Essential Roles of $CD8^+CD122^+$ Regulatory T Cells in the Maintenance of T Cell Homeostasis

Muhaimin Rifa'i,[1,2] Yoshiyuki Kawamoto,[1] Izumi Nakashima,[1] and Haruhiko Suzuki[1]

[1]*Department of Immunology, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan*
[2]*Brawijaya University, Malang 65145, East Java, Indonesia*

Abstract

Regulation of immune system is of paramount importance to prevent immune attacks against self-components. Mice deficient in the interleukin (IL)-2/IL-15 receptor β chain, CD122, are model animals of such immune attacks and characteristically have a high number of abnormally activated T cells. Here, we show that the transfer of $CD8^+CD122^+$ cells into CD122-deficient neonates totally prevented the development of abnormal T cells. Furthermore, recombination activating gene–$2^{-/-}$ mice that received wild-type mice–derived $CD8^+CD122^-$ cells died within 10 wk after cell transfer, indicating that normal $CD8^+CD122^-$ cells become dangerously activated T cells in the absence of $CD8^+CD122^+$ T cells. $CD8^+CD122^+$ cells could control activated $CD8^+$ or $CD4^+$ T cells both in vivo and in vitro. Our results indicate that the $CD8^+CD122^+$ population includes naturally occurring $CD8^+$ regulatory T cells that control potentially dangerous T cells.

Key words: immune regulation • $CD8^+$ T cells • CD122 • T cell control • activated T cells

Introduction

Immune reaction against virulent foreign objects could be harmful for self-organisms. Therefore, a strict regulatory mechanism must exist in the immune system (1–6). Regulatory T cells are key subpopulations of T cells that control immune activity. $CD4^+CD25^+$ (IL-2 receptor α chain) regulatory T cells are a well-documented subpopulation of T cells (1, 2, 7–9). The importance of $CD4^+CD25^+$ regulatory T cells has been confirmed in many types of autoimmune diseases. However, other cells, especially those of $CD8^+$ T cell lineage, have also been suggested as regulatory T cells (5, 10, 11).

CD122 (IL-2/IL-15 receptor β chain)-deficient mice exhibit severe hyperimmunity (12) with augmented granulopoiesis and suppressed erythropoiesis. Severe anemia progresses, and the mice die within 3–4 mo after birth. We found that expansion of abnormally activated T cells is the underlying cause of all phenotypes (13). The mechanism of generation of activated memory-type T cells in CD122-deficient mice is controversial. In the initial investigations by Abbas and others (14–16), the defect of programmed cell death (apoptosis) in IL-2 or IL-2Rα–deficient T cells could be applied to the mechanism for survival of activated CD122-deficient T cells, and such a possibility has not been excluded. Based on our previous experimental results, we proposed that the lack of certain regulatory cells was responsible for the abnormal phenotypes and that a subpopulation of $CD8^+$ T cells could be involved in the control of CD122-deficient abnormal T cells (11). However, in our previous paper, direct evidence of the responsible subpopulation in $CD8^+$ cells was not provided, and it was still unclear whether the lack of regulatory system is sufficient to cause T cell abnormalities in CD122-deficient mice, or whether intrinsic defect of CD122 molecules in T cells is required.

Here, we report a novel observation that the transfer of highly purified $CD8^+CD122^+$ T cells from normal mice into CD122-deficient neonates resulted in normalization of all the phenotypes in these mice. This result indicates that the $CD8^+CD122^+$ T cell population contains novel regulatory T cells that effectively control CD122-deficient T cells. In addition, we show that RAG-$2^{-/-}$ mice that received wild-type mice–derived $CD8^+CD122^-$ cells die within 10 wk after cell transfer, indicating that normal $CD8^+CD122^-$ cells without intrinsic defect of CD122 molecules become dangerously activated T cells in the absence

Address correspondence to Haruhiko Suzuki, Dept. of Immunology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan. Phone: 81-52-744-2135; Fax: 81-52-744-2972; email: k46200a@nucc.cc.nagoya-u.ac.jp

Abbreviation used in this paper: HPRT, hypoxanthine-guanine phosphoribosyltransferase.

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of $CD8^+CD122^+$ T cells, which control not only CD122-deficient T cells but also wild-type T cells.

Materials and Methods

Mice. CD122-deficient mice were originally established by Suzuki et al. (12) and maintained in our animal facility. Breeding pairs of the $C57BL/6^{CD45.1/CD45.1}$ congenic strain were obtained from Taconic and maintained in our animal facility. RAG-2–deficient mice with C57BL/6 genetic background were obtained from The Central Institute for Experimental Animals with permission from the founder F.W. Alt (Harvard Medical School) and maintained in our animal facility. OT-I transgenic mice bearing $H\text{-}2K^b$-restricted OVA-specific TCR (17) were gifts from T. Hirano and M. Murakami (Osaka University, Suita, Japan) The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Nagoya University Graduate School of Medicine.

T Cell Clones. OVA-specific helper-type T cell clones, 35-9D and 35-8H, were gifts from T. Kato and K. Kuribayashi (Mie University School of Medicine, Tsu, Japan; reference 18, 19).

Antibodies. FITC-conjugated anti–mouse CD8α (clone 53-6.7), PE-, or allophycocyanin-conjugated anti–mouse CD4 (clone GK1.5); biotin-conjugated anti–mouse CD122 (clone 5H4); FITC-conjugated anti–mouse CD25 (clone PC61.5); FITC- or biotin-conjugated anti–mouse CD45.1 (clone A20); and biotin-conjugated anti–mouse TER-119 (clone TER-119) antibodies were purchased from eBioscience. FITC-conjugated anti–mouse CD69 (clone H1.2F3), FITC-conjugated anti–mouse CD44 (clone IM7), PE-conjugated anti–mouse CD62L (clone MEL-14), FITC-conjugated anti–mouse Gr-1 (clone RB6-8C5), and PE-conjugated anti–mouse VLA-4 (clone 9C10) antibodies were purchased from BD Biosciences. Biotin-conjugated antibodies were visualized by streptavidin-PE-Cy5 (eBioscience).

Flow Cytometry and Cell Sorting. Analytical flow cytometry was performed using FACS Calibur flow cytometer (BD Biosciences). Preparative cell sorting was performed using FACS Vantage cell sorter (BD Biosciences).

Adoptive T Cell Transfer to Neonates. T cells were hypodermically transferred into 2–3-d-old neonates offspring of CD122-heterozygous mutant parents. After the neonates grew beyond 3 wk, their genotype for CD122 gene was analyzed by PCR from tail DNA (11).

Adoptive T Cell Transfer to $RAG\text{-}2^{-/-}$ Mice. T cells were intravenously transferred into 8–10-wk-old RAG-2–deficient mice that had received a sublethal dose (5.5 Gy) of irradiation.

Hematocrit. Peripheral blood was taken from mice tail vein using heparin-coated capillary tubes. After one end of the capillary was sealed with clay, it was centrifuged, and the length of packed RBC per total blood was measured.

Intracellular Cytokine Staining. Intracellular cytokine staining was performed using a Cytofix/Cytoperm kit (BD Biosciences) according to the protocol provided by the manufacturer. Cells were incubated with biotin-conjugated anti–IFN-γ or anti–IL-2 antibody and visualized with streptavidin-PE-Cy5.

Real-Time RT-PCR. Total cellular RNA was extracted by using RNA-bee reagent (Tel-Test Inc.). The total amount of RNA was reverse transcribed using Superscript II reverse-transcriptase and random hexamer primers (Invitrogen). IFN-γ, TNF-α, and hypoxanthine-guanine phosphoribosyltransferase (HPRT) mRNA levels were quantified by real-time PCR using the ABI/PRISM 7700 sequence detection system (Applied Biosystems). Analyses were performed using primers, an internal fluorescent TaqMan probe specific to IFN-γ, TNF-α, or HPRT, and the TaqMan Universal PCR Master Mix (Applied Biosystems). Premixture of primers and TaqMan probe of IFN-γ and TNF-α were optimized for real-time PCR system by the manufacturers. Standard curves of cDNA from total $CD8^+$ T cells and $CD8^+CD122^-$ T cells were used to calibrate the threshold cycle to relative quantities of HPRT, TNF-α, and IFN-γ cDNA.

Results

Age-related Distribution of $CD8^+CD122^+$ Cells in Wild-Type Mice. In our previous paper, we found that the lack of regulatory T cells is the cause of abnormal phenotype in CD122-deficient mice (11). In the experiment of transfer of a mixture of wild-type T cells and CD122-deficient T cells, wild-type $CD8^+$ T cells more effectively suppressed the abnormal activation of CD122-deficient T cells than wild-type $CD4^+$ T cells (11). Based on this observation, we hypothesized that the regulatory T cells that control $CD122^-$ cells

