

# Critical Role for the Common Cytokine Receptor $\gamma$ Chain in Intrathymic and Peripheral T Cell Selection

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## Summary

The common cytokine receptor  $\gamma$  chain ( $\gamma_c$ ), which is a functional subunit of the receptors for interleukins (IL)-2, -4, -7, -9 and -15, plays an important role in lymphoid development. Inactivation of this molecule in mice leads to abnormal T cell lymphopoiesis characterized by thymic hypoplasia and reduced numbers of peripheral T cells. To determine whether T cell development in the absence of  $\gamma_c$  is associated with alterations of intrathymic and peripheral T cell selection, we have analyzed  $\gamma_c$ -deficient mice made transgenic for the male-specific T cell receptor (TCR) HY (HY/ $\gamma_c^-$  mice). In HY/ $\gamma_c^-$  male mice, negative selection of autoreactive thymocytes was not diminished; however, peripheral T cells expressing transgenic TCR- $\alpha$  and - $\beta$  chains (TCR- $\alpha_T/\beta_T$ ) were absent, and extrathymic T cell development was completely abrogated. In HY/ $\gamma_c^-$  female mice, the expression of the transgenic TCR partially reversed the profound thymic hypoplasia observed in nontransgenic  $\gamma_c^-$  mice, generating increased numbers of thymocytes of all subsets, particularly the TCR- $\alpha_T/\beta_T$  CD8<sup>+</sup> single-positive thymocytes. Despite efficient positive selection, however, naive CD8<sup>+</sup> TCR- $\alpha_T/\beta_T$  T cells were severely reduced in the peripheral lymphoid organs of HY/ $\gamma_c^-$  female mice. These results not only underscore the indispensable role of  $\gamma_c$  in thymocyte development, but also demonstrate the critical role of  $\gamma_c$  in the maintenance and/or expansion of peripheral T cell pools.

Cytokines play an important role during both intrathymic T cell development and postthymic T cell functioning (for review see reference 1). The earliest committed T cell lineage precursors in the thymus, the pro- and pre-T cells (CD3<sup>-</sup>CD4<sup>-</sup>CD8<sup>-</sup> or triple negative [TN]<sup>1</sup> cells), define a compartment characterized by extensive proliferation and cellular expansion. TN thymocytes are responsive in vitro to multiple cytokines, including IL-1, -3, -6, -7, and -12, stem cell factor, flt-3 ligand, and TNF- $\alpha$ , which are produced by thymic stromal cells (1, 2). Thymocyte proliferation continues at the next developmental stage, which is characterized by the surface expression of both the CD4 and CD8 proteins associated with the TCR- $\alpha/\beta$  heterodimer. CD4<sup>+</sup>CD8<sup>+</sup>, or double-positive (DP) thymocytes undergo positive and negative selection (3, 4), during which the vast majority of DP cells die via apoptosis (5). The resultant CD4<sup>+</sup> or CD8<sup>+</sup> single-positive (SP) TCR-

$\alpha/\beta^+$  cells exit the thymus to seed the secondary lymphoid organs as immunocompetent, mature T cells. TCR- $\gamma/\delta$  T cells also derive from TN cells in the thymus, although their developmental pathway and repertoire selection mechanisms are less well defined (6). Peripheral T cells are responsive to multiple cytokines, including but not limited to IL-2, -4, -7, and -12 (1), which are produced in their immediate microenvironment.

Cytokines exert their functions by interactions with specific receptors expressed on the surface of responding cells. The common cytokine receptor  $\gamma$  chain ( $\gamma_c$ ) occupies a unique place in the hierarchy of cytokine receptors, as it forms a functional component of the receptors for IL-2, -4, -7, -9, and -15 (7-10). In humans, mutations in the  $\gamma_c$  gene are found in X-linked severe combined immunodeficiency (SCIDX1; 7), a disease characterized by an early block in thymocyte development and the absence of circulating mature T cells (11). We have recently generated mice with a targeted deletion in the  $\gamma_c$  gene (12). These mice are also immunodeficient, but in contrast to typical SCIDX1 patients, the block in thymopoiesis is only partial, and peripheral mature T cells are detected. Hence T cell develop-

<sup>1</sup>Abbreviations used in this paper: DN, double negative; DP, double positive; HY, Y-chromosome encoded histocompatibility antigen; IEL, intraepithelial lymphocyte;  $\gamma_c$ , common  $\gamma$  chain; SCIDX1, X-linked severe combined immunodeficiency; SP, single positive; TN, triple negative.

ment in  $\gamma_c$  mice appears more similar to the canine X-linked SCID model (13) and to rare "leaky" human SCIDX1 syndromes, which may result from reduced expression of  $\gamma_c$  (14) or partial signal transduction through the  $\gamma_c$ -associated JAK3 kinase (15).

To address the potential role of  $\gamma_c$  during T cell repertoire selection, we analyzed thymocytes and peripheral T cells from  $\gamma_c$ -deficient transgenic mice expressing a TCR specific for the male-specific (Y-chromosome encoded histocompatibility [HY]) antigen (16). This model system allowed us to analyze the effects of  $\gamma_c$  deficiency on positive and negative selection of thymocytes and to follow the further development of intra- and extrathymically derived  $\gamma_c^-$  T cells in the peripheral organs of the transgenic mice.

