Delayed Maturation of CD4-CD8- FcγRII/III+ T and Natural Killer Cell Precursors in FcεRIγ Transgenic Mice

By Véronique Flamand,* Elizabeth W. Shores,* Tom Tran,* Kun Huang,* Eric Lee,* Alexander Grinberg,* Jean-Pierre Kinet,* and Paul E. Love*

From the *Laboratory of Molecular Allergy & Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland 20852; ‡Division of Hematologic Products, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Maryland 20892; and the §Laboratory of Mammalian Genes and Development, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892

Summary

FcεRIγ (γ) is a member of a group of related proteins (the ζ-family dimers) that function as signal-transducing components of both Fc receptors and the T cell antigen receptor (TCR). Analysis of γ expression during fetal thymus ontogeny revealed that it is expressed in early thymocytes, before the initiation of clonotypic TCR-α and TCR-β gene rearrangement but is down-regulated in most adult thymocytes. To explore a possible role for γ in thymocyte development, we generated transgenic mice in which this protein was overexpressed at all stages of ontogeny. Overexpression of γ inhibited the maturation of T cells as well as natural killer (NK) cells. The developmental effects were transgene dose related and correlated with markedly delayed maturation of fetal CD4-CD8- FcRII/III+ thymocytes, cells thought to include the progenitors of both T and NK cells. These results suggest that the ζ and γ chains serve distinctive functions in thymocyte development and indicate that Fc receptor(s) may play an important role in regulating the differentiation of early progenitor cells within the thymus.

Signals transduced by the TCR regulate the stage-specific development and selection (positive and negative) of thymocytes (1, 2). The signal-transducing potential of the TCR complex is conferred by multiple subunits (the CD3-γ, -δ, and -ε, and the ζ, η, or FcεRIγ chains) that share a conserved structural element, the immunoreceptor tyrosine-based activation motif (ITAM) (3). A considerable body of evidence supports the current notion that ITAMs, through their ability to facilitate activation of cytoplasmic protein tyrosine kinases, mediate all known TCR effector functions (4).

The signals that regulate the earliest stages of thymocyte maturation are less understood. Precursor CD4+CD8+ thymocytes populate the murine thymus on or about day 12 of gestation (2). These early thymocytes, which are thought to include the precursors of both T cells and NK cells (5, 6) progress through multiple stages of differentiation before expressing CD4, CD8, and α/β TCR (2, 7).

Although the molecular events that regulate their transit through these various stages remain uncharacterized, recent evidence suggests that they may receive activating signals through cell surface structures (8). The majority of early fetal thymocytes express low affinity receptors for IgG (FcγRII and/or FcγRIII) (5). FcγRIII is also expressed on mature NK cells where it functions as a signal-transducing complex to mediate antibody-dependent cellular cytotoxicity (ADCC), and on a subset of mature T cells (9). These observations have led to speculation that FcγRIII may represent a developmentally important signaling structure in early CD4+CD8+ TCR+ thymocytes (5).

In mice, the FcγRIII α chain forms a complex with a dimer composed of FcεRIγ chains (10, 11). Initially identified as a subunit of the high affinity IgE receptor, FcεRIγ is also a subunit of the high affinity IgG receptor (FcγRI), and in a subset of T cells, functions as a subunit of the TCR complex (12–17). The structural and functional similarity between FcεRIγ and ζ suggests that these proteins are members of a family of signal-transducing proteins whose genes were likely generated by duplication (10, 18). Although FcεRIγ is reportedly expressed in early thymocytes, its potential role in thymocyte development remains controversial. The observation that T cell development is unaffected in mice lacking FcεRIγ argues that its...
function is not critical for T cell maturation (19). However, recent experiments indicate that Fcy receptors on fetal thymocytes may transduce developmentally important signals (20). In this study, we document that FcεRIγ is expressed early in fetal thymic ontogeny, before the rearrangement and expression of the clonotypic TCR-α and TCR-β chains. To investigate the role of FcεRIγ in thymocyte development, we generated transgenic mice that express elevated levels of FcεRIγ at all stages of fetal ontogeny. We found that overexpression of FcεRIγ inhibited the maturation of both T cells and NK cells. The developmental effects were transgene dose related and were correlated with delayed maturation of fetal FcγRII/III+ thymocytes into FcγRII/III+ thymocytes. Collectively, these results show that FcεRIγ can regulate the differentiation of an early progenitor cell population within the thymus.

