

Thymic Selection of CD8⁺ Single Positive Cells with a Class II Major Histocompatibility Complex-restricted Receptor

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Summary

We describe mice that express a transgenic T cell receptor α/β (TCR- α/β) specific for peptide 111–119 from influenza hemagglutinin presented by I-E^d class II major histocompatibility complex (MHC) molecules. The transgenic TCR is expressed on CD4⁺8⁻ as well as CD4⁻8⁺ mature T cells even in mice that are deficient in rearrangement or do not express endogenous TCR- α genes. The CD4⁻8⁺ T cells require I-E^d class II MHC molecules for positive selection and can be activated to proliferate and to kill by I-E^d molecules presenting the relevant peptide. Full maturation of these cells, however, also requires the presence of class I MHC molecules. The results are compatible with the notion that T cell maturation requires multiple receptor-ligand interactions and establish an exception to the rule that class II-restricted TCRs are exclusively expressed by mature CD4⁺8⁻ cells.

Experiments in various TCR- α/β transgenic mice have indicated a strict correlation of CD4 or CD8 coreceptor expression and presence of class II and class I MHC-restricted TCR- α/β 's, respectively, in mature T cells (1–4). However, in the thymus of TCR transgenic mice, CD4⁺8^{low} cells with a class I MHC-restricted TCR could be detected (5). Likewise CD4^{low}8⁺TCR^{int} cells have been seen in both normal and β_2 -microglobulin (β_{2m})¹ deficient mice (6). It was argued that these cells represented immature precursors of mature CD4⁺8⁻ and CD4⁻8⁺ cells, respectively, but that they could not develop further because the coreceptor and the TCR- α/β could not be coengaged by the same MHC molecule. Experiments consistent with this notion showed that a CD4 transgene could apparently rescue a population with heterogeneous levels of CD8 coreceptors expressing a class II MHC-restricted TCR (7). If correct, this hypothesis would indicate that the initial downregulation of either CD4 or CD8 coreceptors by double positive cells undergoing positive selection occurs independently of the specificity of the TCR- α/β , at least in a subset of developing cells.

The initial observations concerning mature T cells in TCR- α/β transgenic mice were confirmed in class I or class II MHC-deficient mice which essentially lacked mature single positive CD4⁻8⁺ and CD4⁺8⁻ cells, respectively, even though small numbers of cells belonging to the respective subsets could be detected (8, 9). The specificity of the TCR- α/β on these cells was unknown.

There were also reports on the existence of CD4⁺ T cells that could recognize class I MHC antigen (10–12). Unfortunately it was not clear whether this was simply due to some crossreactivity of class II MHC-selected cells, or whether these cells were selected by class I MHC molecules.

Here we present another apparent exception to the rule of “matched” specificity of coreceptor and TCR- α/β on mature T cells. We have analyzed TCR transgenic mice with a TCR specific for peptide 111–119 from influenza hemagglutinin presented by I-E^d class II MHC molecules. In these mice, we have detected a small but significant portion of mature CD4⁻8⁺ T cells with the transgenic TCR- α/β , even when the transgenic mice were rearrangement defective or lacked endogenous TCR- α genes. The CD4⁻8⁺ T cells responded with proliferation and differentiation into cytolytic T cells when stimulated by peptide plus I-E^d class II MHC molecules. Positive selection of these cells required I-E^d class II MHC molecules in the thymus. Full maturation of these cells, however, also required the presence of class I MHC molecules.

In accord with previous data, our results indicate that downregulation of the CD4 coreceptor does not require binding of the TCR- α/β to thymic class I MHC molecules, at least in a subset of developing cells; yet class I MHC molecules are required for full maturation of these CD4⁻8⁺ cells bearing a class II MHC-restricted TCR.

Materials and Methods

Mice. BALB/c and C56BL/6 (B6) mice were obtained from IFFA-Credo (France). B10.GD mice, of H-2^{b2} haplotype (13),

¹ Abbreviations used in this paper: β_{2m} , β_2 -microglobulin; HSA, heat-stable antigen; Rag-2, recombination-activating gene 2.