## Materials and Methods

**Mice.** IL-2R $\gamma$ -deficient ( $\gamma_c^-$ ) mice have been previously described (12). Mice expressing the transgenic TCR (TCR- $\alpha_T/\beta_T$ ) reactive with male-specific antigen (HY) backcrossed onto the C57Bl/6 background (H-2<sup>b</sup>) have been described (16). Breeding was done in the animal facilities at the Hôpital Necker, Paris, and at the Institute for Genetics, Cologne. Animals were analyzed at 3–6 wk of age.

**Isolation of Lymphoid Cells.** Single-cell suspensions of thymus, LN, and spleen were lysed of RBC and prepared aseptically in RPMI 1640 + 5% FCS. Preparation of gut-associated intraepithelial lymphocytes (IEL) has been previously described (17).

**Antibodies and FACS<sup>®</sup> Analysis.** The following antibodies directly coupled to FITC, PE, or biotin were used: GK1.5 (anti-CD4) (18), H53-6.7 (anti-CD8 $\alpha$ ) (19), T3.70 (anti-TCR HY clonotype,  $\alpha_T/\beta_T$ ) (20), F23.1 (anti-TCR V $\beta$ 8 reactive with  $\beta_T$ ) (21), Pgp-1 (anti-CD44), (22), and M1/69 (anti-heat-stable antigen [23]).  $10^5$ – $10^6$  cells were washed once in PBS + 2% FCS and stained with saturating amounts of antibodies for 20 min on ice. After washing, biotinylated antibodies were revealed with either streptavidin CyChrome (PharMingen, San Diego, CA) or streptavidin Tricolor (CALTAG Laboratories, South San Francisco, CA) secondary reagents. Labeled cells were analyzed for simultaneous three-color fluorescence using a FACScan<sup>®</sup> flow cytometer (Becton Dickinson, & Co., Mountain View, CA) equipped with Cell Quest software.  $10^4$  events were collected on the viable lymphoid cell population gated using forward and side scatter profiles, which excluded propidium iodide.

**In Vitro Proliferation.** For thymocyte stimulation, 96-well flat-bottom plates were coated with antibody T3.70 (5  $\mu$ g/ml in PBS) for 3 h at room temperature.  $3 \times 10^5$  cells were cultured in RMI 1640 medium supplemented with 5% FCS, antibiotics, and  $5 \times 10^{-5}$  M  $\beta$ -ME. Additional stimuli included PMA (5 ng/ml), ionomycin (500 ng/ml), and IL-2 (20 U/ml). Cells were maintained at 37°C for 3 d before an 8-h pulse with [<sup>3</sup>H]-thymidine (0.5  $\mu$ Ci/well). Results are presented as the incorporation (cpm  $\times 10^{-3}$ ) averaged from triplicate wells.

**Histological Analysis.** Sections of duodenum were snap-frozen in embedding media (Tissue-tek, Miles Scientific, Puteaux, France) cooled in dry ice/isopentane. 5- $\mu$ m sections were fixed in acetone and stained with a rat anti-integrin  $\alpha_E/\beta_T$ -specific antibody (24), followed by biotinylated anti-rat immunoglobulins (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) and revealed with avidin-biotinylated peroxidase complex (Amersham International, Les Ulis, France) and 3-amino-9-ethylcar-

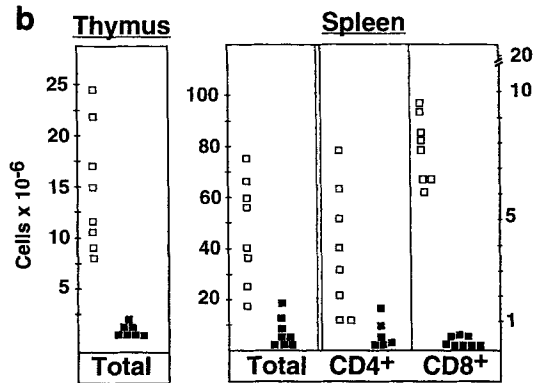
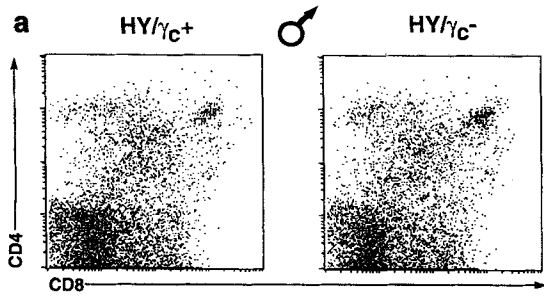
bazole (Aldrich, St. Quentin, France). Sections were counterstained with hematoxylin.

