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Immunology

Biosynthesis of major histocompatibility complex molecules and generation of T cells in Ii TAP1 double-mutant mice

(antigen presentation/major histocompatibility complex class I transport/major histocompatibility complex class II transport/T cell differentiation/T cell selection)

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ABSTRACT **Major histocompatibility complex (MHC) class I and II molecules are loaded with peptides in distinct subcellular compartments. The transporter associated with antigen processing (TAP) is responsible for delivering peptides derived from cytosolic proteins to the endoplasmic reticulum, where they bind to class I molecules, while the invariant chain (Ii) directs class II molecules to endosomal compartments, where they bind peptides originating mostly from exogenous sources. Mice carrying null mutations of the TAP1 or Ii genes ($TAP1^0$ or Ii^0, respectively) have been useful tools for elucidating the two MHC/peptide loading pathways. To evaluate to what extent these pathways functionally intersect, we have studied the biosynthesis of MHC molecules and the generation of T cells in Ii^0TAP1^0 double-mutant mice. We find that the assembly and expression of class II molecules in Ii^0 and Ii^0TAP1^0 animals are indistinguishable and that formation and display of class I molecules is the same in $TAP1^0$ and Ii^0TAP1^0 animals. Thymic selection in the double mutants is as expected, with reduced numbers of both $CD4^+$ $CD8^-$ and $CD4^-$ $CD8^+$ thymocyte compartments. Surprisingly, lymph node T-cell populations look almost normal; we propose that population expansion of peripheral T cells normalizes the numbers of $CD4^+$ and $CD8^+$ cells in Ii^0TAP1^0 mice.**

The function of major histocompatibility complex (MHC) class I and II molecules is similar, yet distinct (for reviews see refs. 1–3). Both bind antigenic peptides and present them to T cells, but class I molecules associate primarily with peptides generated from cytosolic proteins and offer them to $CD8^+$ cytotoxic T cells, while class II molecules interact with peptides derived mostly from exogenous proteins and present them to $CD4^+$ helper T cells. This dichotomy reflects the intracellular pathways travelled by the two classes of MHC molecule. Class I heavy chains assemble with β_2-microglobulin and are loaded with peptides shortly after being synthesized in the endoplasmic reticulum (ER). Most of the peptides are delivered into the ER from the cytosol by the transporter associated with antigen processing (TAP) complex. Class I–peptide complexes exit the ER and are transported along the secretory pathway, via the Golgi to the cell surface. Class II α and β chains assemble in the ER and associate with the invariant chain (Ii). Ii prevents many peptides from binding in the ER and is responsible for directing the exit of class II molecules from the ER and their targeting to endosomal compartments. In the endosomes, class II molecules are dissociated from Ii and loaded with peptides, mostly processed from proteins internalized from the exterior. Class II–peptide complexes are then transported to the cell surface via an as yet poorly defined route.

Evidence is accumulating, however, that this scenario is too neat. Sometimes, class I molecules are capable of presenting exogenously supplied antigens (4–7), and class II molecules can occasionally present endogenous antigens (8–10), although the extent to which such presentation relies on loading of class II molecules in the ER has not been accurately established. Similarly, TAP-independent presentation of exogenous material by class I molecules has been observed, but the extent to which this represents processing akin to that seen for class II molecules remains to be established. It is not known what is responsible for such breakdown in the dichotomy, but some intriguing observations have been made. On the one hand, some protein epitopes are actually presented better in the absence of Ii, provoking speculation that they might be bound to class II molecules in the ER (11). On the other hand, Cerundolo *et al.* (12) and Sugita and Brenner (13) have detected complexes of class I molecules and Ii. These complexes are, in one case, diverted from the secretory pathway and appear in the endosomes (13).

Here, we approach the problem by exploiting mice carrying null mutations in the TAP1 or Ii genes ($TAP1^0$ or Ii^0, respectively). In the former, class I complexes are largely held up in the ER, although a few unstable complexes, probably devoid of peptide, appear at the cell surface; consequently, $CD8^+$ T cells are poorly selected in the thymus, and there is only a paltry peripheral population (14). In the latter, class II $\alpha\beta$ chains are retained in the ER, but, again, some unstable complexes make it to the cell surface, in this case 10–30% the usual numbers; the $CD4^+$ T cell compartments are reduced in the thymus and periphery, but only by severalfold (15–17). We have now mated Ii^0 and $TAP1^0$ mice to produce litters of single and double mutants. To explore any functional intersection of the class I and II loading pathways, we compared the biosynthesis of MHC molecules and the generation of T cells in the different littermates.

