Temporal and spatial pattern of expression of the HDL receptor SR-BI during murine embryogenesis

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Abstract During rodent fetal development, maternal lipoproteins can be sources of cholesterol for the membrane synthesis required for tissue growth in the developing embryo and for steroid hormone production in the extraembryonic tissues. Although the mechanisms underlying the maternal–fetal lipoprotein cholesterol transport system are not well defined, the placenta and yolk sac seem to play major roles in this process, serving as functionally active interfaces between maternal circulation and the embryo. In rodents, the principal cholesterol transporter in the plasma is HDL, and the HDL receptor SR-BI is a physiologically important mediator of cholesterol uptake in adult liver and steroidogenic tissues. To begin to investigate SR-BI's role in maternal cholesterol uptake by the fetus, we used immunofluorescence microscopy to determine the pattern of SR-BI expression during murine embryogenesis. At day E7.5 in gestation, there was significant SR-BI expression in endothelial cells of the decidua, but little in intraembryonic and extraembryonic tissues. By day E8.5, there was a dramatic increase in SR-BI expression in the trophoblast cells which surround the developing embryo. Beginning at day E10, SR-BI was expressed in both the placenta and yolk sac. The expression in these extraembryonic tissues was correlated with significant uptake of fluorescent dye by the yolk sac visceral endodermal cells from DiI-labeled HDL injected into pregnant mice. Within the embryo proper, SR-BI expression appeared by day E14.5 at high levels in the adrenal gland. SR-BI expression was not detected in the embryonic liver through day E17.5 of gestation; however, it could be observed in neonatal livers. These findings suggest that SR-BI may play a role in the rodent maternal–fetal lipoprotein cholesterol transport system, supplying HDL cholesterol for either membrane or steroid hormone synthesis, or both.—**Hatzopoulos, A. K., A. Rigotti, R. D. Rosenberg, and M. Krieger.** Temporal and spatial pattern of expression of the HDL receptor SR-BI during murine embryogenesis. *J. Lipid Res.* 1998. **39:** 495–508.

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The high growth rate of embryos during fetal development imposes an enormous requirement for nutrients, including cholesterol, a critical component in membranes. Fetal development also requires large amounts of cholesterol for steroid hormone synthesis by embryonic and extraembryonic tissues, e.g., the placenta (1–3). Different experimental models have demonstrated the importance of cholesterol during development, particularly with respect to the growth and differentiation of brain tissue (4–6).

Although the rate of cholesterol synthesis in the fetus is elevated compared to that in the adult (7–13), several studies in various mammalian species suggest that endogenous synthesis may not always be able to provide for the considerable cholesterol requirements of the fetal embryonic and extraembryonic tissues (14–18). These studies indicate that a significant fraction of cholesterol may, at least in some species, be derived from lipoproteins in the maternal circulatory system, although there is controversy in this area (18, 19).

One model for cholesterol transfer from maternal circulation to the developing embryo postulates that maternal lipoproteins bind to and are internalized via the yolk sac and the placenta (9, 14–18, 20–23). The yolk sac visceral endoderm cells then apparently repackage the maternal lipids (e.g., cholesterol, fat-soluble vitamins) as well as endogenously synthesized cholesterol into lipoproteins that are secreted into the vitelline circulation and subsequently deliver their lipid to developing embryonic tissues (24). The following observations are consistent with this model. *1*) Several members of the LDL receptor superfamily, including the LDL and VLDL receptors, LDL receptor-related

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; ac-LDL, acetylated LDL; LRP, LDL receptor-related protein; apoA-I, apolipoprotein A-I; apoB, apolipoprotein B; apoE, apolipoprotein E; SR-BI, scavenger receptor class B, type I; TM, thrombomodulin; DiI, D-282: 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate.

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protein (LRP) and gp330/megalin, are expressed in the placenta and the yolk sac (25–31); *2*) placenta and yolk sac can bind and internalize maternal lipoproteins both in vivo and in vitro, indicating the presence of functionally active lipoprotein receptors (9, 17, 18, 21, 22, 32); and *3*) the yolk sac synthesizes apolipoproteins (e.g., apoB, apoE, apoA-I) and apolipoprotein B-containing lipoproteins, presumably for delivery of lipid to embryonic tissues (33–38). Recent studies of the consequences of apolipoprotein-B and gp330/megalin gene disruption are consistent with this model for maternal–fetal lipoprotein cholesterol transport (39–42).

In rodents, most of the plasma cholesterol is transported in high density lipoprotein (HDL). Studies in the hamster have indicated that maternal HDL cholesterol is efficiently removed by the yolk sac and the placenta, and this accounts for a significant fraction of the cholesterol incorporated into growing fetal tissues (18). Furthermore, these studies have suggested that clearance rates of HDL cholesterol by these two organs are much higher than those of LDL cholesterol (18). The mechanism(s) of fetal clearance of maternal HDL cholesteryl has not been established.

In adult rodents, the major mechanism by which the cholesteryl esters of HDL are transferred from the lipoprotein to target cells (liver, steroidogenic tissues) is fundamentally different from the receptor-mediated endocytic pathway used for the delivery of LDL cholesterol to cells (43). This novel mechanism, called selective lipid uptake, involves HDL binding to surface receptors, transfer of lipid to the cell membrane, and subsequent dissociation of lipid-depleted HDL particles from the cells (for reviews see 44–46).

Recent studies have demonstrated that the class B, type I scavenger receptor, SR-BI, is a cell surface HDL receptor that mediates selective lipid uptake (47). In adult mice and rats SR-BI is expressed most abundantly in the liver and steroidogenic tissues, and its expression in vivo is coordinately regulated with HDL binding to tissues and with steroidogenesis (48–50). Adenovirus-mediated hepatic overexpression of SR-BI in mice causes a dramatic decrease in plasma HDL cholesterol and increase in biliary cholesterol (51). Furthermore, targeted disruption of the SR-BI gene in mice leads to substantial increases in plasma HDL cholesterol (30–40% in heterozygous mutants and 2.2-fold in homozygous null mutants) and a decrease in the cholesterol content of the adrenal glands (52). Thus, current evidence strongly supports the proposal that SR-BI is a physiologically relevant receptor that mediates the delivery of HDL cholesterol to tissues in adults (46, 47, 52). Analysis of the genotypes of mice from crosses of heterozygous mutants showed a gene–dose-dependent decrease in mutant animals relative to that expected from Mendelian inheritance, suggesting that SR-BI may play a role during embryonic development, perhaps by transferring maternal HDL cholesterol to the yolk sac and the placenta.

To begin to address this question, we have determined the expression pattern of SR-BI during murine embryonic development using a rabbit polyclonal antibody that specifically recognizes the C-terminus of SR-BI. Analysis of its expression at various embryonic stages, starting at post-gastrulation day E7.5 (birth on day E21), demonstrated that SR-BI is expressed during embryogenesis in a pattern consistent with its playing a role in maternal cholesterol uptake from lipoproteins into the developing embryo.

MATERIALS AND METHODS

Preparation of tissues

Mouse (C57BL6, 129/J, and BALBc) embryos were isolated at various developmental stages on days E7.5, E8.5, E9.5, E10.5, E11.5, E12.5, E14.5, and E17.5 of development (day E0.5 was assigned as the midday of detection of the vaginal plug). The embryos were immediately submerged in Tissue Freezing Medium (Electron Microscopy Sciences) and allowed to freeze on dry ice. Neonates and dissected adult organs were similarly processed. Cryosections of tissues were prepared and stored at −80°C. To control for the specificity of SR-BI antibody staining, day E12.5 embryos from a cross between a homozygous SR-BI$^{-/-}$ male and a heterozygous SR-BI$^{+/-}$ female were isolated, a portion of the embryonic tissue was used for PCR genotyping (52), and the remaining samples were processed as described above. Analysis of the placenta and yolk sac from the mutants confirmed the specificity of the antibody staining. Relative to wild-type embryos, there was reduced signal in heterozygous embryos and no staining of homozygous mutant tissue (data not shown).

