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Expression of HLA class II-associated peptide transporter and proteasome genes in human placentas and trophoblast cell lines

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SUMMARY

Expression of HLA class I antigens is closely controlled in the placental trophoblast cells, which interface directly with maternal cells during pregnancy. In this study, the possibility that peptide transporter (*TAP-1, TAP-2*) or proteasome (*LMP7*) genes might be involved in regulating antigen expression in these or other cells that comprise placentas was investigated. Analysis by Northern blot hybridization showed that transcripts from all three genes were present in samples of first trimester and term placental RNA. *TAP-1* and *TAP-2* messages were consistently more abundant in early than in late gestation placentas, whereas the reverse was observed for *LMP7* mRNA. Further experiments were done on two trophoblast cell lines. One line, Jar, is negative for HLA class I, and the second, JEG-3, expresses HLA-G as well as other HLA class I genes. Both Jar and JEG-3 cells contained *TAP-1*, *TAP-2* and *LMP7* mRNA. With the exception of *LMP7* in JEG-3 cells, message from all three genes was increased by treating the trophoblast cells with interferon-γ. While no evidence was collected to support the postulate that the HLA class I negative status of some trophoblast cell subpopulations could be related to absent or dysfunctional *TAP-1*, *TAP-2* or *LMP7* mRNA, the data are consistent with the postulate that placental cell expression of HLA class I antigens could be influenced by the availability of peptide transporters and proteasome components.

INTRODUCTION

Strict control over HLA expression by trophoblast cells forming the fetal cellular component of the maternal–fetal interface is thought to be required for coexistence of genetically disparate fetal and maternal cells during pregnancy.[1] Several phenotypic variants of these cells have been described. One variant, syncytiotrophoblasts, contains little HLA class I mRNA and no protein.[2–5] In a second, villous cytotrophoblast cells, mRNA is present but protein is absent.[3,6] Both of these subpopulations are refractory to induction of their HLA class I antigens by interferon-γ (IFN-γ).[7] A third subpopulation, extravillous trophoblast cells, also referred to as interstitial trophoblasts, contains transcripts from the novel HLA class I gene, HLA-G, and expresses high levels of HLA class I protein.[6,8,9] While cytotrophoblast cells are prominent in first trimester placentas, syncytiotrophoblasts predominate as gestation progresses to term.

Choriocarcinoma cell lines have been used extensively to study HLA expression in trophoblast cells. The Jar tumour cells resemble syncytiotrophoblasts in that HLA-B, -E, -F and -G transcripts are undetectable.[7,10] HLA class I antigens are absent and cannot be induced with IFN-γ.[7,11] BeWo cells contain HLA-G mRNA,[10] as well transcripts from an HLA-C-related gene,[12] and express HLA class I antigens that are inducible with IFN-γ.[11] The JEG-3 cells are an easily cultured subclone of the BeWo cells, and have the same HLA profile.[7]

Cell-surface expression of HLA class I antigens requires the participation of proteins encoded by genes located in the major histocompatability complex (MHC) class II region.[13–17] Products of *TAP-1* (*Ring 4, Y3, PSF-1*) and *TAP-2* (*Ring 11, Y1, PSF-2*) move fragments of antigenic proteins from the cytosol into the endoplasmic reticulum, there to associate with newly synthesized MHC class I heavy and light chains (reviewed in ref. 18). Peptide-loaded class Ia antigens are subsequently transported and inserted in the cell membrane. It is not yet clear whether or not this same pathway operates for class Ib antigens.[19] As with HLA class I heavy chain genes, transcription of the *TAP-1* and *TAP-2* genes is stimulated by IFN-γ.[15] Two additional genes, *LMP2* and *LMP7*, encode components of the proteasomes that degrade proteins into peptides. Although originally thought to be required for cell-surface expression of MHC class I antigens,[15] recent experiments suggest that this might not be the case.[20]

In this study, we assessed *TAP-1*, *TAP-2* and *LMP7* mRNA in samples of early and late gestation placentas as well as in untreated and IFN-γ-treated Jar and JEG-3 cells in an effort to learn whether or not these genes might participate in regulation of HLA class I expression by cells in human placentas.