## Results

**HY/ $\gamma_c$ -deficient Male Mice.** To determine whether the absence of  $\gamma_c$  can influence T cell selection processes, we crossed  $\gamma_c$ -deficient mice (12) with transgenic mice bearing a TCR- $\alpha/\beta$  specific for HY antigen presented in the context of H-2D<sup>b</sup> class I molecules (16). HY antigens are Y chromosome-encoded proteins whose expression leads to rejection of male tissue by female recipients (25, 26). Using this HY-transgenic mouse system, a number of aspects of T cell development have been analyzed, including intrathymic positive and negative selection (16, 27, 28), peripheral tolerance (20, 29), generation of naive T cells (30), and extrathymic T cell development (31). In  $\gamma_c^+$  HY male mice on the selecting H-2<sup>b</sup> background, most thymocytes undergo strong negative selection by virtue of interactions between the transgenic TCR and the cognate male-specific peptide. As a result, thymi are small ( $1\text{--}2 \times 10^7$  cells) and are comprised mostly of immature cells that fail to progress past the CD4<sup>+</sup>8<sup>+</sup> DP stage (16; Fig. 1, a and b). A small number of TCR- $\alpha_T/\beta_T$  cells can be detected in the peripheral lymphoid compartment, where they express high levels of TCR- $\alpha_T/\beta_T$  but reduced levels of CD8 (CD4<sup>-</sup>CD8<sup>-</sup> or CD4<sup>-</sup>CD8<sup>lo</sup>) (20; Fig. 2). These cells expand (albeit slowly) in response to male antigen in vivo and persist as a potentially autoreactive peripheral cell population (32). In addition, a minor population of CD4<sup>+</sup> T cells expressing rearranged endogenous TCR- $\alpha$  chains (TCR- $\alpha_E/\beta_T$ ) are found in the spleen and LN of HY male mice (20). We analyzed  $\gamma_c$ -deficient male HY-transgenic mice (HY/ $\gamma_c^-$ ) on the H-2<sup>b</sup> background to determine effects of  $\gamma_c$  deficiency on the development of T cells expressing this autoreactive TCR.

HY/ $\gamma_c^-$  male mice demonstrated markedly reduced thymic cellularity ( $0.5 \times 10^6$  cells) in comparison to HY/ $\gamma_c^+$  males, while maintaining a similar distribution of thymocyte subpopulations as defined by CD4 and CD8 expression (Fig. 1, a and b). No significant differences in the surface expression of TCR- $\alpha_T/\beta_T$ , CD44, or heat-stable antigen were detected on thymocytes between the two groups of mice (data not shown). Thus, absence of  $\gamma_c$  does not inhibit intrathymic negative selection, although it clearly reduces total thymocyte numbers. The decrease in thymocyte numbers between  $\gamma_c^+$  and  $\gamma_c^-$  HY male mice (Fig. 1 b) is similar to that previously reported for nontransgenic  $\gamma_c^+$  and  $\gamma_c^-$  mice (12).

HY/ $\gamma_c^-$  male mice completely lacked TCR- $\alpha_T/\beta_T$  cells (CD4<sup>-</sup>CD8<sup>-</sup> or CD4<sup>-</sup>CD8<sup>lo</sup>) in the peripheral LN and spleen (Fig. 1 b and 2 and data not shown). In HY/ $\gamma_c^+$  mice, these cells comprise a large fraction of the total peripheral T cell pool, and their expansion is dependent on male antigen (32). Thus, our results suggest that the CD4<sup>-</sup>CD8<sup>-</sup> and CD4<sup>-</sup>CD8<sup>lo</sup> TCR- $\alpha_T/\beta_T$  peripheral T cells require  $\gamma_c$ -dependent cytokines for their migration and/or expansion in the periphery. CD4<sup>+</sup> TCR- $\alpha_E/\beta_T$  T cells can

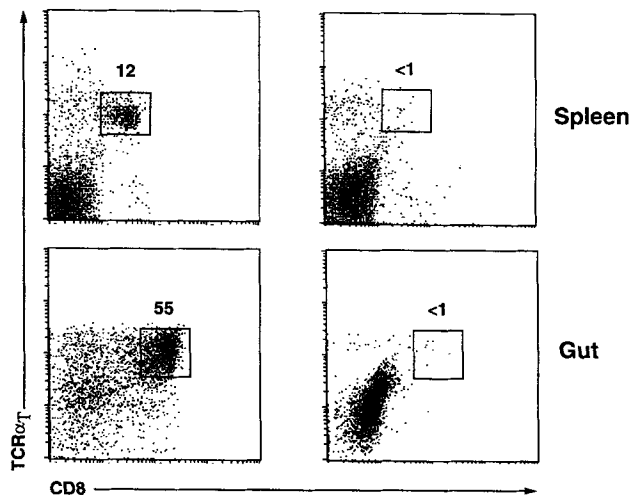
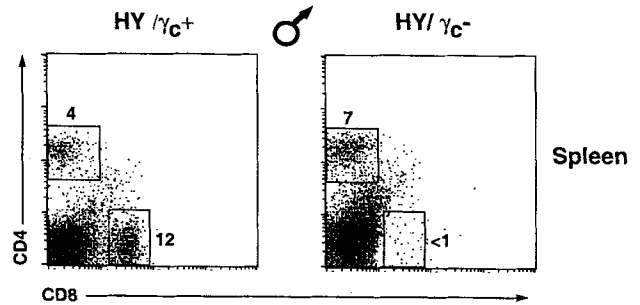


**Figure 1.** (a) No defect in negative selection in the thymus of  $\text{HY}/\gamma_c^-$  male mice. Staining of total thymocytes from  $\text{HY}/\gamma_c^+$  transgenic control male mice ( $\text{HY}/\gamma_c^+$ ; left) and  $\gamma_c$ -deficient  $\text{HY}$  male mice ( $\text{HY}/\gamma_c^-$ ; right) by CD4 and CD8 antibodies. Most thymocytes are deleted at an early (DN) stage in both types of mice. (b) Comparison of thymocyte and spleen lymphocyte cell numbers from  $\text{HY}/\gamma_c^+$  ( $\square$ ) and  $\text{HY}/\gamma_c^-$  ( $\blacksquare$ ) male mice.  $\text{CD4}^+$  or  $\text{CD8}^+$  T cell numbers were determined after staining with appropriate antibodies, and calculations were based on the percentage of positive lymphoid cells.

be detected in the spleen of  $\text{HY}/\gamma_c^-$  male mice, although their numbers are severely reduced as compared to  $\text{HY}/\gamma_c^+$  male mice (Fig. 1 *b* and 2 and data not shown).

