

Reduced expression of IL-12 receptor $\beta 2$ and IL-18 receptor α genes in natural killer cells and macrophages derived from B6-*mi/mi* mice

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The *mi* transcriptional factor (MITF) is a basic helix–loop–helix leucine zipper-type transcriptional factor. The *mi* mutant allele encodes an abnormal MITF, in which one out of four consecutive arginines is deleted in the basic domain. The *VGA-9-tg (tg)* allele is another mutant allele and considered to be a null mutant allele. C57BL/6 (B6)-*mi/mi* mice showed abnormal phenotypes of natural killer (NK) cells and macrophages, whereas B6-*tg/tg* mice did not. The expression levels of the genes for the interleukin-12 receptor (IL-12R) $\beta 2$ and IL-18R α were reduced in both the NK cells and macrophages of B6-*mi/mi* mice, while the expression levels of the corresponding genes in B6-*tg/tg* mice were unaffected. The B6-*mi/mi* NK cells and B6-*mi/mi* macrophages showed impaired responses to stimulation with IL-12, IL-18, and IL-12 plus IL-18 stimulation. The abnormal NK cell and macrophage of B6-*mi/mi* mice appear to be due to decreased expression of the IL-12R $\beta 2$ and IL-18R α genes. *Laboratory Investigation* (2005) 85, 146–153, advance online publication, 11 October 2004; doi:10.1038/labinvest.3700188

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The *mi* locus of mice encodes a member of the basic helix–loop–helix leucine zipper protein family of transcription factors, MITF.^{1,2} Many mutations have been reported for the *mi* locus.^{1,2} Of these, the *mi* mutant allele has been most intensively studied. The *mi* mutant allele encodes an abnormal MITF (*mi*-MITF), in which one out of four consecutive arginines is deleted in the basic domain.^{1,2} The *mi*-MITF protein is defective in DNA binding³ and nuclear localization.⁴ The *tg* mutation represents another mutant allele of the *mi* locus. It has been shown that *tg/tg* mice possess an insertional mutation in the promoter region of the *mi* gene, and they do not express any MITFs.^{1,2} Thus, *tg* is considered to be null mutant allele.

Both B6-*mi/mi* and B6-*tg/tg* mice share certain phenotypic features, such as microphthalmia, white coat color, and abnormal mast cell phenotypes.^{1,2,5–7}

These mice are distinguishable from each other in the following respect; B6-*mi/mi* mice showed, but B6-*tg/tg* mice do not show abnormal phenotypes of the NK cell and macrophage lineage.^{8–13} Both B6-*mi/mi* and B6-*tg/tg* mice showed normal NK 1.1⁺ cell development, but only B6-*mi/mi* mice showed impaired NK activity.^{8–10} Both B6-*mi/mi* and B6-*tg/tg* mice showed normal distributions of osteoclasts, whereas only B6-*mi/mi* mice showed osteopetrosis, which results from the impaired bone-resorption abilities of their osteoclasts.^{11,12} In addition to the osteoclasts, the peritoneal macrophages of B6-*mi/mi* mice have been reported to show some abnormal phenotypes, such as decreased phagocytic capacity and decreased acid phosphatase activity.¹³

Interleukin (IL)-12 and IL-18 are proinflammatory cytokines that are produced mainly by macrophages and dendritic cells in response to pathogens (reviewed in Trinchieri¹⁴ and Nakanishi *et al*¹⁵). IL-12 is a heterodimeric cytokine, which was originally described as NK cell stimulatory factor.¹⁶ The IL-12 receptor is composed of $\beta 1$ subunit (IL-12R $\beta 1$) and $\beta 2$ subunit (IL-12R $\beta 2$).¹⁷ IL-12 is the major cytokine for inducing Th1-type responses.^{14,18} IL-12 activates NK cells and induces IFN- γ production.¹⁸ IL-18 was

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originally identified as interferon (IFN)- γ inducing factor.¹⁹ The receptor for IL-18 is composed of an α subunit (IL-18R α)²⁰ and a β -subunit (IL-18R β).²¹ Both IL-18R α and IL-18R β are belong to the IL-1R superfamily.^{15,20,21} In contrast to IL-12, IL-18 plays important roles in both Th1 and Th2 responses.¹⁵ In synergy with IL-12, IL-18 stimulates Th1 responses by enhancing NK activity and IFN- γ production.²² Both NK cells and macrophages express the receptors for both IL-12 and IL-18, and produce IFN- γ in response to stimulation with IL-12, IL-18, and IL-12 plus IL-18.^{18,22–27}