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MATERIALS AND METHODS

Tissues and cell lines

First trimester placentas were obtained from elective pregnancy terminations, and term placentas were obtained from Caesarean sections performed to avoid fetal distress, in co-operation with the Department of Obstetrics and Gynecology ($n = 3$ each). The protocol was approved by the Human Subjects Committee of the University of Kansas Medical Center. First trimester placentas were separated by manual dissection from other components. Samples of term placentas were taken from between the basal and chorioallantoic plates. Cell pellets and tissues were flash frozen in liquid N_2 and stored at $-80°$ until used for RNA extractions. Jar human choriocarcinoma cells were a gift from R. A. Patillo. JEG-3 human choriocarcinoma cells (HTB-36) and U937 human myelomonocytoic cells (CRL1593) were purchased from the American Type Culture Collection (ATCC, Rockville, MD). Jar, JEG-3 and U937 cells were maintained as described previously.[21] For IFN-γ inductions, subconfluent cultures were treated with 100 U/ml of human recombinant IFN-γ (Endogen, Boston, MA) for 24 hr.

Flow cytometry, cell enzyme immunoassays (ELISA) and MTT assays

HLA class I antigens were identified on Jar and JEG-3 cells in flow cytometry and cell ELISA assays, as described elsewhere.[21,22] Both assays used the mouse anti-human class I monoclonal antibody W6/32 (HB95; ATCC), which recognizes all class I heavy chains, including HLA-G, in combination with β_2-microglobulin.[23] MTT assays, which identify mitochondrial enzyme activity, were used to assess cell proliferation.[24]

Reagents

Restriction enzymes, polymerases, plasmid vectors and DNA ligase were purchased from Promega (Madison, WI). Radiolabelled nucleotides were obtained from DuPont-New England Nuclear (Wilmington, DE). Except where noted otherwise, all other reagents were purchased from Sigma Chemical Co. (St Louis, MO).

Northern blot hybridization analysis

Total RNA was isolated from samples by extraction with guanidine isothiocyanate.[25] RNA samples (10 μg/lane) were fractionated by electrophoresis in 1·2% agarose gels, blotted onto Nytran membranes (Schleicher and Schuell, Keene, NH) and hybridized with ^{32}P-labelled cDNA or cRNA probes, as described previously.[21,26] Non-specific binding of the *TAP-2* and *LMP7-E2* probes was removed by incubating the membranes with 5 μg/ml of RNase A at 37° for 5 min. Membranes were exposed to X-ray film for 2–5 days.[21,26]

Probe preparation

cDNA clones containing the entire coding sequences for *TAP-1* and *TAP-2*[15] and *LMP7-E2*[27] were gifts from Dr T. Spies (Dana-Farber Cancer Institute, Boston, MA). Fragments of the *TAP-1* (381 bp, bases 1504–1841) and *TAP-2* (448 bp, bases 855–1303) coding sequences were subcloned into pGEM-3Zf- plasmids. A fragment of the *LMP7-E2* coding sequence (566 bp, bases 306–872) was subcloned into pSP72. *TAP-2* and *LMP7-E2* cDNAs were used as templates for Sp6 or T7 polymerase-directed synthesis of ^{32}P-labelled cRNA probes. The *TAP-1* cDNA insert was used as a template for the synthesis of ^{32}P-labelled cDNA probes with an oligonucleotide random primer extension labelling kit (Bethesda Research Laboratories, Bethesda, MD). The glucose-3-phosphate dehydrogenase (G3PDH) DNA probe was synthesized from a 1·2 kb *Eco*RI fragment of G3PDH cDNA (R. W. Allen, American Red Cross Blood Services, St Louis, MO) cloned into pGEM3z plasmid (Promega, Madison, WI).

RESULTS AND DISCUSSION

Peptide transporter and proteasome mRNA in human placentas

Figure 1 shows that all samples of human placental RNA contained *TAP-1* and *TAP-2* mRNA. Assessment by scanning densitometer (Fig. 2) indicated that transcripts were more abundant in first trimester than in term placentas. This finding was consistent and reproducible in three samples of first trimester placental RNA and three samples of term placental RNA. Both peptide transporter mRNA and HLA-G were elevated in early placentas and decreased in term placentas, whereas HLA-A, -B were elevated at term.[6,9,28] While the association might be merely coincidental, it suggests the intriguing possibility that loading of HLA-G requires higher transporter activity than HLA-A, -B. If this were the case, control over the proportions of class Ia : Ib expressed by individual cells would reside with the class II-associated genes. This proposition is entirely speculative; the mechanisms underlying preferential expression of class Ia or Ib are unknown.

