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A case of primary immunodeficiency due to a defect of the major histocompatibility gene complex class I processing and presentation pathway

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Abstract

Introduction: We report a case of primary immunodeficiency due to a defect of the TAP transporter, an heterodimeric complex which controls the expression of HLA class I molecule by delivering peptides from the cytosol into the lumen of the endoplasmic reticulum. Since childhood, the 36 year old female suffered from recurrent sinusitis/bronchitis. She later developed bronchiectasis and destructive nasal epitheloid granulomata in conjunction with a generalized vasculitic syndrome that did not improve upon immunosuppression and antibiotics. **Methods**: The class I monomorphic W6/32 was used for cell surface staining and immunoprecipitation of MHC class I molecules. Peptide transport assay was carried out in semi-permeabilized cells with iodinated peptides. Antigen presentation experiments were performed using chromium 51 labelled patient B cell line and EBV specific CTL. TAP1 and TAP2 specific antibodies were used for Western blotting and immunoprecipitation of the TAP complex. **Results and conclusions**: A severe reduction of MHC class I molecules at the cell surface of the B-cell lines was observed, whereas MHC class II expression was not altered. Isoelectric focusing of metabolically labelled MHC class I molecules revealed that class I heavy chains remain unsialylated, consistent with a block of TAP dependent peptide translocation. These conclusions were confirmed by further experiments showing that peptide translocation was completely abolished. We also demonstrated that presentation of viral antigens through endogenous class I molecules was severely impaired. Immunoprecipitation and Western blotting of TAP1/2 complex showed that TAP2 was not detectable. Further experiments are in progress to identify the site of the mutation. © 1997 Elsevier Science B.V.

Keywords: Immunodeficiency; MHC; Peptides

1. Introduction

Major histocompatibility gene complex (MHC) class I molecules present peptides derived from intracellular proteins for recognition by cytotoxic T lymphocytes (CTL) [1,2]. Newly synthesised proteins are degraded in the cytosol and peptides generated from them are transferred into the lumen of the endoplasmic reticulum (ER) by the TAP1/TAP2 complex. Peptides of 8–11 amino acids associate in the lumen of the ER to MHC class I/β-2 microglobulin (β-2m) complexes and are subsequently expressed on the cell surface [3]. We have previously shown that in the absence of functional TAP1 and/or TAP2 proteins, presentation of the majority of antigens through class I molecules is severely compromised. In mutant cell lines with deletions or mutations of TAP1 and/or TAP2 genes class I molecules assemble loosely with β-2m and dissociate readily in vitro, but can be stabilised by addition of appropriate peptide ligands [4–7].

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Here we report a case of primary immunodeficiency due to a defect of the TAP transporter. The patient (AK) was a 36 old woman from a Turkish family. She suffered from recurrent sinusitis and bronchitis since childhood. She later developed bronchiectasis and destructive nasal epithelioid granulomatous lesions in conjunction with a generalised vasculitic syndrome that did not improve upon treatment with immunosuppressives and antibiotics. The patient finally died at the age of 36 under signs of progressive pneumonia. Low level expression of MHC class I molecules was detected at the cell surface of peripheral blood mononuclear cells (PBMC), whereas normal levels of β-2m were measured in the serum. These findings led us to the analysis of the class I presentation pathway using a B-cell line derived from the patient's PBMC and finally to the identification of the molecular defect. The patient's immunological parameters will be compared with a previously-described case of primary immunodeficiency due to a homozygous TAP2 deletion [8].

2. Materials and methods

2.1. Cell lines

The B-cell line AK was generated from EBV-transformed B-cells from the patient AK. Positive control B-cell lines SW and WJ were generated from healthy volunteers. The TAP-negative human cell lines .174 and 2 [5] were used as controls in some experiments. The TAP2B positive B cell line .45 was used as a positive control in the Western blotting experiments with TAP2B specific antisera.

