Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 11284–11288, November 1995
Immunology

A null mutation in the gene encoding a type I interferon receptor component eliminates antiproliferative and antiviral responses to interferons α and β and alters macrophage responses

(gene targeting/viral infection/macrophages/signal transduction)

SEUNG Y. HWANG*†, PAUL J. HERTZOG,*†‡, KERRY A. HOLLAND*, SONY H. SUMARSONO*, MARTIN J. TYMMS*, JOHN A. HAMILTON§, GENEVIEVE WHITTY§, IVAN BERTONCELLO¶, AND ISMAIL KOLA*

*Molecular Genetics and Development Group, Institute of Reproduction and Development, Monash Medical Centre, Monash University, Clayton, Victoria 3168, Australia; §Department of Medicine, University of Melbourne, Royal Melbourne Hospital, Parkville, Victoria 3050, Australia; and ¶Trescowthick Research Laboratories, Peter MacCallum Cancer Institute, East Melbourne, Victoria 3002, Australia

Communicated by J. F. A. P. Miller, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, July 10, 1995

ABSTRACT **To examine the *in vivo* role(s) of type I interferons (IFNs) and to determine the role of a component of the type I IFN receptor (IFNAR1) in mediating responses to these IFNs, we generated mice with a null mutation (−/−) in the IFNAR1 gene. Despite compelling evidence for modulation of cell proliferation and differentiation by type I IFNs, there were no gross signs of abnormal fetal development or morphological changes in adult IFNAR1 −/− mice. However, abnormalities of hemopoietic cells were detected in IFNAR1 −/− mice. Elevated levels of myeloid lineage cells were detected in peripheral blood and bone marrow by staining with Mac-1 and Gr-1 antibodies. Furthermore, bone marrow macrophages from IFNAR1 −/− mice showed abnormal responses to colony-stimulating factor 1 and lipopolysaccharide. IFNAR1 −/− mice were highly susceptible to viral infection: viral titers were undetected 24 hr after infection of IFNAR1 +/+ mice but were extremely high in organs of IFNAR1 −/− mice, demonstrating that the type I IFN system is a major acute antiviral defence. In cell lines derived from IFNAR1 −/− mice, there was no signaling in response to IFN-α or -β as measured by induction of 2′-5′ oligoadenylate synthetase, antiviral, or antiproliferative responses. Importantly, these studies demonstrate that type I IFNs function in the development and responses of myeloid lineage cells, particularly macrophages, and that the IFNAR1 receptor component is essential for antiproliferative and antiviral responses to IFN-α and -β.**

Type I interferons (IFNs) are pleiotropic cytokines important in host defence and in physiological processes such as growth and differentiation (1, 2). These IFNs include 15 subtypes of IFN-α, one IFN-β, one IFN-ω, and in some species several subtypes of IFN-τ (trophoblast proteins) (1–3). The classification into type I IFN is based on similarities in structure and biological activities, a shared receptor or receptor component, and induction in response to virus (with the probable exception of IFN-τ) (1–3). Type II IFN (IFN-γ) differs from type I IFNs in all of the above respects except for some overlap in biological activities. IFNs protect cells against viral infection, regulate cell proliferation and differentiation, and activate immune effector cells such as natural killer cells and macrophages (1). Type I IFNs also may affect hemopoiesis (4–6): for example, IFN-α/β is produced by cultured murine bone marrow (BM) cells in response to colony-stimulating factor 1 (CSF-1) and may suppress or induce differentiation depending on the experimental conditions (6). IFN-α and -β are constitutively produced by hemopoietic cells (6–9), but direct evidence of an *in vivo* role for constitutive IFN in hemopoiesis is lacking. Type I IFNs also may regulate the differentiation of other cell types (2, 10). For instance, the production of and responses to IFNs are repressed in the early embryo and thus are developmentally regulated (2, 11). Furthermore, in ruminants at least, IFN-τ subtypes are important in the maternal recognition of pregnancy (2).

Individual type I IFNs compete for binding to cell surface receptors and therefore probably share a common receptor component (1). The cDNA encoding a component of the human type I IFN receptor (12), designated here as IFNAR1, and its murine homologue (13) have been cloned. Recently another human type I IFN receptor component, designated here as IFNAR2, was cloned and shown by blocking studies to be involved in antiviral responses and by cross-linking to bind several type I IFNs (14). However, the roles of these two type I IFN receptor components in ligand binding and/or signal transduction have yet to be resolved. There is evidence that different biological responses to type I IFNs are mediated by different intracellular pathways: (*i*) certain viruses inhibit antiproliferative but not antiviral responses to IFNs (15), (*ii*) IFN-α subtype J lacks NK-stimulating activity but retains antiviral activity (16), and (*iii*) cells can develop resistance to antiproliferative but not antiviral effects of IFNs (17). However, the point at which these cellular response pathways diverge is unclear. Recent data also suggest that IFN-β may have a signaling pathway (perhaps even a receptor component) distinct from IFN-α subtypes. A mutated cell line does not signal in response to IFN-α but maintains some signaling in response to IFN-β (18). Furthermore, human IFNAR1 expressed in murine cells mediates responses to IFN-α subtype B but not IFN-β (12).

