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Antiviral Defense in Mice Lacking both Alpha/Beta and Gamma Interferon Receptors

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Alpha/beta interferon (IFN) and gamma IFN exert widely overlapping biological effects. Still, mice with individually inactivated alpha/beta or gamma receptors exhibit variably severely reduced resistance to infection and altered immune responses. To investigate to what extent the two IFN systems are functionally redundant, we generated mice with a combined receptor defect (AG129 mice). Like mice with individual mutations, AG129 mice had no apparent anomalies, confirming that in the mouse the IFN system is not essential for normal development. These mice showed an additive phenotype with respect to antiviral defense and exhibited an increased susceptibility to lymphocytic choriomeningitis virus (LCMV) and notably vaccinia virus infection. Because of unlimited replication and subsequent rapid exhaustion of cytotoxic T lymphocyte (CTL) precursors, these mice were unable to mount a CTL response to LCMV. $CD8^+$-mediated immunopathology was absent in LCMV-infected mice, and virus persisted. Vaccinia virus replicated much faster in AG129 mice, and a 10^4-fold lower dose of vaccinia virus was sufficient to prime these mice. With the normal priming dose of 10^6 PFU, cytopathic effects and overwhelming infection possibly causing partial exhaustion of CTL interfered with the anti-vaccinia virus response. Even though global antiviral immunoglobulin G (IgG) titers were within normal ranges, the IgG subclass distribution was heavily biased toward IgG1.

Interferons (IFNs) are well known for their antiviral effects. The IFNs can be divided into two classes: alpha, beta, and omega IFNs (IFN-α, IFN-β, and IFN-ω, respectively), which are encoded by a large family of genes (25), and gamma IFN (IFN-γ), which is structurally unrelated and encoded by a single gene (9). IFN-α, IFN-β, and IFN-ω presumably bind to a common receptor (18) composed of several subunits. One receptor subunit (24), however, seems to be essential for a functional response toward IFN-α, IFN-β, and IFN-ω (10, 23). IFN-γ uses a different, unique receptor which is expressed on many different cell types (1). Both types of IFNs exert pleiotropic effects which partly overlap, including stimulation of major histocompatibility complex antigen expression, inhibition of cell growth including tumor cells, involvement in hematopoiesis, and regulatory functions in the cellular and humoral immune response. However, the physiological significance of IFNs is not completely understood. To study the in vivo role of IFNs, mice lacking the IFN-α, IFN-β, or IFN-ω receptor (R) or the IFN-γ R have been generated by embryonic stem cell gene targeting and have been described previously (10, 17). Mice without a functional IFN-γ R (G129 mice) have been shown to develop normally but have a decreased resistance to infection with *Listeria monocytogenes* and vaccinia virus, despite normal cytotoxic T-lymphocyte (CTL) and helper responses (10). In contrast, mice lacking a functional IFN-α/β R (A129 mice) were unable to cope with an array of viral infections (vaccinia virus, lymphocytic choriomeningitis virus [LCMV], vesicular stomatitis virus [VSV], and Semliki Forest virus [17]), whereas the resistance to *L. monocytogenes* infection was normal. This shows that the two IFN systems are functionally nonredundant, at least for some infections. By crossing A129 mice with G129 mice, double-knockout mice were generated. These double-knockout mice (AG129) were healthy by 12 months of age and showed no gross abnormalities in hematological status, in the major lymphocyte subsets, or in constitutive major histocompatibility complex expression. We analyzed the effect of absence of both types of IFN Rs on antiviral defense against LCMV or vaccinia virus. AG129 mice had the phenotype of A129 mice but exhibited a more profound defect in anti-LCMV and in anti-vaccinia virus response.