A

THYMUS

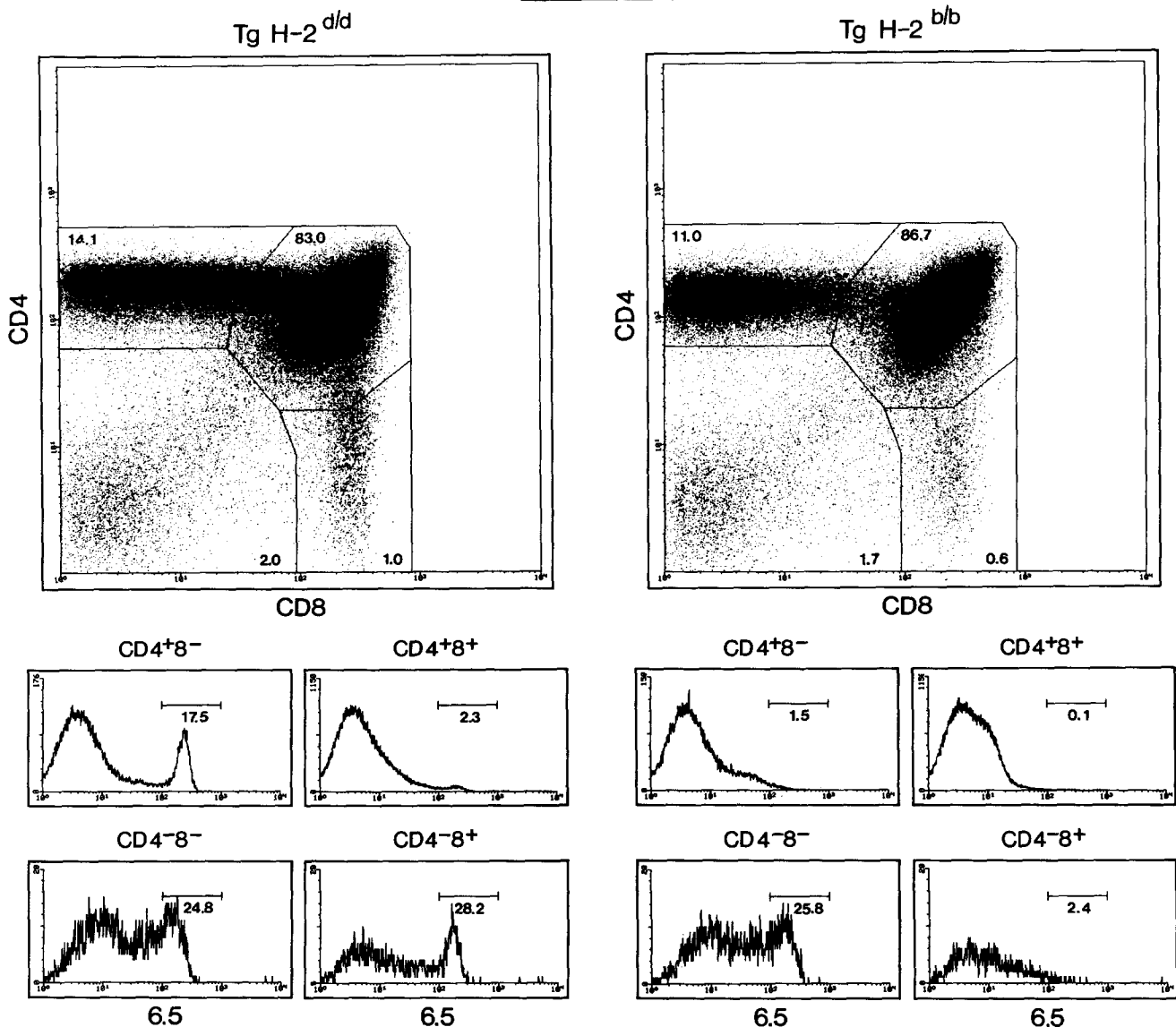


Figure 1. Thymus (A) and lymph nodes (B) of TCR transgenic mice on H-2^d and H-2^b MHC haplotype. Single cell suspensions of thymus or lymph nodes from transgenic BALB/c and B6 (F₂) mice were used for three colour immunofluorescence. Lymphocytes were depleted of sIg⁺ cells before staining. Absolute numbers were 8.3×10^7 and 10.7×10^7 thymocytes and 6.5×10^7 and 7.0×10^7 lymphocytes in H-2^d and H-2^b mice, respectively. All CD4⁺CD8⁻ and CD4⁻CD8⁺ mature T cells stained homogeneously positive with TCR V β ₈-specific mAbs.

were obtained from Dr. van Mourik (Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands). The various genotyped mice used for this study have been described. Homozygous mutant mice, deficient for (a) recombination-activating gene 2 (Rag-2^{-/-}) (14), (b) TCR- α constant region (TCR α _E^{-/-}) (15) or (c) β _{2m}^{-/-} (8) were obtained by intercrossing F1 offspring of homozygous mutant H-2^b (a, c) or H-2^d (b) and TCR transgenic H-2^d mice. Mice expressing hemagglutinin have the hemagglutinin transgene under control of Ig κ promoter and enhancer elements (Rolink, A., manuscript in preparation). All breeding was done in the animal colony of the Basel Institute for Immunology.

Phenotyping of offspring was done by FACS[®] (Becton Dick-

inson & Co., Mountain View, CA) staining of PBLs. TCR transgenic, as well as TCR transgenic mice deficient in either Rag-2 or TCR α _E genes, were identified by staining PBLs with TCR V β ₈- and clonotype-specific mAbs.