To investigate the functions of type I IFNs *in vivo* and their mechanism of action, we have generated mice with a null mutation in the IFNAR1 gene. During the course of this work, another group also published the generation of an IFNAR1 gene "knock-out" mouse and reported a susceptibility to viral infection (19). In the present study, we show that an IFNAR1 null mutation results not only in a complete lack of antiviral responses but also in a loss of antiproliferative response to IFN-α and -β, absence of constitutive IFN activity, and, importantly, abnormalities in the proportions and responses of hemopoietic cells.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: IFN, interferon; IFNAR, interferon (type I) receptor; BM, bone marrow; PBL, peripheral blood leukocytes; LPS, lipopolysaccharide; BMM, BM-derived macrophages; CSF-1, colony-stimulating factor-1; PEF, primary embryo fibroblasts; SFV, Semiliki Forest virus; EMCV, encephalomyocarditis virus; RT-PCR, reverse transcription–PCR; 2′-5′OAS, 2′-5′ oligoadenylate synthetase.

†S.Y.H. and P.J.H. contributed equally to this work.
‡To whom reprint requests should be addressed.

MATERIALS AND METHODS

Generation of Mutant Embryonic Stem (ES) Cells and Mice. A targeting vector was constructed by using a 5.7-kb genomic DNA fragment including exons 3–6 of the IFNAR1 gene (20) isolated from a 129SVJ mouse genomic library (Stratagene). The neomycin-resistance cassette (*neo*) (from pMC1neoPolyA, Stratagene) was ligated into a unique *Sna* BI site (nucleotide 632 of IFNAR1 cDNA) in exon 5, and a herpes simplex virus thymidine kinase cassette (*TK*) (from pMC1-TK) was ligated to the 5′ end of intron 2 (21). ES D3 cells were transfected with linearized targeting vector, selected in gancyclovir (2 μM) and G418 (175 μg/ml). Two positive ES cell clones were identified by the presence of a 5.9-kb band in Southern blots of *Bam*HI-digested DNA hybridized with neo cDNA and a 3.3-kb band after hybridization with IFNAR1 genomic DNA probe (MIR) (Fig. 1*A*). Bands of the predicted sizes were also detected in Southern blots of *Eco*RI-digested DNA probed with MIR and neo (data not shown). ES cell clones containing a targeted IFNAR1 allele were microinjected into Balb/c blastocysts to generate germ-line chimeras, which were bred to Balb/c females to produce heterozygous mice. Northern blots were performed with total RNA extracted from organs by using guanidinium isothiocynate and probed with a 586-bp IFNAR1 cDNA fragment (nucleotides 811–1397) and subsequently with glyceraldehyde-3-phosphate dehydrogenase cDNA (22). Reverse transcription (RT)–PCR was conducted by using primer P1 (958–939 bp) to initiate RT. Thirty-five PCR cycles (60 sec at 93.5°C; 60 sec at 58°C; and 60 sec at 72°C) were then performed with P2 (388–410 bp) and P1 primers.

Viral Infection of Mice. Four-day-old mice were injected i.p. with a suspension of Semliki Forest virus (SFV) equivalent to 100 times the tissue culture 50% infective dose ($TCID_{50}$) on L cells or with encephalomyocarditis virus (EMCV) at 10 times the $TCID_{50}$. Mice were observed several times daily for health status. Five normal mice killed 24 hr after SFV infection and five IFNAR1 −/− mice taken at death, 22–24 hr after infection, were dissected, and their organs were homogenized on ice in phosphate-buffered saline and centrifuged at 10,000 × *g* for 30 min. The supernatants were tested for viral titers as above.

Primary Cell Cultures. Primary embryo fibroblasts (PEF) derived from day 13 embryos from heterozygous crosses were cultured in Dulbecco's modified Eagle's medium containing 10% (vol/vol) fetal calf serum (FCS), 1% penicillin, and 1% streptomycin (P.A. Biological, Sydney, Australia). BM-derived macrophages (BMM) were generated as a relatively pure and homogeneous population of adherent cells from their nonadherent progenitors (23) and were grown to confluence for 5–6 days in RPMI 1640 medium supplemented with 50 μM 2-mercaptoethanol, 20 mM Hepes, 15% FCS, and 20% L cell-conditioned medium (as a crude source of CSF-1). Similar numbers of total cells were harvested from BMs of mice of all three genotypes. BMM were washed twice with phosphate-buffered saline and recultured in growth medium without L cell-conditioned medium for 18–20 hr to render the cells quiescent. These quiescent cells were used for BMM experiments.

