M 3-[*N*-morpholino]propanesulfonic acid [ionic strength = 0.10 (NaCl) (pH 7.0)] and 5% dimethyl sulfoxide containing 20 μM antibody. The concentration of esters **5a** and **5b** was 1 mM, amine **6** was 2 mM, and, because of limited solubility, **5c** was 100 μM. After 20 s and 6 min, an aliquot was withdrawn and quenched with perchloric acid to a final pH of approximately 2.5. The quenched sample was injected into an HPLC column (Waters 600E) equipped with an analytical C_{18} reversed-phase column (VYDAC, Hesperia, CA) and eluted with an acetonitrile-water (0.1% trifluoroacetic acid) gradient with the detector set at 280 nM. We identified the product by comparing the retention time of the product formed during the reaction with that of authentic samples. The relative rates were determined by dividing the amount of **7** formed by the antibody with that formed in the control reaction.

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Homozygous Human TAP Peptide Transporter Mutation in HLA Class I Deficiency

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Human lymphocyte antigen (HLA) class I proteins of the major histocompatibility complex are largely dependent for expression on small peptides supplied to them by transporter associated with antigen processing (TAP) protein. An inherited human deficiency in the TAP transporter was identified in two siblings suffering from recurrent respiratory bacterial infections. The expression on the cell surface of class I proteins was very low, whereas that of CD1a was normal, and the cytotoxicity of natural killer cells was affected. In addition, $CD8^+$ αβ T cells were present in low but significant numbers and were cytotoxic in the most severely affected sibling, who also showed an increase in $CD4^+CD8^+$ T cells and γδ T cells.

Class I molecules of the major histocompatibility complex (MHC) present peptides to $CD8^+$ T cells. These peptides derive from proteolytic degradation in the cytosol. They are subsequently imported by a peptide transporter into the lumen of the endoplasmic reticulum where they associate with class I molecules (*1*). The peptide transporter is a heterodimeric protein formed of two homologous polypeptides encoded by the *TAP1* and *TAP2* genes located in the MHC class II region (*2–4*). In mutant cell lines that do not express this transporter, most β_2-microglobulin (β_2M)–class I heavy chain complexes do not acquire peptides. Such "empty" complexes are unstable at physiological temperature and are inefficiently transported through the Golgi compartment. Consequently, most of the class I heavy chains remain unsialylated. Relatively few peptide-free class I molecules reach the cell surface where they can be stabilized by exogenous class I–specific peptides (*5*). Mice in which the *TAP1* gene has been disrupted by homologous recombination have almost no detectable $CD8^+$ T cells and no alloreactive cells when bred in germ-free conditions (*6*). In this report we describe a human peptide transporter (TAP) genetic defect.

A TAP deficiency was observed in family E which is from Morocco and includes the parents, who are first cousins, and five children. The first case, EFA, was a 15-year-old female who had chronic bacterial sinobronchial infections but no history of viral infections. Human lymphocyte antigen (HLA) serotyping did not detect HLA class I molecules on her peripheral blood mononuclear cells (PBMCs). A deficiency in β_2M was ruled out. A complete HLA typing of the family (Fig. 1A) showed that two children in the family, EFA and a 6-year-old brother, EMO, are HLA-homozygous and express class II but not class I molecules. The other members of the family, who are heterozygous or HLA different (EAH) express both class I and class II antigens. These results suggest that the defect is genetically linked to the MHC.

Because "absence" of class I molecules as determined by serological typing methods does not mean complete absence of these molecules from the cell surface, we labeled PBMCs with the W6/32 class I monomorphic monoclonal antibody (mAb) and analyzed them by flow cytometry (Fig. 1B). The fluorescence intensity was 1% (T cells and monocytes) to 3% (B cells) that of normal PBMCs. Epstein-Barr virus (EBV)–transformed B cell lines (named ST-XXX) were generated from B cells from EMO and EAH. Flow cytometry showed a 99% reduction of class I molecules on the ST-EMO cells compared with the ST-EAH cell line. Because both cell lines expressed equivalent amounts of class I mRNA, detected by Northern (RNA) blot analysis, we looked for defects in processing of class I molecules. The time course of polysaccharide side chain maturation in ST-EMO (class I^-) and SCH10W9013, a control HLA-A3–homozygous cell line, showed that HLA-A3 molecules became resistant to endoglycosidase H (endo H) (Fig. 1C); thus, they were transported to the *cis*-Golgi compartment. However, whereas endo H–resistant molecules remained stable after a 2-hour chase in SCH10W9013, those of ST-EMO decreased with time. In the extracts of ST-EMO, β_2M was poorly detected, showing that it was loosely associated with the class I heavy