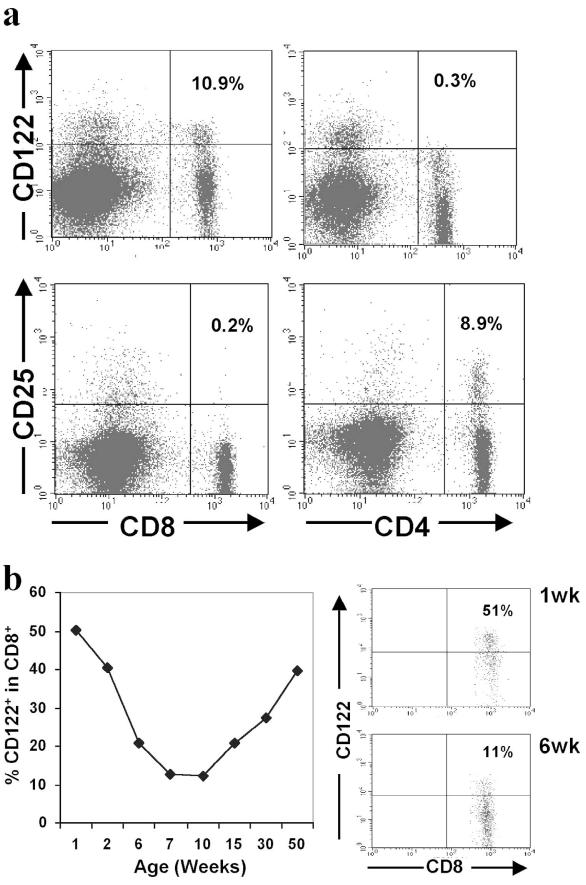

Figure 1. $CD122^+$ cells are more abundant in $CD8^+$ T cells than in $CD4^+$ T cells in wild-type mice spleen. (a) Spleen cells were obtained from 7-wk-old wild-type C57BL/6 mice, stained with indicated fluorescence-conjugated antibodies, and analyzed by flow cytometry. Percentages of $CD122^+$ or $CD25^+$ cells in $CD8^+$ or in $CD4^+$ T cells are shown in each panel. (b) Spleen cells were taken from mice of various ages, and the percentages of $CD122^+$ cells in $CD8^+$ cells were analyzed as in a. Data are averages of two mice at each age. (right) Representative results of flow cytometric analysis obtained from a 1-wk-old mouse, which shows >50% of $CD122^+$ cells in the $CD8^+$ population, and that obtained from a 6-wk-old mouse.

are in the $CD8^+$ T cell population. Spleens (Fig. 1 a) and lymph nodes (not depicted) contain $CD122^+$ cells in the $CD8^+$ population. Because the expression level of CD122 fluctuates continuously among T cells both in $CD8^+$ and $CD4^+$, it may be difficult to distinguish $CD122^+$ from $CD122^-$. However, a population in $CD8^+$ cells expresses obviously high levels of CD122, the same expression level of which is not observed in $CD4^+$ T cells (Fig. 1 a). Therefore, though some $CD4^+$ cells are actually $CD122^{low+}$, we call the cells forming a population with a high CD122 expression level $CD122^+$ cells. The percentage of $CD122^+$ cells in the $CD8^+$ population varied between 10 and 50% depending on mouse age (Fig. 1 b). Generally, the percentage of $CD122^+$ cells in $CD8^+$ population was high in very young mice, decreased to the lowest level of ~10% at 7–10 wk of age, and increased in older mice. In contrast with the considerable proportion of $CD122^+$ cells in the $CD8^+$ population, $CD122^+$ cells were rare in $CD4^+$ population (Fig. 1 a, top) although $CD4^+$ cells contain $CD122^{low+}$ cells, many of which are $CD25^+$ (not depicted). This is in contrast with the fact that $CD25^+$ cells exist in the $CD4^+$ population but not in the $CD8^+$ population (Fig. 1 a, bottom).

Isolation of $CD8^+CD122^+$ Cells and Their Transfer to CD122-deficient Neonates. $CD8^+CD122^+$, $CD8^+CD122^-$, and $CD4^+CD25^+$ cells were isolated from spleens and lymph nodes of 6-wk-old CD45.1 congeneic C57BL/6 mice by cell sorting. Fig. 2 a demonstrates that the purity of specific cell population after cell sorting was >98%. Next, 5×10^4 of these highly purified cells were subcutaneously transferred into CD122-deficient neonates, and the cells obtained from spleens or lymph nodes were analyzed 7 wk later. No donor-type $CD4^+$ T cells were observed at 7 wk after transfer of $CD8^+$ T cells, indicating that phenotypic changes, if any, should not be due to contaminated $CD4^+$ T cells (Fig. 2 b). Donor-type $CD8^+$ T cells were also very few in $CD4^+$ T cell–transferred mice (Fig. 2 b). Analysis of the absolute numbers of each T cell population, derived either from host CD122-deficient mice or transferred wild-type donor cells, showed that transferred donor-type cells, especially $CD8^+$ cells, expanded >20 times (from 5×10^4 to $>10^6$) during the 7 wk (Fig. 2 c). Furthermore, analysis of CD122-deficient neonates after transfer of 10^4 $CD8^+CD122^+$ cells derived from 2-wk-old or 2-yr-old mice showed that the resultant donor-type T cells 7 wk af-

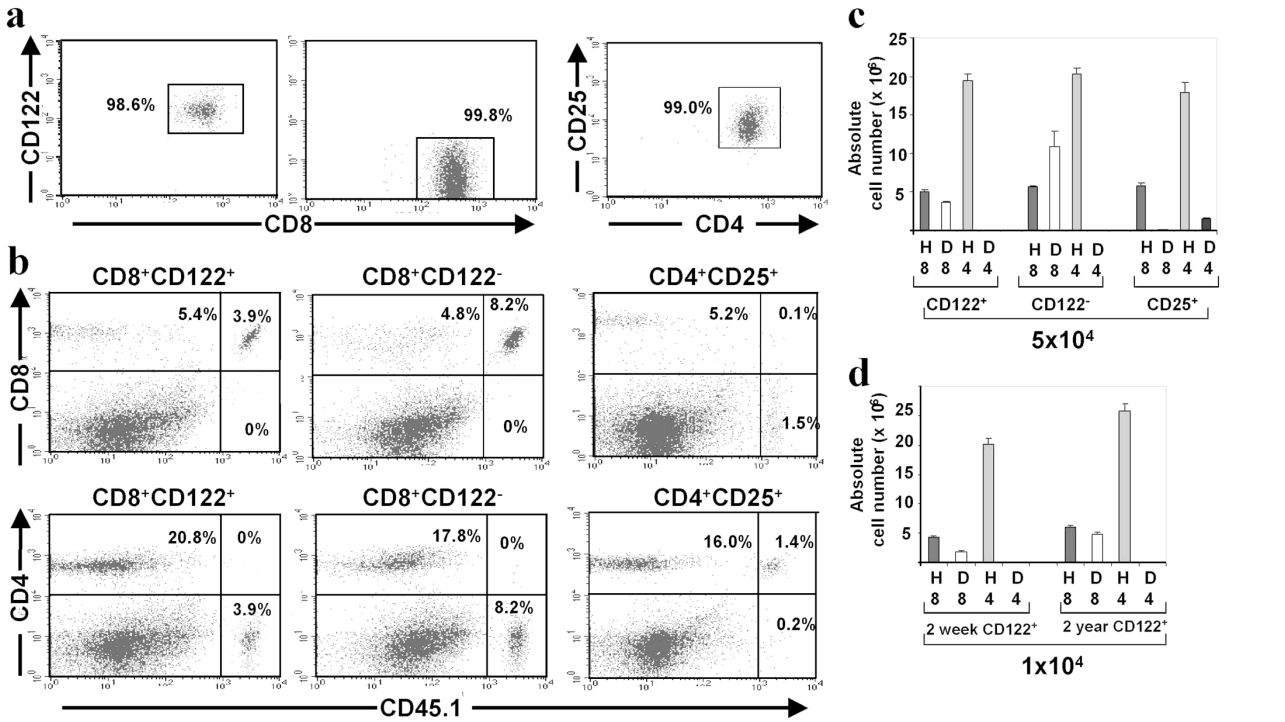

Figure 2. Highly purified T cell subpopulation and successful adoptive transfer to CD122-deficient neonates. (a) Spleen cells obtained from 6-wk-old CD45.1 congeneic C57BL/6 mice were stained with anti-CD8 antibody and anti-CD122 antibody, or were stained with anti-CD25 antibody and anti-CD4 antibody. $CD8^+CD122^+$ T cells, $CD8^+CD122^-$ T cells, and $CD4^+CD25^+$ cells were collected by using a cell sorter, and recovered cells were analyzed by flow cytometry. Percentages of purity for required cells are shown in each panel. (b) 5×10^4 each of purified $CD8^+CD122^+$, $CD8^+CD122^-$, or $CD4^+CD25^+$ T cells were transferred into 2–3-d-old CD122-deficient neonates. 7 wk later, spleen cells were stained with the indicated antibodies and analyzed by flow cytometry. Numbers in each quadrant indicate percentages of cells. (c) Absolute numbers of each T cell subpopulation derived from host CD122-deficient mice and donor wild-type mice (H8: host-derived $CD8^+$ cells; D8: donor-derived $CD8^+$ cells; H4: host-derived $CD4^+$ cells; and D4: host-derived $CD4^+$ cells). Absolute numbers of each T cell subpopulation were calculated by (absolute number of spleen cells) × (percentage of T cell subpopulation determined by FACS analysis). Data are mean ± SD values of three mice in each group. (d) 10^4 each of purified $CD8^+CD122^+$ T cells obtained from 2-wk-old or 2-yr-old CD45.1 congeneic C57BL/6 mice were transferred into 2–3-d-old CD122-deficient neonates. 7 wk later, spleen cells were analyzed. Absolute numbers of each T cell subpopulation derived from host mice and donor mice are shown. Data are mean ± SD values of three mice in each group.