**Gut-associated T Cell Development in  $\text{HY}/\gamma_c$ -deficient Male Mice.** T cell precursors may also differentiate within endodermally derived organs, including the digestive tract. This differentiation pathway appears distinct from mainstream intrathymic development with regard to the use of TCR-associated signal transduction molecules and mechanisms of repertoire selection (for review see reference 33). In  $\text{HY}/\gamma_c^+$  male mice, numerous  $\text{CD8}^{\text{hi}}$   $\text{TCR-}\alpha_T/\beta_T$  T cells are found in the gut as IEL (31; Fig. 2). These self-reactive cells are not clonally deleted, but are positively selected by male antigen presented on  $\text{H-2}^b$ . In contrast,  $\text{CD8}^{\text{hi}}$   $\text{TCR-}\alpha_T/\beta_T$  IEL are not found in the gut of female  $\text{HY}$ -transgenic mice (31).

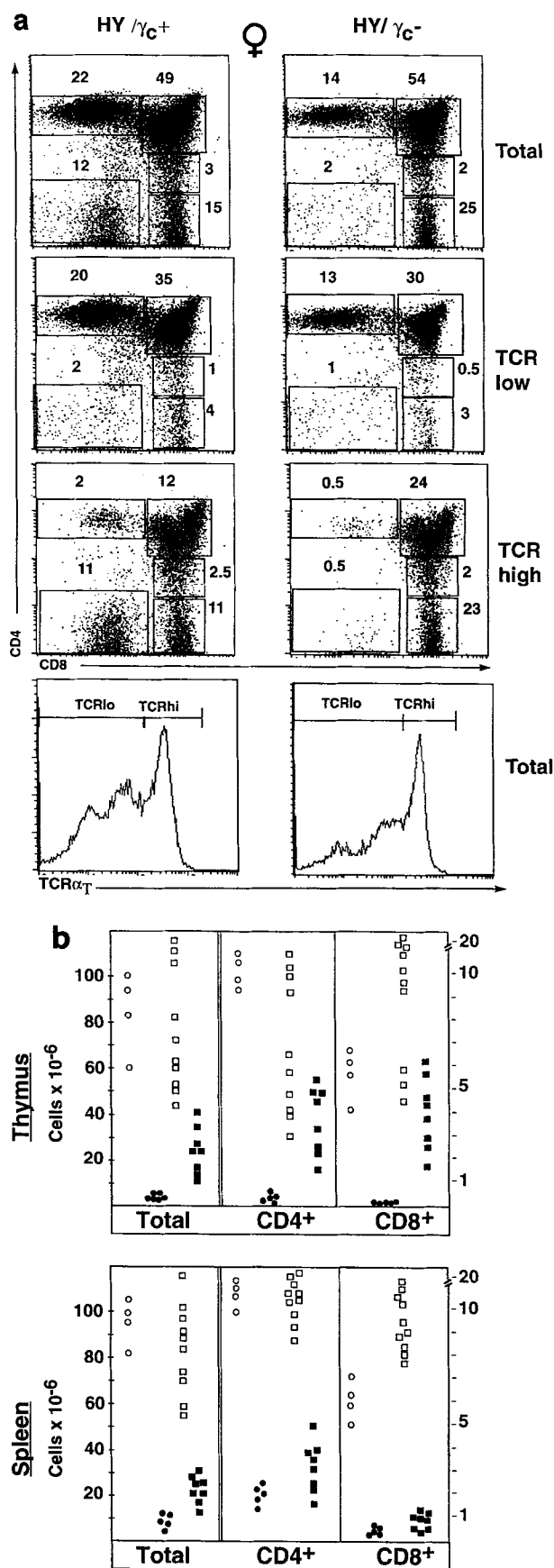
We are unable to detect  $\text{CD8}^{\text{hi}}$   $\text{TCR-}\alpha_T/\beta_T$  intraepithelial T cells in gut lymphocyte preparations from  $\text{HY}/\gamma_c^-$  male mice (Fig. 2). Additional immunohistological studies were performed to confirm this result. Frozen sections from  $\text{HY}/\gamma_c^+$  and  $\text{HY}/\gamma_c^-$  male mice were immunostained using an anti- $\alpha_E/\beta_7$  antibody (24) reactive with all IEL subpopulations (33). Abundant  $\alpha_E/\beta_7^+$  IEL were detected in  $\text{HY}/\gamma_c^+$  male mice, whereas no staining was seen in  $\text{HY}/\gamma_c^-$  male mice (data not shown). These results indicate a



**Figure 2.** Staining of peripheral T cells in  $\text{HY}/\gamma_c^+$  and  $\text{HY}/\gamma_c^-$  male mice. Spleen (top and middle) or gut-associated lymphocytes (bottom) were stained with CD4, CD8, or transgene-specific antibodies ( $\text{TCR-}\alpha_T = \text{T3.70}$ ; see Materials and Methods). The percentage of cells within the boxed regions are indicated.

complete block of the extrathymic T cell differentiation pathway in the absence of  $\gamma_c$ .

