Brief Definitive Report

The Transcription Factor Interferon Regulatory Factor-1 Is Essential for Natural Killer Cell Function In Vivo

By Gordon S. Duncan,* Hans-Willi Mittrücker,*‡ David Kägi,*‡ Toshifumi Matsuyama,*‡ and Tak W. Mak*‡

*From the *Amgen Institute, Toronto, Ontario, Canda M5G 2M9; and ‡Ontario Cancer Institute, Department of Immunology and Department of Medical Biophysics, University of Toronto, Ontario, Canada M4X 1K9*

Summary

The activation of natural killer (NK) cells, cytotoxic lymphocytes capable of major histocompatibility complex (MHC)-unrestricted killing and early antiviral defense, is temporally related to the increased interferon (IFN)-α/β production that is seen in the viral infection of mice. Type I IFN (IFN-α/β) are expressed in many cell types early after primary viral infection and have been shown to mediate resistance against a variety of viruses. In this study, the role of the transcriptional activator IFN regulatory factor-1 (IRF-1) in murine NK cell activity was assessed. IRF-1–deficient mice displayed a normal frequency of NK marker–positive cells, but exhibited greatly reduced NK cell–mediated cytotoxicity after both virus infection and stimulation with the IFN inducer polyinosinic:polycytidilic acid in vivo. In vitro, cytolytic activity in IRF-1–deficient NK cells remained defective after stimulation with IFN-β, IL-2, and IL-12. IRF-1–deficient mice were unable to eliminate syngeneic MHC class I–negative tumor cells in vivo, and had a reduced ability to reject parental semi-allogeneic donor cells from the circulation. Thus, IRF-1 is essential for the induction of NK cell–mediated cytotoxicity and for the in vivo effector functions that are mediated by this activity.

NK cells are cytotoxic lymphocytes that are capable of lysing a variety of target cells, including tumor cells, virus-infected cells, and cells infected with intracellular bacteria. NK cell–mediated killing does not require previous sensitisation and is MHC unrestricted (1), and as such, forms a first line of defense against microbial pathogens or tumor growth. In the case of primary viral infection, NK cell responses are induced early and are temporally distinct from those of T lymphocytes. The early response to viral infection is associated with increases in both systemic and local type I IFN (IFN-α/β) production, peaking at 3–5 d after infection, and closely mirroring the time course of NK cell activation, blastogenesis, and the augmented cytolytic activity that is seen in viral infection (2).

IFN regulatory factor-1 (IRF-1), an IFN-inducible transcriptional activator, binds regions within the promoters of type I IFNs and several IFN-inducible genes, and has been implicated as a mediator of the IFN-induced antiviral state (3, 4). IRF-1 is constitutively expressed in many cell types, and its expression can be strongly enhanced by type I and II IFNs, viral infection, double-stranded RNA, and cytokines such as IL-6 (4, 5). The functional deletion of IRF-1 does not, however, fully ablate the expression of IFN and IFN-inducible genes. We have previously demonstrated in vitro induction of type I IFN genes by polyinosinic:polycytidylic acid (pI:pC) and Newcastle disease virus to be IRF-1 dependent and independent, respectively (6). In addition, IRF-1–dependent antiviral effects of IFNs and the course of viral infection in IRF-1–deficient mice have been shown to be dependent on the type of virus investigated (7), suggesting IRF-1–dependent and –independent pathways in the induction of antiviral states.

To clarify the role of IRF-1 in NK cell function, we have examined the cytolytic activity of NK cells from IRF-1–deficient mice in response to lymphocytic choriomeningitis virus (LCMV) and pI:pC in vivo, and to IFN-β, IL-2, and IL-12 in vitro. Killing of NK-sensitive targets in vitro by spleen NK cells from IRF-1–deficient mice was found to be markedly reduced both after in vivo and in vitro activation of NK cells. A defect was also evident in the clearance of parental donor cells from the circulation of IRF-1–deficient hosts and in the elimination of a syngeneic MHC class I–deficient tumor in vivo, both processes known to be mediated by NK cells. Our results clearly indicate that IRF-1 plays a crucial role in the induction of NK cell–mediated cytotoxic and effector functions in vivo.

