JOURNAL OF VIROLOGY, Sept. 1991, p. 4839–4846
0022-538X/91/094839-08$02.00/0
Copyright © 1991, American Society for Microbiology
Vol. 65, No. 9

Endogenous Interferon Specifically Regulates Newcastle Disease Virus-Induced Cytokine Gene Expression in Mouse Macrophages

RAINER ZAWATZKY,[1]* HERIBERT WURMBAECK,[1] WERNER FALK,[2] AND ANGELA HOMFELD[1]†

Institut für Virusforschung[1] and Institut für Immunologie und Genetik,[2] Deutsches Krebsforschungszentrum, D-6900 Heidelberg, Germany

Received 22 February 1991/Accepted 18 June 1991

In macrophages from inbred mice, the magnitude of the interferon (IFN) response to Newcastle disease virus (NDV) infection is under genetic control of the *If-1* locus, which carries the allele for either high (*h*) or low (*l*) IFN production. Here, we report that the activity of genes within the *If-1* locus is influenced by macrophage-derived endogenous IFN. In addition to various other biological effects, we observed that endogenous IFN specifically downregulated NDV-induced IFN and interleukin 6 production. Preculture of bone marrow-derived macrophages (BMM) from BALB/c (*If-1^l*) mice in macrophage colony-stimulating factor plus anti-IFN-β provoked a 30- to 50-fold increase in NDV-induced cytokine production compared with induced control cultures in macrophage colony-stimulating factor alone, whereas only a 4- to 6-fold increase was observed in anti-IFN-β-treated BMM from C57BL/6 (*If-1^h*) mice. This resulted in nearly complete abrogation of the genetically determined difference in the response to NDV. The increase was specific for NDV and was marked by strong additional activation of IFN-α genes. Studies using BMM from B6.C-H28^c *If-1^l* congenic mice gave results identical to those obtained with BALB/c BMM. Addition of 20 IU of recombinant IFN-α_4 to anti IFN-β-treated macrophages from B6.C-H28^c mice 20 h prior to NDV infection strongly downregulated the IFN-α, IFN-β, and interleukin 6 responses. The genetic difference between macrophages from *If-1^h* and *If-1^l* mice was thus reestablished, since the same treatment caused only weak reduction of NDV-induced cytokine gene expression in BMM from C57BL/6 mice. These data suggest that the *If-1^h* and *If-1^l* alleles harbor IFN-inducible genes that, following activation, specifically suppress subsequent cytokine gene expression in response to NDV.

Virus-induced interferon (IFN) production in a host is under genetic control. Studies using inbred strains of mice have provided evidence for the existence of several loci that specifically influence the amount of alpha/beta interferon (IFN-α/β) produced following infection with one particular virus (for reviews, see references 12 and 13). These loci, named *If* loci, have high- and low-producer alleles which are inducer specific in that they influence IFN production only after contact with the corresponding inducer. The various *If* loci are characterized by their quantitative effects on IFN synthesis that lead to differences in IFN production between 3- and 30-fold, but their mode of action and gene products are not known. The *If-1* locus, which specifically influences IFN production by Newcastle disease virus (NDV), harbors two alleles, *If-1^h* and *If-1^l*, coding for high and low IFN production, respectively. C57BL/6 mice harbor the high-responder allele and BALB/c mice harbor the low-responder allele, resulting in 10- to 20-fold differences in NDV-induced circulating IFN levels. In vitro studies aimed at characterizing the cell populations involved in IFN production revealed that cultures of resident peritoneal macrophages from C57BL/6 and BALB/c mice also expressed the corresponding *If-1* allele when induced by NDV (15). Analysis of IFN production in a variety of congenic lines generated by repeated backcrossing of F_1 progeny with C57BL/6 parental mice led to the identification of the particular line B6.C-H28^c, carrying the *If-1^l* allele and histocompatibility locus *H-28* from BALB/c on a C57BL/6 genetic background, since NDV-induced IFN levels in these mice were similar to those observed in BALB/c mice (14). In the present study, we used cultures of bone marrow-derived macrophages (BMM) from these mouse strains to study in more detail the regulatory mechanisms of the *If-1* locus on NDV-induced IFN production. Our results indicate that the *If-1* locus also influences interleukin 6 (IL-6) production in response to NDV and that the *If-1^h* and *If-1^l* alleles are differentially influenced by macrophage-derived endogenous IFN.

MATERIALS AND METHODS

Mice. Male C57BL/6 and BALB/c mice were purchased from the Zentralinstitut für Versuchstierkunde, Hannover, Germany. Male B6.C-H28^c/By and additional C57BL/6 and BALB/c mice were obtained from the Jackson Laboratories, Bar Harbor, Maine. Throughout the experiments, 10- to 14-week-old animals were used.

Macrophage cultures. BMM were obtained as described previously (23). The cells were cultured in RPMI 1640–10% fetal calf serum (FCS) supplemented with 500 CFU of either crude or purified mouse macrophage colony-stimulating factor (M-CSF) per ml prepared from L-cell conditioned medium (LCM; kindly provided by E. R. Stanley; 42). When indicated, 100 neutralizing units (NU) of either monoclonal anti-mouse alpha interferon (anti-IFN-α) or monoclonal anti-mouse beta interferon (anti-IFN-β) (kindly provided by Y. Watanabe; 26) was added to the culture medium. Experiments were routinely carried out on day 7 or 8, when cultures consisted of more than 95% macrophages as analyzed by F4/80 antigen expression (22). LCM was continuously monitored for the presence of antiviral activity. After fivefold concentration by Amicon ultrafiltration, LCM was

* Corresponding author.
† Present address: Heinrich Pette-Institut, D-2000 Hamburg, Germany.

screened for induction of an antiviral state and of the 2′-5′ oligoadenylate synthetase (OASE) in mouse embryo fibroblasts. Controls included LCM to which 250 NU of monoclonal anti-IFN-α, anti-IFN-β, or both had been added 1 day before. In no case, however, did we obtain evidence for the presence of IFN activity in LCM.

Viruses. NDV strain Ulster (38) was isolated from infected embryonated eggs as previously described (23). Virus titers were determined in a standard hemagglutination assay using chicken erythrocytes and are given as hemagglutination units (HAU). The NDV preparation used in this study had a titer of 3,600 HAU.

Herpes simplex virus type 1 (HSV) strain WAL was prepared from infected RC-37 cells as previously described (48).