The abnormal phenotypes of B6-*mi/mi* NK cells resemble those of mice that are deficient in IL-12 or IL-18 signaling.^{8–10,18,22–27} The administration of IL-12 or IL-18 did not recover the decreased NK activity of B6-*mi/mi* mice (our unpublished data). In this study, we examined the expression of IL-12R β 1, IL-12R β 2, IL-18R α and IL-18R β in NK cells that were derived from B6-+/+, B6-*mi/mi*, or B6-*tg/tg* mice. The expression levels of IL-12R β 2 and IL-18R α were reduced significantly in the NK cells of B6-*mi/mi* mice, but not in the NK cells of B6-*tg/tg* mice, when compared with those of B6-+/+ mice. IFN- γ production in response to stimulation with IL-12, IL-18, or IL-12 plus IL-18 was also impaired in the NK cells of B6-*mi/mi* mice. In addition, the macrophages of B6-*mi/mi* mice also showed decreased expression levels of IL-12R β 2 and IL-18R α , and had impaired IFN- γ production. These data show that the abnormal NK cell and macrophage phenotypes of B6-*mi/mi* mice are due to the decreased expression of the genes for IL-12R β 2 and IL-18R α .

Materials and methods

Mice

The original stock of B6-*mi*/+ mice was purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and was maintained in our laboratory by consecutive backcrosses with our own inbred B6 colony (more than 17 generations at the time of the present experiment). B6-*mi/mi* mice were obtained by mating among B6-*mi*/+ mice. Mice of the *mi/mi* genotype were identified by the white coat color. Most of the B6-*mi/mi* mice died before 4 weeks of age due to the failure of teeth eruption. To ensure the survival of B6-*mi/mi* mice to 6–8 weeks of age, we fed the weaning B6-*mi/mi* mice with powdered chow.¹⁰

The original stock of VGA-9-*tg*/+ mice, in which the mouse vasopressin-*Escherichia coli* β -galactosidase transgene is integrated at the 5' flanking region of the *mi* gene,^{1,2} were kindly provided by Dr H Arnheiter (National Institutes of Health, Bethesda, MD, USA). The integrated transgene was maintained by repeated backcrosses to our own inbred B6 colony. B6-*tg/tg* mice were obtained by mating among the B6-*tg*/+ mice. Mice of the *tg/tg* genotype were identified by their white coat colour.

Reagents

Recombinant mouse (rm) IL-2, IL-12, M-CSF, and IFN- γ were purchased from R&D Systems (Minneapolis, MN, USA). rmIL-18 was purchased from MBL Co. Ltd (Nagoya, Japan). The anti-NK 1.1⁺ antibody (Ab), anti-Mac-1 (CD11b) Ab and anti-CD16/CD32 Ab were purchased from BD Pharmingen (San Diego, CA, USA). The anti-mouse IL-12R β 2 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The anti-mouse IL-18R α was purchased from R&D Systems. Phorbol 12-myristate 13-acetate (PMA) and ionomycin were provided from Dr K Nishida (RIKEN Yokohama Institute, Yokohama, Japan).

NK Cell Preparation

Mice that were 6–8 weeks of age were used to obtain freshly isolated NK 1.1⁺ cells. The mice were killed by decapitation after ether anesthesia, and the spleens were removed. The spleens were passed through a mesh, to prepare spleen cell suspensions. NK cells were isolated by negative selection with the MACS NK cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) following the manufacturer's instructions. More than 95% of the isolated cells derived from B6-+/+, B6-*mi/mi*, and B6-*tg/tg* mice expressed the NK 1.1⁺ antigen as confirmed by flow cytometry. Cultured NK 1.1⁺ cells were obtained by the method described previously.¹⁰

Peritoneal Macrophage Isolation

Mice that were 6–8 weeks of age were used to obtain peritoneal macrophages. A volume of 5 ml of phosphate-buffered saline (PBS) was injected into the peritoneal cavity, and the abdomen was massaged gently. The peritoneal cavity was carefully opened, and the fluid that contained the peritoneal macrophages was aspirated with a Pasteur pipette. The collected cells were cultured for 1 h in a α -minimal essential medium (α -MEM; ICN Biomedicals, Costa Mesa, CA, USA) supplemented with 10% FCS. Nonadherent cells were removed, and the adherent cells were used as peritoneal macrophages. In total, 72 \pm 5% of peritoneal macrophages derived from B6-+/+, 68 \pm 6% of B6-*mi/mi*, or 69 \pm 5% of B6-*tg/tg* mice were Mac-1⁺, as confirmed by immunocytochemical method.

