of *cdc2* kinase. It is possible that certain mitotic substrates for MPF, unlike c-Abl, are not phosphorylated by interphase *cdc2*. Alternatively, critical substrates for MPF may only be present in late G_2 cells, and thus would not be available to *cdc2* kinase in the unsynchronized interphase cells used in our experiments.

Treatment of interphase cells with okadaic acid did induce a morphological change similar to that of mitotic cells (Fig. 5B). The rounding of mitotic cells is likely to be the result of Ser/Thr phosphorylation of certain proteins by MPF. The finding that okadaic acid induced similar morphological change in interphase cells suggested that the phosphorylation state of those MPF substrates might be regulated in the same manner as that of c-Abl. Cells transformed by the v-Abl tyrosine kinase have a rounded morphology. Hyperphosphorylation of c-Abl, although not affecting the measurable kinase activity in vitro, may alter its property in vivo to phosphorylate substrates that regulate cell morphology.

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26. We thank J. L. Maller for purified MPF from *Xenopus* oocytes and A. O. Morla for the insightful observation of c-Abl mobility shift during mitosis. Supported by grants from NIH (CA43054) and NSF (DMB86-1378).

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A Class I Antigen, HLA-G, Expressed in Human Trophoblasts

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The α chain of the human histocompatibility antigen HLA-G was identified as an array of five 37- to 39-kilodalton isoforms by the use of two-dimensional gel electrophoresis. Both cell-associated and secreted HLA-G antigens are prominent in first trimester villous cytotrophoblasts and are greatly reduced in third trimester cytotrophoblasts. Allelic variation was not detected, an indication that HLA-G is not obviously polymorphic in cytotrophoblasts. Among the following choriocarcinoma cell lines studied, HLA-G is expressed in JEG but not in Jar or BeWo. Expression of endogenous HLA-G genes has not been found in normal lymphoid cells. Thus, HLA-G is subject to both cell type–specific and developmental regulation and is expressed in early gestation human cytotrophoblasts.

THE PLACENTAL TROPHOBLAST IS the only fetal cell type that is exposed to the maternal uterine decidua and blood. Because of their position at the maternal-fetal interface, trophoblasts are thought to shield the fetus from rejection by serving as a barrier to maternal effector cells (*1*), by eliciting the local production of maternal suppressor cells (*2*) or protective blocking antibodies (*3*), or by being nonrecognizable. The inability to HLA-type trophoblasts with standard typing allosera has suggested the absence of class I antigens as a possible explanation for maternal tolerance toward placenta of different HLA type. However, some trophoblasts bind reagents, such as the monoclonal antibody (MAb) W6/32 (*4*) or antibody to β-2-microglobulin (β-2m), that recognize monomorphic determinants on class I antigens (*5, 6, 7*). We now present data that may reconcile these apparently contradictory reports. These data show that an apparently nonpolymorphic class I molecule, HLA-G, is expressed by cytotrophoblasts and suggest a function for such molecules in protecting the placenta from rejection.

Human trophoblast cell types have different locations within the placenta and the amount of HLA class I expression appears to depend on location. Syncytiotrophoblasts line the external surface of the chorionic villi and are in direct contact with maternal blood; they do not bind MAb W6/32 (*8*). Beneath this layer are mononuclear cytotrophoblasts, an undifferentiated stem cell population that weakly binds MAb W6/32 (*8, 9, 10*). These cells either fuse to form the syncytia or invade the uterus as extravillous (intermediate) cytotrophoblasts (*11*), in which case they express class I–like proteins (*12*). In this work, we studied the mononuclear stem cell population from chorionic villi.

We and others (*13, 14*) have identified the α chains encoded by the HLA-E, -F, and -G class I genes that were isolated from human lymphoblastoid cell line (LCL) 721 (*15, 16*).

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Transfer of the HLA-G gene into the HLA-A, -B, -C-null LCL .221 (HLA-G$^+$LCL.221) (*13, 17*) resulted in the appearance of a β-2m–associated α chain that could be detected with MAb W6/32 and MAb BBM.1 (*18*) but not with MAbs to polymorphic determinants of HLA-A, -B, or -C.