**$\text{HY}/\gamma_c$ -deficient Female Mice.** In  $\text{HY}/\gamma_c^+$  female mice on the  $\text{H-2}^b$  selecting background, positive selection of  $\text{CD8}^+$   $\text{TCR-}\alpha_T/\beta_T$  thymocytes occurs in the absence of male antigen (16, 27). Thymi from these mice contain  $\sim 10^8$  cells; however, expression of the transgenic TCR and its interaction with  $\text{H-2D}^b$  causes changes in the distribution of major thymocyte subpopulations expressing CD4 and CD8 (16; Fig. 3, *a* and *b*) characterized by increased proportion of  $\text{CD4}^- \text{CD8}^-$  (double negative [DN]) and  $\text{CD8}^+$  SP thymocytes, whereas the fraction of  $\text{CD4}^+ \text{CD8}^+$  (DP) thymocytes are correspondingly decreased (Fig. 3 *a*). The level of  $\text{TCR-}\alpha_T/\beta_T$  expression also differs on cells of these subpopulations, being highest on DN and  $\text{CD8}^+$  SP thymocytes and relatively low on DP and  $\text{CD4}^+$  SP cells (16; Fig. 3 *a*). The  $\text{CD8}^+$   $\text{TCR-}\alpha_T/\beta_T$  T cells migrate to the periphery, where they represent a substantial fraction of splenic and LN T cells (16, 20; Fig. 4). These naive T cells can persist for several months, although they appear to be noncycling (30). Due to the lack of allelic exclusion at the TCR- $\alpha$  locus in DP cells, the remainder of the  $\text{CD8}^+$  and all of the  $\text{CD4}^+$  peripheral cells express  $\text{TCR-}\alpha_E/\beta_T$  (20).

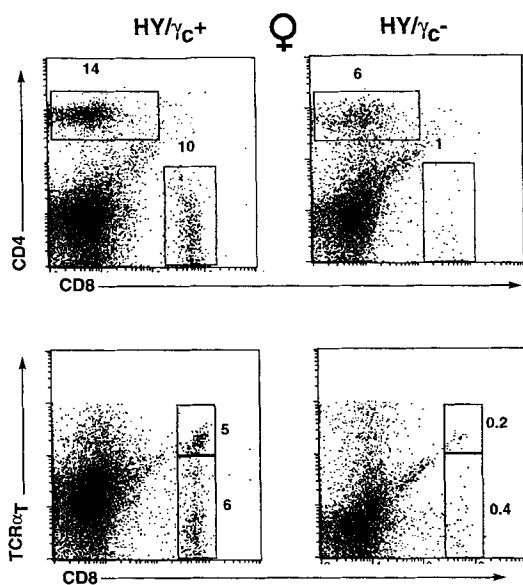


We analyzed  $\gamma_c$ -deficient female HY-transgenic mice (on the H-2<sup>b</sup> background) to determine the effect of  $\gamma_c$  deficiency on intrathymic positive selection and the generation of naive T cells.

According to the previously described thymic hypoplasia in  $\gamma_c$ - mice (12) and the dramatically reduced thymocyte number in HY/ $\gamma_c$ - male mice (Fig. 1 b), we expected to observe reduced thymic cellularity in HY/ $\gamma_c$ - female mice. In contrast, thymic cellularity in these mice was significantly increased ( $1-4 \times 10^7$  cells) when compared with their  $\gamma_c$ - nontransgenic counterparts (Fig. 3 b). Expression of the HY TCR increased cell numbers for each of the different thymocyte subpopulations compared with nontransgenic  $\gamma_c$ - mice, although not in a uniform fashion (Table 1). DN thymocyte numbers were increased roughly 8-fold, and included primarily cells expressing low levels of the transgenic TCR (Fig. 3 a). DN TCR- $\alpha_T/\beta_T$ <sup>hi</sup> thymocytes, which account for 11% of TCR<sup>hi</sup> cells in HY/ $\gamma_c$ <sup>+</sup> female mice, were virtually absent in  $\gamma_c$ -deficient HY females (Fig. 3 a). Absolute numbers of DP and CD4<sup>+</sup> SP thymocytes were also increased 2- and 4-fold, respectively, whereas numbers of CD8<sup>+</sup> SP cells were augmented 25-fold. Positive selection in HY/ $\gamma_c$ - female mice was not diminished; in contrast, it was enhanced, with a 2-fold increase in the percentage of CD8<sup>+</sup> thymocytes expressing high levels of TCR- $\alpha_T/\beta_T$  (Fig. 3 a). However, despite the overall increase in thymic cellularity and positive selection in HY/ $\gamma_c$ - female mice, cell numbers for each thymocyte subpopulation were still reduced compared with HY/ $\gamma_c$ <sup>+</sup> females (Table 1).

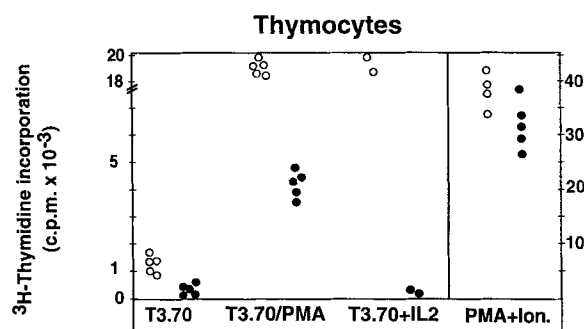
In contrast to HY/ $\gamma_c$ <sup>+</sup> females, the CD8<sup>+</sup> TCR- $\alpha_T/\beta_T$  T cells were severely reduced from the spleen and LN of HY/ $\gamma_c$ - female mice (Fig. 4 and data not shown). These cells are, by definition, naive cells, which are generated in the thymus and, once exported to the periphery, persist without cell division (30). Their absence in HY/ $\gamma_c$ - female mice could therefore result from defects in thymic export and/or peripheral persistence in  $\gamma_c$ -deficient mice. In contrast, small numbers of CD4<sup>+</sup> TCR- $\alpha_E/\beta_T$  T cells can be found in the periphery of HY/ $\gamma_c$ - female mice, although in reduced numbers relative to HY/ $\gamma_c$ <sup>+</sup> controls (Fig. 4 and data not shown).

