Impairment of Natural Killer Cytotoxic Activity and Interferon γ Production in CCAAT/Enhancer Binding Protein γ-deficient Mice

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Summary
We have investigated in vivo roles of CCAAT/ enhancer binding protein γ (C/EBPγ) by gene targeting. C/EBPγ-deficient (C/EBPγ−/−) mice showed a high mortality rate within 48 h after birth. To analyze the roles of C/EBPγ in lymphoid lineage cells, bone marrow chimeras were established. C/EBPγ−/− chimeras showed normal T and B cell development. However, cytolytic functions of their splenic natural killer (NK) cells after stimulation with cytokines such as interleukin (IL)-12, IL-18, and IL-2 were significantly reduced as compared with those of control chimera NK cells. In addition, the ability of C/EBPγ−/− chimera splenocytes to produce interferon (IFN)-γ in response to IL-12 and/or IL-18 was markedly impaired. NK cells could be generated in vitro with normal surface marker expression in the presence of IL-15 from C/EBPγ−/− newborn spleen cells. However, they also showed lower cytotoxic activity and IFN-γ production when stimulated with IL-12 plus IL-18 than control NK cells, as observed in C/EBPγ−/− chimera splenocytes. In conclusion, our study reveals that C/EBPγ is a critical transcription factor involved in the functional maturation of NK cells.

Key words: gene targeting • natural killer cells • C/EBPγ • interleukin 15 • interferon γ

The CCAAT/enhancer binding proteins (C/EBP) belong to a family of basic leucine zipper transcription factors (1, 2). So far six members are isolated and share well conserved COOH-terminal regions, which contain a basic region for DNA binding and a leucine zipper motif for dimerization. Gene targeting revealed that each member plays a critical role in cellular proliferation and differentiation in various tissues. For example, nuclear factor (NF)-IL6, also designated C/EBPβ, is essential for macrophage function (3, 4) and involved in adipocyte differentiation, working coordinately with C/EBPδ (5). C/EBPα is essential for hepatocyte function and adipocyte differentiation (6, 7).

Furthermore, C/EBPε, expressed specifically in myeloid lineage cells, plays a critical role in granulopoiesis (8). C/EBPγ is a member of the C/EBP family. C/EBPγ can bind to the pEBP-E binding sites (E sites) in both the IgH enhancer and the Vh1 promoter and was originally identified as an Ig enhancer binding protein, Ig/EBP-1 (9, 10). C/EBPγ can also bind to the regulatory elements for other genes such as G-CSF and IL-4 (11, 12). Although other members are expressed in a relatively tissue-specific or inducible manner, expression of C/EBPγ is ubiquitous and constitutive (9). In addition, C/EBPγ does not contain transcription activating domains. It can nevertheless interact with other transcription factors through the leucine zipper domain and function as a dominant negative form (10). In some cases, C/EBPγ can augment the DNA binding ability of other transcription factors (12). Thus, C/EBPγ is considered to be a regulator for transcription factors with...
the leucine zipper domain. However, the biological roles of C/EBPγ remain unclear.

To clarify in vivo functions of C/EBPγ, we have generated C/EBPγ-deficient (C/EBPγ−/−) mice by gene targeting. C/EBPγ−/− mice showed a high mortality rate within 48 h after birth, indicating that neonatal survival requires C/EBPγ. By establishing bone marrow chimeras, we found that C/EBPγ is critical for NK cell functions. NK cells are large granular lymphocytes derived from the bone marrow and essential for innate immunity (13, 14). NK cells can mediate cytotoxicity against tumor cells and virus-infected cells and produce immunomodulatory cytokines such as IFN-γ. These activities were impaired in C/EBPγ−/− NK cells, establishing that C/EBPγ is a novel regulator for functional NK cell maturation.