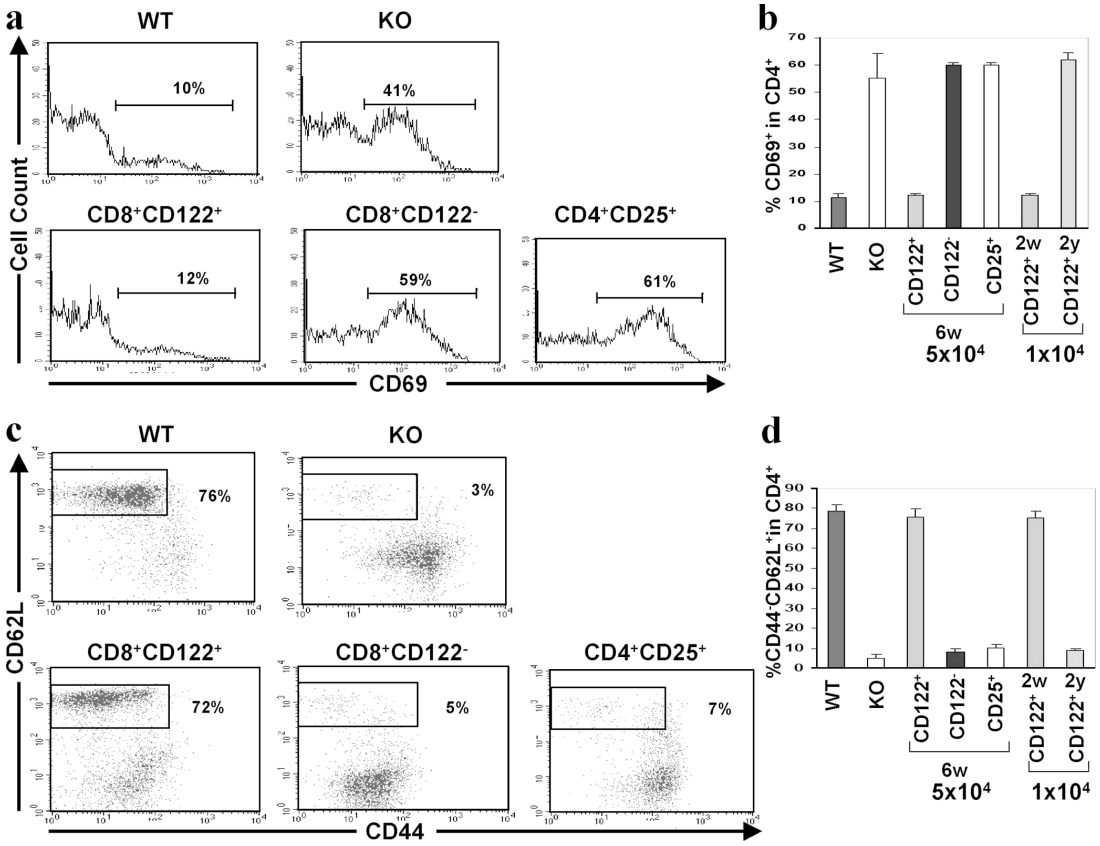

Figure 3. CD122-deficient mice that had received $CD8^+CD122^+$ T cells at neonatal stage do not develop activated memory-type T cells. (a) After 7 wk from the transfer of 5×10^4 of indicated T cell subpopulations into CD122-deficient neonates, spleen cells obtained from the mice were stained with anti-CD69, anti-CD4, and anti-CD45.1 antibodies, and analyzed by flow cytometry. Expression levels of CD69 are shown as histograms for cells gated to $CD4^+CD45.1^-$ population. Numbers in each panel are percentages of $CD69^+$ cells in gated $CD4^+$ cells. Data obtained from a 7-wk-old wild-type (WT) mouse and a 7-wk-old CD122-deficient mouse (KO) are also shown as controls. (b) Percentages of $CD69^+$ cells in gated $CD4^+$ cells. Data are mean ± SD values of three mice in each group. Data obtained from CD122-deficient mice that received 10^4 $CD8^+CD122^+$ cells obtained from 2-wk-old mice or 2-yr-old mice are also shown. (c) After 7 wk from the transfer of 5×10^4 of indicated T cell subpopulations, spleen cells were stained with anti-CD44, anti-CD62L, anti-CD45.1, and anti-CD4 antibodies, and analyzed by flow cytometry. Data are shown as dot-plot analysis for cells gated to $CD4^+CD45.1^-$ population. Numbers in each panel are percentages of naive-type $CD44^-CD62L^+$ cells in gated $CD4^+$ cells. Data obtained from a 7-wk-old wild-type (WT) mouse and a 7-wk-old CD122-deficient mouse (KO) are also shown as controls. (d) Percentages of naive-type $CD44^-CD62L^+$ cells in gated $CD4^+$ cells. Data are mean ± SD values of three mice in each group. Data obtained from CD122-deficient mice that received 10^4 $CD8^+CD122^+$ cells obtained from 2-wk-old mice or 2 yr-old mice are also shown.

ter transfer were mostly $CD8^+$ cells and had expanded >50 times (from 10^4 to $>10^6$) during the 7 wk (Fig. 2 d).

$CD8^+CD122^+$ Cells Prevent Expansion of CD122-deficient Activated T Cells. As shown in Fig. 3 (a and c, top middle), CD122-deficient mice without any treatment had a high percentage of activated memory-type T cells with markers of $CD69^+$, $CD44^+$, and $CD62L^-$. In contrast, CD122-deficient neonatal mice that received 5×10^4 $CD8^+CD122^+$ cells showed no such increase of activated $CD69^+$ T cells (Fig. 3 a, bottom left) and $CD44^+CD62L^-$ activated memory-type T cells (Fig. 3 c, bottom left). Transfer of 5×10^4 $CD8^+CD122^-$ T cells into CD122-deficient neonates did not prevent the development of activated $CD69^+$ T cells (Fig. 3 a, bottom middle) and $CD44^+CD62L^-$ memory-type T cells (Fig. 3 c, bottom middle). Transfer of 5×10^4 $CD4^+CD25^+$ T cells also did not alter the abnormal phenotype of $CD69^+$ T cells (Fig. 3 a, bottom right) and $CD44^+CD62L^-$ T cells (Fig. 3 c, bottom right) in CD122-deficient mice, although transfer of a larger number (2×10^5) of $CD4^+CD25^+$ T cells into neonates normalized all the phenotypes in CD122-deficient mice (not depicted). We also transferred 10^4 $CD8^+$ $CD122^+$ cells obtained from 2-wk-old or 2-yr-old mice into CD122-deficient neonates. This number of $CD8^+$ $CD122^+$ cells from 2-wk-old mice effectively normalized the shift to activated memory phenotype of T cells in CD122-deficient mice, whereas the same number of cells from 2-yr-old mice did not have such activity (Fig. 3, b and d). These results indicate that at least 10^4 of $CD8^+CD122^+$ cells in young mice are sufficient to perform regulatory activity when transferred at neonatal stage, but the same number of $CD8^+CD122^+$ cells in older mice does not contain a sufficient number of regulatory T cells. We analyzed $CD4^+$ T cells and $CD8^+$ T cells separately, and Fig. 3 shows representative results of CD122-deficient $CD4^+$ T cells. Activation and memory status of CD122-

