Interferon-γ Rapidly Increases Peptide Transporter (TAP) Subunit Expression and Peptide Transport Capacity in Endothelial Cells

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Human cytotoxic T lymphocytes (CTL) recognize specific complexes of HLA class I molecules and peptides, which assemble when nascent class I molecules bind peptides transported from the cytoplasm into the endoplasmic reticulum by the heterodimeric transporter associated with antigen processing (TAP). Increased class I molecule expression on the cell surface increases the efficiency of CTL lysis. The kinetics of interferon (IFN)-γ induction of TAP, peptide transport capacity, and HLA class I molecule expression was determined in endothelial cells, which are targets of CTL following transplantation or viral infection. TAP mRNAs are induced rapidly, increasing 20-fold (TAP1) or 10-fold (TAP2) by 12 h, whereas HLA class I mRNA is induced more slowly, increasing 10-fold in 24 h. TAP1 and TAP2 proteins are also induced rapidly, increasing 10-fold in 24 h, whereas HLA class I heavy chain proteins and surface expression increase more slowly. Peptide transport capacity in endothelial and HeLa cells increases within 6 h of IFN-γ treatment, suggesting that the IFN-γ-induced TAP heterodimers are functional. Therefore, the IFN-γ-induced increase in TAP proteins is accompanied by an increased peptide transport capacity, which may be important in supporting the subsequent rise in HLA class I protein expression.

Major histocompatibility complex (MHC) class I molecules (HLA-A, -B, and -C in humans) are highly polymorphic cell surface glycoproteins that bind peptide antigens, thereby forming a structure that can be recognized by antigen-specific cytotoxic T lymphocytes (CTL) (1). MHC class I molecules are normally expressed at low levels by nearly all cells, but expression is strongly induced by certain cytokines, especially the interferons (IFNs) (2), and increased expression of MHC class I molecules correlates with increased CTL function (3). Regulated expression of MHC class I molecules by vascular endothelial cells, because of their location between peripheral tissues and circulating lymphocytes, may be particularly important in the recognition and rejection of vascularized allografts and in the activation of specific T lymphocytes at sites of viral infections (4, 5).

Mature MHC class I molecules consist of a polymorphic transmembrane heavy chain, a soluble light chain (β₂-microglobulin), and a peptide antigen of 8–10 amino acids (for review, see Ref. 6). The peptides usually derive from endogenously synthesized proteins that are proteolyzed, often by the proteasome, and then transported by the transporter associated with antigen processing (TAP) into the lumen of the endoplasmic reticulum (ER) where they bind nascent MHC class I molecules (for review, see Refs. 7–9). TAP consists of two proteins, TAP1 and TAP2, which are encoded by genes located in the class II region of the MHC (10). Mice lacking TAP1 because of insertional mutagenesis upon homologous recombination (TAP1 “knockouts”) are deficient in cell surface MHC class I molecule expression and antigen presentation, but expression is partially restored upon incubation with immunogenic peptides (11). Humans lacking TAP2 have greatly reduced levels of cell surface HLA class I molecules and suffer chronic bacterial respiratory infections (12). The importance of TAP in providing antigenic peptides for presentation by MHC class I molecules is further suggested by the identification of a 9-kDa herpes simplex virus-encoded protein, called ICP47, which binds to TAP and blocks peptide transport, thus greatly reducing surface expression of HLA class I molecules and, perhaps, recognition by specific CTL (13, 14).

The TAP1-TAP2 heterodimer belongs to a large family of integral membrane transporters that possess a cytosolic ATP-binding cassette and multiple hydrophobic regions that are thought to form a transmembrane channel (15). ATP hydrolysis drives transport of various substrates by ATP-binding cassette transporters. Both TAP subunits bind ATP (16) and contribute to the peptide binding site (17), and each possesses six hydrophobic regions, which together may form the transmembrane peptide channel (18). TAP is located predominantly in the ER (19), the site of MHC class I assembly, and binds transiently to nascent MHC class I heavy chain-β₂-microglobulin complexes (20) through a recently characterized molecule called tapasin (21). Increased TAP expression leads to increased class I surface expression (16), and decreased TAP function leads to decreased class I surface expression (14), strongly suggesting that peptides transported by TAP contribute to regulating the surface expression of class I molecules.