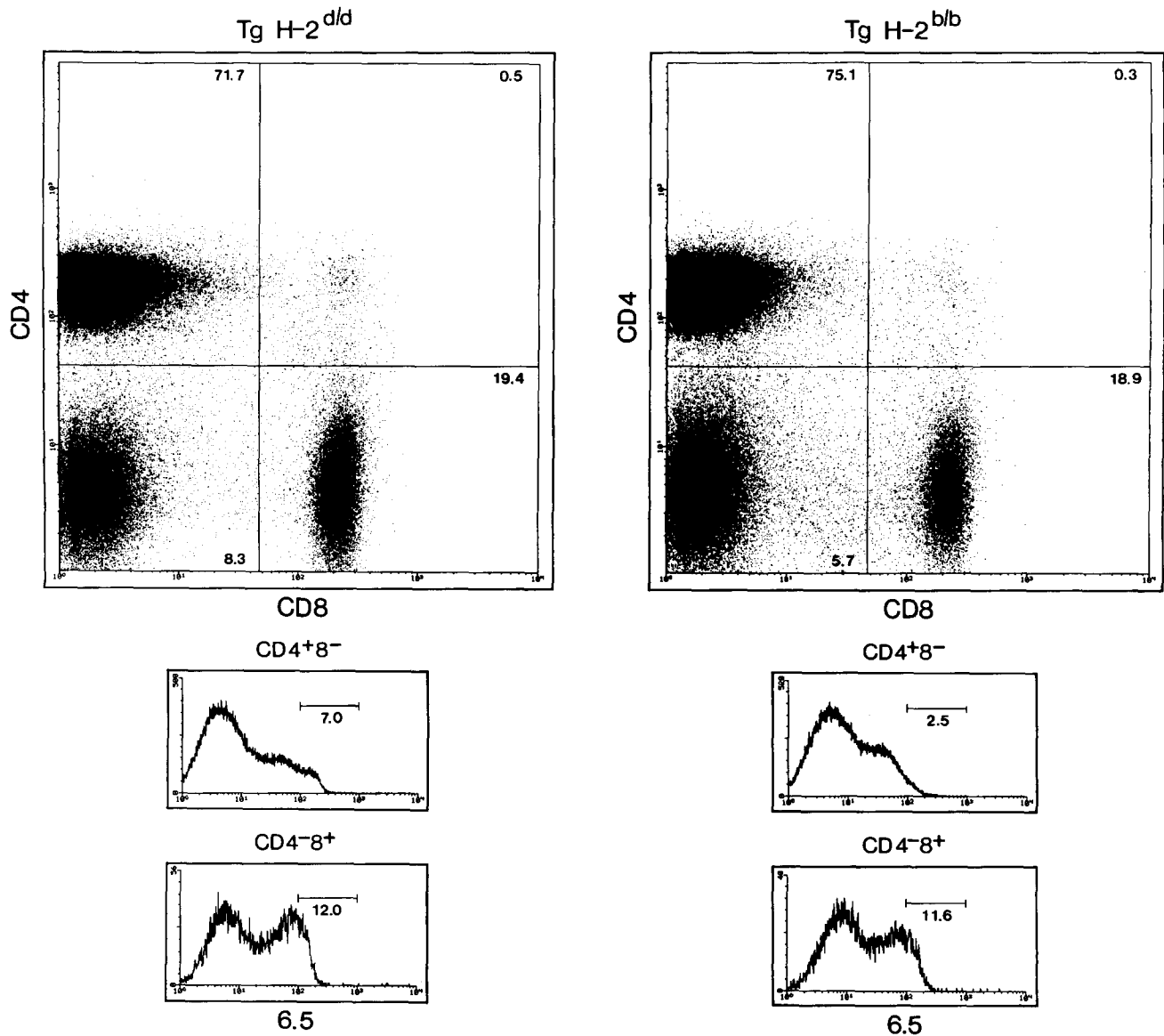
MHC haplotype and β _{2m} deficiency was revealed by staining PBLs with mAbs specific for D^d, K^b, I-A β ^d, or I-A^b molecules. If necessary, TCR transgene presence was determined by Southern blotting.

Mice were killed between 6 and 10 wk of age.

Cell Suspensions and B or T Cell Depletion. Single cell suspensions of thymus, lymph nodes, and spleen (RBCs lysed) were prepared in PBS + 2% FCS. sIg⁺ cells were depleted using Dy-

B

LYMPHNODE



nabeads (Milan Analytica, La Roche, Switzerland). In some experiments T cells were depleted by staining with Thy-1-specific mAbs followed by complement (Low-Tox Rabbit Complement; Cedarlane Laboratories Ltd., Hornby, Ontario, Canada) treatment, as recommended by the manufacturer.

Antibodies and FACS[®] Analysis. Hybridoma supernatants containing mAbs were purified by protein A or protein G (Pharmacia LKB, Uppsala, Sweden) affinity chromatography. mAbs were labeled using biotin- or fluoresceine-succinimidyl-ester (FLUOS) (Boehringer Mannheim, Mannheim, Germany) or FITC (Nordic Immunology, Tilburg, Netherlands) according to the manufacturers instructions. The following mAbs have been used: T19.191 (Anti-D^d) (Hämmerling, G. J., unpublished results), AF6-88.5.3 (anti-K^b) (16), MKD6 (anti-I-A^{βd}) (17), AF6-120.1.2 (anti-I-A^b) (16), T24 (anti-Thy-1) (18), M5/114 (anti-I-A^{b,d,q}, anti-I-E^{b,d,k}) (19), M1/69 (anti-heat-stable antigen [HSA]) (20), F23.1 (anti-TCR V_{β8}) (21), and 6.5 (anticonotypic TCR). The 6.5 mAb was produced

in rats immunized with the soluble TCR obtained as described in (22).

Biotinylated or FITC-labeled F23.1 and FLUOS-labeled 6.5 mAbs were used for TCR detection. Anti-CD8-biotin (Becton Dickinson & Co.), anti-CD8-PE (Caltag Laboratories, S. San Francisco, CA), anti-CD4-PE (Becton Dickinson & Co.) and anti-CD8-Red613 (GIBCO BRL, Gaithersburg, MD) conjugates were obtained commercially. Class II MHC expression was determined by staining with M5/114 mAbs; HSA-specific mAbs M1/69 were biotinylated. With these and second step reagents streptavidin-allophycocyanin (APC, Molecular Probes Inc., Eugene, OR), streptavidin-PE (Southern Biotechnology, Birmingham, AL) or goat anti-mouse Ig-F(ab)₂-FITC (Silenus Laboratories, Pty Ltd., Hawthorne, Victoria, Australia) one-, two-, three-, and four-color flow cytometry was performed on FACSscan[®], FACStar⁺[®] or FACS-Vantage[®] (Becton Dickinson & Co.) instruments equipped appropriately.

Staining was done in 96-well plates (10⁶ cells/well) in 100 μl

PBS + 2% FCS with mAbs at optimal dilution as determined before. Between first and second step reagents cells were washed twice with 200 μ l PBS + 2% FCS as was done after the last step.

Data (up to 5×10^5 events) were stored in list mode and analyzed with the Lysys II software (Becton Dickinson & Co.). Dead cells were excluded using forward- and side-scatter parameters. Data are presented as dot-plots showing all cells fulfilling the gating criteria. Numbers in quadrants, regions, or histograms indicate the proportion of cells in that area. As far as analyzed data from lymph nodes or spleen gave similar results.