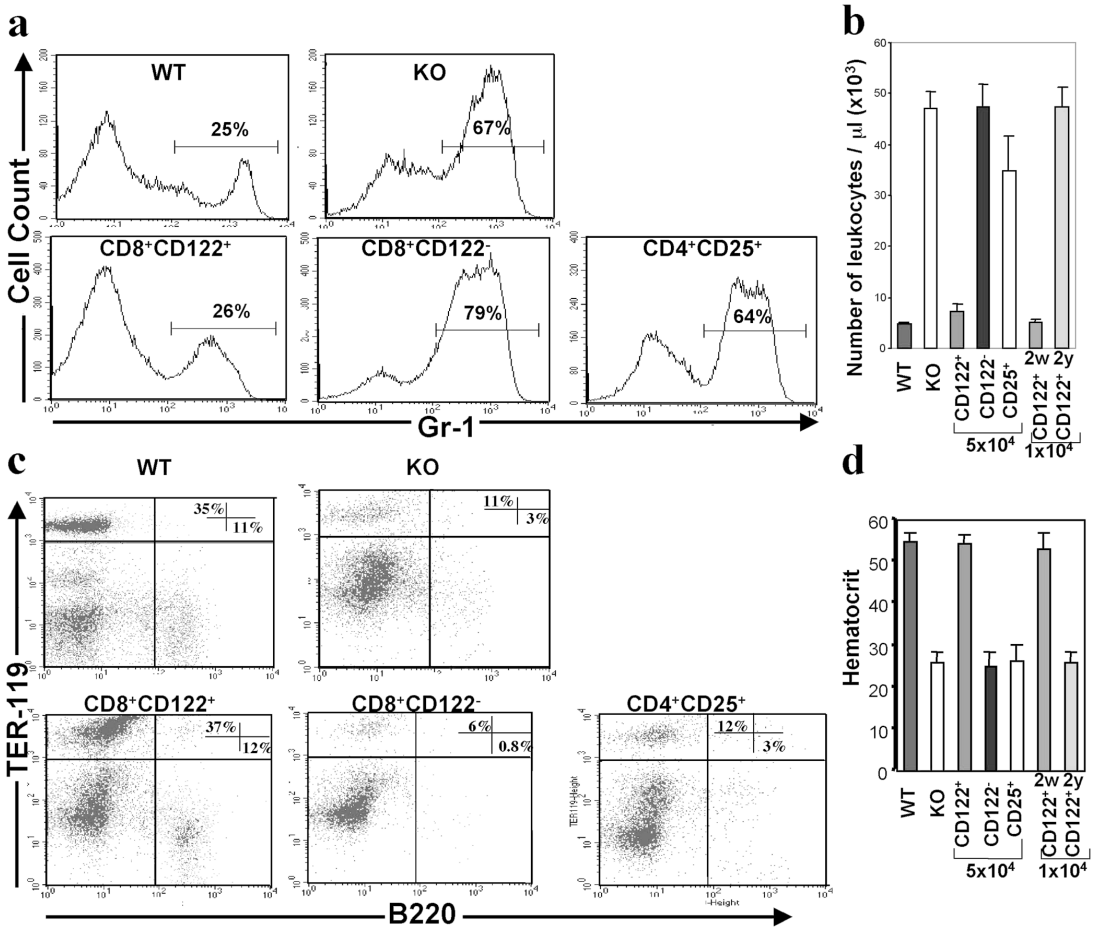

Figure 4. Normalized granulocytes, erythrocytes, and B cells in CD122-deficient mice that had received $CD8^+CD122^+$ T cells. (a) After 7 wk from the transfer of 5×10^4 of indicated T cell subpopulations into CD122-deficient neonates, bone marrow cells obtained from the mice were stained with anti–Gr-1 and anti-CD45.1 antibodies, and analyzed by flow cytometry. Percentages of $Gr\text{-}1^+$ cells are shown for cells gated to $CD45.1^-$ population. Data obtained from a 7-wk-old wild-type mouse (WT) and a 7-wk-old CD122-deficient mouse (KO) are also shown as controls. (b) Numbers of white blood cells in peripheral blood from mice that had received indicated cell populations were counted using cell-counting chamber. Data are mean ± SD values of three or four mice in each group. Data obtained from CD122-deficient mice that received 10^4 $CD8^+CD122^+$ cells obtained from 2-wk-old mice or 2-yr-old mice are also shown. (c) After 7 wk from the transfer of 5×10^4 of indicated T cell subpopulations into CD122-deficient neonates, bone marrow cells obtained from the mice were stained with anti-CD45.1, anti-B220, and anti–TER-119 antibodies, and analyzed by flow cytometry. Data are shown as dot-plot analysis for cells gated to $CD45.1^-$ population. Numbers in each panel are percentages of erythroid lineage cells ($TER\text{-}119^+$) and B lineage cells ($B220^+$) in $CD45.1^-$ bone marrow cells. Data obtained from a 7-wk-old wild-type (WT) mouse and a 7-wk-old CD122-deficient mouse (KO) are also shown as controls. (d) Hematocrit values of mice that had received the indicated cell populations. Data are mean ± SD values of three or four mice in each group. Data obtained from CD122-deficient mice that received 10^4 $CD8^+CD122^+$ cells obtained from 2-wk-old mice or 2-yr-old mice are also shown.

deficient $CD8^+$ T cells analyzed by expression of CD69, CD44, and CD62L were not different from those of $CD4^+$ T cells (unpublished data).

$CD8^+CD122^+$ Cells Normalize Granulocytes, B Cells, and Erythrocytes in CD122-deficient Mice. Examination of granulopoiesis showed that the number of $Gr\text{-}1^+$ cells in the bone marrow was markedly increased in CD122-deficient mice (Fig. 4 a, top middle). This increase was normalized by transferring 5×10^4 $CD8^+CD122^+$ cells into neonates, but not by transferring 5×10^4 $CD8^+CD122^-$ cells or 5×10^4 $CD4^+CD25^+$ T cells (Fig. 4 a, bottom). Changes in the number of $Gr\text{-}1^+$ cells in the bone marrow matched those of leukocyte numbers in peripheral blood. Increased leukocyte number in peripheral blood of CD122-deficient mice was normalized by the transfer of 5×10^4 $CD8^+CD122^+$ cells into neonates but neither by the transfer of 5×10^4 $CD8^+CD122^-$ cells nor by that of 5×10^4 $CD4^+CD25^+$ cells (Fig. 4 b). Transfer of 10^4 $CD8^+CD122^+$ cells from 2-wk-old mice normalized the number of $Gr\text{-}1^+$ cells in the bone marrow (not depicted) and the leukocyte number in peripheral blood in CD122-deficient mice, but transfer of 10^4 $CD8^+CD122^+$ cells from 2-yr-old mice did not (Fig. 4 b). Severe anemia caused by a combination of autoimmune hemolysis and suppression of erythropoiesis is another striking feature of CD122-deficient mice (12). Examination of erythroid lineage cells by expression of TER-119 antigen (20), an erythroid specific marker, showed marked reduction in the number of $TER\text{-}119^+$ cells in the bone marrow of CD122-deficient mice. This erythroid suppression was also

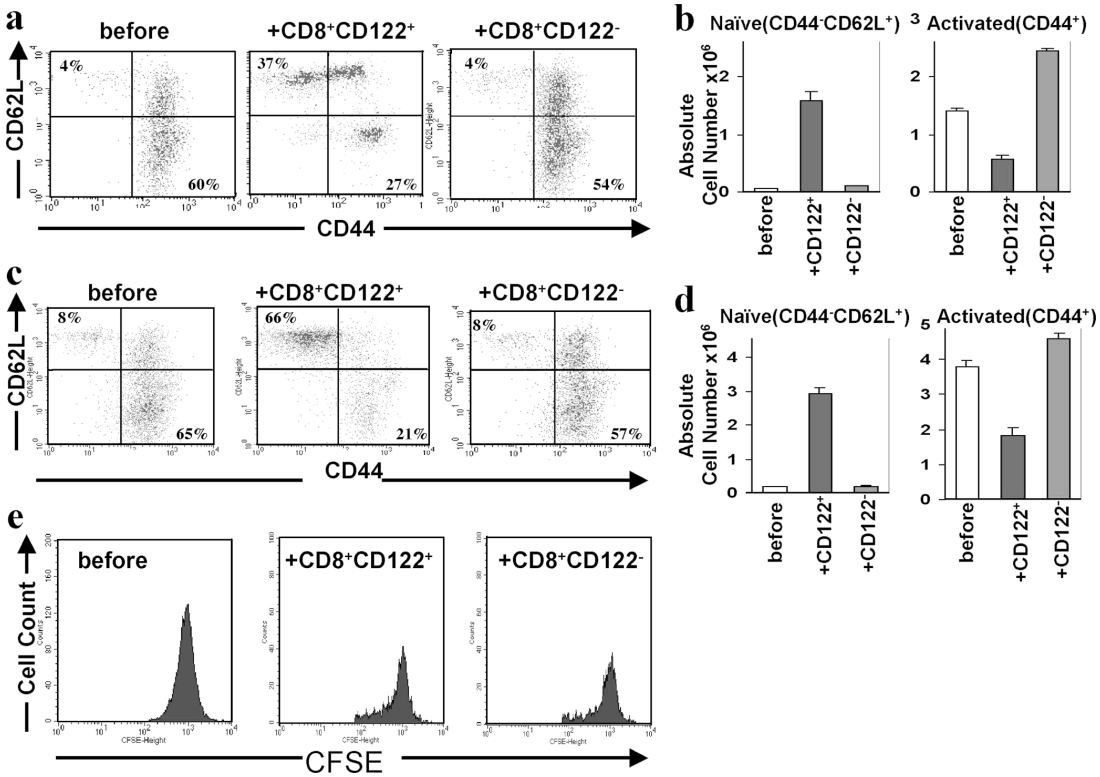

Figure 5. Effects of $CD8^+CD122^+$ T cells on $CD8^+$ and $CD4^+$ T cells. (a) $CD8^+CD122^+$ T cells and $CD8^+CD122^-$ T cells were isolated from $C57BL/6^{CD45.1/CD45.1}$ congeneic mice by cell sorting. The isolated cells (5×10^5) were mixed with T cells from CD122-deficient mice (5×10^6) and transferred into RAG-$2^{-/-}$ mice. The status of $CD8^+$ T cells from CD122-deficient mice before transfer is shown (left). 1 wk later, spleen cells were harvested and examined according to the cell surface marker expression of CD44 and CD62L. Cells that had been cotransferred with $CD8^+CD122^+$ T cells (+$CD8^+CD122^+$) and those cotransferred with $CD8^+CD122^-$ T cells (+$CD8^+CD122^-$) were analyzed. Cells gated to CD122-deficient mice–derived $CD8^+$ T cells ($CD45.1^-CD8^+$) are shown. (b) Mean ± SD of absolute numbers of naive-type ($CD44^-CD62L^+$) and activated-type ($CD44^+$) $CD8^+$ T cells derived from CD122-deficient mice ($CD45.1^-CD8^+$) before transfer (before) and 1 wk after cotransfer with $CD8^+CD122^+$ T cells (+$CD122^+$) or cotransfer with $CD8^+CD122^-$ T cells (+$CD122^-$) ($n = 5$ each). (c) Cell transfer into RAG-$2^{-/-}$ mice was performed as in a, and analyzed for cells gated to CD122-deficient mice–derived $CD4^+$ T cells ($CD45.1^-CD4^+$). (d) Mean ± SD of absolute numbers of naive-type ($CD44^-CD62L^+$) and activated-type ($CD44^+$) $CD4^+$ T cells derived from CD122-deficient mice ($CD45.1^-CD4^+$) for the same groups defined in b. (e) CFSE-labeled $CD122^{-/-}$ T cells (5×10^6) were mixed with $CD8^+CD122^+$ T cells or $CD8^+CD122^-$ T cells and transferred into RAG-$2^{-/-}$ host mice as in a. Dilution of CFSE was analyzed at 1 wk after adoptive transfer (+$CD8^+CD122^+$ and +$CD8^+CD122^-$). Data of CFSE-labeled $CD122^{-/-}$ T cells before transfer are shown as control (before).

rescued by the transfer of 5×10^4 $CD8^+CD122^+$ cells into neonates, whereas the transfer of 5×10^4 $CD8^+CD122^-$ cells or 5×10^4 $CD4^+CD25^+$ cells was ineffective, resulting in the same decrease of TER-119^+ cells as that in nontreated CD122-deficient mice (Fig. 4 c). Analysis of hematocrit in peripheral blood also confirmed the prevention of anemia by the transfer of $CD8^+CD122^+$ cells but not by that of other cell populations (Fig. 4 d). The number of $B220^+$ cells, representing all developing B cells, was also normalized by the transfer of 5×10^4 $CD8^+CD122^+$ cells, but not by that of 5×10^4 $CD8^+CD122^-$ cells or 5×10^4 $CD4^+CD25^+$ cells (Fig. 4 c). Again, transfer of 10^4 $CD8^+CD122^+$ cells from 2-wk-old mice rescued erythropoiesis in the bone marrow (not depicted) and normalized hematocrit in CD122-deficient mice, whereas transfer of 10^4 $CD8^+CD122^+$ cells from 2-yr-old mice did not (Fig. 4 d). As a conclusive result, CD122-deficient mice that received $CD8^+CD122^+$ cells at neonatal period grew normally and remained healthy. No improvement of general condition was observed in mice that received 5×10^4 $CD8^+CD122^-$ or 5×10^4 $CD4^+CD25^+$ cells, and they all died before 3 mo of age. Transfer of a smaller number (10^4) of $CD8^+CD122^+$ cells from 2-wk-old mice at the neonatal stage effectively normalized all phenotypes in CD122-deficient mice, but that of the same number of $CD8^+CD122^+$ cells from 2-yr-old mice did not. 10^4 $CD4^+CD25^+$ cells obtained from 2-wk-old mice were also ineffective in normalizing CD122-deficient mice (unpublished data).