Materials and Methods

Mice. C57BL/6 mice deficient in IRF-1 (6) were genotyped by genomic Southern blotting of tail DNA as described previ-

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ously (6). For experiments involving the injection of F_1 cells, C57BL/6 (H-2^b) IRF-$1^{+/-}$ mice were bred with A/J (H-2^k) IRF-$1^{-/-}$ mice (kindly provided by Dr. J. Penninger, Amgen Institute, Toronto). Mice were housed in specific pathogen–free conditions, and littermate controls were used within experiments.

Induction of NK Activity In Vivo. Mice were injected with 0.1 mg i.v. pI:pC (Pharmacia, Uppsala, Sweden) or 2×10^6 PFU LCMV (Armstrong strain). After 24 h (pI:pC) or 72 h (LCMV), spleens were harvested and mononuclear cells (MNC) isolated by density gradient centrifugation (Lympholite M; Cedarlane, Ontario, Canada).

Induction of NK Activity In Vitro. Spleen MNC were cultured in complete medium (CM; α-MEM containing 10% FCS, 20 mM Hepes, and 10 μM β-mercaptoethanol) within 48-well tissue culture plates at a concentration of 10^7 cells/ml. Wells contained murine rIFN-β (Lee Biomolecular Research, San Diego, CA) or murine rIL-12 (Genzyme, Cambridge, MA). Plates were incubated for 72 h (IFN-β) or 24 h (IL-12) at 37°C/5% CO_2.

Evaluation of IFN-γ in IL-12–stimulated Spleen Cultures. Supernatants were removed from IL-12–stimulated spleen MNC after a 24-h culture, and IFN-γ was measured by ELISA (Intertest-γ; Genzyme).

NK Cytotoxicity Assay. MNC were added to V-bottomed 96-well plates in CM. The NK-sensitive cell line YAC-1 was grown in CM, labeled with $Na_2{}^{51}CrO_4$ (NEN, Boston, MA; 35 μCi/10^6 cells), and added to wells containing dilutions of MNC. Target cell lysis was determined after a 4-h incubation by measurement of ^{51}Cr release using the following formula: *percent of specific lysis* = $(a - b/c - b) \times 100\%$, where a = test release, b = spontaneous release, and c = release in the presence of 1% SDS. Spontaneous release was always <10%.

Analysis of IL-2–stimulated Cells. 10^7 spleen MNC were cultured in CM with 500 U/ml murine rIL-2 in 24-well tissue culture plates. Cells were recovered at day 9, washed twice, counted, and analyzed by flow cytometric analysis.

Tumor Elimination In Vivo. Mice were injected subcutaneously in the right flank with 10^5 RMA-S cells in 0.1 ml PBS (8). Mice were monitored for palpable tumors and were killed when tumors reached a diameter of 1 cm.

Persistence of Parental Donor Cells. Spleen and LN MNC cells were prepared from 6–8-wk-old C57BL/6 (H-2^b) and C57BL/6 × A/J F_1 (H-$2^{b/k}$) mice by density gradient centrifugation. After two washes in CM, the cells were labeled with FITC (Sigma Immunochemicals, St. Louis, MO; 30 μg/ml in PBS) for 18 min at 37°C/5% CO_2. Excess FITC was removed by centrifugation through 6% BSA in PBS, and 2×10^7 cells in 0.2 ml PBS were injected into the lateral tail vein of recipient mice (C57BL/6 × A/J F_1, IRF-$1^{+/-}$, or IRF-$1^{-/-}$). The fate of the donor cells was monitored by flow cytometry of peripheral blood and LN cells. NK activity was induced in recipient mice by injection of 0.1 mg i.p. pI:pC at 1 and 24 h after injection of donor cells.