MATERIALS AND METHODS

Mice. Mice deficient in Ii have been described (15). The $TAP1^0$ mice used in this study were generated independently from those previously reported (14). A 15-kb TAP1 clone was isolated from a 129/OLA genomic phage library and analyzed by restriction mapping and partial sequencing. Two adjacent *Bgl* II restriction fragments (1400 and 700 bp, respectively),

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Abbreviations: MHC, major histocompatibility complex; TAP, transporter associated with antigen processing; Ii, invariant chain; ER, endoplasmic reticulum; mAb, monoclonal antibody; 1D-IEF, one-dimensional isoelectric focusing.

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encoding at least the second and part of the third transmembrane helix, were replaced by a 1.1-kb *Bgl* II–*Bam*HI fragment containing the pMC1neo gene (18, 19). E14 ES cells were electroporated with a 9-kb *Apa* I targeting fragment, consisting of the *neo* gene flanked by 0.5 kb and 7.5 kb of TAP1 genomic sequence at the 5′ and 3′ ends, respectively (Bio-Rad gene pulser, 800V, 3μF, electrode distance 0.4 cm). Transfectants were selected in G418, and 200 drug-resistant colonies were expanded and analyzed for homologous recombination with a 750-bp *Hin*dIII–*Not* I fragment situated 500 bp upstream from the targeting construct. Homologous recombination had taken place in eight clones, and three clones were used for injection into blastocysts. Generation of chimeras and homozygous knockout mice was as described (20).

Single and double mutants resulted from a cross between Ii^0 (2 or 3 generations backcrossed onto C57Bl/6) and $TAP1^0$ (backcrossed 2 or 3 generations onto FVB) individuals. Animals were housed in conventional facilities in either Strasbourg or Cambridge.

Metabolic Labeling and Immunoprecipitations. Freshly isolated spleen cells were incubated for 45 min in methionine-free RPMI medium, labeled for 30 min with 500 μCi of [^{35}S]methionine/cysteine (1 Ci = 37 GBq) (protein labeling mix, NEN/Du Pont), and chased in medium supplemented with 1 mM unlabeled L-methionine and L-cysteine. Cells were lysed in NP40 lysis mixture (0.5% Nonidet P-40/50 mM Tris·HCl, pH 7.4/5 mM $MgCl_2$/0.1 mM phenylmethylsulfonyl fluoride), supplemented with 10 mg of bovine serum albumin per ml. Lysates were precleared twice with normal mouse serum and formalin-fixed *Staphylococcus aureus*, then immunoprecipitated with the indicated MHC class II- and I-specific antibodies and antisera. Immunoprecipitates, adjusted for total amount of incorporation, were analyzed by SDS/PAGE or one-dimensional isoelectric focusing (1D-IEF) PAGE (21) and fluorography.

The antisera and monoclonal antibodies (mAbs) used were as follows: αHC serum, a rabbit polyclonal antiserum directed against free MHC class I heavy chains (22); αp8, a rabbit antiserum directed against the cytoplasmic tail of K^b made in our laboratory essentially as described in ref. 23; mAb Y3, recognizing properly folded K^b complexes (24); mAb B22.249, specific for properly conformed D^b complexes (25), and mAb Y3.P, recognizing I-A^b complexes both in the presence and absence of Ii (26).

Endoglycosidase H Treatment and Immunoblotting. Whole thymus was depleted of thymocytes by gently squeezing the thymic lobes with forceps and rinsing them in phosphate-buffered saline. The remaining capsule was macerated with a razor blade and resuspended in 0.5% SDS/1% 2-mercaptoethanol. This suspension was briefly heated to 95°C, vortexed, and sheared through a 30-gauge needle. The suspension was adjusted to 50 mM sodium citrate (pH 5.5), divided in two parts, and treated or mock-treated with 2000 units of Endo H_f (New England Biolabs) for 2 h at 37°C. Aliquots were fractionated by SDS/PAGE and transferred to nitrocellulose. The blots were incubated with αp8 antiserum followed by horseradish peroxidase-coupled goat anti-rabbit Ig serum (Promega). Bound antibody was detected by a chemoluminescence detection kit (Kirkegaard & Perry Laboratories) and exposure to x-ray films.

Cytofluorimetric Analyses. Cell suspensions from thymus, lymph nodes, or spleens were prepared and stainings made on 3×10^5 cells as previously described (27, 28).