2.2. Fluorescent activated cell sorter analysis

PBMC and B-cell lines were stained by immunofluorescence with the following antibodies: PerCP-anti-CD4, PerCP-anti-CD8, PE-anti-CD56, FITC-anti-CD3 (Becton-Dickinson), FITC-anti-CD8, PE-anti-CD16 (Serotec); and W6/32 (monoclonal anti-HLA class I molecules) and L243 (monoclonal anti-HLA-DR), followed by FITC-goat anti-mouse immunoglobulin (DAKO). Fluorescent activated cell sorter (FACS) analysis was performed on the gated lymphocyte population; negative controls were not exposed to first-layer antibody.

2.3. TAP1 and TAP2 immunoblotting

Cells, 1×10^6, were lysed in NP40 lysis buffer [9]. Total lysate was run on a 10% acrylamide gel and transferred onto a Hybond-C membrane (Amersham). Membranes were incubated with a mouse antiserum raised against the TAP1 C-terminal peptide, or two rabbit antisera raised against the C-terminal peptides of the TAP2A and TAP2B proteins (kindly provided by J. Trowsdale, ICRF London). After incubation with horseradish peroxidase-conjugated anti-mouse or anti-rabbit immunoglobulin antibodies, membranes were treated with chemiluminescent substrate (ECL, Amersham) and exposed to film.

2.4. Isoelectric focusing of HLA class I molecules

Cells, 3×10^7, were labelled with 300 μCi [^{35}S]methionine for 30 min at 37°C and lysed in 1.5 ml of NP40 lysis buffer. Samples were then split into three aliquots. Two aliquots were incubated with either 10 μM of the HLA-A11 binding peptide from EBNA 3B protein (IVTDFSVIK), or with 15 μg of W6/32 added immediately after lysis. Immunoprecipitation was then carried out using W6/32 (15 μg/ml) after overnight preclearing at 4°C. HLA class I molecules were resolved by IEF and detected by autoradiography.

2.5. Peptide translocation assay

Cells, 2.5×10^6, were permeabilised with streptolysin O following a protocol previously described [10]. Radioiodinated peptides and ATP were added to a final sample volume of 100 μl and incubated for 15 min at 37°C. Following lysis in 1% NP40, the transported and glycosylated peptide fraction was isolated with Concanavalin A-Sepharose and quantified by γ-counting.

2.6. TAP2 genomic sequencing

Exon 11 of the TAP2 gene, which encodes the C-terminus of TAP2, was amplified by polymerase chain reaction (PCR) from DNA extracted from AK cells, and sequenced by automated sequencing (Applied Biosystems).

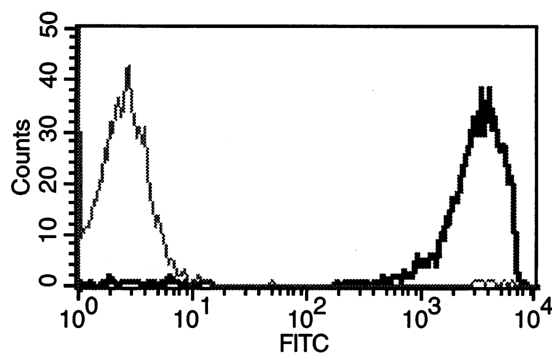
3. Results

3.1. Analysis of MHC class I biosynthetic pathway

Cell surface expression of MHC class I and class II molecules was analysed on both B-cell lines and PBMC from AK. The results of this analysis showed a normal level of expression of MHC class II molecules (data not shown), whereas surface expression of MHC class I molecules was reduced by at least two logs (Fig. 1). Low level of surface expression of MHC class I molecules was confirmed on both T and B lymphocyte sub-populations from PBMC (data not shown). Tissue typing by PCR of AK PBMC showed the patient was homozygous for MHC class I and class II molecules (HLA-A11, -B75, -Cw8, -DR15, -DQB6).