Materials and Methods

DNA Constructs and Microinjection. A 4.8-kb BglI genomic fragment containing the human FcεRIγ (γ) promoter and coding sequences (18) was cloned into the BamHI site of plasmid CD2-B-N2 (21) after removal of the SalI-BamHI fragment containing the human CD2 promoter and minigene. A 9.8-kb fragment composed of the γ gene and the human CD2 3-prime enhancer was then generated by digestion with NotI, separated from vector sequences by gel electrophoresis, purified as described (22), and used for pronuclear injection. Transgenic mice were identified by Southern blotting of tail DNA using the human CD2 enhancer sequences as a probe.

Mice. Transgenic mice were generated by injection of zygotes obtained from matings of inbred FVB/N mice. Timed matings were performed with either non-Tg FVB/N males × non Tg C57BL/6 females or homozygous γ551Tg FVB/N males × non Tg C57BL/6 females. The first day of mating was designated as day 0.5. FVB/N × C57BL/6J matings were performed because staining with mAbs 2.4G2 (FcγRII/III) and PK136 (NK1.1) were determined to be superior in the C57BL/6J background; however, the developmental effects of the γTg were identical regardless of the background.

RNA Extraction and Analysis. Purification of total thymocyte RNA and Northern blot analysis were performed as described previously (22). cDNA probes for TCR-α (Co; 23) and TCR-β (CB; 24) were provided by E. Shevach (National Institutes of Health [NIH]). cDNA was provided by R. Klausner (NIH). Human (18) and murine (10) FcεRIγ cDNAs were isolated as described. Human GAPDH cDNA was purchased from Clontech Laboratories, Inc. (Palo Alto, CA).

Antibodies and Reagents. mAbs used for flow cytometric analysis included: FITC conjugated anti-Thy-1.1 (OX-7), anti-Thy-1.2 (53-2.1), anti-CD4 (RM4.5), anti-TCR-β (H57-597), anti-CD8α (53-6.7), anti-CD3e (145-2C11), anti-CD2 (RM2.5), anti-CD25 (7D4), and anti-HSA (M1/69); PE-conjugated anti-CD4 (RM4.5), anti-CD8α (53-6.7), anti-CD3e (145-2C11), anti-B20 (RA3-6B2), and anti-NK1.1 (PK136); and biotinylated anti-FcγRII/III (2.4G2), anti-CD8α (53-6.7), anti-CD4 (RM4.5), anti-B20 (RA3-6B2), anti-CD3e (145-2C11), and anti-CD5 (53-7.3). Unconjugated mAb 2.4G2 was additionally used to block nonspecific binding and for immunoprecipitation. All of the aforementioned antibodies were purchased from Pharmingen (San Diego, CA). Streptavidin-PE (Pharmingen) and streptavidin-Red 670 conjugate (GIBCO-BRL, Gaithersburg, MD) were used as second step reagents. Biotin or FITC-conjugated anti-human CD3 (mAb SK7, Becton Dickinson and Co., San Jose, CA) were used as control antibodies at a concentration of 10 μl/106 cells. Anti-FcεRIγ was generated in rabbit to a peptide corresponding to amino acids 80-86 of human FcεRIγ (25). Rabbit anti-rat sera were purchased from Jackson Immunoresearch Laboratories (West Grove, PA).

Multicolor Flow Cytometry (FCM). Single cell suspensions (106 cells/ml) of thymi or LN were washed twice in PBS containing 0.5% BSA and 0.05% sodium azide before and after each incubation with antibody. Cell suspensions were preincubated with unconjugated mAb 2.4G2 to inhibit nonspecific staining unless cells were to be stained for FcγRII/III. Incubations with labeled mAb (10 μg/ml), streptavidin-PE or streptavidin-Red 670 were performed at 4°C for 30 min. FCM was performed using a FACScan® flow cytometer (Becton Dickinson Immunocytometry Systems, Mountain View, CA) and data were analyzed using FACScan® research software. Unless stated otherwise, fluorescence data were collected on 5 × 105 cells, gating on viable cells as assessed by forward and side scatter profiles. In Fig. 6, B and C, gates were set on specific thymocyte subpopulations at the time of collection and data were collected on 4-5 × 104 viable cells. Data were displayed as logarithmic overlay histograms or dot plots.