The mAb Y3P was used to detect A^b molecules; K^b and D^b were stained with the K9-178 (29) and B22-329 (25) mAbs, respectively. Texas-red-conjugated anti-IgM was obtained from Jackson ImmunoResearch. CD4 and CD8 were revealed by phycoerythrin (PE)-conjugated YTS 191.1 (Caltag) and biotinylated H35.275 revealed by streptavidin-tricolor (Dako), respectively. BrdUrd incorporation was detected with an anti-BrdUrd antibody (Becton-Dickinson), as described (28). All other reagents are listed in refs. 27 and 28).

RESULTS

MHC Class II Molecule Assembly, Transport, and Cell Surface Expression. Assembly of class II molecules in splenocytes from Ii^0 mice of the H-2^b haplotype is reduced, but class II complexes can still be detected intracellularly and on the cell surface (15–17). To investigate whether peptides delivered to the ER by TAP molecules contribute to the formation and display of these class II complexes, we performed metabolic labeling experiments and cytofluorimetric analyses on splenocytes from $Ii^0/TAP1^0$ single- and double-mutant mice comparing, in particular, Ii^0 and Ii^0TAP1^0 cells.

A pulse–chase analysis was undertaken using a mAb that precipitates the $A\alpha^bA\beta^b$ complex. As depicted in Fig. 1*A*, Aα and Aβ chains can be immunoprecipitated from Ii^0TAP1^0 splenocytes. Their quantity is reduced compared with wild-type and $TAP1^0$ splenocytes but is approximately equal to the amount present in Ii^0 splenocytes. The Aα and Aβ chains from wild-type and $TAP1^0$ splenocytes show a mobility shift during the chase, no doubt due to the acquisition of complex type oligosaccharides in the Golgi. However, as previously reported (15–17), most of the class II complexes in Ii^0 splenocytes remain in the ER, as concluded from the absence of a mobility shift. Complexes in Ii^0TAP1^0 splenocytes also appear to remain largely in the ER. Furthermore, in Ii^0 and Ii^0TAP1^0 cells, most of the α and β chains remain Endo H sensitive, even after 3 h of chase (data not shown).

The class II complexes which do reach the surface of splenocytes from Ii^0 mice do not attain an SDS-stable conformation (15–17), commonly taken as a criterion for peptide occupancy (30, 31). Class II complexes in Ii^0TAP1^0 splenocytes also fail to bind peptides tightly, as no SDS-resistant compact form can be detected at later time points in the chase. Cytofluorimetric analysis (Fig. 1*B*) shows that the number of

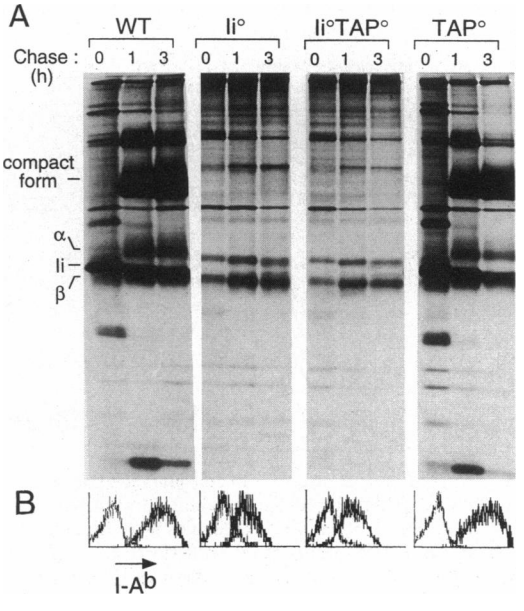


FIG. 1. Intracellular transport and cell surface expression of MHC class II molecules. (*A*) Spleen cells were metabolically labeled with [^{35}S]methionine for 30 min at 37°C and chased with unlabeled methionine for 0, 1, or 3 h at 37°C. A^b molecules were precipitated with Y3.P and analyzed by SDS/PAGE. (*B*) Cytofluorimetric analysis of A^b expression on the cell surface of IgM^+ splenocytes. A^b molecules were detected by using Y3P and visualized by using a goat anti-mouse fluorescein isothiocyanate-conjugated antibody. The thin line in each panel represents background staining (secondary reagent alone).

class II complexes on the surface of Ii^0TAP1^0 cells is similar to that on the surface of Ii^0 cells—i.e., reduced by a factor of ≈6 vis-a-vis wild-type and $TAP1^0$ cells.