Materials and Methods

Generation of C/EBPγ−/− Mice. A BALB/c-derived genomic fragment including the C/EBPγ gene was provided by Dr. S. Nagaoka (O saka University, O saka, Japan). As the long arm of the homology region, an 8.8-kb genomic fragment including the first exon, the first intron, and part of the second exon was used. The neomycin resistance gene derived from pMC1Neo-poly(A) (15) was inserted into the targeting vector to disrupt the basic DNA binding and leucine zipper regions. A short arm of the homology region was amplified by PCR. The M C1 herpes simplex virus thymidine kinase (16) was inserted 5’ upstream of the homologous region. E14.1 embryonic stem cells, which were derived from 129/SvJ (129) mice, were transfected with a linearized targeting vector by electroporation. Homologous recombinants were identified among double-resistant clones against G418 and gancyclovir by PCR and Southern blot analysis. Generation of chimeras and mutant mice was essentially as described (17).

Reverse Transcriptase–PCR for C/EBPγ. Splenic B and T cells were purified from B6 spleen cells using Magnetic Cell Sorter (MACS®; Miltenyi Biotec) with B220 and CD3 microbeads, respectively. For purification of NK cells, DX5+ cells were first prepared from B6 spleen cells using MACS® with biotinylated DX5 and streptavidin microbeads. Then the cells were stained with anti-DX5 and anti-DX5. CD3+ DX5− cells were sorted with EPICS Elite (Coulter Immunology) and used as NK cells for receptor analysis. Generation of chimeras and mutant mice was essentially as described (17).

Flow Cytometric Analysis. Single-cell suspensions from thymus, spleen, or cultured cells were incubated with anti-CD16/32 (Phar-Mingen) to minimize nonspecific staining. They were then stained with cocktails of mAbs conjugated to FITC, PE, biotin, or Cy-Chrome for 20 min at 4°C. The biotinylated Abs were developed with streptavidin conjugated to PE or Cy-Chrome (Pharmingen). All mAbs, with the exception of PE-labeled anti-Id (Southern Biotechnology Associates), were purchased from Pharmingen. Flow cytometric analysis was performed using a FACSCalibur™ with CELLQuest™ software (Becton Dickinson).

Generation of Radiation Chimeras. Fresh splenocytes (106 cells per well) were cultured in complete RPMI 1640 (RPMI 1640 medium supplemented with 10% FCS, 2-ME, penicillin, and streptomycin) with 10 μg/ml anti-IgM (Zymed Labs.) plus 0.5 μg/ml anti-CD4 (Pharmingen). 10 μg/ml LPS (055:B5; Sigma Chemical Co.), 20 ng/ml IL-2 (Genzyme Corp.), 0.1 μg/ml anti-CD3 (Pharmingen) plus 0.1 μg/ml anti-CD28 (Pharmingen), and 2.5 μg/ml Con A (Sigma Chemical Co.) in 96-well plates. 48 h later, they were pulsed with 0.2 μCi of [3H]thymidine (NEN Research Products) and cultured for a further 15 h.

Analysis of NK Cell Activity. Splenocytes from bone marrow chimeras were incubated with 2 ng/ml IL-12, 20 ng/ml IL-18, and 2 ng/ml IL-12 plus 20 ng/ml IL-18 or 500 U/ml IL-2 for 24 h, and their cytotoxic activities against YAC-1 cells were measured as described previously (20, 21). Spontaneous cytotoxic activity was measured by incubating splenocytes with 51Cr-labeled YAC-1 cells in the absence of cytokines for 4 h.

ELISA and RT-PCR for IFN-γ. Splenocytes at 106 cells per well in 96-well plates were cultured in complete RPMI 1640 in the presence or absence of 2 ng/ml IL-12 and/or 20 ng/ml IL-18 for 24 h. Amounts of IFN-γ in harvested supernatants were measured by ELISA using Duoset (Genzyme Corp.) according to the manufacturer’s instructions. The lowest detection limit of ELISA is 10 pg/ml. Semi-quantitative RT-PCR for IFN-γ was performed as described previously (22).