Immunostaining

Cryosections on glass slides were removed from −80°C, air-dried for 10–20 min and fixed at 4°C in a 1:1 mix of acetone–methanol for 5 min. Fixed slides were washed twice for 10 min in PBS (PBS: 100 mm NaCl, 4.5 mm KCl, 3 mm Na_2HPO_4, 3 mm KH_2PO_4) and then blocked with 1% BSA and 0.05% saponin in PBS at room temperature or 4°C for 2–3 h. Subsequently, primary antibodies were added after dilution in blocking buffer. We used a protein A purified rabbit polyclonal anti-mouse SR-BI at 3 μg/ml and a rat monoclonal anti-mouse thrombomodulin (kindly provided by Dr. Steven Kennel) at 30 ng/ml. Incubations were conducted either overnight at 4°C or for several hours at room tem-

perature. The slides were then washed twice for 10 min with PBS and secondary antibodies were applied in blocking buffer for 2–3 h at room temperature. The secondary antibodies were fluorescein (FITC)-conjugated donkey anti-rat IgG (H + L) at 3.75 μg/ml and Cy3-conjugated donkey anti-rabbit IgG (H + L) at 2.5 μg/ml (both from Jackson Immunoresearch Laboratories Inc.). The slides were then washed twice for 10 min in PBS, mounted with Vectashield (VECTOR), and examined using either a standard fluorescence microscope or a confocal microscope.

As additional controls for antibody specificity, adjacent sections of all tissues at all stages of development were incubated in parallel with the anti-mouse SR-BI or with the corresponding pre-immune antibody (3.2 μg/ml). In all cases, the pre-immune staining was negative (data not shown), indicating that the antibody was specific for SR-BI. In addition, a preliminary immunoblot of total protein isolated from whole deciduas at days E7.5 and E8.5 (adult adrenal gland and adult liver were included as positive controls) revealed the same single SR-BI (~82 kD) band in all cases in the molecular weight range resolved in the experiment (~40 kD and higher; not shown). In the immunoblot, there was an approximate 4-fold increase in the intensity of the SR-BI band on day E8.5 relative to E7.5. This correlated with the marked increase in signal seen in the immunofluorescence experiments (see below). The lower absolute increase in intensity seen in the immunoblot relative to the immunofluorescence (50- to 100-fold, see below) appears to be due to the fact that trophoblasts represent only a small portion of the entire decidua tissue.

DiI-HDL uptake

Pregnant mice were injected in a tail vein with 100–200 μl of DiI HDL (0.24 mg protein/ml) in PBS prepared as previously described (47, 53). After 4 h, animals were killed and extensively perfused with PBS. After perfusion, embryos inside the uterine wall were dissected, placed in Tissue Freezing Medium (Electron Microscopy Sciences), and allowed to freeze on dry ice. The frozen embryos were sectioned and observed using a standard fluorescence or a confocal microscope. For costaining with the anti-TM antibody, the same procedure was followed as described above, except that fixation of cryosections was omitted.

RESULTS

Expression of SR-BI in post-gastrulation mouse embryos on day E7.5

On day 7.5 of mouse embryonic development (E7.5), gastrulation is complete and the three germ layers of ectoderm, mesoderm, and endoderm have been established (**Fig. 1**). The cardiovascular and hematopoietic systems have begun to form with the first manifestation of blood islands in the yolk sac (54). At this stage, there is no embryonic circulation and the placenta, which will be derived in part from the chorion, neighboring trophoblasts, and decidua in the region of the ectoplacental cone, has not formed. In the endometrium-derived decidua which surrounds the developing embryo and extraembryonic tissues, newly formed maternal blood vessels supply nutrients and remove waste products. The embryo grows in a pool of maternal blood that fills the lacunae between the trophoblast cells that encircle the embryo. Macromolecules are thought to diffuse directly through Reichardt's membrane (an extracellular matrix membrane, see Fig. 1) to reach the parietal and visceral endoderm of the embryo (55, see also Fig. 1).

SR-BI expression was analyzed by indirect immunofluorescence using a highly specific rabbit polyclonal anti-SR-BI primary antibody. The antibody's specificity was confirmed using three approaches: immunofluorescence staining controls using pre-immune antibody and heterozygous and homozygous mutant embryos with targeted null mutations in the SR-BI gene as well as immunoblotting (see Materials and Methods). **Figure 2** shows that in cryosections of day E7.5 mouse embryos, including surrounding decidual tissue, SR-BI was expressed within the decidua in a pattern that virtually overlapped with that seen in the same sections using a rat monoclonal antibody to the thrombin receptor thrombomodulin (TM), a cell surface marker for endothelial cells (56, 57) (Fig. 2, panels A, B, compare equivalent sites indicated by arrows). Higher magnification revealed that SR-BI and TM colocalized at the surfaces of the endothelial cells in the decidua (Fig. 2, panels C, D). This result was confirmed by superposition of the confocal microscope images (not shown). SR-BI was not uniformly expressed in the decidual endothelium, but rather its expression appeared to be confined to a subpopulation of endothelial cells.

There was no SR-BI staining inside the day E7.5 embryos, including the extraembryonic membranes of the yolk sac, chorion, and the ectoplacental cone (see below). However, low levels of SR-BI were detected in some trophoblast cells, usually at the areas furthest away from the developing embryo (see also Fig. 3 below).

SR-BI expression on days E8.5 and E9.5

On day E8.5 of mouse development, differentiation of mesodermal-derived structures takes place. The blood islands expand and vascular structures develop, including the endocardial tubes, dorsal aorta, and

