Lymphocyte Accumulation in the Spleen of Retinoic Acid Receptor-Related Orphan Receptor γ-Deficient Mice

Nu Zhang, Jian Guo and You-Wen He

*J Immunol* 2003; 171:1667-1675; doi: 10.4049/jimmunol.171.4.1667

http://www.jimmunol.org/content/171/4/1667

---

**References**

This article cites 33 articles, 16 of which you can access for free at:

http://www.jimmunol.org/content/171/4/1667.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
Lymphocyte Accumulation in the Spleen of Retinoic Acid Receptor-Related Orphan Receptor γ-Deficient Mice

Nu Zhang, Jian Guo, and You-Wen He

The hormone nuclear receptor retinoic acid receptor-related orphan receptor γ (RORγ) plays important roles in thymocyte development and lymphoid organogenesis. RORγ and its thymus-specific isoform RORγt are expressed in the thymus, but not in the spleen and bone marrow (BM). However, RORγ−/− mice have 2- to 3-fold more splenocytes than wild-type controls due to an accumulation of conventional resting B lymphocytes. The increase in B lymphocytes in RORγ−/− mice is caused neither by abnormal B cell development in the BM nor by an obvious defect in the peripheral T cell compartment. Furthermore, analyses of BM chimeras using either RORγ−/− or recombinase-activating gene-2−/− mice as recipients and wild-type or RORγ−/− mice as donors, respectively, demonstrate that the splenic microenvironment of RORγ−/− mice is defective, since wild-type T and B lymphocytes accumulated in these chimeric mice. In addition, T lymphocyte homeostasis was altered due to a lowered thymic output in RORγ−/− mice. Collectively, these results suggest that RORγ regulates lymphocyte homeostasis at multiple levels. The Journal of Immunology, 2003, 171: 1667–1675.

A n appropriate homeostasis of lymphocyte populations is essential for the maintenance of an intact immune system. Many different mechanisms, including control of the rate of production, division of mature cells, homing, and cell death, have been shown to regulate lymphocyte homeostasis (1–5). A large body of evidence suggests that the homeostasis of different lymphocyte populations, i.e., T vs B cells or naive vs memory cells, is regulated independently from each other (4). This is because different types of lymphocytes may occupy different niches and use different resources in secondary lymphoid organs. As a part of the homeostatic process, mature lymphocytes recirculate continuously from blood to tissue and back to blood again. Naïve lymphocytes have a distinct trafficking pattern from that of memory/effector cells. Naïve cells recirculate through secondary lymphoid tissues, while memory/effector cells home to both secondary lymphoid organs as well as nonlymphoid tissues (1).

Secondary lymphoid organs such as spleen, lymph nodes (LNs), and Peyer’s patches (PPs) are located at strategic sites to capture and concentrate foreign Ags (6). Their major function is understandably to initiate a productive immune response. Several reports using various mouse models lacking some secondary lymphoid organs demonstrate that these organs may be involved in delayed-type hypersensitivity responses, antiviral immunity, and allograft rejection (7–9). Recently, the role of secondary lymphoid organs has been investigated in the aly/aly mouse model (10). The aly/aly mice lack LNs and PPs due to a mutation in the NF-κB-inducing kinase (11, 12). The naïve CD4+ T cell population in aly/aly and splenectomized aly/aly mice cannot be maintained in the absence of thymic production, suggesting that the circulation of naive CD4+ T cells through secondary lymphoid organs is required for their survival (10).

The orphan nuclear receptor RORγ and its thymus-specific isoform, RORγt, play important roles in thymocyte development (13, 14). RORγ and RORγt differ from each other only in their 5′ exons. Both isoforms are predominantly expressed in CD4+CD8− double-positive (DP) thymocytes (15). Thymocyte maturation depends on the down-regulation of this orphan receptor in CD4+ or CD8+ single-positive (SP) stages (16). Mice lacking both RORγ and RORγt (referred to hereafter as RORγ−/− mice) due to the deletion of the common DNA binding domain show severe impairment in thymocyte maturation (17, 18). The production of mature CD4+ and CD8+ thymocytes in RORγ−/− mice is reduced by 90%. Thymocyte development is impaired at the immature SP to DP transition in RORγ−/− mice (19). Furthermore, DP thymocytes in RORγ−/− mice have dramatically decreased the expression of the antiapoptotic protein Bcl-xL and undergo massive apoptosis (17, 18).

