International Immunology, Vol. 11, No. 10, pp. 1595–1600

Positive selection of thymocytes induced by gene transfer: MHC class II-mediated selection of CD8 lineage cells

Ronald Rooke, Caroline Waltzinger, Christophe Benoist and **Diane Mathis**

Institut de Génétique et de Biologie Moléculaire et Cellulaire (CNRS/INSERM/ULP), 67404 Illkirch, CU de Strasbourg, France

Keywords: adenovirus, gene therapy, knockout mice, lymphocyte repertoire, MHC class II genes, T cell differentiation

Abstract

Recombinant adenovirus vectors are powerful tools for inducing *de novo* gene expression *in vivo*. Here we have exploited them to study the specificity of CD4/CD8 lineage commitment during thymocyte positive selection, transferring MHC class II genes directly into thymi of mice deficient in both class I and II molecules. Expression of class II molecules was induced on cortical stroma, provoking the selection of a large population of mature $CD4^+CD8^-$ cells, as expected, but also of a significant number of $CD4^-CD8^+$ cells. The latter constituted a diverse population, containing both immature precursors and, though less frequent, cells that were mature according to several criteria. $CD4^-CD8^+$ cells appeared with the same kinetics as their $CD4^+CD8^-$ counterparts, but tended to be more prevalent at early times or when thymocyte reconstitution was only modest. These observations, derived from a dynamic selection system, indicate that CD4/CD8 lineage commitment is not irredeemably linked to the class of MHC molecule driving positive selection, a conclusion most compatible with selective models of commitment.

Introduction

The peripheral T lymphocyte repertoire of mature animals consists of two main populations, which share expression of the αβ TCR and can be differentiated by the surface display of either the CD4 or CD8 co-receptor molecule. The precursors of these peripheral populations undergo a highly regulated program of differentiation in the thymus. Chromosome rearrangement occurs at defined stages, resulting in the juxtaposition of germline TCR gene segments, first at the β locus, then at the α, to generate a functional gene that encodes the clonotypic receptor characteristic of each T cell clone, and defining its antigen specificity. At this stage, the thymocytes express both the CD4 and CD8 co-receptors. Double-positive (DP) cells are subjected to a selection process keyed on the specificity of their receptors (1). Cells expressing a TCR capable of recognizing MHC molecules complexed with self-peptides on cortical epithelial cells are positively selected and finish the maturation process, shutting off synthesis of one of the co-receptor molecules to become either CD4 or CD8 single-positive (CD4- or CD8-SP) cells. However, cells expressing a receptor with too high an affinity for self-MHC–peptide ligands are eliminated by negative selection. The net result is a repertoire purged of useless cells unable to recognize foreign antigens presented by the animal's MHC molecules and of harmful cells reactive to self-antigens presented by self-MHC molecules.

The requirements for positive selection have been thoroughly analyzed over the past decade using genetically manipulated mice. Experiments involving TCR transgenic animals have demonstrated that DP thymocytes expressing class I-reactive TCR are generally selected to become $CD8^+$ cells (2–4), while those displaying class II-reactive receptors differentiate into $CD4^+$ cells (5,6). Elimination of MHC class I or class II molecules by homologous recombination has shown that they are essential for the maturation of end-stage CD4- or CD8-SP respectively (7–10). Thus, the positive selection process seems to coincide with lineage commitment, the specificity of the TCR for a class of MHC molecule matching the co-receptor expressed by the mature cell. How this match is achieved, and the nature of the signals that direct commitment towards the CD4 or CD8 lineage, remain unclear. It has been debated whether lineage choice is primarily instructive (directed from the start by the recognition

Correspondence to: C. Benoist

Transmitting editor: T. Sasazuki

Received 27 January 1999, *accepted* 31 May 1999

of MHC class during TCR–MHC interaction) or selective (initial commitment following other cues and confirmed only secondarily after shut-off of one co-receptor), or some combination of the two (for review and references, see 11).