In Vitro Culture of Newborn Spleen Cells. Newborn spleen cells were cultured in complete RPMI 1640 with 300 ng/ml IL-15 (Genzyme Corp.) at 105 cells per well in 24-well plates. 10 d later, cells were harvested, washed four times, and used for further analysis. For cytotoxic activities, harvested cells were incubated with labeled YAC-1 cells in the absence of cytokines for 4 h. Their cytotoxic activities against YAC-1 cells were then measured as described previously (20). For IFN-γ measurement, harvested cells were cultured at 106 cells per well in 96-well plates with 2 ng/ml IL-12 and 20 ng/ml IL-18 for a further 24 h. Then, amounts of IFN-γ in the culture supernatants were measured with ELISA.

RT-PCR Analysis of Cultured Spleen Cells. Total RNAs were purified from 10-d-cultured newborn spleen cells with IL-15, re-
verse transcribed, and amplified. Primers and amplifying conditions for IL-12Rβ1, IL-12Rβ2, and IL-18Rα were described previously (21).

Western Blot Analysis of Spleen Cells. Newborn spleen cells cultured with IL-15 for 10 d were stimulated with 2 ng/ml IL-12 or 20 ng/ml IL-18 for 20 min and lysed in buffer containing 20 mM Tris/HCl, pH 8.0, 137 mM NaCl, 5 mM EDTA, 10% glycerol, 1% Triton X-100, 1 mM PM SF, 20 mg/ml aprotinin, 20 mg/ml leupeptin, 1 mM Na3VO4, 1 mM EGTA, 10 mM NaF, 1 mM Na4P2O7, and 10 mM β-glycerophosphate. For signal transducer and activator of transcription (STAT)4 activation, the cell lysates were immunoprecipitated with anti-STAT4 Ab (Santa Cruz Biotechnology) and Protein A-Sepharose (Amersham Pharmacia Biotech). The immunoprecipitates were separated on SDS-PAGE, transferred onto a nitrocellulose membrane, and blotted with antiphosphotyrosine 4G10 (Upstate Biotechnology Inc.) or anti-STAT4 Ab. For c-Jun NH2-terminal kinase (JNK) activation, the cell lysates were separated on SDS-PAGE, followed by blotting with antiphospho-JNK Ab (Promega Corp.) or anti-JNK1 Ab (Santa Cruz Biotechnology). Proteins bound to the Abs were visualized using the enhanced chemiluminescence system (Dupont).

Results and Discussion

Generation of C/EBPγ−/− Mice. The C/EBPγ genomic locus was disrupted by inserting the neomycin resistance gene into the second exon (Fig. 1 A). This insertion resulted in disruption of the basic and leucine zipper domains, which are essential for DNA binding and dimer formation. Homologous recombinants were obtained through a double selection with G418 and gancyclovir. Targeted clones resulting in disruption of the basic and leucine zipper domains, which are essential for DNA binding and dimer formation. Homologous recombinants were obtained through a double selection with G418 and gancyclovir. Targeted clones were injected into B6 blastocysts to generate chimeric mice, which were bred to achieve germline transmission. By mating heterozygous mutants, homozygous mutants were obtained at a frequency of the expected Mendelian ratio (22.6% of littermates). C/EBPγ obtained at a frequency of the expected Mendelian ratio by mating heterozygous mutants, homozygous mutants were obtained at a frequency of the expected Mendelian ratio (22.6% of littermates). C/EBPγ obtained at a frequency of the expected Mendelian ratio (22.6% of littermates). C/EBPγ obtained at a frequency of the expected Mendelian ratio (22.6% of littermates). C/EBPγ obtained at a frequency of the expected Mendelian ratio.