Primary MLR and Killer Assay. To generate cytolytic effector cells 10^7 responder cells were cultured with 10^7 x-irradiated stimulator cells (2,200 rad) in 8 ml IMDM supplemented with FCS (10%), β -mercaptoethanol (5×10^{-5} M), penicillin (100 IU/ml), and streptomycin (100 mg/ml) (referred to as medium hereafter) in flasks that were kept in an upright position. To some flasks, peptide 110–119 of influenza hemagglutinin (SFERFEIFPK) was added at a final concentration of 7.5 μ M. In other experiments spleen cells from hemagglutinin transgenic mice were used as stimulators or cultures contained a chimeric mAb (2.5 μ g/ml) including the 111–119 epitope (23). Cultures were kept in a water-saturated atmosphere of 6% CO₂ in air at 37°C for 4–5 d.

To separate CD4⁺8⁻ from CD4⁻8⁺ effector cells, cultures were set up in upright big flasks at the same cell concentrations. Effectors were harvested at day 4, dead cells removed by centrifugation over Ficoll (Pharmacia LKB) and cells stained with CD8-FITC (Becton Dickinson & Co.) and CD4-PE specific mAbs followed by cell sorting.

Target LPS blasts were generated from T cell depleted, Con A blasts from sIg⁺ depleted spleen cells. 3×10^7 cells were cultured in 30 ml of medium containing either 50 μ g/ml LPS or 2.5 μ g/ml Con A. Cells were harvested 48 h later. LPS blasts were centrifuged over Ficoll; Con A blasts were treated for 30 min with 50 mM α -D-methylmannopyranoside (Sigma Chemical Co., St. Louis, MO). Blasts were washed, pelleted, and 50–150 μ l Na₂⁵¹CrO₄ was added together with 20 μ l IMDM or 20 μ l of 1 mM peptide 110–119 in IMDM. Cells were loaded for 1 h at 37°C and washed three times before adding to the killer assay.

2×10^4 target cells were incubated with various numbers of responder cells for 4 h at 37°C in 200 μ l medium as duplicates in round-bottom 96-well plates. Effector/target ratios were calculated from the number of responder cells initially cultured, the descendants of which are the effector cells lysing target cells on day 4 or 5. Some wells received only target cells, to others Zap-O-Globin (Coulter Corp., Hialeah, FL) was added, to determine spontaneous and total release, respectively. After the incubation period plates were spun briefly and 100 μ l supernatant was harvested. Data are presented as percent specific ⁵¹Cr release calculated as follows: percent specific release = [(experimental release – spontaneous release)/(total release – spontaneous release)] \times 100. Spontaneous release was below 15% of total release; standard deviations were below 10%.

Results

TCR Transgenic Mice. The productively rearranged TCR- α and β genes were cloned from a T cell hybridoma 14.3.d (derived from a T helper clone V₂₋₁₅) specific for peptide 111–119 of influenza hemagglutinin (24) presented by class II I-E^d MHC molecules as described previously (22, 25). The receptor is encoded by V _{β 8.2}, J _{β 2.1} as well as V _{α 4}, J _{α 2B4}

variable TCR gene segments. Genomic DNA was injected into fertilized (B6 \times DBA/2J)_{F2} eggs. Since initially the transgenic offspring showed little expression of the transgenic TCR- α gene, the TCR- β enhancer was introduced downstream of the C α enhancer. The resulting transgenic mice were backcrossed for four to five generations to mice of the BALB or B6 background.

Expression of TCR- α and β Genes. As expected from previous results, the transgenic mice exhibited an increased ratio of CD4⁺ to CD8⁺ single positive cells in the thymus, even though <20% of the CD4⁺ cells stained with the clonotypic 6.5 antibody. In fact, similarly skewed ratios were already seen in mice that expressed only the TCR- β transgene, irrespective of whether the mice were of the b/b or d/d MHC haplotype (our unpublished results). It is noteworthy that in the thymus of mice with the d/d but not the b/b MHC haplotype, a significant portion not only of the CD4⁺8⁻, but also of the CD4⁻8⁺ cells, bear high levels of the transgenic TCR (Fig. 1 A). In lymph node cells, the proportions of CD4⁺8⁺ T cells with high levels of the transgenic TCR are not really different in MHC b/b vs. d/d mice, presumably because of peripheral selection of cells with endogenous TCR α chains in both types of mice such that most CD4⁺8⁺ express either low or only intermediate levels of the transgenic TCR α chain (Fig. 1 B).

CD4⁻8⁺ T Cells with the Transgenic TCR Are Stimulated to Proliferate and to Kill by Class II MHC Presented Peptide. Spleen cells from the TCR transgenic mice were first stimulated by BALB/c spleen cells in the presence or absence of peptide and tested for cytotoxicity on LPS blasts (class II MHC⁺) and Con A blasts (class II MHC⁻) with or without peptides as targets. While both targets were equally lysed by B6 anti-BALB/c killer cells, only peptide-pulsed LPS targets but not peptide-pulsed Con A targets were lysed (Fig. 2, A and C). We also obtained effector cells by stimulating cells from TCR transgenic mice with spleen cells from a mouse that expresses hemagglutinin as a transgene under the control of Ig κ promoter and enhancer (Rolink, A., manuscript in preparation). From the same mice LPS targets were prepared that were lysed by various effector cells (Fig. 2 C). These data, and other data (see Fig. 2, legend) with LPS blasts from I-E^{d+} and I-E^{d-} mice, showed that all cytolytic T cells were specific for the class II MHC presented peptide 111–119. In Fig. 2 B, CD4⁺8⁻ effector cells were separated from CD4⁻8⁺ effector cells before the killer assay. It can be seen that CD4⁻8⁺ cells give rise to much more potent effector cells than CD4⁺8⁻ cells.