RORγ also plays critical roles in the development of secondary lymphoid organs. RORγ−/− mice lack all LNs and PPs. In addition, the putative lymphoid organ precursors expressing CD45+CD4+CD3− are not detected in fetal mesentery and intestines of RORγ−/− mice, indicating an early role of RORγ in lymphoid organogenesis (17, 18). In analyzing the peripheral lymphoid compartments of RORγ−/− mice, we found that the mutant mice have an enlarged spleen. We found that the increased splenic cellularity is primarily due to an accumulation of conventional resting B cells. Bone marrow (BM) chimeric studies suggest that the altered lymphocyte homeostasis appears to reflect a defect(s) in the host microenvironment.

Materials and Methods

Mice

RORγ−/− mice (17), back-crossed onto a C57BL/6 background for five generations, were housed in a specific pathogen-free facility at the Duke University vivarium. Heterozygous mice were bred to generate homozygous mutant mice and control wild-type or heterozygous littermates. RAG-2−/−/Ly5.2 and C57BL/6Ly5.2 mice were provided by Dr. M. Kondo (Duke University Medical Center) and were similarly housed. All mice...
were used at 3–14 wk of age as indicated. Animal usage was conducted according to protocols approved by the Duke University institutional animal care and use committee.

**Flow cytometric analyses**

Blood were taken retro-orbitally and lysed of RBC using FACS Lysing Solution (BD Biosciences, San Jose, CA). Cells were sequentially incubated with an excess of an FcR blocker (2.4G2); biotinylated mAb; and PE-streptavidin, FITC-, CyChrome-, or APC-labeled Abs on ice and washed with PBS containing 2% FCS. Data for 1–5 × 10^6 cells were collected on a FACScan flow cytometer (BD Biosciences) and analyzed using CellQuest software. The following FITC-, PE-, biotin-, or CyChrome-labeled mAbs were purchased from either eBioscience or BD PharMingen (San Diego, CA): anti-CD3, CD4, CD8, CD5, CD6, CD11C, Ly6C, CD122, CD62L, AA4.1, and allophycocyanin-streptavidin.

**BM transfer**

BM transfer was performed as previously described (20). BM cells from C57BL/6Ly5.2 and RORγt−/− mice were depleted of T cells by biotin-anti-CD3, followed by streptavidin-magnet beads. Cells were transferred i.v. into the indicated hosts within 24 h after lethal irradiation (950 rad). Mice were injected i.p. with 1 ml of PBS or PBS containing 10^8 PHA cells in the spleen of RORγt−/− mice.

**Immunization and immunofluorescence**

Mice were injected i.p. with 1 ml of PBS or PBS containing 1 × 10^8 SRBC. Ten days later, serum was collected by retro-orbital bleeding, and mice were sacrificed. Freshly removed spleens were immersed in TissueTek OCT compound (Sakura Finetek U.S.A., Torrance, CA) and snap-frozen in dry ice. Tissue sections of 5-μm thickness were cut in a cryostat, placed on siliconized glass slides, air-dried, fixed in cold (−20°C) acetone for 15 min, and stored at −20°C. Sections were blocked with BSA and stained with biotin-anti-B220 mAb diluted in PBS containing 1% BSA and 5% FCS in a dark moist chamber for 30 min at room temperature. After washing three times, sections were incubated with rhodamine-conjugated avidin (Molecular Probes, Eugene, OR) and FITC-anti-CD4 or FITC-pea-nut agglutinin (Vector Laboratories, Burlingame, CA) for 50 min and examined under a fluorescence microscope.

**Results**

**Increased splenic cellularity in RORγt−/− mice**

RORγt−/− mice had an ~10-fold reduction of mature CD4^+ and CD8^+ SP thymocytes due to an impairment in the immature SP to DP transition and massive apoptosis of DP thymocytes (17, 19). However, we observed that the spleens of RORγt−/− mice were larger than those of littermate controls (Fig. 1A). On the average, the spleens of RORγt−/− mice contained 2- to 3-fold more cells than controls (Fig. 1B). The increase in splenic cellularity in RORγt−/− mice was evident as early as 3–4 wk after birth and remained constant through 12–14 wk of age (Fig. 1B). We determined the cellular components in the spleen of RORγt−/− mice with surface markers for T, B, and myeloid cells using FACS analyses. The number of B lymphocytes in the mutant mice was increased from an average of 48 × 10^6 to 148 × 10^6 compared to littermate controls. Total cell numbers obtained from spleens of RORγt−/− mice were 2- to 3-fold more cells than controls (Fig. 1B).

