T cell receptor contact to restricting MHC molecules is a prerequisite for peripheral interclonal T cell competition

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Survival of naive T cells depends on the interaction of the TCR with MHC molecules of the same allele/isotype they were restricted to in the thymus (1–7). For most T cell specificities where this was rigorously tested (TCR transgenic Rag-deficient mice), transfer into lymphopenic hosts resulted in the expansion of transferred naive T cells (3, 8–11). This "homeostatic proliferation" is critically dependent on the presence of MHC molecules, thus resembling requirements for survival (1, 3, 5, 10, 12–14). More recently, proliferation of transferred T cells was observed in recipient mice containing a full complement of T cells, provided that they were of different TCR specificity (15, 16). Instead of a pure intraclonal competition toward their MHC ligand only, subsequent work found a "hierarchy" among T cells that were restricted to putatively different peptide–MHC molecule complexes (17). Thus, cells of one specificity proliferated in mice harboring T cells of a different specificity, but not vice versa. However, in these studies, some combinations of transfers were not pursued, and the possibility that peptide–MHC ligands could be cross-recognized by the given TCRs was not investigated (18).

Considering these data, homeostatic proliferation is not simply dependent on lymphokine availability, as interclonal T cell competition appears to depend on the nature of the TCR. Yet, it is unclear whether T cells would compete only for specific peptide–MHC molecule complexes or whether TCR contact with cells bearing the MHC restriction element would
Figure 1. Hierarchical competitiveness in homeostatic proliferation of OT1 and P14 T lymphocytes. (A) Equal numbers of CFSE-labeled lymphocytes from OT1 Rag-2^-/- and P14 Rag-2^-/- donors were cotransferred into B6.Rag-2^-/- recipient mice. Proliferation of donor CD8^+ cells expressing the OT1 or P14-TCR, stained for V^b5 or V^b8, respectively, is shown for the indicated time points after transfer. Two mice were analyzed for each time point and dose; an independent experiment using OT1 donor cells was also performed. (B) CFSE-labeled lymphocytes from OT1 Rag-2^-/- and P14 Rag-2^-/- Ly-5.1^+ mice were cotransferred into P14 Rag-2^-/- and B6.Rag-2^-/- (not depicted) recipients. Note that some CD8^+ non-T cells of the P14 recipient will be included in the population of V^b5^+ CD8^+ Ly-5.1^+ cells used to define OT1 donor T cells. However, division of OT1 cells was clearly evident at all time-points when focusing on cells that are labeled with CFSE and thus donor derived, or by staining for V^b5^+ CD8^+ Ly-5.1^+ as performed from week 2 onward (not depicted). For each time point, two mice per genotype were analyzed. Proliferation of OT1 cells in P14
determine the T cells’ subsequent ability to respond to lymphokines and undergo homeostatic proliferation.

We addressed these hypotheses by comparing the proliferation of transferred T cells in recipient mice expressing the same or different TCRs. We demonstrate a hierarchical competitiveness: OT1 (Kb restricted) > P14 (Db restricted) > F5 (Dp restricted). The ability of transferred OT1 cells to proliferate in P14 recipients was solely caused by OT1 TCR binding to Kb, as transferred OT1 cells failed to proliferate in Kb mutant P14 recipients. Moreover, transferred P14 cells demonstrate homeostatic proliferation in Kb-mutant OT1 recipients when these had been grafted with Kb-expressing thymi to allow peripheral OT1 cell reconstitution. Interestingly, using parabiosis of OT1 and Kb-mutant mice, we demonstrate that homeostatic proliferation of P14 cells is offset even when OT1 cells cannot interact with all MHC-bearing cells.

Thus, peripheral T cells derive a relative competitiveness that is apparently defined by the TCR’s affinity toward restricting MHC molecules. Yet, competition does not operate via direct competition for, or modulation of, the MHC-bearing cell or MHC molecules per se, but by secondary competition for ligands whose availability is restricted both in quantity and location and that are not bound to the MHC-bearing cell.