CD4⁻8⁺ T Cells with the Class II MHC-restricted Transgenic TCR in RAG-2^{-/-} or TCR α E^{-/-} Mice. To rule out the possibility that CD4⁺8⁺ T cells with the class II MHC-restricted, transgenic TCR were positively selected because of additional receptors composed of endogenous TCR α chains associated with transgenic TCR β chains (26–28), the TCR transgenic mice were crossed with RAG-2^{-/-} and TCR α E^{-/-} mice. From the intercrosses we obtained TCR transgenic RAG-2^{-/-} and TCR α E^{-/-} mice that were either of the d/d or b/b MHC haplotypes. It can be seen in

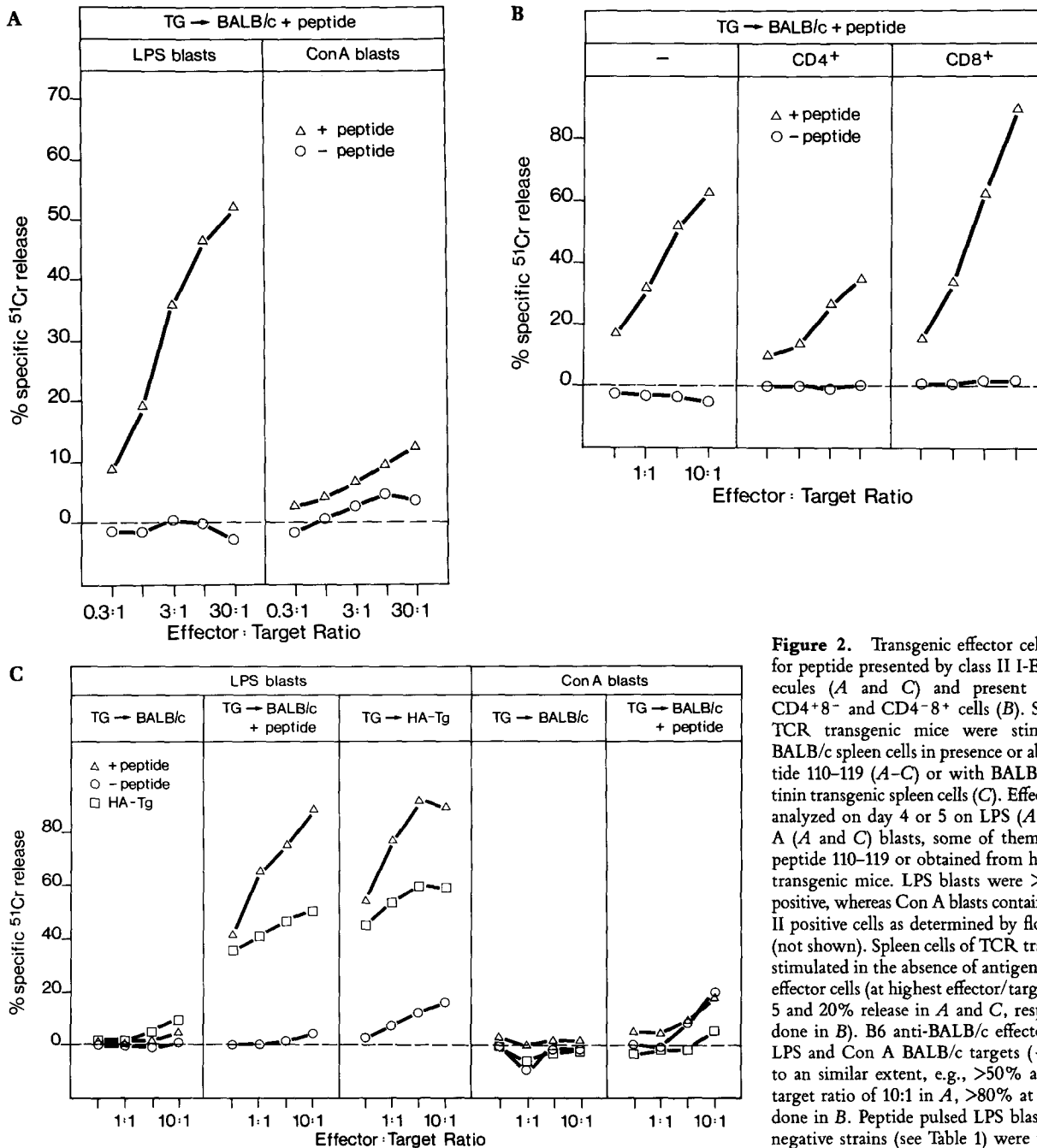


Figure 2. Transgenic effector cells are specific for peptide presented by class II I-E^d MHC molecules (A and C) and present among both CD4⁺8⁻ and CD4⁻8⁺ cells (B). Spleen cells of TCR transgenic mice were stimulated with BALB/c spleen cells in presence or absence of peptide 110-119 (A-C) or with BALB/c hemagglutinin transgenic spleen cells (C). Effector cells were analyzed on day 4 or 5 on LPS (A-C) and Con A (A and C) blasts, some of them loaded with peptide 110-119 or obtained from hemagglutinin transgenic mice. LPS blasts were >90% class II positive, whereas Con A blasts contained <5% class II positive cells as determined by flow cytometry (not shown). Spleen cells of TCR transgenic mice stimulated in the absence of antigen generated no effector cells (at highest effector/target ratio below 5 and 20% release in A and C, respectively; not done in B). B6 anti-BALB/c effector cells killed LPS and Con A BALB/c targets (+/- peptide) to an similar extent, e.g., >50% at an effector/target ratio of 10:1 in A, >80% at 1:1 in C; not done in B. Peptide pulsed LPS blasts from I-E^gd negative strains (see Table 1) were not killed. In B effector cells were generated from transgenic

spleen cells and tested either unseparated or after sorting into CD4⁺8⁻ or CD4⁻8⁺ cells. The effector/target ratio of sorted effectors was adjusted to equal the ratio of that population in the unseparated effectors. The increased lysis by CD4⁻8⁺ sorted effectors compared to unseparated effectors might be due to removal of cold target cells carried over from the primary MLR in case of the latter. Contamination of CD4⁺8⁻ effectors by CD4⁻8⁺ cells can not explain the extent of CD4⁺8⁻-mediated killing as it should exceed 5% to explain the killing. However, cell-sorter reanalysis showed that the actual contamination was well below this level. We also purified CD4⁺8⁻ and CD4⁻8⁺ cells before the primary MLR and obtained similar results (data not shown).

