The Common Cytokine Receptor γ Chain Plays an Essential Role in Regulating Lymphoid Homeostasis

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Summary

In the immune system, there is a careful regulation not only of lymphoid development and proliferation, but also of the fate of activated and proliferating cells. Although the manner in which these diverse events are coordinated is incompletely understood, cytokines are known to play major roles. Whereas IL-7 is essential for lymphoid development, IL-2 and IL-4 are vital for lymphocyte proliferation. The receptors for each of these cytokines contain the common cytokine receptor γ chain (γ_c), and it was previously shown that γ_c-deficient mice exhibit severely compromised development and responsiveness to IL-2, IL-4, and IL-7. Nevertheless, these mice exhibit an age-dependent accumulation of splenic $CD4^+$ T cells, the majority of which have a phenotype typical of memory/activated cells. When γ_c-deficient mice were mated to DO11.10 T cell receptor (TCR) transgenic mice, only the T cells bearing endogenous TCRs had this phenotype, suggesting that its acquisition was TCR dependent. Not only do the $CD4^+$ T cells from γ_c-deficient mice exhibit an activated phenotype and greatly enhanced incorporation of bromodeoxyuridine but, consistent with the lack of γ_c-dependent survival signals, they also exhibit an augmented rate of apoptosis. However, because the $CD4^+$ T cells accumulate, it is clear that the rate of proliferation exceeds the rate of cell death. Thus, surprisingly, although γ_c-independent signals are sufficient to mediate expansion of $CD4^+$ T cells in these mice, γ_c-dependent signals are required to regulate the fate of activated $CD4^+$ T cells, underscoring the importance of γ_c-dependent signals in controlling lymphoid homeostasis.

The common cytokine receptor γ chain (γ_c)[1] is the genetic defect in X-linked severe combined immunodeficiency (XSCID) (1) and is a shared component of the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15 (2–5). In humans with XSCID, the lack of functional γ_c results in profoundly diminished T cell development and an absence of natural killer (NK) cells. In contrast, B cell numbers are normal, although the B cells are nonfunctional with concomitant hypogammaglobulinemia. Mice in which the γ_c gene has been targeted by homologous recombination exhibit a related, but somewhat different phenotype (6–8). Like humans with XSCID, they have hypoplastic thymuses and lack NK cells. Unlike humans with XSCID, conventional B cells are greatly diminished in the bone marrow and the spleens of both young and adult γ_c-deficient mice, likely reflecting the key role of IL-7 as a pre-B cell growth factor in mice, but not in humans (5). In addition, these mice lacked visible gut-associated lymphoid tissue and peripheral lymph nodes were essentially absent. Although splenic T cells are diminished in number at 3 wk of age, $CD4^+$ T cells, but not $CD8^+$ T cells, dramatically accumulate in the spleen in an age-dependent manner. Moreover, the finding of $CD4^+$ T cell infiltrates, particularly in the gut in association with colitis, suggested that the $CD4^+$ T cells might be activated and involved in mediating the pathological changes found in these mice. Given that the mice are compromised in their ability to respond to γ_c-dependent cytokines including IL-2, IL-4, and IL-7 (6–8), this accumulation of $CD4^+$ T cells was unexpected. We now have performed studies to characterize further these $CD4^+$ T cells to understand better the role of γ_c in development of the immune response in vivo.

[1]*Abbreviations used in this paper:* γ_c, common cytokine receptor γ chain; PI, propidium iodide; TUNEL, terminal deoxynucleotide transferase (TdT)-dUTP nick end–labeling; XSCID, X-linked severe combined immunodeficiency.

Materials and Methods

Mice and Genetic Analysis. The γ_c-deficient mice (7) used in these studies were back-crossed to either C57BL/6 (B6) ($H\text{-}2^{b/b}$) or BALB/c mice ($H\text{-}2^{d/d}$) (Jackson Laboratory, Bar Harbor, ME) for three/four generations. Since the γ_c gene is localized on X chro-