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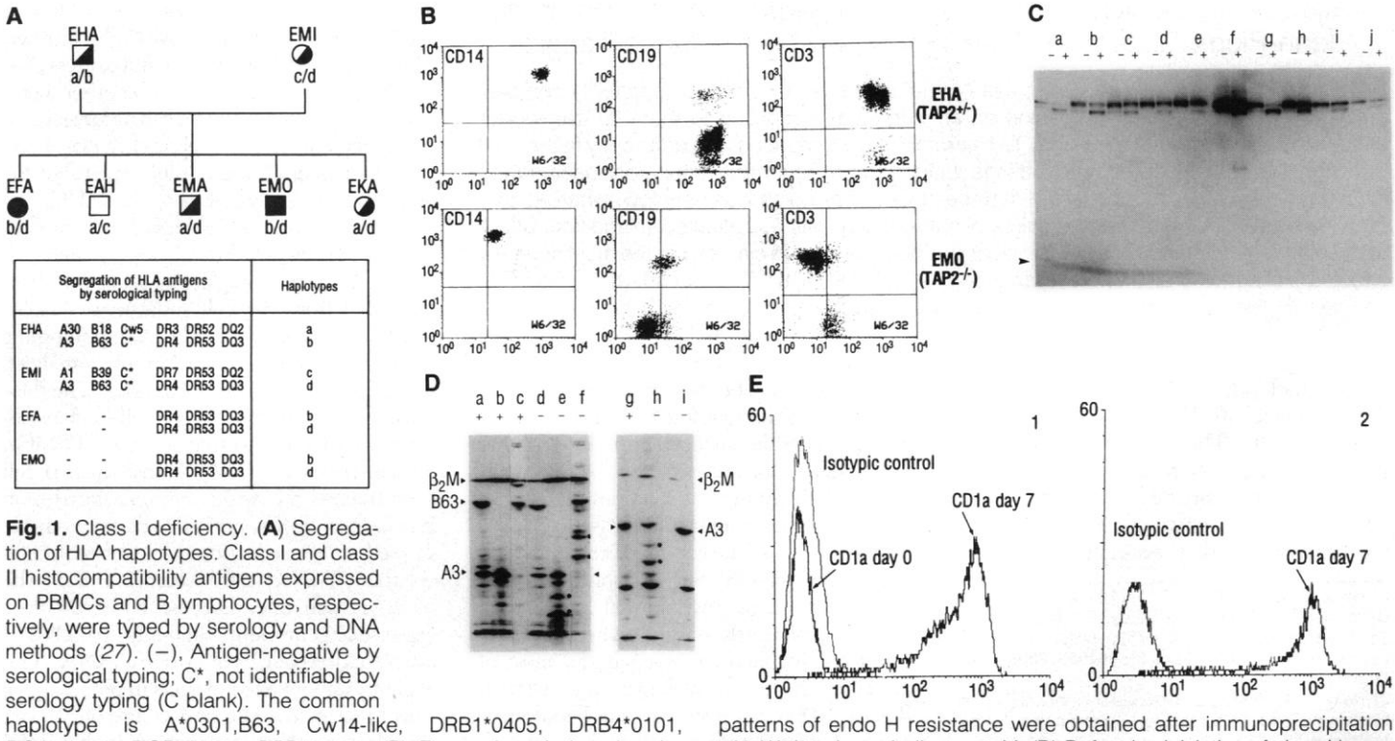
chains and probably dissociated during the immunoprecipitation. Isoelectric focusing of class I molecules immunoprecipitated from ST-EMO with the W6/32 and HLA-A3 mAb GAP A3 (*7*) showed that most class I heavy chains remained unsialylated (Fig. 1D), suggesting that the class I molecules were unstable or poorly transported toward the *trans*-Golgi compartment. These observations are reminiscent of those reported with the TAP-deficient BM36.1 cell line (*3*).