Flow Cytometric Analysis. MNC were preincubated with anti-FcγR mAb (Fc block; Pharmingen, San Diego, CA) for 10 min at room temperature and were subsequently incubated on ice with mAbs [anti-NK1.1 (PK136, PE conjugated), anti-CD3 (145-2C11, FITC-conjugated), anti-CD69 (H1.2F3, biotinylated), anti-CD25 (7D4, biotinylated), and anti-CD11a (M17/4, biotinylated); all mAbs were from PharMingen (San Diego, CA)] for 30 min in 0.1 ml of PBS containing 1% BSA and 0.1% sodium azide. Cells were washed with PBS and analyzed by flow cytometry (FACScalibur®, Becton Dickinson & Co., San Jose, CA) using CellQuest software (Becton Dickinson). Viable lymphocytes were gated on the basis of forward and side scatter characteristics, and 10,000 gated events were analyzed.

Results

NK Cytotoxicity in IRF-1–deficient Mice in Response to LCMV Infection and pI:pC Treatment. The role of IRF-1 in the induction of NK cell cytotoxicity in response to LCMV infection was investigated. 72 h after high dose infection with 2×10^6 PFU LCMV, significant lysis of the prototypic NK-sensitive cell line YAC-1 was observed with spleen cells from IRF-$1^{+/+}$ mice, but not with cells from IRF-$1^{-/-}$ mice (Fig. 1 *A*). Spleen cells from uninfected IRF-$1^{+/+}$ or IRF-$1^{-/-}$ mice displayed no measurable lytic activity (data not shown). Stimulation of NK lytic activity in response to the type I IFN inducer pI:pC was also investigated. 24 h after administration of 0.1 mg i.v. pI:pC, significant lysis of YAC-1 target cells was clearly observed in wild-type mice (Fig. 1 *B*). However, pI:pC-treated IRF-$1^{-/-}$ mice exhibited markedly reduced lytic activity compared to wild-type mice, confirming the results obtained with LCMV.

IFN-β–enhanced Cytolytic Activity In Vitro. To address the possibility that a defect in type I IFN production in IRF-$1^{-/-}$ mice was responsible for the lack of cytotoxicity observed in IRF-$1^{-/-}$ mice, spleen NK cells were incubated in vitro with IFN-β. IFN-β induced a dose-dependent activation of NK-mediated lytic activity in wild-type animals, with a maximal effect obtained in the presence of 1,000 IU/ml IFN-β (Fig. 2), as reported previously (9). IRF-1–deficient splenocytes demonstrated maximal lytic activity at the same dose of IFN-β, but exhibited greatly reduced lytic activity compared to wild-type controls.

IL-12–enhanced Cytolytic Activity In Vitro. Spleen MNC–derived from IRF-$1^{+/-}$ and IRF-$1^{-/-}$ mice were incubated in the presence of 10 ng/ml IL-12. Significant lysis of the target cell line by IRF-$1^{+/-}$ spleen NK cells was induced by IL-12 (Fig. 3 *A*), while IL-12 failed to augment the cytolytic activity of IRF-1–deficient NK cells. Analysis of the supernatants from spleen cells cultured in the presence of

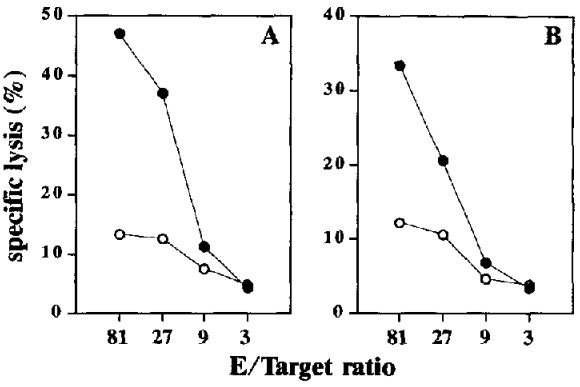

Figure 1. In vitro lysis of the NK-sensitive mouse lymphoma YAC-1 after in vivo induction of NK cell activity by (*A*) LCMV and (*B*) pI:pC. Panels show specific target cell lysis by IRF-$1^{+/+}$ (*closed symbols*) and IRF-$1^{-/-}$ (*open symbols*) spleen NK cells. Data is representative of at least eight mice of each genotype examined in five separate experiments. No lytic activity was detectable in the absence of induction.

