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Expression of a Secreted Transplantation Antigen Gene During Murine Embryogenesis

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We examined the midgestation mouse embryo for transcripts related to the secreted transplantation antigen Q10 and show here that this gene is transcribed in the endoderm of the visceral yolk sac. Its level of expression is highest at day 14 and then declines as development proceeds. Concurrently with the decrease in yolk sac expression, the amount of transcripts accumulating in the fetal liver increases during late embryogenesis.

The murine major histocompatibility complex encodes different classes of proteins that regulate the immune response. The analysis of class I-related cDNA clones derived from adult mouse liver RNA has recently led to the description of a novel class I antigen (5). The cDNA sequence of one clone predicted a protein which would have several polar amino acid substitutions followed by nonsense codons in the normally hydrophobic transmembrane domain and would therefore lack the carboxy-terminal cytoplasmic domain (12). Because of these considerations it was reasoned that if such a protein was synthesized, it would be secreted rather than membrane bound. Using antibodies raised against a peptide that would be derived from the modified transmembrane domain, a class I-related protein of the molecular weight predicted from the sequence was immunoprecipitated from adult mouse serum (15), and its synthesis was shown to be restricted to the liver (6, 15). Other studies mapped the gene to the Q10 locus of the Qa region in the major histocompatibility complex (18, 19).

Since the liver is not well developed until about day 12 in mouse embryonic development and does not mature until later in gestation (21), we were interested in whether another tissue might synthesize the Q10 antigen during embryogenesis. The visceral yolk sac (VYS) seemed a likely candidate since it is one of the first tissues to differentiate (8) and is known to synthesize a number of liver proteins (1, 7, 10, 17). These properties cease in the latter stages of gestation and are then taken over by the developing liver. We report here that RNAs which show homology to a probe specific for the secreted class I antigen, Q10, are present in the VYS of the midgestation mouse embryo.

MATERIALS AND METHODS

Dissection of mouse conceptuses. Mature female CD-1 mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were injected intraperitoneally with 1 IU of pregnant mare serum gonadotropin (Diosynth, Inc., Chicago, Ill.). Two days later, they were injected with 5 IU of human chorionic gonadotropin (Organon, West Orange, N.J.) and mated. The morning that a vaginal plug was found was considered day 1 of pregnancy. This amount of pregnant mare serum gonadotropin synchronized the estrous cycle without inducing superovulation and thereby increased the number of successful matings.

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At the appropriate day, conceptuses were dissected free of the uterus in phosphate-buffered saline. Each conceptus was then separated into placenta, parietal yolk sac (PYS) (trophectoderm and parietal endoderm), and VYS (visceral endoderm [VE] and extraembryonic mesoderm [exM]). In some cases the VYS was separated into mesoderm and endoderm (16).

Isolation and analysis of RNA. The dissected tissues were homogenized in 4 M guanidinium thiocyanate–1 M β-mercaptoethanol. RNA was then purified from the mixture by centrifugation through a cushion of 5.7 M CsCl–0.1 M EDTA, pH 5 (4).

Unless otherwise indicated, 50 μg of total RNA was separated by electrophoresis on a 0.8% agarose gel in a buffer containing 3% formaldehyde (3). The gels were washed for 20 min in water, followed by another 20 min in 20× SSC (1× SSC is 15 mM sodium citrate and 0.15 M sodium chloride), and then blotted onto nitrocellulose (22). The conditions of hybridization and the probes used have been described previously (6).

RESULTS

Owing to the high degree of sequence homology between class I antigens, DNA probes derived from the protein-coding domains cannot discriminate between individual genes. However, portions of the 3′-noncoding region are sufficiently different between class I genes that a DNA fragment derived from these sequences can be used for specific hybridization purposes (5, 13). When RNA from fetal tissues obtained from 12-day-old conceptuses was hybridized to a Q10-specific probe (6), the VYS RNA showed a strongly hybridizing transcript of approximately 1.7 kilobases, along with two more weakly hybridizing bands of approximately 1.6 and 1.4 kilobases (Fig. 1B). The 1.7-kilobase transcript corresponds to an RNA of the appropriate size for the Q10 gene (6). These three transcripts were found only in the VYS and were absent from both the placenta and PYS (Fig. 1B) as well as the fetus proper (data not shown). To confirm that the various tissues contain class I sequences, the same filter was probed with coding-domain sequences (Fig. 1A). In this case, one would expect to see hybridization in each tissue since this probe recognizes sequences common to all class I genes. A 1.8-kilobase RNA characteristic of class I genes was present in the placenta and PYS, as well as the VYS; however, the two smaller Q10-related transcripts observed in the VYS were not detected in this experiment with the coding probe (Fig. 1A).

