis are present in the rabbit blastocoel (31), and protein markers can be transferred via rabbit and rat trophoblast to the blastocoel where they are taken up by endoderm cells (29, 32). Such a mechanism is very likely to account also for the presence of immunoglobulins in the proximal endoderm cells in 6- and 7-day-old mouse embryos.

In the days after implantation the proximal endoderm or endoderm-derived yolk sac cells contain significant amounts of immunoglobulins. The mechanisms of transfer of several proteins through the visceral yolk sac have been the object of extensive studies at later stages of pregnancy in mammals (for review see reference 33) and are probably similar to the mechanisms used to transfer immunoglobulins at the early stages studied in this report. Briefly, protein markers appear to first come into close contact with the microvillous membrane of the endoderm cell and are then taken into vesicles by endo or micropinocytosis at the apex of the cell; some of the markers can subsequently be seen at the base of the cell, and presumably cross the basement membrane, since they can be found in the vitelline vessels endothelium and in the macrophages present in the vessels. Only a small fraction of homologous gamma globulins reaches the fetal circulation when injected into the pregnant rabbit uterine cavity (34). Furthermore, the integrity of the Fc portion of immunoglobulin molecules is essential for the transfer of immunoglobulins to the fetus (5). In the 24-day-old pregnant rabbit, some homologous IgG is bound to the glycocalyx of the visceral yolk sac endoderm cells and engulfed by micropinocytosis, while others remain free in the vesicles lumen (35). According to Brambell's hypothesis (5), only the immunoglobulins bound to visceral yolk sac cell membranes through Fc receptors are protected against degradation by lysosomal enzymes when transported in the cell. IgG from various species can be recovered in the 24-day rabbit fetal circulation after injection into the uterine cavity (36). Their rates of transmission are lower than that of homologous rabbit IgG and vary from species to

species; bovine IgG is almost not transmitted at all. Consistent with Brambell's hypothesis bovine IgG does not bind to purified plasma membrane vesicles of rabbit visceral yolk sac, while homologous IgG does (37). Presumably, depending upon their degree of binding to rabbit yolk sac Fc receptors, IgGs of other species are degraded to a variable extent, thus accounting for their various rates of transmission. It is likely that a similar mechanism occurs in the mouse: Fc receptors binding human and rabbit immunoglobulins as well as homologous mouse immunoglobulins have been detected on the mouse 15 day yolk sac visceral endoderm by EA rosette formation (38). It is quite possible that such receptors also exist on the endoderm of mouse embryos at the earlier stages that we have studied, and that similar mechanisms of IgG uptake and transmission account for the presence of immunoglobulins in the various embryonic cavities as early as 7 and 8 days.

Trophoblast giant cells may pick up immunoglobulins from the adjacent maternal capillaries as they phagocytize erythrocytes and other maternal blood components (39). However, they may also bind immunoglobulins through membrane Fc receptors. Fc receptor-bearing cells are present in both mouse and human placenta (38, 40). It is not known whether some of the immunoglobulins present in the giant cells are degraded, although the latter contain the necessary lysosomal enzymes (19). Trophoblast giant cells are probably transferring at least part of the immunoglobulins they contain into the yolk cavity as suggested by studies with protein markers (41-44).

In the embryo proper, at 9-10 days, immunoglobulins are present in the early embryonic gut. They probably reach it directly through the yolk cavity as seen in Fig. 8.

Immunoglobulins are present in the vitelline vessels at 9 days. This is consistent with the mechanism of transmission at later stages, but the staining of the membrane of some hemocytoblasts is not clearly understood. Since the methods used in this study did not detect any endogenous peroxidase and no intracellular staining was obvious, it seems most likely that some of these hemocytoblasts are able to bind immunoglobulins on their surface. As stated earlier, immunoglobulin-containing cells have been described only at 12 days in the mouse fetal liver (10), although in the 9-10 day yolk sac cells are present which, when transferred into thymectomized and irradiated mice of another strain, are able to produce IgG specific for their own strain (45). Various different cell types can be found in the 10-day mouse vitelline vessels, and many lymphoid cells are present (46). It may be that some of these possess surface receptors for maternal immunoglobulins.

Possible Roles of Maternal Immunoglobulins. A few hypotheses can be presented to account for the role of such an early transmission of immunoglobulins from the mother to the embryo, as described in this paper. This transfer is clearly detectable in significant amounts in the days immediately after implantation, and it occurs in primiparous and inbred matings, where, as far as transplantation antigens are concerned, the embryo and the mother should not differ. Furthermore, it is unlikely that, at these early stages, a significant amount of the antibodies detected here could be elicited by the presence of the embryo. Maternal immunoglobulins as well as other maternal serum proteins are almost surely a source of amino acids and carbohydrates for the embryo;

immunoglobulins may thus be detected here simply as markers of the transfer of maternal serum proteins. The role of the trophoblast giant cells and of the visceral endoderm in the nutrition of the mouse embryo was suggested long ago (47).