Much of the debate has centered on the existence of receptor/co-receptor mismatched cells (e.g. CD4$^+$CD8$^-$ cells expressing a TCR with affinity for MHC class I molecules), either as transitional intermediates or as fully mature lymphocytes. Such cells should not exist according to the instructive model, but are clearly predicted by the selective model. By now, the weight of evidence indicates that 'mismatched' transitional intermediates do exist in thymi of TCR transgenic or MHC-deficient mice (for review, see 11); mismatched mature cells have also been observed in some cases. In particular, examples of TCR transgenic mice expressing class II-restricted TCR and generating significant numbers of CD8-SP in a class II-dependent fashion have been described (12,13). This non-standard commitment was enhanced when co-receptor deficiencies were introduced (13) and full maturation of the class II-restricted CD8$^+$ T cells was enhanced in the presence of class I molecules, but not in an allele-specific fashion.

It has been argued that the latter evidence is not generalizable and merely represents transgenic phenomenology peculiar to these receptors. Indeed, it has repeatedly been asserted that commitment to the CD8 lineage requires an instructive signal that can only be delivered by MHC class I molecules (14–16). Thus, it would be of interest to know whether the ability of class II molecules to mediate selection of CD8-SP extends to a broader repertoire of TCR specificities. In a previous study of thymocyte selection, we used an adenovirus-based gene delivery system to introduce class II molecules into the thymus of animals deficient in both classes of MHC molecules (17). As expected, CD4-SP cells were efficiently selected and exported to the periphery. In the course of these experiments, we also noticed the appearance of a significant population of CD8-SP cells. Here, we focus on the generation and phenotype of these class II-elicited CD8-SP cells, and discuss the implications of our findings for models of CD4/CD8 lineage commitment.

Methods

Mice

Mutant animals were β_2-microglobulin (β2m)-deficient (I°) (10) or MHC class I/II double-deficient (I°II°) (18). All mutant strains were on a mixed C57Bl/6 (B6)×129 genetic background. All mice were bred under specific pathogen-free conditions, and housed and handled under EEC guidelines. Virus injections and subsequent animal husbandry were performed under Ministère de l'Agriculture guidelines (Agrément B67900).

Recombinant viruses and intrathymic (i.t.) injections

The recombinant viruses have been described (17). Briefly, they carry a cDNA segment encoding an MHC class II molecule under the control of the E_α promoter, placed in lieu of the deleted E1A region of the adenovirus 5 mutant *dl234*. Viruses encoding either the $A_\beta{}^b$ or $E_\alpha{}^k$ chains were used—as they yielded identical results, they are often not distinguished in the figures. Viruses were grown as large stocks in 293 cells, and were purified and concentrated as described (17). For every virus preparation, one aliquot was thawed to verify the genomic structure of the amplified virus and to determined its titer. The i.t. injections of 4- to 6-week-old mice were performed as described (17), each thymic lobe receiving 10 μl of either control or recombinant virus, corresponding to ~3×10^8 infectious particles.

Flow cytometry

Single-cell thymocyte suspensions were stained with the following mAb: KT3 (anti-CD3ε), H57-597 (anti-TCR$\alpha\beta$), IM-7 (anti-CD44), anti-CD-69 (PharMingen, San Diego, CA) and anti-CD8 (Caltag, Paris, France) (17). Flow cytometry was performed on a Coulter Elite instrument with 488 nm argon and 590 nm dye laser excitation, fitted with four-decade logarithmic amplifiers, and data were stored as list mode for analysis. Analysis of list-mode files was performed on Elite software (Coulter, Margency, France), generating contour pseudo-dot plots. Live cells were gated on forward versus side scatter profiles.