Fig. 3 A that significant numbers of mature CD4⁻8⁺ cells with high levels of the transgenic TCR were present in the thymus of d/d but not b/b transgenic mice. The few CD4⁻8⁺ cells in the RAG-2^{-/-} b/b mice were mostly HSA^{high} and had lower levels of the TCR and were thus,

by these criteria, very immature cells, not affected by positive selection. Thus, both CD4⁺8⁻ as well as CD4⁻8⁺ cells with high levels of the transgenic TCR required positive selection on H-2^d MHC molecules (Fig. 3 A). I-E^gd class II MHC molecules were required for the selection of

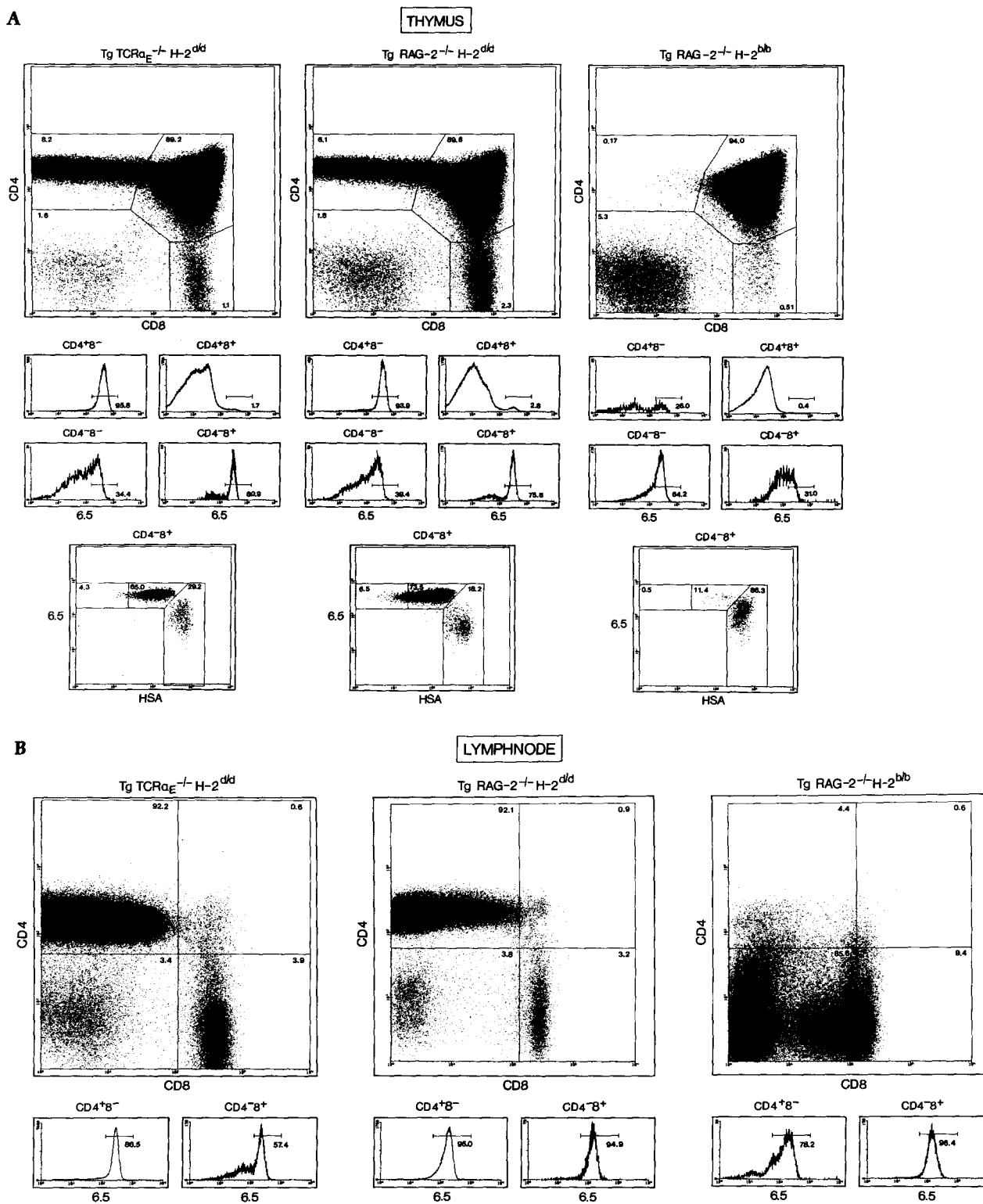


Figure 3. Thymus and lymph nodes in $TCR\alpha_E^{-/-}$ and $RAG-2^{-/-}$ TCR transgenic mice. Thymocytes (*A*) and peripheral cells (from lymph nodes, *B*) of various mice were prepared and used for four-color flow cytometry. $CD4^{-}8^{+}6.5^{+}$ cells in lymph nodes of $H-2^b$ mice repeatedly had lower levels of CD8 as $CD4^{-}8^{+}6.5^{+}$ cells in $H-2^d$ mice.