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MATERIALS AND METHODS

Animals. Mice lacking the IFN-α/β receptor (A129 mice) and those lacking the IFN-γ receptor (G129 mice) have been described previously (10, 17). Mice lacking both types of receptors (AG129 mice) were obtained by breeding A129 × G129 mice. A129, G129, and AG129 mice had a pure 129Sv(ev) genetic background. Control 129Sv(ev) (129), C57BL/6 (B6), and (129 × B6)F_1 mice were obtained from the Institut für Zuchthygiene, Tierspital, Zürich, Switzerland. Male and female mice used were between 6 and 12 weeks of age. All these mice are of an *H-2*^{b} type. For most experiments, 129 mice are used as controls; however, for some experiments with LCMV B6 mice have been used. B6 and 129 mice showed a difference in anti-vaccinia virus CTL responses, because of a variation in the *H-2K*^{b} molecule and because, in *H-2*^{b} mice, killing of vaccinia virus-infected targets is predominantly via *H-2K*^{b}. All other responses were indistinguishable between 129 and B6 mice (data not shown).

Viruses and cell lines. LCMV-WE was originally obtained from F. Lehmann-Grube, Hamburg, Germany (12), and LCMV-Armstrong (ARM) was obtained from M. Oldstone, Scripps Clinic and Research Foundation, La Jolla, Calif. (7). Second-passage virus derived from plaque-purified isolates was propagated on BHK cells (ARM) or L929 fibroblast cells (WE). Vaccinia virus WR was grown on BSC40 cells, and the less virulent vaccinia virus strain Lancy was purchased from the Serum und Impfinstitut, Bern, Switzerland. The recombinant baculoviruses expressing the nucleoprotein (NP) of LCMV or of VSV were a generous gift of D. Bishop, NERC Institute of Virology, Oxford, United Kingdom (3). The recombinant baculoviruses were derived from nuclear polyhedrosis virus and were grown at 28°C in *Spodoptera frugiperda* cells in spinner cultures in TC-100 medium (13).

VSV NP-transfected (N1) and mock-transfected (V1) EL4 cells were originally obtained from L. Lefrançois (20).

Cytotoxicity assays. (i) Primary ex vivo assay. Cytolytic activity of spleen cells was determined by a ^{51}Cr release assay as described previously (19, 26). Briefly, mice were injected intravenously (i.v.) 8 days previously with LCMV or 6 days