Results

We have shown previously that i.t. injection of adenovirus vectors encoding MHC class II molecules (Ad-cII) into MHC-deficient mice (I°II°) induced significant expression of class II molecules on the surface of thymic epithelial cells (17). The vectors encoded either a functional A_β chain, complementing an engineered defect in the MHC-deficient mouse strain, or an E_α chain, complementing a naturally occurring mutation. As anticipated, the restoration of class II molecule expression induced the selection and maturation of a significant CD4-SP population (17) (Fig. 1). Unexpectedly, a small but distinct and reproducible increase in the number of CD8-SP cells could also be detected in the cytofluorimetic profiles of thymocytes from Ad-cII-injected mice (Fig. 1C); such cells were absent from unmanipulated I°II° animals (not shown) and from those injected with the *dl324* control virus (Fig. 1B). The newly generated CD8-SP thymocytes were best visualized when the analysis was focused on cells displaying high surface levels of CD3 (Fig. 1A–C, right panels). Because there is significant variation in the efficiency of i.t. gene delivery by adenovirus vectors (17), the number of CD8-SP recovered after transduction of class II genes fluctuated significantly from mouse to mouse; however, these cells were consistently observed whenever the recovery of CD4-SP signalled efficient infection (see below). $E_{\tilde{\alpha}}$ and $A_{\tilde{\beta}}$ transducing viruses were equally effective at promoting the selection of CD8-SP, and therefore we have pooled results from the two types of injections.

The CD4$^-$8$^+$ thymocytes in Ad-cII-injected I°II° mice were mature by several criteria. First, four-color cytofluorimetry, staining with an anti-$\alpha\beta$TCR mAb and peanut agglutinin (PNA) in addition to anti-CD4 and anti-CD8 reagents, (Fig. 1, lower panels) showed that the newly generated CD8-SP cells had a phenotype typical of mature cells: high expression of the TCR, low levels of PNAr. These cells also expressed mature levels of heat stable antigen (not shown). Cells with this phenotype were essentially absent from I°II° animals injected

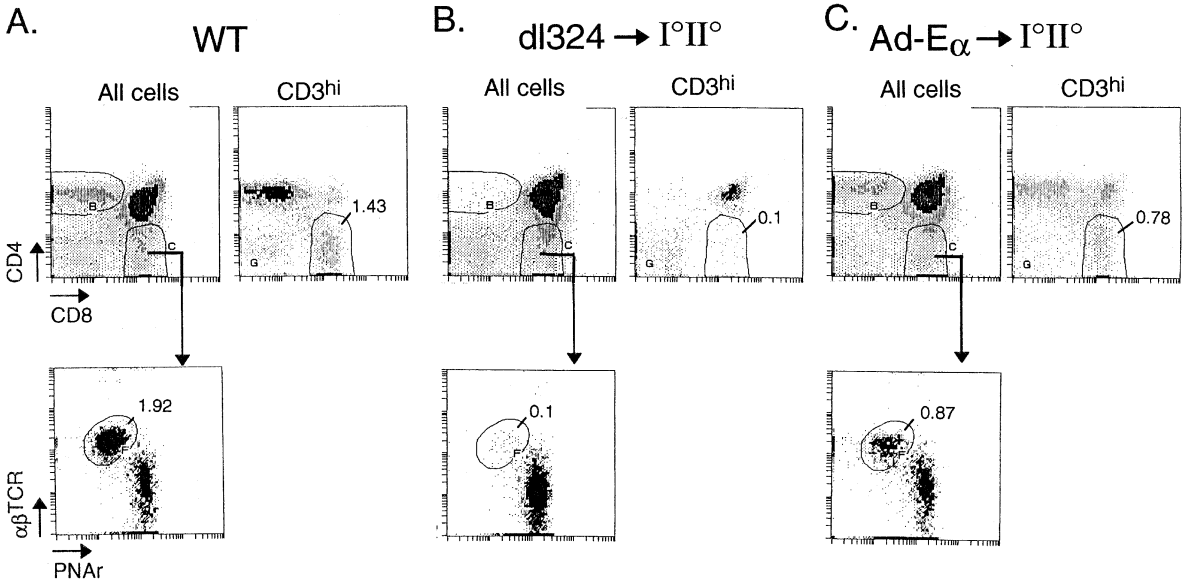

Fig. 1. Selection of CD8-SP after Ad-cII gene transfer. Flow cytometric analysis of thymi from wild-type mice (A), MHC-deficient I°II° animals injected i.t. with the control adenovirus *dl324* (B) or mice injected with the E_α-recombinant virus (C). These profiles are representative of several expressing experiments. The top panels show CD4/CD8 plots of all thymocytes (left panels) or of $CD3^{hi}$ thymocytes (right panels). Cells within the $CD4^-CD8^+$ gate were electronically gated and their PNAr/TCR αβ profiles are shown in the bottom panels. The values represent the percentage of total thymocytes that fall within the gates shown (as total thymocyte numbers are not significantly affected by the type of virus injected, the percentages also reflect absolute numbers).