mosome, DO10 TCR transgenic male mice (H-$2^{d/d}$), specific for ovalbumin (9), were mated with BALB/c $\gamma_c^{+/-}$ heterozygous female mice. These matings yielded four genotypes of male mice ($DO10^-\gamma_c^{+/Y}$, $DO10^+\gamma_c^{+/Y}$, $DO10^-\gamma_c^{-/Y}$, and $DO10^+\gamma_c^{-/Y}$). Similarly, AND TCR transgenic male mice (H-$2^{b/b}$), specific for cytochrome c (10, 11), were mated with B6 $\gamma_c^{+/-}$ female mice. Mice were housed in microisolator cages under pathogen-free conditions. The mice were genotyped by PCR of tail DNA using the following primer pairs: to detect the wild-type γ_c gene, 5′-CTTTATTGATAACGATCTATCCCTCACCC-3′ and 5′-CTCCACTCTGCAGAGTCTATGGAATCC-3′; to detect the γ_c knockout gene, 5′-GCTGACAGCCGGAACACGGCGG-3′ and 5′-GTGCAATCCATCTTGTTCAATGGCCG-3′; to detect the DO10 transgene, 5′-CAGGAGGGATCCAGTGCCAGC-3′ and 5′-TGGCTCTACAGTGAGTTTGGT-3′; to detect the AND transgene, 5′-GACTTGGAGATTGCCAACCCATATCT-AAGT-3′ and 5′-TGAGCCGAAGGTGTAGTCGGAGTTTG-CATT-3′.

Flow Cytometric Analysis. Cells from thymus and spleen were stained and analyzed on a FACSort® (Becton Dickinson, San Jose, CA) using Lysis II software (7). For direct staining, the following conjugated antibodies were purchased from PharMingen (San Diego, CA): anti-CD4 Cy-Chrome (H129.19), anti-CD8 PE (53-6.7), anti-CD25 PE (7D4), anti-CD44 Cy-Chrome (IM7), anti-CD45R (B220) PE (RA3-6B2), anti-CD49d FITC (R1-2), anti-CD62L PE (MEL-14), anti-CD69 PE (H1.2F3), anti-CTLA-4 PE (UC10-4F10-11), anti-erythroid cells PE (TER-119), anti-Fas biotin (Jo2), anti-I-A^b FITC (AF6-120.1), anti-I-A^d PE (AMS-32.1), anti-TCR Vα11 FITC (RR8-1), and anti-Qa2a biotin (1-9-9) (12). KJ1-26 mAb, anti-idiotype for DO10 TCR (13) (provided by Dr. B.J. Fowlkes), was purified from supernatants of hybridoma cells using protein G columns (Pharmacia, Uppsala, Sweden) and conjugated to FITC.

Splenic $CD4^+$ T Cell Purification. Since erythroblastoid cells and macrophages were increased in the spleens of γ_c-deficient mice as compared with wild type mice, single cell suspensions from spleen were treated with ACK lysing buffer to remove red cells, stained with PE-conjugated anti-CD8 mAb (53-6.7, PharMingen) and PE-conjugated anti-erythroid mAb (TER-119, PharMingen) and anti-CD16/CD32 mAb (FcγRIII/II receptor, 2.4G2, PharMingen), washed twice with 1 × PBS containing 1% BSA (PBS, 1% BSA), and applied to T cell enrichment columns (R&D Systems). The resulting cells from both wild-type and γ_c-deficient mice were 88–95% pure $CD4^+$ T cells (average 91.8%) as determined by flow cytometry.

Cell Survival Assay. Purified $CD4^+$ T cells were cultured in RPMI-1640 media supplemented with 10% charcoal-treated fetal bovine serum (Cocalico Biological, Inc., Reamstown, PA), 2 mM glutamine, and antibiotics for 8 to 48 h. Cells were harvested and stained with 5 μg/ml of propidium iodide (PI) (Boehringer Mannheim) and analyzed by flow cytometry.

Annexin V Staining. After cells were stained with anti-CD4 Cy-Chrome and washed twice with PBS, 1% BSA, cells were stained with annexin V FITC (R&D Systems) according to the instructions of the manufacturer. Cells were analyzed on a FACSort®.

TUNEL Method. The terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end–labeling (TUNEL) method was performed according to the instructions of the manufacturer (Boehringer Mannheim). Splenocytes were stained with anti-CD4 Cy-Chrome and washed with PBS, 1% BSA. Cells were then fixed with 70% ethanol for 20 min at 4°C, treated with PBS containing 1% paraformaldehyde and 0.01% Tween-20 for 30 min at 25°C, washed once with PBS, and incubated with the fluorescein-labeled TUNEL reaction mix for 60 min at 37°C. As a negative control, cells were incubated without TdT. Cells were then washed with PBS, 1% BSA and analyzed on a FACSort®.