TAP- and ATP-dependent peptide transport was demonstrated using microsomes prepared from the organs of double-stranded RNA-induced wild-type mice compared with TAP1 knockout mice (22) or else using permeabilized T2 cells (which express neither TAP) compared with TAP1- and TAP2-transfected T2 cells (23) or normal cells compared with TAP-deficient cells (24). Efflux from the ER is prevented in these sys-
tems by intraluminal peptide glycosylation (22, 23) or association with HLA class I molecules (24). Transport occurs in these systems at 37 °C, at low peptide concentrations (<0.5 μM) and in peptide-selective. TAP1 and TAP2 expressed in insect cells display normal affinities for peptides and are capable of ATP-dependent peptide transport, suggesting that no additional molecules are required for TAP-dependent peptide transport (25).

IFN-γ, a product of activated T lymphocytes and natural killer cells, regulates the expression of different components in the pathway of MHC class I-restricted antigen presentation. IFN-γ increases transcription of the HLA class I structural genes in neuroblastoma cells (26) and endothelial cells (EC) (27). IFN-γ also increases the expression of TAP1 mRNA in colon carcinoma cells (28) and HeLa cells (18). We have reported previously that IFN-γ induces TAP1 much more rapidly than HLA class I in EC (29), and we suggested that an increase in TAP-supplied peptides in the lumen of the ER might increase the efficiency of peptide loading into class I molecules, thereby limiting the expression of “empty” class I molecules that have been implicated in the induction of autoimmune diseases (30). The recent availability of TAP2-specific antibodies and the development of a TAP-dependent peptide transport assay have allowed us now to test this proposal by examining more closely the mechanism of IFN-γ-induced HLA class I expression by EC.

EXPERIMENTAL PROCEDURES

Cells and Reagents—Human EC were isolated from umbilical veins by digestion with collagenase and cultured serially on gelatin-coated plastic in 20% fetal calf serum, medium 199, and 2.5 mM l-glutamine (all from Life Technologies, Inc.) and endothelial cell growth factor (Collaborative Biomedical Products, Bedford MA) as described (31). All experiments were conducted on confluent monolayers at passage levels 3 or 4. Such cultures are free of contaminating CD45+ leukocytes and stain uniformly positive for EC markers (von Willebrand factor and CD31). HeLa cells (gift of R. Flavell, Yale University) were cultured in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum, 1 mM glutamine, 100 units/ml penicillin, and 0.1 mM β-mercaptoethanol. RNA (10 μg/ml) was a gift of Biogen (Cambridge, MA) and used at 500 units/ml. Recombinant human IFN-γ (expressed in Escherichia coli, 2.5 × 107 units/mg) was a gift of Biogen (Cambridge, MA) and used at 500 units/ml.

S1 Nuclease Protection Assay of mRNA—Total RNA from EC was isolated using acidic phenol and a nucleic acid-binding resin (Ultraspere II, Biotech Laboratories, Inc., Houston). RNA (10 μg) was hybridized to a mixture of uniformly labeled single-stranded probes; human γ-actin, 164 nucleotides protected; HLA class I, 280 nucleotides protected; TAP1, 350 nucleotides protected; and TAP2, 389 nucleotides protected. The γ-actin, HLA, and TAP1 probes and the details of the S1 assay have been described (29). The HLA class I probe maps to the third domain of the heavy chain, which is relatively conserved among HLA-A, -B, and -C alleles (33). The TAP2 probe was generated by reverse transcription of total RNA from IFN-γ-treated EC using random hexamer primers followed by polymerase chain reaction amplification using nested primers TGCGCCCTTCCTCTCCTCCT and CATAACGCCACCATTTG (both listed 5’–3’). The two peptides used in this study were (i) a variant of an HLA-B27-binding, human histone 3 peptide (RYRQNSTEL, where Asn is substituted for Lys; see Ref. 36), and (ii) a variant of an HLA-A3-binding, HIV nef 7B peptide (QVPLRNMTYK, where Asn is substituted for Thr). Both peptides were synthesized by the Keck Biotechnology Resource Laboratory (Yale University). Peptide transport assays were performed with Na235S (1 μCi/ml) and chloramine T and then desalted through a Sephadex G-10 column. Specific activities ranged from 75 to 90 cpm/nmol.