Mac-1⁺ Cells Preparation

Cultured Mac-1⁺ cells were obtained by the standard techniques.²⁸ Mice that were 3–4 weeks of age were used to obtain cultured Mac-1⁺ cells. Spleen cell suspensions were suspended in α -MEM medium that was supplemented with 10% FCS and 25 ng/ml rmM-CSF. Spleen cells that were derived from B6-+/+, B6-*mi/mi*, or B6-*tg/tg* mice expressed normal levels of the M-CSF receptor gene.²⁹ More

than 95% of the cultured adherent cells derived from B6-+/+, B6-*mi/mi*, or B6-*tg/tg* mice were Mac-1⁺, as confirmed by immunocytochemistry and flow cytometry.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Reverse transcription-polymerase chain reaction (RT-PCR) was used to evaluate the expression levels of IL-12R and IL-18R. Total RNAs (10 μ g) was extracted from freshly isolated NK 1.1⁺ cells and cultured Mac-1⁺ cells that were derived from B6-+/+, B6-*mi/mi* or B6-*tg/tg* mice. The extracted RNAs were subjected to reverse transcription by Superscript (Invitrogen, Carlsbad, CA, USA), and the single-stranded cDNAs were obtained. Subsequently, 0.1 μ l of the reaction mixture was added to 25 μ l of PCR mixture containing 1.25 U of *Taq* DNA polymerase (Roshe Diagnostics, Mannheim, Germany) 25 pmol of each of the primers. The following (forward and reverse) primers were used: IL-12R β 1 5' AATGTGCTCGCCAAAACCTCG and 3' CGCAGTCTTATGGGTCCTCC; IL-12R β 2 5' ACATTACTGCCATCACAGAG and 3' AGGAGATTATCCGTAGGTAG; IL-18R α 5' GTGCACAGGAATGAAACAGC and 3' ATTTAAGGTCCAATTGCGACGA; IL-18R β 5' GGAGTGGGAAATGTCAGTAT and 3' CCGTGCCGAGAAGGATGTAT; β -actin 5' TAAAGACCTCTATGCCAACAC and 3' CTCCTGCTTGCTGATCCACAT. Each PCR cycle parameters were denaturation 20 s at 95°C, annealing 30 s at 52°C and polymerase reaction 1 min at 72°C. For semiquantitative PCR, the fragments of IL-12R β 1, IL-12R β 2, IL-18R α or IL-18R β were amplified with 28 cycles out of RT products derived from NK 1.1⁺ cells and with 32 cycles out of that of Mac-1⁺ cells. The fragments of β -actin were amplified with 22 cycles out of RT products derived from both freshly isolated NK 1.1⁺ cells and cultured Mac-1⁺ cells. Aliquots (10 μ l) of the PCR products were electrophoresed on a 1% agarose gel that contained ethidium bromide.

Flow Cytometry

For the determination of IL-12R β 2 or IL-18R α expression, we incubated freshly isolated NK 1.1⁺ cells and cultured Mac-1⁺ cells for 3 days with or without IFN- γ (20 ng/ml). Cells were harvested and washed with PBS twice. After Fc receptor blocking using anti-CD16/CD32 Ab, the cells were incubated with anti-mouse IL-12R β 2 or anti-mouse IL-18R α followed by FITC-conjugated anti goat IgG Ab. Stained cells were analyzed using FACScan (Becton Dickinson, Los Angeles, CA, USA).

Cytokine Measurements

Freshly isolated (1 \times 10⁵ cells/ml) or cultured NK cells (2 \times 10⁴ cells/ml) were recultured for 5 h

in 24-well plates, together with rmIL-12 (20 ng/ml), rmIL-18 (20 ng/ml), and rmIL-12 plus rmIL-18. Peritoneal macrophages (5 \times 10⁵ cells/ml) or cultured Mac-1⁺ cells (5 \times 10⁴ cells/ml) were recultured for 5 h in 24-well plates with rmIL-12 (20 ng/ml), rmIL-18 (20 ng/ml), rmIL-12 plus rmIL-18. Cultured NK cells (2 \times 10⁴ cells/ml) or cultured Mac-1⁺ cells (5 \times 10⁴ cells/ml) were recultured for 5 h in the presence of 50 ng/ml PMA and 1 μ M ionomycin.³⁰ The culture supernatants were assayed using an ELISA kit for IFN- γ (R&D) according to the manufacturer's instructions.

