Differential requirement of the cytoplasmic subregions of γc chain in T cell development and function

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The common cytokine receptor γ chain (γc), a shared component of the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15, is critical for the development and function of lymphocytes. The cytoplasmic domain of γc consists of 85 aa, in which the carboxyl-terminal 48 aa are essential for its interaction with and activation of the Janus kinase, Jak3. Evidence has been provided that Jak3-independent signals might be transmitted via the residual membrane-proximal region; however, its role *in vivo* remains totally unknown. In the present study, we expressed mutant forms of γc, which lack either most of the cytoplasmic domain or only the membrane-distal Jak3-binding region, on a γc null background. We demonstrate that, unlike γc or *Jak3* null mice, expression of the latter, but not the former mutant, restores T lymphopoiesis *in vivo*, accompanied by strong expression of Bcl-2. On the other hand, the *in vitro* functions of the restored T cells still remained impaired. These results not only reveal the hitherto unknown role of the γc membrane-proximal region, but also suggest the differential requirement of the cytoplasmic subregions of γc in T cell development and function.

The common cytokine receptor γ chain (γc) is a shared subunit of the cytokine receptors, such as IL-2R, IL-4R, IL-7R, IL-9R, and IL-15R (1–3). The importance of γc in lymphocyte development and function has been underscored by the discovery of mutations in the γc gene in patients with X-linked severe combined immunodeficiency (XSCID), which is characterized by an absence or diminished numbers of T cells (3, 4). Consistent with these findings, mice carrying a null mutation in the γc alleles show severe defects in the development of lymphoid cells, such as a severe reduction in the numbers of B cells, as well as T and natural killer cells (5–7).

Although lacking intrinsic kinase activity, cytokine receptors, which use γc as a subunit, couple ligand binding to induction of tyrosine phosphorylation of cellular substrates (8, 9). Jak3 protein tyrosine kinase (PTK), a member of the Jak PTK family, has been found to be specifically and constitutively associated with γc (10–13). Unlike other members of the Jak family kinases that are expressed ubiquitously, the expression of Jak3 is highly restricted to the hematopoietic cell lineage (14, 15). The functional importance of Jak3 in the lymphoid system has been extensively investigated *in vitro*, providing evidence that the Jak3-signaling pathway is essential for the lymphocyte responses to IL-2 and other cytokines (10–12, 16). In fact, patients with mutations of the *Jak3* gene, manifesting similar immunodeficiency to XSCID, have been subsequently identified (17, 18). Consistent with this finding, mice carrying a null mutation in the *Jak3* alleles also were found to show severe defects of lymphocyte development (19–21).

The cytoplasmic domain of human and mouse γc contains 86 aa and 85 aa, respectively (3). It has been shown that the carboxyl-terminal 48 aa of the cytoplasmic domain of human γc is required for its association with and activation of Jak3 (11–13) and for the IL-2-induced transmission of mitogenic signals (10, 22, 23). On the other hand, evidence has been provided that the membrane-proximal region is also required for activation of Jak3 (24). It has been reported that a truncated mutant of γc, lacking the carboxyl-terminal 48 aa, is still capable of invoking some of the intracellular events caused by ligand stimulation in cultured cell lines (23, 24). In fact, the membrane-proximal, proline-rich motif of γc may be critical for Jak3-independent signal transduction (23). These observations raise an interesting issue of how signals mediated by the membrane-proximal region contribute to the development and functions of lymphocytes *in vivo*.

In the present study, we took an *in vivo* approach to analyze the functions of this membrane-proximal cytoplasmic subregion of γc by generating mice expressing the mutant form of a γc transgene on a γc null background. We demonstrate that expression of the mutant form of γc, which lacks the Jak3-binding region but retains the membrane-proximal region, results in significant restoration of T lymphopoiesis. We also show that the restored T cell development is accompanied by strong expression of the Bcl-2 protein in the T cells. However, these T cells cannot proliferate in response to mitogenic stimuli, and their ability to produce cytokines is severely impaired. These results may provide a new insight into γc-mediated signal transduction *in vivo*.

Materials and Methods

Construction of Transgenes and Generation of Transgenic Mice. For the construction of the M1-mutant lacking the C-terminal 79 aa of the mouse γc chain, pCDM8IL2Rγ (25) was amplified by PCR using the synthetic oligonucleotides 5′-GAACTCGAGCTTATCCCTGTTGGCAC-3′ and 5′-AGAGCGGCCGCTGGCTAAATTGGAGGCATTCGTTCC-3′ as primers. The PCR product was digested with *Xho*I and *Not*I and ligated with *Xho*I/*Not*I-digested pBluescript SK+. The resulting plasmid was digested with *Xho*I and *Nco*I and ligated with the cDNA fragment excised from pCDM8IL2Rγ with *Xho*I/*Nco*I to gen-

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Abbreviations: γc, common cytokine receptor γ chain; XSCID, X-linked severe combined immunodeficiency; PE, phycoerythrin; PTK, protein tyrosine kinase; Stat, signal transducer and activator of transcription; WT, wild type.