Intracellular Staining of Bcl-2. Bcl-2 levels in $CD4^+$ T cells were analyzed as previously described (14). In brief, splenocytes (1×10^6) were permeabilized in PBS, 1% BSA containing 0.04% saponin (saponin buffer) and incubated with anti-Bcl-2 mAb (clone 3F11, PharMingen, 2 μg/ml final concentration) or purified hamster IgG as a negative control (4°C for 30 min). Cells were washed twice with saponin buffer, incubated with anti-hamster IgG cocktail conjugated to FITC (clone G70-204, G94-56, G91-140, PharMingen) (4°C for 30 min), washed once with saponin buffer and once with PBS, 1% BSA, then stained with anti-CD62L PE/and anti-CD4 Cy-Chrome, and analyzed on a FACSort®.

In Vivo Bromodeoxyuridine (BrdU) Uptake and BrdU Staining. 8 to 12-wk-old γ_c-deficient or littermate control mice were injected i.p. with 750 μg of BrdU (Sigma) in 250 μl of PBS at time 0 and again at 4 h. Control mice were injected with PBS. 16 h after the second injection, thymocytes and purified splenic $CD4^+$ T cells were fixed with 70% ethanol for 30 min on ice, denatured with 2 N HCl containing 0.5% Triton X-100 for 30 min to produce single-stranded DNA, and neutralized with 0.1 M sodium tetraborate, pH 9.0 (Sigma). Cells were resuspended in PBS, 1% BSA containing 0.5% Tween-20, stained with anti-BrdU FITC (Becton Dickinson) for 30 min at 25°C, washed once with PBS, 1% BSA containing 0.5% Tween-20, resuspended in PBS, 1% BSA, and analyzed on a FACSort®.

Apparent Rates of Replication and Survival. Assuming that cells that have taken up BrdU at 20 h have divided once, the apparent replication rate/day, *r*, can be calculated from the equation: percent BrdU uptake/20 h × 24 h/d = $2r/(100 + r)$. Based on the data in Fig. 1 *D*, this yields values for *r* of 21.43%/d for γ_c-deficient mice and 6.32%/d for wild-type mice. Assuming that no death occurs and that there is no new migration of cells from thymus to periphery (neither assumption is likely to be correct), we can estimate the number of cells that might exist after 63 d of growth (from 3–12 wk of age) as $1.3 \times 10^6 \times (1.2143)^{63} = 2.66 \times 10^{11}$ $CD4^+$ splenic T cells instead of 5.28×10^7 cells for γ_c-deficient mice and $9.1 \times 10^6 \times (1.0632)^{63} = 4.31 \times 10^8$ cells instead of 1.67×10^7 for wild-type mice. Although not accurate, these calculations suggest that survival rates for the cells differ between the wild-type and γ_c-deficient mice. Again, assuming no new migration from the thymus to periphery and a constant replication rate, the survival rate/day can be estimated as ~87% for γ_c-deficient mice and 95% for wild-type mice, suggesting that cells from the γ_c-deficient mice had an increased rate of cell death (see below).

Results and Discussion

Like humans with XSCID, γ_c-deficient mice have hypoplastic thymi and lack NK cells (5–8). However, peripheral T cells are generally not found in humans with XSCID (4), whereas peripheral $CD4^+$ T cells are found in the γ_c-deficient mice (5–8). Although these cells are diminished in numbers in γ_c-deficient mice at 3 wk of age (6, 7), in most mice there is a dramatic age-dependent accumulation of these cells (7) (Fig. 1 *A*), even though the thymus remains small (7). Analysis using a series of different TCR Vβ-specific mAbs (Vβ2, Vβ3, Vβ4, Vβ5.1,5.2, Vβ6, Vβ8.1,8.2,