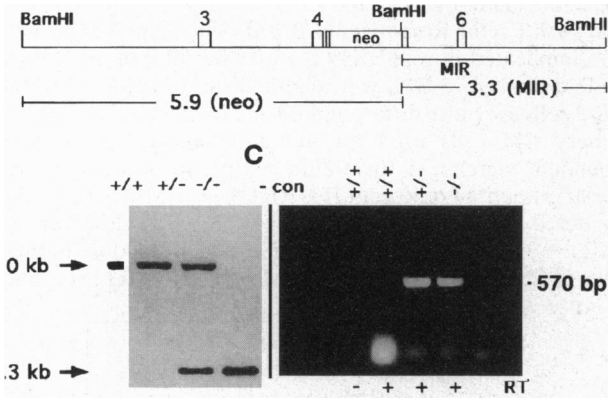

FIG. 1. Gene disruption by homologous recombination at the IFNAR1 locus. (*A*) The targeted allele contained a *neo* gene inserted into exon 5. MIR designates the IFNAR1 genomic DNA probe. (*B*) Southern blot analysis of *Bam*HI-digested DNA (probed with MIR DNA) from IFNAR1 +/+ mice showed an 8.0-kb band only, from IFNAR1 −/− mice showed a 3.3-kb band only, and from IFNAR1 +/− mice showed both 8.0-kb and 3.3-kb bands. (*C*) RT-PCR analysis of IFNAR1 gene expression in the lung of IFNAR1 +/+, +/−, and −/− mice. Negative PCR control (− con) did not contain RNA for the PCR reaction.

2′-5′ Oligoadenylate Synthetase (2′-5′OAS) Assay. Monolayers of PEF were incubated with or without 1000 units of mixed IFN-α or IFN-β subtypes (Lee Biomolecular Laboratories, San Diego, catalogue nos. 22051 and 20171, respectively) per ml of medium for 48 hr. The cells were harvested, lysates were prepared, and 2′-5′OAS specific activity was determined as described (24).

Proliferative Responses of BMM. Quiescent BMM were stimulated for 22 hr with CSF-1 (Chiron; 80–625 μg/ml) and then pulsed with [^{3}H]thymidine for a further 2 hr prior to harvesting. Trichloroacetic acid-precipitable material was assayed for [^{3}H]thymidine incorporation, which was corrected for cellular protein concentrations and expressed as the increase induced by added growth factor. Macrophage lineage-restricted colony-forming cells of low proliferative potential (LPP-CFC; representing small colonies with ≲50 cells) responsive to CSF-1 alone were assayed in a double-layer nutrient agar culture system (25).

Antiviral and Antiproliferative Assays. Antiviral activities of IFNs were determined in quadruplicate as the concentration of IFN that provides protection to 50% of the cells. Antiproliferative activities of exogenous IFNs were determined by using BMM stimulated with high levels of CSF-1 (5000 μg/ml). Inhibition of this incorporation was tested by adding 10–400 units of IFN-α, 1000 units of IFN-γ, or 100 ng of lipopolysaccharide (LPS, Difco) per ml.

Immunophenotyping. Peripheral blood leukocytes (PBL) (previously incubated with 0.83% NH_4Cl for 10 min at 37°C to lyse contaminating erythrocytes) and BM cells were labeled with rat monoclonal antibodies anti-Gr-1 (neutrophils) (26) and anti-Mac-1 (macrophage subsets) (27), followed by fluorescein isothiocyanate (FITC)-conjugated goat anti-rat IgG, each for 30 min at 4°C, and were analyzed on a FACStar^{Plus} cell sorter. The proportion of antigen-positive cells was determined by comparison with cells labeled with FITC-conjugated second antibody alone. There was no significant difference in the concentrations of PBL or BM cells in +/+ (PBL, 5.2 ± 1.0 × 10^6 cells per ml; BMM, 18.4 ± 1.6 × 10^6 cells per ml) or −/− (PBL, 5.0 ± 1.4 × 10^6 cells per ml; BMM, 19.0 ± 2.7 × 10^6 cells per ml) mice.

RESULTS

Generation of Mice with a Null Mutation in IFNAR1. The genotypes of mice resulting from heterozygote crosses were confirmed by Southern blots (Fig. 1*B*). The expression of IFNAR1 was examined by RT-PCR of total RNA from spleen, liver, and lungs of mice; a band of the expected 570 bp was seen in IFNAR1 +/+ and +/− mice but not in IFNAR1 −/− mice (data for lung RNA only is shown in Fig. 1*C*). Northern blot analysis of mRNA from IFNAR1 −/− mice also showed no bands, thus confirming that we have generated a null mutant (data not shown). From breeding of heterozygous mice, all three IFNAR1 genotypes were obtained in approximately the expected proportions. Of 189 pups, 38 were +/+ (20%), 101

were +/− (53%), and 50 were −/− (27%). Male and female IFNAR1 −/− mice were fertile and produced normal-size litters (data not shown). Thus, despite earlier proposals that type I IFNs may be important in reproduction and development, there is no evidence of developmental anomalies in the IFNAR1 −/− mice, nor are there gross macroscopic or microscopic abnormalities in organs of adult mice up to 6 months of age.