Protein Analysis. Thymocytes were lysed in buffer (0.15 M NaCl, 50 mM Tris-HCl, pH 7.4, 20 μg/ml leupeptin, 40 μg/ml aprotinin) containing 1% NP-40 (adult thymocytes) or 1% digitonin (fetal thymocytes), precleared with protein A-Sepharose plus normal rabbit serum, then incubated with protein A-Sepharose plus 10 μl antiserum. Immunoprecipitates were washed, solubilized in leading buffer + 2M E, and resolved by 14% SDS-PAGE. Separated proteins were then transferred to nitrocellulose and blotted with a 1:1,000 dilution of anti-γ. Detection of immunoreactive proteins was done by chemiluminescence using the ECL system (Amer sham Corp., Arlington Heights, IL).

Cytotoxicity Assays. Mice were injected with 100 μg of polyinosinic-polycytidylic acid (poly[IC]) (Sigma Chemical Co., St. Louis, MO). Cytotoxicity assays were performed with splenocytes obtained 36 h after injection. Target cells were labeled with 51Cr (American Radiolabeled Chemicals, Inc., St. Louis, MO) at 100 μCi/106 cells for 1 h at 37°C, washed twice, and used in a standard 6 h 51Cr-release assay with 2,500 target cells/well in 96-well V-bottom plates. Lysis was performed in RPMI 1640 medium containing 10% heat-inactivated FCS, 10 mM Hepes, and 500 U/ml human IL-2 (Hoffman-LaRoche, Nutley, NJ). NK activity was evaluated on the NK-sensitive target cell line, YAC-1. ADCC assays were performed essentially as described (5) with the exception that 3 mM rather than 30 mM 2,4,6-trinitrobenzyl sulfonic acid was used.

Results

Differential Expression of γ and FcεRIγ during Fetal Ontogeny. To examine the normal pattern of FcεRIγ (γ) expression during ontogeny, Northern blot analysis was performed on RNA obtained from thymi on sequential days of fetal development. γ transcripts were present on fetal day 13.5, the earliest stage in which the thymus could clearly be identified in situ (Fig. 1). γ mRNA levels were highest at early stages of development (fetal days 13.5–16.5), decreased progressively during late gestation, and were barely detectable in adult thymocytes (Fig. 1). In contrast, low levels of γ mRNA were first detectable in the thymus on
days 14.5–15.5 of fetal development and increased markedly during gestation to high levels in adult thymocytes (Fig. 1). The pattern of 𝜉 expression coincided temporally with rearrangement and surface expression of the clonotypic (TCR-α and TCR-β) chains (Fig. 1), an observation which is consistent with the central role of 𝜉 in α/β T cell receptor (α/β TCR) surface expression and signal transduction (26–29). On the other hand, 𝜇 predominates in fetal thymocytes during stages that precede expression of the clonotypic TCR-α and TCR-β genes (Fig. 1). The striking difference in expression of 𝜇 and 𝜉 during ontogeny suggested that these proteins might play distinctive roles in thymocyte development. To investigate this possibility, we perturbed the normal pattern of 𝜇 expression by generating transgenic mice in which 𝜇 is overexpressed in early thymocytes and continues to be expressed throughout development.

**Generation of FcεRIγ Transgenic Mice.** The FcεRIγ (γ) transgene was generated from a 4.8-kb BclI genomic fragment containing the human γ promoter and coding sequences and a 5-kb genomic fragment containing the human CD2 3′-enhancer element (18, 21; Fig. 2 A). Six independently derived γ transgenic (γTg) founder lines were obtained by microinjection of DNA into embryos derived from inbred strain FVB/N females.

Transgene expression was detected by Northern blotting of total thymocyte RNA with radiolabeled human γ cDNA. RNA expression correlated with transgene copy number, varying ~12-fold between the lowest (γTg371) and the highest (γTg551) expressing founder lines (Fig. 2 B). Both transgenic and endogenous (murine) transcripts were expressed early in fetal ontogeny; however, unlike endogenous γ mRNA, high level expression of the transgene continued throughout thymocyte development (not shown). The level of
transgene expression relative to endogenous γ was assessed by sequential hybridization of northern blots with human and mouse cDNA probes followed by densitometric quantitation. Transgene expression was estimated to be 50-fold (Tg line γ371) to 600-fold (Tg line γ551) greater than endogenous in adult thymocytes. In fetal day 15.5 thymocytes, the relative increase was less due to the higher level of endogenous γ; 5-fold in Tg line γ371 and 60-fold in Tg line γ551 (not shown).