These results are consistent with a deficiency in the peptide transporter. Therefore, protein immunoblot analysis of protein extracts from ST-EMO (class I^-) was done (Fig. 2A) with polyclonal antibodies to TAP1 and TAP2, generated from immunization with synthetic peptides of the COOH-terminal domain of the two subunits. The TAP1 but not the TAP2 protein was detectable in the cell extracts from ST-EMO, whereas both were present in extracts of the normal B cell line LCL721. Antibodies to LMP2 and LMP7 revealed normal expression of the two genetically linked proteasome subunits. Thus, the TAP2 protein is either missing or truncated, and the deficiency of class I molecules is probably due to a mutation affecting only the *TAP2* gene.

To localize the mutation, we reverse transcribed RNA samples from ST-EMO (class I^-) and ST-EAH (class I^+), and *TAP2* complementary DNA (cDNA) was amplified with three sets of oligonucleotides. Equivalent amounts of cDNA were amplified from each RNA with each set of oligonucleotides, showing that the defect is not due to the absence or instability of TAP2 mRNA. The complete sequence of the *TAP2* cDNA in the ST-EMO cell line was determined. A single mutation was identified, a C to T substitution changing the CGA Arg codon at amino acid 253 into a TGA stop codon. This was confirmed by direct sequencing of polymerase chain reaction (PCR) amplification products from genomic DNA obtained from different members of the family (*8*). The two affected children displayed the C to T transition. The TAP2 sequence of EAH was normal and the heterozygous genotypes of EHA and EMA included both sequences. Thus, the genetic defect resulted from a premature stop mutation in the *TAP2* gene.

To determine whether β_2M–class I heavy chain complexes could be stabilized on the surface of the ST-EMO ($TAP2^-$) cells by appropriate synthetic peptides, as observed on TAP-deficient cells (*5*), we incubated ST-EMO cells that express the *HLA*0301* allele in serum-free medium supplemented with human β_2M and peptides that bind to HLA*0301 (pn2a) or HLA-A2 (*9*). Flow cytometry with mAb GAP A3 showed that addition of pn2a, but not control peptide, stabilized the HLA-A3 molecules (Fig. 2B). Thus, the absence of class I antigens on the cell surface appears to be the sole consequence of a failure to load β_2M–class I heavy chain complexes with peptides.

We assessed the effect of the TAP deficiency on the expression of class Ib molecules by investigating the presence of CD1a on epidermal Langerhans cells of a patient. Results of immunochemical staining techniques on a skin biopsy indicated that CD1a mole-



Segregation of HLA antigens by serological typing						Haplotypes
EHA	A30 A3	B18 B63	Cw5 C*	DR3 DR52 DQ2 DR4 DR53 DQ3		a b
EMI	A1 A3	B39 B63	C* C*	DR7 DR53 DQ2 DR4 DR53 DQ3		c d
EFA	- -	- -		DR4 DR53 DQ3 DR4 DR53 DQ3		b d
EMO	- -	- -		DR4 DR53 DQ3 DR4 DR53 DQ3		b d

Fig. 1. Class I deficiency. (**A**) Segregation of HLA haplotypes. Class I and class II histocompatibility antigens expressed on PBMCs and B lymphocytes, respectively, were typed by serology and DNA methods (*27*). (−), Antigen-negative by serological typing; C*, not identifiable by serology typing (C blank). The common haplotype is A*0301,B63, Cw14-like, DRB1*0405, DRB4*0101, DQA1*0301, DQB1*0302, DPB1*0301. (**B**) Expression of class I molecules on PBMCs. The PBMCs from EHA ($TAP2^{+/-}$) and from EMO ($TAP2^{-/-}$) were double labeled with mAb W6/32 and mAbs to CD14 (monocyte specific), CD19 (B cell–specific), or CD3 (T cell–specific) and analyzed by flow cytometry. Similar results as for EMO were obtained for EFA. The *y*-axis data are for the antibody named in the upper left, and the *x*-axis data are for W6/32. (**C**) Endo H resistance of HLA-A3 heavy chains and stability of HLA-A3 molecules. Homozygous HLA-A3 SCHU10W9013 EBV cells (lanes a to e) and ST-EMO ($TAP2^-$) cells (lanes f to j) were labeled 30 min with ^{35}S-methionine and chased with cold methionine for 0 (a and f), 1 (b and g), 2 (c and h), 3 (d and i), and 4 hours (e and j). Cell extracts were immunoprecipitated with HLA-A3 mAb (GAP A3), treated (+) or not treated (−) with endo H, and analyzed by SDS-PAGE. Similar patterns of endo H resistance were obtained after immunoprecipitation with W6/32. Arrow indicates β_2M. (**D**) Defect in sialylation of class I heavy chains. ST-EMO cells (lanes a, d, and i), HLA-A3$^+$ (SCHU10W9013 cell line) (lanes b, e, g, and h), and B63$^+$ (peripheral blood lymphocytes from a normal donor) (lanes c and f) control cells were labeled for 4 hours with ^{35}S-methionine, and class I molecules were immunoprecipitated with mAb W6/32 (lanes a to f) or GAP A3 (lanes g, h, and i), treated (+) or not treated (−) with neuraminidase, and analyzed by gel isofocusing electrophoresis (*28*). Sialylated heavy chains are indicated by dots, unsialylated heavy chains by arrows. (**E**) Induction of the expression of CD1a on monocyte-derived dendritic cells. Monocytes from EMO (histogram 1) and a normal donor (histogram 2) were isolated by plastic adherence and incubated 7 days in the presence of GM-CSF and IL-4 (*29*), and expression of CD1a was determined by cell cytometry.