RESULTS AND DISCUSSION
Hierarchical competition across MHC restriction elements
We initially compared the ability of different class I-restricted TCR transgenic CD8+ T cells to proliferate in a lymphopenic environment using cells from TCR transgenic Rag-/- mice. As reported by others (9–11), most class I-restricted transgenic cells demonstrate homeostatic proliferation under lymphopenic condition (OT1, 2C, A18, and F5), whereas others (HY) do not (Fig. 1A and not depicted). For TCRs demonstrating homeostatic proliferation, increasing the dose of transferred cells resulted in a decrease in proliferation (Fig. 1A). Apparently, when numbers increase, T cells are signaled to slow or cease proliferation. This could be caused by the progressive consumption of a soluble factor present in limited supply, accumulation of an inhibitory factor, and/or by increasing competition for restricting MHC molecules/MHC-bearing cells.

To distinguish between these possibilities, we cotransferred CFSE-labeled OT1 and P14 cells into P14 recipients. OT1 and P14 TCRs differ in the isotype of the restricting MHC molecule. Initially, this experiment was prompted by the idea that a separate niche of peptide–MHC complexes might exist for each clone expressing a given TCR specificity. T cell proliferation within a given niche would be possible so long as the number of T cells occupying it remained low. T cells occupying different niches would not interfere with each other (15, 16). Indeed, Kb-restricted OT1 cells divided in mice that had normal numbers of Dp-restricted P14 cells, whereas cotransferred P14 cells did not divide (Fig. 1B). Because the only genetic difference between these two T cell populations is their TCR, proliferation of OT1 cells and lack of proliferation of P14 cells must be caused by a difference in TCR signaling. This differential ability to proliferate excludes the possibilities that proliferation is regulated exclusively by a factor becoming limiting, or the accumulation of an inhibitory factor acting on a receptor expressed by T cells or the APCs, as this should affect both populations. If the model proposing TCR-specific niches was correct, the complementary experiment should result in a similar outcome: P14 cells should divide in OT1 hosts. Surprisingly, with a preformed population of OT1 cells, neither P14 nor OT1 donor cells divided (Fig. 1C). Interestingly, both proliferated in an environment prefilled with F5 cells (Fig. 1D).

We interpret these results as showing that homeostatic proliferation is not a simple issue of TCR-specific niches being filled. This conclusion relies on the implicit assumption that the OT1 TCR, selected in the thymus on Kb molecules (19), does not cross-react with Db molecules. This will be addressed experimentally below. However, it is evident that there is a hierarchy in the ability of T cells to undergo homeostatic proliferation within the prefilled environment of a recipient differing in TCR specificity in the order OT1 > P14. Our finding is similar to the hierarchical competition described for OT1 and 2C; these TCRs are, however, both restricted to Kb (17). Our result is contrary to those reported by Troy and Shen despite using the same TCRs. However, these authors used TCR transgenic cells from Rag-proficient mice in which TCR-α chain rearrangement can occur (16).

We found a significant increase in the number of memory T cells (CD44hi), or recently activated CD62L+ T cells (not depicted), in OT1 mice compared with P14 or F5 mice (Fig. 2A). As overrepresentation of memory OT1 cells in the inoculum might favor the proliferation of OT1 cells in adoptive hosts, we also performed cotransfer experiments with purified naive OT1 cells (CD44-122-). However, this led to similar results; naive OT1 cells did not proliferate in OT1 recipients, but they proliferated in P14 mice (Fig. 2B). We also determined whether hierarchical competition could be observed between T cell populations that have similar numbers of naive and memory T cells, i.e., from P14 and F5 mice. We found that naive P14 cells (CD44-) divide in F5 recipients (Fig. 2C). However, as for the OT1 and P14 combination before, the reverse experiment, i.e., the transfer of F5 cells into P14 hosts, did not result in proliferation (Fig. 2C). Thus, we found a second case of hierarchical competitiveness.