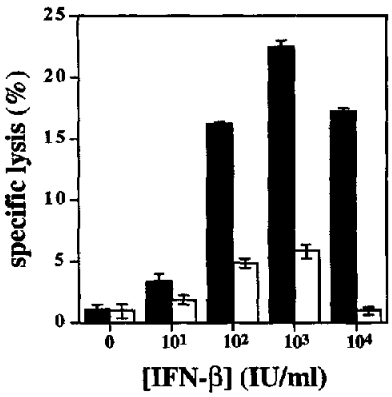

Figure 2. NK-specific lysis of YAC-1 target cells after in vitro induction of NK activity by IFN-β. Black bars represent lysis by IRF-$1^{+/-}$ spleen NK cells, and white bars represent lysis by IRF-$1^{-/-}$ spleen NK cells at an E/T ratio of 100.1. Data are shown as mean ± SEM for a single experiment performed in quadruplicate in which cells from two mice of each genotype were pooled, and are representative of two experiments.

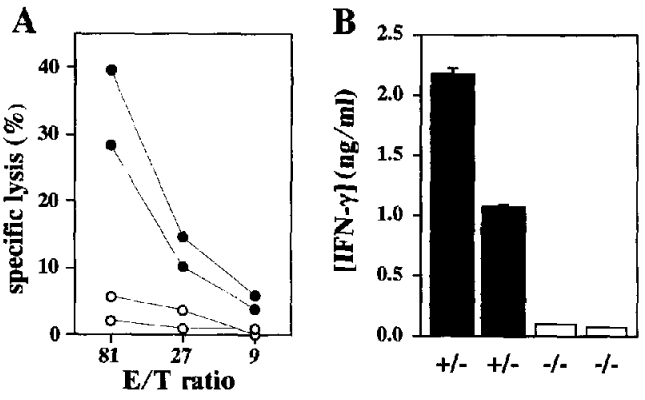

Figure 3. IL-12–augmented lysis of YAC-1 target cells, and IL-12–dependent IFN-γ production is defective in IRF-1–deficient mice in vitro IL-12 stimulates significant lysis of YAC-1 target cells in IRF-$1^{+/-}$ mice, while cytolytic activity is defective in IRF-$1^{-/-}$ mice (*A*). Two mice of each genotype are shown (closed symbols represent heterozygous control effector cells, open symbols represent homozygous IRF-1–deficient effector cells), representative of six animals examined in three other experiments. Analysis of supernatants taken from IL-12–stimulated spleen cell culture (10 ng/ml) revealed a defect in IFN-γ production (*B*) Black bars represent IFN-γ production by IRF-$1^{+/-}$ mice, and white bars represent IRF-$1^{-/-}$ mice. Data are shown as mean ± SEM of an experiment with two mice in each group performed in triplicate, and are representative of six animals.

10 ng/ml IL-12 revealed a defect in IFN-γ production in IRF-$1^{-/-}$–derived spleen cells (Fig. 3 *B*).

IL-2–stimulated Proliferation and Cytolytic Activity In Vitro. Incubation in the presence of 500 U/ml IL-2 for 9 d induced preferential proliferation of NK cells, with $NK1.1^+/CD3^-$ cells comprising 61.2 ± 1.2% of recovered IRF-$1^{+/-}$–derived cells, compared to 44.5 ± 3.6% in the case of IRF-$1^{-/-}$–derived spleen cells (mean ± SEM for three mice in each group). Both IRF-$1^{+/-}$– and IRF-$1^{-/-}$–derived cells contained approximately equal percentages (2.5–3.0%) of $NK1.1^+/CD3^-$ cells at the start of the 9-d culture. Calculation of absolute numbers of $NK1.1^+$ and $CD3^+$ cells revealed a more profound deficit in IRF-$1^{-/-}$–derived NK cell proliferation in response to IL-2 (Fig. 4 *B*), with a five-fold decrease in viable $NK1.1^+$ cells present after 9 d. The cytolytic activity of day 9 IL-2–stimulated NK cells was evaluated against YAC-1 target cells (Fig. 4 *C*) and revealed that although IRF-$1^{-/-}$ LAK cells are clearly capable of YAC-1–directed cytolytic activity, their lytic activity is reduced compared to IRF-$1^{+/-}$ controls.