$CD8^+CD122^+$ Cells Control Both $CD8^+$ and $CD4^+$ Cells. From the aforementioned experimental results, it is clear that $CD8^+CD122^+$ cells control CD122-deficient T cells. To elucidate whether $CD8^+CD122^+$ cells can control already-activated T cells, we transferred a mixture of $CD8^+CD122^+$ cells and already-activated T cells obtained from CD122-deficient mice into RAG-$2^{-/-}$ mice and analyzed the status of T cells 1 wk later. Activated memory-type cells ($CD44^+CD62L^-$) were dominant among $CD8^+$ and $CD4^+$ T cells from CD122-deficient mice (Fig. 5, a and c, before). After 1 wk of coexistence with $CD8^+CD122^+$ cells, naive-type cells ($CD44^-CD62L^+$)

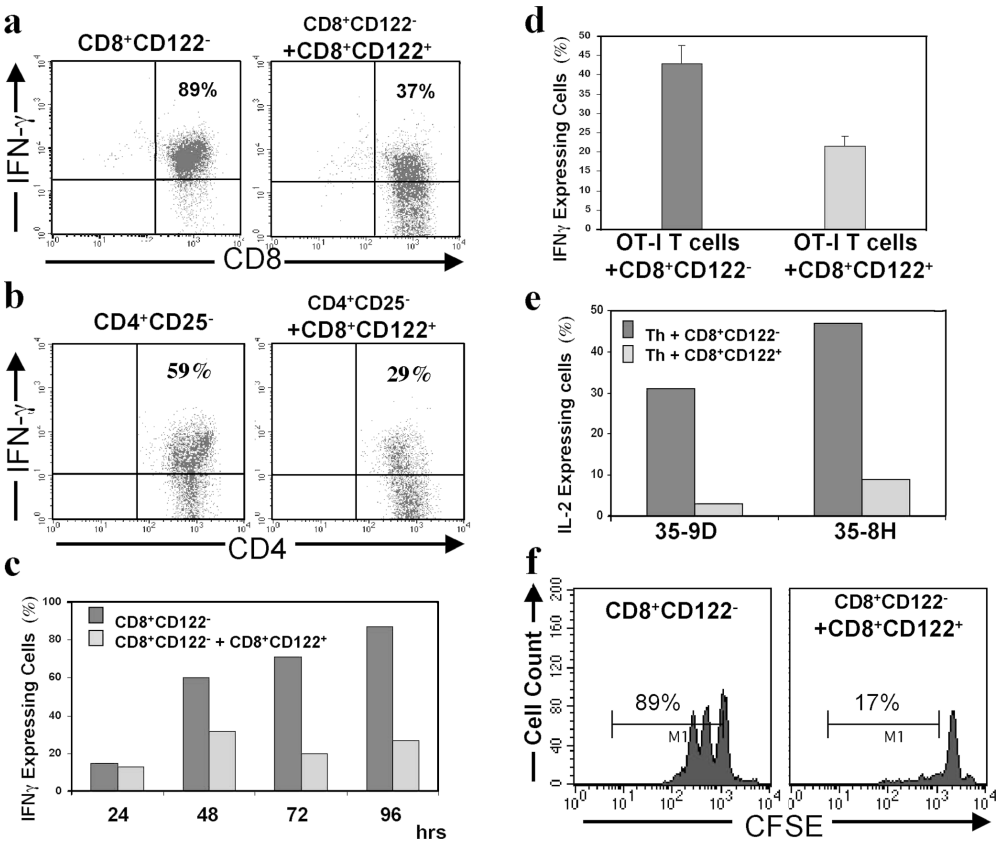

Figure 6. (a) Purified $CD8^+$ $CD122^-$ T cells (CD45.1, 4×10^4) and $CD8^+CD122^+$ T cells (CD45.2, 10^4) were cocultured in 96-well plates coated with anti-CD3 antibody for 72 h in a medium containing rIL-2 (25 U ml^{-1}). After culture, cells were stained with anti-CD8 and anti-CD45.1 antibodies and subjected to intracellular cytokine staining. Simple culture of $CD8^+CD122^-$ T cells alone (5×10^4) under the same condition as coculture was set as a control ($CD8^+CD122^-$). Cells gated to $CD45.1^+$ region are shown. Percentage of cells positively stained with intracellular IFN-γ is presented in the panels. (b) Purified $CD4^+CD25^-$ T cells (CD45.1, 4×10^4) and $CD8^+CD122^+$ T cells (CD45.2, 10^4) were cocultured and analyzed as in a, except that anti-CD4 antibody was used instead of anti-CD8 antibody. Simple culture of $CD4^+CD25^-$ T cells alone (5×10^4) was set as a control ($CD4^+CD25^-$). Cells gated to $CD45.1^+$ region are shown. Percentages of cells positively stained with intracellular IFN-γ are presented in the panels. (c) Purified $CD8^+CD122^-$ T cells were cultured alone or cocultured with $CD8^+CD122^+$ T cells as in a for the indicated time intervals, and their IFN-γ expression was measured. Percentages of IFN-γ–expressing cells determined by FACS analysis as shown in a are presented. (d) $CD8^+CD122^-$ T cells (2×10^5) were isolated from OT-I transgenic mice and stimulated with OVA peptide 257-264 (100 μg/ml) plus 2×10^5 T cell–depleted and γ-irradiated (30 Gy) spleen cells in 96-well flat-bottom plates for 48 h. Next, 5×10^4 of $CD8^+CD122^+$ or $CD8^+CD122^-$ T cells isolated from C57BL/6-CD45.1 mice were added to each well, and the culture was continued for 48 h. Expression of IFN-γ on OT-I transgenic T cells was assessed by intracellular cytokine staining and analyzed by flow cytometry. Data are averages of three independent experiments ± SD. (e) OVA-specific Th1 clones, 35-8H or 35-9D (5×10^4 each) were cocultured with $CD8^+CD122^+$ T cells or $CD8^+CD122^-$ T cells (5×10^4 each) under stimulation with OVA peptide presented on syngeneic APCs (C57BL/6 spleen cells). IL-2 production was assayed by intracellular cytokine staining after 72 h of culture. Percentages of Th1 clone cells expressing IL-2 are shown. (f) Purified $CD8^+CD122^-$ T cells (4×10^4) were labeled with CFSE and cocultured with $CD8^+CD122^+$ T cells (10^4) in 96-well plates coated with anti-CD3 antibody for 48 h without the addition of exogenous IL-2. After culture, cells were analyzed for their content of CFSE. Simple culture of CFSE-labeled $CD8^+CD122^-$ T cells alone (5×10^4) under the same condition as coculture was set as a control ($CD8^+CD122^-$). Percentage of cells that have proliferated is presented in the panels.

became dominant both in $CD8^+$ and $CD4^+$ cells (Fig. 5, a and c, $+CD8^+CD122^+$). In contrast, coexistence with $CD8^+CD122^-$ cells did not alter the ratio between naive-type and activated memory-type cells (Fig. 5, a and c, $+CD8^+CD122^-$). The absolute number of naive-type cells increased, but that of activated-type cells rather decreased when $CD8^+$ and $CD4^+$ T cells coexisted with $CD8^+CD122^+$ cells (Fig. 5, b and d). CD122-deficient T cells that had been labeled with CFSE and coexisted with $CD8^+CD122^+$ cells for 7 d showed the same pattern of CFSE intensity as those that had coexisted with $CD8^+CD122^-$ cells (Fig. 5 e), indicating that naive-type cells that appeared after coexistence with $CD8^+CD122^+$ cells were not the cells expanded from small number of naive-type cells or stem cells. These results (Fig. 5, b, d, and e) may better explain the phenomenon observed in Fig. 5 (a and c) as the phenotypic change of cells from activated type to naive type than by simple expansion of naive cells or deletion of activated cells. Although the only action to the CD122-deficient mice–derived T cells has been demonstrated eventually, these results suggest that $CD8^+CD122^+$ cells control already-activated T cells of both $CD8^+$ and $CD4^+$ types.