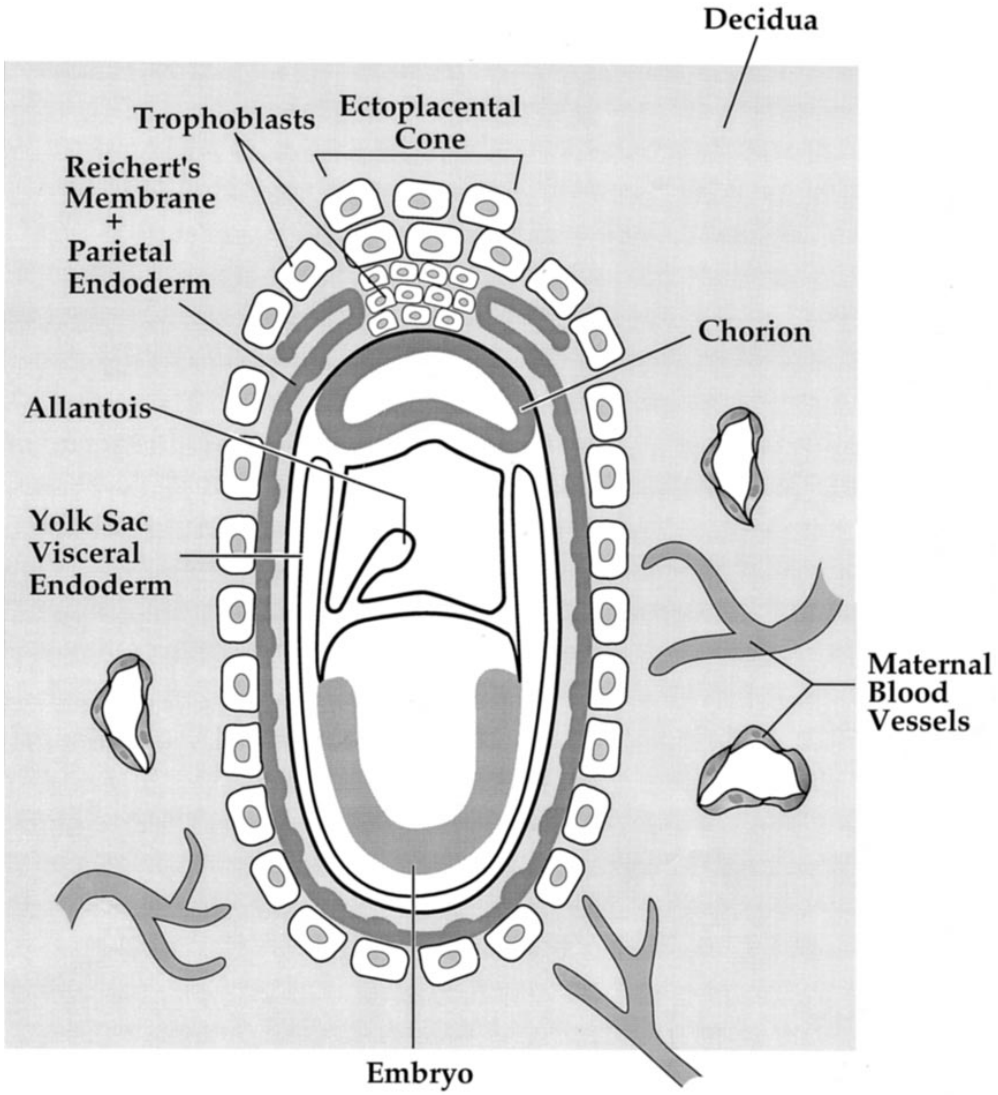

Fig. 1. Schematic drawing of a section of a day 7.5 mouse embryo. The embryonic and extraembryonic tissues are embedded in the endometrium-derived decidua tissue (see text).

heart. Somitogenesis also takes place from paraxial mesoderm, and at this stage 4–5 somites have formed. The developing heart myocardium begins to contract but no embryonic circulation takes place.

On day E8.5, there was a very strong induction of SR-BI expression in the trophoblast cells (**Fig. 3**, panel A, arrows) that surround the developing embryo (Em). The trophoblast cells also expressed high levels of TM (58) (panel B), but the intensity of staining was similar on days E7.5 and E8.5. Superposition revealed that, as was the case for decidual endothelial cells, the two molecules colocalized (not shown). Based on visual inspection, we estimate that there was an approximately 50- to 100-fold increase in SR-BI expression on day E8.5 relative to E7.5 (Fig. 3, panels C and D). We are not aware of any functional changes in trophoblasts between days E7.5 and E8.5 that might provide insight into the physiological basis for this massive increase in SR-BI expression. Figure 3 also shows that SR-BI expression persisted in the endothelial cells inside the decidua. As seen for E7.5, there was no detectable SR-BI expression inside the embryo or the yolk sac.

Analysis of day E9.5 embryos revealed that SR-BI continued to be highly expressed in trophoblast cells surrounding the embryo (not shown). Overall, the pattern of SR-BI expression and its relation to endothelial-cell markers such as TM and von Willebrand Factor (59) were very similar to those of day E8.5; however, it appears that expression in endothelial cells of the decidua was diminished (not shown).

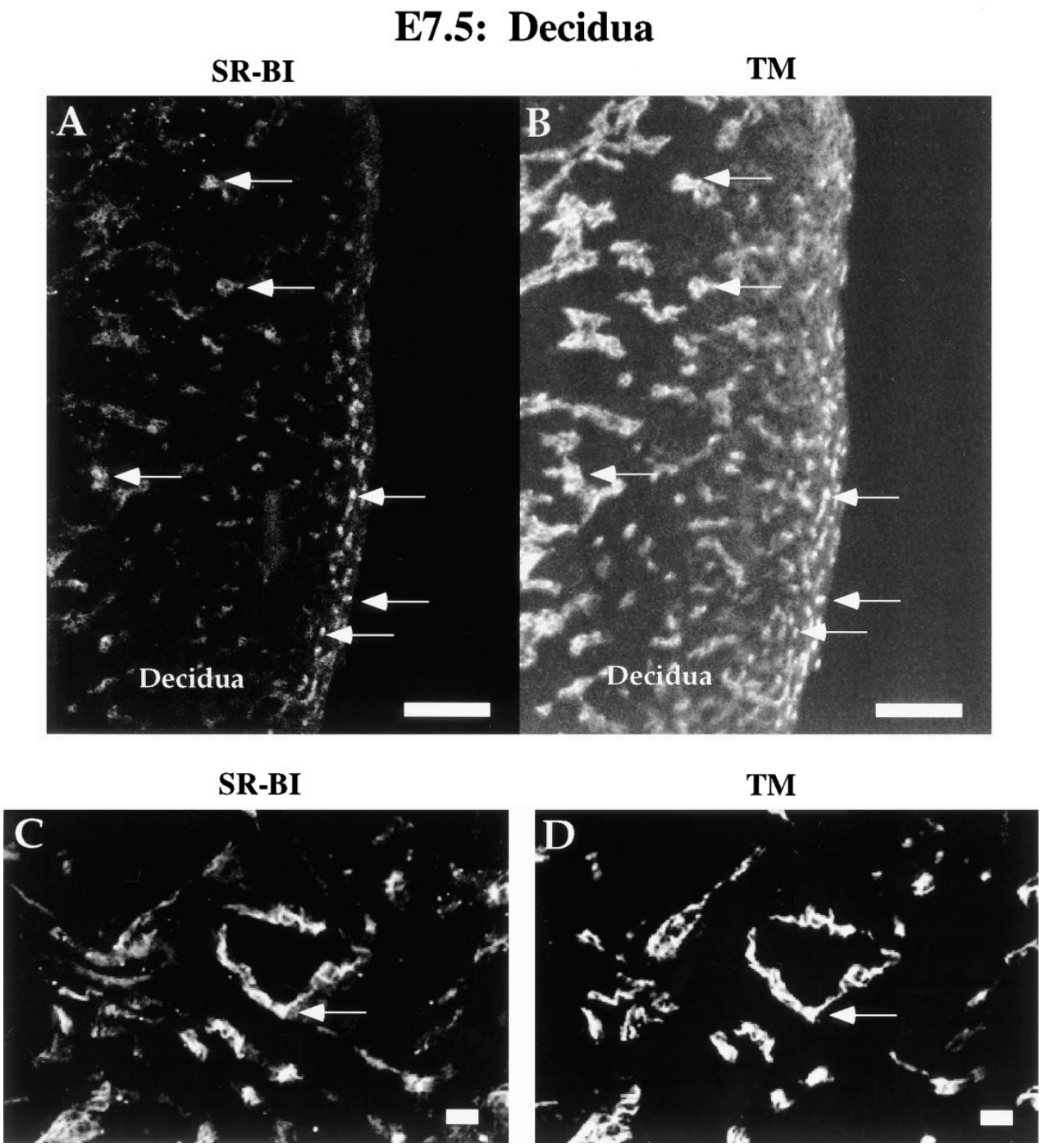

Fig. 2. SR-BI and TM expression in day E7.5 maternal decidual tissue. Cryosections of decidua surrounding a day E7.5 embryo were costained with an anti-SR-BI rabbit polyclonal antibody (anti-rabbit Cy3 conjugated secondary antibody, panels A and C, SR-BI) and a rat monoclonal anti-TM antibody (anti-rat FITC conjugated secondary antibody, panels B and D, TM) and observed using confocal fluorescence microscopy as described in Materials and Methods. The embryo proper was located to the left, out of the field of view in panels A and B (not shown). The bars represent either 100 μm (panels A and B) or 20 μm (panels C and D). The arrows in panels A and B (also in C and D) indicate examples of colocalization of the staining patterns on the surface of maternal endothelial cells.

