

Localization of Major Histocompatibility Complex Class I and II mRNA in Human First-Trimester Chorionic Villi by In Situ Hybridization

By Jennifer A. Lata,* Rocky S. Tuan,† Kenneth J. Shepley,‡
Mary M. Mulligan,‡ Laird G. Jackson,*§ and J. Bruce Smith*

From the Departments of *Medicine, †Orthopedic Surgery, and ‡Medical Genetics, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

Summary

Maternal immune recognition of pregnancy occurs despite the nonexpression of classical major histocompatibility complex (MHC) antigenic determinants by chorionic villous trophoblast, which comprise the major surface area where maternal blood contacts fetal-derived cells. cDNA-mRNA in situ hybridization was used to probe expression of transcripts corresponding to nonpolymorphic MHC determinants in first-trimester chorionic villus samples. The HLA-B7 probe hybridization signals were localized to syncytiotrophoblast and to cells of the mesenchyme but not to villous cytotrophoblast. HLA-G mRNA was found only in syncytiotrophoblast. A DR β clone hybridized to both villous cytotrophoblast and syncytiotrophoblast. The results suggest that expression of trophoblast class I and class II determinants early in gestation (10 wk) may be regulated by posttranscriptional events. This also suggests the potential for maternal antifetal alloimmune responses.

The human placenta is formed from embryonic tissue and therefore expresses gene products of both maternal and paternal derivation. In the first trimester of pregnancy, villi of the developing chorion frondosum are bathed in maternal blood and the villous trophoblast serves as the cellular interface where maternal exposure to fetal alloantigens occurs. It is now clear that during the course of normal human gestation, maternal immune responses are evoked that are of fetal specificity and may be required for pregnancy success (1, 2). The lack of detectable expression of conventional MHC Class I and II determinants by villous trophoblast throughout gestation (3) is of obvious significance since classical immune responsiveness to allogeneic tissues results from host recognition of these molecules on the cell surface. Although it remains to be established whether effector mechanisms play a role in pregnancy success or failure, trophoblast expression of MHC determinants would clearly potentiate cellular antifetal responses. To further understand mechanisms involved in the maternal-fetal immunologic relationship, we have examined chorionic villus samples obtained at 9–10 wk of pregnancy for RNA transcripts specific for class I and II determinants. We demonstrate here that in the apparent absence of expression of the corresponding antigens on the cell surface, villous trophoblast contain mRNA specific for nonpolymorphic MHC antigenic determinants.

Materials and Methods

Preparation of Placental Tissue. Chorionic villi were obtained by chorionic villus sampling (CVS) performed at 9–10 wk of gestation by the Division of Medical Genetics of Thomas Jefferson University ($n = 5$). The tissue studied was not required for diagnostic testing and would have otherwise been discarded. Villi were washed and processed immediately after sampling. Villi were fixed, embedded, sectioned, and processed for in situ cDNA-mRNA hybridization as described (4), or were snap frozen and stored at -80°C for immunohistologic analysis.

Preparation of cDNA Probes. An HLA-B7 cDNA clone (1.4-kb insert in pSP64) that hybridizes with HLA-A,B,C mRNA (5) was a gift from Dr. Sherman M. Weissman (Yale University, New Haven, CT). A locus-specific clone homologous to 0.25 kb of the 3' untranslated region of HLA-G (6) was a gift of Dr. Harry Orr (University of Minnesota, Minneapolis, MN). A cDNA clone encoding a nonpolymorphic region of HLA-DR β chains (1.32-kb insert in pcDV1-p12) (7) was obtained from American Type Culture Collection (Rockville, MD). pMCP, a cloned cDNA to the mouse placental calcium-binding protein (0.7-kb insert in pBR322) (8), and a 2.5-kb cDNA of the *Onchocerca volvulus* paramyosin gene (9) were used as control probes. Isolation of plasmid DNA and restriction analysis was performed following standard protocols (10). Total plasmid DNA was biotinylated by nick translation (11).