**FIGURE 1.** Increased splenic cellularity in RORγt−/− mice. A. Comparison of spleen size between a 3-wk-old RORγt−/− mouse and a littermate control mouse. B. Total cell numbers obtained from spleens of RORγt−/− and littermate controls. Single-cell suspensions from spleens of mutant and control mice at the indicated ages were prepared, and cell number was determined by trypan blue exclusion. C–G, Total number of B220^+ , CD4^+ , CD8^+ , and Mac-1^+ cells in the spleen of RORγt−/− and littermate control mice. Single-cell suspension of spleens from mutant and control mice were labeled and analyzed by FACS. Total numbers were derived by multiplying the percentage of cells by the total splenic cellularity. *, p < 0.05; **, p < 0.01.
with littermate controls and remained significantly higher in all groups of different ages (Fig. 1C). In contrast, the numbers of CD4+ and CD8+ T lymphocytes and Mac-1+ myeloid cells were only marginally increased in a majority of the mutant mice and were significantly increased in a few groups (Fig. 1, D–F). In addition, RORγ−/− heterozygote mice contained comparable numbers of total splenocytes and other subsets as those in wild-type controls (+/+), suggesting that there is no gene dosage effect on the accumulation of B cells in the spleen by RORγ (Fig. 1G). These data demonstrate that B lymphocytes are the major cellular component contributing to the enlarged spleen in RORγ−/− mice.

To fully characterize the homeostasis of the lymphoid and myeloid compartments, we analyzed blood cells in RORγ−/− mice. The number of nucleated cells in the blood of RORγ−/− mice was slightly reduced at the age of 5 wk and was significantly reduced at the age of 10 wk (Fig. 2A). This reduction was primarily due to a decreased number of CD4+ and CD8+ T lymphocytes (Fig. 2, B and C). In contrast to the increased number of splenic B cells, peripheral blood of RORγ−/− mice contained a slightly reduced number of B lymphocytes and Mac-1+ myeloid cells (Fig. 2, D and E). Similar to the data for spleen, no difference was observed in the numbers of PBL from heterozygote and wild-type mice (Fig. 2F). These results show that lymphocyte numbers in the spleen and blood of RORγ−/− mice were skewed differentially and indicate that the homeostasis of both T and B lymphocytes was perturbed in RORγ−/− mice.

**B cell maturation in RORγ−/− mice**

One explanation for the increase in splenic B lymphocytes in RORγ−/− mice is that B cell development was affected by the deletion of RORγγt, even though B cells themselves express neither RORγ nor RORγγt (15). We first examined B cell maturation in the BM of RORγ−/− mice. The total numbers of nucleated cells and B cells at different stages of development in the BM of the mutant mice were comparable to those in littermate controls (Fig. 3A). Furthermore, no difference was observed for B cell development in older mutant and control mice (Fig. 3B). B lymphocyte subsets defined by the expression of B220, CD43, and IgM were similarly detected in the BM of both RORγ−/− and control mice, indicating that early B cell differentiation in the BM of RORγ−/− mice is not obviously altered (Fig. 3C).

B lymphocyte maturation was further investigated in the spleen of RORγ−/− mice. The percentage of B220+ IgM− cells in the mutant mice was slightly higher than that in the control mice (Fig. 4A). The percentage of follicular B cells (CD21intCD23int) in RORγ−/− spleen was slightly, but consistently, higher, while that of marginal zone B cells (CD21highCD23−) was at a comparable level compared with controls (Fig. 4A). B lymphocytes in the mutant mice expressed similar levels of activation markers, including CD44, CD69, CD80, and MHC class II to those on control B cells (Fig. 4B). Importantly, the expression of CD62L on these B cells was normal as well (Fig. 4B). In addition, no abnormal expansion of some minor subpopulations, such as the B220+ plasmacytoid dendritic cells (22) and the CD5− B-1 B cells, in the mutant mice was observed (Fig. 4, C and D). A recent report using B220/AA4.1/CD23/IgM to define peripheral B cell maturation showed that multiple selection points may exist among immature peripheral B cells (23). These transitional B cell subsets as defined by AA4+CD23−slgMhigh, AA4+CD23+slgMhigh, and AA4+CD23−slgMlow were similarly detected in the spleen of RORγ−/− and control mice (Fig. 5A).