Cell Surface HLA Class I Molecule Expression (Fluorescence Flow Cytometry)—Approximately 5 × 106 cells were cultured with saturating amounts (1 μM) of mouse monoclonal antibodies specific for monomorphic epitopes on HLA class I molecules, W6/32 (38) or BH9 (fluorescein isothiocyanate-conjugated, Bisoro International, or an irrelevant antibody 6B/16, 150 μg/ml of 2% fetal calf serum, 0.01% Na235S phosphate-buffered saline for 30 min, washed twice, and then the W6/32 and K16/16 samples were stained with saturating amounts (1 μM) of a fluoresceinated secondary antibody (Fab')2 goat anti-mouse IgG (H+L), Boehringer Mannheim) in 50 μl for 30 min. After washing twice and fixation in 1% paraformaldehyde, phosphate-buffered saline, 2,000 intact cells (gated on forward and side light scatter parameters) were measured on a fluorescence flow cytometer (FACSort, Becton Dickinson, Sunnyvale, CA). Relative expression = (normalized counts in treated cells) – (normalized counts in untreated cells). Normalized counts = (band of interest – background) ÷ (γ-actin band – background).

Immunoblot Measurement of Protein—Confluent EC cultures (approximately 2 × 106 cells) were left untreated or treated with IFN-γ for the indicated times and then harvested with trypsin, lysed in 60 μl of Triton X-100 containing 1% nonidet P-40, 0.1 mM 1-β-mercapto-ami- no-2-heptanone, 0.5 mM phenylmethylsulfonyl fluoride, and quantified by spectrophotometry at 280 nm (Beckman). Lysates (0.9 μg) were separated by 8% reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto a polyvinyl membrane (Immobilon-P, Millipore). The membrane was then blocked overnight at 4 °C with 5% bovine serum albumin in Tris-buffered saline, 0.2% Tween 20, stained with the appropriate antibody (see below), and developed using a biotin-coupled antibody (horse anti-mouse IgG, or rabbit anti-rat as noted) with avidin-coupled horseradish peroxidase enhancement (Vectorstain ABC Elite, Vector Laboratories, Burlingame, CA) and ECL detection reagents (Amersham). The autoradiographs were quantified by densitometry (Computing Densitometer, Molecular Dynamics) using the area integration function (ImageQuant). Data are presented as relative expression levels (relative expression = (band area of treated samples) ÷ (band area of untreated samples)). The antibody H4A8B13 is a monoclonal mouse anti-human TAP1 antibody (34). The anti-human TAP2 monoclonal mouse antibody 435.3 was derived by immunizing mice with a 280-amino acid COOH-terminal fragment of TAP2 (25). The antibody 3B10.7 is a conformation-independent monoclonal rat anti-human class I heavy chain antibody (35).

Peptide Transport Assay—Peptide transport was measured in permeabilized EC and HeLa cells as described previously for lymphoid cells (24). Briefly, untreated and IFN-γ-treated cells were treated with trypsin, washed in Hank’s balanced salt solution (Life Technologies, Inc.), and suspended in Isevo’s modified Dulbecco’s medium (107 cells/ml on ice). Streptolysin O (Murex, Norcross, GA) was activated with dithiothreitol (4 μl for 10 min at 37 °C) and then added to the cell suspension (2 units/ml final for 15 min at 4 °C). Cells were washed twice in cold Isevo’s modified Dulbecco’s medium to remove unbound streptolysin O and resuspended in warm intracellular transport buffer (50 mM Hepes, pH 7.0, 7.8 mM KCl, 4 mM MgCl2, 8.37 mM CaCl2, 1 mM EGTA, 1 mM dithiothreitol, 1 mM ATP) for 5 min to initiate pore formation. Approximately 80% of the cells were permeabilized as assessed by trypan blue exclusion. For each time point, translocation was started by adding 4 × 106 cells in 600 μl of intracellular transport buffer to a tube containing a radiolabeled peptide (40 pmol, 5 μl), and incubation was continued at 37 °C. The reaction was stopped after the indicated intervals by addition of cell lysis buffer (50 mM Tris-HCl, pH 7.4, 450 mM NaCl, 15 mM iodoacetamide, 1.5 mM phenylmethylsulfonyl fluoride). The glycosylated, radiolabeled peptides were isolated from the postnuclear supernatant (12,000 × g, 5 min) using concanavalin A-Sepharose beads (30 μl, Sigma, 50% suspension in 10 mM Tris, pH 7.4, 150 mM NaCl). The Sepharose pellets were washed three times in 1 ml of wash buffer (10 mM Tris-HCl, 150 mM NaCl, 0.5% Triton X-100) and counted in a γ-counter (LKB Compu- gamma CS). The two peptides used in this study were (i) a variant of an HLA-B27-binding, human histone 3 peptide (RYRQNSTEL, where Asn is substituted for Lys; see Ref. 36), and (ii) a variant of an HLA-A3-binding, HIV nef 7B peptide (QVPLRNMTYK, where Asn is substituted for Pro (37)), which were modified to include a consensus N-linked glycosylation site (N-X-T/S, where X ≠ P). Both peptides were synthesized by the Keck Biotechnology Resource Laboratory (Yale University). Peptide radiolabeled with Na235S (1 μCi/ml) and chloramine T and then desalted through a Sephadex G-10 column. Specific activities ranged from 75 to 90 cpm/nmol.
RESULTS