In conclusion, the formation of class II complexes and their display at the surface are indistinguishable in Ii^0 and Ii^0TAP1^0 splenocytes. Therefore, peptides delivered to the ER by TAP do not measurably contribute to expression of class II molecules in mice lacking Ii.

Assembly, Intracellular Transport, and Cell Surface Expression of MHC Class I Molecules. In human cells, Ii can bind to MHC class I molecules, and these complexes are dissociated by inclusion of appropriate class I-binding peptides (12). Class I–Ii complexes can be transported along the endocytic route (13), but it is not clear whether they eventually reach the cell surface or are simply degraded in the endosomes. To evaluate the influence of Ii on formation and display of class I molecules in murine cells, we performed metabolic labeling and cytofluorimetry on the set of Ii^0TAP1^0 mutants. We considered the comparison of $TAP1^0$ and Ii^0TAP1^0 cells to be potentially the most revealing because any effect of Ii on class I expression might be exaggerated due to the reduced quantity of class I complexes in $TAP1^0$ mice (14).

A pulse–chase analysis was performed by using a mixture of two mAbs that recognize properly folded D^b and K^b molecules (Fig. 2*A*). The immunoprecipitates were analyzed on 1D-IEF gels, where the transport of glycoproteins can be conveniently visualized as a shift in isoelectric point due to acquisition of sialic acids in the trans-Golgi network. Properly folded D^b and K^b molecules are detected in $TAP1^0$ cells, but, unlike their counterparts in wild-type and Ii^0 cells, most of these complexes remain unmodified and thus are probably confined to the ER. The class I complexes in Ii^0TAP1^0 cells display similar behavior. Cytofluorimetric analysis (Fig. 2*B*) shows that the number of folded class I molecules that eventually reach the surface is similar in wild-type versus Ii^0 cells and $TAP1^0$ versus Ii^0TAP1^0 cells. Thus, in murine cells, the presence of Ii has no measurable influence on the number of class I molecules expressed at the cell surface.

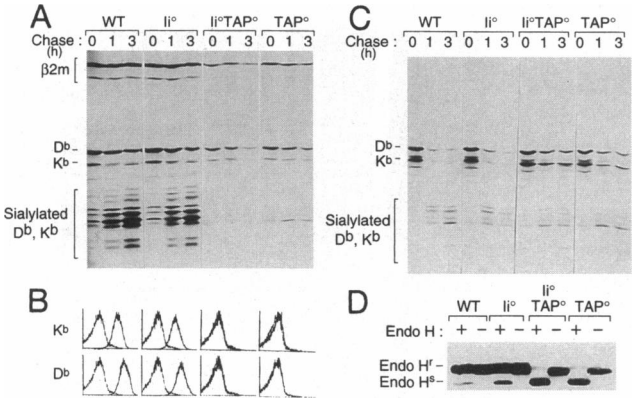


FIG. 2. Intracellular transport and cell surface expression of MHC class I molecules. (*A*) Spleen cells were labeled and chased as described in the legend to Fig. 1. K^b and D^b molecules were precipitated with a mix of Y3 and B22-249 and analyzed on a 1D-IEF gel. (*B*) Cytofluorimetric analysis of the cell surface expression of K^b and D^b molecules on splenocytes. K^b and D^b were detected by using the K9-178 and B22-249 mAbs, respectively, and visualized by using a goat anti-mouse fluorescein isothiocyanate-conjugated antibody. The thin line in each panel shows background staining (secondary reagent alone). (*C*) Spleen cells were labeled and chased as in *A*. The free heavy chains of the MHC class I molecules were immunoprecipitated with the αHC antiserum and analyzed by ID-IEF PAGE. (*D*) Lysates prepared from the thymic capsules were treated or not treated with endo H, resolved by SDS/PAGE, and immunoblotted with the αp8 antiserum directed against the cytoplasmic tail of the K^b molecule.