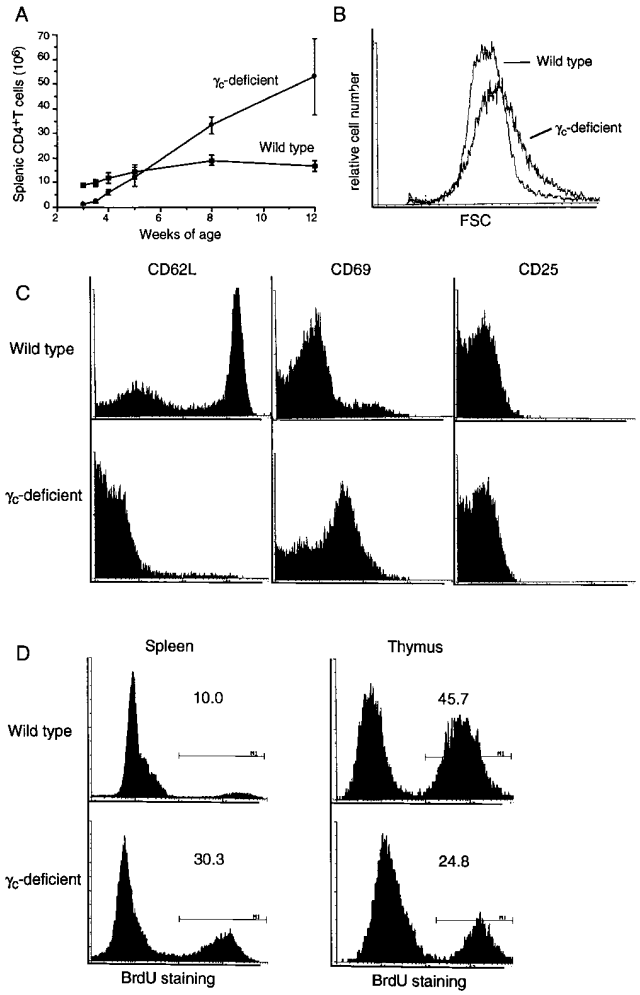

Figure 1. Activated $CD4^+$ T cells accumulate in an age-dependent fashion in γ_c-deficient mice and exhibit increased BrdU uptake. (*A*) The number of splenic $CD4^+$ T cells in γ_c-deficient (*closed diamonds*) and wild-type (*open squares*) mice was calculated as the product of the number of splenocytes and the percent of $CD4^+$ T cells, based on staining with anti-CD4 Cy-Chrome and analysis on a FACSort® (Becton Dickinson) using Lysis II software. Shown are mean ± SEM (n = 4–9 at each timepoint). (*B*) Size of $CD4^+$ T cells. Splenocytes from 6-wk-old mice were stained with anti-CD4 Cy-Chrome and PI, and cell size of viable cells was assessed by forward light scatter (*FSC*); data are shown on a linear scale. (*C*) Activation markers expressed on $CD4^+$ T cells. Splenocytes were stained with anti-CD4 Cy-Chrome and either PE conjugated anti-CD62L (L-selectin), anti-CD69, or anti-CD25. Data were gated on $CD4^+$ cells and displayed on a log scale. The data shown are representative of six experiments. (*D*) BrdU uptake of thymocytes and splenic $CD4^+$ T cells. Mice were injected twice with BrdU (see Material and Methods). 16 h after the second injection, purified splenic $CD4^+$ T cells and thymocytes were stained with anti-BrdU FITC according to the Becton Dickinson protocol and analyzed on a FACSort®. Histograms show BrdU staining (log scale); the numbers above the gate represent the mean percentage of BrdU-positive cells from four experiments. When mice were injected with PBS instead of BrdU, less than 0.1% of thymocytes and splenic $CD4^+$ T cells were stained with anti-BrdU FITC (data not shown).

Vβ10, and Vβ11) indicated that the $CD4^+$ T cell accumulation was polyclonal (unpublished data). Interestingly, the splenic $CD4^+$ T cells in γ_c-deficient mice were increased in size (Fig. 1 *B*). The large size of the cells, coupled with the finding of $CD4^+$ T cell infiltrates, particularly in the gut in association with colitis (5, 7), suggested that the $CD4^+$ T cells might be activated and involved in mediating the pathological changes found in these mice. Therefore, we investigated the basis for the $CD4^+$ T cell expansion.

A striking difference between splenic $CD4^+$ T cells from γ_c-deficient and wild type mice was that the cells from the γ_c-deficient mice exhibited a $CD62L^{low}CD69^{high}$ phenotype typical of memory/activated cells, whereas those from wild-type mice exhibited a $CD62L^{high}CD69^{low}$ phenotype typical of naive cells (Fig. 1 *C*) (15). Interestingly, $CD4^+$ T cells in mice deficient in IL-2 (16, 17), IL-2Rα (18), and IL-2Rβ (19) also exhibited activated phenotypes. Expression of CD49d (VLA-4 α chain) and CD44 was slightly increased in γ_c-deficient $CD4^+$ T cells (data not shown), whereas no significant difference was seen in expression of IL-2Rα (CD25) (Fig. 1 *C*), or in CD40L, CD28, CTLA-4, or in Fas (data not shown). The lack of increase in these activation markers suggested that these cells were partially rather than fully activated and that γ_c-dependent signals are required for completing T cell activation. In contrast with the different phenotypes in splenic T cells, $CD4^+CD8^-$ thymocytes from γ_c-deficient mice and wild type mice expressed similar levels of CD62L, CD69, and $Qa\text{-}2^a$ (data not shown).