Rag-2+/− recipients was also detected in further experiments (Fig. 2B, Fig. 3A, and Fig. 5, B and C). (C and D) Equal numbers of CFSE-labeled lymphocytes from OT1 Rag-2+/− Ly-5.1+ and P14 Rag-2+/− Ly-5.1+ mice were cotransferred into OT1 Rag-2+/− (C), F5 Rag-1+/− (D), or B6.Rag-2+/− (not depicted) recipients. For each time point, two mice per genotype were analyzed. A similar experiment, without the Ly-5 allotype marker, was also performed. A, B, and C and D were derived from independent experiments.
and extended the order to: OT1 > P14 > F5. The observed hierarchy is most likely based on the avidity of each TCR for its restricting MHC molecule loaded with self-/environmental peptides. Unfortunately, comparative affinity measurements that would confirm such a notion are not available. In fact, binding affinities have been determined for a limited number of TCR and MHC/peptide ligands, but the chosen peptides may not reflect the full complement of self-/environmental peptides presented by each MHC molecule as recognized by a given TCR (20, 21).

Hierarchical competitiveness across MHC isotype is not caused by TCR cross-reactivity

Because we observed that P14 cells, restricted to D\textsuperscript{b}, failed to divide in hosts that are prefilled with OT1 cells, nominally restricted to K\textsuperscript{b}, one might deduce that the OT1-TCR has cross-reactivity to D\textsuperscript{b}. This has recently been concluded using a different experimental setting (18). We decided to determine whether any presumed cross-reactivity of the OT1-TCR could account for the observed hierarchical competitiveness.

We first determined whether the OT1-TCR could be selected on a class I molecule other than K\textsuperscript{b} by breeding OT1 Rag-2\textsuperscript{-/-} mice onto the K\textsuperscript{b}-deficient allele (6, 22). In the absence of K\textsuperscript{b}, the OT1-TCR was not positively selected in the thymus and no CD8\textsuperscript{+} cells were present in the periphery (Fig. S1, A and B, available at http://www.jem.org/cgi/content/full/jem.20070467/DC1) (19). Thus, in the absence of K\textsuperscript{b} there is no positive selection of the OT1-TCR by other MHC isotypes present in H-2\textsuperscript{b} mice. Also, K\textsuperscript{b} is essential for OT1 proliferation in response to Ova257-264 (Fig. S1 C) (19).

Second, we determined whether division of OT1 cells in P14 recipients was mediated by K\textsuperscript{b}. To this end, we bred P14 K\textsuperscript{b}-/- Rag-2\textsuperscript{-/-} mice. These were then used as recipients for CFSE-labeled OT1 and P14 donor cells. 2 wk after transfer, OT1 cells showed only limited division in K\textsuperscript{b}-deficient P14 recipients compared with K\textsuperscript{b}-sufficient P14 recipients (Fig. 3 A).

Third, we wanted to determine whether the competence of OT1 cells to block division of P14 cells resides in the ability of the OT1-TCR to bind to K\textsuperscript{b}. P14 cells proliferated in OT1 Rag-2\textsuperscript{-/-} K\textsuperscript{b}-deficient recipients when these recipients had previously been transplanted with thymi from K\textsuperscript{b}-sufficient B6.Rag-2\textsuperscript{-/-} embryos to allow for the reconstitution of CD8\textsuperscript{+} cells expressing the OT1-TCR (Fig. 3 B, right).