In Situ Hybridization. Pre-hybridization and hybridization procedures were those described by Tuan et al. (4). The location of biotinylated probes in villus sections was detected using the

streptavidin-alkaline phosphatase procedure as described by Lo (11) and Enzo Biochem, Inc. (New York, NY). The streptavidin conjugate was used as a 1:100 dilution, and the chromogenic substrates were 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Sigma Chemical Co., St. Louis, MO). Hybridization signals were detected by examination of sections with an Olympus BH-2 microscope using bright field, phase-contrast and Nomarski differential interference contrast optics. Kodak Panatomic-X film was used for photography.

Results

A phase-contrast photomicrograph of an unstained villus section (Fig. 1) demonstrates the histologic architecture of a 10-wk chorionic villus. At this early stage of gestation, villous trophoblast consists of the external multinucleate syncytiotrophoblast and the mononucleated ovoid cells, which comprises the cytotrophoblast. The underlying mesenchyme forms the villous core. Histologic morphology was preserved in villus sections prepared for cDNA-mRNA hybridization, thus allowing localization of transcripts to particular cellular layers. The typical hybridization signal generated on a 10-wk villus section with the HLA-B7 cDNA probe is shown in Fig. 2 A. An intense hybridization signal is seen at the syncytiotrophoblast layer along with scattered signals on the mesenchymal cells. Hybridization of the cDNA clone specific for DR β chain message localized to both the syncytiotro-

phoblast and cytotrophoblast of a section from the same villus sample (Fig. 2 B). A (control) cDNA probe for placental calcium binding protein expressed during late gestation (11) did not hybridize to villus sections, as evidenced by lack of signal on any villous cells (Fig. 2 C). Fig. 3 demonstrates an additional panel of results obtained with an alternate control probe and a class I locus specific cDNA clone (HLA-G). Comparison of Fig. 3, A and B reveals that the HLA-G-specific clone generated a weak, scattered hybridization signal on the syncytiotrophoblast layer only (Fig. 3 B), whereas, as also seen in Fig. 2 A, the HLA-B7-specific clone hybridized to both syncytiotrophoblast and scattered mesenchymal cells (Fig. 3 A). The signal generated with the HLA-B7 clone was stronger than the HLA-G signal. In both cases, however, the signals were uniformly scattered over the syncytiotrophoblast layer and no hybridization signals were observed on cytotrophoblasts. Similar to the results displayed above, the DR β cDNA sequence hybridized to both the syncytiotrophoblast and cytotrophoblast in the 10-wk villus sample (Fig. 3 C). As expected, no signal was observed on the sections treated with the *O. volvulus* paramyosin probe (Fig. 3 D).

Discussion

The human placenta is comprised of specialized cells that enable this organ to perform a diversity of functions critical