Wild-type and $TAP1^0$ splenocytes have been shown to express considerable quantities of free class I heavy chains at the cell surface (22, 32, 33). These molecules can bind peptides, are recognized by class I-restricted, $CD8^+$ T cells, and may be involved in selection of $CD8^+$ T cells in β_2-microglobulin null mice. It was of interest, then, to compare intracellular transport and cell surface expression of free heavy chains in our panel of mice. Free heavy chains were immunoprecipitated with a rabbit antiserum raised against D^b and K^b inclusion bodies (22), and the immunoprecipitates were analyzed on 1D-IEF gels (Fig. 2*C*). Unsialylated chains are present at the onset of the chase in all four types of cell; their amount decreases rapidly with time, particularly in wild-type and Ii^0 cells, which is most likely due to a chase into heavy chain–β_2-microglobulin complexes as well as degradation. Sialylated chains are immunoprecipitated from all cell types analyzed after 1 and 3 h of chase. These heavy chains may have reached the trans-Golgi network either as free heavy chains (22) or as part of unstable class I complexes that dissociated after reaching the trans-Golgi network or after cell lysis. The amount of sialylated free heavy chains that can be immunoprecipitated is similar in all cell types examined, as is the amount detected on the cell surface by cytofluorimetry (data not shown).

Finally, we compared class I molecule expression in thymic stroma, a source of cells directly relevant for the selection of T cells. Thymuses were first depleted of thymocytes [We had previously established that the thymocytes released in the course of the preparation of thymic capsules contributed little to total thymic class I content (data not shown).] The remnants were macerated and boiled in a buffer containing 2-mercaptoethanol and SDS. These suspensions were treated or mock-treated with Endo H, resolved by SDS/PAGE, and immunoblotted with an antibody specific for the cytoplasmic tail of the K^b molecule (Fig. 2*D*). Only high-mannose-containing N-linked glycans are sensitive to cleavage by Endo H. Consequently, acquisition of an Endo H-resistant carbohydrate chain is indicative of exit from the ER and transport at least through the medial Golgi. Most of the K^b molecules in wild-type and Ii^0 thymuses are endo H resistant and thus likely to be expressed on the cell surface. In $TAP1^0$ and Ii^0TAP1^0 thymuses, the converse is true; almost all K^b molecules are sensitive to Endo H and therefore still reside in the ER. There was no measurable difference in Endo H sensitivity between the single and double mutant.

We conclude that the assembly, transport, and cell surface expression of class I molecules are the same in $TAP1^0$ and Ii^0TAP1^0 cells, regardless of whether the class I molecules are in folded complexes or free heavy chains and whether they are in splenic cells or thymic stroma. Thus, Ii does not appreciably influence class I molecule biosynthesis even in a situation where low levels of class I molecule surface expression might exaggerate any potential effects.

T-Cell Selection. Any differences in loading or display of MHC class I and II molecules in the single versus double mutants could well be reflected in changes in the T-cell repertoire. Therefore, we prepared thymocytes from the four types of littermate, stained them with reagents specific for CD4, CD8, and CD3, and analyzed them by cytofluorimetry. Typical dot plots are shown in Fig. 3. Ii^0 and Ii^0TAP1^0 mice have similarly reduced numbers of $CD4^+$ $CD8^-$ cells, while $TAP1^0$ and Ii^0TAP1^0 animals have a similar deficiency in the $CD4^-$ $CD8^+$ population. This conclusion is valid both for total thymocytes (Fig. 3*A*) and for cells expressing high levels of CD3 and thus presumably mature (Fig. 3*B*). Fig. 3*C* generalizes this observation to several sets of littermates.

Surprisingly, different results were obtained from cytofluorimetric analysis of lymph node cells. As illustrated in Fig. 4*A*, Ii^0 mice have a reduced $CD4^+$ compartment, and $TAP1^0$ animals a diminished $CD8^+$ compartment, but in double

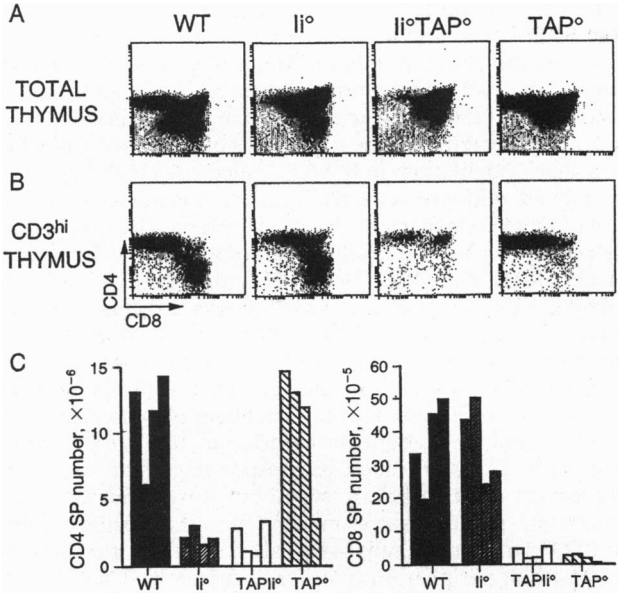


FIG. 3. Phenotype of thymocytes from mice lacking Ii and/or TAP molecules. Total thymocytes (*A*) or only those expressing high levels of CD3 (*B*) were stained for CD4 and CD8. The number of $CD3^{hi}$ $CD4^+$ and $CD8^+$ T cells found in the thymus of several wild-type (WT), Ii^0, Ii^0TAP1^0, and $TAP1^0$ mice is shown in *C*.