Expression of SR-BI after placentation on days E10 to E12.5

On day E10, dramatic changes take place inside the embryo and in the extraembryonic membranes. The embryo itself has grown considerably and direct diffusion of nutrients and gases apparently cannot support further growth (2). The embryo has a functional circulation and all the major organ primordia are in place. From this

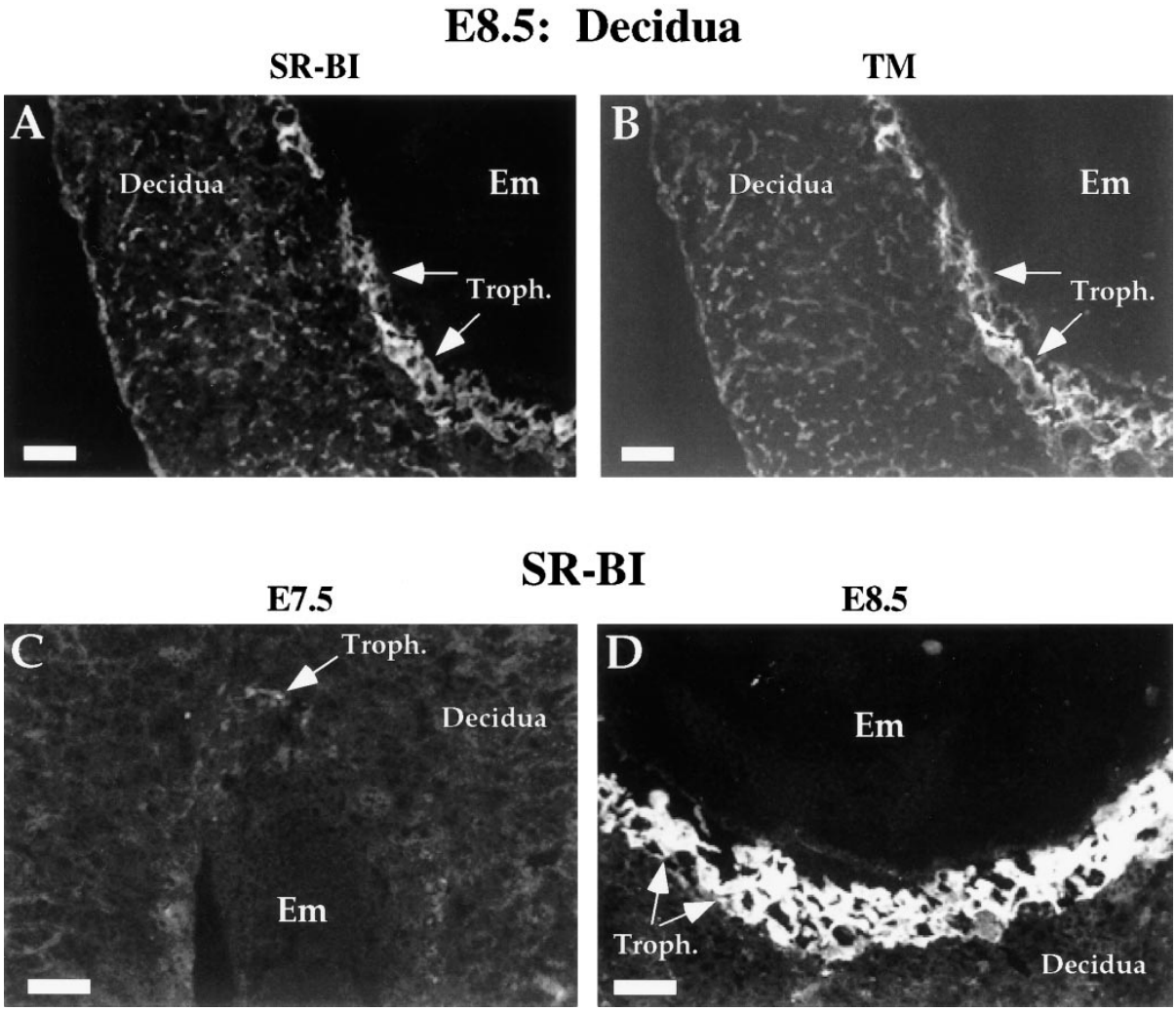

Fig. 3. SR-BI and TM expression on day E8.5 and comparison with day E7.5 staining. Cryosections of day E8.5 (panels A, B, and D) and day E7.5 (panel C) embryos were costained with anti-SR-BI (panels A, C, and D, SR-BI) and anti-TM (panel B, TM) antibodies and observed using confocal fluorescence microscopy as described in Materials and Methods. The locations of the decidua, trophoblast cells (arrows, Troph.) and the embryos (Em) are indicated. The bars represent 100 μm. The images in panels C and D were photographed under identical settings to permit direct comparison of the staining intensities.

point on, there will be dramatic embryonic growth and organ differentiation until birth. At this time, placentation is completed and the embryo receives nutrients through the interface between the maternal and embryonic regions of the placenta (see **Fig. 4**). The maternal vessels bring blood that passes through a layer of spongiotrophoblasts and fills lacunae between the folds of the chorionic labyrinth which are formed by the chorionic villi. On the embryonic side of the chorionic villi (Fig. 4, panel B), extensions of the umbilical vessels arrive and facilitate exchange of nutrients and gases between the mother and the fetus. The placenta takes over as the main site for transport of nutrients and for communication between the mother and the fetus (2). Mutations that affect formation of a functional placenta result at embryonic lethality at this stage (2).

The expression patterns of SR-BI and TM were examined in cryosections of day E10.5 and E12.5 mouse embryos including the placenta and the yolk sac. The expression patterns on these days were virtually identical. Here we only present the results from day E12.5. On these days, TM is expressed in endothelial cells and in spongiotrophoblast cells in the placenta (58). In the extraembryonic tissues on day E12.5, SR-BI was observed in subpopulations of endothelial cells (not shown) and decidual (TM negative) cells on the maternal side of the placenta [**Fig. 5A**, SR-BI (red fluorescence, see arrows, also see Fig. 4A, box 1), TM (green fluorescence, see ar-

