Increase in Positive Selection of CD8+ T Cells in TAP1-Mutant Mice by Human β2-Microglobulin Transgene

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Summary

Mice harboring a deletion of the gene encoding the transporter associated with antigen presentation-1 (TAP1) are impaired in providing major histocompatibility complex (MHC) class I molecules with peptides of cytosolic origin and lack stable MHC class I cell surface expression. They consequently have a strongly reduced number of CD8+ T cells. To examine whether selection of CD8+ T cells is dependent on TAP1-dependent peptides, we partially restored MHC class I cell surface expression in TAP1-deficient mice by introduction of human β2-microglobulin. We show that selection of functional CD8+ T cells can be augmented in vivo in the absence of TAP1-dependent peptides.

Materials and Methods

Mice. TAP1-deficient mice have been described previously (3). HLA-A2/β2m- and HLA-B27/β2m-transgenic mice have been described (6). Mice were kept in the animal facilities of the Massachusetts Institute of Technology. Mice 6–10 wk of age were used in all experiments.

Flow Cytometric Analysis. The following monoclonal antibodies and reagents were used: PA2.1 (HLA-A2) (a gift from Dr. H. N. Eisen, Massachusetts Institute of Technology, Cambridge, MA); ME1 (HLA-B27) (a gift from Dr. J. L. Strominger, Harvard University, Cambridge, MA); Y3 (Kb, α1 + α2) (American Type Culture Collection, Rockville, MD) and B22-249.R1 (D3, α1) (American Type Culture Collection); PE-coupled anti-CD4, FITC-coupled anti-CD8-α, biotin-coupled anti-V3 antibodies and PE-coupled streptavidin (PharMingen, San Diego, CA); FITC-labeled goat anti-mouse IgG antiserum (Southern Biologicals, Birmingham, AL). Single-cell suspensions from spleen, lymph nodes, and thymus were prepared, stained, and ana-

CD8+ T lymphocytes are positively selected by MHC class I molecules to ensure self-restriction, a process that requires proper surface expression of MHC class I molecules (1). MHC class I molecules present peptides, derived mainly from cytosolic proteins, to CD8+ T cells. Most MHC class I molecules rely on these peptides, as provided by the heterodimeric transporter associated with antigen processing (TAP) complex, for efficient expression at the cell surface (2). Mice in which the gene encoding the TAP1 subunit is deleted have strongly reduced MHC class I cell surface expression and are impaired in positive selection of CD8+ T cells (3). However, HLA-A2 can be expressed at intermediate levels on the cell surface of TAP-deficient cells, due to its ability to bind signal sequence-derived peptides, a TAP-independent source of peptides (4). We crossed mice transgenic for HLA-A2 and human β2-microglobulin (β2m) (TAP1+β2A) onto a TAP1-deficient background to examine whether TAP-independent peptides are essential for development of CD8+ T cells, or whether TAP-independent peptides suffice. We also crossed mice transgenic for HLA-B27 and β2m (TAP1+β2B) with TAP1−-mutant mice. HLA-B27 is inefficiently expressed at the cell surface of TAP-mutant cells (5), and the TAP1−β2B7 mice were intended as controls. However, whereas only HLA-A2 was expressed at the cell surface in a TAP1-deficient background, TAP1−β2A and TAP1−β2B mice showed a similar increase in percentage of CD8+ T cells compared with TAP1− mice. Rescue of CD8+ T cells is therefore independent of the ability of the transgenic human MHC class I molecules to bind signal sequence-derived peptides and must be due to a feature shared by the transgenic animals, to wit, the presence of β2m.
lyzed on a FACScan® flow cytometer (Becton Dickinson & Co., Mountain View, CA) as described previously (3). Blood was stained for CD8-expressing cells with the FITC-coupled anti-CD8 antibody. Red blood cells were subsequently lysed with FACS® lysis solution (Becton Dickinson & Co.), and lymphocytes were analyzed on a FACScan®.

Metabolic Labeling and Immunoprecipitations. Spleen cells were cultured for 2 d in DME containing 10% FCS and 2.5 μg/ml Con A. Cells were incubated for 30 min in methionine-free RPMI medium and labeled with [35S]methionine/cysteine (protein labeling mix; DuPont/NEN, Boston, MA) for the indicated time and, where appropriate, chased in presence of 1 mM cold l-methi-
ionine and t-cysteine. Cells were lysed in NP-40 lysis mix (0.5% NP-40, 50 mM Tris, pH 7.4, 5 mM MgCl₂, 0.1 mM PMSF). Immunoprecipitations, neuraminidase digestion, and one-dimensional isoelectric focusing (1D-IEF) were performed as previously described (7).