with control virus. A second approach to document the maturation of the $CD4^-CD8^+$ thymocytes in Ad-cII-injected mice was to evaluate their sensitivity to glucocorticoids, which eliminate immature thymocytes but tend to spare the mature populations (19). While virtually no CD8-SP cells remained in the thymus of a control virus-injected I°II° mouse after dexamethasone treatment, a clear population resisted in animals injected with Ad-cII, ~10% of the numbers found in the wild-type control; as expected, these cells display high levels of TCR (Fig. 2). Thus, the newly generated CD8-SP cells appeared to be *bona fide* mature thymocytes.

Figure 3(A) shows the time-course of appearance of mature thymocytes after i.t. injection of Ad-cII. As previously reported (17), CD4-SP cells began to accumulate at day 9–10 post-injection, reflecting the time required for virus entry and decapsidation, transcription of the viral genome, translation and transport of the class II gene products, and transition of the newly selected DP cells into the SP compartment. The timing of appearance of CD8-SP cells was very similar, significant numbers again first appearing after day 8 post-injection; if anything, accumulation was slightly accelerated vis-à-vis CD4-SP. By these criteria, the CD8-SP seemed to be elicited directly by class II molecules and not indirectly, somehow in response to the sudden appearance of CD4-SP.

Interestingly, there seemed to be a cap on the total number of $CD4^-CD8^+$ cells selected after Ad-cII injection. This point is illustrated in Fig. 3(B), a plot of the relative reconstitution of the CD4- and CD8-SP populations in individual mice. As noted above, the efficacy of adenovirus-mediated gene transfer after i.t. injection is quite variable from mouse to mouse: the number of CD4-SP cells ranged from only slightly above background to comparable with those of wild-type

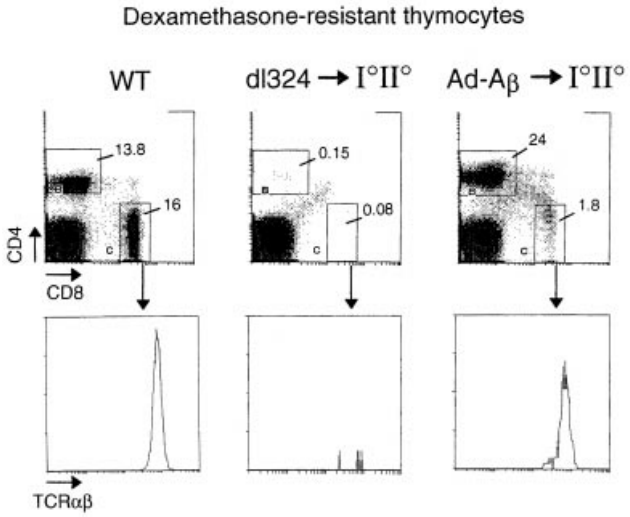

Fig. 2. Dexamethasone resistance of CD8-SP induced by Ad-cII gene transfer. Flow cytometric analysis of thymi from wild-type (left) or MHC-deficient mice injected i.t. either with control virus (center) or E_α-expressing recombinant virus (right), treated with dexamethasone (125 mg/kg body weight) 25 days after viral transfer, eliminating the bulk of immature cells. These profiles are representative of several experiments. The values represent the percentage of total thymocytes that fall within the gates. MHC II-selected $CD8^+$ T cells

controls. In contrast, the number of CD8-SP cells induced by *de novo* expression of class II molecules never went beyond 20% of the normal, even for mice showing a full complement of CD4-SP cells. The reconstitution of the CD8-SP compart-