![Figure 2](image-url)  
**Figure 2.** Decreased T cell populations in the peripheral blood of RORγ−/− mice. A, Total number of circulating nucleated cells in RORγ−/− and control mice. Erythrocytes were lysed, and the numbers of nucleated cells were counted and expressed as number per microliter. B–F, Numbers of CD4+, CD8+, B220+, and Mac-1+ cells in the blood of RORγ−/− and littermate control mice. Peripheral blood was labeled and analyzed by FACS as described in Fig. 1. *, p < 0.05; **, p < 0.01.
Collectively, these results demonstrate that B lymphocyte development in the BM and spleen of RORγ−/− mice was apparently normal, and that the majority of splenic B lymphocytes in the mutant mice were resting conventional B lineage cells.

**Phenotype of peripheral T cells in RORγ−/− mice**

The accumulation of resting B cells in the spleen of RORγ−/− mice may be caused by the lack of LNs and PPs. However, B lymphocyte homeostasis in other genetically manipulated mouse models lacking secondary lymphoid organs suggests that the lack of LNs and PPs alone could not result in a 3-fold increase in the number of splenic B cells (24, 25). Thus, the perturbed homeostasis of B cells may be due to a secondary effect from an altered T lymphocyte development and/or function in the mutant mice. To address this issue, we first analyzed thymocyte-negative selection by crossing the H-Y TCR Tg (26) mice into the RORγ−/− model. Importantly, the number of splenic B cells was not reduced compared with littermate controls/H9253 blood of RORγ−/− mutant mice were resting conventional B lineage cells.

**Normal splenic structure and B cell function in RORγ−/− mice**

We next examined the splenic structure of RORγ−/− mice. Consistent with the increased B cells in RORγ−/− mice, the spleen of RORγ−/− mice had larger B cell follicles (Fig. 6A). The majority of T lymphocytes were localized in the T cell zones, with some T cells being present in the B cell follicles in the RORγ−/− spleen (Fig. 6A). To determine whether the formation of follicular dendritic cell clusters and germinal centers (GC) is normal in RORγ−/− mice, we immunized mutant and control mice with SRBC by i.p. injection. Seven to 10 days later the spleen was harvested and examined for GC formation. As shown in Fig. 6A, the number and size of GCs in the spleens of RORγ−/− mice were comparable to those in the control mice. Furthermore, anti-SRBC Ab production in RORγ−/− mice after the primary immunization was comparable to that in control mice (Fig. 6B). Taken together, these results demonstrate that B cells in RORγ−/− mice can be induced to form GC and generate a normal Ab response.

**Lymphocyte homeostasis in BM chimeras**

To examine whether lymphocyte accumulation in RORγ−/− spleens is due to a defective host environment rather than intrinsic defects in T or B lymphocyte compartments, we generated BM chimeric mice using either RORγ−/− or RAG-2−/−Ly5.2 mice as recipients. The CD45 congenic marker was used to distinguish between donor- and host-derived cells. Lethally irradiated RORγ−/− mice receiving BM from normal C57BL/6Ly5.2 mice had significantly more donor-derived cells in the spleen than similarly reconstituted littermate control recipients 6–12 wk after BM transfer (Fig. 7A). Interestingly, the same was true for T lymphocytes, whereas Mac-1+ donor-derived cells were at comparable

If you require any further assistance or have more questions, please let me know!
levels in RORγ−/− and control recipients (Fig. 7A). This is in contrast with the spleens of unmanipulated RORγ−/− mice, in which T lymphocyte numbers were only slightly higher than in controls (Fig. 1), suggesting that an increased output from the thymus of RORγ−/− mice reconstituted with normal BM may account for the increase in T lymphocytes in the spleen of RORγ−/− mice. Moreover, in contrast to the lowered number of T cells in the blood of RORγ−/− mice, T lymphocytes in the peripheral blood of these BM chimeric mice were comparable to those in control chimeras (Fig. 7B), further supporting the role of thymic output in regulating the homeostasis of peripheral T cells.