cules were normally expressed, whereas no class I molecules could be detected on the whole skin section (*10*). Moreover, expression of CD1a molecules could be induced at normal levels on dendritic cells obtained by differentiation of monocytes with granulocyte-macrophage colony-stimulating factor and interleukin-4 (IL-4) (Fig. 1E). Thus, in contrast to HLA class I molecules, the class Ib CD1a molecule does not require TAP for surface expression.

Blood cell populations of EFA and EMO were analyzed by flow cytometry and cytotoxicity tests. Flow cytometry showed that the number of $CD3^-CD16^+CD56^+$ natural killer (NK) cells was normal in both children as was the number of $CD8^+$ NK cells (Table 1). However, no cytotoxic activity against the class I–negative K562 cell line was observed (Fig. 3A). Thus, this class I deficiency does not affect the formation of $CD8^+$ NK cells but, like the murine β_2M deficiency (*11*), affects NK activity. T cell population numbers were compared with the numbers observed in normal subjects (*12*). Normal numbers of $CD4^+CD8^-$ T cells were observed but, contrary to the $TAP1^-$ or the β_2M-deficient mice (*6*, *11*), $CD8^+CD4^-$ T cells were found in PBMCs from EMO (7% of T cells, normal range 30%) and EFA (20% of T cells). The peripheral blood of EFA contained high numbers of $CD4^+CD8^+$ double-positive T cells (10% of T cells, normal range <2%), which have been observed in only a few individuals (*13*). The presence of these $CD4^+CD8^+$ T cells may be the consequence of a thymic failure, but their absence in the PBMCs of the younger brother suggests other factors. Compared with healthy subjects (*14*), a high proportion of γδ-positive T cells was observed in EFA (33% T cells, normal range 5 to 10%), among which 33% were $CD8^+$ and 1% $CD4^+$. Lower numbers of γδ T cells were observed in EMO, but one-third were also $CD8^+$. Thus, the higher number of $CD8^+$ T cells in EFA results from an expansion of γδ T cells, the numbers of αβ $CD8^+$ T cells being low in the two siblings. Because the percentage of $CD8^+$ γδ T cells was normal (*14*), their development seems not to be as strictly dependent on the expression of class I antigens, unlike the positive selection of $CD8^+$ αβ T cells. Nonclassical class I antigens, such as CD1a, that are expressed normally in these TAP-deficient individuals, may be involved in their selection. The expansion of γδ T cells in EFA may be a consequence of her lung disease or may compensate for the low numbers of $CD8^+$ αβ T cells.

Functions of helper T cells and cytotoxic T cells were tested in mixed lymphocyte culture (MLC) and cell-mediated lympholysis (CML) assays, respectively. In MLC tests, EFA and