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erate pMγM1. For the construction of the M2-mutant lacking the C-terminal 48 aa of the mouse γc chain, pCDM8IL2Rγ was amplified by PCR using the synthetic oligonucleotides 5′-TCCCTCGAGCAAGAATCTAGAGGA-3′ and 5′-TCAGCG-GCCGCTGGCTACAGACTCTC-3′ as primers. The PCR product was digested with *Xho*I and *Not*I and ligated with pBluescript SK+. The resulting plasmid was digested with *Xho*I and *Xba*I and ligated with the cDNA fragment excised from pCDM8IL2Rγ with *Xho*I/*Xba*I to generate pMγM2. The cDNA of the wild-type (WT) mouse γc, M1-, or M2-mutant γc was excised from pCDM8IL2Rγ, pMγM1, or pMγM2 with *Xho*I/*Bam*HI, *Xho*I/*Not*I, or *Xho*I/*Not*I, respectively, blunted at both ends with T4 DNA polymerase, and ligated into *Sma*I-digested human *CD2* expression cassette (26) to generate transgenic mice, or ligated into *Eco*RI-digested pEF vector (27) whose ends were blunted, for the COS cell experiments. All of these constructs were verified by DNA sequencing. Transgenic mice were generated as described previously (28, 29). Each transgenic mouse was subsequently mated with the null mutant mice to generate mice of the desired genotype. The generation and maintenance of the *γc* and *Jak3* null mice were described previously (6, 20).

cDNA Transfection, Immunoprecipitation, and Immunoblot Analysis. These experiments were performed essentially according to previously published procedures (23, 27).

Flow Cytometric Analysis. Cells were incubated on ice with mAbs for 30 min and analyzed by flow cytometry (FACScalibur; Beckton Dickinson) using the CELLQUEST software (Beckton Dickinson). Intracellular staining of Bcl-2 was performed as described in a previous report (31). The mAbs used in this study were as follows: anti-mouse γc [phycoerythrin (PE)-conjugated, clone 4G3], anti-mouse CD4 (FITC-, PE-, and Cy-Chrome-conjugated, clone RM4–5), anti-mouse CD8α (PE- and Cy-Chrome-conjugated, clone 53–6.7), anti-mouse CD3 (PE-conjugated, clone 145–2C11), anti-mouse CD69 (FITC-conjugated, clone H1.2F3), anti-mouse TCRβ (PE-conjugated, clone H57–597), anti-mouse CD25 (FITC-conjugated, clone 7D4), anti-mouse Bcl-2 (clone 3F11), and anti-hamster IgG mixture-FITC. All of the mAbs were purchased from PharMingen.

Cytokine Production. Splenocytes (1×10^6 cells/ml) were incubated in either media without or with anti-CD3 (10 μg/ml) for 48 h (for IL-2) or 72 h (for IFNγ and IL-4). The supernatants were subsequently collected and the levels of cytokines were determined by ELISA (OptEIA, PharMingen).

Results

Generation of Mice Expressing the Mutant γc cDNA on a *γc* Null Background. To examine the functions of the cytoplasmic subregions of γc *in vivo*, a transgene encoding the mouse WT γc or mutants lacking either the carboxyl-terminal 79 aa (M1-mutant) or 48 aa (M2-mutant) were generated (Fig. 1*A*). We first confirmed that, as in the human γc, the deletion of 48 aa of mouse γc also eliminates the γc-Jak3 association (12, 23), by coimmunoprecipitation assay. COS cell lysates transfected with Jak3 cDNA, together with the WT or each mutant γc cDNA, were immunoprecipitated with the anti-γc antibody and followed by immunoblotting with the anti-Jak3 antiserum. As shown in Fig. 1*B*, immunoprecipitates from cells expressing WT γc indicated coprecipitation of the 115-kDa Jak3; however, this coprecipitation was not detected in the immunoprecipitates from cells expressing the M1- or M2-mutant γc (Fig. 1*B*). Thus, neither M1- nor M2-mutant can bind Jak3, an observation consistent with the previous data on human γc (12, 23).

Transgenic mice expressing the WT or mutant γc cDNAs were generated by using a human *CD2* expression cassette (26) to ensure expression in cells of the lymphoid lineage on a *γc* null background (6) (Tg-γc− lines). As shown in Fig. 1*C*, cell surface expression of the transgene-encoded WT, M1-mutant, or M2-mutant protein can be detected on CD4+ CD8+ (double positive: DP) thymocytes from each of the Tg-γc− lines (WT-γc−, M1-γc−, and M2-γc− mice, respectively). Cell surface expression of the WT or mutant γc also was detected on splenic and peripheral blood T cells (data not shown). Experiments were performed on at least three different Tg founder lines, and the data were highly reproducible.