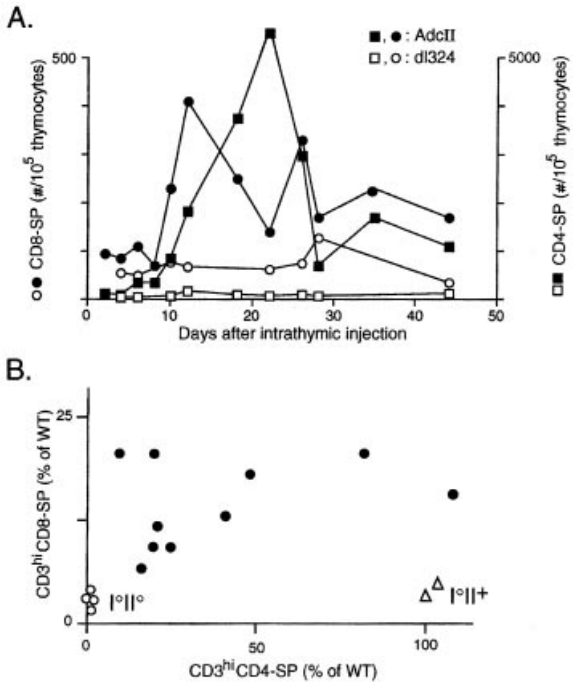

Fig. 3. Kinetics and numbers of CD8-SP after Ad-cII injection. (A) Large numbers of I°II° mice were injected i.t. with Ad-cII or control viruses (data from E_α- and A_β-encoding viruses were similar, and are therefore pooled), and the proportion of CD4- and CD8-SP was measured by flow cytometry at various times after injection. Each point represents the average from four to seven mice (except for the very early time points when there was no reconstitution, when there were two mice each). (B) Filled circles: numbers of CD3^{hi} CD4- versus CD8-SP (expressed as percent of wild-type controls) in thymi of individual I°II° mice transferred i.t. with Ad-cII virus. Each dot represents an individual mouse. Open circles: uninjected or *dl324* control virus-injected I°II° mice. Open triangles: control β_2m-deficient I°II$^+$ mice.

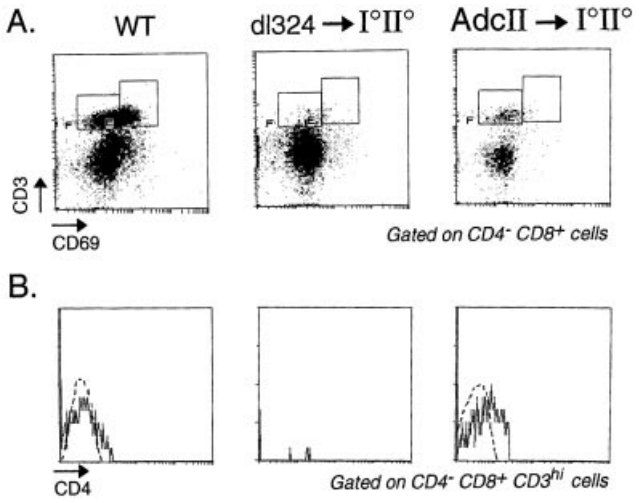

Fig. 4. Unusual characteristics of CD8-SP elicited by Ad-cII gene transfer. (A) CD69 expression analyzed by four-color flow cytometry. Cells from thymi similar to those of Fig. 1 were stained with anti-CD4 and anti-CD8 (not shown), and the CD4$^-$CD8$^+$ population electronically gated; the CD69/CD3 profiles of these gated cells are shown. The boxes help to visualize the proportion of cells with high levels of CD3 which do, or do not, express CD69. Profiles representative of four similar experiments. (B) Cells from the same thymi were stained with anti-CD3, anti-CD4 and anti-CD8. The CD4 intensity of CD3^{hi}CD8-SP (gated as in Fig. 1, top panels) is displayed in these histograms. The overlay dotted line represents negative staining.

ment was relatively better when that of the CD4-SP was only partial. Figure 3(B) also shows that thymi of unmanipulated I°II$^+$ mice host very few CD8-SP.