**Figure 3.** (a) Enhanced positive selection and absence of DN TCR- $\alpha_T/\beta_T$  thymocytes in HY/ $\gamma_c$ - female mice. Staining of total thymocytes from HY-transgenic control female mice (HY/ $\gamma_c$ <sup>+</sup>; left) and  $\gamma_c$ -deficient HY female mice (HY/ $\gamma_c$ <sup>-</sup>; right) by CD4 and CD8 antibodies (top three sets of panels). CD4 and CD8 dot plots of cells expressing low (TCR low; second set of panels) or high (TCR high; third set of panels) levels of the transgenic TCR are shown. Histograms of TCR- $\alpha_T$  expression on total thymocytes (bottom) indicate the gates used. The percentages of cells within the boxed regions are indicated. (b) Increased thymic cellularity in HY/ $\gamma_c$ - female mice. Comparison of thymocyte and spleen lymphocyte cell numbers from  $\gamma_c$ - nontransgenic (○) or  $\gamma_c$ - nontransgenic (●) mice and HY/ $\gamma_c$ <sup>+</sup> (□) or HY/ $\gamma_c$ <sup>-</sup> (■) female mice. CD4<sup>+</sup> or CD8<sup>+</sup> cell numbers were determined after staining with appropriate antibodies, and calculations were based on the percentage of positive lymphoid cells.



**Figure 4.** Staining of peripheral T cells in HY/γ<sub>c</sub><sup>+</sup> and HY/γ<sub>c</sub><sup>-</sup> female mice. Spleen cells were stained with CD4 and CD8 (*top*) or CD8 and transgene-specific antibodies (TCR-α<sub>T</sub> = T3.70). The percentages of cells within the boxed regions are indicated.

**Proliferative Capacities of HY/γ<sub>c</sub><sup>-</sup> Thymocytes.** The absence of peripheral CD8<sup>+</sup> transgenic T cells could result from the failure of CD8<sup>+</sup> SP thymocytes to respond to TCR-α<sub>T</sub>/β<sub>T</sub> engagement. To test this hypothesis, thymocytes from HY/γ<sub>c</sub><sup>-</sup> female mice were cultured in the presence of aggregated antibody to the HY TCR with or without various costimuli, and proliferative responses were measured (Fig. 5). Unfractionated thymocytes were tested since the vast majority of potentially responsive TCR<sup>hi</sup> thymocytes were the positively selected CD8<sup>+</sup> SP cells (Fig. 3 *a*). Thy-



**Figure 5.** Positively selected thymocytes from HY/γ<sub>c</sub><sup>-</sup> female mice respond *in vitro* to mitogenic stimulation. Proliferation of HY/γ<sub>c</sub><sup>+</sup> (○) or HY/γ<sub>c</sub><sup>-</sup> (●) female thymocytes to the indicated stimuli. Each dot represents the average response from the single mouse.

mocytes from HY/γ<sub>c</sub><sup>-</sup> female mice responded to anti-TCR-α<sub>T</sub>/β<sub>T</sub> stimulation in the presence of phorbol ester, albeit less effectively than their HY/γ<sub>c</sub><sup>+</sup> counterparts, and the proliferative response of HY/γ<sub>c</sub><sup>-</sup> female thymocytes was not augmented by IL-2, in agreement with previous observations (12). Thymocytes from both mice responded to the combination of phorbol ester and ionomycin (Fig. 5). Thus, the absence of peripheral CD8<sup>+</sup> TCR-α<sub>T</sub>/β<sub>T</sub> cells in γ<sub>c</sub>-deficient HY female mice does not result from a global inability of the positively selected CD8<sup>+</sup> thymocytes to proliferate.

## Discussion

The common cytokine receptor γ<sub>c</sub> plays a critical role in the formation and function of the receptors for IL-2, -4, -7, -9, and -15 (7-10). Mutations in γ<sub>c</sub> in humans cause SCIDX1, an immunodeficiency disease characterized by

**Table 1.** Absolute Cell Numbers and Relative Ratios of Different Thymocyte Subpopulations in Transgenic and Nontransgenic γ<sub>c</sub><sup>+</sup> and γ<sub>c</sub><sup>-</sup> Mice

Mouse	Absolute cell numbers			
	DN	DP	CD4 <sup>+</sup> SP	CD8 <sup>+</sup> SP
γ <sub>c</sub> <sup>-</sup> (M or F)	0.16 ± 0.05	6.8 ± 2.1	0.8 ± 0.3	0.16 ± 0.04
γ <sub>c</sub> <sup>+</sup> (M or F)	2.3 ± 0.5	90 ± 14	10.2 ± 2.7	3.3 ± 0.8
HY/γ <sub>c</sub> <sup>-</sup> (F)	1.2 ± 0.8	14.5 ± 6.6	3.5 ± 1.5	4.0 ± 1.6
HY/γ <sub>c</sub> <sup>+</sup> (F)	9.2 ± 4.7	49 ± 18	6.9 ± 3.4	10.4 ± 5.3
	Relative ratios			
γ <sub>c</sub> <sup>+</sup> /γ <sub>c</sub> <sup>-</sup>	14.0	14.0	12.5	21.0
HYγ <sub>c</sub> <sup>+</sup> /HYγ <sub>c</sub> <sup>-</sup>	7.7	3.4	2.0	2.5
HYγ <sub>c</sub> <sup>-</sup> /γ <sub>c</sub> <sup>-</sup>	7.5	2.0	4.0	25.0