**Figure 1.** Generation of C/EBPγ−/− mice and expression of C/EBPγ MrNA. (A) Schematic representation of the C/EBPγ targeting vector, the C/EBPγ genomic locus, and the targeted C/EBPγ allele. The targeting vector contains the herpes simplex virus thymidine kinase (HSV-TK) gene 5′ upstream of the long arm homology region and the neomycin resistance gene (NEO) in the middle of the second exon. (B) RT-PCR analysis of newborn spleen and liver RNAs from C/EBPγ−/+ and C/EBPγ−/− mice. Total RNAs were isolated and analyzed for C/EBPγ and β-actin expression by RT-PCR. M, PhiX174/HaeIII digest marker. (C) RT-PCR analysis for C/EBPγ in lymphoid lineage cells. Total RNAs were isolated from splenic CD3+DX5+(NK), B220+(B), and CD3+ (T) cells and analyzed for C/EBPγ and β-actin expression by RT-PCR.

Table I. Postnatal Survival of C/EBPγ−/− Mice

| Time   | Alive (%) | N.o. dead |
|--------|-----------|-----------|
| 0–12   | 18 (100)  | 0         |
| 12–24  | 12 (66.7) | 6         |
| 24–36  | 9 (50.0)  | 3         |
| 36–48  | 3 (16.7)  | 6         |
| 48–60  | 2 (11.1)  | 1         |
| >60    | 2 (11.1)  | 0         |

The C/EBPγ−/− mice showed normal population of CD4+ T cells and CD8+ T cells (13, 14), also expressed C/EBPγ mRNA (Fig. 1 C). To investigate in vivo roles of C/EBPγ in these lymphoid lineage cells, we generated bone marrow chimeric mice by transferring newborn spleen cells into lethally irradiated, RAG2−/− B6 mice. Thymocytes from C/EBPγ−/− chimeras showed normal T cell development (Fig. 2 A). Splenocytes from C/EBPγ−/− chimeras also showed normal population of CD4+8− and CD4+8+ T cells (Fig. 2 A). Furthermore, analysis of surface markers such as B220, IgM, and IgD revealed that B cell maturation was not disturbed in C/EBPγ−/− chimeras (Fig. 2 A). In homozygous mutants could survive >60 h, although they were healthy until 12 h after they were born (Table I). These results indicate that C/EBPγ is involved in early neonatal survival but not embryonic survival. By histological examination, C/EBPγ−/− mice at 24 h showed emphysematous changes in their lungs (data not shown). However, it is not clear at the time of this writing whether the lung lesions alone can account for the high mortality of homozygous mutants at the early neonatal stage.
addition to surface phenotype, proliferative responses to mitogens or stimulating Abs were not significantly different between C/EBP\(\gamma^{+/+}\) and C/EBP\(\gamma^{-/-}\) chimeras (Fig. 2 B). Taken together, these results suggest that C/EBP\(\gamma\) is not essential for functional T and B cell development.

Impaired Splenic NK Cell Activity in C/EBP\(\gamma^{2/2}\) Chimeras. NK cells can be identified as CD3\(^{-}\)IL-2R\(^{b+}\), CD3\(^{-}\)NK1.1\(^{+}\), or CD3\(^{-}\)DX5\(^{+}\) cells by flow cytometry. However, the NK1.1 analysis in the chimeras is limited, because only some mice in a mixed genetic background of 129 and B6 carried the NK1.1 allele, of which expression is detected on B6 but not 129 NK cells. Therefore, the NK cell population was analyzed for IL-2R\(^{b}\) and DX5 expression. Splenic CD3\(^{-}\)IL-2R\(^{b+}\) cells in C/EBP\(\gamma^{2/2}\) chimeras were detected at levels equivalent to those in C/EBP\(\gamma^{1/1}\) chimeras (Fig. 3 A). Furthermore, the frequency of CD3\(^{-}\)DX5\(^{+}\) cells was also comparable between C/EBP\(\gamma^{1/1}\) and C/EBP\(\gamma^{2/2}\) chimeras (Fig. 3 A). Percentages of CD3\(^{-}\)DX5\(^{+}\) cells were larger than those of CD3\(^{-}\)IL-2R\(^{b+}\) cells in both control and C/EBP\(\gamma^{-/-}\) chimeras, as described previously (23).