Steady State Distribution of Kb Molecules in the Thymus. Thymuses were isolated from 6-8-wk-old mice. Thymocytes were removed by gently squeezing the thymic lobes with forceps and rinsing with PBS. The remaining capsule was macerated and dissolved in IEF sample buffer by repeated shearing through a 25G1/2 needle. Samples were separated on 1D-IEF and blotted to nitrocellulose paper. The blot was incubated with a rabbit antiserum raised against a peptide derived from the cytoplasmic tail of K⁺ (exp8) followed by a horseradish peroxidase-coupled goat anti-rabbit antibody (Amersham, Arlington Heights, IL). Bound antibody was detected by a chemiluminescence detection kit (Amersham) and exposure to films (X-OMAT AR; Eastman-Kodak Co., Rochester, NY).

Stability Assay. Freshly isolated spleen cells were labeled by lactoperoxidase-catalyzed iodination (see reference 18). Cells were lysed in 2 ml NP-40 lysis mix. Lysates were divided into four equal parts and were incubated for 15 min on ice after addition of 0.5 ml lysis mix with or without 60 μM YAPGNFPAL peptide. Samples were kept on ice or incubated at 39°C for 45 min. Lysates were precleared twice, followed by immunoprecipitation of H-2b com-
plexes with a conformation-dependent anti-H-2\(^b\) serum (a gift from Dr. S. Nathenson, Albert Einstein College of Medicine, Bronx, NY). Immunoprecipitates were analyzed by 12.5\% SDS-PAGE.

**Peptide-binding Assay.** YAPGNFPAL peptide was labeled by chloramine T-catalyzed iodination (see reference 11). 10\(^6\) splenocytes were incubated in PBN buffer (PBS + 1\% BSA + 0.01\% NaNO\(_3\)) with the indicated concentrations of 12\(^s\)I-YAPGNFPAL for 1 h at 23\(^\circ\)C. Cells were lysed in NP-40 lysis mix containing 100 \(\mu\)M cold FAPGNYPAL. Lysates were precleared once, and K\(^b\) molecules were precipitated with the \(\alpha\)p8 serum. Immunoprecipitates were counted in a \(\gamma\) counter. Values are the mean of triplicates.

**Results.**

Spleen cells from TAP1\(^{-}\)\(\beta A2\) mice were stained with an mAB directed against HLA-A2. They express an intermediate level of HLA-A2 on the cell surface (Fig. 1 a) compared with cells from TAP1\(^{+}\)\(\beta A2\) mice. This is in agreement with observations on TAP-deficient cell lines (8). The cell surface expression of HLA-A2 is paralleled by an approximately two-fold higher percentage of CD8\(^+\) cells in the thymus (Fig. 2 a) and an approximately sixfold higher percentage of CD8\(^+\) T cells in blood (Fig. 2 b) in TAP1\(^{-}\)\(\beta A2\) mice, as compared with TAP1\(^{-}\) mice.

HLA-B27 cannot be detected either at the cell surface in TAP1-deficient animals, in contrast to HLA-A2 (Fig. 1 b). A 3-h metabolic labeling of TAP1\(^{-}\)\(\beta B27\) spleen cells followed by immunoprecipitation with the conformation-dependent antibody W6/32 and analysis of the immunoprecipitates on a 1D-IEF gel shows that a substantial fraction of the HLA-B27 molecules fail to assemble with \(\beta 2\)m (Fig. 1 g). Parallel immunoprecipitation with an antiserum raised against denatured free heavy chains reveals that most B27 molecules are present as free heavy chains (Fig. 1 g). The few complexes that are present do not carry sialic acids, the acquisition of which is indicative of proper intracellular transport. These data underscore the reliance of HLA-B27 on TAP for a suitable source of peptides required for assembly and surface expression. Nonetheless, the TAP1\(^{-}\)\(\beta B27\) mice show a similar increase in the percentage of CD8\(^+\) T cells in the periphery, as do the TAP1\(^{-}\)\(\beta A2\) mice (Fig. 2 b).

By examination of the surface expression of H-2K\(^b\) and D\(^b\), this paradox may be satisfactorily explained. Surface expression of H-2K\(^b\) and D\(^b\) class I molecules in a murine TAP2-deficient cell line can be increased by transfection of this cell line with h\(\beta 2\)m (5). Indeed, K\(^b\) shows a fivefold and D\(^b\) a twofold increase in cell surface expression on spleen cells from both TAP1\(^{-}\)\(\beta A2\) and TAP1\(^{-}\)\(\beta B27\) mice compared with TAP1\(^{+}\) mice (Fig. 1, c-f). Pulse-chase analysis shows that these K\(^b\) and D\(^b\) heavy chains preferentially associate with h\(\beta 2\)m and are transported to the cell surface, as judged also by acquisition of sialic acids (Fig. 1 h).

Relative levels of MHC class I surface expression in the thymus were determined at steady state. Thymic lobes were depleted of thymocytes, and extracts of the remaining thymic capsules were resolved on 1D-IEF and analyzed by immunoblotting. In TAP1\(^{-}\) animals, the majority of K\(^b\) heavy chains remains unmodified (Fig. 1 i), but in TAP1\(^{-}\)\(\beta A2\) animals (and TAP1\(^{-}\)\(\beta B27\) animals, data not shown) modification of K\(^b\) is observed, indicating that the K\(^b\) molecules have

![Figure 3](image-url)
traversed the trans-Golgi network. Thus both in the periphery and in the thymus, surface expression of endogenous MHC class I molecules is increased in the presence of hβ2m. This increase in MHC expression may explain selection of CD8+ T cells in the TAP1-βA2 and TAP1-βB27 mice.