Susceptibility to Viral Infection. To examine the importance of type I IFNs in host responses to viral infection, we challenged neonatal IFNAR1 −/− mice with viruses. After SFV infection, wild-type mice had a mean survival time of about 70 h (Fig. 2*A*). The survival of heterozygous mice was similar to controls (Fig. 2*A*). By contrast, the IFNAR1 −/− mice died rapidly: all were alive but moribund 21 hr after inoculation with virus, and all were dead 3 hr later (Fig. 2*A*). Viral replication was extremely high in organs of IFNAR1 −/− mice: titers of 10^4 to 10^{10} were detected in the lung, spleen, brain, liver, and kidney, but no virus was detectable in organs of normal mice (Fig. 2*B*). We then tested the response of mice to a different type of virus, EMCV, a picornavirus. Again, there was sudden death of all IFNAR1 −/− mice—on this occasion within 38 h of inoculation of virus, whereas normal mice had a mean survival time of ≈60 hr as did heterozygous mice (data not shown).

The importance of the IFNAR1 receptor component in determining antiviral activity of IFN-α and -β was studied with PEF cell lines. IFNAR1 +/+ cell lines were protected against SFV and EMCV by low levels of IFN-α and -β (between 3 and 58 units/ml) (Table 1). In contrast, IFNAR1 −/− cell lines showed no protection against viral infection when as much as 3000 units of IFN-α or IFN-β per ml were used; there was at least a 500-fold difference in the sensitivity of IFNAR1 +/+ and −/− cell lines to the antiviral actions of these type I IFNs. The antiviral activities of IFN-α and -β in IFNAR1 +/− cells were less potent by a factor of 2–6 than in IFNAR1 +/+ cells, presumably because of reduced receptor expression (see below). These studies show the importance of an intact type I IFN system in the primary protection against acute infection by a broad range of viruses. Furthermore, these results show that the IFNAR1 component of the type I IFN receptor is essential for mediating antiviral responses *in vivo* and *in vitro* to IFN-α and -β.

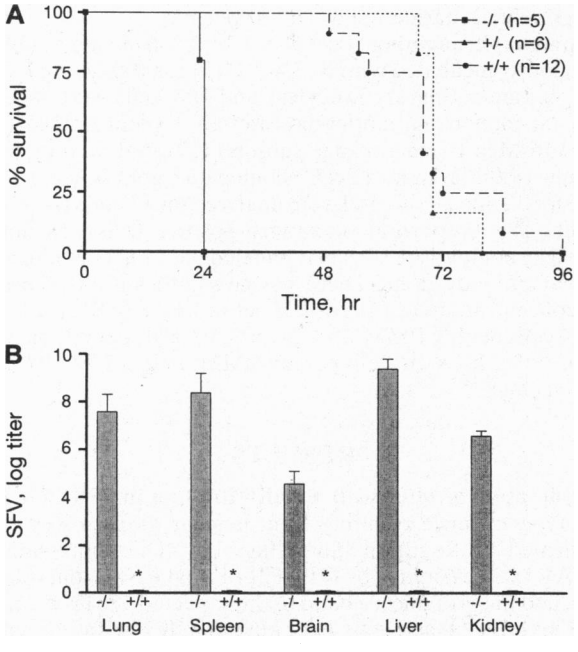

FIG. 2. (*A*) Survival curve of newborn mice after infection with SFV. Data are the mean percent of surviving mice among 5 IFNAR1 −/−, 6 IFNAR1 +/−, and 12 IFNAR1 +/+ mice. (*B*) Viral titers in extracts of organs of mice killed about 24 hr after inoculation of SFV. Data are the mean $\log_{10}$ titers ± SEM for at least four samples. Stars indicate no virus detection even at log 0.5 dilution.

Table 1. Antiviral responses of PEF to type I IFNs

Virus	IFN	+/+	+/−	−/−
SFV	α	7 ± 2	13 ± 3	>3000
	β	3 ± 1	18 ± 8	>3000
EMCV	α	58 ± 16	103 ± 48	>3000
	β	28 ± 8	50 ± 25	>3000

Data are means ± SEM of IFN activity (units/ml) for 50% reduction in cytopathic effect of at least four cell lines of each genotype.