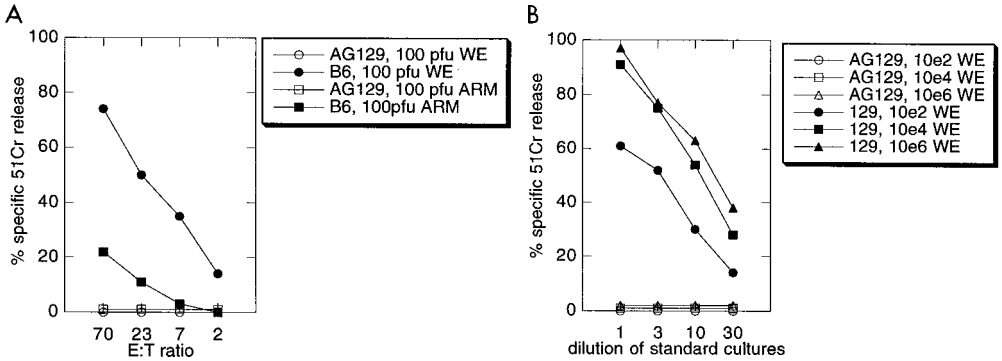

FIG. 1. (A) LCMV-specific CTLs are undetectable in AG129 mice after LCMV infection by a primary ex vivo CTL assay. Mice were infected 8 days previously with LCMV as indicated in figure. Splenocytes (E) were incubated with 10^4 LCMV-infected, ^{51}Cr-labelled MC57G target cells (T) for 5 h. Killing is expressed as percent specific ^{51}Cr release of duplicate cultures. Killing of uninfected MC57G fibroblasts was <5%. Spontaneous release of infected and uninfected targets was 12 and 11%, respectively. (B) LCMV-specific CTLs are undetectable in AG129 mice after LCMV infection by a secondary CTL assay. Mice were infected 30 days previously with LCMV as indicated in figure. Splenocytes (4×10^6/ml) were restimulated with LCMV-infected or control macrophages (0.2×10^6/ml) in 1-ml cultures for 5 days. Cultures were diluted as indicated and tested for cytotoxicity on 10^4 LCMV-infected MC57G target cells in a 5-h assay. Killing is expressed as percent specific ^{51}Cr release of duplicate cultures. Lysis of uninfected MC57G cells was <5%. Lysis of infected MC57G cells by cells derived from cultures stimulated with uninfected macrophages was <10%. Spontaneous release from infected and uninfected targets was 15 and 13%, respectively.

previously with vaccinia virus. Doses and virus strains used are indicated in the results. Spleen cells were suspended at 7×10^6/ml in minimal essential medium supplemented with 2% fetal calf serum (FCS). MC57G (*H-2*^{b}) fibroblast target cells were infected with LCMV (multiplicity of infection = 0.01) 48 h or with vaccinia virus (multiplicity of infection = 5) 3 h before they were used as target. MC57G cells were labelled with 1 μCi of ^{51}Cr-sodium chromate for 2 h at 37°C (1×10^6 to 10×10^6 cells in 1 ml of minimal essential medium–2% FCS) and suspended at 10^5/ml. Threefold dilutions of spleen cells were incubated with 100 μl of targets in 96-well microtiter round-bottom plates for 5 h (LCMV) or 6 h (vaccinia virus). A total of 70 μl of the supernatant was assayed for released ^{51}Cr. The percent specific release of ^{51}Cr was calculated as [(experimental release − spontaneous release) × 100/(total release − spontaneous release)].

(ii) Secondary CTL assay. Spleen cells (4×10^6/ml) of mice infected with LCMV (at least 4 weeks ago) or with vaccinia virus (strain Lancy, 6 days ago) were restimulated in vitro in 1-ml cultures with virus-infected, thioglycolate-elicited macrophages (2×10^5/ml) in Iscove's modification of Dulbecco's modified medium supplemented with 10% FCS, 10^{-5} M β-mercaptoethanol, and antibiotics as described previously (19). In the case of vaccinia virus, the cultures were supplemented with 5% concanavalin A supernatant to support CTL proliferation and activation. After 5 days, cells were resuspended in 0.5 ml of minimal essential medium–2% FCS and 100 μl was tested in threefold dilutions on 10^4 ^{51}Cr-labelled targets as described above.

For a virus-independent secondary CTL assay, mice were primed i.v. with 10 μg of Baculo-VSV NP. Two weeks later, spleen cells were restimulated in 1-ml cultures as described above with irradiated VSV NP-transfected EL4 cells (N1) or with mock-transfected EL4 cells (V1) as control for 5 days. Undiluted cultures were assayed for cytotoxicity in a 5-h assay with ^{51}Cr-labelled (see above) V1 and N1 cells as targets.

Local immunopathology (footpad swelling). Mice were injected in both footpads of the hind legs with 30 μl of virus (indicated in Results) as described elsewhere (14, 16). Footpad thickness was measured daily from day 5 on with a spring-loaded caliper. Results are expressed as the percent increase compared with preinfection values. Data represent the mean of at least four footpads. Standard deviations were within 10%.

LCMV-specific antibodies. Antibodies were measured with an enzyme-linked immunosorbent assay (ELISA) as described previously (5). Briefly, ELISA plates were coated overnight with 10 ng of Baculo-LCMV NP in carbonate buffer (pH 9.6). Sera and peroxidase-labelled anti-mouse immunoglobulin G1 (IgG1), IgG2a, IgG2b, IgG3, and IgM (PharMingen) were diluted in 1% bovine serum albumin–phosphate-buffered saline (PBS). Mice were bled 4 weeks after infection. Results are expressed as the dilution required to obtain an optical density at 405 nm of 0.5 in a standard assay. All sera were tested in the same assay, and pooled serum from at least five mice was tested for each experimental condition.