Purification of monoclonal anti-IFN antibodies. Monoclonal antibodies to mouse IFN-α and IFN-β were prepared from culture supernatants of hybridoma lines 4EA1 and 7FD3, respectively. The cell lines were a generous gift of Y. Watanabe (26). Dense, confluent hybridoma cultures were obtained after growth in RPMI 1640–10% FCS and incubated for an additional 3 days in fresh RPMI 1640 without serum. The medium was then recovered and concentrated 100-fold following ammonium sulfate precipitation. The antibodies were then purified by high-performance liquid chromatography (Beckman System Gold) using rabbit anti-rat immunoglobulin G coupled to Diasorb AC 1500 (Diagen). The monoclonal antibodies eluted at pH 3. The neutralization titers of the purified preparations from 4EA1 and 7FD3 supernatants were 1:8,000 against 4 IU of recombinant IFN-α_4 (rIFN-α_4) and 1:24,000 against 4 IU of natural mouse IFN-β, respectively.

Mouse rIFN-α_4 was kindly provided by E. C. Zwarthoff (46), and a preparation of natural mouse IFN-β (lot no. 82011; specific activity, 1.3×10^7 IU/mg) was purchased from Stratech Scientific Ltd.

Induction and determination of IFN and IL-6 activities in cultures of BMM. On day 7 of culture, BMM were detached from petri dishes with a rubber policeman following incubation with ice-cold saline, reseeded into 24-well plates at a density of 2.5×10^5 per well, and allowed to attach for 4 to 6 h. They were then incubated with NDV or HSV preparations for 1 h and refed, after removal of the inducer, with fresh, prewarmed RPMI 1640–5% FCS. After overnight incubation, supernatants were removed for IFN and IL-6 assays. For these experiments, purified mouse M-CSF (kindly provided by E. R. Stanley; 42) was used in general, but identical results were obtained with crude LCM as a source of M-CSF. IFN and IFN neutralization tests to determine the relative proportions of IFN-α and IFN-β were performed as previously described (23, 49). IL-6 activity was determined in a colorimetric proliferation assay (35) using subclone B9.9 isolated from hybridoma cell line B13.29, kindly provided by L. A. Aarden (1). One unit of IL-6 was defined as giving half-maximal proliferation of B9.9 cells. Supernatants to be assayed for IL-6 were preincubated with excess antibodies to mouse IFN-α/β to neutralize the antiproliferative effect of mouse IFN on B9.9 cells.

DNA probes. Genomic DNA probes for mouse IFN-α_1, IFN-α_2, and IFN-α_4 and cDNA probes for IFN-β and IL-6 were kindly provided by E. Zwarthoff (50), Y. Kawade (21), and J. van Snick (47), respectively. A 2.5-kb probe coding for the F protein and part of the HN protein of NDV was kindly provided by P. T. Emmerson (8, 9). For RNA blot analysis, the probes were labeled with [α-^{32}P]dCTP by random priming (17).

Preparation of RNA and blot hybridization. Total cellular RNA was extracted from mouse BMM with 4 M guanidinium thiocyanate as described by Chirgwin et al. (10). Denatured RNA was electrophoresed through agarose gels in the presence of formaldehyde (31) and transferred to nylon filters. Hybridization was carried out as previously described (23). For monitoring of identical amounts of cellular RNA per slot on the filter membrane, probes were removed by shaking the filters in a water bath at 70°C in 1.5 mM NaCl–0.01% sodium dodecyl sulfate, followed by rehybridization to labeled β-actin cDNA (23).

Assay of OASE. IFN-induced, double-stranded RNA-dependent OASE activity was determined as described previously (23). Briefly, the cells were lysed in a buffer containing 0.5% Nonidet P-40 and protein extracts were bound to poly(IC) agarose (Pharmacia) and incubated with buffer containing ATP (43). The 2′-5′ oligo(A) produced was quantitated by a competition radioactivity binding assay (27).

Vesicular stomatitis virus (VSV) infection and virus titration. Macrophages were detached from petri dishes on day 7 of culture and plated on 35-mm-diameter petri dishes at a concentration of 5×10^5 per dish. At 6 h later, the cells were washed and infected with VSV (strain Indiana) in serum-free medium by using a multiplicity of 2 PFU per cell. After adsorption for 1 h at 37°C, the cell monolayer was washed again to remove nonadsorbed virus particles and fresh, prewarmed RPMI 1640 plus 5% FCS was added. To account for residual input virus, an aliquot of the culture fluid was removed 1 h later. At 14 h later, culture dishes were frozen at −70°C to allow for approximately one replicative cycle of VSV. Virus titrations were done by using a standard viral plaque assay on a monkey kidney cell line as previously described (29). The amount of input virus varied between 0.5×10^3 and 1.5×10^3 PFU per culture and was deduced from the titers in the experimental groups.

RESULTS

Detection of endogenous IFN in cultures of BMM in the presence of M-CSF. In cultures of BMM, endogenous IFN is secreted constitutively in low amounts, and mostly its detection has been possible only by indirect means, i.e., induction of IFN-induced OASE or establishment of an antiviral state (23, 29, 36, 37). When we compared IFN levels in culture supernatants from C57BL/6, BALB/c, and B6.C-H28^c macrophages, we observed significant differences in that BALB/c macrophages consistently secreted between 12 and 54 IU of IFN whereas macrophages from the two other strains produced low-to-undetectable amounts (Table 1). As expected, the IFN levels increased with time of culture and thus correlated with the number of mature macrophages differentiating from myeloid precursor cells in the presence of M-CSF. The highest titers were found on day 7, 1 day after the cultures were supplied with fresh medium. The antiviral activity was completely neutralized by monoclonal antibodies to IFN-β. No differences were observed between crude LCM and purified mouse M-CSF as medium supplements (data not shown).

We also studied IFN gene expression at the RNA level by Northern (RNA) blot analysis. As shown in Fig. 1, a hybridization signal was observed for IFN-β mRNA in BALB/c BMM (lanes C) whereas no expression was detectable in BMM from C57BL/6 (lanes B) or B6.C-H28^c congenic mice (data not shown). These results were in agreement with IFN levels measured in the corresponding culture supernatants and indicate that expression of endogenous

TABLE 1. Secretion of endogenous IFN in M-CSF-cultured BMM

Culture conditions[a]		Day of culture[b]	Range[c] of IFN titers (IU/ml) with the following macrophage donor:		
Anti-IFN-α (NU)	Anti-IFN-β (NU)		C57BL/6	BALB/c	B6.C-H28[c]
		3	0	0–4	0
		6	0	6–18	0–4
		7	0–4	12–54	0–4
100		3	0	0–4	0
		6	0	6–12	0
		7	2–4	18–60	0–4
	100	3	0	0	0
		6	0	0	0
		7	0	0	0

[a] BMM were cultured for 7 days in RPMI 1640 medium containing 10% FCS and 500 CFU of purified mouse M-CSF per ml with or without antibodies to IFN-α or IFN-β as indicated.
[b] Day of removal of culture supernatant.
[c] From five different petri dishes.