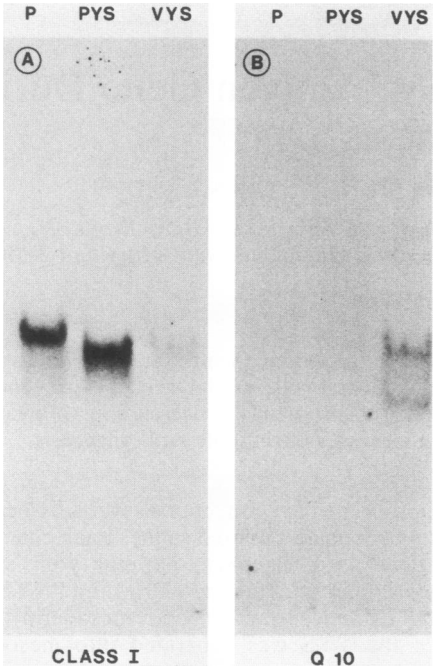

FIG. 1. Expression of major histocompatibility complex transcripts in 12-day-old conceptuses. The tissues examined were placenta (P), PYS, and VYS. (A) Northern analysis with the same blot shown in panel B rehybridized to a ~570-base-pair class I-specific cDNA probe which spans amino acids 151 to 236. (B) Northern blot of extraembryonic tissues hybridized with a ~170-base-pair 3'-noncoding fragment from the Q10 cDNA clone.

(Although the PYS class I RNA migrates faster in this particular gel, it was not a reproducible phenomenon and should not be regarded as indicating a difference in size.) These data demonstrate that the Q10-specific probe is hybridizing to Q10-like sequences and not to class I RNAs in general, since only the VYS contained RNA bearing significant homology to the Q10 probe.

At midgestation the VYS is bilaminar, consisting of an outer cell layer of VE and an inner layer of exM. The VYS endoderm is in many ways functionally analogous to the adult liver. To determine whether the endoderm layer was responsible for synthesis of the Q10 transcripts, we separated the VYS into its constituent cell types, VE and exM. The VE preparation was highly enriched for Q10 transcripts, whereas the exM appeared to contain few, if any, of these gene-specific transcripts (Fig. 2B). Because the technique used to separate the VE from the exM results in some cross-contamination, the filter was reprobed with a cDNA clone for α-fetoprotein which is a known marker for VE (7). Strong hybridization of the α-fetoprotein probe was observed in the VE lane, whereas a minimal signal was seen with exM (data not shown). Since the Q10 clone also hybridizes with a similar pattern to these two RNA preparations (Fig. 2B), this suggests that Q10 is made solely by the VE cells.

The relative amounts of *H-2*-related transcripts that were present in the VE and exM were examined by hybridizing the blot to a class I-coding probe. As is the case with Q10, the VE also expressed much more class I RNA than the exM (Fig. 2A). The apparent bias in class I gene expression between the two cell types is not due to differences in the amount of RNA present in each lane (data not shown).

As the fetus approaches parturition, the VYS begins to deteriorate so that by late gestation it may be considered nonfunctional (9). Concomitant with the VYS decline, the fetal liver begins to increase the synthesis of proteins previously produced by the VYS (23). To determine whether a similar shift in expression might occur for Q10, we examined the VYS and fetal liver at various times during development. Maximal accumulation of this RNA occurred at day 14 in the VYS and then declined rapidly (Fig. 3B). Unlike the VYS, expression of Q10 RNA in the liver steadily increases during the latter half of gestation. When the blot is examined for the presence of class I mRNAs, a slightly different result is obtained. In this case, class I transcripts were found to gradually increase in both the VYS and liver late in development (Fig. 3A).

DISCUSSION

The Q10 hybridization probe, derived from the 3'-noncoding region of the cDNA, has been used successfully in a number of studies to detect Q10-specific transcripts (6, 13). However, the probe hybridizes to two smaller transcripts in the VYS, which are also seen in the fetal liver. One possibility for the origin of these RNAs is that differential splicing of the Q10 primary transcript occurs (14). It is also possible that these two transcripts represent degradation products, since with different preparations of RNA, the ratio among the three Q10-specific transcripts varied. Furthermore, their hybridization to a coding probe which spans the conserved C-2 domain, from amino acids 151 to 236, was also variable. At present, their exact structure is unknown.

During early embryogenesis, the VYS produces a number of proteins usually associated with the liver. Some of these can be detected in the VYS endoderm as early as day 8 of gestation (1), a time well before the fetal liver cords have formed. This suggests that the yolk sac endoderm may

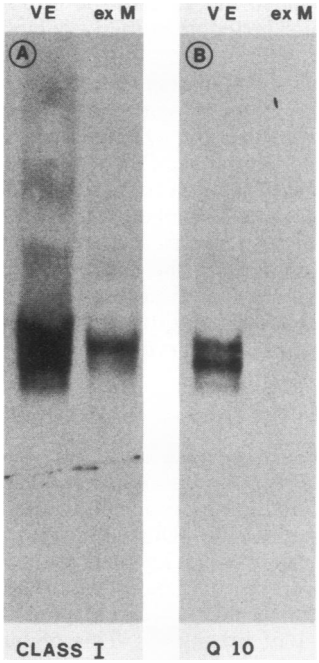

FIG. 2. Characterization of transcripts in the VYS. The yolk sac was separated into VE and exM, and the RNA was isolated. (A) Rehybridization of the same blot shown in panel B with the class I-specific cDNA probe. (B) Hybridization with the Q10-specific 3'-noncoding cDNA probe.

