Upon entry in the thymus at the cortico-medullary junction, T cell progenitors migrate to the outer cortex in response to CCR7, CXCR4 and CCR9 signals. Positively selected DP cells are attracted to the medulla through CCR7 signals, where negative selection occurs. Expression of S1P_1 allows for proper export in the periphery.

Adapted from Takahama Nat Rev Immunol 2006
Figure 2: Apoptotic cell death during T cell development

IL-7, pre-TCR and TCR signals are essential survival signals during thymopoiesis. The lack of γc cytokines, the inefficient rearrangement of the TCRs, the absence of positive selection or the elimination through negative selection regulate the survival outcome of the thymocytes.

Adapted from Hogquist Nat Rev Immunol 2005 and Bouillet Nat Rev Immunol 2009
Figure 3: IL-7 signal transduction and homeostasis

IL-7 signals through two main pathways: the Jak/Stat and the PI3K/AKT pathway. Key downstream targets involved in T cell survival, homeostasis, growth and metabolism are depicted.

From Takada and Jameson Nat Rev Immunol 2009
Figure 4: Phases of the primary immune response

Viral peptides presented by mature DCs to naive CD8 T cells trigger expansion and differentiation into effector CD8 T cells. 5-10% of the cells detected at the peak of the expansion phase survive the contraction phase and generate a stable memory T cell pool.

Adapted from Haring et al. Immunity 2006
Figure 5: Antigen presentation by MHC class I and II

MHC class I molecules present peptides that are derived from endogenous proteins degraded in the cytosol or from exogenous proteins through cross-presentation. MHC class II molecules acquire exogenous and endogenous peptides generated by proteolytic degradation in endosomal compartments. Adapted from Villadangos Nat Rev Immunol 2007
Pathogens
Cytokines
T cells

Sample environment
Receive danger signals
Capture antigen

Migration to lymph node
Antigen presentation
Costimulation
Secretion of cytokines and chemokines

Figure 6: Dendritic cell maturation

Immature DCs integrate pathogen-derived, T-cell derived or cytokine signals in the periphery in order to undergo maturation and migration to secondary lymphoid organs, where they are essential for the initiation of an adaptive immune response.

Adapted from Banchereau, Steinman Nature 1998
Figure 7: Immunological synapse

Through key receptor-ligand interactions, a DC and a naive CD8 T cell form an immunological synapse.

From Huppa Nat Rev Immunol 2003
Figure 8: Three signals for CD8 T cell expansion and differentiation

DC-derived signals transmitted to a naive T cell at the time of priming (signal 1: MHC-peptide/TCR, signal 2: costimulatory molecules, signal 3: inflammatory cytokines). IL-2 derived signals are also depicted.

Adapted from Malek Nat Rev Immunol 2004
Figure 9: CD4 help in $T_H$ dependent and independent responses

DCs might acquire cellular antigens through phagocytosis. Phagocytosed antigens are presented to CD4 T cells which in turn activate the CD through CD40/CD40L interactions. The activated DC can then promote CD8 T cell responses. Conversely, infectious agents circumvent the need for CD4 T cell recognition of the antigen by stimulating TLRs directly or by causing the release of inflammatory cytokines, promoting the adequate maturation of the DC and the subsequent CD8 immune response.

From Bevan Nat Rev Immunol 2004
Figure 10: $\gamma_c$ cytokine receptor subunit expression during an immune response

Differential expression of each cytokine-receptor upon infection.

From Schlums Nat Rev Immunol 2003
Figure 11: IL-2 versus IL-15 trans-presentation

IL-2 and IL-15 share the IL2Rβ and the γ_γ_c chain. IL-2 is a secreted cytokine and binds pre-formed high-affinity heterotrimeric receptors, while IL-15 is membrane-bound to the IL15Rα that present IL-15 in trans to cells expressing the intermediate-affinity IL2Rβ/γ_c receptor.