Tumor Elimination In Vivo. Wild-type and IRF-1–deficient mice were injected subcutaneously with 10^5 RMA-S cells. RMA-S cells have been shown to be eliminated in mice with fully functional NK cells, while mice deficient in NK cell–mediated cytotoxicity are unable to clear 10^5 RMA-S cells (8, 10). No palpable tumors were detected in wild-type mice during the experiment (20 d), but tumor growth developed in all IRF-1–deficient mice between days 12 and 15 (Fig. 5).

Persistence of Parental Donor Cells in the Circulation of IRF-1–deficient Mice. It is known that rejection of a parental bone marrow graft in F_1 recipients is mediated by NK cells that are activated by the absence of the full complement of MHC class I molecules (11). To investigate this hybrid resistance in IRF-1–deficient mice, FITC-labeled F_1 (C57BL/6 × A/J, H-$2^{b/k}$) or C57BL/6 (H-2^b) cells were injected into the circulation of F_1 IRF-$1^{+/-}$ and IRF-$1^{-/-}$ mice (H-$2^{b/k}$). NK activity was induced in recipient mice by the injection of pI:pC at the time of donor cell injection and at 24 h after injection. Persistence of FITC-labeled donor cells in the LN of recipient animals was followed by flow cytometry (Fig. 6). While F_1 syngeneic donor cells persisted in F_1 hosts, C57BL/6-derived parental donor cells (H-2^b) were eliminated in F_1 IRF-$1^{+/-}$ mice. In contrast, F_1 IRF-$1^{-/-}$ mice were unable to eliminate the parental donor cell graft.

Normal Numbers and Surface Markers of IRF-$1^{-/-}$ $NK1.1^+$ NK Cells. The potential role of IRF-1 in the ontogeny and activation of murine NK cells was addressed by flow cytometric analysis of spleen MNC that were obtained from pI:pC–treated mice (Fig. 7). No significant difference in the number of $NK1.1^+/CD3^-$ cells was evident between heterozygous and homozygous IRF-1–deficient mice, and the number of $CD3^-/NK1.1^+$ cells expressing the early activation marker CD69 was similar in both groups of mice. Comparable numbers of $CD3^-/NK1.1^+$ cells also expressed the α chain of the IL-2 receptor (CD25) and CD11a, the α chain of LFA-1, in both heterozygous and homozygous IRF-1–deficient mice (data not shown).

Discussion

From the data presented in this study, it is clear that IRF-1 plays an essential role in the augmented lytic activity of murine NK cells after viral infection, stimulation with type I IFNs, or stimulation with IL-12. Flow cytometric analysis of spleen MNC from heterozygous and homozygous IRF-1–deficient mice indicates that IRF-1 does not play a role in the ontogeny of NK cells, and that the defect seen in NK cell–mediated cytotoxicity is not caused by a