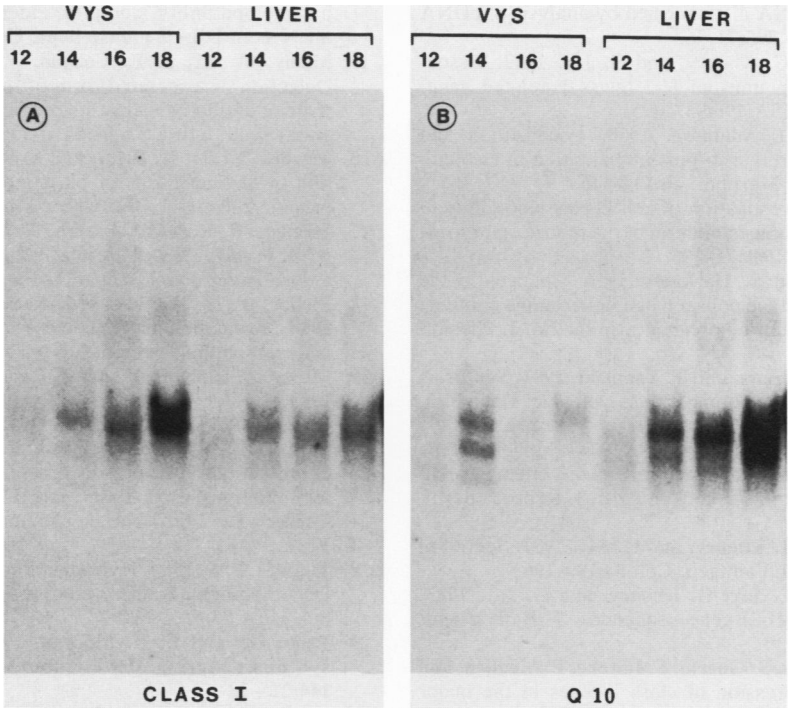

FIG. 3. Time course of Q10 mRNA expression in the VYS and fetal liver. Total RNA (30 μg) was loaded in each lane. The day in fetal development from which tissues were isolated is indicated at the top of each lane. (A) Rehybridization of the same blot shown in panel B with the class I-specific cDNA probe. (B) Hybridization with the Q10-specific 3'-noncoding cDNA probe.

functionally serve as an early embryonic liver (21). Analysis of Q10 expression showed that transcripts in the VYS are highest at day 14 and then sharply decline. At the same time, the liver supplants the VYS as the major site of synthesis of Q10 since the amount of liver RNA hybridizing to the Q10 probe increases rapidly between days 12 and 18.

It is thought that the VYS begins to degenerate around day 17 in gestation, although it is present until parturition (9); toward the end of development there is also a decrease in the total number of proteins synthesized by the VYS (10). However, our analysis indicated that the overall amount of class I-specific RNA increases in the VYS even at late stages of gestation. Therefore, the decrease in Q10 production may not be due to destruction of the VYS but rather to the selective repression of the gene in this tissue. Since there are few classical transplantation antigens present on the surface of late-gestation VYS (20), one possibility is that the some of the transcripts are derived from the Qa and Tla regions of the major histocompatibility complex.

During the latter stages of development, the PYS has degenerated to an extent such that the VYS is in direct contact with the uterine wall. At this time, the VYS could potentially elicit an immune response since it is known that isolated VE cells are antigenic and react vigorously in an in vitro cell-mediated cytotoxicity assay (2, 11). However, the VYS displays an unusual graft response when transplanted to the dorsal thorax of the host. If the mesodermal side of the yolk sac is placed on the graft bed, vascularization occurs rapidly and at the same time the yolk sac is destroyed by the allograft reaction. On the other hand, if the endodermal cells are placed in contact with the graft bed, they resist vascularization and the allograft rejection is less vigorous (2). This may be partly explained by the fact that the VE cells may not be good targets for the cell-mediated immune responses (20). Such observations are consistent with the idea that endodermal cells may play a role in tolerance of the embryo by the host immune system.

One level at which tolerance might be controlled is by active suppression of effector T cells through the Q10 antigen. In many cases, recognition of both self *H-2* class I and foreign antigens is necessary to mount an immune response. The Q10 molecule might serve to block the *H-2* class I receptor on maternal immunoreactive cells, preventing recognition of the nonself (paternally derived) surface antigens present on the embryo. This hypothesis is conceptually similar to speculations put forth to explain the paucity of liver rejections during transplants (6). However, the exact role of a secreted class I molecule in the embryo remains to be determined.

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