Viral persistence. LCMV persistence was measured in spleens and livers of mice 20 days after infection with different doses of LCMV-WE or -ARM by a focus-forming assay on MC57G fibroblasts (4). Each value represents the mean of at least two mice. Standard deviations were within 15%. Vaccinia virus persistence was measured in lungs of mice 6 days after infection by a standard plaque assay on BSC40 cells (10).

RESULTS AND DISCUSSION

CTL responses to LCMV. In general, low doses of LCMV induce a potent CTL response peaking on days 8 to 10; high doses (>10^6 PFU) may induce all CTLs, which then are deleted (15). Primary ex vivo (Fig. 1A) CTL responses from mice infected with 100 PFU of LCMV-WE or -ARM were absent in AG129 mice, whereas splenocytes from similarly primed B6 mice killed LCMV-infected MC57G targets to a considerable extent. Infection with a high dose of LCMV (10^6 PFU) also did not lead to detectable cytotoxic activity in AG129 mice, whereas in control B6 mice the killing was even higher than that after infection with 100 PFU (data not shown). The relatively low rate of killing seen in control mice infected with 100 PFU of LCMV-ARM is due to the low replication of this virus isolate, compared with LCMV-WE, which leads to less extensive CTL priming. Also, secondary responses in AG129 mice primed with 10^2, 10^4, or 10^6 PFU of LCMV-ARM (Fig. 1B) or -WE (not shown) were undetectable. Together with virus persistence, this suggested complete exhaustion of antiviral CTL precursors (CTLp) even by low doses of a relatively slowly replicating virus (15).

Infection of G129 mice with various doses (10^2 to 10^6 PFU) of LCMV-WE or -ARM induced primary and secondary CTL responses which were comparable with those in infected control mice (data not shown and reference 10), whereas infection of A129 mice with LCMV did not induce any detectable CTL activity as was the case in AG129 mice (data not shown and reference 17). Therefore, the IFN-α/β R seems to play a more important role than the IFN-γ R in controlling virus replication, which in turn regulates the CTL response against LCMV (15).

CTL response to vaccinia virus. Primary ex vivo CTL responses after infection with the low-virulence vaccinia virus strain Lancy were measurable in control 129, A129, and G129 mice after infection with 2×10^6 PFU but not after infection with a much lower dose (2×10^2 PFU). In contrast, vaccinia virus-specific cytotoxic activity could also be detected in AG129 mice after infection with a low dose (2×10^2 PFU) of vaccinia virus Lancy (Fig. 2A), whereas the response after 2×10^6 PFU of vaccinia virus Lancy was lower compared with 129, A129, or G129 mice. Even in a secondary CTL assay (Fig. 2B), no CTLs were detected after priming with 2×10^2 PFU in 129, A129, or G129 mice, whereas they were present in AG129 mice. In immunocompetent mice (129) and in mice lacking only one type of IFN R (A129, G129), a dose of 200 PFU of vaccinia virus Lancy is apparently too low to prime. Infection