TAP1 and TAP2 mRNAs Are Induced More Rapidly by IFN-γ Than Is HLA Class I mRNA—To determine the kinetics of IFN-γ induction in EC, mRNAs encoding TAP1, TAP2, and HLA class I heavy chains were measured in an S1 nuclease protection assay. TAP1, TAP2, and HLA class I mRNAs are expressed constitutively at low levels in untreated EC (Fig. 1a, lanes 1 and 7; HLA class I mRNA is seen upon longer exposure of the gel). IFN-γ rapidly increases TAP1 mRNA (lanes 2–6) and TAP2 mRNA (lanes 8–12) and slowly increases HLA class I mRNA (probe included in both time courses). The signals are specific since no protected fragments are detected in the control hybridization to yeast tRNA (lane 13). Also, protected fragments from the HLA class I probe but neither the TAP1 nor TAP2 probes are detected in hybridizations to RNA from mutant 721.174 lymphoblastoid B cells, from which the TAP1 and TAP2 genes are deleted (data not shown).

The results of three (TAP2) or four (TAP1 and class I) independent S1 assays were quantified, and the expression of TAP1, TAP2, and HLA class I mRNAs was normalized with the unregulated γ-actin in each lane to correct for small differences in sample handling (Fig. 1b; 48- and 72-h values are from a single experiment). Following IFN-γ treatment, the kinetics of TAP2 mRNA induction is similar to that of TAP1: both are rapidly induced (9-fold TAP1, 4-fold TAP2 at 3 h), reach peak expression levels in approximately 12 h (>20-fold and 10-fold), and decrease gradually thereafter. In contrast, HLA class I mRNA increases slowly and only reaches maximal expression levels after 48 h (>15-fold induction). Although the kinetics of TAP1 and of TAP2 are similar, the TAP2 mRNA signal was consistently less intense than that of TAP1. Reduced signals may result from less target mRNA or from less efficient hybridization between the mRNA and the single-stranded probe (the specific activities of the probes are similar, data not shown). Although the exact cause is not known, similar results were obtained with two different (nonoverlapping) S1 probes for TAP2, suggesting that there is less TAP2 mRNA than TAP1 mRNA.

TAP1 and TAP2 Proteins Accumulate More Rapidly in Response to IFN-γ Than Does HLA Class I Protein—Immunoblots were performed to measure the accumulation of TAP1, TAP2, and HLA class I heavy chain proteins induced by IFN-γ. Untreated EC contain detectable levels of TAP1, TAP2 (Fig. 2a, upper and middle panels, lanes 2) and HLA class I proteins (lower panel, lane 1). IFN-γ induces a rapid and prolonged increase in both TAP subunits in EC (upper panels, lanes 3–9) and a more gradual increase in HLA class I heavy chains. Neither TAP1 nor TAP2 proteins are detected in the lysate of the deletion mutant 721.174 (lane 1), demonstrating that the immunoblots specifically detect TAP. Additional experiments with the normal parental cell line demonstrated the presence of TAP1 and TAP2 proteins that comigrate with the bands identified in EC (data not shown).

The results of two independent experiments were quantified by densitometry and the values plotted (Fig. 2b). TAP1 and TAP2 increase rapidly and coordinately following IFN-γ treatment and continue to increase, reaching a maximum induction of approximately 17-fold at 48 h. IFN-γ induces a slow and prolonged accumulation of HLA class I heavy chains, which are increased slightly at 12 h (1.5-fold) and reach a maximum induction of nearly 6-fold at 48 h.