Thus, class II molecules transduced by adenoviral transfer into MHC-deficient thymi were capable of selecting and maintaining a population of mature CD4$^-$CD8$^+$ cells. Two observations suggested that this process was slightly peculiar, though. First, CD69 was under-expressed on these cells. In normal mice, this early activation marker is transiently displayed on most thymocytes engaged in positive selection, although CD4-SP cells tend to be more uniformly positive than their CD8$^+$ counterparts (20,21). In the virally transduced thymi, the CD8-SP cells expressed very little CD69 (Fig. 4A), even though CD4-SP from the same thymi showed normal levels (data not shown). Second, the Ad-cII-elicited CD8-SP population was enriched for early intermediates over fully mature cells. This peculiarity is visible in the analysis of dexamethasone-resistant thymocytes of Fig. 2, but is perhaps better appreciated by comparing CD4 levels in Fig. 4(B): in wild-type mice, most of the CD8-SP had completely down-regulated CD4 expression; in contrast, in Ad-cII-injected I°II° mice, there was a higher proportion of cells with residual levels of CD4.

Discussion

Intrathymic injection of an MHC class II gene into MHC-deficient mice provoked selection of a substantial cohort of CD8-SP thymocytes, in addition to the expected CD4-SP cells. The CD8-SP resulted from positive selection, as indicated by their TCR^{hi}PNA^{lo} dexamethasone-resistant phenotype, their kinetics of appearance after transduction of MHC class II genes and their absence after injection of control virus. One might raise the caveat that these CD8-SP might not be selected by class II molecules themselves but by stabilization of unstable MHC class I molecules by class II-derived peptides; this seems unlikely since there is no precedent of an influence of class II on class I maturation or stability, and since both E_α- and A_β-carrying vectors have the same effect. It might have been argued that i.t. injection of adenovirus particles somehow leads to the appearance of CD8-SP thymocytes when MHC class II molecules are present, through some non-specific response to viral particles or the cytokines they induce. This possibility was ruled out by injecting control or MHC class II-expressing viruses into thymi of I°II$^+$ mice: no CD8-SP appeared under such circumstances (data not shown).

As previously reported (9,10), such a population of CD8-SP thymocytes is not found (or in nowhere near the same frequencies) in I°II$^+$ mice, which have the same complement of MHC genes as Ad-cII-injected animals, and this point is brought home by the direct comparison in Fig. 3(B). The difference probably lies in the timing of class II molecule expression in the two cases and in the ensuing competition between different thymocyte populations. In the I°II$^+$ mice,

class II molecules are expressed from fetal life onwards and the compartment of mature thymocytes is filled with abundant CD4-SP. In contrast, class II molecules appear suddenly in Ad-cII-injected I°II° animals and the first wave of selected cells finds itself in an empty compartment with no immediate competitors. Class II-restricted CD8-SP are thus free to accumulate, at least until CD4-SP fill the available space. It is easy to understand why class II-restricted CD8-SP cells, whose selection may not be the most robust, would be visible after a punctual turn-on of MHC class II molecules but not in a steady-state situation. In addition, there is some evidence for this interpretation in the ceiling reached by CD8-SP even after the best i.t. transfers of virus (Fig. 3B).

Competition for positive selection has been described (22,23). Not all cells potentially able to be positively selected actually do, as seen in mixed reconstitution experiments with transgenic or wild-type precursors restricted by the same MHC molecule (22,23), perhaps due to a limiting number of niches capable of supporting selection of a particular lineage (24). It is interesting that the competition hypothesized here would take place after the initial TCR–MHC interaction: the initial commitment and selection events would generate both receptor/co-receptor-matched and -mismatched intermediates, but competition would modulate the survival and progression of maturing populations. CD4-SP would have an advantage over class II-restricted CD8-SP, most likely because they maintain CD4 expression at the cell surface, allowing optimal signals from co-engaged TCR and co-receptor. These optimal signals would make for winners in the competition for space in the thymic niches.