The accumulation and activation of the $CD4^+$ T cells appeared to result from a defect in T cells, because the defect was corrected in mice in which γ_c expression was selectively reconstituted in T cells (transgenic mice in which γ_c is under control of the CD3δ enhancer and promoter) but not in mice in which γ_c expression was selectively reconstituted in B cells (transgenic mice in which γ_c is under control of the mb-1 promoter and Eμ heavy chain enhancer) (data not shown). A similar activated phenotype in Jak3-deficient mice was corrected when these mice were reconstituted with Jak3 under control of the Lck promoter (19a), a finding consistent with the similar phenotypes in mice and humans deficient in either γ_c or Jak3 and with the conclusion that Jak3 is the major mediator of γ_c-dependent signals (4, 5).

Next, we investigated whether the $CD62L^{low}CD69^{high}$ phenotype of the γ_c-deficient splenic $CD4^+$ T cells reflected active proliferation and found that bromodeoxyuridine (BrdU) uptake in these cells was greater than in wild-type mice (Fig. 1 *D*, left). In contrast, γ_c-deficient thymocytes exhibited lower BrdU uptake than wild-type thymocytes (Fig. 1 *D*, right). Thus, there was more active cell division in the spleens than in the thymi of adult γ_c-deficient mice, correlating with the activated phenotype of the γ_c-deficient splenocytes. It is important to recognize that because these mice were not subjected to thymectomy, some splenic BrdU uptake could have occurred in thymocytes before $CD4^+$ T cell migration to the periphery; however, because the thymi in γ_c-deficient mice are very small (0.5–5% of littermate controls) and the BrdU uptake in thymocytes in these mice was lower than in wild-type mice, it is likely that the majority of BrdU uptake occurred in the periphery.