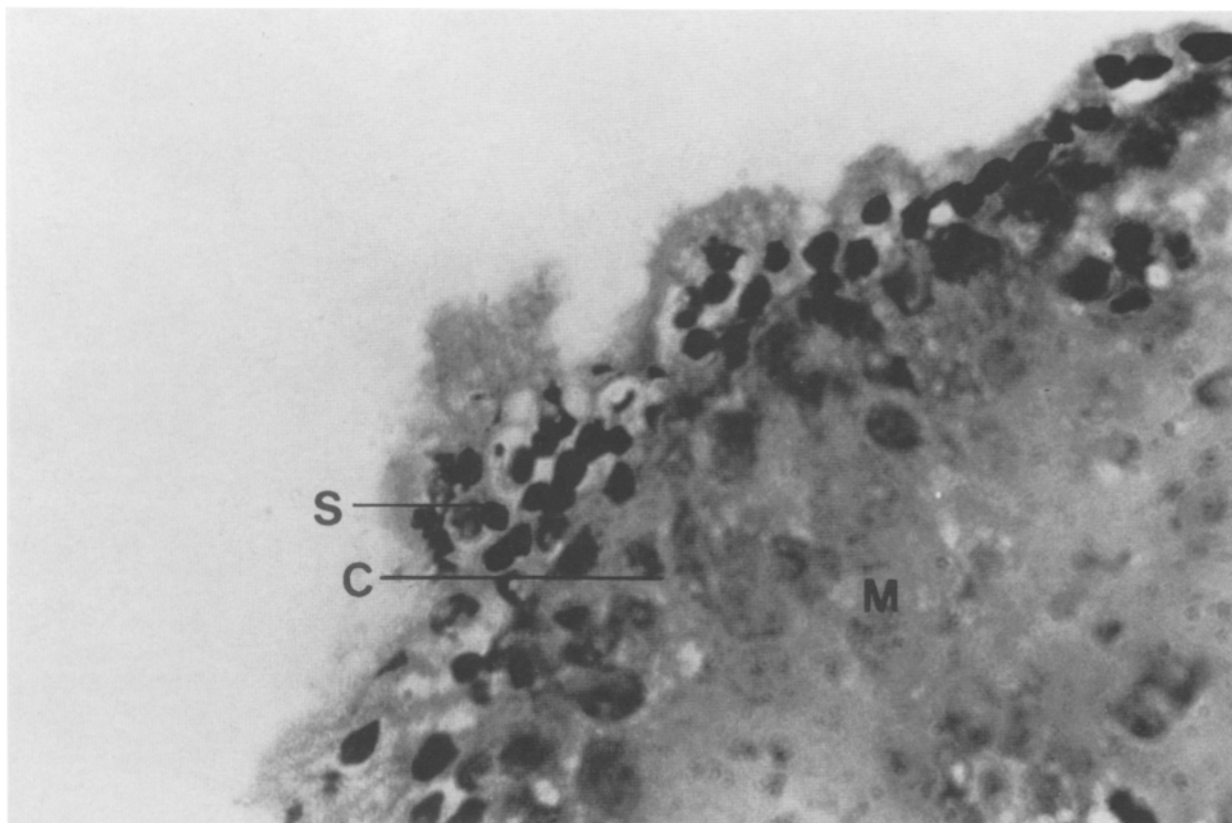


Figure 1. Phase-contrast micrograph demonstrating the histology of a 10-wk chorionic villus sample ($\times 400$). At high power, a cross-section of the first-trimester chorionic villus exhibits the multinucleate syncytiotrophoblast (S) and cytotrophoblast (C) surrounding a mesenchymal core (M).