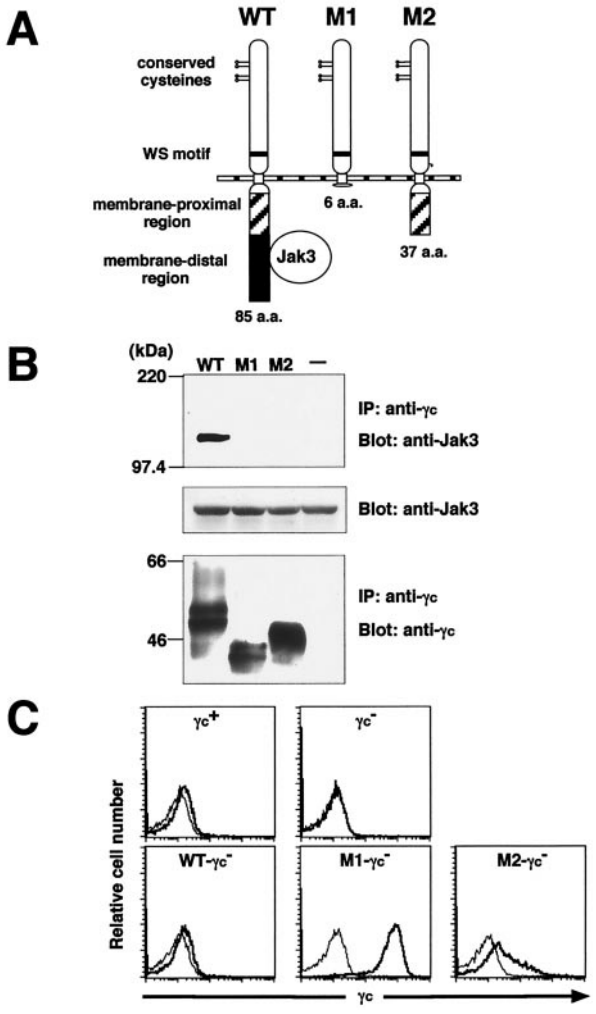

Fig. 1. Generation of mice expressing the mutant γc cDNA on a γc null background. (*A*) Schematic view of the murine γc chain and its mutants. WT, M1-, and M2-mutant γc retain 85, 6, and 37 aa of the cytoplasmic domain, respectively. The M2-mutant lacks the carboxyl-terminal 48 aa essential for Jak3 binding. (*B*) Absence of Jak3 association with the M2-mutant. COS cells were transfected with Jak3 and the indicated cDNA by using Lipofectamine Plus reagents (GIBCO/BRL). The cell lysates were immunoprecipitated (IP) with anti-γc (mixture of antibodies derived from 4G3 and TUGm3 clones, PharMingen). Immunoprecipitates were separated by 7.5% SDS/PAGE and subsequently immunoblotted (Blot) with anti-Jak3 (06–342, Upstate Biotechnology) (*Top*) or anti-γc antibody (sc-669, Santa Cruz Biotechnology) (*Bottom*). Whole cell lysates were separated by 7.5% SDS/PAGE and immunoblotted with anti-Jak3 antibody to determine the Jak3 protein levels (*Middle*). (*C*) Expression of γc on thymocytes. Thymocytes from 4-week-old Tg-γc− mice were stained with mAbs against γc, CD4, and CD8. Data were gated on CD4+ CD8+ cells. Profiles for unstained (fine line) and anti-γc-stained (bold line) thymocytes are shown in the histograms.