IFN Signaling in Response to Exogenous IFNs. We next examined signal transduction in response to type I IFNs in the absence of the IFNAR1 component—i.e., whether signaling could occur through other receptor components. The activity of the IFN-induced enzyme, 2′-5′OAS, which is involved in both antiviral and antiproliferative activities of type I IFNs (1), was assayed because of its sensitivity (in the absence of exogenous IFNs, even basal levels can be quantitated). Basal levels of 2′-5′OAS activity in PEF in pmol/μg of protein were 189 ± 54 in IFNAR1 +/+ cells, 130 ± 64 in IFNAR1 +/− cells, and 23 ± 9 in IFNAR1 −/− cells (Fig. 3) and correlated with levels of receptor expression (the ratio of IFNAR1/glyceraldehyde-3-phosphate dehydrogenase mRNA by Northern blots of IFNAR1 +/− cells was 66% of IFNAR1 +/+ cells). The reduced basal activity in IFNAR1 −/− cells is consistent with the idea that in cells from normal mice, constitutive production of type I IFNs acts in a paracrine or autocrine manner to stimulate "basal" enzyme activity. Treatment of PEF with IFN-α resulted in a mean 7- to 8-fold induction of enzyme activity in IFNAR1 +/+ and +/− cell lines but no induction in IFNAR1 −/− cell lines (Fig. 3). IFN-β also failed to induce 2′-5′OAS activity in IFNAR1 −/− PEF (before IFN treatment, 6 ± 4 pmol/μg; after IFN, 3 ± 1), whereas it did induce this enzyme in IFNAR1 +/+ PEF as expected (before IFN treatment, 86 ± 44 pmol/μg; after IFN, 365 ± 120). Therefore, the receptor component encoded by IFNAR1 is essential for transducing signals responsible for induction of 2′-5′OAS in response to exogenous (and possibly also endogenous) IFN-α and -β.

Growth Factor Responses of BMM. Since type I IFNs have been implicated in regulating the proliferation of cells of the myeloid lineage in BM, we examined the function of BMM. These cells are more differentiated and homogeneous than the primary BM cells used for immunophenotyping. A dose-dependent increase in thymidine incorporation was detected after treatment of quiescent IFNAR1 +/+ BMM with graded low doses (80–625 μg/ml) of CSF-1 (Fig. 4). However, IFNAR1 −/− BMM show a markedly reduced response to these doses of CSF-1 (Fig. 4). Thus, BMM from IFNAR1 −/− mice

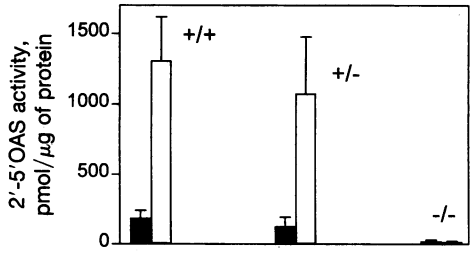

FIG. 3. Specific activities of 2′-5′OAS before (dark bars) and after (open bars) treatment of PEF with 1000 units of IFN-α per ml. Data are means ± SEM of three to six different cell lines of each genotype.

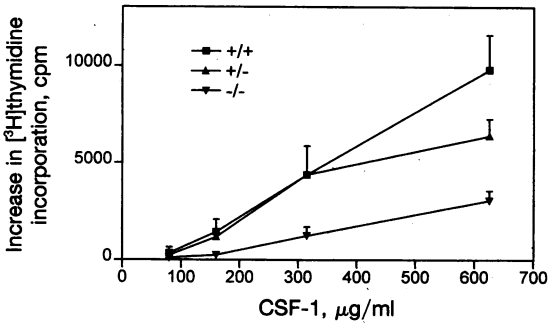

FIG. 4. Increase in [^{3}H]thymidine incorporation after addition of different concentrations of CSF-1. Data are means ± SEM of triplicate cpm measurements.

displayed a functional abnormality, even in the absence of exogenously added IFN-α or -β.

Antiproliferative Activity of Exogenous IFNs on BMM. We chose the proliferation of BMM in response to high levels of CSF-1 (5000 μg/ml) as a sensitive system to examine the inhibitory activities of exogenous type I IFNs and other factors, since IFNAR1 −/− BMM showed approximately normal stimulation of thymidine incorporation in response to this very high level. IFNAR1 +/+ BMM were inhibited by 95% in response to 12.5 units of mixed IFN-α subtypes per ml and by 99% in response to higher levels (Fig. 5*A*). IFN-γ and LPS also strongly inhibited proliferation. IFNAR1 −/− BMM were resistant to the antiproliferative effects of up to 400 units of IFN-α per ml, but IFN-γ was still active (Fig. 5*B*). Surprisingly, LPS also did not inhibit the CSF-1-stimulated thymidine incorporation into IFNAR1 −/− BMM (Fig. 5*B*), indicating that this effect of LPS on BMM is mediated through IFNAR1. Indeed, in several experiments, IFN levels of 400–1000 units/ml were detected in supernatants of LPS-treated BMM in a cytopathic effect reduction bioassay. Responses of BMM from heterozygous mice were similar to those from IFNAR1 +/+ mice (data not shown), indicating no detrimental effect of reduced IFNAR1 gene expression on these responses.