Based on the BrdU uptake studies, the γ_c-deficient mice

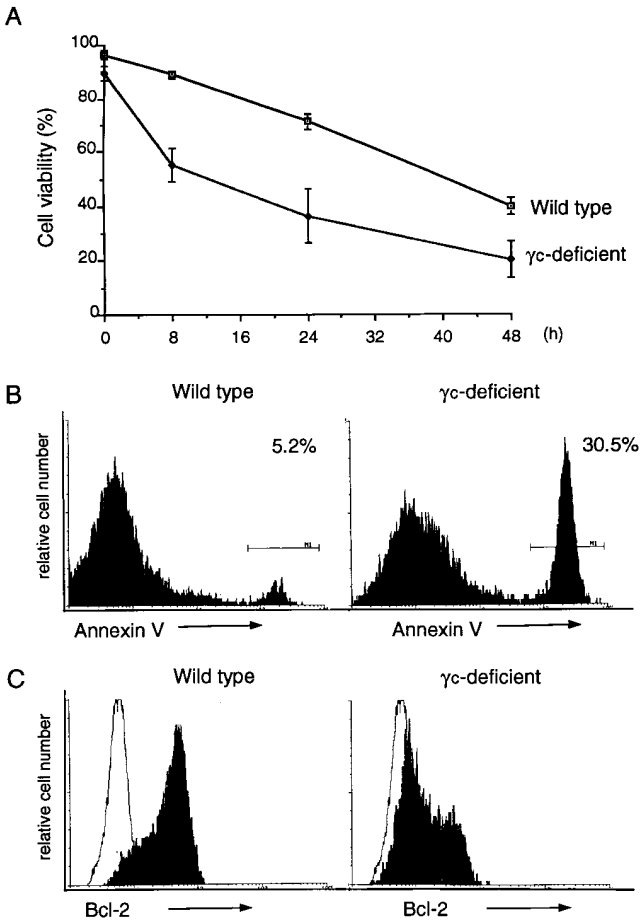

Figure 2. Augmented cell death and diminished Bcl-2 expression in γ_c-deficient $CD4^+$ T cells. (*A*) Spontaneous cell death in vitro. Purified splenic $CD4^+$ T cells from 6-wk-old mice were cultured in RPMI medium containing 10% charcoal-treated fetal bovine serum (Cocalico Biological, Inc.) for 8–48 h, harvested, and cell survival determined by staining with 5 μg/ml of PI, followed by analysis using a FACSort®. Data for each mouse were analyzed in duplicate. Shown are the means ± SD of three mice in each group. At time 0, approximately 98% of cells from wild-type and 93% of cells from γ_c-deficient mice were PI negative. (*B*) Annexin V binding to $CD4^+$ T cells. Splenocytes from 6-wk-old mice were stained with anti-CD4 Cy-Chrome and then with annexin V FITC. Annexin V binding (log scale) is shown for $CD4^+$ PI^- cells. (*C*) Bcl-2 expression in splenic $CD4^+$ T cells was analyzed approximately as previously described (14). The histograms show profiles for staining with anti-Bcl-2 (*closed histograms*) and control IgG (*open histograms*) in wild-type and γ_c-deficient mice (log scale).

had an apparent replication rate much greater than wild-type mice (21.43%/d for γ_c-deficient mice and 6.32%/d for wild-type mice; see Materials and Methods). Such an increased rate for the γ_c-deficient mice should have yielded a much greater accumulation of cells than actually seen (Fig. 1 *A*), suggesting that $CD4^+$ T cells from the γ_c-deficient mice had an increased rate of cell death (see Materials and Methods). Indeed, when cultured without stimulation, purified splenic $CD4^+$ T cells from γ_c-deficient mice exhibited a higher level of death than cells from wild-type mice, with the greatest difference occurring in the first 8 h (Fig. 2 *A*). Increased apoptosis was confirmed both by TUNEL analysis (data not shown) and by staining with annexin V (Fig. 2 *B*), which allows the detection of apoptotic cells at an early stage because of its selective affinity for phospholipids, especially phosphatidylserine (PS), which is exposed on the cell surface in apoptotic cells, while normally PS is restricted to the inner cell membrane (20–22). The rapid increase in death seen at 8 h in γ_c-deficient mice (Fig. 2 *A*) presumably is accounted for by the large number of cells that stained with annexin V at time 0 (Fig. 2 *B*), suggesting that splenic $CD4^+$ T cells of γ_c-deficient mice may be primed to die by apoptosis in vivo. Bcl-2 levels correlate with the survival of lymphoid cells (23, 24) and are induced by γ_c-dependent cytokines that protect against apoptosis (25–28). Therefore, it was interesting that γ_c-deficient splenic $CD4^+$ T cells expressed very low levels of Bcl-2 (Fig. 2 *C*) (including both $CD62L^{high}$ and $CD62L^{low}$ subpopulations; data not shown), suggesting that γ_c is required for maintaining the normal levels of Bcl-2 expression in both activated and naive peripheral $CD4^+$ T cells.