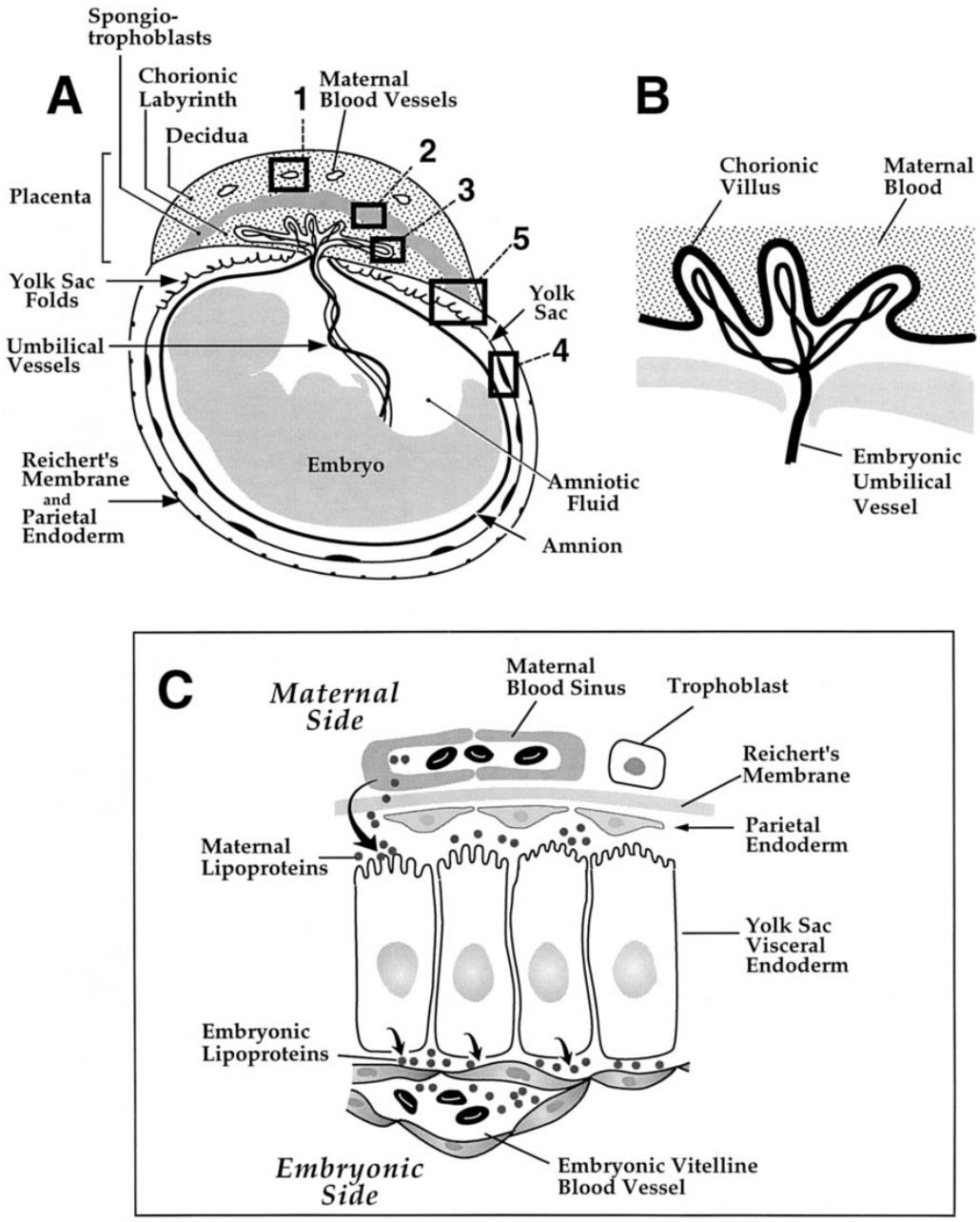

Fig. 4. Schematic drawing of a day E12.5 mouse embryo with surrounding extraembryonic tissues. Panel A: overview of fetal tissues. Boxes 1–5 indicate approximate locations of fields shown in Figs. 5 (boxes 1–3), 6 (box 4), and 7 (box 5). Expanded views of the areas indicated in boxes 3 and 4 are shown in panels B and C, respectively. Suggested movements of maternal lipoproteins toward the apical surfaces of the yolk sac visceral endoderm and secretion of embryonic lipoproteins into the embryonic (vitelline) circulation are indicated in panel C. Panel C is a slightly modified version of Figure 7 from the work of Robert Farese and colleagues (ref. 23), used with permission of these investigators.

rowheads)], as well as in a band of spongiotrophoblasts between the maternal and embryonic sides of the placenta (see Fig. 4A, box 2), where it colocalized with TM [Fig. 5B, colocalized SR-BI (red) and TM (green) appear as yellow]. SR-BI expression was not observed in all of the spongiotrophoblast cells at this stage (Fig. 5B and data not shown). SR-BI expression was also observed in the chorionic labyrinth on the embryonic side of the placenta (Fig. 5C, arrows, also see Fig. 4, panel A box 3 and reference 60). SR-BI was on the cell surfaces facing the

maternal blood and not those facing the embryonic blood vessels. As can be seen at high magnification in Fig. 5C, TM (green, arrowhead) and SR-BI (red) were on opposite sides of the chorionic labyrinth in contrast to their colocalization both at earlier stages of development and in the spongiotrophoblasts (compare with Figs. 2, 3, and 5B).

On E10.5 SR-BI was first detected in the yolk sac, starting at the yolk sac folds and extending further down the visceral yolk sac (not shown). This same pattern was also observed on day E12.5 (**Fig. 6**, red fluorescence) when expression of SR-BI on the apical surface of the visceral endodermal cells of the yolk sac was observed. Interestingly, the expression was confined to the cell surface facing the maternal tissues but was not seen on the basal membrane surfaces that were in contact with the embryonic yolk sac blood vessels (Bl. Ves., marked by TM antibody staining, Fig. 6, green fluorescence, see also Fig. 4A, box 4 and panel C). It is again noteworthy that there was virtually no SR-BI expression inside the embryo proper (not shown).

DiI-HDL uptake by placenta and yolk sac

The presence of SR-BI in the placental labyrinth and the yolk sac suggests that this receptor might be involved in HDL binding and lipid uptake. To begin to address this question, we introduced fluorescent HDL (DiI-HDL) into the maternal circulation by injection in a tail vein of pregnant mice on day E12.5 of gestation. Previous studies in vitro and in vivo have established that SR-BI mediates uptake of the lipophilic DiI from DiI-HDL to cells (47, 48, 51). Four hours after injection the embryos were isolated and frozen, and subsequently sectioned, stained with the anti-TM antibody, and the anti-TM staining (green fluorescence representing blood vessels and trophoblasts) and DiI fluorescence (red) were observed. **Figure 7** (equivalent site in schematic diagram can be seen in Fig. 4A, box 5) shows that there was a very high level of DiI uptake by the yolk sac endoderm, especially around the area of the folds close to the placenta. Very little DiI accumulation was detected in the maternal blood vessels and trophoblasts. A lower level of DiI accumulation (not visible at the magnification shown in Fig. 7) was also observed in the placenta. The stained areas coincided with the sites of SR-BI expression; however, relative to the immunostaining of SR-BI, there was significantly more DiI accumulation in the yolk sac folds than in the placenta. To verify that this pattern of DiI staining was specific for DiI carried in HDL and not simply a consequence of