Figure 2.  Hierarchical competitiveness in homeostatic proliferation of naive OT1, P14, and F5 T lymphocytes. (A) Total lymphocyte numbers in the same pool of lymph nodes and from spleen of individual animals of the indicated genotype were determined. The proportion of CD8\textsuperscript{+} TCR-β+ or CD8\textsuperscript{+} CD44+ TCR-β+ cells was determined by FACS. Individual animals are each indicated by a symbol; the horizontal bar indicates the arithmetic mean. (B) Sorted CD44\textsuperscript{+}122\textsuperscript{-} naive T cells, or unseparated cells, from OT1 Rag-2\textsuperscript{-/-} mice were mixed with cells from P14 Rag-2\textsuperscript{-/-} mice, labeled with CFSE, and cotransferred into OT1 Rag-2\textsuperscript{-/-}, P14 Rag-2\textsuperscript{-/-}, or B6.Rag-2\textsuperscript{-/-} recipients. Lymphocytes were analyzed 3 wk after transfer as previously described in Fig. 1. Two individual P14 Rag-2\textsuperscript{-/-} recipients were analyzed for each of the donor cell mixtures. *, there is a predominant CFSE-negative population within the gate of OT1 T cells that is host derived in OT1 recipients (shaded area) that was excluded from the calculation of percentages. (C) Sorted CD44\textsuperscript{+} naive T cells from P14 Rag-2\textsuperscript{-/-} Ly-5.1+ and F5 Rag-1\textsuperscript{-/-} mice were labeled with CFSE and cotransferred into P14 Rag-2\textsuperscript{-/-}, F5 Rag-1\textsuperscript{-/-}, or B6.Rag-2\textsuperscript{-/-} recipients. Analysis was performed after 2 (not depicted) and 4 wk after transfer as previously described in Fig. 1. *, there is a predominant CFSE-negative population within the gate of F5 cells that is host derived in F5 recipients.
We conclude from these experiments that the ability of OT1 cells to proliferate in P14 recipients and the ability of OT1 cells to block homeostatic proliferation of P14 cells in OT1 recipients are both mediated by OT1-TCR binding to K\(^b\) molecules. Thus, there is no functional cross-reactivity of the OT1-TCR to other MHC isotypes that could account for its observed hierarchical competitiveness.

**Hierarchical competitiveness operates beyond competition for the MHC-bearing cell**

Two hypotheses could explain the data so far. T cells could compete for the MHC-bearing cell, either by competition for physical access or by modulation of the ability of this cell to stimulate homeostatic proliferation. Alternatively, T cells could derive a graded ability to compete for some (diffusible) ligands, whereas they transit through the peripheral lymphoid organs (23). These ligands would be available in limited supply and would not be bound to the APCs; the level of competitiveness for these ligands would be determined by the affinity of the TCR toward restricting MHC molecules. It would be possible to differentiate these possibilities by creating a situation where T cells of “higher” hierarchy could not interact with a proportion of the MHC-bearing cells. To generate a balanced situation in which only some APCs express K\(^b\), we performed parabiosis between K\(^b\)-mutant or control K\(^b\)-sufficient mice and OT1 mice. After several weeks of parabiosis, CFSE-labeled OT1 and P14 cells were transferred into the animals. Interestingly, P14 donor cells did not divide more in K\(^b\)-mutant and OT1 parabiosis pairs compared with control K\(^b\)-sufficient and OT1 parabiosis pairs (Fig. 4). Thus, P14 cells are not able to obtain sufficient signals to allow for their division, even in the presence of K\(^b\)-deficient APCs that OT1 cells cannot interact with (Fig. S2, available at http://www.jem.org/cgi/content/full/jem.20070467/DC1). Similar parabiosis experiments with K\(^b\)-sufficient, K\(^b\)-mutant, and OT1 mice were also performed, resulting in identical outcomes (unpublished data).

To determine which factors may rule the level of competitiveness downstream of the TCR, we analyzed cytokine receptor expression before and after transfer. No differences were detected before transfer (Fig. S3, available at http://www.jem.org/cgi/content/full/jem.20070467/DC1). Upon transfer into a lymphopenic environment, IL-7R\(\alpha\) and -2R\(\beta\) receptor expression was similar for OT1 and P14 cells (Fig. 5, A and B). However, we observed differences between OT1 cells proliferating in T cell–proficient P14 recipients and OT1 or P14 cells undergoing lymphopenia–induced proliferation in immunodeficient recipients in respect to IL-7R\(\alpha\) down-modulation and gradual increase in IL-2R\(\beta\) expression (Fig. 5, A and B). Thus, the mechanism of OT1 cell dominance over P14 cells is different to the lymphopenia induced proliferation of both TCR specificities in lymphopenic Rag-2\(^-/-\) recipients. Proliferation in the latter may be lymphokine-only driven, whereas in P14 recipients, lymphokines may not be as abundant and TCR signaling is, as shown above, essential for OT1 cells to dominate over P14 cells. Indeed, we also observed reduced CD5 expression on OT1 cells in P14 Rag-2\(^-/-\)K\(^b\)-/- versus P14 Rag-2\(^-/-\) recipients (unpublished data).