Perforin (Pfn) Expression Assay

The cultured NK 1.1⁺ cells were recultured for 24 h at 1 \times 10⁵ cells/200 μ l in 96-well plates, together with rmIL-12 (0.1, 1, 10, 100, or 1000 ng/ml) and rmIL-18 (0.1, 1, 10, 100, or 1000 ng/ml). Total RNA samples were extracted from the cultured cells, and RT-PCR was performed as described above. The following PCR primers were used: Pfn 5' TGCCACTCGGTCAGAATGCAAGC and 3' CTTCCAGTAATGTGTGCAGGGGC. Cycle parameters were denaturation 30 s at 95°C, annealing 30 s at 50°C and polymerase reaction 1 min at 72°C. The fragments of Pfn were amplified with 30 cycles. The fragments of β -actin were amplified with 22 cycles, and used as control. Aliquots (10 μ l) of the PCR products were electrophoresed on 1% agarose gel that contained ethidium bromide.

Results

Reduced Expression of the IL-12R and IL-18R Genes in NK Cells and Macrophages of B6-*mi/mi* Mice

We analyzed the expression of the IL-12R and IL-18R genes in freshly isolated NK 1.1⁺ cells and cultured Mac-1⁺ cells that were derived from the spleen of B6-+/+, B6-*mi/mi*, and B6-*tg/tg* mice, using semiquantitative RT-PCR. The levels of IL-12R β 2 and IL-18R α mRNA were reduced significantly in cells from B6-*mi/mi* mice, as compared to cells from B6-+/+ and B6-*tg/tg* mice (Figures 1a and 2a). In contrast, the levels of IL-12R β 1 and IL-18R β mRNA were comparable in the cells of these mutant and control mice (Figures 1a and 2a).

The expression of IL-12R β 2 and IL-18R α was analyzed at the protein level by flow cytometry. As shown in Figures 1b and 2b, the surface expression levels of IL-12R β 2 and IL-18R α were reduced significantly in freshly isolated NK 1.1⁺ NK cells and cultured Mac-1⁺ cells that were derived from B6-*mi/mi* mice. IFN- γ has been reported to enhance expression of IL-12R (reviewed in Trinchieri¹⁴). But IFN- γ administration did not increase the expression of IL-12R β 2 gene on freshly isolated NK 1.1⁺ NK cells or cultured Mac-1⁺ cells derived from B6-*mi/mi* mice (Figures 1b and 2b). And cultured NK cells and peritoneal macrophages (about 70% Mac-1⁺)

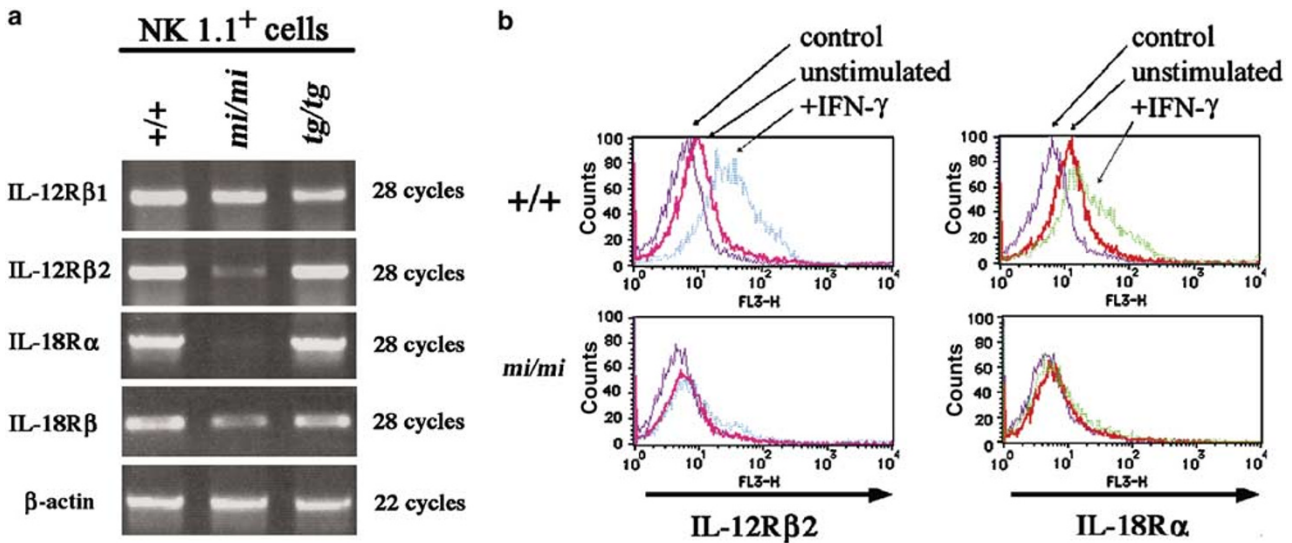


Figure 1 Expression of IL-12R and IL-18R genes in NK cells that were derived from B6-*+/+*, B6-*mi/mi*, or B6-*tg/tg* mice. **(a)** Expression of IL-12R β 1, IL-12R β 2, IL-18R α , and IL-18R β genes in freshly isolated NK 1.1⁺ cells derived from B6-*+/+*, B6-*mi/mi*, or B6-*tg/tg* mice was examined, as measured by RT-PCR. Three independent experiments were performed, with comparable results: a representative experiment is shown. **(b)** Flow cytometric analysis of the expression of IL-12R β 2 and IL-18R α genes in freshly isolated NK 1.1⁺ cells with or without IFN- γ treatment that were derived from B6-*+/+* or B6-*mi/mi* mice. Three independent experiments were performed, with comparable results: a representative experiment is shown.