Because an HLA-G–specific antibody is not yet available, we used immunoprecipitation of methionine-labeled HLA-G$^+$LCL.221 transferent cells (*13*) with MAb W6/32 and then two-dimensional (2-D) gel electrophoresis to identify an autoradiographic spot array that is characteristic of the HLA-G α chain and distinct from the spot arrays of HLA-A, -B, -C, -E, and -F. Because HLA-A, -B, and -C are absent from LCL.221, it is easily seen that the HLA-G α chain occurs as an array of five cell-associated 37- to 39-kD isoforms, with isoelectric points from about 4.5 to 5.5 (Fig. 1A).

First trimester villous cytotrophoblasts pooled from four individuals, labeled with methionine in vitro, and immunoprecipitated with MAb W6/32 also showed the isoform array characteristic of HLA-G (Fig. 1B). When immunoprecipitates that contained like amounts of label (counts per minute) (Fig. 1, A and B) were mixed prior to electrophoresis, the HLA-G α chains in HLA-G$^+$LCL.221 transferent cells comigrated exactly with those in the villous cytotrophoblasts (Fig. 1C), thereby providing evidence for their identity. The vertical array of spots of increasing molecular size associated with each HLA-G isoform in cytotrophoblasts may be due to the addition of N-linked polylactosamines of variable size (4 to 10 kD); this type of glycosylation is characteristic of human proteins, such as fibronectin, that appear in the first trimester of gestation (*19*). Immunoprecipitation with MAb W6/32 indicates that the HLA-G α chains in cytotrophoblasts are associated with β-2m (*4*).

HLA-G was also prominent in unpooled preparations of whole first trimester placental villi obtained from four unrelated individuals (Fig. 1D). The 43- to 45-kD molecules that were detected are derived from fetal blood and fibroblasts that are also present in such preparations (see below).

Allelic variation in HLA-G might be detected as shifts in isoelectric point of the α chain. No apparent variation in position of the HLA-G isoforms was observed in first trimester placental cells from the four individuals studied or in cytotrophoblasts from pooled preparations. These observations with a total of 13 individuals suggest that most individuals express the HLA-G α chain in first trimester cytotrophoblasts and that the HLA-G α chain is not obviously polymorphic.

The sub–HLA-A, -B, -C size of HLA-G present in HLA-G$^+$LCL.221 transferent cells is due to an in frame stop codon in exon 6 that truncates the cytoplasmic tail to six amino acids (*16*). Our observations suggest that the same difference in size occurs in the HLA-G expressed in the cytotrophoblasts studied here and, thus, is not a special characteristic of the gene cloned from LCL 721 (*16*). Maintenance of a shortened cytoplasmic tail may be functionally significant.

In three different experiments, we looked for the HLA-G α chain in third trimester trophoblasts isolated from the villi of individual term placentas and labeled during the first 24 hours of culture, when mononuclear cytotrophoblasts predominate. The amount of cell-associated HLA-G α chain in such third trimester cytotrophoblasts is greatly reduced in comparison to that in corresponding first trimester cytotrophoblasts (Fig. 1E). The marked difference in expression of cell-associated HLA-G α chain between first and third trimester cytotrophoblasts implies that HLA-G expression is linked to the developmental stage of the placenta.

The array of spots in the 43- to 45-kD size range in the cytotrophoblasts (Fig. 1, B to E) is characteristic of HLA-A, -B, and -C α chains. Experiments in which contaminating lymphocytes and monocytes were depleted from the cell preparation with a monoclonal antibody specific for CD45 have shown that these α chains are derived from the CD45$^+$ contaminating cells and not from the cytotrophoblasts (*20*).

The acidic 37-kD isoform of HLA-G (Fig. 1, A to D) is also secreted from HLA-G$^+$LCL.221 transferent cells during metabolic labeling and can be immunoprecipitated from the culture medium in association with β-2m by MAb W6/32 (Fig. 2A). After cellular and secreted proteins were partitioned into aqueous and detergent-soluble fractions with Triton X-114, the 37-kD isoform was present only in the aqueous fraction (*14*). Labeling of HLA-G$^+$LCL.221 transferent surface membrane proteins with ^{125}I has revealed that the 37-kD isoform is not expressed on the cell surface (*14*). Loss of the hydrophobic transmembrane domain would explain both the decreased size and water-soluble nature of the secreted HLA-G α chain.