IFN in macrophages is under genetic control. Similarly, we also found endogenous IL-6 gene expression in M-CSF-cultured BALB/c BMM (Fig. 1, lanes C). Interestingly, however, no IFN-β and IL-6 mRNAs were visible in RNA preparations from M-CSF-cultured macrophages kept in the presence of anti-IFN-β (Fig. 1, lanes C). This finding suggests that constitutive secretion of endogenous IFN-β in BALB/c macrophages is maintained by a positive feedback mechanism.

Autocrine biological effects of endogenous IFN. (i) Cell growth inhibition. Since IFN has antiproliferative effects on hematopoietic cells, we compared the influence of endogenous IFN on M-CSF-driven growth of macrophages from C57BL/6, BALB/c, and congenic B6.C-H28[c] mice. Cultures of bone marrow cells were set up in the presence of 500 CFU of mouse M-CSF per ml with or without addition of 100 NU of monoclonal anti-IFN-α or anti-IFN-β. The cell number per petri dish on day 7 revealed a significantly reduced growth rate in BALB/c BMM cultures (Table 2). Addition of monoclonal anti-IFN-β, however, caused a pronounced increase in BALB/c macrophage counts but had only little influence on cell growth in C57BL/6 and B6.C-H28[c] BMM cultures. The observed reduced growth rate of BALB/c macrophages in M-CSF thus does not reflect a lower sensitivity to M-CSF but rather is due to enhanced secretion of endogenous IFN-β.

(ii) Induction of OASE activity. It has been shown that endogenous IFN is responsible for elevated levels of OASE in cultures of BMM (23, 29, 37). In view of the observed differences in endogenous IFN levels, we also determined the levels of OASE in BMM from all three of the mouse strains under study. As can be seen additionally from Table 2, there was clear-cut induction of the enzyme in M-CSF-cultured control macrophages from all of the mouse strains compared with those treated with anti-IFN-β. Addition of monoclonal anti-IFN-α had no effect. OASE activity was also more elevated in macrophages from BALB/c mice than in those from C57BL/6 and B6.C-H28[c] mice, probably also

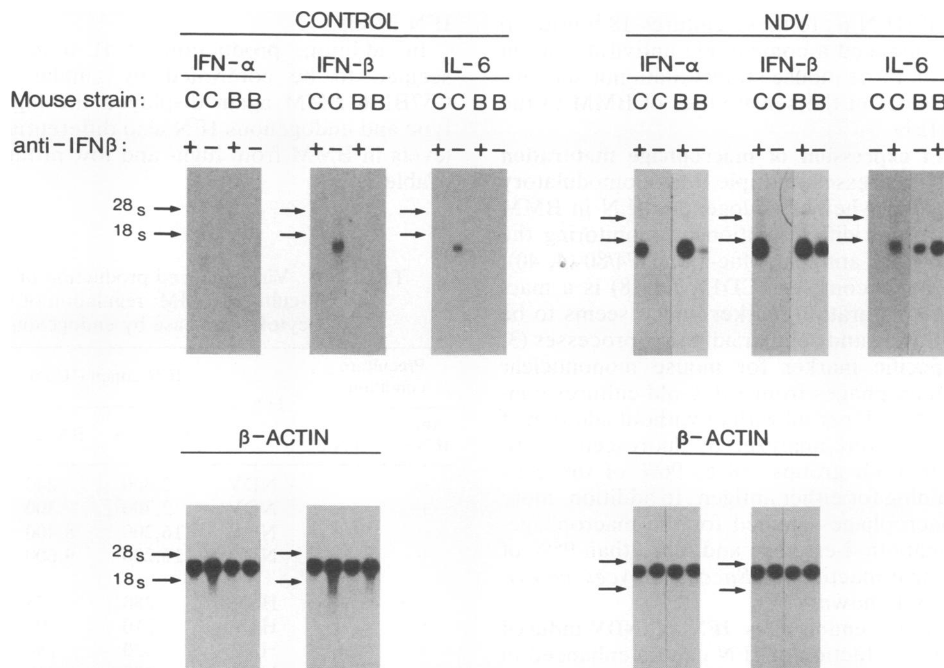

FIG. 1. Endogenous and NDV-induced cytokine gene expression in BMM from C57BL/6 and BALB/c mice. Bone marrow cells were cultured in mouse M-CSF (500 CFU/ml) with or without addition of 100 NU of anti-IFN-β. Total RNA was extracted from 7-day-old cultures of pure BMM, either noninfected (control) or 8 h after infection with NDV (100 HAU/10^6 cells). Agarose gels with 10 μg of RNA per lane were run, blotted onto nylon filters, and hybridized with the indicated cDNA probes. Filters hybridized to IFN-α probes were rehybridized first to IL-6 cDNA and subsequently to β-actin cDNA. Filters hybridized to the IFN-β probe were rehybridized to β-actin cDNA only. Mouse strains: C, BALB/c; B, C57BL/6. The exposure times for the filters were 96 h for controls, 16 h for B NDV, 40 h for C NDV, and 8 h for β-actin.

TABLE 2. Secretion of endogenous IFN by M-CSF-cultured BMM and its effect on cell growth, virus replication, and OASE activity

Mouse strain	Culture conditions[a]		VSV replication (PFU/culture)[b]	Macrophage growth (cell no., 10^6)[c]	OASE activity [pmol of 2'-5' oligo(A) mg^{-1} h^{-1}][d]
	Anti-IFN-α	Anti-IFN-β			
C57BL/6	–	–	3.40	1.25	20,620
	+	–	3.8	1.30	12,800
	–	+	6.18	1.35	1,500
BALB/c	–	–	3.08	0.76	98,000
	+	–	3.60	0.90	92,000
	–	+	6.40	1.25	980
B6.C-H28[c]	–	–	3.60	1.20	ND[e]
	+	–	3.71	1.25	ND
	–	+	6.3	1.35	ND

[a] BMM were cultured for 7 days in RPMI 1640 medium containing 10% FCS and 500 CFU of purified mouse M-CSF per ml with or without 100 NU of anti-IFN-α or anti-IFN-β as indicated.
[b] VSV titers are given as $\log_{10}$ values.
[c] Cell numbers are means of six petri dishes. All assays were carried out on day 7 of culture.
[d] Enzyme activity was measured in cell extracts.
[e] ND, not done.

because of the higher levels of endogenous IFN released from BALB/c macrophages.