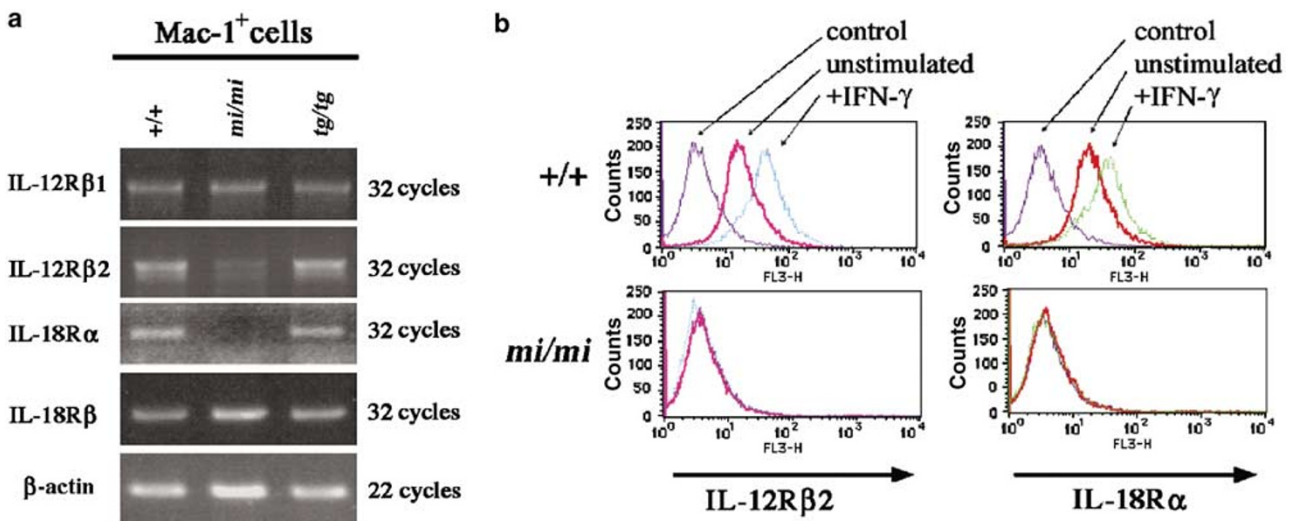


Figure 2 Expression of IL-12R and IL-18R genes in the macrophages of B6-*+/+*, B6-*mi/mi*, or B6-*tg/tg* mice. **(a)** RT-PCR analysis of the expression of IL-12R β 1, IL-12R β 2, IL-18R α , and IL-18R β genes in cultured Mac-1⁺ cells that were derived from B6-*+/+*, B6-*mi/mi*, or B6-*tg/tg* mice. Three independent experiments were performed, with comparable results: a representative experiment is shown. **(b)** Flow cytometric analysis of the expression of IL-12R β 2 and IL-18R α genes in cultured Mac-1⁺ cells with or without IFN- γ treatment that were derived from B6-*+/+* or B6-*mi/mi* mice. Three independent experiments were performed, with comparable results: a representative experiment is shown.

show essentially similar expression pattern of the IL-12R and IL-18R genes as well (data not shown).

Impaired IFN- γ Production of NK Cells and Macrophages Derived from B6-*mi/mi* Mice

IL-12 and IL-18 enhance synergistically the production of IFN- γ by NK cells and macrophages.^{18,22–27}

Therefore, we analyzed the IFN- γ production in NK cells and macrophages from B6-*+/+* and B6-*mi/mi* mice. An enzyme-linked immunosorbent assay (ELISA) was used to measure the IFN- γ production levels of freshly isolated or cultured NK 1.1⁺ cells, peritoneal macrophages and cultured Mac-1⁺ cells. IL-12 and IL-18 induced IFN- γ production of freshly isolated and cultured NK 1.1⁺ cells derived from B6-*+/+* mice, and these cytokines functioned

synergistically (Figure 3a,b). In contrast, the cells that were derived from B6-*mi/mi* mice showed significantly impaired IFN- γ production (Figure 3a,b). In contrast to IL-12 and IL-18 stimulation, PMA-ionomycin-induced IFN- γ production was comparable among cultured NK cells derived from B6-*+/+* or *mi/mi* mice (Figure 3b). Essentially similar results were observed for the macrophages. When B6-*mi/mi* peritoneal macrophages and cultured Mac-1⁺ cells were stimulated with IL-12 plus IL-18, the level of IFN- γ production was significantly lower than that of the control (Figure 3c,d). IFN- γ production by PMA-ionomycin stimulation was comparable among cultured Mac-

1⁺ cells derived from B6-*+/+* or *mi/mi* mice (Figure 3d).