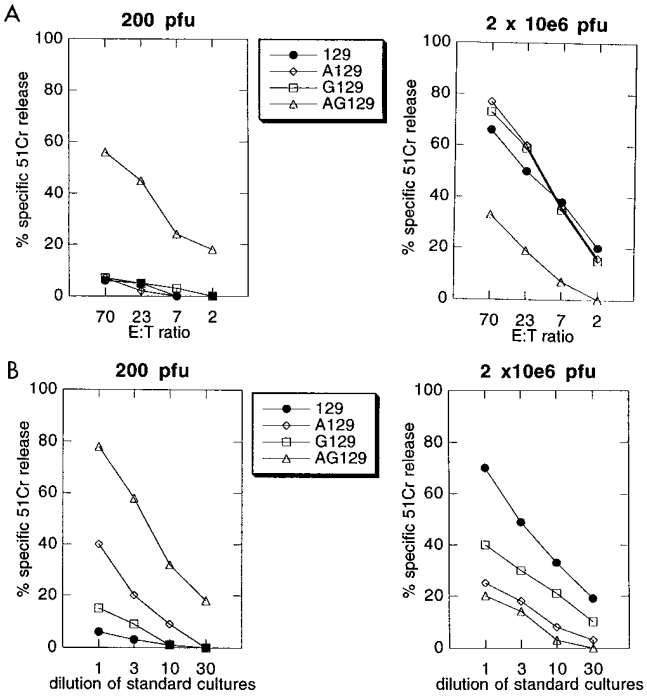

FIG. 2. (A) Vaccinia virus-specific CTLs were present in AG129 mice after infection with a low and a high dose of vaccinia virus Lancy by a primary ex vivo CTL assay. Mice were infected 6 days previously with the low-virulence vaccinia virus strain Lancy as indicated in figure. Splenocytes (E) were incubated with 10^4 vaccinia virus-infected, ^{51}Cr-labelled MC57G target cells (T) for 6 h. Killing is expressed as percent specific ^{51}Cr release of duplicate cultures. Killing of uninfected MC57G targets was <5%. Spontaneous release of infected and uninfected targets was 11 and 19%, respectively. (B) Vaccinia virus-specific CTLs are present in AG129 mice after infection with vaccinia virus Lancy by a secondary CTL assay. Mice were infected 6 days previously with vaccinia virus as indicated in the figure. Splenocytes (4×10^6/ml) were restimulated with vaccinia virus-infected (UV-inactivated) macrophages (0.2×10^6/ml) for 5 days in the presence of 5% concanavalin A supernatant. Cultures were diluted as indicated and tested for cytotoxicity on 10^4 vaccinia virus-infected MC57G targets in a 6-h assay. Killing is expressed as percent specific ^{51}Cr release from duplicate cultures. Lysis of uninfected MC57G cells was always <3%. Lysis of infected MC57G targets by cells derived from cultures stimulated with uninfected macrophages was <10%. Spontaneous release from uninfected and infected targets was 16 and 18%, respectively.

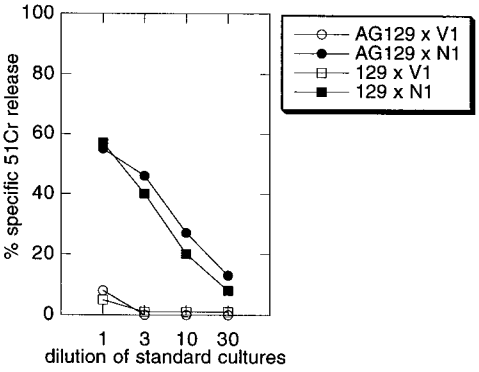

FIG. 3. Normal levels of CTLs are present in AG129 mice after priming with a nonreplicating agent. Mice were primed i.v. with 10 μg of Baculo-VSV NP. Two weeks later, splenocytes were restimulated (4×10^6/ml) with VSV NP-transfected (N1) or mock-transfected (V1) EL4 cells (0.2×10^6/ml) in 1-ml cultures for 5 days. Cultures were tested for cytotoxic activity on 10^4 ^{51}Cr-labelled N1 targets in a 5-h assay. Killing is expressed as percent specific ^{51}Cr release of duplicate cultures. Killing of V1 by N1- or V1-stimulated cultures was <20% (not shown); killing of N1 by V1-stimulated cultures was <5%. Spontaneous release of V1 and N1 targets was 17 and 18%, respectively. (AG)129 × V1 and (AG)129 × N1, spleen cells from (AG)129 mice restimulated with V1 or N1 in vitro.