IFN-γ Increases Peptide Transport Capacity in EC—Increased TAP expression has been measured following IFN-γ treatment of colon carcinoma cells (28) and HeLa cells (18) or following expression of an Epstein-Barr virus gene (encoding latent membrane protein-1) in lymphoblastoid B cells (39), but any contribution of the increased expression to changes in peptide transport capacity has not been directly assessed. To test whether the newly synthesized, IFN-γ-induced TAPs are functional, a measurement of TAP-dependent peptide transport capacity was performed. The transport assay was conducted for different periods (0.5, 2, and 5 min) to see whether relative transport rates change over the time of assay. IFN-γ increases the rate at which two different peptides are transported, with a significant increase observed as early as 6 h (Fig. 3a, human histone 3-derived peptide; panel b, HIV nef-derived peptide). The relative transport rates were nearly constant over the time of the assay (0.5–5 min), although transport slowed in all samples during the assay. The kinetics of IFN-γ-
induced transport capacity is remarkably similar for these peptides (Fig. 3), each of which binds a different HLA class I gene (36, 37), suggesting a general increase in peptide transport capacity in IFN-γ-treated EC. Similar increases in peptide transport capacity were observed in IFN-γ-treated melanoma cells (data not shown) and HeLa cells (below).

Kinetics of Increased HLA Class I Surface Expression on IFN-γ-treated EC—Greatly increased expression of TAP1 and TAP2 following infection by vaccinia-virus expression vectors (16) or increased peptide translocation following infection by flavivirus (40) increases the expression of MHC class I molecules on the cell surface, suggesting that the supply of peptides in the ER limits MHC class I expression in these cases. It seemed therefore possible that the rapid, IFN-γ-induced increase in TAP proteins and transport activity could lead to a rapid increase in cell surface HLA class I expression preceding increases in mRNA or protein. The expression of HLA class I molecule on the surface of IFN-γ-treated EC was measured by flow cytometry using two different monoclonal antibodies specific for monomorphic determinants on HLA class I. The different levels of fluorescence are attributable to a greater number of fluorescein moieties conjugated to the secondary antibody (detecting W6/32) than to the primary antibody (B-H9). Surface HLA class I heavy chain expression increases slightly at 8 h and increases more than 2-fold in 12 h (Fig. 4), somewhat more slowly than peptide transport capacity (Fig. 3) and much more slowly than HLA class I heavy chain mRNA (Fig. 1) but roughly in parallel with increases in total HLA class I heavy chain protein (Fig. 2).

Peptide Transport Capacity Is Increased in IFN-γ-treated EC and HeLa Cells More Rapidly Than Is HLA Class I Surface Expression—IFN-γ increases TAP mRNA, HLA class I heavy and light chain mRNA, and HLA class I surface expression in
IFN-γ synthesized molecules bind peptides efficiently (46). The regulated expression of HLA class I molecules is important because only newly synthesized molecules bind peptides efficiently. CD8+ memory T cells (4), is likely to be particularly important because of their anatomical position between circulating lymphocytes and the peripheral tissues. The regulated expression of HLA class I molecules in EC. TAP1 and TAP2 mRNAs and proteins are induced by IFN-γ (data not shown). To compare more closely the induction by IFN-γ of peptide transport and surface HLA class I expression at early times, HeLa cells and EC were left untreated or treated with IFN-γ for 6 or 12 h. Both cell types show more rapid induction of TAP-dependent peptide transport capacity than HLA class I surface expression (Fig. 5).

**DISCUSSION**

HLA class I molecules are critical mediators in host defense against intracellular pathogens, including bacteria and noncytolytic viruses (43, 44), and can stimulate strong alloreactions against transplanted tissue (45). HLA class I molecule expression by vascular EC, which act as antigen-presenting cells for monomorphic determinants on HLA class I molecules (W6/32) or tested irrelevant antibody, K1616). Panel b, the mean fluorescence intensity versus time of IFN-γ treatment was also calculated and plotted (lower panel).

HeLa cells (41, 42). TAP-dependent peptide transport capacity in HeLa cells is also increased by IFN-γ (data not shown). To compare more closely the induction by IFN-γ of peptide transport and surface HLA class I expression at early times, HeLa cells and EC were left untreated or treated with IFN-γ for 6 or 12 h. Both cell types show more rapid induction of TAP-dependent peptide transport capacity than HLA class I surface expression (Fig. 5).