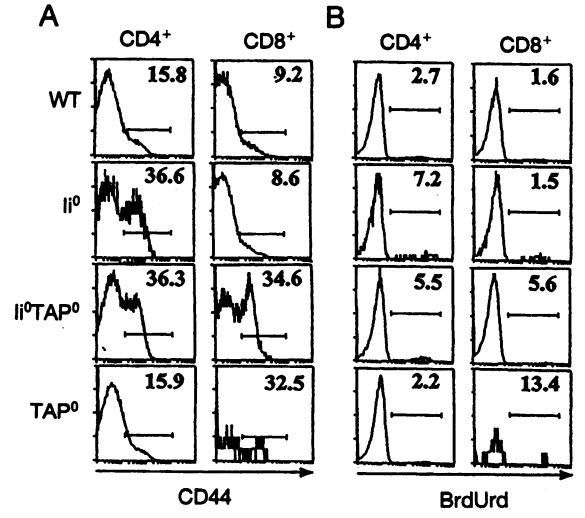


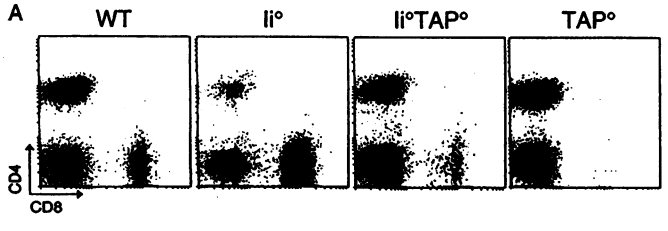
FIG. 5. Peripheral T cells in Ii^0TAP1^0 double-mutant mice. (*A*) Expression of CD44 molecules on the surface of $CD4^+$ and $CD8^+$ T cells in the lymph nodes. Values on the panels represent the proportion of cells positive for staining (gated as shown). (*B*) Mice were intraperitoneally injected with BrdUrd, and 48 h later, incorporation of BrdUrd was detected by using a mAb. Values on the panels represent the proportion of cells positive for staining (gated as shown).

mutants, there appears to have been mutual compensation, such that both the $CD4^+$ and the $CD8^+$ populations appear much more normal. For reasons we do not yet understand, normalization seems more effective for the $CD4^+$ compartment than for the $CD8^+$ compartment. Data, whether as percentages, as cell numbers, or as ratios from $CD4^+$ to $CD8^+$ cells, from several littermate sets are plotted in Fig. 4*B* and serve to generalize this finding.

Given the disparity between the results with thymic cells and lymph node cells, we considered it likely that the compensation in double mutants is due to peripheral expansion rather than increased thymic selection. This idea is supported by the fact that a high proportion of peripheral $CD4^+$ and $CD8^+$ cells in Ii^0TAP1^0 mice express the late activation marker CD44 (Fig. 5*A*) and incorporate BrdUrd, indicative of ongoing division (Fig. 5*B*). To determine whether a particular set of T cells is expanded in Ii^0TAP1^0 mice, we looked for skewing of the Vβ repertoire. Some of the data from triple stainings with anti-CD4, -CD8 and -Vβ reagents are tabulated in Fig. 6. No over- or under-representations particular to the double mutants were detected. However, the values for certain Vβs do shed light on the origin of the $CD8^+$ populations in $TAP1^0$ and Ii^0TAP1^0 mice. Similar to what has been reported (34), $V\beta5^+$ T cells are selected more efficiently on MHC class I than on class II molecules: 10.4% versus 2.4% in the CD8 versus CD4 compartment of wild-type mice. The $CD8^+$ T cells in $TAP1^0$ and double-mutant mice show reduced levels of $V\beta5^+$ T-cell receptors, indicating that class II molecules were probably involved in their selection.