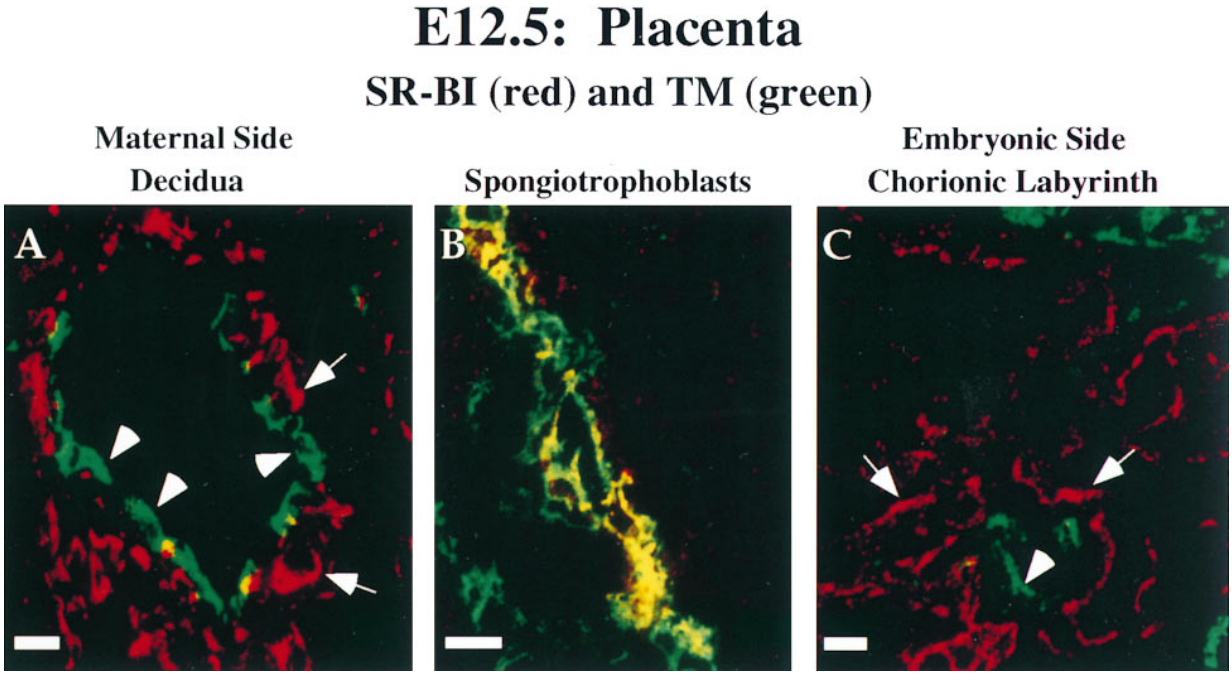

Fig. 5. SR-BI and TM expression in the placenta on day E12.5. Cryosections of day E12.5 specimens were costained with anti-SR-BI (red fluorescence, arrows) and anti-TM (green fluorescence, arrowheads) antibodies and observed using confocal fluorescence microscopy as described in Materials and Methods. Colocalization is indicated by yellow. Panel A: decidua tissue surrounding a maternal blood vessel at the maternal side of the placenta (see box 1 in Fig. 4A); panel B: area of spongiotrophoblasts that reside between the maternal and embryonic sides of the placenta (see box 2 in Fig. 4A); panel C: chorionic labyrinth on embryonic side of the placenta (see box 3 in Fig. 4A and Fig. 4B). The bars in panels A, B, and C represent 20, 100, and 20 μm, respectively.

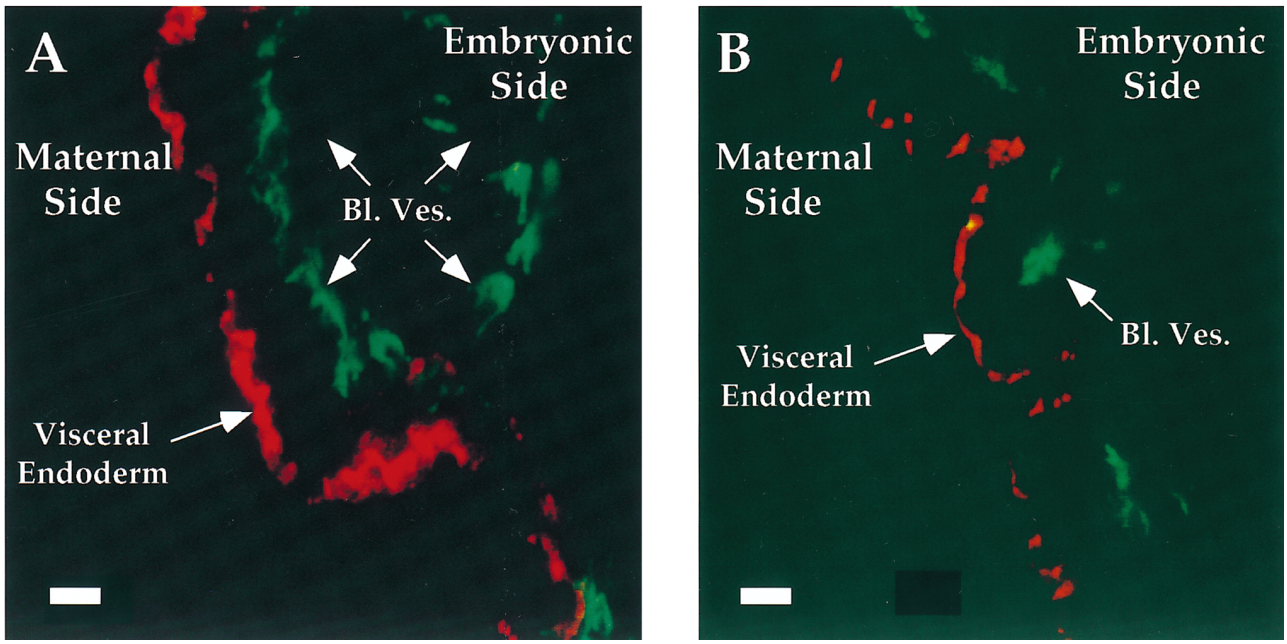

Fig. 6. SR-BI and TM expression in the yolk sac on day E12.5. Cryosections of day E12.5 specimens were costained with anti-SR-BI (red fluorescence) and anti-TM (green fluorescence) antibodies and observed using confocal fluorescence microscopy as described in Materials and Methods. Each of the two independent fields shown in panels A and B corresponds to regions indicated in box 4 in Fig. 4A and Fig. 4C (refer to Fig. 4 legend). SR-BI was seen on the apical surface (maternal side) of the visceral endoderm while TM marked the endothelial cells of the blood vessels (Bl. Ves.) of the vitelline circulation on the embryonic side of the yolk sac. The bars represent 10 μm.

the intrinsic properties of the dye itself, DiI-labeled acetylated LDL (DiI-AcLDL) was injected into control mice. Scavenger receptors that can bind and mediate the endocytosis of DiI-AcLDL are expressed on macrophages and endothelial cells (61). There was no detectable DiI accumulation in the yolk sac or the placenta of the fetuses in the DiI-AcLDL-injected mothers (not shown). Instead, there was DiI accumulation in endothelial cells in the placenta and inside the uterine wall (not shown), a result consistent with previous studies that have established that endothelial cells express scavenger receptors that can mediate the endocytic accumulation of DiI from DiI-AcLDL (62). These data suggest that SR-BI may be directly involved in the efficient transfer of lipids from HDL to the placenta and yolk sac.

Intraembryonic SR-BI expression

Up to day E12.5, no intraembryonic expression of SR-BI was observed. We first detected SR-BI protein expression inside the embryo on day E14.5 when there was very strong expression in the developing embryonic adrenal gland (**Fig. 8A**). It is possible that SR-BI may have been expressed earlier (the adrenal gland begins forming around day E11 of gestation (63); we have not conducted a detailed analysis of SR-BI expression in this rather small tissue between days E11 and E14.5. We were unable to detect SR-BI expression in the embryonic liver (E10–E17) although we did observe expression in neonatal liver (not shown). Thus, it appears that the onset of hepatic SR-BI expression, which has been previously described in adults (47, 48), may coincide with the induction of several key enzymes and receptors of hepatic cholesterol metabolism (13). The only other site at which we observed significant intraembryonic staining with the anti-SR-BI antibody was the hindgut on day E17 (Fig. 8B), where the staining outlined the folds of the gut epithelium. The significance of this hindgut staining is currently unknown.