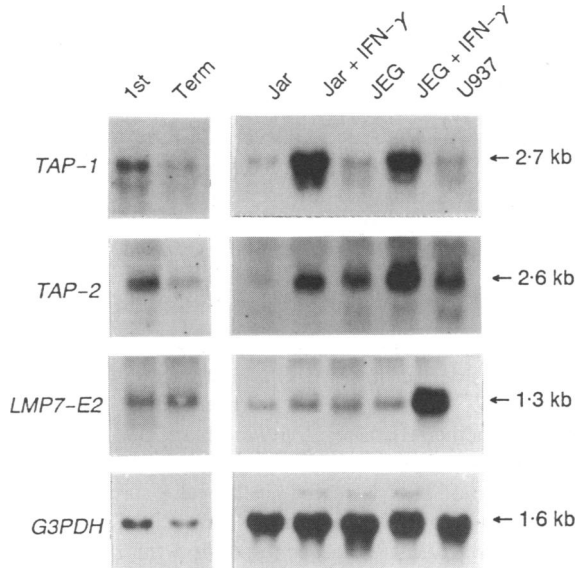

Figure 1. Northern blot hybridization analysis of *TAP-1*, *TAP-2*, *LMP7-E2* and *G3PDH* mRNA in samples of total cell RNA obtained from first trimester placenta (1st), term placenta (Term), Jar and JEG-3 choriocarcinoma cells, and Jar and JEG-3 cells treated with 100 U/ml of human recombinant IFN-γ for 24 hr. Molecular sizes of the messages obtained by comparison with standards on the same gels are shown on the right side of the figure, and are essentially the same as the sizes reported by other investigators.

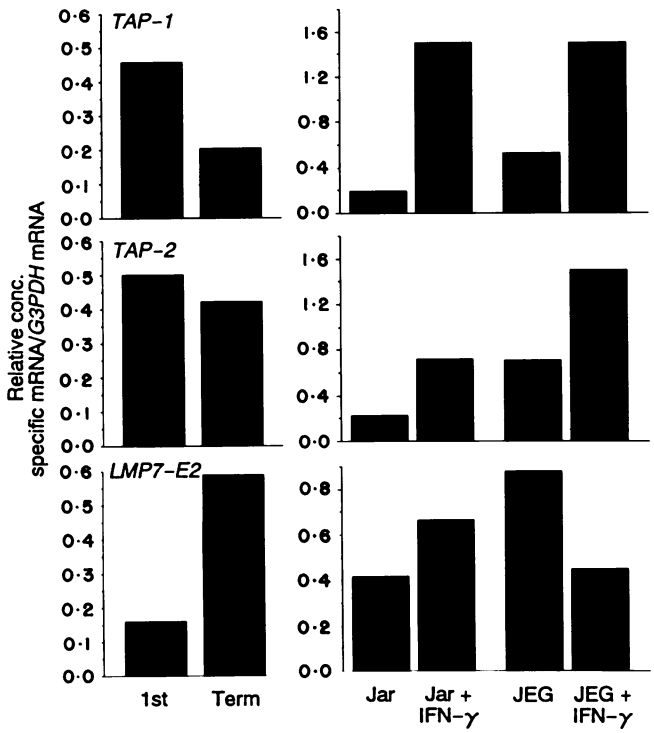

Figure 2. Concentrations of *TAP-1*, *TAP-2* and *LMP7-E2* mRNA in samples of placental and choriocarcinoma cell RNA. Values were obtained by using a scanning densitometer to assess hybridization signals (same blots as shown in Fig. 1), then establishing ratios between specific mRNA and *G3PDH* mRNA densitometer values.

The reduced *TAP* mRNA in term placentas was probably not due to a lesser degree of overall metabolic activity as parturition approached; *LMP* mRNA in term placentas was elevated in comparison with first trimester placentas (Figs 1 and 2). Message could have been derived from placental macrophages, which are abundant and activated in term placental villi.[29] This viewpoint is supported by the finding, reported below, that the macrophage lineage U937 cells exhibited particularly strong LMP7 hybridization signals.

Peptide transporter and proteasome mRNA in trophoblast cell lines

Two cell lines derived from trophoblastic tumours, Jar (HLA null) and JEG-3 (HLA-G+), were tested in an effort to determine whether HLA negativity in cells of this lineage might be related to lack of *TAP-1*, *TAP-2* or *LMP7-E2* mRNA. Untreated and IFN-γ-treated cells were tested by flow cytometry and cell ELISA using a monoclonal antibody to HLA class I, W6/32,[23] in order to confirm the reported HLA phenotypes of the Jar and JEG-3 cells. Table 1 shows that W6/32 did not bind to Jar cells and treatment with IFN-γ did not increase binding. By contrast, cell-surface class I antigens were detected on JEG-3 cells by flow cytometry and cell ELISA. Binding of W6/32 was modestly increased in IFN-γ-treated JEG-3 cells. Culture with IFN-γ did not alter cell numbers. MTT values for Jar cells were 0·45 (A_{450}, untreated) and 0·41 (IFN-γ-treated), and for JEG-3 cells 0·74 and 0·72, respectively.