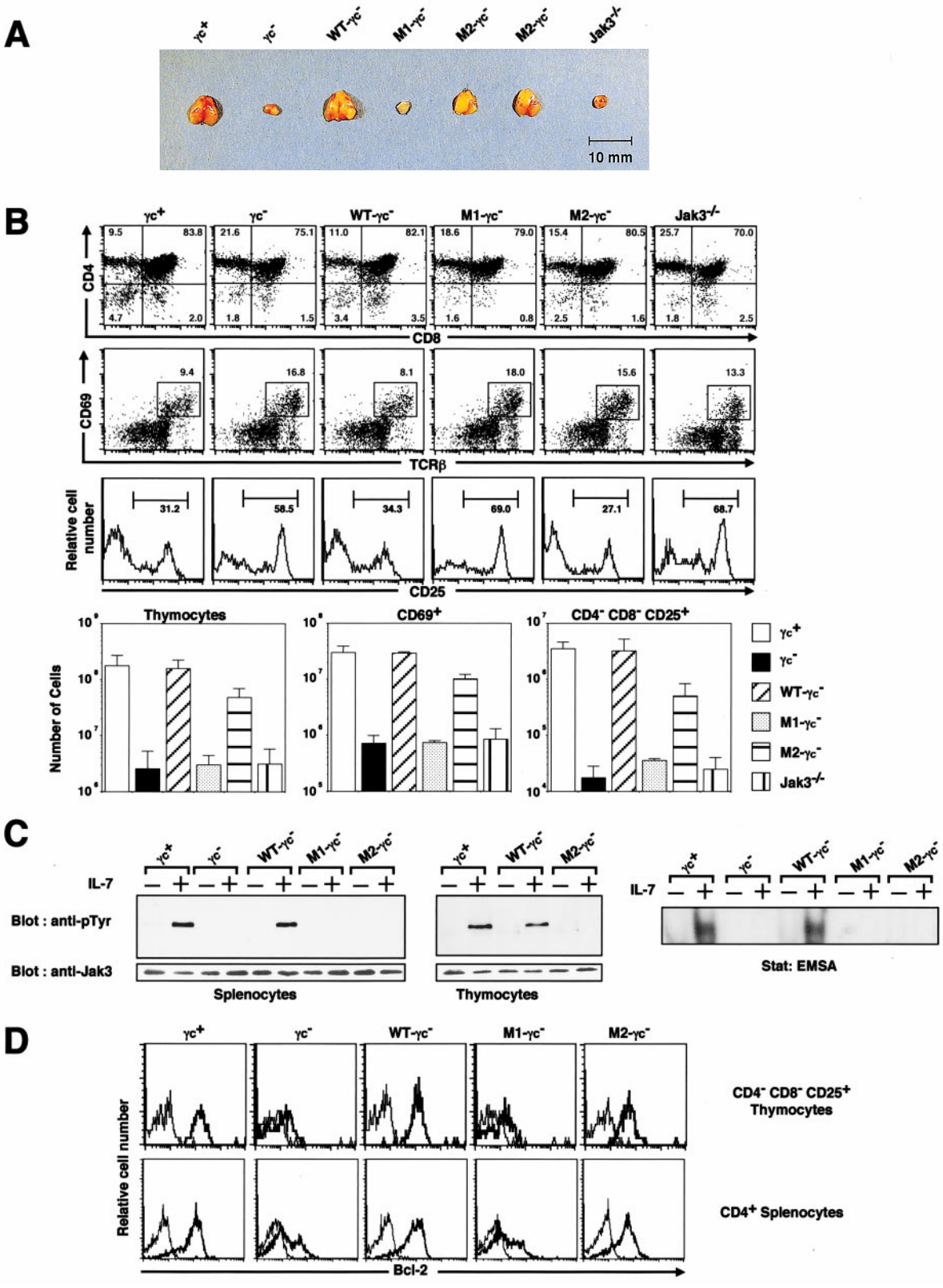

Fig. 2. Thymocyte development in transgene-reconstituted γc null mice. (*A*) Size comparison of the thymi from 5-week-old Tg-γc− mice. (*B*) CD4/CD8, CD69/T cell receptor β, and CD25 expression profiles of thymocytes. The absolute total numbers of thymocytes, CD69+ thymocytes, and CD4− CD8− CD25+ thymocytes are also shown. Hemocytometric counts were performed on single-cell suspensions prepared from the thymus of the indicated mice. The numbers of CD69+ and CD4− CD8− CD25+ cells in the thymus were calculated by determining their frequency by immunofluorescence staining and flow cytometric analysis and multiplication by the total number of thymocytes. The data represent the arithmetic mean ± SD of six 4-week-old mice of each type. (*C*) IL-7-induced Jak3 activation and Stat activation in primary lymphocytes. (*Left*) IL-7-induced tyrosine phosphorylation of Jak3 in splenocytes and thymocytes. Cells were starved for growth factors for 20 h and stimulated with IL-7 (5 ng/ml, Genzyme) for 15 min. The cell lysates were immunoprecipitated with an antibody against the N-terminal

T Cell Development Mediated by the Membrane-Proximal Region of γc. It has been shown that the thymus in γc− mice is markedly diminished in size and cell number (5–7). As shown in Fig. 2*A*, the expression of WT γc successfully restored the size of the thymus. In contrast, expression of the M1-mutant was not associated with an increase in the size of the thymus, confirming that the cytoplasmic domain of γc plays an essential role in thymocyte development. As reported previously, the size of the thymus in Jak3-deficient (Jak3−/−) mice also was severely diminished, to a level comparable to that in γc− mice (19–21). Interestingly, it was found that expression of the M2-mutant of γc, which lacks the Jak3-binding region, was associated with a significant increase in the size of the thymus (Fig. 2*A*). The ability of the M2-mutant γc to support thymocyte development also was demonstrated when the total numbers of thymocytes were examined (Fig. 2*B*). In addition, the number of CD69+ thymocytes that underwent positive selection (33–35) and that of early thymocyte precursors (CD4− CD8− CD25+ cells) were significantly increased in M2-γc− mice, as compared with those of γc−, M1-γc− or Jak3−/− mice (Fig. 2*B*). Furthermore, the number of splenic T cells also was restored in M2-γc− mice, and flow cytometric analysis revealed that they represent αβ T cells (data not shown). These results collectively indicate that the membrane-proximal region of γc, lacking the Jak3 binding site, indeed contributes to T lymphopoiesis.

We next examined IL-7-induced activation of Jak3 in T cells from these transgenic mice. As shown in Fig. 2*C*, the expression of WT γc successfully restored tyrosine phosphorylation of Jak3 in response to IL-7; however, such restoration was not observed in splenocytes from M1-γc− and M2-γc− mice. Essentially, a similar observation was made with thymocytes from these mice (Fig. 2*C*). Thus, the carboxyl-terminal 48 aa is essential for the IL-7-induced activation of Jak3 in primary lymphocytes, an observation consistent with previous results using cultured cell lines (22–24). Consistently, the activation of the signal transducer and activator of transcription 5 (Stat5) in response to IL-7 was not observed in splenocytes from γc−, M1-γc−, or M2-γc− mice (Fig. 2*C*, *Right*).

Bcl-2 Protein Expression Mediated by the Membrane-Proximal Region of γc. The above results raised the interesting issue of how signals mediated by the membrane-proximal region of γc support T cell development. In this regard, it has been suggested that γc supports T lymphopoiesis, at least in part, by transmitting signals that support the expression of the anti-apoptotic protein Bcl-2 (36–39). To clarify whether Bcl-2 protein expression is regulated by the membrane-proximal region of γc, we analyzed the expression level of Bcl-2 in splenic CD4+ T cells and developing CD4− CD8− CD25+ thymocytes by intracellular staining of Bcl-2 protein (31). In splenic CD4+ T cells from γc− mice, Bcl-2 expression was significantly lower than that in the CD4+ T cells from γc+ mice (Fig. 2*D*), as previously described (39). As expected, Bcl-2 expression was restored by expression of the WT γc transgene. Furthermore, transgenic expression of the M2-mutant, which retains the membrane-proximal region, could also significantly restore the expression of Bcl-2 protein in splenic CD4+ T cells (Fig. 2*D*). In contrast, expression of the M1-mutant transgene could not restore the expression of Bcl-2. Expression of Bcl-2 protein also was significantly restored in CD4− CD8− CD25+ thymocytes (Fig. 2*D*) and splenic CD8+ T cells (data not shown) in M2-γc− mice. These data indicate that Bcl-2 expression in T cells, which is known to promote T lymphopoiesis (36–39), can be supported by the membrane-proximal region of γc.