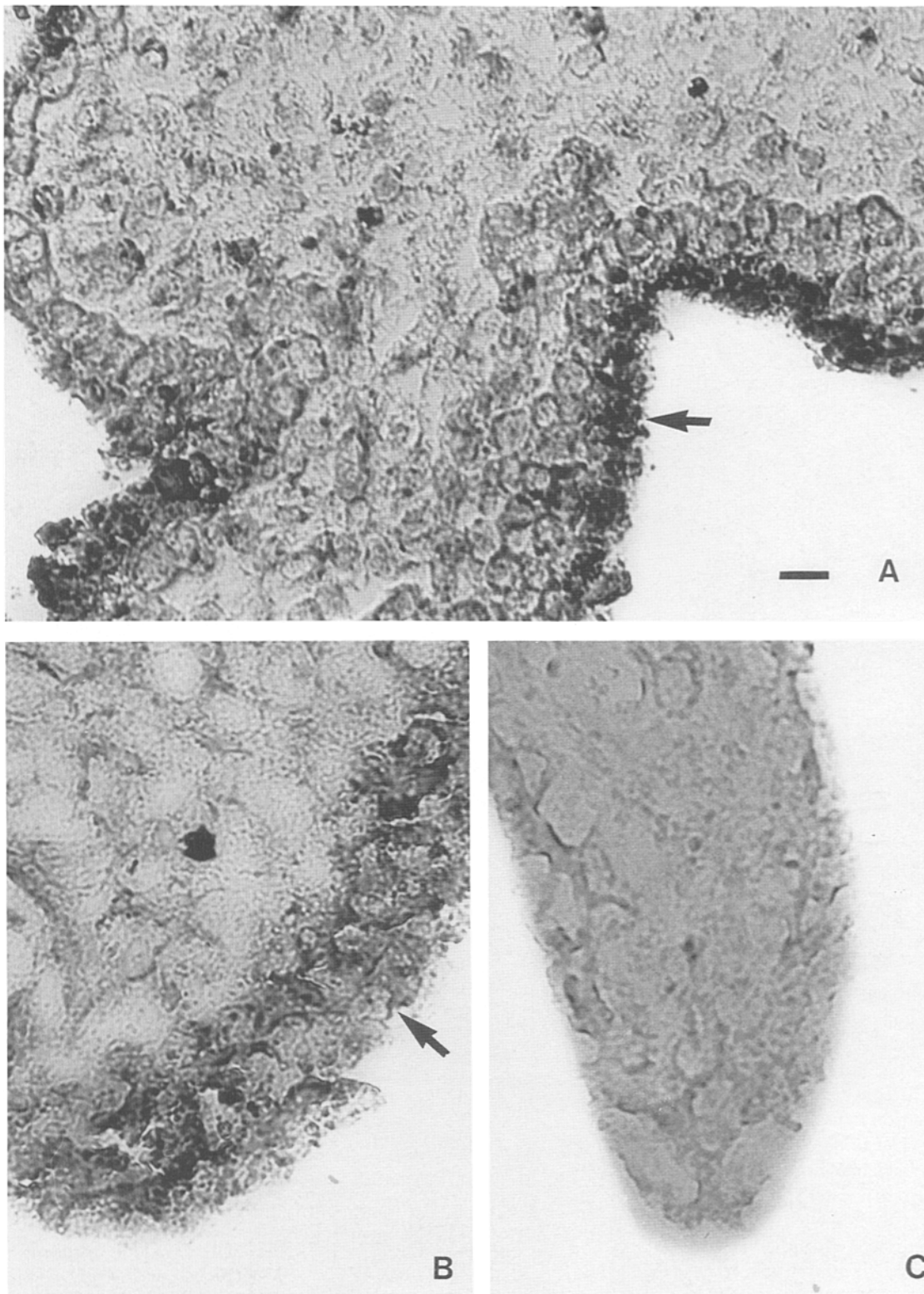


Figure 2. Localization of in situ hybridization signal in 10-wk villus sections. Examination by bright field optics demonstrated hybridization of an HLA-B7 probe to syncytiotrophoblast (*arrow*) and cells of the mesenchyme (*A*). Hybridization signals generated with a DR β clone localized DR β mRNA to syncytiotrophoblast and cytotrophoblast (*arrow*) (*B*). No hybridization signals were observed with control cDNA probe pMCP (*C*) (bar = 3 μ m).