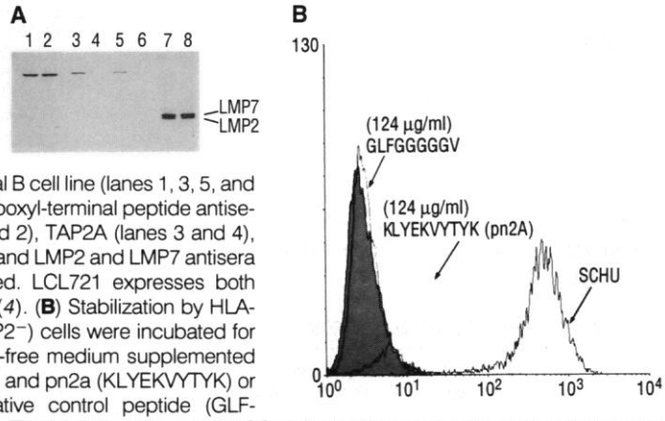


Fig. 2. (**A**) Protein immunoblot analysis of MHC-encoded transporter and proteasome subunits. Protein extracts from the ST-EMO cell line (lanes 2, 4, 6, and 8) and from the LCL721 normal B cell line (lanes 1, 3, 5, and 7) were analyzed (*30*). Carboxyl-terminal peptide antisera for TAP1 (*3*) (lanes 1 and 2), TAP2A (lanes 3 and 4), TAP2B (*4*) (lanes 5 and 6), and LMP2 and LMP7 antisera (lanes 7 and 8) were used. LCL721 expresses both TAP2A and TAP2B alleles (*4*). (**B**) Stabilization by HLA-A3 peptides. ST-EMO ($TAP2^-$) cells were incubated for 18 hours at 37°C in serum-free medium supplemented with human β_2M (10 μg/ml) and pn2a (KLYEKVYTYK) or an HLA-A2–specific negative control peptide (GLFGGGGGV) (124 μg/ml) (*9*). The HLA-A3 homozygous SCHU10W9013 cell line was similarly incubated without peptide. Expression of HLA-A3 was followed by flow cytometry with the mAb GAP A3. Shaded curve:isotypic control. The single letter abbreviations for amino acid residues are as follows: E, Asp; F, Phe; G, Gly; K, Lys; L, Leu; T, Thr; V, Val; and Y, Tyr.

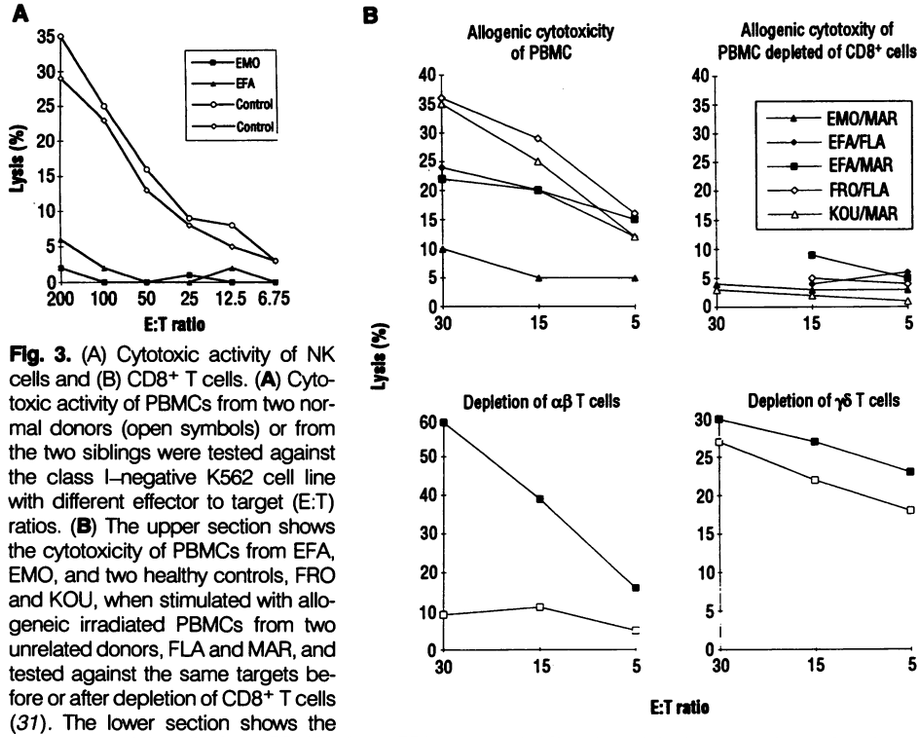


Fig. 3. (A) Cytotoxic activity of NK cells and (B) $CD8^+$ T cells. (**A**) Cytotoxic activity of PBMCs from two normal donors (open symbols) or from the two siblings were tested against the class I–negative K562 cell line with different effector to target (E:T) ratios. (**B**) The upper section shows the cytotoxicity of PBMCs from EFA, EMO, and two healthy controls, FRO and KOU, when stimulated with allogeneic irradiated PBMCs from two unrelated donors, FLA and MAR, and tested against the same targets before or after depletion of $CD8^+$ T cells (*31*). The lower section shows the allogeneic cytotoxicity from PBMCs from EFA against MAR before (black squares) or after (open squares) depletion of αβ or γδ T cells. Percentage of specific lysis obtained for effector to target ratios (E:T) (30:1, 15:1, and 5:1) is given. Autologous specific lysis was <10% (*32*).