This demonstration of class II-mediated selection of CD8-SP thymocytes extends reports of class II-restricted CD8-SP in TCR transgenic systems (12,13). It indicates that these prior results were not due to quirks of the TCRs under study, but can be generalized to broad, randomly generated, repertoires. Interestingly, and as in the TCR transgenic systems (12,13), we found very few corresponding CD4$^-$CD8$^+$ lymphocytes in the peripheral lymphoid organs of the reconstituted mice; even in the best cases, mice with sizeable CD8-SP thymic populations only had rare peripheral cells, barely and inconsistently above the background of cells in unmanipulated I°II° mice. The most straightforward explanation for this paradox may lie in recent observations that the survival of naive CD8$^+$ lymphocytes requires continued engagement by MHC molecules involved in their selection (25). The class II-restricted CD8$^+$ lymphocytes selected after thymic class II gene transfer cannot survive in the periphery because they cannot find class II molecules there, while the rare CD8-SPs already present in I°II° lymph nodes without manipulation are probably stabilized by the self-molecules that selected them (non-classical class I, residual D^b?).

These observations are not consistent with models of thymocyte lineage commitment that contend that commitment to the CD8 lineage requires instructive signals that can only be delivered through class I molecules (14–16): the CD8-SP observed here in substantial numbers were selected without intervention of class I molecules, in keeping with the recent demonstration that CD8 lineage commitment can be induced *in vitro* without class I (26). These observations are consistent with a model in which initial lineage commitment is chosen independently of the class of MHC molecule, but depends on the affinity/kinetic characteristics of the signal received through the TCR, subsequent survival of the cells requiring that this choice provide a match between the remaining co-receptor and the specificity of the receptor (for review, see 11).

Abbreviations

B6	C57Bl/6
β_2m	β_2-microglobulin
DP	double positive
i.t.	intrathymic
PNA	peanut agglutinin
SP	single positive

References

1 Jameson, S. C., Hogquist, K. A. and Bevan, M. J. 1995. Positive selection of thymocytes. *Annu. Rev. Immunol.* 13:93.
2 Teh, H. S., Kisielow, P., Scott, B., Kishi, H., Uematsu, Y., Bluthmann, H. and von Boehmer, H. 1988. Thymic major histocompatibility complex antigens and the alpha beta T-cell receptor determine the CD4/CD8 phenotype of T cells. *Nature* 335:229.
3 Sha, W. C., Nelson, C. A., Newberry, R. D., Kranz, D. M., Russell, J. H. and Loh, D. Y. 1988. Positive and negative selection of an antigen receptor on T cells in transgenic mice. *Nature* 336:73.
4 Pircher, H., Burki, K., Lang, R., Hengartner, H. and Zinkernagel, R. M. 1989. Tolerance induction in double specific T-cell receptor transgenic mice varies with antigen. *Nature* 342:559.
5 Berg, L. J., Pullen, A. M., Fazekas de St Groth, B., Mathis, D., Benoist, C. and Davis, M. M. 1989. Antigen/MHC-specific T cells are preferentially exported from the thymus in the presence of their MHC ligand. *Cell* 58:1035.
6 Kaye, J., Hsu, M. L., Sauron, M. E., Jameson, S. C., Gascoigne, N. R. and Hedrick, S. M. 1989. Selective development of CD4$^+$ T cells in transgenic mice expressing a class II MHC-restricted antigen receptor. *Nature* 341:746.
7 Cosgrove, D., Gray, D., Dierich, A., Kaufman, J., Lemeur, M., Benoist, C. and Mathis, D. 1991. Mice lacking MHC class II molecules. *Cell* 66:1051.
8 Grusby, M. J., Johnson, R. S., Papaioannou, V. E. and Glimcher, L. H. 1991. Depletion of CD4$^+$ T cells in major histocompatibility complex class II-deficient mice. *Science* 253:1417.
9 Zijlstra, M., Bix, M., Simister, N. E., Loring, J. M., Raulet, D. H. and Jaenisch, R. 1990. β_2-microglobulin deficient mice lack CD4$^-$8$^+$ cytolytic T cells. *Nature* 344:742.
10 Koller, B. H., Marrack, P., Kappler, J. W. and Smithies, O. 1990. Normal development of mice deficient in β_2M, MHC class I proteins, and CD8$^+$ T cells. *Science* 248:1227.
11 Chan, S., Correia-Neves, M., Benoist, C. and Mathis, D. 1999. CD4/CD8 lineage commitment: matching fate with competence. *Immunol. Rev.* 165:195.
12 Kirberg, J., Baron, A., Jakob, S., Rolink, A., Karjalainen, K. and von Boehmer, H. 1994. Thymic selection of CD8$^+$ single positive cells with a class II major histocompatibility complex-restricted receptor. *J. Exp. Med.* 180:25.
13 Matechak, E. O., Killeen, N., Hedrick, S. M. and Fowlkes, B. J. 1996. MHC class II-specific T cells can develop in the CD8 lineage when CD4 is absent. *Immunity* 4:337.
14 Suzuki, H., Punt, J. A., Granger, L. G. and Singer, A. 1995. Asymmetric signaling requirements for thymocyte commitment to the CD4$^+$ versus CD8$^+$ T cell lineages: a new perspective on thymic commitment and selection. *Immunity* 2:413.
15 Lucas, B., Vasseur, F. and Penit, C. 1995. Stochastic coreceptor shut-off is restricted to the CD4 lineage maturation pathway. *J. Exp. Med.* 181:1623.
16 Sebzda, E., Choi, M., Fung-Leung, W. P., Mak, T. W. and Ohashi, P. S. 1997. Peptide-induced positive selection of TCR transgenic thymocytes in a coreceptor-independent manner. *Immunity* 6:643.