Next, we investigated the in vitro action of $CD8^+$ $CD122^+$ cells. Wild-type mice–derived $CD8^+CD122^-$ cells cocultured with $CD8^+CD122^+$ cells showed lower expression of IFN-γ than those cultured alone (Fig. 6 a). Wild-type mice–derived $CD4^+CD25^-$ cells cocultured with $CD8^+CD122^+$ cells also showed low expression of IFN-γ (Fig. 6 b) and IL-2 (not depicted), suggesting that $CD8^+CD122^+$ cells can potentially suppress cytokine expression in both $CD8^+$ and $CD4^+$ cells derived from wild-type mice. Time-course study of IFN-γ expression indicated that IFN-γ expression was induced in a time-dependent manner in $CD8^+CD122^-$ cells cultured alone with plate-bound anti-CD3 and rIL-2, whereas that in $CD8^+CD122^-$ cells cocultured with $CD8^+CD122^+$ cells was clearly suppressed through the culture during 2–5 d

(Fig. 6 c). In this in vitro experiment, only purified $CD8^+$ and $CD4^+$ T cells were used and no APCs were added to the culture. When purified $CD8^+CD122^-$ cells from OT-I transgenic mice that express OVA-specific TCR were stimulated with OVA peptide presented by MHC class I H-$2K^b$ on syngeneic APCs for 4 d and cocultured with CD45.1-congenic $CD8^+CD122^+$ T cells during the latter 2 d of culture, $CD8^+CD122^+$ T cells obtained from C57BL/6-CD45.1 mice suppressed IFN-γ production from transgenic T cells (Fig. 6 d). In addition, $CD8^+CD122^+$ cells suppressed IL-2 production from Th1-type clones that were stimulated with antigen and OVA presented by MHC class II I-A^b on syngeneic APCs (Fig. 6 e). In these cases, using OVA peptide–responding T cells, $CD8^+CD122^+$ cells were not stimulated with anti-CD3 antibody, indicating that nonspecific activation was unnecessary for performing the regulatory action in $CD8^+CD122^+$ cells. We also examined the activity of $CD8^+CD122^+$ cells in suppressing proliferation of regulated cells. The results showed that $CD8^+CD122^+$ cells suppressed the proliferation of anti-CD3–stimulated $CD8^+$ $CD122^-$ cells when these cells were cocultured without exogenous IL-2 (Fig. 6 f). This suppression of proliferation could be due to reduced production of IL-2 from regulated cells or to direct suppression effect on cell proliferation. The cytotoxic activity of $CD8^+CD122^+$ cells against anti-CD3–activated $CD8^+CD122^-$ cells or $CD4^+CD25^-$ in a culture condition, similar to that used for detecting suppression of cytokine production, was not detected (unpublished data), suggesting that cytotoxic activity against the regulated cell is not involved in the regulatory action of $CD8^+CD122^+$ cells.

Normal $CD8^+CD122^-$ Cells without $CD8^+CD122^+$ Cells Cause Death of RAG-$2^{-/-}$ Mice. As the aforementioned in vitro experiment suggested a regulatory activity of $CD8^+$ $CD122^+$ cells to normal T cells, we examined whether $CD8^+CD122^+$ cells control normal T cells and how normal T cells behave in the absence of $CD8^+CD122^+$ cells in vivo. The $CD8^+$ cell population of normal 6-wk-old C57BL/6 mice consists of ~10% $CD122^+$ cells and 90% $CD122^-$ cells (Fig. 7 a). We collected $CD8^+CD122^-$ cells and total $CD8^+$ cells, each with a purity of >99%, by cell sorting and transferred 5×10^5 of these cells into lymphocyte-deficient RAG-$2^{-/-}$ host mice (Fig. 7 a). Surprisingly, all mice that received $CD8^+CD122^-$ cells died within 10 wk after cell transfer, whereas all mice that received total $CD8^+$ cells were alive and healthy for >20 wk after cell transfer (Fig. 7 b).

Normal $CD8^+CD122^-$ Cells Become Highly Activated Cells in the Absence of $CD8^+CD122^+$ Cells. We examined the mice at 7 wk after cell transfer into RAG-$2^{-/-}$ mice. All tissue of mice that had received $CD8^+CD122^-$ cells appeared pale and anemic. Total cell numbers in the spleen and bone marrow of bilateral femurs of mice that received total $CD8^+$ cells were not significantly different from those that received $CD8^+CD122^-$ cells (spleen: $1.83 \pm 0.08 \times 10^7$ vs. $1.64 \pm 0.26 \times 10^7$; bone marrow: $5.01 \pm 0.25 \times 10^7$ vs. $4.80 \pm 0.28 \times 10^7$, respectively,

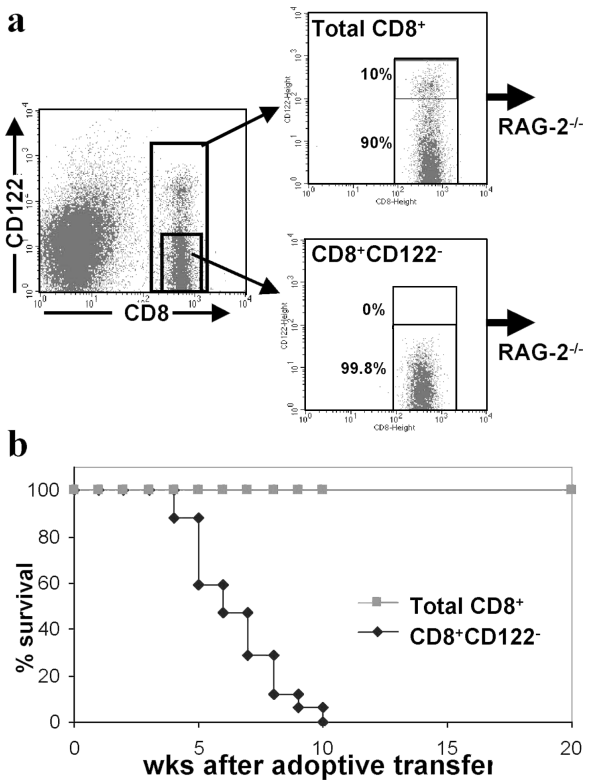

Figure 7. Lethal effect of $CD8^+CD122^-$ T cells transferred into RAG-$2^{-/-}$ mice. (a) Spleen cells from a 6-wk-old C57BL/6 mouse were stained with anti-CD8 and anti-CD122 antibodies, and analyzed by flow cytometry (left). Rectangle frames represent the regions to collect the desired cells by cell sorting. Collected cells by cell sorting were reanalyzed by flow cytometry and their purities were confirmed (right). 5×10^5 of such purified T cells were injected into each RAG-$2^{-/-}$ mouse. (b) Survival of RAG-$2^{-/-}$ mice that received either $CD8^+CD122^-$ T cells or total $CD8^+$ cells was monitored for 20 wk ($n = 17$ each). All surviving mice that received total $CD8^+$ cells showed no signs of any illness at 20 wk after transfer.

$n = 5$ each, mean ± SD). We analyzed the status of transferred $CD8^+$ cells. Although the numbers of $CD8^+$ cells recovered from spleens were not different between the two groups (Fig. 8 a), the percentage of activated $CD69^+$ cells was higher in $CD8^+CD122^-$ cell transfer than in total $CD8^+$ cell transfer (Fig. 8 b). We also transferred 5×10^5 $CD8^+CD122^-$ cells mixed with 5×10^4 $CD8^+CD122^+$ cells or 5×10^3 $CD8^+CD122^+$ cells into RAG-$2^{-/-}$ mice. In the case of transfer of $CD8^+CD122^-$ cells mixed with 1/10 the number (5×10^4) of $CD8^+CD122^+$ cells, the pattern of total $CD8^+$ cell number and the percentage of $CD69^+$ cells in the spleen were similar to those in total $CD8^+$ cell transfer (Fig. 8, a and b). In contrast, the pattern of total $CD8^+$ cell number and the percentage of $CD69^+$ cells in spleen were similar to those in $CD8^+CD122^-$ cell transfer in the case of transfer of $CD8^+CD122^-$ cells mixed with 1/100 number (5×10^3) of $CD8^+CD122^+$ cells. The activated cells that had originally been $CD122^-$ became $CD122^+$ (Fig. 8 c). In the bone marrow, the difference between $CD8^+CD122^-$ cell transfer and total $CD8^+$ cell transfer was more evident. The number of $CD8^+$ T cells in