We examined polyclonality of the CD8+ T cell population present in TAP1-βA2 mice. Cell suspensions, made from lymph nodes of TAP1-βA2 and TAP1+βA2 mice, were stained with antibodies against CD8 and different TCR Vβ chains. Of all the Vβ chains tested, those used by CD8+ T cells in TAP1+βA2 mice are also used by CD8+ T cells in TAP1-βA2 mice, indicating that the CD8+ T cell population in TAP1-βA2 mice is polyclonal (Fig. 2 c). These CD8+ T cells are able to respond in a primary mixed lymphocyte reaction. After 5 d of culture, strong CD8+ T cell-dependent cytotoxicity against H-2Kd targets is observed, similar to that in TAP1+βA2 mice (data not shown).

MHC molecules from TAP-deficient cells (9–11) and mice (3) have been proposed to be devoid of peptide based on their failure to be expressed efficiently at the cell surface, their thermolability, and their increased peptide-binding capacity. Do MHC class I molecules in TAP1-βA2 mice display similar properties? Detergent lysates of cell surface–iodinated splenocytes were incubated at 4°C or 39°C in the presence or absence of the 9-mer peptide YAPGNFPAL, a variant of the Sendai virus peptide FAPGNYPAL (12, 13) that contains the anchor residues for both Kβ and Dβ. H-2Kβ class I complexes were then immunoprecipitated with a conformation-specific anti H-2Kβ serum. Labeled MHC class I complexes are absent from lysates of TAP1- cells, but they are detected in lysates from TAP1+βA2 cells (Fig. 3 a) in amounts in accordance with the data obtained by flow cytometric analysis (Fig. 1, c and e). Exposure of lysates of TAP1+βA2 cells to 39°C results in a strong decrease in reactivity with the conformation-specific anti H-2Kβ antiserum, but addition of YAPGNFPAL peptide prevents thermal unfolding of the H-2Kβ molecules (Fig. 3 a). No such loss of immunoreactive material is observed in extracts from TAP1+ and TAP1+βA2 cells.

To determine the pool size of Kβ molecules on the cell surface that are available for binding class I peptides, a peptide-binding assay was performed on freshly isolated splenocytes by use of radiolabeled 125I-YAPGNFPAL. Kβ molecules on splenocytes from both TAP1- and TAP1+βA2 mice bind considerably more 125I-YAPGNFPAL than Kβ molecules on TAP1+ and TAP1+βA2 cells (Fig. 3 b), despite lower levels of Kβ cell surface expression (Fig. 1 c). An approximately twofold difference in binding capacity between TAP1- and TAP1+ cells is observed, whereas TAP1-βA2 cells express approximately fivefold more Kβ molecules on the cell surface. A significant proportion of Kβ molecules on TAP1-βA2 cells may have bound peptides from a TAP-independent source (15). These peptides presumably bind with a lower affinity than TAP-dependent peptides, since they fail to stabilize H-2Kβ complexes in vitro (Fig. 3 a).

Discussion

In both β2m− and TAP1− mice, small numbers of CD8+ T cells are present that can be expanded in vivo and vitro (16–19). Our data show that selection of CD8+ T cells in TAP1− mice can be augmented in vivo by increasing cell surface expression of H-2Kβ and Dβ through heterodimerization with hβ2m. In vivo selection of CD8+ T cells is therefore not strictly dependent on peptides of cytosolic origin and can be mediated by MHC class I molecules that are either devoid of peptide, have bound peptides from a TAP-independent source, or both. The pool of CD8+ T cells selected by these molecules is polyclonal and alleloreactive.

In fetal thymic organ cultures (FTOCs), an in vitro model for thymic selection, peptides contribute to the specificity of positive selection (20–23). In FTOCs derived from β2m− mice, cell surface expression of MHC class I molecules is not detectable, and structural similarity between the selecting peptide and the nominal antigen is required for positive selection of a monoclonal T cell population bearing a Kβ-restricted, ovalbumin-specific TCR (22). Selection in TAP1−FTOCs of a Dβ-restricted lymphocytic choriomeningitis virus peptide–specific T cell can be accomplished by the nominal antigen at low peptide concentrations (30 μM) (23). However, at a 10-fold higher peptide concentration, and therefore at much higher MHC class I density (21), the same T cell can be selected by a Dβ-binding peptide (influenza NP366–374) structurally unrelated to the nominal antigen. At physiological densities of MHC class I/peptide complexes, selection of a given CD8+ T cell may be less dependent on a specific peptide and may be accomplished by MHC class I molecules bearing a heterogeneous set of peptides not necessarily related to the nominal antigen other than by their ability to bind to the restriction element in question.

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