To further analyse the class I biosynthetic pathway and to rule out a transcriptional defect in the class I synthesis, AK B-cells were metabolically labelled and class I molecules were immunoprecipitated with the monoclonal antibody W6/32 and separated by IEF (Fig. 2). A deficiency of β-2m was ruled out by its co-precipitation with newly synthesised class I molecules (middle arrow). Furthermore, normal levels of β-2m were measured in the patient serum (4.8 mg/l). The majority of HLA-C w8 class I molecules were unstable (Fig. 2, top arrow) and were stabilised by adding W6/32 at the time of lysis (Fig. 2, lane 1 and lane 3). In contrast, a large proportion of A11 (bottom arrow in Fig. 2, lane 1 and 2) and B75 molecules (band above A11) were stable at 4°C (Fig. 2, lane 1 and lane 2). Both A11 and B75 molecules failed to mature during a 30 min pulse with [^{35}S]methionine and remained unsialyated. This result demonstrates that class I molecules in AK cells fail to leave the ER and to reach the medium- and *trans*-Golgi. Lack of maturation of class I molecules in AK cells was confirmed by pulse-chase experiments (data not shown).

control subject



AK

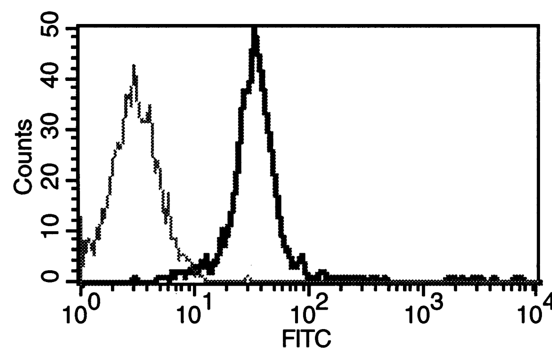


Fig. 1. MHC class I surface expression on lymphocytes. PBMC from AK and a healthy volunteer were stained for class I expression using the monoclonal antibody W6/32. Negative control stainings are shown with thin lines.

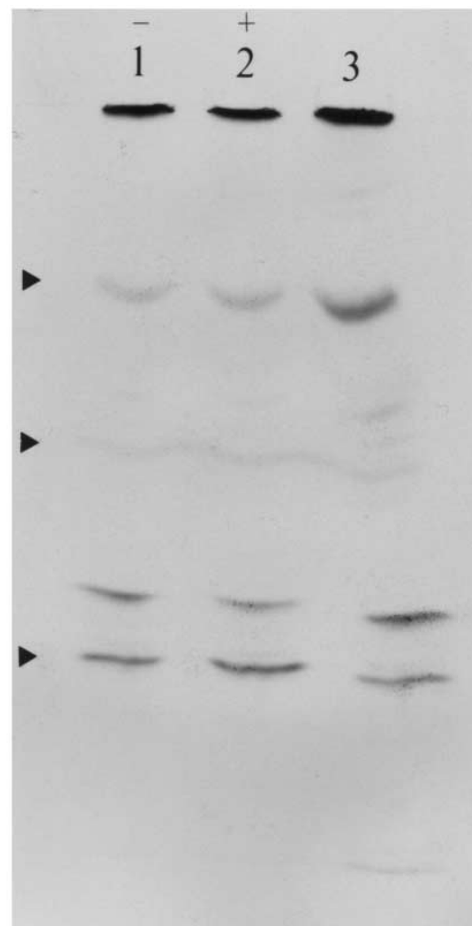


Fig. 2. Detection of HLA class I molecules by IEF. HLA class I antigens were immunoprecipitated from lysates of metabolically labeled AK cells using the monoclonal antibody W6/32. Cells were lysed in the presence (lane 2) or absence (lane 1) of an HLA-A11-binding peptide. In lane 3 W6/32 was added at the time of lysis. Top arrow corresponds to HLA-C0801/3 molecules, middle arrow shows β-2m and bottom arrow shows HLA-A11 molecules. HLA-B75 molecules focus between β-2m and HLA-A11. + and − symbols correspond to the presence or absence of the A11 binding peptide.