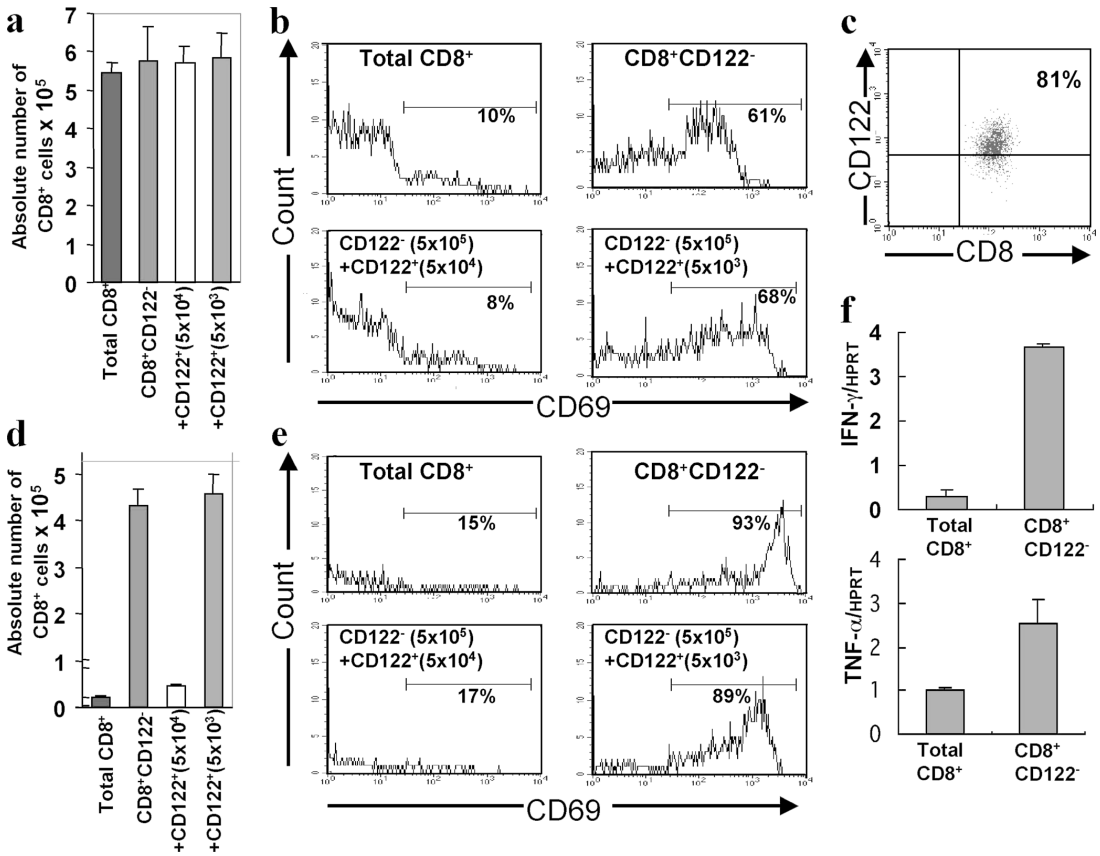

Figure 8. Highly activated T cells infiltrate the bone marrow in RAG-2$^{-/-}$ mice that received CD8$^+$CD122$^-$ T cells. (a) Absolute number of CD8$^+$ T cells in spleen was measured at 7 wk after transfer of 5×10^5 total CD8$^+$ T cells, 5×10^5 CD8$^+$CD122$^-$ T cells, 5×10^5 CD8$^+$CD122$^-$ T cells mixed with 5×10^4 CD8$^+$CD122$^+$ T cells, or 5×10^5 CD8$^+$CD122$^-$ T cells mixed with 5×10^3 CD8$^+$CD122$^+$ T cells into RAG-2$^{-/-}$ mice. Data are mean ± SD of three to six mice per group. There were no significant differences among the groups ($P > 0.5$). (b) Expression of CD69 on CD8$^+$ T cells in the spleen. Histogram analysis gated to CD8$^+$ cells and percentages of CD69$^+$ cells in total CD8$^+$ cells are shown. (c) Expression of CD122 in originally CD8$^+$CD122$^-$ T cells after transfer into RAG-2$^{-/-}$ mice. Spleen cells were harvested from a CD8$^+$CD122$^-$ T cell–transferred RAG-2$^{-/-}$ mouse at 1 wk after transfer and analyzed by staining with indicated antibodies and flow cytometry. Only cells gated to CD8$^+$ region are shown, and the percentage of CD122$^+$ cells in total CD8$^+$ cells is shown. (d) Absolute number of CD8$^+$ T cells in bilateral femoral bone marrow measured at 7 wk after transfer of CD8$^+$CD122$^-$ T cells into RAG-2$^{-/-}$ mice ($n = 6$) was significantly higher than that in mice that received total CD8$^+$ T cells ($P = 10^{-5}$). Data are mean ± SD. The absolute number of CD8$^+$ T cells in bone marrow after transfer of 5×10^5 CD8$^+$CD122$^-$ cells mixed with 5×10^4 CD8$^+$CD122$^+$ cells ($n = 3$) was not significantly higher ($P > 0.5$), but that after transfer of 5×10^5 CD8$^+$CD122$^-$ cells mixed with 5×10^3 CD8$^+$CD122$^+$ cells ($n = 3$) was significantly higher than that in mice that received total CD8$^+$ T cells ($P = 10^{-5}$). (e) Expression of CD69 on CD8$^+$ T cells in bone marrow. Histogram analysis gated to CD8$^+$ cells and percentages of CD69$^+$ cells in total CD8$^+$ cells. (f) RNA samples were isolated from CD8$^+$ cells recovered from mice that had received either total CD8$^+$ T cells or CD8$^+$CD122$^-$ T cells and subjected to real-time RT-PCR analysis. Normalized value for IFN-γ or TNF-α mRNA expression in each sample was calculated as the relative quantity of IFN-γ or TNF-α divided by the relative quantity of HPRT. All samples were performed in triplicate. Data are mean ± SD.

the bone marrow of CD8$^+$CD122$^-$ cell-transferred mice was ~20 times greater than that of total CD8$^+$ cell-transferred mice (Fig. 8 d). The CD8$^+$ cells in the bone marrow of CD8$^+$CD122$^-$ cell-transferred mice expressed high levels of the activation marker, CD69 (Fig. 8 e), which was higher than that of T cells in spleens of the same mice (Fig. 8, b and e). Again, the total CD8$^+$ cell number and the expression pattern of CD69 in the bone marrow of mice that received 5×10^5 CD8$^+$CD122$^-$ cells mixed with 5×10^4 CD8$^+$CD122$^+$ cells was similar to that in total CD8$^+$ cell-transferred mice, whereas that in the bone marrow of mice that received 5×10^5 CD8$^+$CD122$^-$ cells mixed with 5×10^3 CD8$^+$CD122$^+$ cells was similar to that in CD8$^+$CD122$^-$ cell-transferred mice (Fig. 8, d and e). Real-time RT-PCR analysis showed higher expression levels of IFN-γ and TNF-α in T cells from CD8$^+$CD122$^-$ cell-transferred mice than those in total CD8$^+$ cell-transferred mice (Fig. 8 f). These features of T cells in CD8$^+$ CD122$^-$ cell-transferred mice were similar to those in CD122-deficient mice, except that the absolute numbers of T cells in lymph nodes and spleens of the former mice were smaller than those of the latter mice.

Hematopoietic Disorders in CD8$^+$CD122$^-$ Cell-transferred Mice. The percentage of granulocytic Gr-1$^+$ cells was increased to >90% in the bone marrow of CD8$^+$CD122$^-$ cell-transferred mice compared with ~60% in total CD8$^+$ cell-transferred mice (Fig. 9 a). Alternately, cells in erythroid lineage that expressed TER-119 almost disappeared in CD8$^+$CD122$^-$ cell transfer (Fig. 9 a). Most of the TER-119$^+$ cells in the bone marrow were VLA4$^+$ erythroblasts

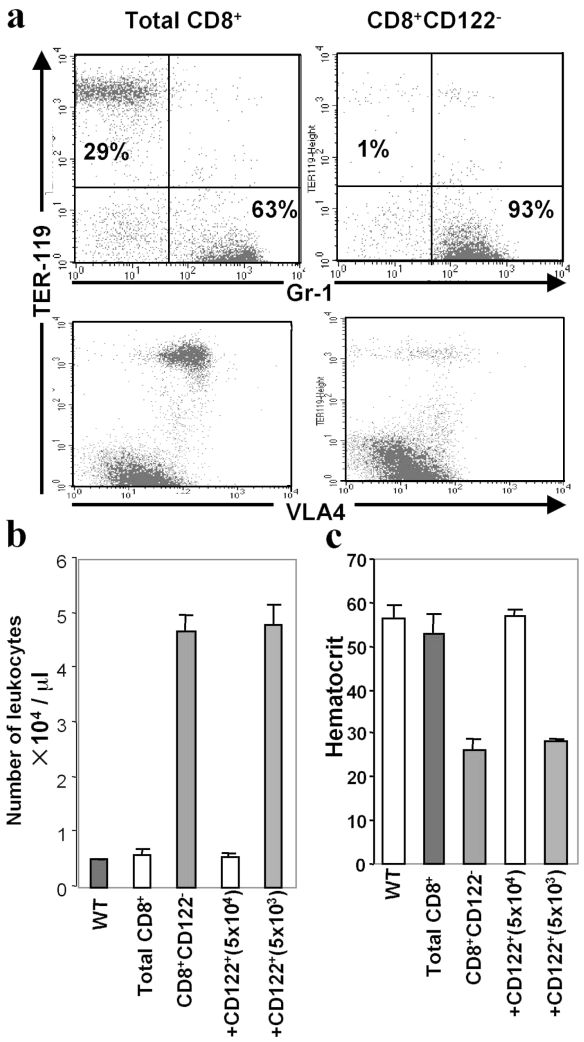

Figure 9. Increased granulopoiesis and reduced erythropoiesis in RAG-$2^{-/-}$ mice that received $CD8^+CD122^-$ T cells. (a) Bone marrow cells obtained from RAG-$2^{-/-}$ mice that had received either total $CD8^+$ cells or $CD8^+CD122^-$ T cells were stained with anti–Gr-1 and anti–TER-119 antibodies (top). Percentages of cells in myeloid lineage ($Gr\text{-}1^+TER\text{-}119^-$) and cells in erythroid lineage ($Gr\text{-}1^-TER\text{-}119^+$) are shown. The same cells were stained with anti–VLA-4 and anti–TER-119 antibodies (bottom). (b) Numbers of leukocytes in peripheral blood of wild-type (WT) mice and RAG-$2^{-/-}$ mice that had received 5×10^5 total $CD8^+$ T cells, 5×10^5 $CD8^+CD122^-$ T cells, 5×10^5 $CD8^+CD122^-$ T cells mixed with 5×10^4 $CD8^+CD122^+$ T cells, or 5×10^5 $CD8^+CD122^-$ T cells mixed with 5×10^3 $CD8^+CD122^+$ T cells were counted using cell-counting chamber. Data are mean ± SD of three to six mice in each group. (c) Hematocrit values of wild-type (WT) mice and RAG-$2^{-/-}$ mice that had received the indicated cell population. Data are mean ± SD of three to six mice in each group.