In EC, TAP1 and TAP2 mRNAs and proteins are induced by IFN-γ more rapidly than are the HLA class I heavy chains (Figs. 1 and 2). The mRNA and protein signals of TAP2 are noticeably weaker than those of TAP1. Although this difference does not influence the measurements of induction kinetics or magnitudes, it may explain the failure to detect TAP2 in uninduced cells (47). In experiments designed to determine the relative expression of the TAP subunits, we detected small amounts of residual TAP1 but not TAP2 in the supernatant following quantitative depletion of heterodimers by immunoprecipitation with antibodies specific for other subunit (data not shown), suggesting that small amounts of TAP1 are not complexed with TAP2. This could be due to higher levels of TAP1 mRNA and higher rates of TAP1 synthesis. However, even when expressed from identical vaccinia virus constructs, more TAP1 than TAP2 is often recovered by immunoprecipitation (16), suggesting that TAP2 may be less stable than TAP1.

The function of rapidly induced TAP is unclear. However, it has been shown in the vaccinia virus expression system that increased levels of TAP itself increase MHC class I expression on the cell surface (16), presumably as a result of the increased availability of peptides in the lumen of the ER which bind surplus MHC class I molecules. Similarly, decreased TAP function leads to decreased MHC class I surface expression (14). Consistent with this interpretation is the finding that flavivirus infection increases (TAP-independent) peptide translocation into the ER, thereby increasing the availability of peptides in the ER and increasing MHC class I surface expression (40). HLA class I surface expression (Fig. 4) increases roughly in parallel with total heavy chain protein (Fig. 2) and well after mRNA induction (Fig. 1) or peptide transport (Figs. 3 and 5), suggesting that peptide availability does not strongly limit the maturation of HLA class I molecules in EC.

There is a greater IFN-γ induction of TAP proteins than peptide transport at all times tested. After 24 h of IFN-γ treatment, by which time both TAP1 and TAP2 have increased more than 10-fold, peptide transport is increased approximately 3-fold (Figs. 2b and 3c). This discrepancy may reflect an important regulation of peptide transport. Alternatively, several limitations in the peptide transport assay system may contribute to artifactually low values. First, the limiting component in vitro may be the radiolabeled peptide, which is present in relatively low concentration. Peptide transport in vivo is probably not limited by peptide availability (48). However, increased peptide concentration in vitro may lead to TAP-independent peptide transport (49). Second, peptides immedi-
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by HLA class I molecules may be unavailable for glycosylation and be therefore missed in this assay. Third, peptides containing glycosylation signals appear to be better competitors in the peptide transport assay (25), suggesting that glycosylation may be a limiting step. Finally, it should be noted that although a mixture of IFNs was reported to inhibit N-linked glycosylation of viral proteins (50), subsequent studies found that the effect of IFN on viral glycoproteins is negligible (51) and should therefore not complicate significantly the interpretation of the peptide transport data. Taken together, however, these considerations suggest that the peptide transport assay may underestimate peptide transport capacity in untreated or IFN-γ-treated cells. Nevertheless, the kinetics of increase may be fairly compared with mRNA and protein levels.

IFN-γ regulates other components of the class I synthesis pathway. For example, IFN-γ increases the expression of two alternative β-subunits of the 20 S proteasome, LMP2 and LMP7 (52, 53). Although LMP2 (54) or LMP7 (55) knockout mice are deficient in CD8+ T lymphocytes or class I expression, neither LMP2 nor LMP7 is required for antigen presentation in several systems in vitro (56–58). IFN-γ also induces the expression of an activator of 20 S proteasome named PA28 (or 11 S) (59). A 3-fold increase in PA28α expression, comparable to that obtained after 72 h of IFN-γ treatment, increases CTL efficiency without increasing cell surface expression of MHC class I (60). This may result from the more efficient generation of MHC class I-binding peptides by PA28-associated 20 S proteasomes (61). The kinetics of these IFN-γ-induced changes in the proteasome is unknown, but it is likely that LMP2 and TAP1 are induced with similar kinetics because they share a bidirectional promoter (42, 62).

In conclusion, IFN-γ rapidly increases the expression by EC of both TAP subunits followed by peptide transport capacity and then HLA class I surface expression. These results are consistent with our previous interpretation of the kinetics of IFN-γ-induced TAP expression and suggest that TAP-dependent transport function is coordinately regulated to support subsequent class I molecule assembly and expression.

Acknowledgments—We thank Louise Benson and Gwendolyn Davis for expert assistance in EC culture.

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