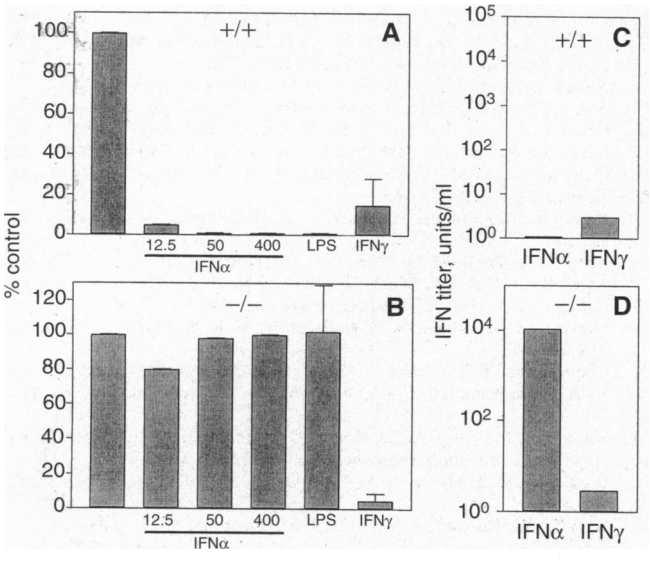

FIG. 5. Antiproliferative activities of IFNs and LPS on BMM from IFNAR1 +/+ (*A*) and IFNAR1 −/− mice (*B*). Data are expressed as the percent of proliferation measured in the absence of additives (100%) and presented as means ± SEM of triplicate measurements. Antiviral activities of IFNs on BMM from IFNAR1 +/+ (*C*) and IFNAR1 −/− mice (*D*) are expressed as the activity of IFN (units/ml) required to attain 50% inhibition of SFV-induced cytopathic effect.

To ascertain whether different biological responses of IFNs can be differentially regulated in the same cell type, we also examined the antiviral activities of IFNs in BMM. While IFNAR1 +/+ cells developed an antiviral response to as little as 1 unit of IFN-α per ml (Fig. 5*C*), the IFNAR1 −/− cells showed no responses to as much as 10,000 units/ml (Fig. 5*D*). The antiviral activity of IFN-γ was similar (≈3 units/ml) in BMM of both genotypes (Fig. 5 *C* and *D*). Similar trends were noted in responses to IFN-β (data not shown). Interestingly, studies of SFV growth in BMM showed that virus replication was better in IFNAR1 −/− cells than in IFNAR1 +/+ cells (data not shown). This result is also consistent with the different basal 2′-5′OAS activities in cells of the three genotypes, which together support the idea that constitutive production of type I IFN(s) induces a basal antiviral state in these primary cell lines.

Immunophenotyping of Hemopoietic Cells. In view of the altered response of BMM of IFNAR1 −/− mice to CSF-1 and their resistance to the antiproliferative effects of IFNs, we examined the proportions of hemopoietic cells in BM and PBL. Immunofluorescence staining and flow cytometric analysis of PBL and BM aspirates revealed abnormalities in cells of the myeloid lineage. The percent of cells staining positive for Mac-1 in PBL increased from 13.9 ± 2.8 in IFNAR1 +/+ cells (mean ± SEM, n = 5) to 42.1 ± 13.9 in IFNAR1 −/− cells (n = 4; $P < 0.05$ by Mann–Whitney test) (Fig. 6*A*). The percentage of cells staining with Gr-1 also increased from 8.4 ± 1.8 in IFNAR1 +/+ cells (n = 5) to 33.2 ± 13.9 in IFNAR1 −/− cells (n = 4; $P < 0.05$) (Fig. 6*B*). When fresh BM aspirates were examined, similar trends were observed with an increase in Mac-1-positive cells from 48.2 ± 6.9 to 60.5 ± 8.8 (Fig. 6*C*) and an increase in Gr-1-positive cells from 40.6 ± 5.1 to 53.0 ± 10.8 (Fig. 6*D*) (differences marginally significant). In addition to the higher proportion of Gr-1-positive cells in PBL and BM of IFNAR1 −/− mice, there was also an increase in the expression of this antigen per cell (i.e., a shift to the right of the peak in Fig. 6 *C* and *D*). Colony assays using fresh BM aspirates demonstrated that in IFNAR1 −/− mice, there was a 50% increase in the number of colonies of low proliferative potential obtained in the presence of CSF-1 (6.0 ± 1.1 in IFNAR1 +/+ cells compared with 9.1 ± 1.3 in IFNAR1 −/− cells). The staining of PBL and BM with markers for CD4, CD8, Thy-1, and B220 was essentially unaltered in IFNAR1 −/− mice, except for a trend towards fewer B220-positive cells in BM aspirates (data not shown).