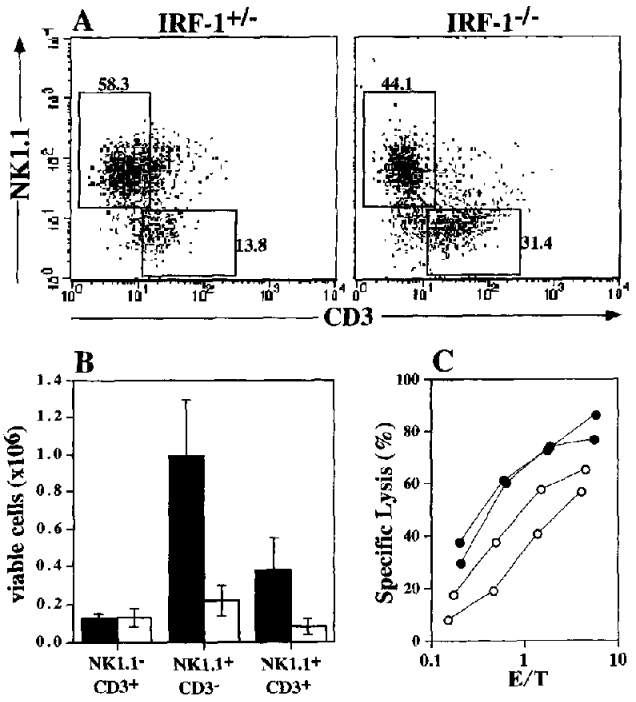

Figure 4. IL-2–stimulated proliferation and augmented killing of NK cells is partially impaired in IRF-1–deficient mice. During a 9-d culture of spleen cells in the presence of 500 U/ml IL-2, $NK1.1^+/CD3^-$ cells proliferate preferentially. (*A*) FACS® analysis (with the percent of $NK1.1^+$ and $CD3^+$ cells shown in their respective quadrants) of day 9 IL-2–stimulated cells from single representative heterozygous and homozygous IRF-1–deficient mice, selected from three of each genotype that was examined in this experiment. Absolute numbers of $NK1.1^+$ and $CD3^+$ cells at day 9 (*B*) were determined from total viable cell counts and the percent of cells obtained from FACS® analysis. Solid bars represent cells from heterozygous mice, while open bars represent cells from homozygous IRF-1–deficient mice. Data are shown as the mean number of viable cells ± SEM for three mice in each group. Lytic activity of day 9 IL-2–stimulated cells was assessed using YAC-1 target cells (*C*). E/T ratios were calculated using absolute numbers of viable $NK1.1^+/CD3^-$ cells present in the killing assay. Data are shown as percent specific lysis by NK cells derived from two $IRF\text{-}1^{+/-}$ (*solid symbols*) and two $IRF\text{-}1^{-/-}$ (*open symbols*) mice.

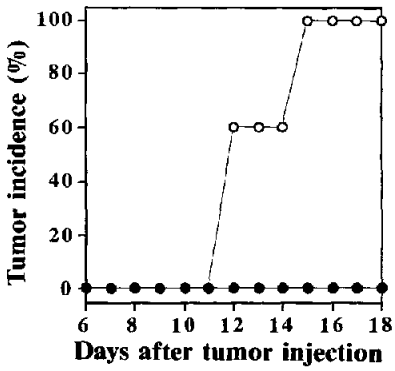

Figure 5. Elimination of MHC class I–negative syngeneic tumor is impaired in IRF-1–deficient mice. $IRF\text{-}1^{+/+}$ (*closed symbols*) and $IRF\text{-}1^{-/-}$ (*open symbols*) mice were injected subcutaneously with 10^5 RMA-S cells and monitored for palpable tumors. Five age- and sex-matched mice were analyzed in each group.

lack of NK cells. In addition, the expression of the surface molecules CD25, CD69, and LFA-1 by NK cells was similar in IRF-1–deficient mice compared to heterozygous control mice.

One explanation for our findings is that an inherent defect in the production of type I IFNs exists in $IRF\text{-}1^{-/-}$ mice, leading to the insufficient activation of NK cells. The absolute role of IRF-1 in the induction of type I IFNs is controversial, and the effect of IRF-1 deficiency on type I IFN production remains unclear. IRF-1 has been shown to bind regions within the promoter of type I IFNs (3, 4), and overexpression of IRF-1 results in IFN-β production in some cell lines (4, 12). However, although the induction of type I IFN mRNAs by pI:pC has been shown to be dramatically reduced in vitro in IRF-1–deficient embryonic fibroblasts compared to wild-type controls (6), Reis et al. have shown that in vivo, pI:pC injection does not reveal any significant difference in IFN-α or IFN-β production between wild-type and IRF-1–deficient mice (13). In our study, the addition of exogenous IFN-β to spleen cells in vitro clearly does not fully restore the lytic activity of $IRF\text{-}1^{-/-}$ NK cells to that seen in wild-type cells. This implies that the diminished NK activity seen upon in vivo induction may not be caused by a defect in type I IFN production, and that other factors intrinsic to NK cells and essential for lytic activity are disrupted in $IRF\text{-}1^{-/-}$ mice.