We next generated BM chimeras using RAG-2−/−Ly5.2 or C57BL/6Ly5.2 mice as recipients. BM from RORγ−/− and littermate control mice had similar numbers of T and B lymphocytes in the spleen of RORγ−/− BM. We found that both T and B lymphocyte homeostasis was perturbed in the spleen and BM of normal mice. We observed several


Discussion
In this report we investigated lymphocyte homeostasis in mice lacking the orphan nuclear receptor RORγ and RORγt. We found that both T and B lymphocyte homeostasis was perturbed in RORγ−/− mice. This is unexpected, since RORγt is not detectable in the spleen and BM of normal mice. We observed several
defects in the homeostasis of T and B cell compartments. First, the number of T lymphocytes in peripheral blood of the mutant mice was significantly reduced, while the number of T cells in the spleen was comparable to that in controls. Second, the number of B lymphocytes in the spleen of RORγ−/− mice was 2- to 3-fold greater than that in controls, while B cells in the blood were at a comparable level as controls.

What are the underlying mechanisms for the perturbed homeostatic pattern of T and B lymphocytes in RORγ−/− mice? Several explanations could account for these defects. First, the lack of LNs and PPs in RORγ−/− mice could result in a loss of niches for T and B lymphocytes. As a consequence, these lymphocytes might migrate to the spleen. However, several other mouse models lacking LNs and PPs do not have significantly increased splenic B lymphocytes, arguing against such an idea (24, 25). In addition, CD62L-deficient mice, in which lymphocytes lack the capability to migrate into LNs, have only a 30% increase in splenic cellularity, suggesting that even if all LN and PP B cells find their way to the spleen, they can not account for the 2- to 3-fold increase in B cells in RORγ−/− mice (28, 29). Second, splenic B cell accumulation could be due to an indirect effect of T cells on B cell development. This is unlikely based on the fact that B lymphocyte development proceeds apparently normally in the BM and spleen of mutant mice. These mice do not have any sign of lymphoproliferative disease or defects in the deletion of autoreactive TCR+ T cells. Furthermore, B cells derived from normal C57BL/6 BM also accumulated in increased numbers in RORγ−/− spleen, suggesting that the accumulation of B cells is not intrinsic to RORγ−/− BM. Third, the increased B cells in the spleen of RORγ−/− mice could reflect a defect(s) in the splenic microenvironment. Consistent with this, B lymphocytes derived from C57BL/6 mice accumulated in the spleen of RORγ−/− mice, while the number of B cells derived from RORγ−/− BM was not increased in the spleens of RAG-2−/− or C57BL/6 hosts. Furthermore, T cells in the spleen of RORγ−/− mice are retained longer based on the observations that RORγ−/− mice receiving normal C57BL/6 BM have increased CD4+ and CD8+ T cells in the spleen.

The exact defect(s) in the splenic microenvironment of RORγ−/− mice remains unclear. Lymphocyte migration is actively controlled by multiple factors, including adhesion molecules and chemokines (1, 3). Investigations of the migration of T and B cells into secondary lymphoid organs have shed important insights into the molecular mechanisms that regulate these processes. However, how lymphocytes migrate out of secondary lymphoid organs is largely unknown. Recent work has suggested that chemokines and their receptors play roles in the exit of mature thymocytes into the periphery (30, 31). This chemorepulsion process has been termed fugetaxis (31). It is conceivable that peripheral naive and memory lymphocytes may require signals for efficient exit from the spleen or LN. These signals would greatly facilitate the movement of these lymphocytes and therefore the recirculation of the lymphocyte pools. The splenic structure in RORγ−/− mice is not
grossly altered at the level of our analysis. However, the analysis may not be sensitive enough to detect defects in splenic stromal elements. Since RORγt expression is not detected in the normal spleen (15), one might speculate that RORγt is required for the normal development of some splenic stromal elements at the primitive stage of spleen organ formation. In support of this, RORγt is detected in the putative CD4+CD45−CD3− lymphoid organ precursors but not in LNs (15, 17). The fact that both T and B cells accumulate in the spleen of RORγt−/− mice suggests that the efficient exit of these cells from the spleen may be regulated by a common mechanism.