Table 1. Lymphocyte subpopulations from peripheral blood. The PBMCs were isolated on Ficoll Hypaque, labeled with mAb, and analyzed on FACSort (Becton-Dickinson). Results are expressed as percent of lymphocytes. The number of lymphocytes in blood were 2.1×10^6 per milliliter (EMO) and 3.9×10^6 per milliliter (EFA) (standard values are 1.9 to 4.8×10^6 per milliliter).

Lymphocyte subpopulations	EFA	EMO
NK cells		
$CD3^-CD16^+CD56^+$	11	5
$CD3^-CD8^+$	5.5	3.5
T cells		
$CD3^+CD8^+$	22.5	5
$CD3^+CD4^+$	42.5	60.5
$CD8^+CD4^+$	7.5	0.3
αβ T cell receptor		
$CD8^-CD4^-$	0.3	0.6
$CD8^+$	11	2.4
γδ T cell receptor		
$CD8^-CD4^-$	18.3	3.5
$CD8^+$	9.7	1.2
Total number	27	5

EMO lymphocytes had normal proliferation indexes when cultured with unrelated stimulatory cells carrying different class II types, suggesting that their $CD4^+$ T cells were normal (*15*). In contrast, EFA and EMO T cells differed in CML tests, and unlike TAP1 knock-out mice, an allogeneic cytotoxicity activity from $CD8^+$ cells was observed in PBMCs from EFA (Fig. 3B). Depletion of $\alpha\beta$ or $\gamma\delta$ T cells in PBMCs from EFA demonstrated that the allogeneic response was mediated by $CD8^+$ $\alpha\beta$ T cells but not by $\gamma\delta$ T cells. Local development of cytotoxic T cells was observed in β_2M-deficient mice after intraperitoneal injection of allogeneic MHC class I–positive cells (*16*), and $CD8^+$ T cells appeared in the spleens after viral infections (*17*) or rejection of skin grafts (*18*). The presence of these $CD8^+$ T cells may be explained by low cell surface expression of class I molecules (*11*), which may allow the positive selection of T cells expressing T cell receptors with a high affinity for the class I molecules (*19*). Our observations show that, similar to the class I β_2M–deficient mice, $CD8^+$ T cells can develop in a peptide transporter–deficient background where cell surface expression of class I molecules is low. The differences between the TAP^- humans and TAP^- mice in this regard could be that the two children had more extensive exposure to pathogens than the laboratory animals did. In agreement with this hypothesis, EFA, the eldest child, had developed four times more $\alpha\beta$ $CD8^+$ T cells than her brother.

With a low number of $CD8^+$ $\alpha\beta$ T cells, the immune response is likely to be less efficient. Nevertheless, antibody titers in serum show that the patients were infected by several viruses. Indeed, although they have never been vaccinated against herpes, measles, varicella, mumps, or cytomegalovirus, EFA had high titers of antibodies against all of these viruses and EMO had antibodies against the first three of them (*20*). These observations suggest that antibody-dependent responses may be essential in the antiviral defense of these patients. NK cells, although they are unable to lyse the K562 cells, may also be involved because complete absence of NK cells may lead to severe infections by herpes, cytomegalovirus, or varicella (*21*), a pathology not observed in the two siblings. Finally, because antigen presentation in peptide transporter–deficient cell lines occurs in some cases (*22*), the significant numbers of $CD8^+$ T cells that persist may still play a role.