(iii) Induction of resistance to VSV infection. Cultures of BMM set up in parallel were used to study the antiviral effect of endogenous IFN on infection by VSV. In M-CSF-cultured macrophages from all three mouse strains, VSV replication was low, independently of the observed endogenous IFN levels in the culture supernatants prior to infection (Table 2). Anti-IFN-α treatment of macrophages caused a slight (fivefold) rise in VSV titers, whereas in anti-IFN-β-treated cells there was a 3-order-of-magnitude increase in virus yield. Addition of 20 IU of rIFN-α_4 to these cultures 18 h prior to infection, however, induced a comparable antiviral state in macrophages from all three mouse strains (data not shown), indicating no differences in the sensitivities of BMM to the antiviral effects of IFN.

(iv) No change in expression of macrophage maturation markers. Since IFN possesses multiple immunomodulatory effects, we investigated whether endogenous IFN in BMM influenced M-CSF-driven differentiation by monitoring the expression of the surface antigens Mac-1 and F4/80 (4, 40). The Mac-1 glycoprotein complex (CD11b/CD18) is a macrophage-granulocyte maturation marker which seems to be involved in degranulation and cellular adhesion processes (3, 39). F4/80 is a specific marker for mouse mononuclear phagocytes (25). Macrophages from 8-day-old cultures containing 500 CFU of M-CSF per ml with or without addition of monoclonal anti-IFN-β were analyzed by fluorescence-activated cell sorter. In both groups, 88 to 96% of the cells showed specific staining for either antigen. In addition, more than 90% of all macrophages stained for the macrophage-specific enzyme α-naphthyl-esterase and more than 95% of the cells ingested heat-inactivated *Saccharomyces cerevisiae* particles (data not shown).

Specific regulation by endogenous IFN of NDV-induced cytokine production. Production of IFN can be enhanced in cell cultures by pretreatment of the cells with low doses of IFN, a mechanism termed priming (18, 44). We were therefore interested to see whether endogenous IFN also exerted a priming effect on subsequent NDV-induced IFN production in BMM from C57BL/6 and BALB/c mice as high and low responders, respectively. BMM were obtained by culture in the presence of M-CSF plus neutralizing antibodies to IFN-α or IFN-β and induced on day 7 with NDV or HSV. The results are depicted in Table 3. To our surprise, neutralization of endogenous IFN by anti-IFN-β pretreatment did not suppress but markedly enhanced subsequent IFN production in response to NDV, whereas anti-IFN-α had no effect. The enhancing effect was much more pronounced in macrophages from BALB/c mice, resulting in nearly complete abrogation of the genetically determined differences in IFN production between the two mouse strains. The effect was also specific for NDV, since pretreatment of macrophages with anti-IFN-β had little influence on HSV-induced IFN levels.

In addition, production of IL-6 in response to NDV seemed to be controlled by similar host genes, since C57BL/6 BMM again displayed the high-producer phenotype and endogenous IFN also differentially influenced IL-6 levels in BMM from high- and low-producer mouse strains (Table 3).

TABLE 3. Virus-induced production of IFN and IL-6 in M-CSF-cultured BMM: regulation of virus-induced cytokine release by endogenous IFN

Preculture condition[a]		Inducer	IFN concn (IU/ml)		IL-6 concn (U/ml)	
Anti-IFN-α	Anti-IFN-β		C57BL/6	BALB/c	C57BL/6	BALB/c
–	–	NDV	2,400	240	1,800	60
+	–	NDV	2,700	300	1,500	60
–	+	NDV	16,200	8,400	3,600	2,500
+	+	NDV	16,200	9,600	3,200	1,800
–	–	HSV	280	75	ND[b]	ND
+	–	HSV	210	90	ND	ND
–	+	HSV	320	180	ND	ND
+	+	HSV	400	210	ND	ND

[a] Macrophages were cultured for 7 days in purified mouse M-CSF alone (500 CFU/ml) or M-CSF with or without 100 NU of either monoclonal anti-IFN-α or monoclonal anti-IFN-β. The cells were then transferred to 24-well plates at 2.5 × 10^5 per well and induced with NDV (125 HAU/2.5 × 10^5 cells) or HSV at a multiplicity of 2. Culture supernatants were harvested for cytokine assays 18 h later.
[b] ND, not done.

Similar results were obtained when the amounts of NDV-induced IFN-α, IFN-β, and IL-6 gene expression were analyzed on the RNA level by Northern blot hybridization (Fig. 1). This is in agreement with earlier results showing that NDV-induced IFN production is regulated at the level of transcription (24). RNA was extracted from macrophages 8 h after NDV infection, when maximal levels of IFN-α, IFN-β, and IL-6 mRNAs had accumulated in macrophages from all of the mouse strains tested, as analyzed in control experiments (data not shown) and in previous studies (23, 24). As expected, steady-state levels of NDV-induced IFN and IL-6 mRNAs were much higher in C57BL/6 BMM than in BALB/c BMM when precultured in M-CSF alone. It is also obvious that these cells almost exclusively expressed IFN-β mRNA, whereas IFN-α mRNA was hardly detectable. Preculture in M-CSF plus anti-IFN-β, however, not only caused a strong increase in IFN-β mRNA levels in response to NDV but, in particular, activated IFN-α gene expression in BMM from both C57BL/6 and BALB/c mice. Characterization of the corresponding culture supernatants revealed a good correlation with mRNA levels: M-CSF-cultured macrophages produced mainly IFN-β in response to NDV, whereas a composition of about 50% IFN-α and 50% IFN-β was found in NDV-induced BMM precultured in M-CSF plus anti-IFN-β (see Table 5). Analysis of NDV-induced IL-6 mRNA levels in C57BL/6 and BALB/c macrophages revealed strong parallels to the regulation of IFN-β gene expression. Again, there was a dramatic increase in steady-state levels of IL-6 mRNA in BALB/c macrophages precultured in the presence of anti-IFN-β, whereas in C57BL/6 macrophages, IL-6 mRNA levels remained almost unaffected.