Immunoblotting with antiserum that detects both human and murine γ chains demonstrated the presence of human γ chain in adult thymocytes from Tg mice (Fig. 2 C). After prolonged exposure, a far less abundant protein of identical mobility, representing endogenous murine γ, was also observed in thymocytes from nontransgenic mice (not shown). Given the high level of sequence conservation between human and murine γ chains (18) it was expected that these proteins would be functionally equivalent. Consistent with this idea, transgene-encoded human γ chains assembled both as homodimers and as heterodimeric forms with endogenous murine ξ chain (not shown). Analysis of proteins from both adult and fetal (day 17.5) thymocytes by two-dimensional SDS-PAGE revealed that both ζγ and γγ dimers were present in thymocytes from γTg mice and their relative abundance predictably increased with transgene copy number. In contrast, ζζ and γζ dimers were virtually undetectable in adult nontransgenic mice (not shown).

**Figure 3.** Overexpression of γ chain impairs thymocyte development. Thymocytes (left) and LN cells (right) from 4-wk-old nontransgenic (Non Tg) mice or from 4-wk-old γ transgenic lines (γ514, γ549, γ551) expressing varying levels of human γ chain were stained and analyzed by two or one color FCM. Two-color plots (columns 1 and 3) show staining of cells with FITC anti-CD4 vs. biotin anti-CD8 plus avidin-PE. The frequencies of CD4+CD8-, CD4+CD8+, CD4+CD8-, and CD4+CD8+ thymocytes, and CD4+CD8+ and CD4+CD8- LN T cells are shown within their respective quadrants. Numbers on the left show total numbers of thymocytes for each line and are representative of multiple samples. Single-color plots (columns 2 and 4) were obtained by staining with FITC anti-TCR-β mAb. Dotted lines indicate staining with control antibody. For lymph node plots, numbers shown represent the mean fluorescence on TCR-β+ cells.
progressive reduction in thymocyte number with increasing transgene copy number (Fig. 3; Table 1). Analysis of CD4 and CD8 expression on thymocytes from γ Tg mice revealed an impairment of thymocyte maturation. Low copy number transgenic lines exhibited minimal developmental defects whereas high copy number transgenic lines exhibited a progressive loss of mature CD4⁺CD8⁻ (single positive, SP) and CD4⁺CD8⁺ (double positive, DP) thymocytes and a relative increase in the number of immature CD4⁺CD8⁻ (double negative, DN) thymocytes (Fig. 3; Table 1). γ overexpression also resulted in a corresponding reduction in the number of peripheral T cells (Fig. 3). Both DP and SP thymocytes from γ Tg mice expressed α/βTCR (Fig. 3). In fact, though reduced in number, DP thymocytes from adult γ Tg mice expressed higher levels of α/βTCR than DP thymocytes from non-Tg mice (Fig. 3, data not shown). Moreover, despite their reduced numbers, peripheral CD4⁺ and CD8⁺ T cells from γ Tg mice expressed α/βTCR at levels comparable to controls (Fig. 3).

Overexpression of γ did not result in complete developmental arrest, as all thymocyte subsets (DN, DP, and SP) as well as peripheral T cells were present in adult γ Tg mice. The phenotype of adult γ Tg mice additionally suggested a block in early thymocyte development. Numbers of DP thymocytes were markedly reduced in high copy number (γ551) Tg mice (DN/DP ratio of 1.0 ± 0.26; n = 5 compared to 0.03 ± 0.01; n = 5 for controls). In contrast, the transition of DP thymocytes into mature, SP thymocytes appeared less affected as the SP/DP ratio in γ551 Tg mice remained DN (DN/DP ratio of 0.03 ± 0.01; n = 5 for controls). Interestingly, although both CD4⁺CD8⁻ SP and CD4⁺CD8⁺ SP thymocyte numbers were reduced in γ Tg mice, CD4⁺CD8⁻ thymocytes appeared to be more severely affected (Fig. 3; Table 1). A skewed ratio of CD4⁺CD8⁻ to CD4⁺CD8⁺ cells was also seen in the lymph nodes of γ Tg mice indicating that the development of CD4⁺CD8⁻ T cells may be selectively impaired (Fig. 3).