DISCUSSION

Previous work has suggested that, in mammals, maternal lipoproteins may deliver cholesterol to embryos

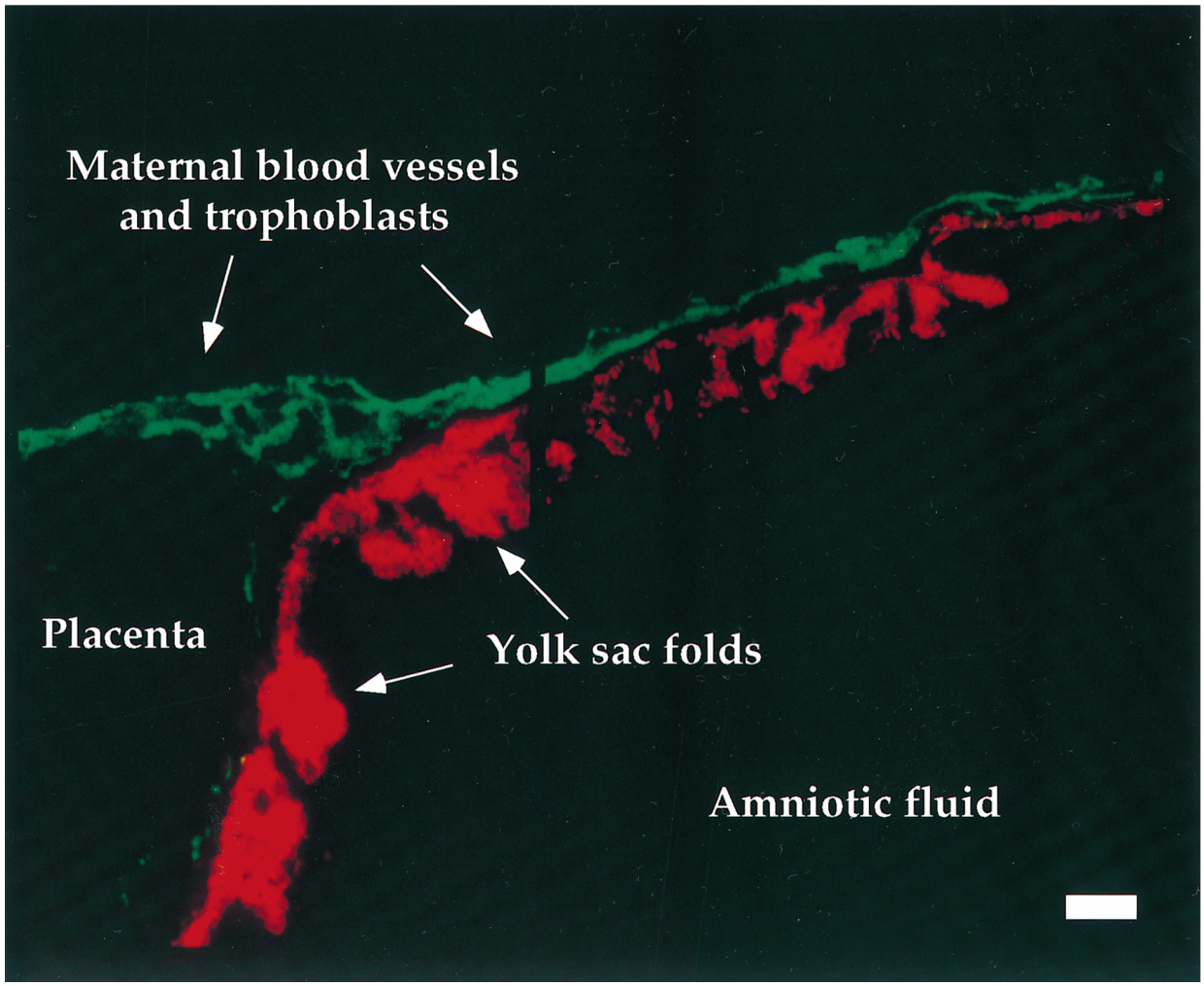

Fig. 7. Accumulation of the lipophilic fluorescent dye DiI in day E12.5 yolk sac from DiI-HDL in the maternal circulation. Pregnant mice were injected with 100–200 μl of DiI-HDL (0.24 mg protein / ml), killed 4 h later, and extensively perfused with PBS. After perfusion, embryos (day E12.5) were harvested and frozen. Cryosections were stained with an anti-TM antibody (endothelial cells of maternal blood vessels and trophoblasts) and the antibody (green fluorescence) and DiI (red fluorescence) staining patterns were observed using confocal fluorescence microscopy as described in Materials and Methods. The locations of the placenta and the amniotic fluid space are indicated. The bar represents 100 μm.

to support both the nutritional and the steroidogenic requirements of the developing organism (14–18; however, see 9, 19 for alternative views). The molecular mechanisms of lipoprotein cholesterol uptake and transfer across the embryonic membranes are poorly understood. Recently, we have shown that the class B, type I scavenger receptor SR-BI is a cell surface HDL receptor that plays an important role both in HDL-mediated transport of cholesterol to the liver and in providing cholesterol as a substrate for steroidogenesis in endocrine tissues (47–49, 51, 52 and reviewed in 46). To determine whether SR-BI might also be involved in the delivery of maternal HDL cholesterol to the developing embryo, we have used immunofluorescence microscopy to study the pattern of SR-BI expression during murine embryogenesis. We examined embryonic as well as maternal and fetal extraembryonic tissues.

Inside the embryo, the first manifestation of SR-BI expression was detected in the developing adrenal gland on day E14.5, although expression may have begun earlier as this tissue begins to form on E11 (63). SR-BI was not detected in the fetal liver up to day