HLA class I deficiency, the so-called type I bare lymphocytes syndrome (BLS), is a rare disease that, in most of the cases described so far, has not been linked to the MHC (*23, 24*). BLS may be lethal in early childhood, but in some instances, such as these two individuals with a TAP transporter deficiency, the disease may manifest itself later in life. EFA suffered from chronic colonization of lung by bacteria. EMO was initially healthy for 6 years until he presented with a pulmonary impairment similar to his sister. A similar pathology has been observed in cases with an HLA class I deficiency, where the expression of class II molecules was not clearly determined (*24, 25*). This pathology may be an indication of the physiological significance of the presentation of bacterial antigens by class I molecules that occurs in macrophages (*26*). Why the pathology is restricted to lungs is not understood: it may reflect an important role of macrophages in this tissue. Moreover, previous viral infections may have injured lung tissue and favored susceptibility to bacterial pathogens. This last hypothesis is compatible with the observation that the TAP deficiency may go unnoticed in early childhood and develop later, depending on the incidence of viral infections. These observations may be useful in identifying other cases of class I deficiency associated with absence of TAPs and in characterizing the immune responses that are invoked to compensate for defects in lymphocyte subpopulations.

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27. Serological typing was performed as described [P. I. Terasaki and J. D. MacClelland, *Nature* **204**, 998 (1964); F. Vardal *et al.*, *Tissue Antigens* **28**, 301 (1986)]. Molecular biology typing was determined as described [J.-M. Tiercy, C. Goumaz, B. Mach, M. Jeannet, *Transplantation* **51**, 1110 (1991); A. Kimura and T. Sasazuki, in *HLA 1991*, K. Tsuji, M. Aizawa, T. Sasazuki, Eds. (Oxford Univ. Press, Oxford, 1992), vol. 1, pp 397–419; H. Zetterquist and O. Olerup, *Hum. Immunol.* **34**, 64 (1992); O. Olerup, A. Aldener, A. Fogdell, *Tissue Antigens* **41**, 119 (1993); M. J. Browning *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2842 (1993)]. The A*3002 subtype was determined by sequencing PCR fragments with the use of 5′-CGGAATGTGAAGGCCCAC-3′ and 5′-TCTCAACTGCTCCGCCCG-3′ oligonucleotides. The cDNA of HLA-C was amplified with the use of 5′-CATCTCAGGGTGAGGGGCTC-3′ and 5′-CCCACTCCATGAGGTATTTC-3′ oligonucleotides and sequenced. This typing showed that both parents expressed two complete sets of class I and class II histocompatibility molecules and shared a common HLA haplotype. EFA and her 6-year-old brother, EMO, were homozygous for this haplotype and expressed only class II antigens. Children heterozygous for this haplotype (EMA and EKA), or carrying the other parental haplotypes (EAH), expressed both class I and class II molecules. The HLA-C antigen of the common haplotype was not identifiable by serology (HLA-C blank) but was determined by DNA sequencing. Its sequence is available, accession number of the sequence in National Center for Biotechnology Information, U07230. The subtype is identical to HLA-Cw14*1401 except for two amino acid substitutions (Thr to Ser and Val to Met at position 178 and 248). DNA methods were used to confirm serological typing and demonstrated that class II genes and HLA-A alleles common to the two siblings were inherited without any recombination.
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then with antibodies to murine IgG–coated immunomagnetic beads. Depletions were confirmed by flow cytometry.
32. H. de la Salle *et al.*, data not shown.
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Gem: An Induced, Immediate Early Protein Belonging to the Ras Family

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A gene encoding a 35-kilodalton guanosine triphosphate (GTP)–binding protein, Gem, was cloned from mitogen-induced human peripheral blood T cells. Gem and Rad, the product of a gene overexpressed in skeletal muscle in individuals with Type II diabetes, constitute a new family of Ras-related GTP-binding proteins. The distinct structural features of this family include the G3 GTP-binding motif, extensive amino- and carboxyl-terminal extensions beyond the Ras-related domain, and a motif that determines membrane association. Gem was transiently expressed in human peripheral blood T cells in response to mitogenic stimulation; the protein was phosphorylated on tyrosine residues and localized to the cytosolic face of the plasma membrane. Deregulated Gem expression prevented proliferation of normal and transformed 3T3 cells. These results suggest that Gem is a regulatory protein, possibly participating in receptor-mediated signal transduction at the plasma membrane.