Many W6/32-precipitable proteins apparently are secreted during labeling (Fig. 2B). The protein indicated by the unmarked arrow migrates to the same position as the secreted form of HLA-G. The 45- to 55-kD molecules (larger than actin) are similar to those present in third trimester cytotrophoblasts (Fig. 1E) and in choriocarcinoma cell lines (Fig. 3). These spots are reproducible and could originate in several ways. They could be class I–related, be coincidentally bound to the protein A immunoprecipitates, or be molecules that are specifically associated and coprecipitated with HLA-G by MAb W6/32.

Choriocarcinoma cell lines, derived from tumors of trophoblast origin, are used as examples of trophoblasts arrested at particular stages of differentiation. We looked for HLA-G α chain in three such lines: BeWo (*21*), Jar (*22*), and JEG (*23*). Cell-associated HLA-G was detected only in JEG (Fig. 3); the secreted form of HLA-G is also present

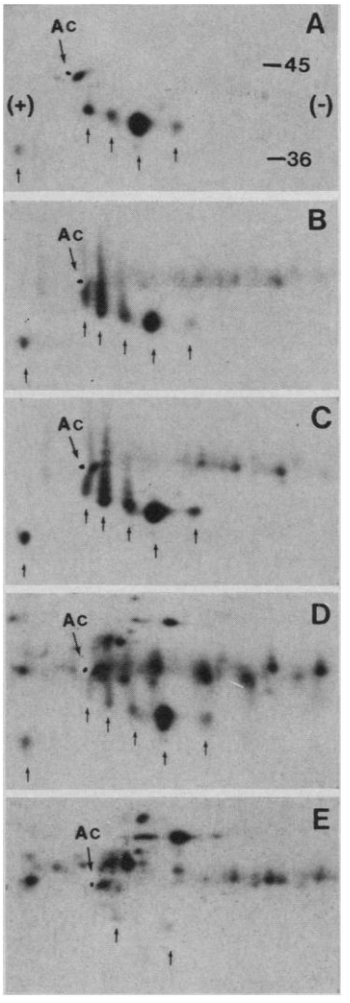

Fig. 1. HLA-G α chain can be detected with MAb W6/32 in normal first trimester cytotrophoblasts. (**A**) HLA-G$^+$LCL.221 transferent; (**B**) purified first trimester cytotrophoblasts pooled from four individuals; (**C**) an equal mixture of the immunoprecipitates shown in (A) and (B); (**D**) first trimester villi from one individual; (**E**) purified third trimester cytotrophoblasts from one individual. "Ac" indicates the position of actin; the gels are oriented from acidic (+) to basic (−). Arrows point to the HLA-G isoforms. Metabolic labeling, immunoprecipitation of α chains with MAb W6/32, and 2-D gel electrophoresis were done as described (*34*).

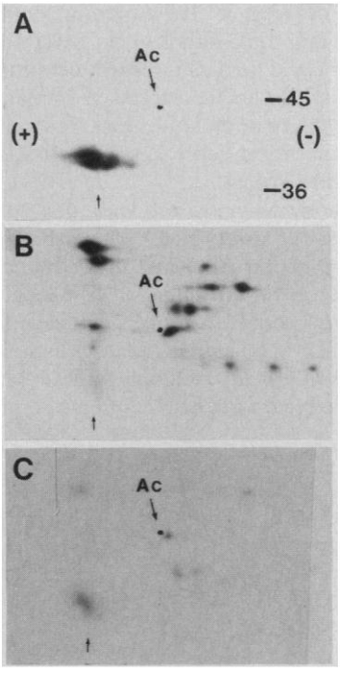

Fig. 2. The 37-kD isoform of HLA-G is secreted. (**A**) HLA-G$^+$ LCL.221 transferent; (**B**) purified first trimester cytotrophoblasts; (**C**) choriocarcinoma cell line JEG. "Ac" indicates the position of actin; (+) acidic, (−) basic. Unlabeled arrows indicate the 37-kD isoforms of HLA-G. After cells were metabolically labeled, the culture medium was collected by centrifugation. Immunoprecipitation of α chains with MAb W6/32 and 2-D gel electrophoresis were done as described (*34*).