γ Overexpression Delays Maturation of α/βTCR⁺ T Cells. To determine when the developmental impairment was first evident in γ Tg mice we next analyzed progressive stages of fetal thymocyte maturation. Examination of fetal thymocytes from high copy number (γ551) Tg mice revealed an early perturbation of thymocyte maturation (Figs. 4 and 5). Total numbers of thymocytes were equivalent in control and γ551 Tg mice on day 15, but at all subsequent stages, the number of thymocytes in γ Tg mice was reduced relative to nontransgenic mice (Fig. 4). By fetal day 17.5, significant numbers of "transitional," CD4⁺CD8⁻ and DP thymocytes were detectable in normal mice, whereas most thymocytes in the γ551 Tg mice remained DN (DN/DP ratio of 0.03 ± 0.01; n = 5 for controls). DP and SP thymocytes began to accumulate in γ551 Tg mice only after birth and the relative number of DN thymocytes remained high even in adult mice (Figs. 3 and 5). Thymocytes from γ551Tg mice were immature relative to nontransgenic mice of the same gestational age by several other criteria. For example, most thymocytes from γ551 transgenic newborn mice resembled fetal thymocytes in that they were IL2-Rα⁺ and expressed low levels of surface CD2, CD5, and α/βTCR (Fig. 5 B).

Fetal Thymocyte Development Is Delayed at the CD4⁺CD8⁻ FcRII/III⁺ Stage in γ Tg Mice. Since our analysis of fetal thymocytes from γ Tg mice revealed a block at the DN→DP transition stage, we examined the phenotype of early DN thymocyte populations in γ Tg mice. Four distinct subsets of precursor "triple negative" (CD3⁻CD4⁻CD8⁻) thymocytes have been identified on the basis of CD44 and CD25 expression that represent progressive stages of development: CD44⁺CD25⁻→CD44⁺CD25⁺→CD44⁻CD25⁺→CD44⁺CD25⁻ (30). FACS® analysis of DN thymocyte subsets from newborn γ Tg mice revealed a specific block in development at the CD44⁺CD25⁺ stage (Fig. 5 C). The reduction in CD44⁺CD25⁻ triple negative thymocytes was
consistent with the low numbers of DP thymocytes observed in γTg mice as these cells are the immediate precursors of DP thymocytes (30). Since γ chain is known to associate with FcγRIII we next examined expression of Fcγ receptors on early thymocyte populations. Staining of fetal day 15.5 thymocytes with mAb 2.4G2 (31), which recognizes the FcγRIlb1, FcγRIlb2, and FcγRIII receptor isoforms, revealed that the majority of thymocytes from control and γ551 Tg mice expressed FcγRII and/or FcγRIII (Fig. 6 A, and reference 5). The specificity of staining with mAb 2.4G2 was established by demonstrating that it could be blocked by unconjugated 2.4G2, but not by unconjugated isotype-matched mAb (not shown). In nontransgenic mice, the percentage of FcγRII/III + (2.4G2 +) thymocytes decreased rapidly during gestation (Fig. 6 A, and reference 5). In contrast, the percentage of FcγRII/III + thymocytes remained high in γ551 Tg mice throughout fetal development (Fig. 6 A). Because total cellularity was reduced in γ551 Tg mice (Fig. 4), the total number of FcγRII/III + thymocytes was nevertheless equivalent to that of controls. Moreover, FcγRII/III + thymocytes from fetal day 17.5 control and γ551 Tg mice appeared phenotypically similar (i.e., Thy-1+CD4 CD8 CD3+ HSA−; data not shown). These findings suggested that the high percentage of FcγRII/III + thymocytes in γTg fetal mice reflected their failure to progress further in development.

Recent work has shown that loss of FcγRII/III surface expression precedes the differentiation of early CD3−CD4−CD8− thymocytes to the DP stage (i.e., DN FcγRII/III +→DN FcγRII/III +→DP FcγRII/III −[32]). To determine if thymocyte development was blocked at the DN FcγRII/III +→DN FcγRII/III −transition stage in γ551Tg mice, we examined the surface expression of FcγRII/III on fetal DN thymocytes. In nontransgenic mice, the percentage of DN thymocytes that express FcγRII/III decreased from 61% at fetal day 17.5 to 32% at fetal day 17.5, and those cells that remained positive expressed reduced levels of FcγRII/III (Fig. 6 B). Significant numbers of DP thymocytes were first detected in nontransgenic mice on fetal day 17.5 (Fig. 6 B). In contrast, during the