Enhanced sensitivity to a particular biological effect of IFN encoded by the *If-1^l* locus. Since we had observed higher levels of endogenous IFN in BMM from BALB/c mice, we wanted to know whether the NDV-specific low-responder phenotype of these cells resulted from dose-dependent downregulation of cytokine gene expression by endogenous IFN. To this end, we made use of the congenic line B6.C-H28^c. BMM from these mice produced very low or undetectable amounts of endogenous IFN comparable to those of BMM from the C57BL/6 parental strain (Table 1). Levels of NDV-induced IFN, in contrast, were close to those of BALB/c BMM (Table 4), since B6.C-H28^c macrophages express the *If-1^l* allele (15). However, preculture of B6.C-H28^c macrophages in M-CSF plus anti-IFN-β resulted in enhancement of the IFN response to NDV infection similar to that observed for BALB/c. This indicates that the differences in endogenous IFN production are not responsible for the low-responder phenotype of these cells. The data in Table 4 also reveal that overnight exposure to 20 IU of rIFN-α_4 in the presence of anti-IFN-β also mediated strong inhibition of NDV-induced IFN production in B6.C-H28^c and BALB/c macrophages, which both carry the *If-1^l* allele, whereas this inhibition was only marginal in C57BL/6 macrophages expressing the *If-1^h* allele. The inhibitory effect mediated by exogenous or endogenous IFN was also specific for NDV, since pretreatment of BMM with anti-IFN-β caused little changes in the HSV-induced IFN response, and addition of 20 IU rIFN-α_4 prior to HSV infection even resulted in slight enhancement of IFN titers (Table 4).

In addition, Northern blot analysis was performed in parallel with RNAs from C57BL/6 and congenic B6.C-H28^c macrophages 8 h after NDV infection, and the results are depicted in Fig. 2. Steady-state levels of IFN-α, IFN-β, and IL-6 mRNAs were considerably enhanced by pretreatment of the macrophages with anti-IFN-β (lanes 3) compared with cells cultured in M-CSF alone (lanes 2). The enhancing effect was much more pronounced for IFN-α than for IFN-β or IL-6 mRNA. Upon exposure of macrophages to 20 IU of rIFN-α_4, we also observed strong inhibition of NDV-induced cytokine expression on the RNA level (lanes 4), and the inhibitory effect was most evident for IFN-α mRNA, which became hardly detectable in these cells. In contrast, there was little change in IFN-β or IL-6 mRNA expression in NDV-induced BMM from C57BL/6. The culture supernatants corresponding to these RNA preparations were analyzed in a neutralization assay using monoclonal anti-IFN-α and anti-IFN-β. The results, summarized in Table 5, revealed that the relative proportions of IFN-α and IFN-β were in agreement with the observed steady-state mRNA levels.

TABLE 4. Regulation by endogenous and exogenous IFN of NDV- and HSV-induced IFN production in mouse macrophages

Preculture conditions[a]			IFN titer (IU/ml) at 18 h postinduction with the following macrophage donor:					
Anti-IFN-α	Anti-IFN-β	Amt of rIFN-α_4 (IU)	C57BL/6		BALB/c		B6.C-H28^c	
			NDV	HSV	NDV	HSV	NDV	HSV
−	−		2,400	350	180	40	250	320
+	−		2,100	450	180	72	320	320
−	+		16,200	320	9,600	96	10,800	360
−	+	20	5,400	840	30	110	250	480
−	+	200	4,800	260	<3	24	90	220

[a] Macrophages were cultured for 7 days in mouse M-CSF (500 CFU/ml) with or without monoclonal anti-IFN-α or anti-IFN-β (100 NU/ml). Macrophages were then transferred to 24-well plates at 2.5 × 10^5 per well, and 20 or 200 IU of rIFN-α_4 was added to some of the cultures for 20 h in the presence of anti-IFN-β. The cells were then induced by NDV (125 HAU/2.5 × 10^5 cells) or HSV at a multiplicity of 2.

Influence of anti-IFN-β treatment of BMM on NDV replication. Partial primary transcription of the viral genome and formation of double-stranded RNA intermediates after infection with single-stranded RNA viruses are considered one mechanism that leads to efficient IFN induction by viruses (16, 32, 33). We therefore investigated the possibility that enhanced viral replication accounted for the high NDV-specific IFN response in anti-IFN-β-treated macrophages. No hemagglutination activity, however, was found in culture supernatants from infected BMM, and similarly, no NDV transcripts coding for the viral F protein were detected in total RNA preparations from these cells (data not shown). This indicates that nonpermissiveness of mouse macrophages to NDV infection is not due to an antiviral state induced by endogenous IFN-β.

DISCUSSION

The present report deals with two major independent findings: (i) macrophages from inbred mouse strains differ in the capacity to produce endogenous IFN-β during culture in M-CSF, and (ii) the regulatory effect of the *If-1* gene locus on NDV-induced cytokine release in macrophages is influenced by endogenous IFN.

Secretion of low amounts of antiviral activity in cultures of mouse BMM was initially described by Moore et al. (34), and subsequently, indirect evidence was also obtained for the secretion of endogenous IFN by macrophages in vivo (6, 20, 36). Our studies have documented significant differences in endogenous IFN production between BMM from BALB/c