These results are consistent with a defect of the TAP complex. The finding that a large proportion of A11 and B75 molecules were stable in AK cells does not rule out this possibility, since it has previously been shown that A11 molecules are stable in TAP-negative cells [11].

3.2. Analysis of TAP complex

To analyse the nature of the block in maturation of class I molecules in AK cells, we measured the efficiency of TAP dependent peptide translocation into the ER using a peptide transport assay [10]. The results of these experiments demonstrated a severe impairment of TAP-dependent peptide transport in AK cells,
an efficient peptide translocation was obtained with a positive control B-cell line (Fig. 3). Consistent with a

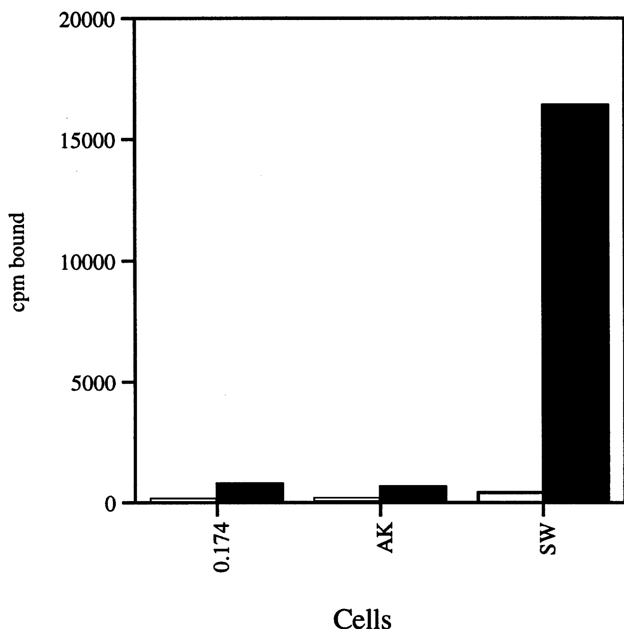


Fig. 3. Peptide transport across the ER membrane. AK cells, SW cells, and the TAP-negative cell line .174, were permeabilised and TAP dependent peptide translocation analysed using the radiolabelled peptide RRYQNSTEL (closed symbol). As a negative control, the peptide RRYQKSTEL was used in which the glycosylation site at position 5 (N) was mutated (open symbol).

lack of functional TAP complex, we demonstrated that AK cells failed to present A11 restricted epitopes to EBV-specific CTL clones (data not shown).

Finally expression of TAP1 and TAP2 was analysed in AK cells by Western blot. The results of these experiments demonstrated that TAP1 molecules were normally expressed (data not shown) whereas no TAP2 molecules were detectable with either anti-TAP2A (data not shown) or anti-TAP2B antibodies (Fig. 4). Sequencing of genomic DNA showed that the patient AK was homozygous for the TAP2B gene.

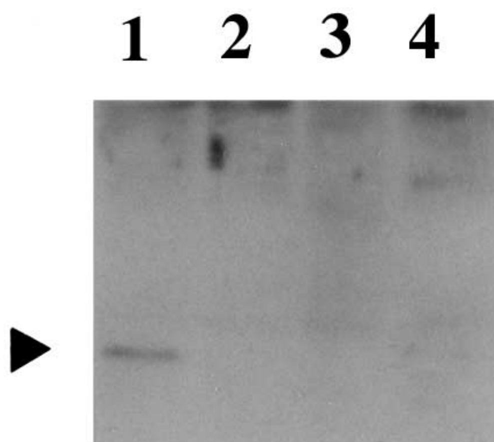


Fig. 4. Western blot analysis of TAP2 molecules. Protein extracts from B-cell lines and from the TAP-negative cell line T2 were analysed with an anti serum raised against the C-terminal peptide of TAP2B. This antiserum is specific for the TAP2B allele and it does not cross react with the TAP2A protein. Lane 1: TAP2B positive cell line .45; lane 2: TAP2A positive cell line WJ; lane 3: AK cells (which were shown to be homozygous for the TAP2B allele by sequencing of genomic DNA); lane 4: TAP negative cells T2. Arrow corresponds to 76 K marker.