Reduced Pfn mRNA Induction by IL-12 and/or IL-18 in B6-*mi/mi* NK Cells

We have reported previously that the cytotoxic activity of splenic NK cells correlates well with the Pfn mRNA expression level in these cells.¹⁰ In addition, it has been reported that IL-12 enhances the expression of Pfn mRNA.³¹ Therefore, we examined the effects of IL-12 on the expression of Pfn mRNA in B6-*mi/mi* splenic NK cells. We

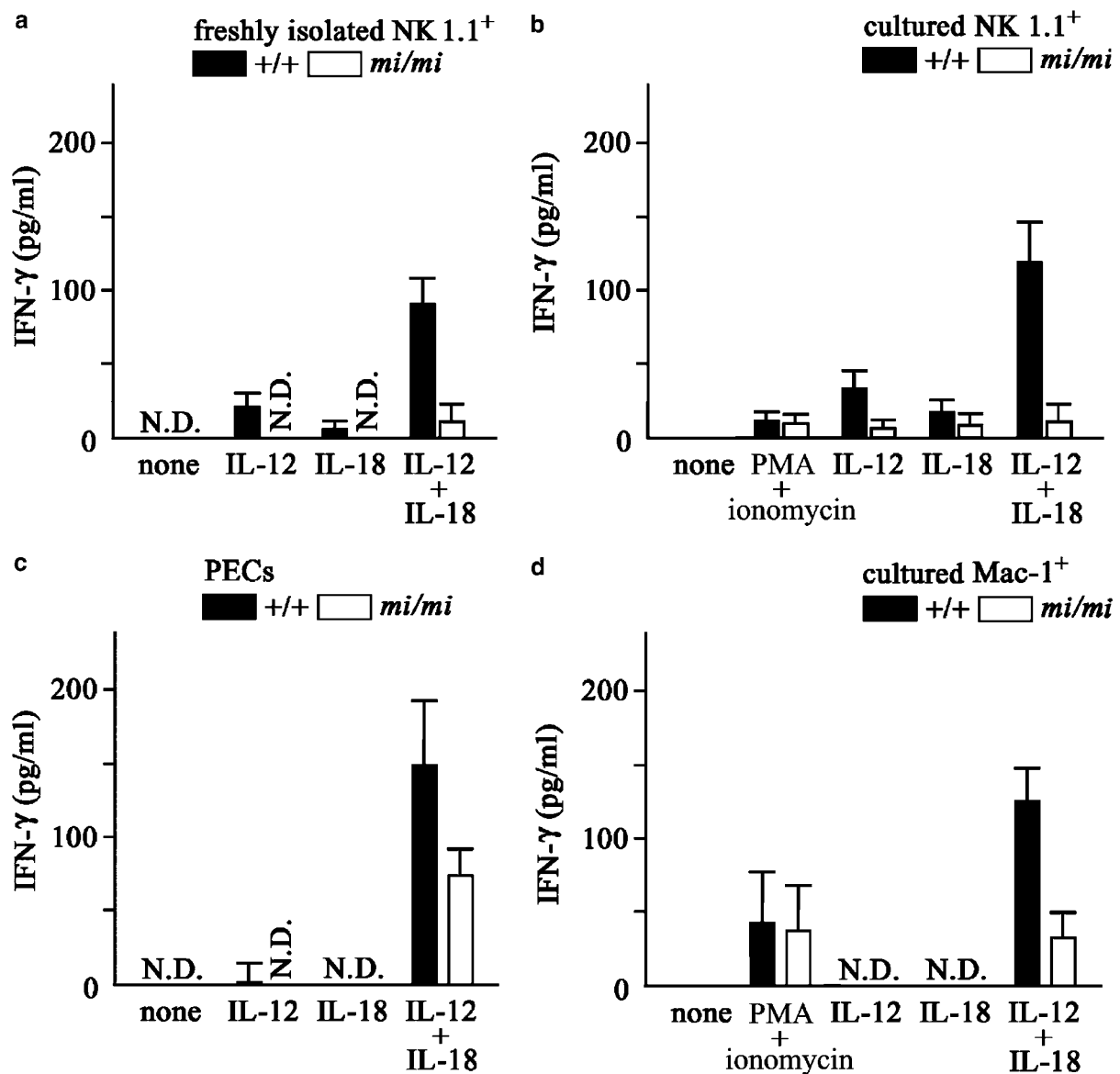


Figure 3 IFN- γ production by NK cells and macrophages that were derived from B6-*+/+*, or B6-*mi/mi* mice. Freshly isolated NK 1.1⁺ cells (a), cultured NK cells (b), peritoneal macrophages (c), and cultured Mac-1⁺ cells (d) were cultured with IL-12, IL-18, or IL-12 plus IL-18 for 5 h. Cultured NK cells (b) and cultured Mac-1⁺ cells (d) were also cultured in the presence of PMA-ionomycin. The culture supernatants were subjected to ELISA.

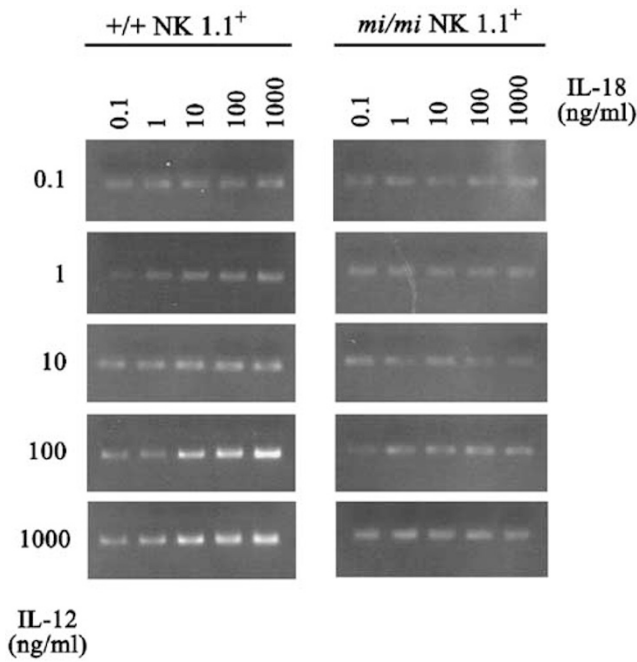


Figure 4 Expression of the Pfn gene in cultured NK 1.1⁺ cells that were derived from B6-^{+/+} or B6-*mi/mi* mice. RT-PCR analysis of Pfn gene expression in cultured NK 1.1⁺ cells that were derived from B6-^{+/+} or B6-*mi/mi* mice. Each of the specimens used for estimating Pfn gene expression contained comparable amounts of the RT products, as assessed by the β -actin levels (data not shown). Three independent experiments were performed, with comparable results: a representative experiments is shown.