DISCUSSION

By all criteria examined, biosynthesis of MHC class II molecules was the same in Ii^0 and Ii^0TAP1^0 mice, and that of class I molecules was indistinguishable in $TAP1^0$ and Ii^0TAP1^0 animals. Thus, our data provide no evidence for functional intersection of the class I and class II loading pathways. Yet, breakdowns in the dichotomy of class II molecules presenting



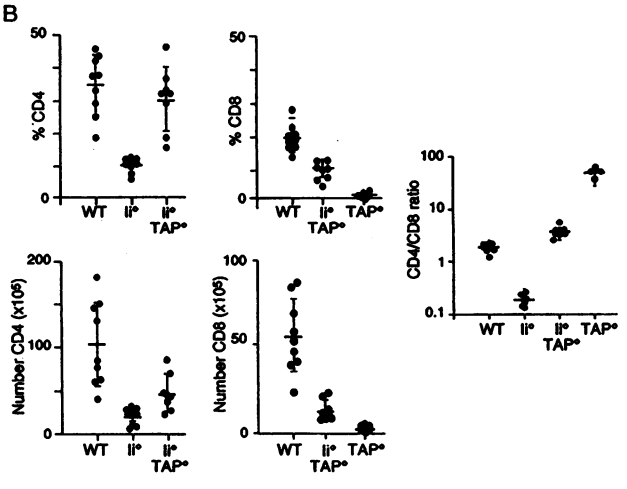


FIG. 4. Phenotype of peripheral T cells from mice deficient for Ii and/or TAP molecules. (*A*) Dot plots of CD4/CD8 stainings of lymph node cells. (*B*) Percentages (upper panels) or numbers (lower panels) of $CD4^+$ or $CD8^+$ T cells or ratios of $CD4^+$ to $CD8^+$ cells (right most panel) found in the lymph nodes of several wild-type (WT), Ii^0, Ii^0TAP1^0, or $TAP1^0$ mice.

		Vβ4			Vβ6			Vβ8			Vβ5		
CD4	WT	6.2	6.2	6.7	9.0	9.9	11.2	16.8	17.5	19.5	3.5	2.2	1.6
		7.2	7.4	**6.7**	11.0	9.6	**10.1**	18.9	18.4	**18.2**	2.3	-	**2.4**
	Ii°	6.8	12.6	5.6	8.1	9.1	8.7	19.8	17.0	18.0	3.5	3.3	4.5
		10.5	10.0	**9.1**	9.9	8.5	**8.9**	19.7	19.1	**18.7**	4.7	4.1	**4.0**
	Ii°TAP°	7.0	8.0	7.3	6.1	9.8	7.8	21.2	17.7	18.5	4.3	4.4	5.7
		7.0	12.8	**8.4**	8.7	11.4	**8.8**	18.5	23.3	**19.8**	5.9	4.1	**4.9**
	TAP°	6.4	7.0	7.5	10.0	10.3	10.6	18.6	20.1	20.6	1.8	2.5	1.5
		7.5	7.6	**7.2**	9.8	12.3	**10.6**	19.6	14.7	**18.7**	2.6	-	**2.1**
CD8	WT	4.8	4.6	3.5	7.1	9.5	7.6	17.4	15.7	17.7	12.4	10.1	9.2
		3.6	4.2	**4.1**	7.8	6.0	**7.6**	17.2	15.5	**16.7**	10.0	-	**10.4**
	Ii°	4.1	3.4	5.2	8.3	7.9	8.4	17.7	16.4	15.5	14.7	15.2	16.4
		5.2	3.8	**4.3**	8.3	9.2	**8.4**	16.3	15.1	**16.2**	15.6	14.8	**15.3**
	Ii°TAP°	6.4	4.6	6.4	6.3	4.8	6.0	21.2	18.4	18.7	8.9	5.1	5.9
		5.2	6.6	**5.8**	7.0	6.2	**6.1**	22.4	16.1	**19.4**	6.4	6.2	**6.5**
	TAP°	6.4	8.5	-	6.7	7.9	6.6	12.9	16.5	17.7	4.0	6.2	-
		-	-	**7.4**	8.1	-	**7.3**	17.8	13.7	**15.7**	-	-	**5.1**

FIG. 6. Vβ repertoire of peripheral T cells. Lymph node cells were stained with anti-CD4, -CD8, and -Vβ antibodies. The table represents the percentages of $CD4^+$ and $CD8^+$ T cells that express a T-cell receptor bearing the Vβ4, Vβ5, Vβ6, or Vβ8 segments. For each Vβ, values for several individual mice are shown; the underlined value in boldface is the mean.

exogenous peptides versus class I molecules offering cytosolic peptides have repeatedly been cited (4–10). Moreover, single Ii^0 or $TAP1^0$ mutant mice do have "leaky" phenotypes: a substantial number of surface class II molecules and $CD4^+$ T cells in the former (15, 16), a few surface class I molecules and $CD8^+$ T cells in the latter (14). We are led to conclude that TAP1 or Ii do not contribute in a major way to such phenomena.