There are at least two possible explanations for why peripheral $CD4^+$ T cells in γ_c-deficient mice were already activated without exogenous stimulation. In the first model, the activated phenotype does not require TCR stimulation (TCR-independent activation model); instead, γ_c-dependent signals are required for keeping mature $CD4^+$ T cells in a naive stage and, that without such signals, these cells are activated nonspecifically. In the second model, peripheral $CD4^+$ T cells in γ_c-deficient mice respond to self- or environmental antigens (TCR-dependent activation model). We investigated these possibilities using mice expressing MHC class II–restricted transgenic TCRs specific for ovalbumin (DO10 mice) or cytochrome c (AND mice). In these experiments, the mice were not exposed to the specific antigens. In DO10 male mice deficient in γ_c ($DO10^+\gamma_c^{-/Y}$ mice), far fewer splenocytes were found than in $DO10^-\gamma_c^{-/Y}$ mice (6.4×10^6 versus 79×10^6 cells/spleen) (Fig. 3 *A*). Analogous differences were also seen between $AND^+\gamma_c^{-/Y}$ and $AND^-\gamma_c^{-/Y}$ mice (2.7×10^6 versus 50×10^6 cells/spleen). These data suggested that endogenous TCR expression might be important in regulating $CD4^+$ T cell accumulation. Using the $DO10^+\gamma_c^{-/Y}$ mice, we compared the relative frequency of memory ($CD62L^{low}$) and naive ($CD62L^{high}$) phenotypes in splenic $CD4^+$ T cells expressing transgenic ($KJ1\text{-}26^+$) versus endogenous ($KJ1\text{-}26^-$) TCRs. Approximately 70% of $CD4^+$ T cells from $DO10^+\gamma_c^{-/Y}$ mice express the transgenic TCR (Fig. 3 *B*, fourth histogram), analogous to $DO10^+\gamma_c^{+/Y}$ mice (second histogram). Surprisingly, although relatively high numbers of $KJ1\text{-}26^+CD4^+$ T cells in $DO10^+\gamma_c^{-/Y}$ mice were $CD62L^{high}$ (naive phenotype), similar to the finding for $DO10^+\gamma_c^{+/Y}$ mice (84.9% versus 84.6%) (Fig. 3 *C*, bottom), relatively few of the endogenous $KJ1\text{-}26^-$ $CD4^+$ T cells in $DO10^+\gamma_c^{-/Y}$ mice were $CD62L^{high}$ (10%), similar to the finding for $DO10^-\gamma_c^{-/Y}$ mice (8.3%) (Fig. 3 *C*, top). Thus, the $CD62L^{low}$ phenotype correlated with the presence of endogenous TCRs. Analogous results were found in AND TCR transgenic γ_c-deficient ($AND^+\gamma_c^{-/Y}$) mice. In $AND^+\gamma_c^{-/Y}$ mice,