17 Rooke, R., Waltzinger, C., Benoist, C. and Mathis, D. 1997. Targeted complementation of MHC class II deficiency by intrathymic delivery of recombinant adenoviruses. *Immunity* 7:123.
18 Chan, S. H., Cosgrove, D., Waltzinger, C., Benoist, C. and Mathis, D. 1993. Another view of the selective model of thymocyte selection. *Cell* 73:225.
19 Scollay, R., Bartlett, P. and Shortman, K. 1984. T cell development in the adult murine thymus: changes in the expression of the surface antigens Ly2, L3T4, and B2A2 during development from early precursor cells to emigrants. *Immunol. Rev.* 82:79.
20 Bendelac, A., Matzinger, P., Seder, R. A., Paul, W. E. and Schwartz, R. H. 1992. Activation events during thymic selection. *J. Exp. Med.* 175:731.
21 Swat, W., Dessing, M., von Boehmer, H. and Kisielow, P. 1993. CD69 expression during selection and maturation of CD4$^+$8$^+$ thymocytes. *Eur. J. Immunol.* 23:739.
22 Huesmann, M., Scott, B., Kisielow, P. and von Boehmer, H. 1991. Kinetics and efficacy of positive selection in the thymus of normal and T cell receptor transgenic mice. *Cell* 66:533.
23 Freitas, A. A., Agenes, F. and Coutinho, G. C. 1996. Cellular competition modulates survival and selection of CD8$^+$ T cells. *Eur. J. Immunol.* 26:2640.
24 Merkenschlager, M., Benoist, C. and Mathis, D. 1994. Evidence for a single-niche model of positive selection. *Proc. Natl Acad. Sci. USA* 91:11694.
25 Tanchot, C., Lemonnier, F. A., Pérarnau, B., Freitas, A. A. and Rocha, B. 1997. Differential requirements for survival and proliferation of CD8 naïve or memory T cells. *Science* 276:2057.
26 Albert Basson, M., Bommhardt, U., Cole, M. S., Tso, J. Y. and Zamoyska, R. 1998. CD3 ligation on immature thymocytes generates antagonist-like signals appropriate for CD8 lineage commitment, independently of T cell receptor specificity. *J. Exp. Med.* 187:1249.