From Waldmann Nat Rev Immunol 2006
Figure 12: Perforin-dependent granzyme release

Granzyme B entry into the target cell could be mediated through three independent or complementary processes. Perforin molecules can polymerize to form pores in the target cell membrane, allowing for granzyme B entry. Granzyme B can also bind to the membrane directly or through association with a receptor (mannose-6-phosphate). Perforin would thus be essential for release of sequestered granzyme B molecules in endosomes.

From Barry Nat Rev Immunol 2002
Figure 13: Fas-dependent apoptotic cell death

Engagement of Fas (CD95) on a target cell with the FasL receptor expressed on the effector CD8 T cell leads to activation of a caspase-dependent, Bid/Bax-dependent cascade resulting in target cell death.

From Barry Nat Rev Immunol 2002
Figure 14: Contraction-associated apoptotic pathways

Cytokine withdrawal deprives the cell from important survival signals and leads to Bim-dependent and Puma-dependent T cell death. Fas-mediated apoptosis might also contribute to CD8 T cell contraction.

Adapted from Strasser Nat Rev Immunol 2005
Figure 15: Bcl2 over-expression rescues peripheral naive γc−/− P14 CD8 T cells.

(A) Flow cytometry analysis of P14, P14 γc−/− and P14 Bcl2 γc−/− thymocytes (upper panel) and splenocytes (lower panel) using a combination of CD4, CD8, GP33-41 tetramer and Bcl2 antibodies. Numbers in dot plots indicate the percentage of each correspondent population.

(B) Size, MHC class I expression and intracellular Bcl2 staining of P14 and P14 γc−/− thymocytes and splenocytes. (C) Absolute numbers of total thymocytes, CD8 single positive (CD8 SP) thymocytes and splenic CD8+ Tetramer+ T cells in P14, P14 γc−/− and P14 Bcl2 γc−/− mice. Five to eleven mice (5-9 weeks of age) of each genotype were analyzed (* p<0.05, ** p<0.005, *** p<0.0005). (D) Cell surface expression of the indicated molecules by P14 Bcl2 (shaded) and P14 Bcl2 γc−/− (line) CD8 T cells at baseline. All results are representative of at least three separate experiments.
Transfer of $10^5$ CD8$^+$ splenocytes from
- Rag$^{-/-}$ P14$^+$ Bcl2$^+$ CD45.2$^+$ donors or
- Rag$^{-/-}$ P14$^+$ Bcl2$^+ \gamma_c^{-/-}$ CD45.2$^+$ donors

C57BL/6
CD45.1$^+$
recipients

Intraperitoneal infection of $2 \times 10^5$
CFU of LCMV
Armstrong

Day -1

Day 0

Day +3

Day +5

Day +7

Day >60

Phenotypic analysis (blood, spleen)
In vivo proliferation assay

Phenotypic analysis (blood, spleen)
In vivo cytotoxicity assay

Phenotypic analysis (blood, spleen, bone
marrow)
Single-cell sort

Reinfection with
$2 \times 10^5$ CFU of LCMV Armstrong

Figure 16: Experimental approach.