***In Vitro* Response of T Cells in M2-γc− Mice.** To examine further how the M2-mutant can contribute to the functions of T cells, we first examined the growth of the primary T cells in response to mitogenic stimulations *in vitro*. The thymocytes from γc− mice and Jak3−/− mice showed severe proliferative defects in response to anti-CD3 stimulation in combination with IL-2, IL-4, or IL-7, and these observations are consistent with previous reports (Fig. 3*A*) (5, 6). Interestingly, the mitogenic responses of thymocytes from M2-γc− mice were severely impaired, whereas thymocytes from WT-γc− mice showed normal mitogenic responses to these stimuli (Fig. 3*A*). Essentially the same results were obtained when thymocytes were stimulated with Con A in combination with IL-2, IL-4, or IL-7 (data not shown). The splenic T cells from M2-γc− mice also showed markedly impaired proliferative responses to anti-CD3 or Con A stimulation even in the presence of exogenously added IL-2 (Fig. 3*B*). Thus, the M2-mutant could not support T cell proliferation *in vitro*.

Next, we examined whether splenic T cells from Tg-γc− mice have the ability to produce cytokines after engagement of the T cell receptor. As shown in Fig. 3*C*, splenic T cells from γc−, M1-γc−, and M2-γc− mice showed a defect in producing IFNγ after anti-CD3 stimulation. In addition, IL-4 production by splenic T cells from these mice also was severely impaired (Fig. 3*C*). The production level of IL-2 by M2-γc− T cells was similar to that by T cells from γc− or M1-γc− mice and significantly lower than those by γc+ and WT-γc− T cells (Fig. 3*C*). These results demonstrate that expression of the M2-mutant does not rescue the defective cytokine production by splenic T cells. Taken together, the carboxyl-terminal region of γc, critical for the Jak3 activation, is essential for the function of T cells, and these observations are consistent with the previous reports (3, 4, 17–21).

Discussion

It has been shown that the carboxyl-terminal 48 aa of γc is essential for the association of this receptor subunit with Jak3 PTK and the activation of Jak3 PTK and is consequently important for the transmission of downstream signals (10–13, 22, 23). On the other hand, it has been reported that the membrane-proximal region of γc could also transmit signals by ligand stimulations (22–24). In the present study, we took an *in vivo* approach to study the functions of these regions.

Here, we provide evidence that expression of a mutant form of γc, which lacks the Jak3-binding region but retains the membrane-proximal region, on a γc null background results in significant restoration of T lymphopoiesis (Fig. 2 *A* and *B*). This finding suggests that the membrane-proximal region of γc can transmit signals which can support T cell development. In this regard, it has been shown that deletion of the membrane-distal Jak3-binding region causes the loss of the Jak3-activating ability of γc in cultured cell lines (22–24) and primary lym-

portion of mouse Jak3 (30). The immunoprecipitates were separated by 7.5% SDS/PAGE, followed by immunoblotting with anti-phosphotyrosine (4G10). To determine the Jak3 expression levels, whole cell lysates were separated by 7.5% SDS/PAGE and immunoblotted with anti-Jak3 antibody. (*Right*) Detection of IL-7-induced DNA-binding activity of Stat5 in splenocytes from WT or each line of the transgenic mice. Cells were stimulated (+) or not (−) with IL-7 (5 ng/ml) for 15 min. Whole cell lysates were prepared and subjected to an electrophoretic mobility-shift assay, using a ^{32}P-labeled *IRF-1*-derived IFN-γ-activated site (GAS) element as the probe DNA (27). The Stat complex, induced by IL-7, mainly consists of Stat5 (ref. 43; data not shown). (*D*) Expression of Bcl-2 in CD4− CD8− CD25+ thymocytes and CD4+ splenic T cells. Thymocytes of 4-week-old mice were stained with mAbs to Cy-Chrome-conjugated antibodies (CD4 and CD8), CD25-PE, and Bcl-2 followed by FITC-conjugated anti-hamster IgG. Splenoyctes of 4-week-old mice were stained with mAbs to CD4 and to Bcl-2 and gated on CD4+ cells. Control stainings with hamster IgG (fine line) and anti-Bcl-2-stainings (bold line) are shown in the histograms.