Figures 1 and 2 show that concentrations of *TAP-1* and *TAP-2* mRNA were very low in the HLA null Jar cells, but were readily elevated by treatment with IFN-γ. Levels of *TAP-1* and *TAP-2* messages relative to *G3PDH* mRNA were higher in the HLA-G+ JEG-3 cells than in the Jar cells, and were increased by treating the cells with IFN-γ. As reported earlier by Bahram *et al.*,[15] the human myelomonocytic cell line, U937, contained *TAP-1* and *TAP-2* mRNA.

These data suggest that differential expression of the HLA class I antigens in subpopulations of trophoblasts, as represented by Jar and JEG-3 cells, may be entirely unrelated to either constitutive levels of transcripts or IFN-γ-inducibility of their *TAP-1* and *TAP-2* genes. However, our experiments would not have identified subtle changes such as single base pair insertions, deletions or substitutions that could result in dysfunctional proteins. Nor have any *in situ* hybridization experiments been done as yet to learn whether or not the various subpopulations of normal trophoblast cells contain *TAP-1* and *TAP-2* transcripts.

Mechanisms underlying reduced expression of the HLA class I antigens by certain trophoblast subpopulations are believed to include hypermethylation of the heavy chain genes (reviewed in ref. 1), diminished heavy chain mRNA[2] and lack of enhancer A DNA-binding proteins.[30] Yet to be explored in humans trophoblast cells are reduced light chain synthesis, which has been reported in mouse trophoblast cells,[31] and negative repressor elements such as those that prevent MHC expression in mouse embryonal carcinoma cells.[32] Organ- and tissue-specific elements are also clearly of great importance; sequences located more than 1 kb upstream from the start of initiation site regulate HLA-G expression in transgenic mouse placentas.[33,34]

The finding that HLA class I-negative trophoblast cells contain *TAP-1* and *TAP-2* transcripts suggests that the transporter proteins might be useful in processes other than those related to peptide loading of nascent class I antigens. However, as mentioned above, there is as yet no evidence that functional proteins are translated from the peptide transporter mRNA.

Table 1. HLA class I expression by uninduced and IFN-γ-treated Jar and JEG-3 choriocarcinoma cells*[7]

		Flow cytometry		Cell ELISA	
Cell line	IFN-γ	%	Peak channel	A_{570}	P†
Jar	0	<1	0	0·13 ± 0·02	
Jar	+	<1	2	0·15 ± 0·01	NS
JEG-3	0	79	96	1·25 ± 0·03	
JEG-3	+	102	99	1·41 ± 0·02	<0·01

*HLA class I antigens were identified on uninduced cells and cells treated for 48 hr with IFN-γ by using W6/32 (flow cytometry, 1 : 500 dilution; cell ELISA, 1 : 800). Percentage positive cells and peak channel of fluorescence obtained after subtracting values for normal mouse IgG are shown. Background binding averaged <2% in flow cytometry and <0·20 in cell ELISA. Means and SD for four replicate microwells are shown for the cell ELISA.

†Significance (*P*) between untreated and IFN-γ-treated cells was calculated using the Student's *t*-test.

LMP7-E2 mRNA was present in both Jar and JEG-3 cells (Fig. 1). As expected, levels of message were increased in IFN-γ-treated Jar cells (Fig. 2). Unexpectedly, but reproducibly in several different harvests of the cells, *LMP7-E2* mRNA concentrations were reduced in IFN-γ-treated JEG-3 cells (Fig. 2). The significance of this finding is unknown. Consistent with its role as an antigen-presenting cell, the U937 human myelomonocytic cell line contained high levels of *LMP7-E2* mRNA.

In summary, the results of this study document the presence of *TAP-1*, *TAP-2* and *LMP7-E2* mRNA in placentas and trophoblast-derived choriocarcinoma cell lines. Gestation-related differences in concentrations of specific transcripts were identified which suggest that HLA class II-associated genes may exert some control over placental cell HLA class I gene expression during pregnancy.

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