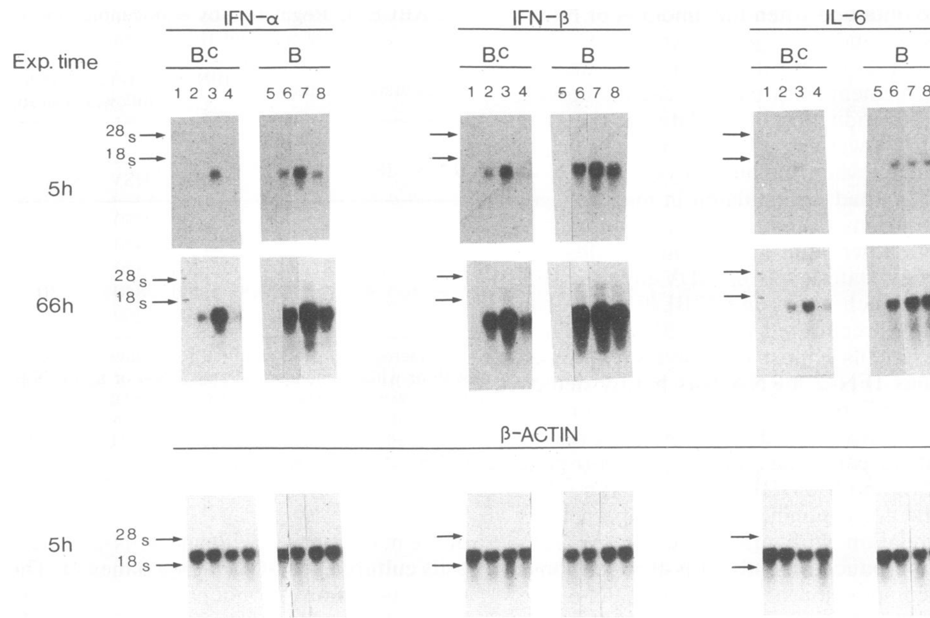

FIG. 2. Regulation by the *If-1* locus of NDV-induced cytokine gene expression in BMM. Total RNA was extracted from BMM and analyzed by Northern blot hybridization as described in the legend to Fig. 1. Lanes: 1 and 5, noninfected BMM cultured in M-CSF alone; 2 and 6, NDV-infected BMM precultured in M-CSF alone; 3 and 7, NDV-infected BMM precultured in M-CSF plus anti-IFN-β; 4 and 8, NDV-infected BMM precultured in M-CSF, anti-IFN-β, and 20 IU of rIFN-α_4 for the last 20 h prior to NDV infection. Mouse strains: B, C57BL/6; B.^{c}, B6.C-H28^c. Two exposure (Exp.) times of the same filters are presented to give a better impression of the differences in the steady-state levels of individual cytokine mRNAs.

and C57BL/6 mice. The IFN was identified as IFN-β, and the differences between the two mouse strains were visible on the RNA and protein levels, suggesting that host genes regulate the amount of endogenous IFN gene expression. Among the autocrine effects of endogenous IFN, we observed pronounced growth inhibition in cultures of BALB/c BMM compared with C57BL/6 BMM, and this was probably due to the higher levels of endogenous IFN, since in the presence of monoclonal anti-IFN-β, proliferation rates were identical in the two cultures. It has also been reported that BMM from BALB/c mice exhibit higher sensitivity to the antiproliferative effect of exogenously added IFN-α/β (11), and this higher sensitivity may further enhance the observed growth-inhibitory effect in these cells. On the other hand, no differences in IFN-mediated antiviral resistance to VSV was detectable among BMM from C57BL/6, B6.C-H28^c, and BALB/c mice. This observation may be explained by the fact that growth inhibition in general requires larger amounts of IFN than does establishment of an antiviral state (45).

When we studied the regulation of cytokine release in response to NDV, we found that BMM from BALB/c and B6.C-H28^c mice secreted considerably lower levels of IFN and IL-6 than did BMM from C57BL/6 mice that were high producers of both cytokines. Although coexpression of IFN-β and IL-6—for which the term IFN-β_2 had been used previously—following induction by virus is a known phenomenon (7, 30), these data additionally indicate that the *If-1* locus regulates the production of both cytokines in response to NDV. In addition, we made the striking observation that endogenous IFN-β caused not priming but blocking of IFN-α, IFN-β, and IL-6 gene expression after NDV infection of macrophages. The blocking effect was much more pronounced in BMM from BALB/c and B6.C-H28^c mice than in those from C57BL/6 mice. This indicates that the amount of blocking does not correlate with the level of endogenous IFN secreted by these macrophages but rather with their *If-1^l* genotype, since BMM from congenic B6.C-H28^c mice also produced low or undetectable levels of endogenous IFN-β, like cells from the C57BL/6 parental strain. This hypothesis was further supported by the finding that addition of identical amounts of mouse rIFN-α_4 in the presence of monoclonal anti-IFN-β also exerted a suppressive effect on subsequent NDV-induced cytokine production, which was much more pronounced in BALB/c and B6.C-H28^c BMM with the *If-1^l* genotype.

Taken together, there is evidence that the *If-1* locus contains a gene(s) which is activated by IFN and that the two alleles respond differently to endogenous IFN-β or exogenous IFN-α. Considering specific inhibition of cytokine gene expression as the only function attributable to these genes up to now, we propose that genes within the *If-1^l* allele display higher activity when stimulated by IFN.

We can only speculate about the mechanism by which IFN-activated genes within the *If-1* locus regulate cytokine

TABLE 5. Variation of NDV-induced levels of mouse IFN-α and IFN-β in macrophages

Cells	% IFN-α	% IFN-β	IFN titer[a]
Peritoneal macrophages	<10	>90	8–15
BMM cultured in:			
M-CSF	<10	>90	8–15
M-CSF + anti-IFN-β	40–50	50–60	1.8–2.5
M-CSF + anti-IFN-β + rIFN-α	<10	>90	8–20

[a] *If-1^h*/*If-1^l* (high- versus low-responder) ratio.

gene expression in macrophages. In view of the specificity for NDV, the possibility that *If-1*-derived gene products in general downregulate IFN and IL-6 production can be excluded. They seem, rather, to interfere with NDV-specific elements responsible for cytokine induction. In our study, replication of the viral genome as assessed by F protein cDNA could not be monitored in macrophages deprived of endogenous IFN-β. It may be, however, that under these conditions primary transcription of viral genes occurs, leading to formation of double-stranded RNA intermediates which, in turn, induce IFN production. Activation by endogenous IFN of either $If\text{-}1^l$ or $If\text{-}1^h$ would consequently result in more- or less-pronounced inhibition of viral transcription and double-stranded RNA formation, depending on the *If-1* allele involved.

The physiological role of *If-1* remains to be elucidated. Since the natural hosts of NDV are fowl, and mice are not susceptible to this virus, it seems likely that the assumed interference of mouse *If-1* gene products with NDV which leads to downregulation of cytokine induction in host cells does not represent its only function. Highly significant individual variations in NDV-induced IFN titers have also been observed in cultures of human peripheral blood leukocytes (5), suggesting that regulatory gene loci similar to *If-1* are also present in the human genome. This finding points to a possible conservation of *If-1* during evolution and also argues for additional biological functions of its gene products.