with 2×10^6 PFU of vaccinia virus Lancy induced specific CTLs in all four strains tested. However, in all knockout mice the response was reduced compared with wild-type 129 mice. This may be explained by the fact that vaccinia virus persists in all knockout mice (Table 1) as follows: G129 < A129 < AG129. Because of this in vivo persistence, replicating vaccinia virus is transferred to the cultures set up for restimulating spleen cells in vitro and may interfere with restimulation. Under standard conditions, vaccinia virus used to infect stimulator cells in secondary cultures is UV inactivated to prevent this interference. The low primary ex vivo CTL response observed in AG129 mice after priming with 2×10^6 PFU of vaccinia virus may be explained by the virus-induced cytopathology, destroying among other things the integrity of secondary lymphoid organs. That this is observed in the double knockouts only is probably due to a higher replication rate of vaccinia virus compared with the wild type and single-knockout mice.

TABLE 1. Vaccinia virus persistence in wild-type and IFN $R^{-/-}$ mice[a]

Inoculum	PFU/lung			
	129	A129	G129	AG129
2×10^2	$<10^2$	1.0×10^5	$<10^2$	9.0×10^5
2×10^6	$<10^2$	5.5×10^5	1.0×10^3	2.0×10^6

[a] Mice were infected with indicated doses of vaccinia virus Lancy. Indicated are the number of PFU per lung 6 days after infection. The values represent the mean of two individual mice. Standard deviation was <15%. 129, wild type; A129, IFN-α/β $R^{-/-}$; G129, IFN-γ $R^{-/-}$; AG129, IFN-α/β and IFN-γ $R^{-/-}$.

CTL response to a nonreplicating agent. To evaluate whether AG129 mice were able to mount detectable CTL responses at all, we had to avoid the problem of exhaustion of CTL by overwhelming virus infection. Therefore, mice were primed with Baculo-VSV NP that has been shown to prime CTL responses in vivo in mice and their splenocytes were restimulated in vitro with VSV NP-transfected (N1) or mock-transfected (V1, control) EL4 cells. These cultures were subsequently tested for cytolytic activity against N1 and V1 targets. 129 and AG129 mice exhibited killing of N1 to a similar extent after restimulation with N1 in vitro (Fig. 3), as is true for A129 and G129 mice under similar conditions (data not shown). Thus, CTL responses per se are not defective in mice lacking one or both IFN Rs. Killing of N1 cells by V1-restimulated cultures was below 10% in 1×-diluted standard cultures (Fig. 3). Killing of control V1 cells by any of the cultures was always <20% (data not shown).

Local immunopathology induced by LCMV. Local inflammation resulting from infection into the foot with LCMV is mediated initially by activated $CD8^+$ cells (16). If B6 control mice were injected with different doses of LCMV-ARM (data not shown) or -WE (Fig. 4), a dose-dependent footpad swelling was observed. However, in AG129 mice no footpad swelling developed after injection of any of the viruses or doses, indicating rapid exhaustion of CTLp by rapidly replicating virus. Similar data had also been obtained previously with A129 mice (data not shown and reference 17), whereas G129 mice were normal in this respect (data not shown and reference 10). It has been shown (16) that both $CD4^+$ and $CD8^+$ cells contribute to local immunopathology. Our data therefore may suggest also a deleterious effect on $CD4^+$ cells in AG129 and A129 mice of