DISCUSSION

We have generated mice with a null mutation in the IFNAR1 component of the type I IFN receptor. Importantly, we show

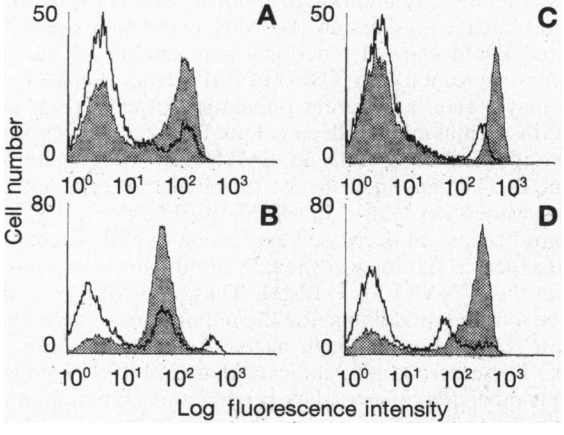

FIG. 6. Immunofluorescence flow cytometry of PBL (*A* and *B*) and BM aspirates (*C* and *D*) using antibodies to Mac-1 (*A* and *C*) or Gr-1 (*B* and *D*).

that cells from these mice lack both antiproliferative and antiviral responses to exogenous IFN-α and -β; the *in vivo* consequences are abnormalities in hemopoietic cells of myeloid lineage, abnormal responses of BMM, and a dramatic susceptibility to viral infection.

Neonatal mice were highly susceptible to infection with SFV or EMCV and died within 24–38 hr. Organs from IFNAR1 −/− mice showed unrestricted viral replication (titers up to 10^{10}) 24 hr after inoculation of SFV, whereas in +/+ mice there was no detectable virus. Thus, a type I IFN response appears to be essential for restricting acute viral infection. Similar susceptibility was reported for type I IFN receptor knock-out mice infected with viruses including SFV (19). Our results confirm the previous report on SFV infection and emphasize the acute nature of this protection. Our data on infection with EMCV, a picornavirus, extend the range of virus types for which an IFNAR1-mediated antiviral response is essential. This *in vivo* data is consistent with studies in PEF and BMM from IFNAR1 −/− mice, which were insensitive to the antiviral effects of at least 3000 units of type I IFNs per ml. Data on 2′-5′OAS activity provides new evidence that the basal level of this IFN-regulated enzyme is lower in IFNAR1 −/− cells than in IFNAR1 +/+ or +/− cells. This supports the concept of an autocrine/paracrine action of constitutively produced type I IFN (7, 8) maintaining basal levels of this enzyme. The absence of such constitutive IFN action may also explain the higher viral titers observed in IFNAR1 −/− cells. Furthermore, the rapid mortality of IFNAR1 −/− mice after exposure to virus may also be due partly to the inactivity of constitutive IFN, which might prime cells for an immediate response.

Importantly, this study demonstrates abnormalities of hemopoietic cells in IFNAR1 −/− mice. While increases in surface marker (Gr-1 and Mac-1)-positive cells were detected in PBL and BM samples, differential cell counts on samples obtained at other times appeared to be normal in both morphology and proportions. For example, the proportion of myeloblasts + polymorphs in BM was 50 ± 16 in IFNAR1 −/− and 49 ± 10 in IFNAR1 +/+ mice. This data could be interpreted as meaning that cells were abnormal by molecular criteria despite being of normal appearance—for example, either increased marker expression on normal cells or expression of markers on atypical cells. Indeed BM shows increased expression of Gr-1 and Mac-1 per cell (Fig. 6 *C* and *D*). Furthermore, the Mac-1 antigen can be up-regulated on a wide variety of cells, including neutrophils and B and T lymphocytes. These results could represent either changes to mature hemopoietic cells or a change in hemopoiesis resulting in a buildup of immature cells with surface marker expression different to that of cells normally found in the BM and peripheral blood. In addition to abnormalities in expression of myeloid lineage markers on BM cells, experiments on differentiated BMM showed functional abnormalities. The lower proliferative responses to CSF-1 of BMM from IFNAR1 −/− mice may reflect a different population of cells from those found in normal mice. Indeed colony-forming assays show an increased number of cells in BMM with low proliferative potential. This may indicate the prevalence of cells with low responsiveness to CSF-1 in BMM of IFNAR1 −/− mice, perhaps because of decreased expression of CSF-1 receptors. An unexpected finding was the lack of inhibitory responses to LPS in the IFNAR1 −/− BMM. Thus, IFNAR1 −/− mice may be a useful model to study the importance of type I IFNs in host responses to LPS, in bacterial infection, sepsis, and shock. These results also indicate that this LPS response is entirely mediated by secondary production and action of type I IFNs.

Although two type I IFN receptor components, IFNAR1 (12, 13) and IFNAR2 (14), have been cloned, their roles in binding and signaling responses to various type I IFN ligands remain unclear. However, it is apparent that the functional type I receptor is composed of multiple components (28, 29). These studies show that in the absence of the IFNAR1 component, there is no signaling as measured by 2′-5′OAS, antiviral, or antiproliferative responses (at least in BMM). Thus, in the absence of IFNAR1, the IFNAR2 and/or other receptor components appear to be unable to transduce signals as measured in this study. Furthermore, despite prior evidence that IFN-β may utilize some factors or pathways independent of IFN-α, these studies show that IFNAR1 is essential for signal transduction in response to IFN-α and IFN-β. The reported divergence in cellular pathways mediating antiviral and antiproliferative responses (15, 17) must lie downstream of receptors—at least in BMM. However, the precise roles of the two receptor components in ligand binding and signal transduction, particularly in response to multiple type I IFN subtypes, need to be further elucidated.