However, such an early and intimate presence of immunoglobulins around the embryo, in the trophoblastic giant cells and in all cavities and membranes of the embryo, could be of immunological significance. For the reasons mentioned above, it is unlikely that these antibodies are elicited by the presence of the embryo. However, naturally occurring antibodies directed against embryonic antigens or cross-reacting with embryonic components could be part of the immunoglobulins detected in our study. High levels of naturally occurring autoantibodies directed against F9, a line of embryonal carcinoma cells, are present in various strains of mice (48). Antibodies of that kind may be present in the trophoblast giant cells and around the embryo. These naturally occurring autoantibodies could be potentially harmful to the embryo. If this is the case, our results indicate that several lines of defense against maternal immunoglobulins are present, the first one being trophoblast giant cells. Selective destruction in the trophoblast of possibly harmful antibodies directed against fetal antigens has been suggested by Morisada et al. (49). Our results show that maternal antibodies are indeed present in early mouse trophoblast giant cells, and that they exist in two forms, globular and diffuse, in the cytoplasm. One of these forms might correspond to harmful antigen-antibody complexes which will be degraded by proteolytic enzymes as suggested by Morisada et al., while the others are protected and transmitted to the yolk cavity. A similar control of potentially harmful antibodies might occur at the visceral yolk sac and/or gut level for the antibodies having crossed the trophoblast and Reichert's membrane.

On the other hand, since cytotoxic activity of antibody in the mouse is not well demonstrated *in vivo*, the naturally occurring antibodies may have a blocking role similar to the one supposed to occur in hybrid matings (2, 50). In hybrid matings, postimplantation embryonic sacs can clearly be immunogenic to the mother (51, 52); although the exact antigenic status of the surrounding trophoblast is not yet clearly established (52-57), even within inbred matings, placenta or trophoblast cells may express stage or tissue-specific antigens which could elicit an immune response from the mother after one or two pregnancies (58, 59). Enhancing factors able to prevent the killing of embryonic cells by sensitized lymphocytes are present in the sera of female mice when they are pregnant by males of the strain used for sensitizing the effector cells (50). In the human, the IgG fraction of sera from pregnant women has been claimed to protect trophoblast cells from lysis by maternal leukocytes (60). Enhancing antibodies might contribute to protecting the embryo by masking antigenic sites which could be the target of cytotoxic reactions from the mother in a way similar to that possibly occurring in tumor-bearing animals (61). If some maternal immunoglobulins actually provide enhancing effects in normal pregnancies, and if the results described in this paper apply to hybrid matings as well, the early transport of immunoglobulins from the mother to the embryo would give the enhancing antibody population access to the embryonic antigens. On the surface of the trophoblast cells proper, specifically bound antibodies could be internal-

ized, thus providing an antigenic modulation as hypothesized by Allison (62). Alternatively, since part of the binding of antibodies may be due to Fc receptors, it is quite possible that circulating antibodies and/or antigen-antibody complexes are bound to the membrane and prevent recognition of nearby antigenic sites by a nonspecific sterical masking.

Embryos homozygous for t^{1073} , a recessive lethal gene at the T/t locus, fail to grow beyond the 6-day stage and die a few days later without developing a normally invasive trophoblast (63). Studies are in progress to test whether their failure to thrive is in some way related to an abnormality in the transfer of nutritive serum proteins or a lack of immunoglobulin protection.

In conclusion, the distribution of maternal immunoglobulins as presented in our study may have various meanings: (a) it may simply be the reflection of the transfer of maternal serum proteins as a means of nutrition for the embryo; (b) nonspecific binding of serum immunoglobulins and/or antigen-antibody complexes through Fc receptors could mask antigens at the surface of the trophoblast cell; (c) trophoblast cells may ingest and degrade potentially harmful antibodies; and (d) lastly, in hybrid matings enhancing antibodies could gain access to the foreign antigenic sites on the trophoblast and in the embryo proper.

Summary

The distribution of maternal immunoglobulins in the mouse uterus and embryo in the days after implantation has been studied on sections incubated with sheep Fab anti-mouse immunoglobulins labeled with peroxidase. At the time of implantation the blastocyst is already surrounded by immunoglobulins that are also present in the blastocoel and early endoderm; uterine glands contain large amounts of immunoglobulins. Later, immunoglobulins are concentrated in the vacuolated endoderm, then the visceral yolk sac and the embryonic gut. They are also present in the various cavities of the embryo. Trophoblast cells progressively contain increasing amounts of immunoglobulins. In the decidua, immunoglobulins coat the cells and also occasionally appear as cytoplasmic granules. The early presence of maternal immunoglobulins may represent the transfer of serum proteins as a means of nutrition for the embryo. It is also very likely to have an immunological significance in the protection of the embryo.

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