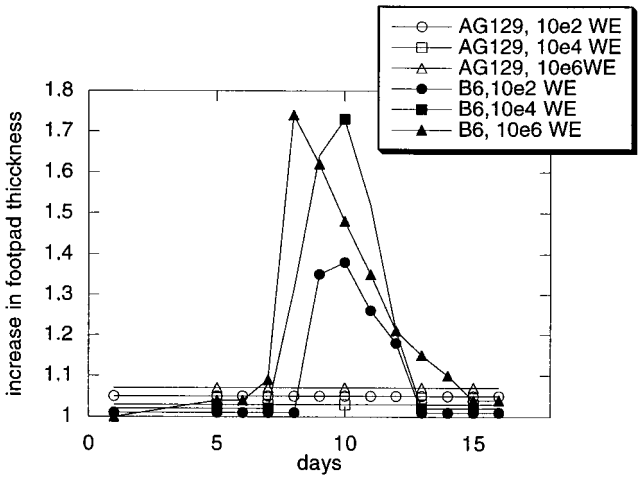

FIG. 4. Local, LCMV-induced, CD8-mediated immunopathology is absent in AG129 mice. Mice were injected in the footpad of both hind legs with LCMV as indicated in figure. Footpad swelling was measured daily from day 5 on. Results are expressed as fold increase compared with noninfected feet. Data represent the mean of four footpads; standard deviation was within 10%.

LCMV infection. However, two arguments are against this interpretation. First, exhausting conditions in immunocompetent mice ($>10^6$ PFU of LCMV-DOCILE in B6 mice) do not affect the function of LCMV-specific $CD4^+$ cells as measured by proliferation, B-cell help, and lymphokine production (24a), and second, perforin knockout mice which have a normal $CD4^+$ compartment do not display footpad swelling after local infection with LCMV. The apparent CD8 dependence of footpad swelling may be explained by the fact that viral persistence at a high level prevents immunopathology by exhausting $CD8^+$ T cells. This reduces freeing of cell internal viral antigen and therefore also the class II presentation of viral epitopes to $CD4^+$ cells.

Viral persistence. Viral persistence was measured in spleen and liver of mice infected 20 days previously with different doses of LCMV-ARM or -WE (Table 2). All control B6 mice had cleared the virus below detection level (titers of <100 PFU/g of tissue), whereas AG129 mice were unable to clear the virus. The rapidly replicating virus (LCMV-WE) led to higher virus titers in spleen compared with the somewhat more slowly replicating strain (LCMV-ARM). It has been shown (10) that G129 mice eliminated LCMV-WE from most tissues,

TABLE 2. LCMV persistence in IFN-α/β and IFN-γ $R^{-/-}$ (AG129) and control B6 mice[a]

LCMV strain (PFU)	PFU/g (10^4)			
	Spleen		Liver	
	B6	AG129	B6	AG129
WE (10^2)	$<10^2$	9.8×10^4	$<10^2$	1.8×10^4
WE (10^4)	$<10^2$	2.9×10^5	$<10^2$	8.0×10^3
WE (10^6)	$<10^2$	3.3×10^5	$<10^2$	2.7×10^4
ARM (10^2)	$<10^2$	5.8×10^4	$<10^2$	1.7×10^4
ARM (10^4)	$<10^2$	1.0×10^5	$<10^2$	2.1×10^4
ARM (10^6)	$<10^2$	5.0×10^3	$<10^2$	5.0×10^3

[a] Mice were infected with indicated doses of LCMV-ARM or -WE, and organs were ground and plaqued on MC57G fibroblasts 4 weeks later. Indicated are the number of PFU per gram of tissue. The values represent the mean of two individual mice. Standard deviation was always <15%.

TABLE 3. LCMV NP-specific antibodies: dependence of IgG isotype pattern on the presence of IFN Rs[a]

Ig	129	A129	G129	AG129
IgG1	800	1,600	51,200	51,200
IgG2a	6,400	6,400	3,200	<50
IgG2b	800	1,600	12,800	50
IgG3	<50	<50	<50	<50

[a] Results are given as the dilution needed to yield a signal of 0.5 in a standard ELISA. Results are obtained with pools of five serum samples per mouse strain. Mice were bled 4 weeks after infection with 200 PFU of LCMV-WE. 129, control; A129, IFN-α/β $R^{-/-}$; G129, IFN-γ $R^{-/-}$; AG129, IFN-α/β and IFN-γ $R^{-/-}$.