Table 1. *H-2 Haplotypes and Effect on Selection in TCR Transgenic Mice*

	Alleles at H-2 loci:						Positive selection of CD4 ⁺ 8 ⁻ and CD4 ⁻ 8 ⁺ 6.5 ^{hi} cells
	K	A β	A α	E β	E α	D	
H-2 ^d	d	d	d	d	d	d	+
H-2 ^d \times b	d	d	d	d	d	d	-
H-2 ^b	b	b	b	b	b	b	-
H-2 ^d \times g ²	d	d	d	d	b	b	+
H-2 ^{g²}	d	d	d	d	b	b	-

The crossover in E β ^{g²} has been localized to the intron between the E β 1 and E β 2 exons (35). The peptide presenting domain of the E β ^{g²} molecule is therefore of E β ^d origin and E β ^{g²} is likely to behave like E β ^d as shown in this table. Positive selection of CD4⁻8⁺ 6.5^{hi} cells was never observed in the absence of positive selection of CD4⁺8⁻ 6.5^{hi} cells.

both subsets of T cells because they were absent in H-2^b, H-2^{g²}, (H-2^d \times H-2^b)_{F1} but not (H-2^d \times H-2^{g²})_{F1} TCR transgenic mice (data not shown). The nonselecting mice are deficient in I-E^d molecules either because of the absence of I-E β ^d or I-E α genes or because I-E β ^d does not come to the cell surface in sufficient quantity like in (H-2^b \times H-2^d)_{F1} hybrids in which the I-E β ^b outcompetes I-E β ^d because of

its strong association with I-E α molecules (29, 30, and our unpublished results). Thus, neither K^d nor D^d molecules are sufficient for positive selection of the CD4⁻8⁺ cells with the class II MHC-restricted transgenic TCR, but I-E β ^d cell surface expression is required (see Table 1).

With regard to lymph nodes of the various mice, the picture is slightly different from that of the thymus; whereas

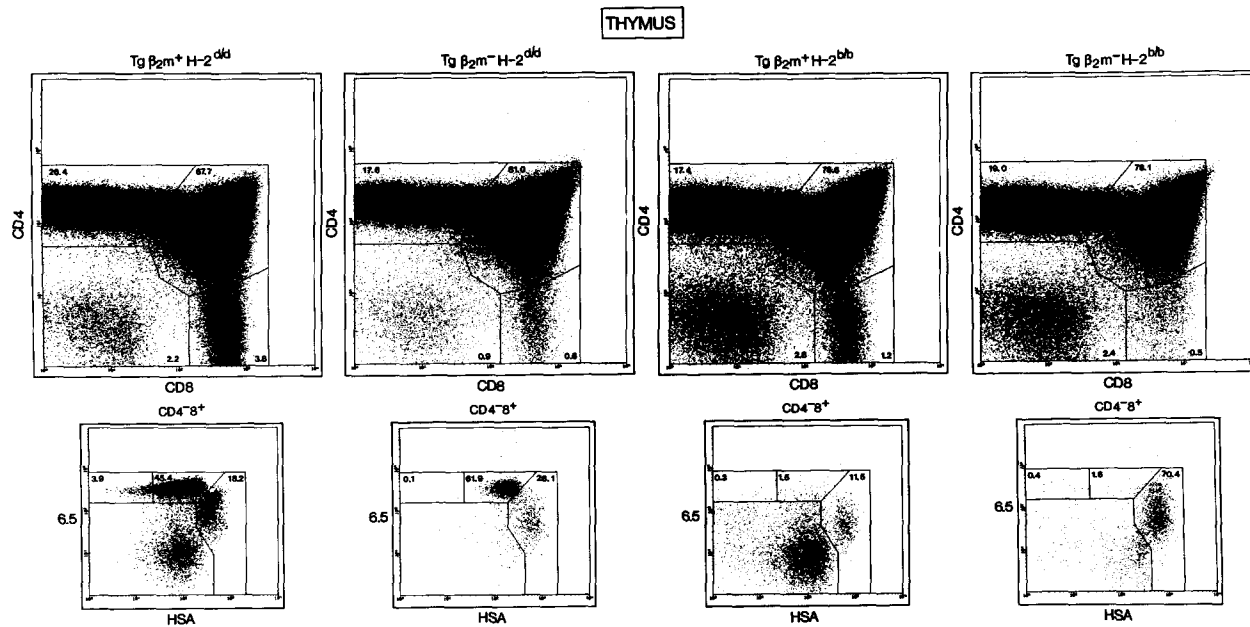


Figure 4. Thymocytes from β 2m⁺ and β 2m⁻ TCR transgenic mice of H-2^d or H-2^b MHC haplotype. Single cell suspensions of thymocytes were prepared from 8–10-wk-old mice and stained and analyzed by four-color flow cytometry. Total numbers of thymocytes ($\times 10^7$) were 7.5 and 9.0 for H-2^b and 14.4 and 11.6 for H-2^d mice expressing or lacking β 2m, respectively.

there are very few CD4⁺8⁻ cells in the lymph node of RAG-2^{-/-} H-2^b TCR transgenic mice, there are few cells with low CD8 levels that express the transgenic receptor (2×10^6) (Fig. 3 B). These cells may represent a different lineage of T cells that do not require positive selection and are more abundant in TCR transgenic than normal mice (31).