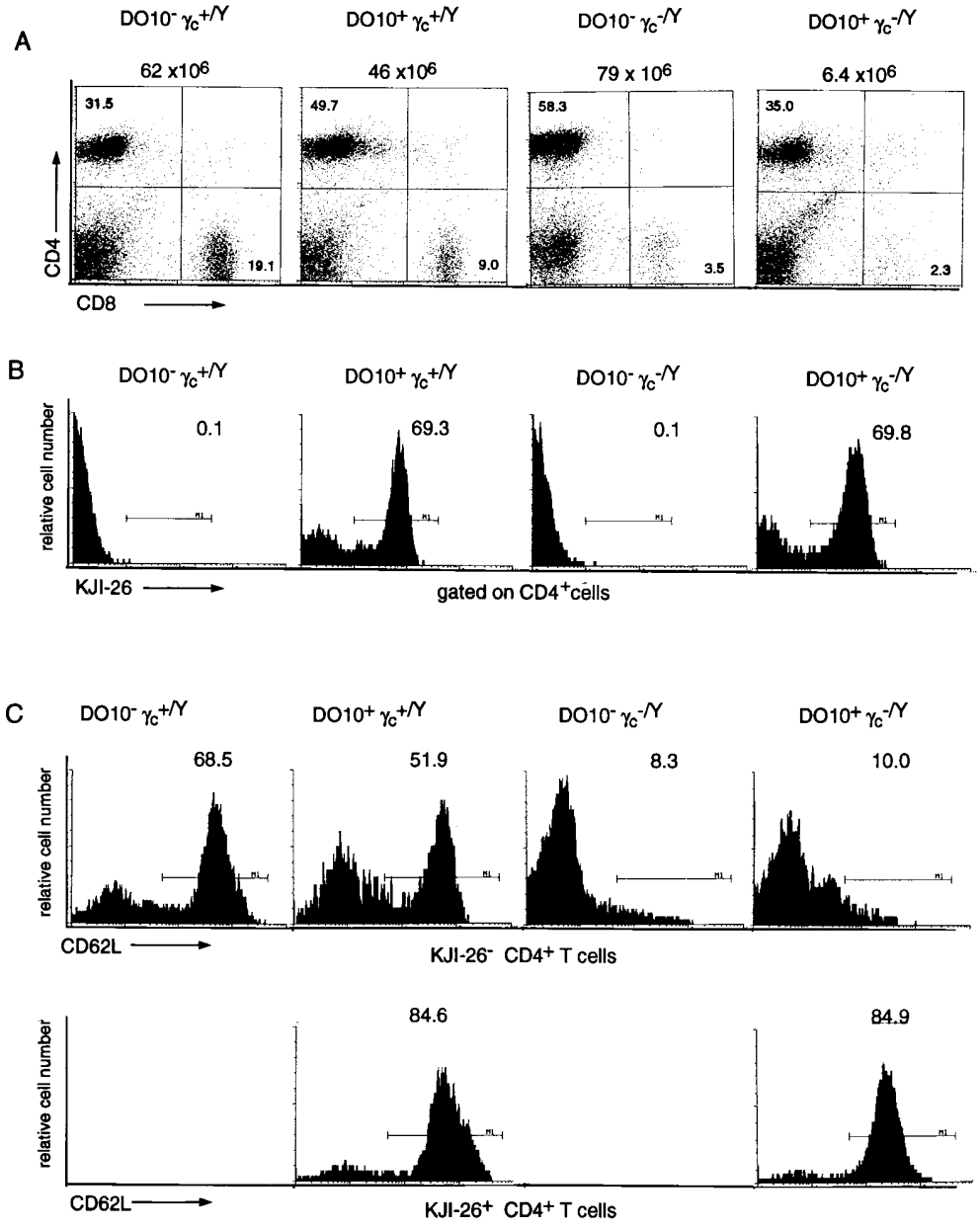

Figure 3. Activation of splenic $CD4^+$ T cells in γ_c-deficient mice is TCR dependent. γ_c-deficient mice were back-crossed to BALB/c mice ($H\text{-}2^{d/d}$, Jackson Laboratory) for three generations. Since the γ_c gene is located on the X chromosome, DO10 TCR transgenic male mice ($H\text{-}2^{d/d}$) were mated with BALB/c $\gamma_c^{+/-}$ heterozygous female mice ($H\text{-}2^{d/d}$). These matings yielded four genotypes (assessed by PCR of tail DNA) of male mice ($DO10^-\gamma_c^{+/Y}$, $DO10^+\gamma_c^{+/Y}$, $DO10^-\gamma_c^{-/Y}$, and $DO10^+\gamma_c^{-/Y}$) (*A*). In (*A*), also shown are the total number and CD4/CD8 staining of splenocytes from 5-wk-old mice. As previously reported (32), $DO10^+$ male mice expressing wild-type γ_c ($DO10^+\gamma_c^{+/Y}$ mice) exhibit an increased CD4/CD8 ratio as compared with $DO10^-\gamma_c^{+/Y}$ mice (first two panels), but consistent with the increased CD4/CD8 ratios previously observed in γ_c-deficient mice (6, 7), $DO10^+\gamma_c^{-/Y}$ mice exhibited an even greater CD4/CD8 ratio (fourth panel). Results are representative of three separate experiments. (*B*) Histograms of KJ1-26 mAb (anti-idiotype TCR) staining (gated on $CD4^+$ T cells). (*C*) Splenocytes were stained by three color flow cytometric analysis for expression of KJ1-26, CD62L, and CD4. Data were gated on KJ1-26^- $CD4^+$ (endogenous TCR) (*top histogram*) or KJ1-26^+CD4^+ (transgenic TCR) (*bottom histogram*) T cells. The histograms show profiles for CD62L expression (log scale); the percent of $CD62L^{high}$ cells is indicated.

93% of transgenic TCR^+ ($V\alpha11^+$) $CD4^+$ T cells were $CD62L^{high}$, similar to $AND^+\gamma_c^{+/Y}$ mice (94%). These results support the TCR-dependent activation model and suggested that TCR(s) expressed on expanding peripheral $CD4^+$ T cells in γ_c-deficient mice are specific for self-antigen or environmental antigens. The finding that over 80% of adult γ_c-deficient mice (C57BL/6 background) develop an inflammatory bowel disease (data not shown) is consistent with the possibility of self-reactive $CD4^+$ T cells in these mice.

In the immune response, there is a careful balance between clonal expansion and clonal deletion (29–31). Clonal expansion is believed to be regulated by mitogenic cytokines, especially IL-2. In γ_c-deficient mice, it was therefore surprising that although splenic T cells exhibit diminished in vitro responsiveness to mitogens (5), there was TCR-dependent activation and a marked expansion of $CD4^+$ T cells in vivo. In addition to this in vivo proliferation, we also detected an augmented rate of apoptosis in γ_c-deficient $CD4^+$ T cells, a finding consistent with the known action of γ_c-dependent cytokines as survival factors (25–28); nevertheless, it was evident that the overall rate of death was inadequate for the degree of proliferation. Although the $CD4^+$ T cells were activated, they expressed only low levels of CD25, CTLA-4, and Fas. Therefore, it is conceivable that γ_c-dependent signals are required to achieve induction of pathways that are required to negatively regulate immune responses. Thus, γ_c-dependent signals are essential components of a carefully controlled homeostatic mechanism that regulates the fate of activated cells.

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