The conclusion concerning Ii deserves further comment. Given the close structural similarity between class I and class II MHC products (35), it is perhaps not surprising that Ii can interact with both. Mouse class I molecules were shown to be capable of interacting with human Ii (12, 13), and this interaction was sensitive to the presence of appropriate class I-binding peptides. These observations raised two important points. First, class I–Ii interactions do occur and could divert a portion of newly synthesized class I molecules to the endocytic pathway. Class I molecules loaded with peptide in the endosomes might contribute to selection of the few $CD8^+$ T cells in $TAP1^0$ mice; if so, the additional presence of the Ii^0 mutation should result in reduced numbers of such cells. This was not observed in our experiments. Second, "empty" class I molecules interact more strongly with Ii than do peptide-loaded class I molecules (12, 13). In cells that are Ii positive, such as the epithelial cells involved in T-cell selection, class I molecules in $TAP1^0$ mice would be predicted to interact more efficiently with Ii. Given the ER-retention and -targeting signals present on Ii, these interactions might be responsible, in part, for the failure to express quantities of class I molecules at the cell surface sufficient to allow positive selection of $CD8^+$ T cells. Were this argument to apply, elimination of Ii would allow an increase in surface expression of empty class I molecules. We have shown earlier that an increase of surface class I in TAP1-deficient animals, as brought about by the introduction of a human β_2-microglobulin transgene, suffices to increase the numbers of $CD4^-$ $CD8^+$ thymocytes and of $CD8^+$ T cells in the periphery (36). The data presented here definitively exclude the idea that Ii affects maturation and intracellular transport of class I molecules in either thymus or spleen. In the absence of both Ii and TAP, no increase in maturation of class I molecules nor in numbers of $CD4^-$ $CD8^+$ thymocytes was observed. Consequently, we are of the opinion that, while class I–Ii interactions are demonstrable in cultured cells, they are unlikely to be of importance in controlling the fate of class I molecules under physiological conditions.

Given the above results on MHC biosynthesis, it was expected that selection of thymocytes in the double mutant would reflect the deficiencies of the single mutants. What was more unexpected were the expansions of the $CD4^+$ and $CD8^+$ peripheral populations in Ii^0TAP1^0 mice, such that they almost resembled wild-type animals. That there is homeostatic control of these compartments has been observed before, perhaps most graphically in recently generated knockout lines. Mice lacking MHC class II (27), CD4 (37), or Ii (15, 16) molecules all have reduced numbers of $CD4^+$ T cells and compensatorily increased numbers of $CD8^+$ cells; animals devoid of β_2-microglobulin (38, 39), CD8α (40), CD8β (41, 42), or TAP1 (14) show the inverse. Interestingly, mice with double MHC deficiencies (43) have reduced numbers of both $CD4^+$ and $CD8^+$ populations, quite in contrast to our present results using the Ii^0TAP1^0 mice. Corresponding data on double coreceptor deficiencies are scant, but it was claimed that at least some animals have as much as 50% the normal numbers of $CD3^+$ lymph node cells (44). Taken together, these findings suggest that peripheral expansion is driven by T-cell interaction with MHC molecules (at least partially coreceptor independent) rather than as part of an "innate" program which dictates a specified number of divisions.

It will certainly be of interest to examine the functional potential of the peripheral T cells in the double mutants. There will probably be similarities with Ii^0 mice in the $CD4^+$ and with $TAP1^0$ animals in the $CD8^+$ compartment, as already suggested from the analysis of Vβ usage. Thus, one might expect the $CD4^+$ cells to include specificities for only a subset of class II-restricted peptides (45), as well as for a substantial CD1-restricted fraction (28). The $CD8^+$ cells should include a significant allo- or self-reactive component (46). However, the functional capabilities of the T cells in Ii^0TAP1^0 mice might also reflect distortions introduced by the peripheral amplification process and thereby give clues to its mechanisms.

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