To determine if other molecules were regulating this clonal competition beyond IL-7 (24, 25), we treated recipient mice with anti–IL-7R\(\alpha\) antibodies: OT1 cell proliferation in P14 mice was not affected (Fig. 5 C). This does not exclude that OT1 cells act as a “sink” for IL-7, thereby hindering P14 cell proliferation in OT1 recipients. Unobstructed proliferation of OT1 cells despite IL-7R blockage appears to contradict studies involving IL-7-deficient recipient mice. In contrast to these results, however, our experiments lasted longer (day 19 vs. day 5 or 7) and another study, using similar IL-7R blockage, showed limited OT1 cell proliferation on day 6, which was further diminished when recipient mice were IL-15 deficient (26). Thus, other cytokines may have allowed for later homeostatic proliferation in our experiments.

Collectively, our results suggest that competition based on TCR triggering is mediated by ILs, such as IL-7, -2, and/or -15 as their receptor expression is modulated, yet other soluble factors (e.g., trophic factors) probably play a role and remain to be characterized (23).

**Concluding remarks**

Several important conclusions can be made on basis of our results. First, hierarchical competitiveness operates beyond competition for MHC molecules. Thus OT1 cells, whose TCR is restricted by K\(^b\), hinder the homeostatic proliferation of P14 cells, restricted to D\(^\beta\). A similar conclusion had been made based on the finding of hierarchical competitiveness among OT1 and 2C-TCRs (17). However, even though the antigenic peptides recognized by these K\(^b\)-restricted TCRs are distinct, one does not know whether they compete for overlapping niches of self-peptide–MHC molecules. Based on our results, we can formally exclude such a mechanism of competition for the OT1 and P14-TCRs because in the absence of K\(^b\) expression the hierarchical dominance of OT1 cells is no longer seen.

Second, we demonstrate that hierarchical competitiveness is not caused by the (simple) model of cellular competition for the MHC-bearing cell, or alternatively, by modulation of the stimulating ability of this cell. This conclusion is derived from our experiments using parabiotic pairs of K\(^b\)-deficient and OT1 mice as recipients for OT1 and P14 cells. Even though in such parabiotic pairs OT1 cells cannot interact with K\(^b\)-deficient APCs, representing half of the APCs in the system, these cells were not able to stimulate homeostatic proliferation of P14 cells. Thus, OT1 cells were still able to dominate P14 cells in trans. Therefore, TCR binding to restricting MHC molecules appears only as a primary requirement for homeostatic competitiveness. Here, the TCRs’ affinity toward self-MHC molecules, as well as the kinetics of DC interactions, while T cells migrate through the lymphoid organs, would result in a TCR-characteristic level of signaling within the lymphocyte. The overall signal received in this way, however, would only define the cells’ relative ability to
Figure 3. In the absence of restricting Kéro molecules, OT1 cells cannot compete successfully, resulting in homeostatic proliferation of P14 cells. (A) CFSE-labeled lymphocytes from OT1 Rag-2^{-/-} and P14 Rag-2^{-/-} mice were cotransferred into P14 Rag-2^{-/-}, P14 Rag-2^{-/-} Kéro^{-/-}, and OT1 Rag-2^{-/-}, B6.Rag-2^{-/-}, or Kéro^{-/-} Rag-2^{-/-} (not depicted) recipients. Lymphocytes were recovered after 15 d and analyzed as in Fig. 1. * The prominent population of recipient P14 cells (shaded) was excluded from the calculation of percentages of cells that had not divided or divided 1–4 times. Results presented in Fig. 5 (A and B) were obtained from the same mice. Additional experiments comparing P14 Rag-2^{-/-} (n = 3), P14 Rag-2^{-/-} Kéro^{-/-} (n = 3), OT1 Rag-2^{-/-}
secondarily compete for trophic ligands essential for homeostatic proliferation, which are available in limited supply and not bound to the stimulating APC.