3.3. Phenotype of peripheral blood lymphocyte subsets

PBMC from AK were analysed by FACS with antibodies specific to different lymphocyte subsets and frequencies were compared with values obtained from healthy volunteers. The patient had ~ 30% lymphocytes with a total white cell count of 3.6×10^9/l. $CD4^-CD8^+$ T-cells were in the normal range (Table 1), suggesting that despite the low surface expression of MHC class I molecules and the lack of a functional TAP complex, a large number of $CD8^+$ T-cells were positively selected. Experiments are in progress to analyse the T-cell receptor repertoire of the $CD4^-CD8^+$ T-cells in order to address whether they derive from an expansion of an oligoclonal population. It is of interest that the level of expression of CD8 was very heterogeneous and ~ 50% of $CD4^-CD8^+$ T-cells expressed low level of CD8 ($CD8^{low}$), a far greater proportion than in normal controls (Table 1). We have also shown that $CD8^{low}$ cells were stained by anti-CD56 and anti-CD16 antibodies (data not shown). We also observed an increase of $CD56^+CD8^-$ cells and $CD16^+CD8^-$ cells, which is consistent with an expansion of NK cells (Table 1). Finally, numbers of $CD4^+CD8^-$ T-cells appear to be reduced in AK (Table 1). The relationship between this finding and the TAP2 defect is unclear.

4. Discussion

We have described a case of primary immunodeficiency associated with a defect of the class I antigen presentation pathway. The patient had a history of recurrent viral and bacterial respiratory infections and later developed destructive nasal epithelioid granulomatous lesions in conjunction with a generalised vasculitic syndrome. We have shown that class I molecules and β-2m are normally synthesised and that the reduced surface expression of class I molecules is due to a block in the maturation of newly-synthesised class I molecules. We traced this defect back to the lack in antigenic peptide supply to class I molecules using a peptide translocation assay in streptolysin-permeabilised cells. In normal cells, peptide translocation across the ER membrane is dependent on a functional TAP complex. We therefore examined the expression of TAP1 and TAP2 molecules by immunoblotting and showed that TAP2 protein was not recognised by C-terminal TAP2-specific antibodies.

Table 1
Lymphocyte subpopulations from peripheral blood

	$CD4^-CD8^+$	$CD4^-CD8^{low}$	$CD56^+CD8^-$	$CD16^+CD8^-$	$CD4^+CD8^-$
AK	28%	14%	23%	25%	23%
Control	25%	4%	10%	8%	45%

The PBMC were labelled with monoclonal antibodies and analysed on FACS. Results are expressed as percent of lymphocytes.

A primary immunodeficiency linked to a defect in TAP2 protein has previously been described in two siblings of a Moroccan family [8]. In the patient described here, as well as in the two Moroccan patients, $CD4^-CD8^+$ T-cells were detected despite the significantly reduced level of surface expression of class I molecules. In contrast, very few $CD4^-CD8^+$ T-cells were detected in TAP1 knock out mice. This discrepancy could be a result of longer exposure to antigens in humans. Consistent with this possibility, it has been shown that the few $CD4^-CD8^+$ T-cells detected in the periphery of TAP1 deficient mice are capable of mounting epitope-specific responses against defined class I-restricted epitopes [12]. It is also possible that $CD4^-CD8^+$ T-cells in AK have been selected through stable class I molecules (HLA-A11 and -B75), perhaps loaded by TAP-independent peptides. The patient studied here also displayed an expansion of cells with an NK phenotype. Although we do not know whether this NK population is functional, one provocative possibility is that NK cells, freed from class I inhibitory signals, could be responsible for the destructive granulomatous lesions and generalised vasculitic syndrome.

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