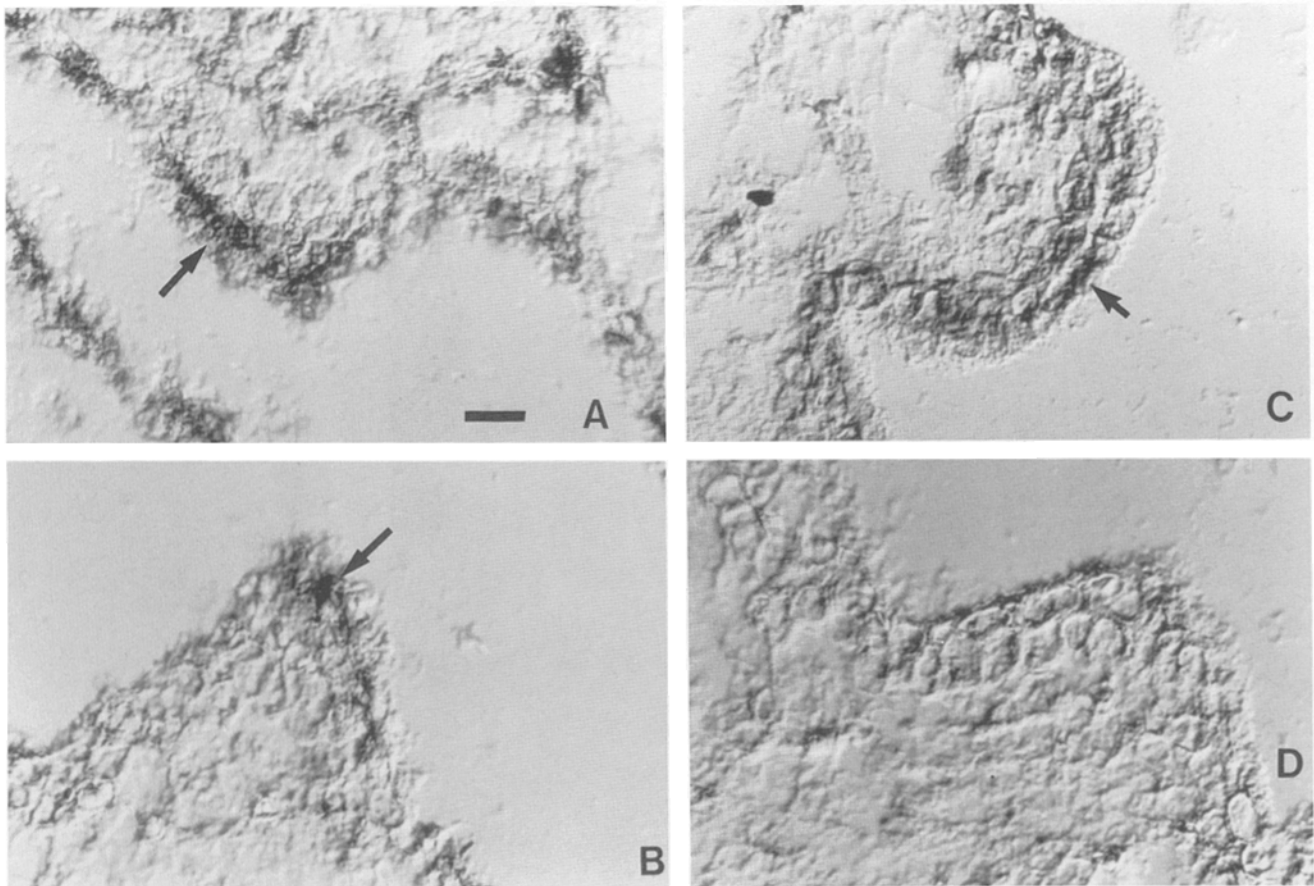


Figure 3. Localization of in situ hybridization signals in 10-wk villus sections. Hybridization of the HLA-B7 probe is localized to syncytiotrophoblast (arrow) and some cells of the mesenchyme (A), while HLA-G signal is confined to syncytiotrophoblast (arrow) (B). The DR β probe hybridized with mRNA in both cyto- and syncytiotrophoblast (arrows) (C), and no signal was observed with the *O. volvulus* paramyosin control probe (D) (bar = 5 μ m).

to successful pregnancy. The chorionic villus trophoblast is the placental cell layer that forms the interface between the allogeneic fetal placental unit and maternal tissues. Immunohistochemical analyses of chorionic villi obtained from term placenta and first-trimester terminations have demonstrated that cytotrophoblast and syncytiotrophoblast fail to express conventional class I and class II determinants (3). This characteristic of trophoblast is thought to contribute to successful pregnancy in that it is one mechanism that may allow the apparent evasion of classical transplantation rejection responses. However, increasing evidence indicates that maternal immunological recognition of fetal alloantigens is necessary for pregnancy success since first-trimester spontaneous abortions, in certain instances, are associated with an insufficient maternal immune response against fetal alloantigens (12–14). This “protective” response may well be due to the absence of fetal MHC determinant expression.

Our studies of trophoblast antigen regulation have been conducted on early-stage villi provided by chorionic villus sampling. We have been unable to detect cytoplasmic (J. A. Lata, and J. B. Smith, unpublished observations) or cell surface (15) expression of classical class I or II antigens by villous trophoblast of any CVS-derived villi. A small subpopulation

of these early-stage cells with the immunohistologic characteristics of villous trophoblast were found to express a class I monomorphic determinant uniquely recognized by the mAb W6/32, but not by additional anti-class I monomorphic determinant monoclonals. Class I-like cell surface antigens, distinct biochemically from classical class I determinants, have been demonstrated on extravillous trophoblast and choriocarcinoma cell lines (16, 17). These are now known to be the nonpolymorphic gene products of the HLA-G class I locus (18).