We thank Professor Don Metcalf (Walter and Eliza Hall Institute, Melbourne) for performing differential cell counts and for valuable discussions and Janine Mison for typing the manuscript. This work was supported by National Health and Medical Research Council of Australia Grant 930398.

1. Pestka, S., Langer, J. A., Zoon, K. C. & Samuel, C. E. (1987) *Annu. Rev. Biochem.* **56,** 727–777.
2. Hertzog, P. J., Hwang, S. Y. & Kola, I. (1994) *Mol. Rep. Dev.* **39,** 226–232.
3. Weissmann, C. & Weber, H. (1986) *Prog. Nucleic Acid Res. Mol. Biol.* **33,** 251–300.
4. Quesada, J. R. & Gutterman, J. U. (1983) *J. Natl. Cancer Inst.* **70,** 1041–1046.
5. Michalevicz, R. & Revel, M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 2307–2311.
6. Resnitzky, D., Yarden, A., Zipori, D. & Kimchi, A. (1986) *Cell* **46,** 31–40.
7. Greenway, A. L., Overall, M. L., Sattayasai, N., Rowley, M. J., Hertzog, P. J., McMullen, G. L., Cheetham, B. F. & Marzuki, S. (1992) *Immunology* **75,** 182–188.
8. Hwang, S. Y., Greenway, A. L., McMullen, G. L. & Hertzog, P. J. (1994) *Immunol. Cell Biol.* **72,** 234–240.
9. Tovey, M. G., Streuli, M., Gresser, I., Gugenheim, J., Blanchard, B., Guymarho, J., Vignaux, F. & Gigou, B. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5038–5042.
10. Clemens, M. J. & McNurlan, M. A. (1985) *Biochem. J.* **226,** 345–360.
11. Harada, H., Willson, K., Sakakibara, J., Miyamoto, M., Fujita, T. & Taniguchi, T. (1990) *Cell* **63,** 303–312.
12. Uzé, G., Lutfalla, G. & Gresser, I. (1990) *Cell* **60,** 225–234.
13. Uzé, G., Lutfalla, G., Bandu, M. T., Proudhon, D. & Mogensen, K. E. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 4774–4778.
14. Novick, D., Cohen, B. & Rubinstein, M. (1994) *Cell* **77,** 391–400.
15. Åman, P. & von Gabain, A. (1990) *EMBO J.* **9,** 147–152.
16. Ortaldo, J. R., Herberman, R. B., Harvey, C., Osheroff, P., Pan, Y. C., Kelder, B. & Pestka, S. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4926–4929.
17. Fleischmann, C. M. & Fleischmann, W. R., Jr. (1988) *J. Biol. Regul. Homeost. Agents* **2,** 173–185.
18. Velazquez, L., Fellous, M., Stark, G. R. & Pelligrini, S. (1992) *Cell* **70,** 313–322.
19. Müller, M., Steinhoff, U., Reis, L. F. L., Hemmi, S., Pavlovic, J., Zinkernagel, R. M. & Aguet, M. (1994) *Science* **264,** 1918–1921.
20. Lutfalla, G. & Uzé, G. (1994) *Gene* **148,** 343–346.
21. Mansour, S. L., Thomas, K. K. & Capecchi, M. R. (1988) *Nature (London)* **336,** 348–352.
22. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **62,** 156–159.
23. Vairo, G. & Hamilton, J. A. (1985) *Biochem. Biophys. Res. Commun.* **132,** 430–437.
24. Hertzog, P. J., Emery, P., Cheetham, B. F., Mackay, I. R. & Linnane, A. W. (1988) *Clin. Immunol. Immunopathol.* **48,** 192–201.
25. Bradley, T. R., Hodgson, G. S. & Rosendall, M. (1978) *J. Cell Physiol.* **97,** 517–522.
26. Hestdal, K., Ruscetti, F. W., Ihle, J. N., Jacobsen, S. E., Dubois, C. M., Kopp, W. C., Longo, D. L. & Keller, J. R. (1991) *J. Immunol.* **147,** 22–28.
27. Springer, T., Galfre, G., Secher, D. S. & Milstein, C. (1979) *Eur. J. Immunol.* **9,** 301–306.
28. Hertzog, P. J., Hwang, S. Y., Holland, K. A., Tymms, M. J., Iannello, R. & Kola, I. (1994) *J. Biol. Chem.* **269,** 14088–14093.
29. Soh, J., Mariano, T. M., Lim, J. K., Izotova, L., Mirochnitchenko, O., Schwartz, B., Ianger, J. A. & Pestka, S. (1994) *J. Biol. Chem.* **269,** 18102–18110.