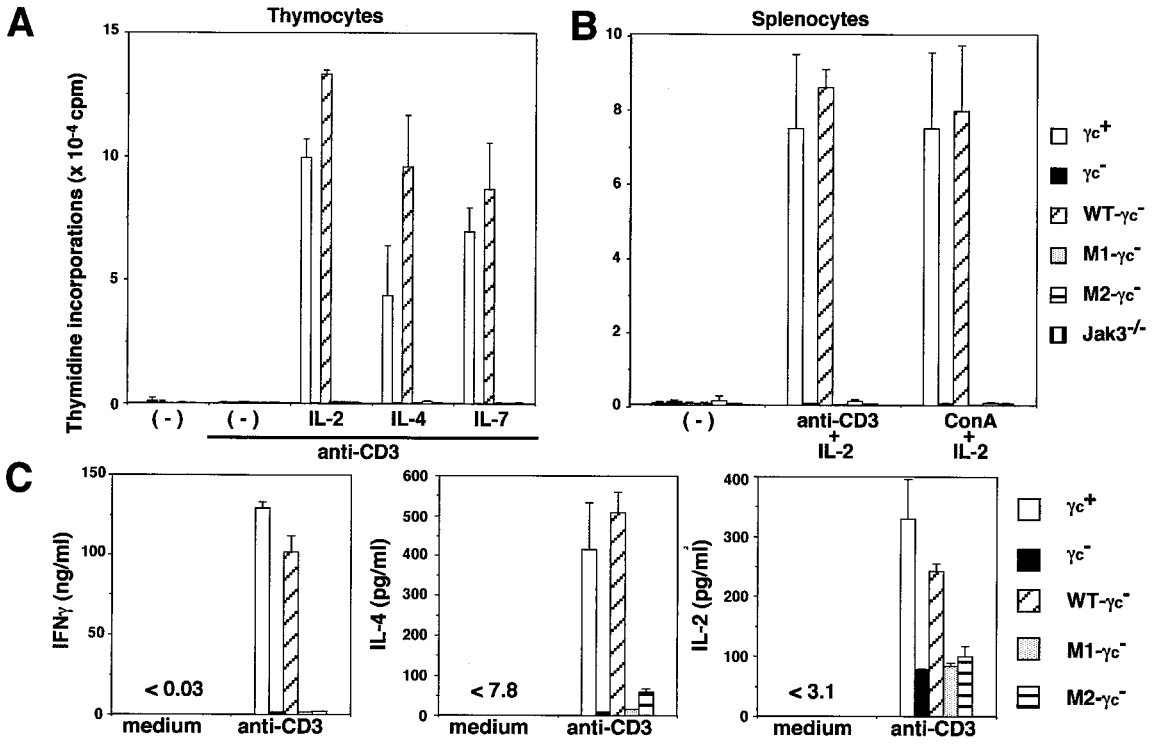

Fig. 3. Impaired functions of thymocytes and splenocytes from M2-γc− mice. Thymocytes (*A*) or splenocytes (*B*) (1×10^5) were cultured in triplicate in flat-bottomed 96-well plates, stimulated with RPMI, anti-CD3 antibody (145–2C11, 10 μg/ml), anti-CD3 antibody plus human IL-2 (1 nM, Takeda, Osaka), mouse IL-4 (10 ng/ml, Genzyme), mouse IL-7 (10 ng/ml), or Con A (5 μg/ml) plus human IL-2 (2 nM). Cells were pulsed with 0.5 μCi of [^{3}H]thymidine (Amersham) for the last 16 h of the 88-h culture period, and the [^{3}H]thymidine uptake levels were measured by using Top Count (Packard). Growth assays were performed in triplicate as described (32). The assay was performed several times, and the results were highly reproducible. (*C*) Cytokine production of splenic T cells from transgene-reconstituted γc null mice. Splenocytes (1×10^6 cells/ml) were incubated in either media without or with anti-CD3 antibody (10 μg/ml) for 72 h (for IFNγ and IL-4) or 48 h (for IL-2). The supernatants were subsequently collected, and the levels of cytokines were determined by ELISA.

phocytes (Fig. 2*C*). Therefore, it is likely that thymocyte development occurs in the absence of the γc-mediated Jak3 signals (see below).

It has been suggested that γc supports T lymphopoiesis, at least in part, through Bcl-2 expression in developing thymocytes (36–40). In the present study, we demonstrated that Bcl-2 expression in T cells is restored by expression of the M2-mutant (Fig. 2*D*). This result suggests that the membrane-proximal region of γc induces T lymphopoiesis by supporting the expression of Bcl-2. In view of the previous study, demonstrating that the induction of bcl-2 mRNA by IL-2 is Jak-independent (16), it is possible that the signal(s) emanating from the membrane-proximal region of γc is independent of the Jak pathway. On the other hand, it also has been demonstrated that Jak1 activation by IL-2 is induced, albeit weakly, in the absence of Jak3 activation in cell lines expressing the M2-mutant (23). Therefore, it cannot be ruled out that Jak1, in the absence of Jak3 activation, may contribute to Bcl-2 expression. Further work will be required to elucidate the molecular mechanism(s) of how the membrane-proximal region supports Bcl-2 expression. In addition, it remains to be clarified whether or not any additional targets, induced by γc in the absence of Jak3 activation, may also contribute to T lymphopoiesis (see ref. 41 for review).