Average absolute numbers were calculated based on percentages of cells having CD4<sup>-</sup>CD8<sup>-</sup> (DN), CD4<sup>+</sup>CD8<sup>+</sup> (DP), or SP CD4<sup>+</sup> or CD8<sup>+</sup> cell surface phenotypes. Average total thymocyte numbers (× 10<sup>-6</sup>) were 8.0 ± 3.0 (γ<sub>c</sub><sup>-</sup>), 105 ± 19 (γ<sub>c</sub><sup>+</sup>), 23 ± 9.7 (HY/γ<sub>c</sub><sup>-</sup> female), and 76 ± 27 (HY/γ<sub>c</sub><sup>+</sup> female).

thymic aplasia and the absence of circulating mature T and NK cells (11). In contrast, partial T cell development has been observed in mice rendered  $\gamma_c$  deficient (12, 34) and in dogs with SCID and a  $\gamma_c$  mutation (13). Still, it is not known whether the growth abnormalities of murine or canine  $\gamma_c$ -deficient T cells are accompanied by changes in intrathymic positive and negative selection. To better comprehend the role of  $\gamma_c$  in T cell development, we have analyzed  $\gamma_c^-$  mice made transgenic for the male-specific TCR (HY).

In the thymus, negative selection results in cell death via apoptosis, a process initiated by interaction of the TCR with a tolerogenic peptide-MHC complex. In HY/ $\gamma_c^-$  male mice, negative selection of TCR- $\alpha_T/\beta_T$  thymocytes was not inhibited, arguing against the critical development of  $\gamma_c$  in the TCR-driven apoptosis in the thymus. In fact, thymic clonal deletion may have been improved in the absence of  $\gamma_c$ . HY/ $\gamma_c^-$  male mice had extremely small thymi, consistent with enhanced negative selection (as well as their reduced proliferative capacity). Moreover, HY/ $\gamma_c^-$  male mice completely lacked peripheral CD8<sup>+</sup> TCR- $\alpha_T/\beta_T$  T cells. A small number of these cells migrate to the periphery in HY/ $\gamma_c^+$  male mice, whereby they become activated and expand upon encounter with cognate antigen (29). One can argue that the absence of these cells in HY/ $\gamma_c^-$  male mice might result from improved clonal deletion. Alternatively, male-specific antigen-induced proliferation of peripheral CD8<sup>+</sup> TCR- $\alpha_T/\beta_T$  T cells may be largely dependent on  $\gamma_c$ -dependent cytokines, including IL-2, -4, and -7. Therefore, these cells would be not found in  $\gamma_c^-$  mice, as  $\gamma_c$ -deficient lymphocytes do not respond to these cytokines (12).

Our observation of efficient negative selection in  $\gamma_c$ -deficient mice would suggest that the CD4<sup>+</sup> T cells seen in  $\gamma_c^-$  mice do not result from defective clonal deletion. These cells may derive from a small pool of peripheral T cells that undergo antigen-driven expansion because the majority of these cells are in cycle, and most lack naive cell surface markers (DiSanto, J.P., unpublished observations). As such, one might predict that the diversity of TCRs expressed by CD4<sup>+</sup> T cells from  $\gamma_c^-$  mice may be limited. Similar observations have been reported in an atypical SCIDX1 patient characterized by reduced  $\gamma_c$  expression (14), in which a highly redundant, oligoclonal TCR- $\beta$  repertoire was observed, consistent with expansion of a restricted number of peripheral T cell clones. Additional analyses of  $\gamma_c^-$  mice transgenic for CD4-dependent TCRs reactive with class II-presented peptides should help to address these issues.

Using HY TCR female mice, the role of  $\gamma_c$  during intrathymic positive selection and the subsequent generation of naive cells can be analyzed. Unexpectedly, the expression of the HY TCR was able to partially compensate for the absence of  $\gamma_c$  in female mice. We found a 3- to 6-fold increase in thymic cellularity and 25-fold increase in number of the CD8<sup>+</sup> SP cells in HY/ $\gamma_c^-$  female mice when compared with nontransgenic  $\gamma_c^-$  mice. A number of explanations can be forwarded for the observed increase in thymocyte number. During the late DN stage, thymocytes