Full Maturation of Class II MHC-restricted CD4⁻8⁺ Cells Requires Class I MHC Molecules. To test whether CD4⁻8⁺ T cells with a class II MHC-restricted TCR could be generated in the absence of class I MHC molecules, we analyzed the thymus of TCR transgenic, $\beta_2m^{-/-}$ mice of the d/d or b/b MHC haplotype (Fig. 4). CD4⁻8⁺ cells with high levels of the transgenic TCR were detected in both β_2m^+ or $\beta_2m^{-/-}$ H-2^d mice, but not in β_2m^+ or $\beta_2m^{-/-}$ H-2^b mice. However, there was a clear-cut difference between the CD4⁻8⁺ T cells with high levels of the transgenic TCR in H-2^d β_2m^+ and $\beta_2m^{-/-}$ mice: in the former they were quite heterogeneous with regard to HSA expression, whereas in the latter they all expressed higher levels of HSA. (Note that most CD4⁻8⁺ cells that were selected through the receptor composed of transgenic β and endogenous TCR α chains in β_2m^+ mice were absent in $\beta_2m^{-/-}$ mice because of the lack of class I MHC molecules.) These results indicate that the CD4⁻8⁺ T cells with the class II MHC-restricted transgenic TCR are undergoing positive selection when their receptor binds to class II MHC molecules in the thymus, but that the full maturation into CD4⁻8⁺ HSA⁻ cells requires the presence of class I MHC molecules.

For that reason CD4⁻8⁺ cells with high levels of the transgenic TCR were virtually absent in lymph nodes of H-2^d $\beta_2m^{-/-}$ mice, as were cells with additional receptors containing endogenous TCR α chains (Table 2). In H-2^b mice we are able to detect very few CD4⁻8⁺6.5^{hi} cells. These cells may have been selected through receptors containing endogenous TCR α chains (H-2^b β_2m^+ mice) or may belong to an abnormal subset as described above (H-2^b $\beta_2m^{-/-}$ mice).

Discussion

Our results document the existence of mature CD4⁻8⁺ cells with a class II MHC-restricted TCR. These cells re-

quire positive selection by class II MHC molecules. Because CD4⁻8⁺ thymocytes with a class II MHC-restricted TCR are also present in $\beta_2m^{-/-}$ mice, our data indicate that downregulation of CD4 coreceptors on developing CD4⁺8⁺ thymocytes does not necessarily require ligation of the TCR- α/β to class I MHC molecules, at least in a fraction of developing cells. These data are in accord with the previously published data that demonstrated the existence of CD4⁺8^{low} cells with a class I MHC-restricted TCR as well as CD4^{low}8⁺ cells in class II MHC-deficient mice (5). It has previously been assumed that the full maturation of single positive T cells requires coengagement of MHC molecules by the TCR- α/β as well as the respective coreceptor (3-7, 32-34). Our data suggest that there may be exceptions to this rule; we find that the presence of class I MHC molecules can induce the full maturation of CD4⁻8⁺ HSA^{high} T cells into CD4⁻8⁺ HSA⁻ cells, even though they express a class II MHC-restricted TCR. One reason for this might be that the transgenic class II MHC-restricted TCR has some affinity for both class II and class I MHC molecules. We think that this is not a very likely explanation for several reasons. First, positive selection of cells with the transgenic TCR necessarily requires class II MHC molecules. Neither K^d nor D^d MHC molecules were sufficient for positive selection of cells with the transgenic TCR. Second, in very sensitive binding assays the soluble transgenic TCR failed to bind to class I MHC molecules, although binding to class II MHC molecules, even in the absence of the specific peptide, could easily be visualized (22, Karjalainen, K., unpublished results). It could thus be that for full maturation of some α/β T cells, it suffices when the TCR- α/β binds to one class of MHC molecules (in our case class II) and the coreceptor to the other class (in our case class I). This may be sufficient with TCRs of relatively high affinity that can induce activation of mature T cells in the absence of coreceptors, as has been reported for the transgenic TCR studied here (Karjalainen, K., unpublished results). One might assume that some of these receptors also have higher affinity for self MHC molecules in the absence of the specific peptide. Our data are consistent with the notion that the full maturation of α/β T cells requires more than a single interaction of the TCR- α/β or

Table 2. Cell Numbers (10^6) in Lymph Nodes from β_2m^+ and β_2m^{-} TCR Transgenic Mice of H-2^d and H-2^b Haplotype

	Total	CD4 ⁺ 8 ⁻		CD4 ⁻ 8 ⁺			
			6.5 ^{hi}		6.5 ^{hi}	6.5 ^{hi} HSA ^{lo}	6.5 ^{hi} HSA ⁻
Tg β_2m^+ H-2 ^d	22.2	20.0	1.30	2.06	0.28	0.07	0.21
Tg β_2m^{-} H-2 ^d	27.1	26.8	1.75	0.04	0.01	0.01	<0.01
Tg β_2m^+ H-2 ^b	25.8	22.8	0.39	1.81	0.14	n.d.*	0.13
Tg β_2m^{-} H-2 ^b	23.5	22.7	0.30	0.05	0.03	n.d.	0.03

* n.d., not detectable as too few cells were acquired to give an exact number.

Absolute cell numbers of indicated cell populations in lymph nodes were calculated from total cell numbers and percentages of cells determined by four-color immunofluorescence as shown in Fig. 4.

CD4/CD8 coreceptors with intrathymic ligands (5). They also provide an exception to the rule that class II and class I MHC-restricted receptors are exclusively expressed by mature CD4⁺ and CD8⁺ single positive T cells, respectively. It remains to be seen how frequent these exceptions are which can only be rigorously tested in TCR transgenic mice that do not produce endogenous TCR proteins. Our results show further that CD4⁻8⁺ T cells can be activated by class

II MHC-presented peptides to become potent killer cells. One could imagine that the activation of CD4⁻8⁺ lytic T cells by class II MHC-presented peptides could be of significance in regulating immune responses, for instance by destroying class II positive antigen-presenting cells. Such cells could also be of pathophysiological importance either in protective immune responses or in autoimmunity.

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