confirmed that IL-12 stimulation dose-dependently induced the transcription of Pfn mRNA in B6-^{+/+} NK cells. Although IL-18 alone induced only weak expression of Pfn mRNA. IL-18 synergized with IL-12, to enhance the expression of Pfn mRNA (Figure 4). In contrast, neither IL-12 nor IL-18 increased the expression level of Pfn gene in B6-*mi/mi* NK cells. Furthermore, the stimulation of B6-*mi/mi* NK cells with IL-12 plus IL-18 enhanced only weakly the expression of Pfn mRNA in B6-*mi/mi* NK cells (Figure 4).

Discussion

Several cytokines play important roles in NK cell development and activity (reviewed in Williams *et al*³² and Colucci *et al*³³). For example, Flt3 (FMS-like tyrosine kinase 3) ligand,³⁴ c-kit tyrosine kinase ligand,³⁵ and IL-7³⁶ are important for the early stages of NK cell development. IL-2 and IL-15 play pivotal roles in NK cell maturation.^{37–39} Notably, IL-12 and IL-18 enhance NK activity.^{18,22} Neither IL-12 nor IL-18 seem to be essential for NK cell development, since both IL-12-deficient and IL-18-deficient mice show normal NK 1.1⁺ cell development.³²

As is the case for IL-12-deficient and IL-18-deficient mice, the B6-*mi/mi* mice showed normal

NK 1.1⁺ cell development and significantly reduced NK activity.^{9,10} However, in contrast to the IL-12-deficient and IL-18-deficient mice, the NK activity of B6-*mi/mi* mice was not recovered even after daily intraperitoneal injection of IL-18 (1000 ng) or IL-12 (100 ng) for 2 days,²² (our unpublished data).