**MATERIALS AND METHODS**

**Mice.** B6.Rag2tm1Alt (Rag-2\(^{-/-}\)) mice were a gift from A. Rolink (Basel Institute for Immunology, Basel, Switzerland). B6.Rag2tm1Alt- ptprc a (Rag-2\(^{-/-}\)) (n = 2), and B6.Rag-2\(^{-/-}\) (n = 2) recipients analyzed donor cell division 2 and 4 wk after transfer. (B) CFSE-labeled lymphocytes from OT1 Rag-2\(^{-/-}\) and P14 Rag-2\(^{-/-}\) mice were cotransferred into OT1 Rag-2\(^{-/-}\)K\(^{b+}\) heterozygote or OT1 Rag-2\(^{-/-}\)K\(^{b+}\) homozygote recipients. Of the latter, some mice had earlier (1 mo) received 5–10 fetal (day 14.5) thymus lobes of K\(^{b+}\) sufficient origin (B6.Rag-2\(^{-/-}\)) to allow for the reconstitution of CD8\(^{+}\) peripheral T cells. As judged upon analysis, reconstitution of host CD8\(^{+}\) OT1 cell numbers was up to >70% compared with host CD8\(^{+}\) OT1 cell numbers observed in K\(^{b+}\) sufficient OT1 Rag-2\(^{-/-}\) recipients; the accumulation of host OT1 cells in thymus grafted OT1 Rag-2\(^{-/-}\) recipients reduces the relative proportion of donor P14 and OT1 cells (identified as CFSE\(^{+}\) cells) to <1%, similar to their proportion in OT1 Rag-2\(^{-/-}\)K\(^{b+}\) recipients, compared with 38% in nongrafted recipients. Histograms show the CFSE profile of OT1 and P14 donor cells that were gated for as shown in the dot-plots. Mice were analyzed 14 d after cell transfer (for each group n = 2). A similar experiment was performed using K\(^{m+}\)Rag-2\(^{-/-}\) mice in place of K\(^{b+}\) Rag-2\(^{-/-}\) mice (in the same order as above: n = 7, 3, 2 parabiotic pairs, respectively).

**Figure 4.** Homeostatic dominance of OT1 cells operates beyond the MHC-bearing cell, indicating a graded, TCR-based competition for a limiting ligand not bound to APCs. The following parabiotic couples of mice were made: [OT1 Rag-2\(^{-/-}\) and K\(^{b+}\) Rag-2\(^{-/-}\)] (n = 5), [OT1 Rag-2\(^{-/-}\) and K\(^{m+}\) B6.Rag-2\(^{-/-}\)] (n = 2), and [K\(^{b+}\) B6.Rag-2\(^{-/-}\) and K\(^{b+}\) Rag-2\(^{-/-}\)] (n = 2). 3 wk after surgery, CFSE-labeled lymphocytes from OT1 Rag-2\(^{-/-}\) and P14 Rag-2\(^{-/-}\) mice were cotransferred into the parabions. Analyses shown were performed 14 d later. Detailed analysis of donor T cell and host DC exchange for individual pairs of parabiotic mice is shown in Fig. S2. All parabiotic couples were prepared at the same time and were injected with the same mixture of donor cells. The experiment was repeated using K\(^{m+}\)Rag-2\(^{-/-}\) mice in place of K\(^{b+}\) Rag-2\(^{-/-}\) mice (in the same order as above: n = 7, 3, 2 parabiotic pairs, respectively).
Ly-5.1 mice to obtain TCR transgenic Rag-2−/−Ly-5.2 or TCR transgenic Rag-2−/−Ly-5.1/2 mice. Kbm1 Rag-2−/− and Kbm2 Rag-2−/− mice were derived by breeding the MHC donor strain (B6.C-H2Kbm1 [Jax] or B6.129P2-H2Kbm1 [6]) to Rag-2−/− mice. Likewise, Kbm1 mutant OT1 or P14 Rag-2−/− mice were obtained.

Parabiosis of 4–10-wk-old sex-matched mice was performed as previously described (30). For thymus transplantation, fetal B6.Rag-2−/− thymus lobes were isolated at day 14.5–15.5 of gestation, and 5–10 lobes were transplanted under the kidney capsule of the indicated recipients. Studies on animals were approved by the Regierungspräsidium Freiburg (regional council of the federal state of Baden-Württemberg, Germany) and the Comité Local des Animaleries Institut National de la Santé et de la Recherche Médicale/CEA with the approval of the Direction Départementale des Services Vétérinaire, Grenoble (local committee and regional representative of the Ministry of Agriculture, France).