whereas A129 mice did not. Persistence of the low-virulence strain vaccinia virus Lancy that normally does not replicate measurably in C57BL/6 mice was measured in lungs of mice 6 days after infection in mutant mice (Table 1): wild-type 129 and G129 mice cleared the virus after infection with 2×10^2 PFU, whereas AG129 and A129 were not able to cope with this low dose. Infection with 2×10^6 PFU invariably led to persistence in all knockout mice. This is in agreement with our previous observations (10, 17) that G129 mice were able to cope with infection with the virulent strain vaccinia virus WR, whereas A129 mice succumbed to it. AG129 mice even died (>14 days) after infection with a high dose of vaccinia virus Lancy.

LCMV NP-specific antibodies. Antibodies were measured in sera from mice infected i.v. 4 weeks earlier with 100 PFU of LCMV-WE. No differences among 129, A129, G129, and AG129 mice with respect to total IgG anti-LCMV NP could be detected (not shown). When IgG isotypes were analyzed, however, clear differences were found (Table 3): compared with control 129 mice, both G129 and AG129 mice had significantly more virus-specific IgG1, whereas IgG2a levels were comparable in 129 and A129 mice and somewhat reduced in G129 mice but undetectable in AG129 mice. IgG3 was low in all four strains, and IgG2b was significantly enhanced in G129 mice. This suggests a role for IFN-γ in the downregulation of virus-specific IgG1 and IgG2b responses, whereas the lack of IFN-α/β apparently does not influence the subclass pattern significantly. However, if both types of IFN were lacking, the IgG2a and IgG2b responses were reduced to very low levels. Although IFN-γ has been implicated in promoting isotype switch to IgG2a (21), the absence of IFN-γ resulted only in a reduction, not the complete absence, of IgG2a (this study and reference 10). IFN-α/β has also been shown to enhance IgG2a secretion (8). It is interesting that the IgG2a production is abolished only when both IFN systems are inactive, suggesting a synergistic action for IFN-γ and IFN-α/β in promoting IgG2a production. In contrast, the enhanced IgG2b production in the absence of IFN-γ seems to be reversed if the receptor for IFN-α/β is absent.

Taken together, AG129 (IFN-α/β and IFN-γ $R^{-/-}$) mice display generally an additive phenotype of A129 (IFN-α/β $R^{-/-}$) and G129 (IFN-γ $R^{-/-}$) with respect to antiviral defense. As in A129 mice, CTLs and CTLp against LCMV are readily deleted in AG129 mice because of overwhelming virus replication and exhaustive CTL activation (14, 15). This results in a lack of virus-specific CTLp leading to lifelong viral persistence of LCMV. In vaccinia virus infection, both IFN Rs seem to be additive: IFN-α/β R is the most crucial but the IFN-γ R certainly contributes to anti-vaccinia virus control. In some viral infections, studied previously (17), the absence of a functional IFN-α/β and IFN-ω system proved to have a major

impact on antiviral defense, whereas no effect was observed when the IFN-γ system was deficient. The present findings illustrate that in some viral infections the two systems act cooperatively. This may particularly be relevant in cases in which viruses evolved mechanisms to escape IFN-α, IFN-β, and IFN-ω action (2, 6, 11, 22). Recent observations with Theiler's virus (7a) also illustrate the cooperation of the two systems and support the notion that the IFN-α, IFN-β, and IFN-ω system mainly inhibits early spread from the primary site of infection, whereas the IFN-γ system may play a more important role in latter stages of viral infection, notably in controlling persistence. Because of the virtual absence of innate defense which contributes to the subsequent exhaustion of acquired immune responses, mice with the combined deficiency of both IFN systems may provide unique conditions to isolate infectious agents that normally cannot escape immune surveillance.

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