Figure 5. Delayed development of α/β T cells in γ Tg mice. Thymocytes were obtained from fetuses (fd 15.5, fd17.5), newborn mice (d1), 9-d-old mice (d9), or 4-wk-old mice (adult) generated by timed matings. For each time point other than d9 or adult, cells from multiple thymi were combined and analyzed by two-color FCM. Data shown are representative of at least three individual experiments. (A) Staining of thymocytes with anti-CD4 and anti-CD8. The frequencies of CD4−CD8−, CD4−CD8+, CD4+CD8+, and CD4+CD8− thymocytes are indicated in their respective quadrants. (B) Immaturity of thymocytes from newborn γTg mice as assessed by FCM. Total thymocytes from a pool of 15 Non Tg newborn mice or 7 γS51 Tg newborn mice were analyzed by FCM for expression of markers of thymocyte maturaton. Single-color plots represent staining with anti-TCR-β, anti-CD2, anti-CD5 (Ly-1), or anti-IL2Rα (CD25). Dotted lines show staining with control antibody. (C) Comparison of CD4−CD8− thymocyte subsets in newborn Non Tg and γS51 Tg mice. Total thymocytes were stained with biotinylated anti-CD3, -CD4, -CD8, -B220 plus avidin-FITC, anti-CD44, and PE-anti-CD25. CD44 and CD25 surface expression was determined on CD3−, 4−, 8−, B220− (max) negative thymocytes by gating. Mix-negative thymocytes were determined to be Thy-1.2+ by separate staining.
same interval (fetal day 15.5–17.5), DN thymocytes from γTg mice failed to differentiate into DP thymocytes, remained predominantly FcγRII/III+ and continued to express high levels of FcγRII/III (Fig. 6 B). Appreciable numbers of DN FcγRII/III− thymocytes were first detectable in newborn γ551Tg mice, indicating that their formation was delayed relative to control mice (Fig. 6 C). The appearance of DN FcγRII/III− thymocytes in newborn mice.
Impaired NK Cell Development in γTg Mice. In view of recent data indicating that at least some NK cells may originate from early FcyRIIIα thymocyte progenitors (5, 6), we assessed whether NK cell development was compromised in γTg mice. Analysis of splenocytes (Fig. 8 A) and PBL (not shown) demonstrated that the number of NK 1.1+ cells was reduced in γ551Tg mice relative to nontransgenic controls. In contrast, B cell numbers were normal or elevated in these mice (Fig. 8 A). As with T cells (indicated by staining with anti-Thy 1.2), the reduction in NK 1.1+ cells was transgene dose dependent (Fig. 8 A). To assess NK activity, nontransgenic and γ551 Tg mice were injected with poly(IC) and spleen cells were assayed for cytotoxicity against the NK-sensitive target cell, YAC-1, and for ADCC. Consistent with the FACS® analysis, NK activity and ADCC were negligible in γ551 Tg mice (Fig. 8 B). Taken together, these results demonstrate that the number of NK cells is markedly reduced in γ551 Tg mice.

Discussion

The present study was directed at exploring a possible role for FcεRγ in thymocyte development. Northern blot analysis of fetal and adult thymocytes demonstrate that γ is expressed in the fetal thymus; expression is highest at early stages (fetal day 13.5–15.5), and decreases progressively with gestational age. This pattern contrasts sharply with that of the structurally and functionally related ξ chain, which is expressed later in development and functions primarily as a signal-transducing subunit of the α/β TCR complex. In view of these results, we generated transgenic mice that overexpress γ, reasoning that a perturbation of thymocyte maturation, if observed, might yield insight into its function in early thymocytes. Analysis of multiple transgenic founder lines revealed that γ overexpression does indeed impair early thymocyte development. The transgenic effects are dose related and result in delayed maturation of fetal DN thymocytes, with less severe effects on later stages of development.

Analysis of fetal thymocytes from high copy number γ551Tg mice revealed that γ chain overexpression specifically blocked thymocyte development at the DN CD25+ FcεRII/III−→DN CD25− FcεRII/III+ transition stage. As DN FcεRII/III1 fetal thymocytes are thought to include common precursors of T lymphocytes and NK cells (5), it is notable that NK cell development was likewise impaired in γTg mice. Further support for the idea that T and NK cells share a common precursor is provided by the observation that perturbation of both T cell and NK cell development was also observed in transgenic mice in which human CD3ε was overexpressed in early thymocytes (33).