**FACS analysis.** Single-cell suspensions of pooled lymph node and spleen (RBCs lysed) were each prepared in PBS with 2% FCS. Antibodies were purchased commercially (BD) or prepared by protein G affinity chromatography of tissue culture supernatants, followed by labeling with fluorochrome or biotin: 104.2.1 (Ly-5.2), A20-1.7 (Ly-5.1), F23.1 (TCR-Vβ), MR9-4 (TCR-β), R20.1 (TCR-β), B20.1 (CD24), M1/69 (CD19), M1/70 (CD11b), A7R34 (CD127), PECy5 (CD5), CD5 (BD), and CD69 (BD). SF1 ascites (Kbm1 specific, not-reactive to Kbm2) was a gift of Nathenson (Albert Einstein College of Medicine, Bronx, NY). Biotinylated mAbs were revealed with streptavidin-allophycocyanin (Innogenit), -PE (SouthernBiotech) or -PerCP (BD); others with sheep F(ab)2, anti-mouse Ig-FITC (Silenus) or R33-18 (mouse IgG1). As applicable, cells were incubated with tissue culture supernatant of 2.4G2 (CD16/32) hybridoma cells to block unspecified staining. Cells were stained with mAbs or 2nd reagents at predetermined optimal dilution. Flow cytometry was done on FACSCalibur and LSRII (BD) instruments, using CellQuest, Diva (BD), or FlowJo (FlowJo) software. Except where indicated, data shown are from lymph node cells. However, cells from spleen always gave similar results.

**CFSE labeling and adoptive transfers.** Cells were obtained from pooled lymph nodes, washed into PBS, and labeled with CFSE (5 μM CFDA-[SE]; Invitrogen) for 8 min at room temperature while mixing. FCS was added to 20% incubating for an additional 2 min, and cells were washed into PBS. Except where noted, 1–5 × 10^6 cells were transferred into various 6–15-wk-old recipient mice i.v.

To block IL-7 cytokine signaling, mice were given multiple injections of blocking antibodies specific for the IL-7Rα chain (A7R34, CD127) or isotype-matched control antibodies i.p. (Fig. S4, available at http://www.jem.org/cgi/content/full/jem.20070467/DC1).

**Online supplemental material.** Fig. S1 shows lack of positive selection of OT1 cells in the absence of Kbm. Fig. S2 shows unobstructed donor T cell exchange in OT1 Rag-2−/− and Kbm1 Rag-2−/− parabiotic couples, as well as DC chimerism for one exemplary couple. Fig. S3 shows CD127, CD25, CD5, and CD69 expression on CD8+ lymph node cells from B6, P14 Rag-2−/−, P14 Rag-2−/− Kbm1, and OT1 Rag-2−/− mice. Fig. S4 shows effective blocking of IL-7 cytokine signaling by injecting antibodies specific for the IL-7Rα chain, resulting in >10-fold decreased thymocyte numbers and >30-fold reduction in CD19+ pre−/−pro-B cell percentages in the bone marrow. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20070467/DC1.

We thank Y. Joos for expert technical assistance; G. Besin and G. Turchinovich for breeding Kbm1 Rag-2−/− mice and FACS; Dr. Nathenson for SF1 ascites; M. Dessing, T. Kline, H. Kohler, S. Meyer, and A. Würch for help with flow cytometry; the animal
care takers for animal husbandry; H.-P. Stahlberger, B. Pfeiffer, and H. Spalinger for art work/photography; F. McBlane, R. Ceredig, H. Jacob, and D. van Essen for critical reading of the manuscript; and all people from Bill for the pleasurable time there. F. A. is greatly indebted to Dr. R.-P. Sekaly for hosting him at Institut National de la Sante et de la Recherche Medicale U743. The Basel Institute for Immunology was founded and supported by F. Hoffmann-La Roche Ltd, Basel, Switzerland.

The authors declare that they have no conflicting financial interests.

Submitted: 6 March 2007
Accepted: 17 October 2008

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