Concerning virus-specific action and inducibility by IFN, some interesting parallels between *If-1* and the mouse *Mx* gene emerge. This gene confers resistance to influenza virus infection and is inducible by IFN-α/β (for a review, see reference 41). Its functional allele, Mx^+, encodes a protein which accumulates in the nucleus and specifically interferes with influenza virus replication (28). Meanwhile, homologs of the mouse Mx protein have also been identified in human cells and their inducibility by IFN has been proven (2, 19). In this respect, further studies concerning the alleles of *If-1* will also be of significant interest for the characterization of one or more new genes activated differentially by endogenous IFN.

ACKNOWLEDGMENTS

We are indebted to O. Haller for helpful discussions, Y. Watanabe for providing hybridoma lines 4EA1 and 7FD3, and E. R. Stanley for the gift of purified mouse M-CSF. The DNA probes for mouse IFN-α_1, IFN-α_2, IFN-α_4, IFN-β, and IL-6 were kindly provided by E. C. Zwarthoff, Y. Kawade, and J. van Snick. We also thank P. T. Emmerson for the NDV F-protein cDNA. The excellent secretarial assistance of M. Kulka is gratefully acknowledged.

This work was supported in part by a grant from the Deutsche Forschungsgemeinschaft (Za 98/2-2).

REFERENCES

1. **Aarden, L. A., E. R. De Groot, O. L. Schaap, and P. M. Landsdorp.** 1987. Production of hybridoma growth factor by human monocytes. Eur. J. Immunol. **17:**1411–1416.
2. **Aebi, M., J. Fäh, N. Hurt, C. E. Samuel, D. Thomis, L. Bazzigher, J. Pavlovic, O. Haller, and P. Staeheli.** 1989. cDNA structures and regulation of two interferon-induced human Mx proteins. Mol. Cell. Biol. **9:**5062–5072.
3. **Anderson, D. C., L. J. Miller, F. C. Schmalstieg, R. Rothlein, and T. A. Springer.** 1986. Contributions of the Mac-1 glycoprotein family to adherence-dependent granulocyte functions: structure-function assessments employing subunit-specific monoclonal antibodies. J. Immunol. **137:**15–27.
4. **Austyn, J. M., and S. Gordon.** 1981. F4/80, a monoclonal antibody directed specifically against the mouse macrophage. Eur. J. Immunol. **11:**805–815.
5. **Bacon, T. H., A. De Vere-Tyndall, D. A. J. Tyrell, A. M. Denman, and B. M. Ansell.** 1983. Interferon system in patients with systemic juvenile chronic arthritis: in vivo and in vitro studies. Clin. Exp. Immunol. **54:**23–30.
6. **Belardelli, F., F. Vignaux, E. Proietti, and I. Gresser.** 1984. Injection of mice with antibody to interferon renders peritoneal macrophages permissive for vesicular stomatitis virus and encephalomyocarditis virus. Proc. Natl. Acad. Sci. USA **81:**602–606.
7. **Billiau, A., J. Van Damme, J. Ceuppens, and M. Baroja.** 1989. Interleukin-6, a ubiquitous cytokine with paracrine as well as endocrine functions. Colloq. INSERM **179:**133–142.
8. **Boursnell, M. E. G., P. F. Green, J. I. A. Campbell, A. Deuter, R. W. Peters, F. M. Tomley, A. C. R. Samson, P. Chambers, P. T. Emmerson, and M. M. Binns.** 1990. Insertion of the fusion gene from Newcastle disease virus into a nonessential region in the terminal repeats of fowlpox virus and demonstration of protective immunity induced by the recombinant. J. Gen. Virol. **71:**621–628.
9. **Chambers, P., N. S. Millar, R. W. Bingham, and P. T. Emmerson.** 1986. Molecular cloning of complementary DNA to Newcastle disease virus, and nucleotide sequence analysis of the junction between the genes encoding the hemagglutinin-neuraminidase and the large protein. J. Gen. Virol. **67:**475–486.
10. **Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18:**5294–5299.
11. **Dandoy, F., E. De Maeyer, and J. De Maeyer-Guignard.** 1981. Antiproliferative action of interferon on murine bone-marrow derived macrophages is influenced by the genotype of the marrow donor. J. Interferon Res. **1:**263–270.
12. **De Maeyer, E., and J. De Maeyer-Guignard.** 1979. Considerations on mouse genes influencing interferon production and action, p. 75–100. *In* I. Gresser (ed.), Interferon I. Academic Press, Inc. (London), Ltd., London.
13. **De Maeyer, E., and J. De Maeyer-Guignard.** 1988. Interferons and other regulatory cytokines. John Wiley & Sons, Inc., New York.
14. **De Maeyer, E., J. De Maeyer-Guignard, and D. W. Bailey.** 1975. Effect of mouse genotype on interferon production. Lines congenic at the If-1 locus. Immunogenetics **1:**438–445.
15. **De Maeyer, E., M. C. Hoyez, J. De Maeyer-Guignard, and D. W. Bailey.** 1979. Effect of mouse genotype on interferon production. III. Expression of If-1 by peritoneal macrophages in vitro. Immunogenetics **8:**257–263.
16. **Falcoff, R., and E. Falcoff.** 1970. Induction de la synthèse d'interféron par des RNA bicaténaires. II. Etude de la forme intermédiaire de réplication du virus Mengo. Biochim. Biophys. Acta **199:**147–158.
17. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132:**6–13.
18. **Fujita, T., and S. Ohno.** 1981. Studies on interferon priming: cellular response to viral and nonviral inducers and requirement of protein synthesis. Virology **112:**62–69.
19. **Goetschy, J.-F., H. Zeller, J. Content, and M. A. Horisberger.** 1989. Regulation of the interferon-inducible IFI-78K gene, the human equivalent of the murine *Mx* gene, by interferons, double-stranded RNA, certain cytokines, and viruses. J. Virol. **63:**2616–2622.
20. **Gresser, I., F. Vignaux, F. Belardelli, M. G. Tovey, and M.-T. Maunoury.** 1985. Injection of mice with antibody to mouse interferon α/β decreases the level of 2'-5' oligoadenylate synthetase in peritoneal macrophages. J. Virol. **53:**221–227.
21. **Higashi, Y., Y. Sokowa, Y. Watanabe, Y. Kawade, S. Ohno, C. Takaoka, and T. Taniguchi.** 1983. Structure and expression of a cloned cDNA for mouse interferon-beta. J. Biol. Chem. **258:**9522–9529.
22. **Hirsch, S., J. M. Austyn, and S. Gordon.** 1981. Expression of the macrophage-specific antigen F4/80 during differentiation of mouse bone marrow cells in culture. J. Exp. Med. **154:**713–725.