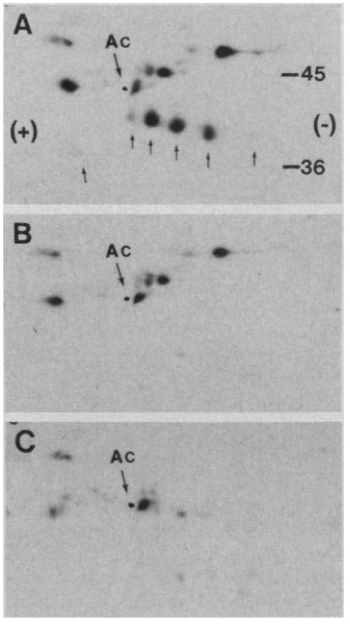

Fig. 3. HLA-G α chains can be detected with MAb W6/32 in the choriocarcinoma line JEG but not Jar or BeWo. (**A**) JEG; (**B**) Jar; (**C**) BeWo. "Ac" indicates the position of actin; (+) acidic, (−) basic. Arrows indicate the HLA-G isoforms. Labeling of cellular proteins with methionine, immunoprecipitation, and 2-D gel electrophoresis were performed as described (*34*).

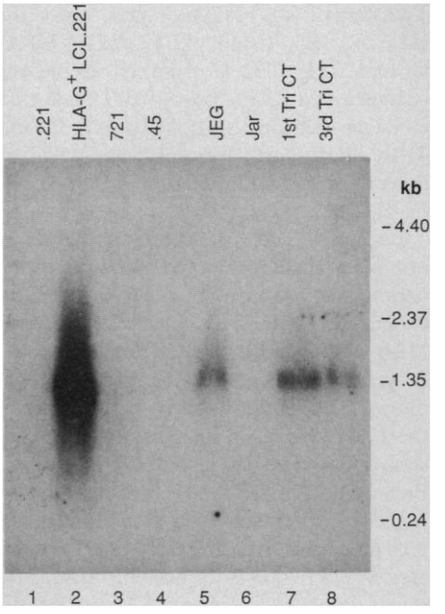

Fig. 4. HLA-G mRNA is present in first and third trimester cytotrophoblasts and JEG. RNA blotting analysis was performed as described (*35*). Cytoplasmic RNA (15 μg per lane except lane 7, which contained 4 μg) was probed with the locus-specific subclone p60-37 (*16*), which is homologous to 250 bp of the 3′ untranslated region of the predicted HLA-G mRNA. Controls for probe specificity include LCL 721 (HLA-A1, A2, B5, B8, C) and LCL .45 (A2, B5, C). (Lane 1) .221; (lane 2) HLA-G$^+$LCL.221 transferent; (lane 3) 721; (lane 4) .45; (lane 5) JEG; (lane 6) Jar; (lane 7) first trimester cytotrophoblast; (lane 8) third trimester cytotrophoblast.

in the culture medium after labeling of JEG (Fig. 2C).

The paucity of class I α chains that we found with MAb W6/32 in these choriocarcinoma lines agrees with the previous analyses of class I antigen density on the surface of these lines (*24–26*). Our 2-D gel analysis of BeWo cell lysates (Fig. 3C) does not reveal the 40-kD class I molecule found previously on the cell surface of BeWo (*7*). JEG is derived from a later passage of the tumor from which BeWo was isolated; the recent isolation of an HLA-G cDNA clone from BeWo (*27*) suggests that the HLA-G gene has been derepressed during in vitro passaging of BeWo.

RNA blotting analysis indicates that HLA-G expression in cytotrophoblasts and choriocarcinoma lines is transcriptionally regulated (Fig. 4). HLA-G–specific mRNA is present in HLA-G$^+$LCL.221 transferent cells, in JEG, and in the first and third trimester cytotrophoblasts (lanes 2, 5, 7, and 8), but is absent in the LCLs .221, 721, .45, and in Jar (lanes 1, 3, 4, and 6). The reduced amount of HLA-G mRNA in third trimester trophoblasts, relative to the amount in first trimester trophoblasts, is consistent with the decrease in HLA-G α chain in third trimester trophoblasts.

Previous studies have also indicated that HLA class I gene expression in human trophoblasts and choriocarcinoma lines is transcriptionally regulated. Class I α chain mRNA is reduced or absent in third trimester trophoblasts and some choriocarcinoma lines; β-2m mRNA is reduced in amount but is not limiting (*8, 9, 24*).