Next, NK cytotoxicity was measured by YAC-1 cell-killing activity in the absence or presence of various cytokines (Fig. 3 B). Spontaneous cytotoxicity of C/EBP\(\gamma^{-/-}\) chimeras splenocytes (1.0% at 100:1) was impaired as compared with that of control chimeras (6.4% at 100:1; Fig. 3 B). IL-12 and/or IL-18 act on NK cells and can enhance their cytotoxic activity (21). When stimulated with these cytokines, C/EBP\(\gamma^{-/-}\) chimeras also showed impaired killing activity as compared with control splenocytes (Fig. 3 B). IL-2 is another stimulatory cytokine for NK cell activity (24). Decreased killing activity of C/EBP\(\gamma^{-/-}\) chimeras was also observed in the presence of IL-2 (Fig. 3 B). Poly (I:C)-stimulated C/EBP\(\gamma^{-/-}\) chimeras also showed lower cytotoxic activity than control splenocytes (data not shown).

Reduced Ability of C/EBP\(\gamma^{-/-}\) Chimera Splenocytes to Produce IFN\(\gamma\) in Response to IL-12 and/or IL-18. NK cells constitutively express both functional IL-12R and IL-18R, whereas naive T cells do not (21, 25, 26). Therefore, splenic IFN\(\gamma\) production by stimulation with IL-12 and IL-18 is dependent on NK but not T cells. To evaluate the ability of NK cells from chimeric mice to produce IFN\(\gamma\), splenocytes were cultured with or without IL-12 and/or IL-18. 24 h later, cell-free supernatants were harvested and assayed for IFN\(\gamma\) production with ELISA. Under this condition, C/EBP\(\gamma^{-/-}\) chimeras splenocytes produced much lower amounts of IFN\(\gamma\) than C/EBP\(\gamma^{+/+}\) chimeras splenocytes (Fig. 4 A). IL-12 or IL-18 can induce IFN\(\gamma\) production at the transcriptional level in NK cells (27). Consistent with reduced IFN\(\gamma\) production, induction of IFN\(\gamma\) mRNA was markedly decreased in C/EBP\(\gamma^{-/-}\) chimeras splenocytes (Fig. 4 B). Taken together, these results suggest...
that C/EBPγ is required for induction of IFN-γ by IL-12 and/or IL-18 in NK cells.

IL-15-induced NK cells from C/EBPγ−/− newborn spleens showed impairment of both cytotoxic activity and IFN-γ production. NK cell population was analyzed in newborn spleens. C/EBPγ1 and/or IL-12. (A) Single-cell suspensions from the chimera splenocytes were stained with FITC-anti-CD3 and PE-anti-IL-2Rβ Ab (top panels) or with FITC-anti-CD3 and biotin-DX5 followed by PE-streptavidin (bottom panels). The percentages of NK cell population are shown. (B) YAC-1 killing analysis of spleen cells. C/EBPγ+/+ (●) and γ−/− (○) chimera spleen cells were incubated without any cytokines for 4 h or with 2 ng/ml IL-12, 20 ng/ml IL-18, 2 ng/ml IL-12 plus 20 ng/ml IL-18, or 500 U/ml IL-2 for 24 h, and their cytotoxic activities against YAC-1 cells were determined. E/T ratios are shown on the x-axis. Six independent experiments were performed with similar results. The data indicate mean ± SD of triplicate samples of one representative experiment.