23. **Hoss, A., E. C. Zwarthoff, and R. Zawatzky.** 1989. Differential expression of interferon alpha and beta induced with Newcastle disease virus in mouse macrophage cultures. J. Gen. Virol. **70**:575–589.
24. **Hoss-Homfeld, A., E. C. Zwarthoff, and R. Zawatzky.** 1989. Cell type specific expression and regulation of murine interferon alpha and beta genes. Virology **173**:539–550.
25. **Hume, D. A., and S. Gordon.** 1983. Mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigen F4/80. J. Exp. Med. **157**:1704–1709.
26. **Kawade, Y., and Y. Watanabe.** 1987. Characterization of rat monoclonal antibodies to mouse interferon alpha and beta, p. 197–201. *In* K. Cantell and H. Schellekens (ed.), Proceedings of the Third International TNO Meeting on the Biology of the Interferon System. The Biology of the Interferon System. Nijhoff, The Hague, The Netherlands.
27. **Knight, M., P. J. Cayley, R. H. Silverman, D. H. Wreschner, C. S. Guilbert, R. E. Brown, and I. M. Kerr.** 1980. Radioimmune, radiobinding and HPLC analysis of 2-5 A and related oligonucleotides from intact cells. Nature (London) **288**:189–192.
28. **Krug, R. M., M. Shaw, B. Broni, G. Shapiro, and O. Haller.** 1985. Inhibition of influenza viral mRNA synthesis in cells expressing the interferon-induced *Mx* gene product. J. Virol. **56**:201–206.
29. **Kruse, A., H. Kirchner, R. Zawatzky, and I. Domke-Opitz.** 1989. In vitro development of bone marrow derived macrophages. Influence of mouse genotype on response to CSF and autocrine IFN induction. Scand. J. Immunol. **30**:731–740.
30. **Le, J., and J. Vilcek.** 1989. Interleukin 6: a multifunctional cytokine regulating immune reactions and the acute phase protein response. Lab. Invest. **61**:588–602.
31. **Lehrach, H., D. Diamond, J. M. Wozney, and H. Boedtker.** 1977. RNA molecular weight determinations by gel electrophoresis under denaturing conditions: a critical reexamination. Biochemistry **16**:4743–4751.
32. **Marcus, P., and F. J. Fuller.** 1979. Interferon induction by viruses. II. Sindbis virus interferon induction requires one-quarter of the genome-genes G and A. J. Gen. Virol. **44**:169–177.
33. **Marcus, P. I.** 1984. Interferon induction by viruses: double-stranded ribonucleic acid as the common proximal inducer molecule. Interferon **3**:113–175.
34. **Moore, R. N., H. S. Larsen, D. W. Horohov, and B. T. Rouse.** 1984. Endogenous regulation of macrophage proliferative expansion by colony-stimulating factor-induced interferon. Science **223**:178–180.
35. **Mosmann, T.** 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods **65**:55–63.
36. **Proietti, E., S. Gessani, F. Belardelli, and I. Gresser.** 1986. Mouse peritoneal cells confer an antiviral state on mouse cell monolayers: role of interferon. J. Virol. **57**:456–463.
37. **Resnitzky, D., A. Yarden, D. Zipori, and A. Kimchi.** 1986. Autocrine beta-related interferon controls c-myc suppression and growth arrest during hematopoietic cell differentiation. Cell **46**:31–40.
38. **Russel, P. H.** 1984. Newcastle disease virus: the effect of monoclonal antibody in the overlay on virus penetration and the immunoselection of variants. J. Gen. Virol. **65**:795–798.
39. **Schleifenbaum, B., R. Moser, M. Patarroyo, and J. Fehr.** 1989. The cell surface glycoprotein Mac-1 (CD11b/CD18) mediates neutrophil adhesion and modulates degranulation independently of its quantitative cell surface expression. J. Immunol. **142**:3537–3545.
40. **Springer, T., G. Galfre, D. S. Secher, and C. Milstein.** 1979. Mac-1: a macrophage differentiation antigen identified by monoclonal antibody. Eur. J. Immunol. **9**:301–306.
41. **Staeheli, P., and O. Haller.** 1987. Interferon-induced Mx protein: a mediator of cellular resistance to influenza virus. Interferon **8**:1–23.
42. **Stanley, E. R., and L. J. Guilbert.** 1981. Methods for the purification, assay, characterization and target cell binding of a colony stimulating factor (CSF-1). J. Immunol. Methods **42**:253–284.
43. **Stark, G. R., R. E. Brown, and I. M. Kerr.** 1981. Assay of (2′-5′)-oligoadenylic acid synthetase levels in cells and tissues: a convenient poly (I)-poly (C) paper-bound enzyme assay. Methods Enzymol. **79**:194–199.
44. **Stewart, W. E., II, L. B. Gosser, and R. Z. Lockart, Jr.** 1971. Priming: a nonantiviral function of interferon. J. Virol. **7**:792–801.
45. **Taylor-Papadimitriou, J., F. Balkwill, N. Ebsworth, and E. Rozengurt.** 1985. Antiviral and antiproliferative effects of interferon in quiescent fibroblasts are dissociable. Virology **147**:405–412.
46. **Van Heuvel, M., I. J. Bosveld, A. T. A. Mooren, J. Trapman, and E. C. Zwarthoff.** 1986. Properties of natural and hybrid murine alpha interferons. J. Gen. Virol. **67**:2215–2222.
47. **Van Snick, J., S. Cayphas, J.-P. Szikora, J.-L. Renauld, E. Van Roost, T. Boon, and R. J. Simpson.** 1988. cDNA cloning of murine interleukin-HP1: homology with human interleukin-6. Eur. J. Immunol. **18**:193–200.
48. **Zawatzky, R., I. Gresser, E. De Maeyer, and H. Kirchner.** 1982. The role of interferon in the resistance of C57BL/6 mice to various doses of herpes simplex virus type 1. J. Infect. Dis. **146**:405–410.
49. **Zawatzky, R., H. Kirchner, J. De Maeyer-Guignard, and E. De Maeyer.** 1982. An X-linked locus influences the amount of circulating interferon induced in the mouse by herpes simplex virus type 1. J. Gen. Virol. **63**:325–332.
50. **Zwarthoff, E. C., A. T. A. Mooren, and J. Trapman.** 1985. Organization, structure and expression of murine interferon alpha genes. Nucleic Acids Res. **13**:791–804.