Our results show that first-trimester villous trophoblast express mRNA transcripts for both class I and class II monomorphic determinants. However, the steady state expression of these mRNAs is not reflected by the production of corresponding polypeptides. It is likely that the signal generated on villous syncytiotrophoblast with the nearly full-length HLA-B7 probe resulted from hybridization to transcripts encoded by HLA-G. This is consistent with the recent finding that HLA-G expression in extraembryonic tissues changes as gestation proceeds with HLA-G transcripts present on placental tissues, but not extravillous cells, during the first trimester. This pattern is then reversed as gestation proceeds to term (19). It is notable that HLA-G message was not de-

tected in cytotrophoblasts or mesenchyme of the early villus samples, as reported by Yelavarthi et al. (20), who demonstrated *in situ* HLA-G transcript expression in cytotrophoblasts of first-trimester elective abortion samples. These conflicting observations may reflect differences in placental sampling procedures. Chorionic villus sampling may provide the more peripheral recent outgrowths of villi, which could be lost in the termination procedure.

The detection of DR β transcripts in first trimester syncytiotrophoblast and cytotrophoblast described here was unexpected. MHC class II determinants have not been detected on any cells of the normal villous trophoblast. However, Labarere and Faulk (21) have demonstrated trophoblast DR expression in localized areas of villitis, indicating that upregulation of DR gene expression may occur. In that circumstance, immune responses to fetal alloantigens could occur via conventional T cell activation pathways.

It is likely that tissue/cell-specific or stage-specific post-transcriptional or translational regulatory events, which remain to be elucidated, are involved in trophoblast MHC antigen expression. It is not yet known if the nonpolymorphic HLA-G antigens are recognized or serve as class I restriction determinants. However, a recent report shows that CD8 can bind HLA-G (22), and thus an interaction between trophoblasts and cytotoxic cells is possible. Likewise, if class II determinant expression could be induced on trophoblast, it seems likely that they could serve as target structures for alloreactive CD4⁺ T cells. Modulation of trophoblast expression of MHC gene transcripts and the corresponding polypeptides may occur in normal human pregnancy as a mechanism to ensure the survival of the fetal allograft, by preventing a destructive cellular immune attack or by allowing a beneficial maternal immune response.

We thank Claire Jesinkey and James Kirwin for their excellent technical assistance, and gratefully acknowledge the staff of Thomas Jefferson University Medical Genetics Laboratory for their cooperation with these studies.

This work was supported by National Institutes of Health grants HD-25629 (J. B. Smith), HD-15822, and HD21355; March of Dimes Birth Defects Foundation grant 1-1146; and the U.S. Department of Agriculture grant 88-37200-3746 (R. S. Tuan).

Address correspondence to J. Bruce Smith, Department of Medicine, Thomas Jefferson University, 613 Curtis Building, 1015 Walnut Street, Philadelphia, PA 19107.

Received for publication 7 October 1991.