Genes that are transcribed early after mitogenic activation of resting cells are thought to be crucial for subsequent cell proliferation and expression of differentiated effector functions. We have cloned mitogen-induced genes from human peripheral blood T cells on the basis of differential expression of mRNA between resting and stimulated cells (*1*). One such clone, pAT 270, is now shown to encode a protein we have termed Gem because it binds GTP and is induced by mitogens. Human Gem is encoded by a single copy gene (*2*), and its 2127–base pair (bp) complementary DNA (cDNA) was similar in size to the corresponding mRNA (2200 bp) and predicted an open reading frame of 296 amino acids (Fig. 1). The Gem protein contains a core sequence (amino acids 75 to 240) that is highly related to members of the Ras superfamily of small GTP-binding proteins; the flanking NH_2- and COOH-terminal sequences are unrelated to Ras. Gem is most closely related to Rad (~60% identity) (Fig. 1), a protein encoded by a gene that is overexpressed in skeletal muscle from individuals with Type II diabetes relative to skeletal muscle from normal or Type I diabetic individuals (*3*). The greatest similarity between Gem and Rad exists in regions that correspond to the guanine nucleotide–binding domains of Ras. Gem and Rad diverge in the putative effector, or G2, region, suggesting that they interact with distinct targets.

Gem initiates from the first ATG codon (nucleotide 175) whereas Rad has been predicted to initiate from an internal ATG. The predicted start site for Gem was confirmed by immunoprecipitation with antibodies to the predicted NH_2-terminus and by in vitro transcription and translation analyses (*2*).

Mutational analyses of GTP-binding proteins and crystallographic studies of the H-*ras* oncogene product have defined regions of sequence consensus that interact with various positions of the guanine nucleotide (*4–6*). The guanine specificity consensus sequences NKXD and EXSA (X represents any amino acid) are perfectly conserved in Gem, with appropriate spacing, as NKSD (residues 191 to 194) and ETSA (residues 219 to 222), respectively. The consensus sequence GXXXXGK, which participates in interactions with the α and β phosphates of the guanine nucleotide, is also conserved in Gem as the sequence GEQGVGK (residues 82 to 88).

Gem contains an unusual motif in the G3 (DXXG) region, which putatively participates in binding and hydrolysis of the GTP γ phosphate (*5*). The sequence, ENKG (residues 134 to 137), contains the invariant glycine residue but has a conser-

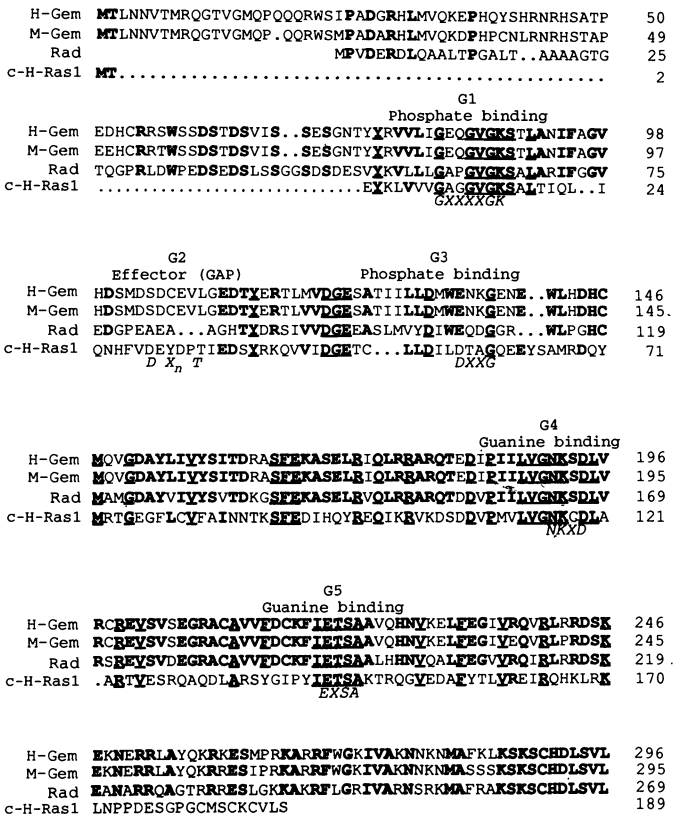

Fig. 1. Predicted amino acid sequences encoded by cDNAs for human and murine Gem (H- and M-Gem, respectively) and comparison to human Rad (*3*) and human c-H-Ras1 (*18*). The open reading frame was determined for two independent human and a single murine Gem cDNA clone. Amino acids conserved in at least three proteins are in bold type and those conserved in all four proteins are bold and underlined. Dots indicate gaps inserted to allow for optimal alignment of the sequences. Numbers on the right indicate residue number. Consensus sequences for GTP-binding regions are indicated in italics (*4, 5*). Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. GenBank accession numbers for the human and murine Gem cDNA sequences are O10550 and U10551, respectively.

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