HLA-G is distinct from the human allotypic trophoblast-lymphocyte cross-reactive (TLX) antigens (*28*) but may be similar to the rat di-allelic class I antigen (PA), which is associated with pregnancy and is present on the surface of basal trophoblasts (*29*). A recent study with nucleic acid probes has shown that murine Qa and Tla mRNAs are not present in placental cells (*30*); therefore, HLA-G and Qa-2 antigens may be functionally dissimilar.

Expression of HLA-G is limited, so far, to cytotrophoblasts: we have not detected HLA-G α chains in normal B cells, resting and phytohemagglutinin-stimulated peripheral T cells, spleen cells, thymocytes, T cell leukemia lines, fibroblasts, or the HepG2 hepatoma cell line (*14*). The fact that evolution has resulted in repressed expression of polymorphic HLA-A, -B, -C molecules specifically in villous and extravillous cytotrophoblasts while permitting expression of nonpolymorphic HLA-G molecules in the same cells suggests that HLA-G has a function in the placenta. A nonpolymorphic class I molecule on cytotrophoblasts would not stimulate major histocompatability complex (MHC)–restricted rejection by maternal effector cells and might protect the placenta from maternal non–MHC-restricted cytolytic lymphoid cells present in the decidua (*31*). HLA-G expressed on the cell surface of cytotrophoblasts or secreted into the surrounding cellular environment might activate the suppressor cells found in the human decidua (*2*), or soluble HLA-G might directly suppress maternal cytotoxic responses by binding to the receptors of the cytotoxic cells and blocking the recognition of non-MHC target structures on trophoblasts. Alternatively, HLA-G, because it is nonpolymorphic, might be involved in growth or function of placental cells by association with other molecules, such as insulin receptors (*32*).

Here, we have shown that not only is the HLA-G mRNA present in normal cytotrophoblasts and in a choriocarcinoma-derived cell line, but the mRNA is translated into an HLA-G α chain that is associated with β-2m. Our results suggest that most reports of class I antigen expression by trophoblasts can be interpreted as detection of HLA-G by nonspecific probes for class I

molecules. The demonstration of HLA-G expression in cytotrophoblasts identifies a molecule and a gene that could be involved in placental-maternal interaction and can be studied quantitatively and qualitatively in connection with certain types of unexplained infertility and spontaneous abortion.

Note added in proof: In agreement with observations described above, a survey of human tissues detected HLA-G mRNA only in first trimester placental cells and, subsequently, only in extra-embryonic membranes upon completion of gestation (*33*).

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A Promoter That Drives Transgene Expression in Cerebellar Purkinje and Retinal Bipolar Neurons

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A genomic clone encoding the Purkinje cell–specific L7 protein has been isolated and utilized to drive the expression of β-galactosidase in mice. Three independent transgenic lines, germ line transformed with an L7–β-galactosidase fusion gene, exhibit β-galactosidase expression in both cerebellar Purkinje cells and retinal bipolar neurons. This distribution is the same as that previously determined for the L7 protein by immunohistochemistry. The transgenic murine lines can be used to obtain populations of marked Purkinje and bipolar neurons. Similar L7 promoter constructs can be used to express other foreign genes specifically in these two classes of neurons.

MUCH OF THE PRESENT UNDERstanding of mammalian neural development has relied on the availability of markers that identify specific nerve cell populations. Although such markers are useful for analyzing cell lineage and fate in complex nervous systems, they can also serve as probes with which to elucidate the cellular and transcriptional mechanisms that orchestrate neuroembryogenesis. For example, by examining the expression of a panel of marker genes, it may be possible to define specific genetic regulatory elements and transcription factors that confer cell type specificity. Such regulatory sequences could then be used to drive the expression of exogenous genes that encode proteins that might be involved in, or interfere with, the normal maturation of a particular set of neurons.

The properties of two neural structures, the cerebellum and the retina, make them particularly suitable for an investigation of neurodevelopment. Such properties include their late and well-characterized developmental progression (*1*), their relatively simple and ordered structure (*2*), and the availability of viable murine mutants that affect their development (*3*). This has led us, and others, to identify and characterize molecules that mark specific sets of neurons and glia within these areas (*4*). One of the most restricted markers identified in these studies is L7, which is expressed only in cerebellar

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