References

1. Rocklin, R.E., J.L. Kitzmiller, C.B. Carpenter, M.R. Garovoy, and J.R. David. 1976. Maternal-fetal relation: absence of an immunologic blocking factor from the serum of women with chronic abortions. *N. Engl. J. Med.* 295:1209.
2. Faulk, W.P., and J.A. McIntyre. 1981. Trophoblast survival. *Transplantation (Baltimore)*. 32:1.
3. Head, J.R., B.L. Drake, and F.A. Zuckermann. 1987. Major histocompatibility antigens on trophoblast and their regulation: implications in the maternal-fetal relationship. *Am. J. Reprod. Immunol. Microbiol.* 15:12.
4. Tuan, R.S., B.T. Lamb, and C.B. Jesinkey. 1988. Mouse placental 57kDa calcium-binding protein. II. Localization of mRNA in mouse and human placenta by *in situ* cDNA hybridization. *Differentiation*. 37:198.
5. Sood, A.K., D. Pereira, and S.M. Weissman. 1981. Isolation and partial nucleotide sequence of a cDNA clone for human histocompatibility antigen HLA-B by use of an oligodeoxynucleotide primer. *Proc. Natl. Acad. Sci. USA*. 78:616.
6. Koller, B.H., D.E. Geraghty, R. DeMars, L. Duvick, S.S. Rich, and H.T. Orr. 1989. Chromosomal organization of the human major histocompatibility complex class I gene family. *J. Exp. Med.* 169:469.
7. Long, E.O., C.T. Wake, M. Strubin, N. Gross, R.S. Accolla, S. Carrel, and B. Mach. 1982. Isolation and expression of distinct cDNA clones encoding HLA-DR chains by use of an expression assay. *Proc. Natl. Acad. Sci. USA*. 79:7465-7469.
8. Tuan, R.S., and J.J. Kirwin. 1988. Mouse placental 57 kDa calcium-binding protein. I. Cloning of cDNA and characterization of developmental expression. *Differentiation*. 37:98.
9. Limberger, R.J., and L.A. McReynolds. 1990. Filarial paramyosin: cDNA sequences from *Dirofilaria immitis* and *Onchocerca volvulus*. *Mol. Biochem. Parasitol.* 38:271.
10. Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl. 1987. Current Protocols In Molecular Biology. 1987. John Wiley & Sons, Inc., New York. 1.7.1-1.7.4.
11. Lo, C. 1986. Localization of low abundance DNA sequence in tissue sections by *in situ* hybridization. *J. Cell Sci.* 81:143.
12. Beer, A.E., J.F. Quebbeman, Y. Hamazaki, and A.E. Semprini. 1987. Immunotherapy of recurrent spontaneous abortion. In *Immunoregulation and Fetal Survival*. T.J. Gill, and T.G. Wegmann, editors. Oxford University Press. New York. 286-299.
13. Smith, J.B., and F.S. Cowchock. 1988. Immunological studies in recurrent spontaneous abortion: effects of immunization of women with paternal mononuclear cells on lymphocytotoxic and mixed lymphocyte reaction blocking antibodies and corre-

- lation with sharing of HLA and pregnancy outcome. *J. Reprod. Immunol.* 14:99.
14. Laberrere, C.A., W.P. Faulk, J.A. McIntyre, and O.H. Althabe. 1989. Materno-trophoblastic immunological balance. *Am. J. Reprod. Immunol.* 21:16.
 15. Lata, J.A., F.S. Cowchock, L.G. Jackson, and J.B. Smith. 1989. Cell surface antigen expression of first trimester chorionic villus samples. *Am. J. Reprod. Immunol.* 22:18.
 16. Redman, C.W.G., A.J. McMichael, G.M. Stirrat, C.A. Sunderland, and A. Ting. 1984. Class I major histocompatibility complex antigens on human extravillous trophoblast. *Immunology.* 52:457.
 17. Ellis, S.A., M.S. Plamer, and A.J. McMichael. 1990. Human trophoblast and choriocarcinoma cell line BeWo express a truncated HLA Class I molecule. *J. Immunol.* 144:731.
 18. Ellis, S. 1990. HLA-G: at the interface. *Am. J. Reprod. Immunol.* 23:84.
 19. Wei, X., and H.T. Orr. 1990. Differential Expression of HLA-E, HLA-F, and HLA-G transcripts in human tissue. *Hum. Immunol.* 29:131.
 20. Yelavarthi, K.K., J.L. Fishback, and J.S. Hunt. 1991. Analysis of HLA-G mRNA in human placental and extraplacental membrane cells by in situ hybridization. *J. Immunol.* 146:2847.
 21. Laberrere, C.A., and W.P. Faulk. 1991. Modulation of cytotrophoblastic phenotypic markers. *Am. J. Reprod. Immunol.* 25:67(Abstr.)
 22. Sanders, S.K., P.A. Giblin, and P. Kavathas. 1991. Cell-cell adhesion mediated by CD8 and human histocompatibility leukocyte antigen G, a nonclassical major histocompatibility complex class I molecule on cytotrophoblasts. *J. Exp. Med.* 174:737.