commence TCR- $\beta$  chain rearrangements (35). Thymocyte survival during this process is likely dependent on cytokines, principally IL-7, which is a maintenance factor for immature thymocytes (36) and can prolong survival of DN cells already committed to the TCR recombination process (37). Once a functional TCR- $\beta$  chain is expressed, a further maturation signal can be provided by the pre-TCR complex (38). Nonfunction TCR- $\beta$  rearrangements result in immature thymocyte cell death. In  $\gamma_c^-$  mice, thymocytes are not responsive to IL-7 (12); therefore, decreases in thymocyte numbers are a likely result of poor maintenance of the DN compartment. Consistent with this hypothesis, we have observed a severe block precisely at the transition from late DN to DP stage in  $\gamma_c^-$  mice (DiSanto, J.P., and B. Rocha; manuscript in preparation). The expression of a functional TCR- $\alpha/\beta$  during this stage in HY/ $\gamma_c^-$  female thymocytes, and its potential interaction with intrathymic ligands might deliver a signal sufficient to enhance survival and thereby partially bypass the DN block. Alternatively, this compensatory effect may be mediated through the pre-TCR complex (38), and as such would be observed on the nonselecting (H-2<sup>d</sup>) background or in  $\gamma_c^-$ /TCR- $\beta$ -only transgenic mice. Regardless of the exact mechanism, DP numbers would be augmented, as well as later stages of thymocyte development. The expression of a class I-restricted TCR would offer a greater increase in CD8 SP thymocytes, and the positive selection in HY/ $\gamma_c^-$  female mice might also be more efficient in the setting of the reduced numbers of selectable thymocytes (28). The ability of thymocytes to expand in the absence of  $\gamma_c$  is supported by our finding that thymocyte proliferation from HY/ $\gamma_c^-$  females can be induced in vitro with anti-TCR- $\alpha_T/\beta_T$  antibody in combination with PMA. Although the cytokines responsible for  $\gamma_c^-$  thymocyte proliferation remain undefined, our results suggest that the TCR- $\alpha/\beta$  expressed by  $\gamma_c^-$  thymocytes are functionally competent.

Despite unaltered (or even more efficient) positive selection in the thymic of HY/ $\gamma_c^-$  female mice, CD8<sup>+</sup> TCR- $\alpha_T/\beta_T$  and CD8<sup>+</sup> TCR- $\alpha_E/\beta_T$  T cells are severely reduced in spleen and LN of these mice. The failure of these cells to emerge in HY/ $\gamma_c^-$  mice could result from potential defects in thymic export and/or peripheral lymphoid survival. Thymic emigration follows the final maturation of SP thymocytes in the medulla. Whether this step requires thymocyte division is the subject of current debate (39). Although little is known about the cytokine requirements of medullary thymocytes, the absence the  $\gamma_c$  could result in a generalized defect in thymic emigration. However, CD4<sup>+</sup> TCR- $\alpha_E/\beta_T$  T cells are found in HY/ $\gamma_c^-$  mice (albeit at much lower numbers than their control HY/ $\gamma_c^+$  counterparts). This observation rules out an absolute block in thymic export in the absence of  $\gamma_c$ , but does not exclude a selective defect in the export of CD8<sup>+</sup> T cells. Independently of any defect in thymic egress, peripheral lymphoid cell persistence may also be compromised in  $\gamma_c^-$  mice. This hypothesis is supported by the recent finding that exposure to  $\gamma_c$ -dependent cytokines, including IL-2, -4, or -7, can enhance peripheral lymphocyte survival in the absence of cell division (40).

Therefore, CD4<sup>+</sup> T cells may be less susceptible to cell death in the absence of  $\gamma_c$ , because in contrast to CD8<sup>+</sup> T cells, they may retain the capacity to proliferate in response to  $\gamma_c^-$  independent cytokines. Since the lack of T cells is not restricted exclusively to CD8<sup>+</sup> cells that express the transgenic TCR, the observed reduction of peripheral CD8<sup>+</sup> T cells may be unrelated to TCR specificity and could reflect a more generalized defect in the maintenance of the peripheral pool of CD8<sup>+</sup> T cells in the absence of  $\gamma_c$ .

The absence of  $\gamma_c$  results in selective perturbations of different subpopulations of T/NK lineage lymphocytes. We have previously reported that  $\gamma_c^-$  mice lack NK cells (12), whereas the development of TCR- $\gamma/\delta$  cells in the skin (34), thymus, and spleen (DiSanto, J.P., unpublished observations) are also completely suppressed in these mice. The absence of TCR- $\gamma/\delta$  cells in  $\gamma_c^-$  mice may provide some explanation for the failure to observe DN TCR- $\alpha_T/\beta_T^H$  thymocytes in HY/ $\gamma_c^-$  female mice and DN TCR- $\alpha_T/\beta_T$  T cells in the periphery of HY/ $\gamma_c^-$  male mice. These two transgenic cell populations do not easily fit into the current models of T cell selection and tolerance, and the hypothesis

has been advanced that these peculiar transgenic T cells might actually be TCR- $\gamma/\delta$  cells with aberrant expression of the transgenic TCR (32). Although no marker exists to define transgenic T cells that might derive from the TCR- $\gamma/\delta$  lineage, our results strongly support the notion that these two transgenic T cell populations are related to the TCR- $\gamma/\delta$  lineage. We also demonstrate a complete block in the development of gut-associated T cells in HY/ $\gamma_c^-$  male mice. Since a subpopulation of IEL bearing TCR- $\alpha/\beta$  or TCR- $\gamma/\delta$  use an extrathymic differentiation pathway for their development (33) and share characteristics of T and NK cells (Guy-Grand, D., and P. Vassalli, manuscript in preparation), these observations suggest a close ontogenic relationship between NK cell, TCR- $\gamma/\delta$  T cell, and IEL subpopulations with respect to cytokine use. An in-depth analysis of T/NK cells in other cytokine/receptor-mutant mice, such as those deficient in IL-7 (41), IL-7R $\alpha$  (42), and the IL-2R $\beta$  chain (43), should help clarify the roles of  $\gamma_c$ -dependent cytokines in T/NK lineage development (44).

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