IL-12 was first identified as an inducer of NK cell–mediated cytotoxicity (14), presumably through the induction of genes involved in target cell lysis, such as perforin or granzyme B (15). Another important function of IL-12 is the induction of IFN-γ production in resting and activated NK cells (16), a key step in the innate response to acute infection. In this study, we report that IRF-1–deficient NK cells are incapable of responding appropriately to IL-12 stimulation in vitro, since both enhanced lytic activity and IFN-γ production are defective in splenocytes derived from $IRF\text{-}1^{-/-}$ mice. Our data suggest that IL-12–mediated signaling itself is defective in the NK cells of IRF-1–deficient mice, correlating with a recent report that IL-12–deficient mice exhibit a defect in both NK cell cytolytic activity and IFN-γ production (17). IL-12 has also been implicated as a central mediator of acute GVHD in BDF_1 mice injected with B6 donor splenocytes. Neutralization of endogenous IL-12 moderated acute GVHD and resulted in decreased splenic NK activity and IFN-γ production (18). We therefore postulate that the defective rejection of semiallogeneic, hematopoetic donor cells by $IRF\text{-}1^{-/-}$ mice is, at least in part, the result of aberrant IL-12–mediated signaling.

The functional response of NK cells to IL-2 stimulation in vitro was also found to be disrupted in IRF-1 knockout mice. Both proliferation and augmentation of lytic activity of IL-2–stimulated NK cells were defective, although the partial reduction in lytic activity seen in $IRF\text{-}1^{-/-}$ compared to $IRF\text{-}1^{+/-}$ controls was not as profound as that seen after activation with type I IFNs or IL-12. It appears, therefore, that IL-2–mediated effects on NK cell function are not fully dependent on IRF-1.

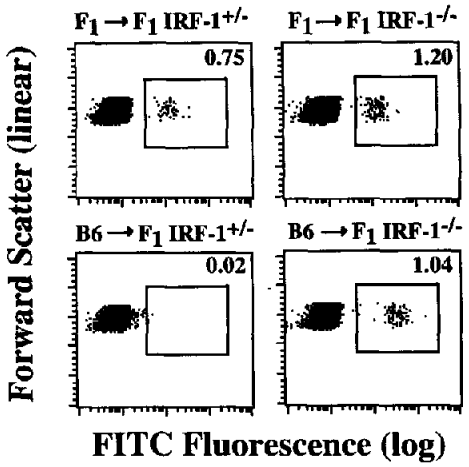

Figure 6. Persistence of parental lymphoid donor cells in IRF-$1^{+/-}$ and IRF-$1^{-/-}$ mice. (C57BL/6 × A/J) F_1 IRF-$1^{+/-}$ (*left panels*) or IRF-$1^{-/-}$ (*right panels*) mice were injected intravenously with either 2×10^7 FITC-labeled (C57BL/6 × A/J) F_1 (F_1, *upper panels*) or C57BL/6 (*B6, lower panels*) parental donor cells. 3 d later, LN cells were analyzed for the persistence of FITC-labeled cells by flow cytometry. Figures in bold type show the percentage of persisting donor cells for single representative animals, selected from six per group. Identical results were obtained in spleen and peripheral blood cells (data not shown).