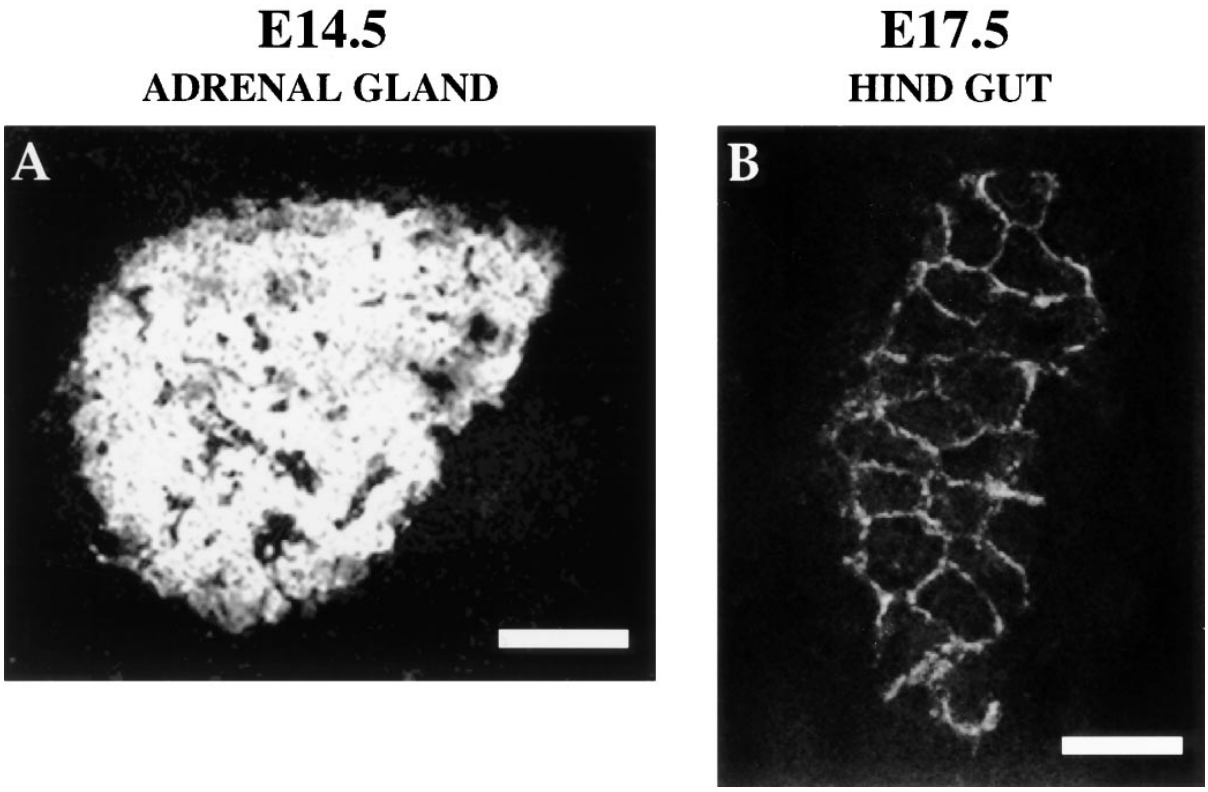

Fig. 8. SR-BI expression in the adrenal gland (E14.5) and hind gut (E17.5) of murine embryos. Cryosections of embryos from day E14.5 (adrenal gland, panel A) and day E17.5 (hind gut, panel B) were stained with an anti-SR-BI antibody and observed using confocal fluorescence microscopy as described in Materials and Methods. The bars represent 100 μm.

E17.5, but did appear shortly after birth, a time which coincides with the induction of synthesis of many liver specific enzymes (13). In the late stages of embryogenesis (E17.5), staining with the anti-SR-BI antibody was observed near the apical surfaces of the epithelium of the hindgut. The significance of this observation is unclear; however, it may be related to the previous observation of the expression of enzymes involved in the steroid hormone synthetic pathway in this area of the embryonic gut (64).

In the extraembryonic tissues, the timing and distribution of SR-BI expression were consistent with the proposal that SR-BI may play a role in embryonic uptake of maternal HDL cholesterol. Before embryonic circulation is established and prior to placentation (days E7.5–E10 of gestation), diffusion of nutrients is sufficient to sustain the developing embryo [(2); see Figs. 1 and 4 for schematic drawings of embryos]. Early on, most SR-BI expression was observed in the decidua which surrounds the embryo, but not in the extraembryonic or intraembryonic tissues. Initially (day E7.5), SR-BI expression was exclusively observed in maternal endothelial cells in the decidua. Shortly thereafter (between days E7.5 and E8.5), there was a dramatic increase in SR-BI expression in the giant trophoblast cells that surround the developing embryo (Fig. 1). The expression of SR-BI in a subpopulation of maternal endothelial cells in the decidua surrounding the embryo in the early stages of embryogenesis (E7.5–E9.5) suggests that it may be involved in nutrient storage in the decidua. This observation was unexpected, because substantial expression of SR-BI in endothelial cells has not been reported previously. Additional studies will be required to determine whether SR-BI is expressed in other endothelia and to define the mechanisms that underlie its regulated expression in endothelial cells. The dramatic increase in SR-BI expression in trophoblasts cells on day E8.5 may be related to endothelial-specific SR-BI expression, because trophoblast cells resemble endothelial cells in that they form channels that enclose maternal blood and they express other endothelial marker genes such as TM, flk-1, flt-1, and VE-cadherin (58, 65–67).

The high expression of SR-BI in the trophoblasts persisted until the formation of the placenta (E10). At this point, SR-BI expression was also detected in decidua cells as well as on the apical surface of chorionic villi, which surround the maternal blood, in the labyrinth area of the embryonic part of the placenta (see

Fig. 4). In addition, SR-BI was expressed in the yolk sac, where it extended to the visceral endoderm that surrounds the embryo. Expression of SR-BI on days E10.5 and E12.5 was seen on the apical surfaces of the visceral endodermal cells that face the maternal tissues surrounding the embryo. As the distance from the placenta increased, the level of SR-BI yolk sac expression decreased. This pattern of SR-BI expression was similar to the pattern of accumulation at these sites of the lipophilic fluorescent dye DiI when pregnant females were injected with DiI-labeled HDL. It seems likely that SR-BI was responsible for the DiI uptake. [Previous studies have suggested that SR-BI-mediated cellular uptake of DiI from DiI-HDL is similar to cholesteryl ester uptake from HDL (47)]. The onset of SR-BI expression in the visceral yolk sac coincided with the development of the vitelline circulation, which serves the intraembryonic tissues. No SR-BI expression was observed prior to day E10.5 in the yolk sac, suggesting that SR-BI is not involved directly in cholesterol uptake by the yolk sac when diffusion of nutrients takes place. Instead, the role of SR-BI in early embryogenesis appears to be confined to the extraembryonic endothelial and trophoblast cells. It is interesting to note that some studies suggest that cholesterol synthesis in the embryo can account for its needs but that synthesis is not adequate if one also considers the requirements of extraembryonic tissues, i.e., placenta and yolk sac, for both growth and steroid hormone (e.g., progesterone) synthesis (18). At this early stage, SR-BI might provide cholesterol to the extraembryonic cells for growth or storage until it can be subsequently transferred to the embryo. Alternatively, SR-BI might function in these extraembryonic cells as it appears to function in adult steroidogenic endocrine cells (e.g., in the adrenal gland, ovaries and testis); it may provide cholesterol to serve as a substrate for steroid hormone synthesis, rather than as a nutrient for cellular membrane synthesis.

The pattern of SR-BI expression was dynamic, in that it was switched on and off at different levels in different types of cells and tissues at various stages of embryonic development. The highest levels of expression were seen in the embryonic adrenal gland and the trophoblasts between days E8.5–E12.5. Analysis of the *cis* and *trans* regulatory elements responsible for the temporal changes in its tissue-specific expression should be instructive. There was, however, a feature of SR-BI expression that was common for those sites at the interface of the maternal circulation and the embryo. In every case, SR-BI was expressed on the side of the tissue that faces the maternal blood (e.g., decidua endothelial cells, trophoblast cells, apical surface of visceral endoderm in the yolk sac, and the chorionic villi). This suggests that SR-BI may play a role in binding maternal HDL and mediating cholesterol uptake, the importance of which might depend on both environmental and genetic factors. Thus, SR-BI-mediated cholesterol uptake might provide an efficient, but not absolutely essential, mechanism for supplying cholesterol to developing embryonic and extraembryonic tissues. If so, this could explain the lower than expected yield of SR-BI null homozygous and heterozygous murine mutants in gene targeting experiments (52), results which raised the possibility that SR-BI may play a role in embryonic development. Additional analysis of these mutant and wild-type mice will hopefully shed further light on the roles of maternal lipoprotein-cholesterol and endogenously synthesized cholesterol during gestation.

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