Figure 3. NK cell population and cytolytic activity of C/EBPγ+/+ and C/EBPγ−/− chimera spleen cells. (A) Single-cell suspensions from the chimera splenocytes were stained with FITC-anti-CD3 and PE-anti-IL-2Rβ Ab (top panels) or with FITC-anti-CD3 and biotin-DX5 followed by PE-streptavidin (bottom panels). The percentages of NK cell population are shown. (B) YAC-1 killing analysis of spleen cells. C/EBPγ+/+ (●) and γ−/− (○) chimera spleen cells were incubated without any cytokines for 4 h or with 2 ng/ml IL-12, 20 ng/ml IL-18, 2 ng/ml IL-12 plus 20 ng/ml IL-18, or 500 U/ml IL-2 for 24 h, and their cytotoxic activities against YAC-1 cells were determined. E/T ratios are shown on the x-axis. Six independent experiments were performed with similar results. The data indicate mean ± SD of triplicate samples of one representative experiment.

Figure 4. IFN-γ production by chimera splenocytes in response to IL-12 and/or IL-18. (A) Spleen cells from C/EBPγ+/+ (filled column) and C/EBPγ−/− (open column) chimera splenocytes were cultured in the absence (med) or presence of 2 ng/ml IL-12 and/or 20 ng/ml IL-18 for 24 h. Amounts of IFN-γ in the culture supernatants were measured by ELISA. Experiments were independently performed six times with similar results. The data indicate mean ± SD of triplicate samples of one representative experiment. N.D., not detected. (B) RT-PCR analysis of C/EBPγ+/+ and C/EBPγ−/− chimera splenocytes cultured with 2 ng/ml IL-12 and 20 ng/ml IL-18 for 24 h. Total RNAs were isolated and analyzed for IFN-γ and β-actin expression by RT-PCR. Experiments were independently performed three times with similar results.

Figure 5. NK cell population and IFN-γ production in C/EBPγ−/− newborn splenocytes. Both CD3-IL-2Rβ+ and CD3-DX5+ cells were equivalently detected in C/EBPγ+/+ and C/EBPγ−/− newborn splenocytes (Fig. 5 A). IL-15 can stimulate NK cell activity and proliferation and is essential for NK cell development (28, 29). Adult bone marrow cells can generate NK cells when cultured with IL-15 (28, 30). Wild-type newborn spleen cells could also give rise to CD3-IL-2Rβ+ DX5+ cells in the presence of IL-15 (Fig. 5 B). In this culture condition, harvested cell numbers from control (C/EBPγ+/+, n = 5, and C/EBPγ−/−, n = 3) and C/EBPγ−/− (n = 7) mouse spleen cells were 5.7 ± 4.1 × 10^3 and 4.9 ± 6.7 × 10^3 per well, respectively. Surface phenotype of cultured C/EBPγ−/− cells was identical to that of wild-type cells (Fig. 5 B). However, NK cells generated from C/EBPγ−/− spleens showed impaired cytotoxic activity against YAC-1 cells as compared with those from C/EBPγ+/+ spleens (Fig. 5 C). Furthermore, C/EBPγ−/− NK cells produced lower amounts of IFN-γ in response to IL-12 plus IL-18 than C/EBPγ+/+ and C/EBPγ−/− NK cells (Fig. 5 D). Taken together, two major NK cell activities, cytotoxic activity and IFN-γ production, were impaired in both C/EBPγ−/− chimera splenocytes and C/EBPγ−/− NK cells generated in the presence of IL-15 in vitro.

IL-12 and IL-18 Signaling Analysis of C/EBPγ−/− NK Cells. To determine if impaired induction of IFN-γ by IL-12 and IL-18 is caused by decreased expression of these receptors, we examined their mRNA expression in C/EBPγ−/− NK cells by IL-12Rβ1, IL-12Rβ2, and IL-18R expression was equivalent in C/EBPγ−/+. IL-12 can
induce tyrosine phosphorylation and activation of STAT4 (31, 32). This pathway was not impaired in C/EBPγ−/− NK cells (Fig. 6 B). IL-18 can cause phosphorylation and activation of JNK (33). Equivalent JNK phosphorylation was observed in C/EBPγ1+/+ and C/EBPγ−/− NK cells when stimulated by IL-18 (Fig. 6 C). Taken together, these results suggest that signaling pathways proximate to these cytokine receptors are intact in C/EBPγ−/− NK cells.