We also examined the responses of B6-*mi/mi* NK cells to IL-12 and IL-18. We reported previously that NK activity correlates with the expression level of Pfn mRNA in spleen-derived mononuclear cells after intraperitoneal injection of polyinosinic-polycytidylic acid (poly I:C).¹⁰ In comparison with B6-^{+/+} mice, the B6-*mi/mi* mice showed significantly reduced levels of NK activity and Pfn gene expression.^{9,10} Since poly I:C stimulated the production of IL-12 by the macrophages,⁴⁰ the decreased expression of the Pfn gene in B6-*mi/mi* NK cells may be attributed to the reduced production of IL-12 by poly I:C-stimulated macrophages. NK cells produce IFN- γ in response to stimulation with IL-12 and IL-18, and IL-12R β 1-deficient, IL-12R β 2-deficient, and IL-18R α -deficient mice have been reported to show impaired IFN- γ production following IL-12 or IL-18 stimulation.^{23–25} Similarly, the B6-*mi/mi* NK cells showed decreased IFN- γ production in response to stimulation with IL-12, IL-18, or IL-12 plus IL-18. PMA-ionomycin stimulation also induces IFN- γ production.³⁰ B6-*mi/mi* NK cells secreted a comparable level of IFN- γ with that of B6-^{+/+} NK cells by PMA-ionomycin administration. These data show that the reduced production of IFN- γ by B6-*mi/mi* NK cells depends on the impaired responsiveness to IL-12 and IL-18, rather than the decreased IFN- γ expression of B6-*mi/mi* NK cells. Taken together, our results suggest that the abnormal phenotype of B6-*mi/mi* NK cells is due to the impaired responses to IL-12 and IL-18.

The macrophage-lineage cells of B6-*mi/mi* mice showed abnormal phenotypes, such as osteopetrosis, decreased phagocytic capacity and decreased acid phosphatase activity.^{11–13} Macrophages produce IFN- γ in response to stimulation with IL-12 and IL-18.^{26,27} In this study, we observed another abnormal phenotype of B6-*mi/mi* macrophages, that is, poor responses to IL-12 and IL-18. It seems likely that this phenotype is due to the reduced expression of IL-12R β 2 and IL-18R α genes in B6-*mi/mi* macrophages.

The expression levels of IL-12R and IL-18R were reduced significantly in NK cells and macrophages that were derived from B6-*mi/mi* mice. These data indicate that the decreased responses to IL-12 and IL-18 of B6-*mi/mi* NK cells and B6-*mi/mi* macrophages are due to the reduced expression of the IL-12R β 2 and IL-18R α genes. It is known that IL-12R is enhanced by IFN- γ , and IL-18R expression is upregulated by IL-12 signaling.^{14,15,41} Reduced expression of IL-12R and IL-18R was not recovered by exogenous IFN- γ in both B6-*mi/mi* NK cells and B6-*mi/mi* macrophages. Therefore, the decreased expression levels of IL-12R and IL-18R on B6-*mi/mi*

mice is not due to the impaired production of IFN- γ , but is intrinsic to the *mi* mutation. In contrast to the B6-*mi/mi* cells, B6-*tg/tg* NK cells and B6-*tg/tg* macrophages showed normal expression patterns of the IL-12R β 2 and IL-18R α genes. This discrepancy indicates that although the simple absence of MITF does not affect the expression of the IL-12R β 2 and IL-18R α genes, the presence of *mi*-MITF results in reductions in the expression of these genes. The *mi*-MITF showed an inhibitory effect on the expression of various genes in cultured mast cells,^{7,42–44} as well as the loss of transactivation activity.³ Considering the inhibitory effects of the *mi*-MITF on mast cells, it is conceivable that the *mi*-MITF inhibits via similar molecular mechanisms the expression of the IL-12R β 2 and IL-18R α genes in NK cells and macrophages.

The inhibitory effect of *mi*-MITF may be explained by the functional cooperation of *mi*-MITF with polyomavirus enhancer binding protein 2/core-binding factor (PEBP2/CBF). We have reported previously that *mi*-MITF interacts with PEBP2/CBF, thereby inhibiting its transactivational activity.⁴⁴ The database of the National Center for Biotechnology Information shows that PEBP2/CBF binding motifs are located in the promoters of the IL-12R β 2 and IL-18R α gene. Thus, *mi*-MITF may inhibit PEBP2/CBF function, which results in the decreased expression of the IL-12R β 2 and IL-18R α genes.

In summary, the abnormal phenotypes that were observed for the NK cells and macrophages of B6-*mi/mi* mice may be attributed to impaired responses to IL-12 and IL-18. This impairment appears to be due to the decreased expression of the IL-12R β 2 subunit and IL-18R α genes in B6-*mi/mi* NK cells and B6-*mi/mi* macrophages.

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