(Fig. 9 a, bottom). These bone marrow changes matched changes in the numbers of red and white blood cells in peripheral blood. The number of leukocytes was markedly increased (Fig. 9 b), and hematocrit was significantly reduced (Fig. 9 c) in $CD8^+CD122^-$ cell-recipient mice, indicating progression of anemia in these mice. RAG-$2^{-/-}$ mice that had received 5×10^5 $CD8^+CD122^-$ cells mixed with 5×10^4 $CD8^+CD122^+$ cells had a normal $TER\text{-}119^+/Gr\text{-}1^+$ cell ratio in the bone marrow (26% of $TER\text{-}119^+$ cells and 62% $Gr\text{-}1^+$cells; not depicted), normal leukocyte number in peripheral blood (Fig. 9 b), and normal hematocrit (Fig. 9 c). In contrast, RAG-$2^{-/-}$ mice that had received 5×10^5 $CD8^+CD122^-$ cells mixed with 5×10^3 $CD8^+CD122^+$ cells had a $TER\text{-}119^+/Gr\text{-}1^+$ cell ratio skewed toward $Gr\text{-}1^+$ cells (2% of $TER\text{-}119^+$ cells and 91% of $Gr\text{-}1^+$ cells; not depicted), increased leukocyte number in peripheral blood (Fig. 9 b), and low hematocrit (Fig. 9 c).

Discussion

Regulatory T cells are classified into two groups; i.e., naturally occurring regulatory cells and regulatory cells induced during immune responses (5). A representative example of the former type is in the $CD4^+CD25^+$ population. In this paper, we clearly demonstrated that $CD8^+$ cells contain regulatory T cells reminiscent of naturally occurring type, and that such regulatory cells are relatively rich in $CD8^+CD122^+$ population, but are rare in the $CD8^+CD122^-$ population.

In recent studies by Malek et al. (21, 22), the defect of $CD4^+CD25^+$ regulatory T cells was proposed to be responsible for the increase of deregulated T cells in CD122-deficient mice. Based on the fact of the presence of very few $CD4^+CD25^+$ T cells in CD122-deficient mice, this idea might well explain the development of abnormalities in CD122-deficient mice. However, in the present paper, transfer of highly purified $CD4^+CD25^+$ cells failed to rescue the phenotypes in CD122-deficient mice (Figs. 3 and 4). These experimental results were incompatible with those of Malek et al. (21). In their work, CD122-deficient mice in which $CD4^+CD25^+$ T cell population had been transferred at neonatal stage were rescued from the development of abnormal phenotypes. There are several possibilities to explain the different results of the two papers. In our experiment, the number of effective $CD4^+CD25^+$ regulatory cells transferred by subcutaneous injection of 5×10^4 cells might not be sufficient to induce regulatory activity. Actually, transfer of a higher number (2×10^5) of $CD4^+CD25^+$ in our experiment was effective in normalizing all the phenotypes in CD122-deficient mice. Thus, the regulatory activity of $CD4^+CD25^+$ cells for CD122-deficient T cells was also confirmed in our paper. However, effective normalization of CD122-deficient mice by transfer of $CD8^+CD122^+$ cells but not by that of the same number of $CD4^+CD25^+$ cells under the same experimental condition (Figs. 3 and 4) allows us to make a definite conclusion that $CD8^+CD122^+$ cells contain stronger regulatory T cells that can control CD122-deficient T cells compared with $CD4^+CD25^+$ cells.

The $CD8^+CD122^+$ regulatory cells are exclusively important in the control of $CD8^+CD122^-$ cells. $CD4^+CD25^+$ regulatory cells do not seem to control normal $CD8^+CD122^-$ cells similar to $CD8^+CD122^+$ cells because transfer of a mixture of $CD4^+CD25^+$ cells (10^5) and wild-type mice–derived $CD8^+CD122^-$ cells (4×10^5) into RAG-$2^{-/-}$ mice resulted in the same lethal phenotype as that of simple $CD8^+CD122^-$ cells (5×10^5; unpublished

data). A mixture of one tenth the number (5×10^4 vs. 5×10^5) of $CD8^+CD122^+$ cells in $CD8^+CD122^-$ cells prevented the development of lethal phenotype caused by deregulated activation of $CD8^+$ T cells, whereas a mixture of one hundredth the number (5×10^3 vs. 5×10^5) of $CD8^+CD122^+$ cells in $CD8^+CD122^-$ cells did not (Figs. 8 and 9), indicating that $CD8^+CD122^+$ cells ranging from one hundredth to one tenth of the number of $CD8^+CD122^-$ cells are necessary to maintain homeostasis in activation of $CD8^+$ T cells. $CD8^+CD122^+$ T cells, along with $CD8^+CD122^-$ cells, do not express Foxp3 (unpublished data), also suggesting that $CD8^+CD122^+$ cells are different cell lineage from $CD4^+CD25^+$ cells. Although the precise mechanism of regulation by $CD8^+CD122^+$ cells is not clear at present, the experimental results of in vitro cocultures (Fig. 6, a–c) suggest a direct interaction between the regulatory cells and regulated cells without intervention of APCs. This feature is different from that of $CD4^+CD25^+$ regulatory T cells, which require the interposition of APCs for regulatory action (23–25). Another exclusive feature of $CD8^+CD122^+$ cells may be that they possibly change the character of already-activated T cells, at least in case of CD122-deficient mice–derived T cells (Fig. 5, a–d).

Apart from the aforementioned difference between $CD4^+CD25^+$ regulatory T cells and $CD8^+CD122^+$ cells, it is quite an interesting correlation that CD25 (IL-2 receptor α chain) and CD122 (IL-2 receptor β chain) could be markers for regulatory T cells in $CD4^+$ populations and $CD8^+$ populations, respectively. IL-2 seems to be essential for maintaining already-developed $CD4^+CD25^+$ regulatory T cells (21). For the development of $CD4^+CD25^+$ cells, IL-2 receptor β chain is not essential because CD122-deficient mice that were cured by inoculating $CD8^+CD122^+$ regulatory T cells at neonate developed $CD4^+CD25^+$ cells that were not genetically affected (unpublished data). Alternately, IL-2 receptor β chain may be rather required in the development of $CD8^+CD122^+$ regulatory T cells in the thymus because the exogenous expression of CD122 only in the thymus is sufficient to normalize the phenotype of CD122-deficient mice (21). IL-2 does not seem to be indispensable for the development of $CD8^+CD122^+$ regulatory T cells because IL-2–deficient mice do possess $CD8^+CD122^+$ cells (unpublished data) that could possibly be maintained by the compensatory action of other cytokines, including IL-15. The $CD8^+CD122^+$ regulatory T cells cannot be induced from $CD8^+CD122^-$ cells even if they restored the expression of CD122 molecule. Actually, some $CD8^+CD122^-$ cells transferred into CD122-deficient neonates (unpublished data) or into RAG-$2^{-/-}$ mice (Fig. 7 c) become $CD122^+$, suggesting that expression of CD122 molecule is not sufficient to provide regulatory activity.

In the same way that CD25 (which is also expressed in activated T cells) is not an exclusive marker for $CD4^+$ regulatory T cells, CD122 cannot be an ideal marker for $CD8^+$ regulatory T cells. $CD8^+CD122^+$ populations have been considered memory-type T cells and their number actually increases in old mice. The recently identified subpopulations of memory T cells called "central memory T cells" have a cell surface marker of $CD8^+CD122^+CD62L^+$ (26–32). Alternately, $CD8^+CD122^+$ cells, which also express CD62L, are relatively abundant in neonatal mice (>50% of total $CD8^+$ in 1-wk-old mice; Fig. 1 b). The change in percentage of $CD8^+CD122^+$ cells in $CD8^+$ cells, which show two age-related phases (Fig. 1 b), may suggest two different components in this population. As the number of memory T cells is increased in older mice, the increased ratio of $CD8^+CD122^+$ cells at old age may be due to the increase of central memory T cells (29, 30, 33). Instead, a higher rate of regulatory T cells may contribute to the high ratio of $CD8^+CD122^+$ cells in younger mice. Thus, the $CD8^+CD122^+$ population may include both the regulatory T cells and the central memory T cells. These results emphasize the need for markers that distinguish $CD8^+CD122^+$ regulatory T cells from the central memory T cells.

We noted extreme activation of $CD8^+$ cells in RAG-$2^{-/-}$ mice that received $CD8^+CD122^-$ cells. How such activated T cells are generated and which antigens they are responding to remain unclear at present. The profile of TCR Vβ usage of activated $CD8^+$ T cells in $CD8^+CD122^-$ cell-transferred mice was not significantly different from that of nonactivated T cells in total $CD8^+$ cell-transferred mice (unpublished data), suggesting that the activated T cells were polyclonal cells responding to multiple antigens. Similar activation of T cells was observed in CD122-deficient mice, in which the activated T cells were also polyclonal (13), further supporting polyclonal activation of $CD8^+$ T cells in $CD8^+CD122^-$ cell-transferred mice. The activated T cells in $CD8^+CD122^-$ cell-transferred mice, and possibly those in CD122-deficient mice as well, could be responding to multiple self-antigens, foreign antigens, or both. Whatever the antigens are, activated T cells may disappear in a usual condition with $CD8^+CD122^+$ regulatory cells but remain without such regulatory cells (Fig. 5, a–d).

In this paper, we demonstrated that the regulatory cells that control $CD122^-$ T cells are in the $CD8^+CD122^+$ population, but not in the $CD8^+CD122^-$ population. We propose here that $CD8^+CD122^+$ T cells include a new type of regulatory T cells that effectively regulates other T cells. Normal $CD8^+CD122^-$ T cells become dangerously activated cells that cause lethality unless the regulatory T cells in $CD8^+CD122^+$ population properly regulate them.

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