As discussed above, restored thymocyte development and Bcl-2 expression in M2-γc− mice suggest the presence of a γc-mediated, Jak3-independent signaling pathway for T cell development. On the other hand, it should be noted that such a Jak3-independent signaling pathway, mediated by the membrane-proximal region of γc, should be intact in Jak3−/− mice. However, in contrast to M2-γc− mice, the immunological phenotype of Jak3−/− mice is similar to that of γc− mice, both showing severe defects in the development of lymphocytes (19–21). In addition, Bcl-2 expression was very low in the residual T cells from Jak3−/− mice (42). These observations imply that Jak3 plays a new role in T cell development, in addition to serve as a signal transducer of γc; Jak3 may be activated by other receptors or in a spontaneous manner during T cell development. In this context, one may postulate that γc-independent Jak3 activation pathway is intact in the M2-γc− thymocytes, and that this γc-independent Jak3 activation could contribute to T lymphopoiesis. On the other hand, it cannot be strictly ruled out that the M2-γc-mediated Jak3 activation may still occur at an undetectable level in our present assay. Obviously, it is most critical to elucidate the molecular nature of the M2-γc-mediated signaling pathway.

In sharp contrast to the restored T cell development in M2-γc− mice, thymocytes and splenocytes from these mice could not proliferate in response to mitogenic stimuli (Fig. 3 *A* and *B*). These results further underscore the critical role of the membrane-distal Jak3-binding region of γc in T cell proliferative functions, as also suggested by previous studies (10, 22, 23). The observation that thymocytes in M2-γc− mice do not proliferate in response to cytokines including IL-7 is consistent with the idea that γc supports T cell development not by promoting cell proliferation but by inhibiting apoptosis. This idea is consistent with the recent reports that transgenic expression of anti-apoptotic Bcl-2 protein could restore T cell development in

IL-7R−/− and γc− mice (36–38). Our result, showing the absence of IL-7-induced activation of Jak3 in the primary T cells from M2-γc− mice, underscores further the critical role of the carboxyl-terminal 48 aa for the Jak3 activation for T cell functions.

Although further mechanistic insights will be required to assess how the membrane-proximal region of γc controls lymphocyte development, our current work reveals the hitherto unknown roles of this region *in vivo*. In addition, these results also reveal the differential requirement of the cytoplasmic subregions of γc in T cell development and function, as well as emphasize the merit of the *in vivo* approach in dissecting the role of the cytoplasmic subregion of cytokine receptors.