P14, P14 Bcl2$^+$ and P14 Bcl2$^+ \gamma_c^{-/-}$ chimeric mice are generated by adoptive transfer of $10^5$
MACS-purified naive CD45.2 TCR Tg CD8 T cells into naive CD45.1 B6 recipients 24 hours prior
to infection. Mice are then infected by intraperitoneal injection with $2 \times 10^5$ PFU of LCMV.
Distinct assays are performed throughout the immune response and are presented in the result
section.
10^5 P14 or P14 IL2Rβ^-/- CD45.2 CD8 T cells were adoptively transferred into naive CD45.1 mice that were subsequently infected with 2 X 10^5 PFU of LCMV Armstrong. Expansion and contraction of antigen-specific splenocytes were analyzed by flow cytometry at specific time points. (A) Total number of antigen-specific CD8 T cells was calculated based on GP33-41 tetramer and CD45.2 congenic marker staining. Data represent the mean ± SEM of eleven to twenty mice per time point, from more than five experiments (* p<0.05, *** p<0.0005, NS p≥0.05) (B) Cell surface or intracellular expression of the indicated molecules by P14 (shaded) and P14 IL2Rβ^-/- (line) CD8 T cells at day 7 post-infection. (C) MPEC and SLEC subsets were analyzed at day 7 post infection based on KLRG1 and CD127 expression. Numbers in dot plots indicate the percentage of each correspondent population from a representative experiment. (D) Splenocytes from day 7 LCMV-infected chimeric mice were stimulated with GP33-41 peptide and analyzed for IFNγ, TNFα and IL-2 production by intracellular cytokine staining. Data represent the mean ± SEM of six to ten mice per time point, from five experiments (* p<0.05, NS p≥0.05). (E-F) P14 or P14 IL2Rβ^-/- CD8 T cells were adoptively transferred into naive perforin knock-out (Pfp^-/-) mice that were subsequently infected with 2 X 10^5 PFU of LCMV Armstrong. Killing function and development of hemophagocytic lymphohistiocytosis were followed over time. (E) In vivo CTL assay comparing day 8 P14 (filled circle) and P14 IL2Rβ^-/- (opened circle) CD8 effector T cells, to infected (filled square) and uninfected/naive (opened square) Pfp^-/- cells. The individual percent killing over 3 hours represents the combined results of two independent experiments (*** p<0.0005, NS p≥0.05). (E) Survival of Pfp^-/- (filled square) chimeric mice transferred with P14 (filled circle) and P14 IL2Rβ^-/- (opened circle) CD8 T cells.
Figure 18: Memory cell generation is dramatically reduced in the absence of IL2Rβ signals.

P14 and P14 IL2Rβ⁻/⁻ chimeric mice were generated as described in Fig. 16, and were followed longitudinally. (A) The frequency of GP₃₃-₄₁⁺ CD45.2⁺ cells from P14 (filled circle) and P14 IL2Rβ⁻/⁻ (opened circle) CD8 T cells is evaluated by flow cytometry over a 90 day time course. Results are representative of five to ten mice from three independent experiments. (B) Absolute number of GP₃₃-₄₁⁺ CD45.2⁺ T cells in the spleen and bone marrow of P14 (black bars) and P14 IL2Rβ⁻/⁻ (white bars) chimeric mice at 90 days post infection (n=4, * p<0.05, ** p<0.005).
Figure 19: Secondary immune responses are preserved in the context of IL2Rβ deficiency.

P14 and P14 IL2Rβ−/− chimeric memory mice (>70 days post infection) are reinfected intraperitoneally with 2 × 10^5 PFU of LCMV Armstrong. Antigen-specific CD8 T cells were analyzed in the blood by flow cytometry. (A) Total number of antigen-specific CD8 T cells was calculated based on GP33-41 tetramer and CD45.2 congenic marker staining, at day 0 and 6 post-reинфекtion (left panel). Bar graph (right panel) represents the mean fold expansion of six to seven mice from two independent experiments (NS p≥0.05). (B) Cell surface expression of the indicated molecules by P14 and P14 IL2Rβ−/− CD8 T cells at day 0 and 6 post-infection. (C-E) Blood from day 7 LCMV-reinfected memory mice were stimulated with GP33-41 peptide and analyzed for IFNγ, TNFα, IL-2 and granzyme B production by intracellular cytokine staining. (C) Bar graph represents the mean ± SD for six to seven mice (NS p>0.05). (D) Cytokine production presented by flow cytometry plots from a representative experiment; numbers indicate the percentage of cells in each correspondent quadrant. (E) Bar graph represents the corresponding percentage of cells secreting only IFNγ, IFNγ and TNFα, or simultaneously IFNγ, TNFα and IL-2 for each genotype (mean ± SEM, * p<0.05, NS p>0.05).
Figure 20: Energy metabolism in naive CD8 T cell

Cytokines regulate the growth and metabolism through activation of the PI3K/AKT and JAK/STAT pathways. AKT regulates mTOR, while STATs regulate PIM1 and PIM2. Together, they repress key inhibitors of T cell survival and stimulate the uptake of extracellular nutrients in the cell.

From Fox and Thompson Nat Rev Immunol 2005