One interpretation of our observations is that the γ transgene–induced effects are mediated by FcεRIII (CD16) complexes on early thymocytes. Although the mAb used to assess FcεRII/III surface expression does not distinguish between these isoforms and therefore does not enable specific quantitative evaluation of FcεRIII surface expression, Western blot analysis is consistent with the idea that γ is associated with FcεRIIIα in both nontransgenic and γTg fetal thymocytes. However, fetal day 17.5 thymocytes from γ551 Tg mice, which express high surface levels of FcεRII/III, con-
Figure 8. (A) Reduction in NK1.1+ cells in γTg mice. Spleenocytes from adult nontransgenic (NonTg), γ514Tg, and γ551Tg mice were stained with anti-Thy 1.2, anti-B220, or anti-NK1.1, and analyzed by FCM. Single-color plots are shown. Dotted line represents staining with control antibody. Numbers indicate percentage of total. Data are representative of three independent experiments. Total NK 1.1+ cells: Non Tg, 3.0 × 10⁶; γ514 Tg, 3.7 × 10⁶; γ551 Tg, 0.6 × 10⁶.

(B) Reduction in functional NK cells in the periphery of γTg mice. NK activity was assessed by cytotoxicity of spleen cells from poly(IC)-treated Non Tg (open circles, n = 2) or γ551 Tg (closed squares, n = 3) 10-wk-old C57BL/6 inbred mice against the NK-sensitive target cell line, YAC-1. ADCC was analyzed by determining cytotoxicity of splenocytes (obtained from the same mice used to determine NK activity) against TNP-derivatized EL-4 cells coated with anti-TNP antibody (EL-4-TNP) or non-TNP derivatized EL-4 cells (EL-4). Results shown are the mean ± SEM of triplicate samples.

Flamand et al.

1733 Flamand et al.

Downloaded from on April 13, 2017
Published November 1, 1996
of hematopoietic cell activation (35); therefore its failure to be down-regulated in early thymocytes could be inhibitory. Since mAb 2.4G2 does not distinguish between FcγRII and FcγRIII we were unable to discern the relative expression of these receptors in non-Tg and γ Tg mice. However, both we (data not shown) and others (5) have detected FcγRIIB expression during fetal thymocyte development by reverse transcription-PCR. Notwithstanding, two lines of evidence indicate that the developmental effects observed in γ Tg mice are specific to γ chain. First, we have previously shown that overexpression of both η chain or a truncated form of η chain that, like γ, contains only a single ITAM does not inhibit thymocyte maturation (22). Second, reconstitution of ζ-deficient (ζ-/-) mice with γ transgenes revealed that the phenotypes of γ Tg mice is identical regardless of the presence of endogenous ζ chain (data not shown). These results indicate that the developmental effects of γ overexpression are not the result of its association with ζ chain or due to competition with ζ chain for association with other molecules.

The phenotype of γ Tg and ζ Tg mice also provides insight into the function of these molecules in developing thymocytes. Although T cell development is adversely affected in both ζ and γ transgenic mice, their phenotypes are strikingly different. γ chain overexpression delays, but does not arrest, thymocyte development at the DN FcγRII/III+ stage, whereas ζ chain overexpression results in a near complete arrest at a later stage of development (22). Fetal thymocytes from ζ Tg mice but not γ Tg mice are further distinguished by their inappropriate expression of activation markers and premature down-regulation of Rag1 and Rag2 (22). These data suggest that γ and ζ may couple to different signaling pathways in early thymocytes. One possibility is that γ and ζ associate preferentially with different cell surface structures, e.g., ζ chain along with the CD3 subunits as part of the pre-TCR and ζ/β/TCR complexes, and γ chain with the FcγRIII complex. Indeed, the distinctive patterns of γ and ζ expression during gestation would be consistent with this idea.

We thank Drs. A. Singer, D. West, and B.J. Fowlkes for critical review of the manuscript.

Address correspondence to Paul E. Love, Building 6B, Room 2B-210, Laboratory of Mammalian Genes and Development, National Institutes of Health, Bethesda, MD 20892. J.-P. Kinet’s present address is Harvard Medical School, Beth Israel Hospital, Research North Building, 99 Brookline Avenue, Boston, MA 02215.

Received for publication 11 June 1996 and in revised form 8 August 1996.

References