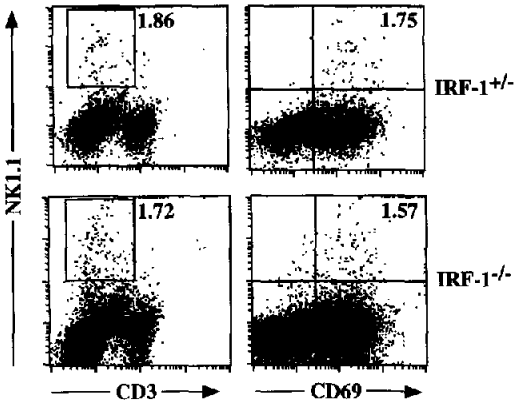

Figure 7. Flow cytometric analysis of spleen lymphocytes obtained from pI:pC–treated IRF-$1^{+/-}$ and IRF-$1^{-/-}$ littermate mice. Numbers in bold type indicate the percentage of $NK1.1^+/CD3^-$ cells (*left panels*) and $NK1.1^+/CD69^+$ cells (*right panels*) for single representative mice. Similar results were obtained in other analyses of at least five animals of each genotype in two separate experiments.

The expression of some genes is critically dependent on IRF-1, for example, inducible nitric oxide synthase (iNOS) or guanylate-binding protein (19). It has recently been suggested that NO may play a role in human NK cell–mediated cytotoxicity (20), and given the dependence of iNOS on IRF-1 for induction, the possibility that the defect in NK cell–mediated cytotoxicity in IRF-$1^{-/-}$ mice is caused by the reduced production of NO was addressed in our laboratory. The nonspecific NO synthase inhibitor *N*-monomethyl-L-arginine and the iNOS-specific inhibitor aminoguanidine exhibited no inhibitory effect (at doses ranging from 10 nM to 1 mM) on pI:pC–induced, NK-mediated killing of YAC-1 target cells in vitro (data not shown). This suggests that NO does not play a role in NK cell–mediated cytotoxicity, and supports the finding that perforin is predominately responsible for NK cell–mediated cytotoxicity (21, 22).

The expression of many IFN-inducible genes is mediated through IRF-1–independent pathways, including double-stranded RNA–dependent protein kinase and 2′-5′ oligoadenylate synthetase (6). It is possible that the residual NK-mediated lytic activity of IRF-$1^{-/-}$ cells observed in our experiments may be mediated via other transcription factors. Candidates include IFN-γ–inducible γ-activated factor, via its association with the IFN-γ activation site, or IFN-stimulated gene factor 3 (ISGF3), which shares overlapping sequence recognition with IRF-1, and may cooperate with IRF-1 in the induction of type I IFN-inducible genes in a nonredundant fashion (23). Although NK cells both produce and respond to IFN-γ, experiments using IFN-γ–deficient mice show that enhanced splenic NK cell activity from pI:pC–treated mice is independent of type II IFN, and that induction of type I IFNs can at least compensate for an absence of IFN-γ (24).

The data obtained in our in vivo studies of hybrid resistance to a hematopoietic graft, as well as the rejection of an MHC class I–negative tumor cell from a peripheral site of injection, confirm the in vitro data showing a lack of NK cell–mediated cytotoxicity in IRF-$1^{-/-}$ mice. While type I IFNs were induced in the in vitro models of NK activity by biological response modifiers, the rejection of allogeneic grafts and MHC class I–negative tumor cells are models for which the role of endogenous IFNs remains to be elucidated. Our data does, at least indirectly, support findings that IL-12 plays a prominent role in the acute rejection of hematopoetic allografts and MHC class I–negative cells in the mouse.

In conclusion, the absence of the transcription factor IRF-1 leads to a defect in NK cell cytolytic activity induced in response to LCMV infection, pI:pC, IFN-β, IL-12, or IL-2, which is not the result of reduced NK cell number, or of NK cell activation as assessed by the expression of the activation markers CD69 and CD25. In addition, MHC class I–deficient tumor cells are not eliminated, and clearance of parental donor cells is impaired in IRF-1–deficient mice. IRF-1 is therefore an essential mediator of normal NK cell function in the mouse.

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Address correspondence to T.W. Mak, Amgen Institute, 620 University Avenue, Toronto, Ontario, Canada M5G 2M9.

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