The number of peripheral T cells is regulated by RORγt at the production level in the thymus. The significantly reduced numbers of CD4+ and CD8+ T cells in peripheral blood of RORγt−/− mice is probably due to a 90% reduction in thymocyte production, since the low numbers of these cells are readily corrected by adaptive transfer of normal BM. A recent report using thymectomized aly/aly mice shows that the maintenance of naive CD4+, but not CD8+, T cell pools depend on LNs and PPs (10). In comparison, the numbers of both CD4+ and CD8+ T cells in the blood of RORγt−/− mice are significantly reduced. The differences in the maintenance of T cell pools between RORγt−/− and aly/aly mice may relate to a difference in splenic structure (8, 11).

The defects in lymphoid organogenesis in RORγt−/− mice are most similar to those in mice lacking the helix-loop-helix protein Id2. Both mouse strains lack LNs and PPs and have a relatively normal splenic structure (17, 32). Both strains can produce normal levels of anti-SRBC Abs (Fig. 6) (32). However, Id2−/− mice have increased numbers of mature B cells, while the marginal zone B cell population is almost absent in the spleen (33). In contrast to the disturbed B cell homeostasis in RORγt−/− mice, the defect in B cell differentiation in Id2−/− mice is intrinsic, since Id2 is expressed in developing B cells (33).

In conclusion, our results demonstrate that RORγt regulates T and B lymphocyte homeostasis at multiple levels. The accumulation of lymphocytes in the spleen of mutant mice raises the possibility that RORγt regulates the efficient exit of lymphocytes from

FIGURE 6. Splenic structure and Ab production after immunization in RORγt−/− mice. A, Immunofluorescence staining of spleen sections with or without immunization with SRBC. Tissue sections were labeled with Abs as indicated and examined under a fluorescence microscopy. B, Ab production in RORγt−/− mice 10 days after immunization of SRBC cells. SRBC-specific IgG was measured using ELISA. Data represent the mean ± SEM of triplicate determinations from three mice in each group. RU, relative units.
and B220/H11001 for investigating this process.

2 chimeric mice. Lethally irradiated ROR/B220, CD4+, CD8+, and Mac-1+ cells in the peripheral blood of RORγ−/− and RORγ+−/− mice. C. Numbers of donor-derived total splenocytes, B220+, CD4+, CD8+, and Mac-1+ cells in the spleen of RAG-2−/− or C57BL/6 mice that received RORγ−/− BM. RAG-2−/− Ly5-2 or C57BL/6Ly5.2 mice were used as recipients, and RORγ−/− and RORγ+−/− mice were BM donors. Mice were analyzed 6–14 wk after BM transfer. Data from RAG-2−/− Ly5.2 and C57BL/6Ly5.2 recipients were grouped together, since no difference was observed between these two groups.

FIGURE 7. Lymphocyte homeostasis in BM chimeric mice. A. Numbers of donor-derived T and B cells in the spleen of RORγ−/−/C57BL/6 BM chimeric mice. Lethally irradiated RORγ−/− and RORγ+−/− mice were adaptively transferred with BM of C57BL/6Ly5.2 mice. Six to 12 wk later, the donor-derived lymphocyte compartment was analyzed by FACS using anti-Ly5.2 mAb. ** p < 0.01. B. Numbers of donor-derived total nucleated cells and B220+, CD4+, CD8+, and Mac-1+ cells in the peripheral blood of RORγ−/− and RORγ+−/− mice. C. Numbers of donor-derived total splenocytes, B220+, CD4+, CD8+, and Mac-1+ cells in the spleen of RAG-2−/− or C57BL/6 mice that received RORγ−/− BM. RAG-2−/− Ly5-2 or C57BL/6Ly5.2 mice were used as recipients, and RORγ−/− and RORγ+−/− mice were BM donors. Mice were analyzed 6–14 wk after BM transfer. Data from RAG-2−/− Ly5.2 and C57BL/6Ly5.2 recipients were grouped together, since no difference was observed between these two groups.

References

the spleen. RORγ-deficient mice may represent an excellent model for investigating this process.

Acknowledgments
We thank Dr. Mike Cook in the Flow Cytometry Facility of Duke University Medical Center for FACS analysis, Dr. Garnett Kelsoe’s laboratory and Hongmei Li for help with histology, and Drs. Michael Krangel and Douglas Steeber for critical review of this manuscript.