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1. Giri, J. G., Ahdieh, M., Eisenman, J., Shanebeck, K., Grabstein, K., Kumaki, S., Namen, A., Park, L. S., Cosman, D. & Anderson, D. (1994) *EMBO J.* **13,** 2822–2830.
2. Leonard, W. J., Noguchi, M., Russell, S. M. & McBride, O. W. (1994) *Immunol. Rev.* **138,** 61–86.
3. Sugamura, K., Asao, H., Kondo, M., Tanaka, N., Ishii, N., Ohbo, K., Nakamura, M. & Takeshita, T. (1996) *Annu. Rev. Immunol.* **14,** 179–205.
4. Noguchi, M., Yi, H., Rosenblatt, H. M., Filipovich, A. H., Adelstein, S., Modi, A. S., McBride, O. W. & Leonard, W. J. (1993) *Cell* **73,** 147–157.
5. Cao, X., Shores, E. W., Hu-Li, J., Anver, M. R., Kelsall, B. L., Russell, S. M., Drago, J., Noguchi, M., Grinberg, A., Bloom, E. T., *et al.* (1995) *Immunity* **2,** 223–238.
6. Di Santo, J. P., Müller, W., Guy-Grand, D., Fischer, A. & Rajewsky, K. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 377–381.
7. Ohbo, K., Suda, T., Hashiyama, M., Mantani, A., Ikebe, M., Miyakawa, K., Moriyama, M., Nakamura, M., Katsuki, M., Takahashi, K., *et al.* (1996) *Blood* **87,** 956–967.
8. Ihle, J. N. (1995) *Nature (London)* **377,** 591–594.
9. Taniguchi, T. (1995) *Science* **268,** 251–255.
10. Asao, H., Tanaka, N., Ishii, N., Higuchi, M., Takeshita, T., Nakamura, M., Shirasawa, T. & Sugamura, K. (1994) *FEBS Lett.* **351,** 201–206.
11. Boussiotis, V. A., Barber, D. L., Nakarai, T., Freeman, G. J., Gribben, J. G., Bernstein, G. M., D'Andrea, A. D., Ritz, J. & Nadler, L. M. (1994) *Science* **266,** 1039–1042.
12. Miyazaki, T., Kawahara, A., Fujii, H., Nakagawa, Y., Minami, Y., Liu, Z.-J., Oishi, I., Silvennoinen, O., Witthuhn, B. A., Ihle, J. N., *et al.* (1994) *Science* **266,** 1045–1047.
13. Russell, S. M., Johnston, J. A., Noguchi, M., Kawamura, M., Bacon, C. M., Friedmann, M., Berg, M., McVicar, D. W., Witthuhn, B. A., Silvennoinen, O., *et al.* (1994) *Science* **266,** 1042–1045.
14. Johnston, J. A., Kawamura, M., Kirken, R. A., Chen, Y.-Q., Blake, T. B., Shibuya, K., Ortaldo, J. R., McVicar, D. W. & O'Shea, J. J. (1994) *Nature (London)* **370,** 151–153.
15. Witthuhn, B. A., Silvennnoinen, O., Miura, O., Lai, K. S., Cwik, C., Liu, E. T. & Ihle, J. N. (1994) *Nature (London)* **370,** 153–157.
16. Kawahara, A., Minami, Y., Miyazaki, T., Ihle, J. N. & Taniguchi, T. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 8724–8728.
17. Macchi, P., Villa, A., Gillani, S., Sacco, M. G., Frattini, A., Prota, F., Ugazio, A. G., Johnston, J. A., Candotti, F., O'Shea, J. J., *et al.* (1995) *Nature (London)* **377,** 65–68.
18. Russell, S. M., Tayebi, N., Nakajima, H., Riedy, M. C., Roberts, J. L., Aman, M. J., Migone, T. S., Noguchi, M., Markert, M. L., Buckley, R. H., *et al.* (1995) *Science* **270,** 797–800.
19. Nosaka, T., van Deursen, J. M. A., Tripp, R. A., Thierfelder, W. E., Witthuhn, B. A., McMickle, A. P., Doherty, P. C., Grosveld, G. C. & Ihle, J. N. (1995) *Science* **270,** 800–802.
20. Park, S. Y., Saijo, K., Takahashi, T., Osawa, M., Arase, H., Hirayama, N., Miyake, K., Nakauchi, H., Shirasawa, T. & Saito, T. (1995) *Immunity* **3,** 771–782.
21. Thomis, D. C., Gurniak, C. B., Tivol, E., Sharpe, A. H. & Berg, L. J. (1995) *Science* **270,** 794–797.
22. Nelson, B., Lord, J. D. & Greenberg, P. D. (1996) *Mol. Cell. Biol.* **16,** 309–317.
23. Tsujino, S., Miyazaki, T., Kawahara, A., Maeda, M., Taniguchi, T. & Fujii, H. (1999) *Genes Cells* **4,** 363–373.
24. Nelson, B. H., McIntosh, B. C., Rosencrans, L. L. & Greenberg, P. D. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 1878–1883.
25. Kobayashi, N., Nakagawa, S., Minami, Y., Taniguchi, T. & Kono, T. (1993) *Gene* **130,** 303–304.
26. Zhumabekov, T., Corbella, P., Tolaini, M. & Kioussis, D. (1995) *J. Immunol. Methods* **185,** 133–140.
27. Fujii, H., Nakagawa, Y., Schindler, U., Kawahara, A., Mori, H., Gouilleux, F., Groner, B., Ihle, J. N., Minami, Y., Miyazaki, T. *et al.* (1995) *Proc. Natl. Acad. Sci. USA* **92,** 5482–5486.
28. Brinster, R. L., Chen, N. Y., Trumbauer, M. E., Yagle, M. K. & Palmiter, R. D. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4438–4442.
29. Koike, S., Taya, C., Kurata, T., Abe, S., Ise, I., Yonekawa, H. & Nomoto, A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 951–955.
30. Chen, M., Cheng, A., Chen, Y.-Q., Hymel, A., Hanson, E. P., Kimmel, L., Minami, Y., Taniguchi, T., Changelian, P. S. & O'Shea, J. J. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 6910–6915.
31. Veis, D. J., Sentman, C. L., Bach, E. A. & Korsmeyer, S. J. (1993) *J. Immunol.* **151,** 2546–2554.
32. Fujii, H., Ogasawara, K., Otsuka, H., M, S., Yamamura, K.-i., Yokochi, T., Miyazaki, T., Suzuki, H., Mak, T. W., Taki, S. & Taniguchi, T. (1998) *EMBO J.* **17,** 6551–6557.
33. Swat, W., Dessing, M., von Boehmer, H. & Kiesielow, P. (1993) *Eur. J. Immunol.* **23,** 739–746.
34. Yamashita, I., Nagata, T., Tada, T. & Nakayama, T. (1993) *Int. Immunol.* **5,** 1139–1150.
35. Brandle, D., Muller, S., Muller, C., Hengartner, H. & Pircher, H. (1994) *Eur. J. Immunol.* **24,** 145–151.
36. Akashi, K., Kondo, M., von Freeden-Jeffry, U., Murray, R. & Weissman, I. L. (1997) *Cell* **89,** 1033–1041.
37. Kondo, M., Akashi, K., Domen, J., Sugamura, K. & Weissman, I. L. (1997) *Immunity* **7,** 155–162.
38. Maraskovsky, E., O'Reilly, L. A., Teepe, M., Corcoran, L. M., Peshon, J. J. & Strasser, A. (1997) *Cell* **89,** 1011–1019.
39. Nakajima, H., Shores, E. W., Noguchi, M. & Leonard, W. J. (1997) *J. Exp. Med.* **185,** 189–195.
40. Di Santo, J. P., Alfantis, I., Rosmaraki, E., Garcia, C., Feinberg, J., Fehling, H.-J., Fischer, A., von Boehmer, H. & Rocha, B. (1999) *J. Exp. Med.* **189,** 563–573.
41. Di Santo, J. P. & Rodewald, H.-R. (1998) *Curr. Opin. Immunol.* **10,** 196–207.
42. Suzuki, K., Nakajima, H., Saito, Y., Saito, T., Leonard, W. J. & Iwamoto I. (2000) *Int. Immunol.* **12,** 123–132.
43. Nakajima, H., Liu, X-W, Wynshaw-Boris, A., Rosenthal, L. A., Imada, K., Finbloom, D. S., Hennighausen, L. & Leonard, W. J. (1997) *Immunity* **7,** 691–701.