In this study, we demonstrate that C/EBPγ−/− NK cells have defects in IFN-γ production and cytotoxicity. It has been shown that several regulatory elements such as the activator protein (AP)-1 or NF-κB sites are essential for IFN-γ gene expression by IL-12 and/or IL-18 (34, 35). Although NF-IL6 is a candidate for heterodimerizing with C/EBPγ (36), it seems unlikely that the heterodimer plays an essential role. First, no C/EBP sites have been shown to be important for IFN-γ induction. Second, splenic IFN-γ production in response to IL-12 and/or IL-18 is not impaired in NF-IL6−/− mice (our unpublished data). It is possible that C/EBPγ plays a critical role in IFN-γ gene regulation by dimerizing with AP-1 components. AP-1 components are activated by IL-12 or IL-18 and essential for both IL-12- and IL-18-induced IFN-γ promoter activation (34). Fos or Jun is shown to require C/EBPγ in order to efficiently bind to the regulatory element in the IL-4 promotor (12). Although further studies are necessary, our results suggest that C/EBPγ regulates IFN-γ gene expression. At present, the possibility that C/EBPγ is necessary for expression of other gene(s) critical for IFN-γ gene induction cannot formally be excluded.

IFN-γ does not seem to be involved in cytotoxic activity of NK cells, because NK activity is not remarkably impaired in IFN-γ−/− mice (21). Therefore, impaired IFN-γ production cannot account for decreased cytotoxic activity of C/EBPγ−/− NK cells. Although CD18 is important for NK cells to recognize target cells (37), surface expression of CD18 was not decreased in C/EBPγ−/− chimera splenocytes (data not shown). Furthermore, IL-12 and IL-18 could induce expression of a critical cytolytic mediator, perforin, in C/EBPγ−/− chimera splenocytes (data not shown). These results indicate the presence of other target(s) of C/EBPγ that are involved in the cytotoxicity of NK cells.
one of these components caused reduction of NK cell num-
bers in vivo and impaired NK cell expansion in vitro (43–45).
Janus kinase (JAK)-3 and STAT5 are essential for IL-15
signaling components. JAK-3−/− or STAT5−/− mice also
showed impairment of NK cell generation (23, 46–49).
Furthermore, IFN regulatory factor (IRF)-1 was found to
be critical for expansion of NK cells (19). This can be ex-
plained by impaired IL-15 production in the bone marrow
microenvironment (19). In these mutants, deficient NK
cell generation is caused by disturbance of IL-15 signaling
or decreased IL-15 production. Membrane lymphotoxin
(LT)x also plays essential roles for NK cell development by
acting independently of IL-15/IRF-1 or upstream of the
IL-15/IRF-1 pathway (50). NK cell generation was se-
verely impaired in LTx−/− mice. Furthermore, NK cells,
which were generated in vitro with IL-15 from LTx−/−
bone marrow cells, showed intact cytotoxic activity. These
characteristics in LTx−/− mice are distinct from those in
C/EBPγ−/− mice. In addition, there are two more mutants
with impaired NK cell development, the mechanism of which
is not yet clear. One is deficiency of a winged helix-turn-
helix transcriptional factor, Ets-1 (51). The other is defi-
ciency of Id2, an inhibitor for transcription factors with
helix-loop-helix domains (52).

At present, little is known about the molecular mecha-
nisms that regulate NK cell functions or development. Our
current study clearly reveals that C/EBPγ is critically involved in
functional NK cell maturation. Identification of the target
genes regulated by C/EBPγ will clarify the molecular
mechanism of NK cell functions.

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