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The In Vivo Antiviral Activity of Interleukin-12 Is Mediated by Gamma Interferon

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The injection of 20 ng of mouse interleukin-12 (IL-12) protects mice from a lethal infection with encephalomyocarditis virus. In vitro, an anti-gamma interferon (anti-IFN-γ) monoclonal antibody but not an anti-IL-12 monoclonal antibody neutralizes the antiviral activity present in the supernatants of splenocytes stimulated with IL-12. Finally, IL-12 fails to protect 129 Sv/Ev IFN-γR$^{0/0}$ mice against encephalomyocarditis virus infection. These results demonstrate that IL-12 exerts its antiviral activity through the induction of endogenous IFN-γ.

Interferons (IFNs) were described as cytokines that make cells resistant to viral infection. Both viral proteins and nucleic acids stimulate the production of alpha and beta IFNs (IFN-α and IFN-β) which are able to induce an antiviral state before the onset of immunity (4). Moreover, IFN-α and IFN-β were shown to activate NK cells and to promote the maturation of Th1 cells and in turn cell immunity (14). IFN-γ, produced by activated NK cells, Th1 cells, and cytotoxic T lymphocytes (CTL), protects cells from viruses and triggers the progression of immunity against virus-infected cells (10, 17, 18). Recently, low doses of interleukin-12 (IL-12) were shown to be active in vivo against lymphocytic choriomeningitis virus infections. The injection of this cytokine enhanced the CTL response against virus-infected cells, reduced viral spreading, and rescued the infected mice (11). IL-12 is produced by activated macrophages and is considered to be the link between the nonspecific immune response and adaptive immunity (16). It stimulates NK cells to release IFN-γ (2), induces Th1 cell and CTL proliferation, and promotes the progression of cell-mediated immunity (7, 8). In vivo, IL-12 is effective in the treatment of experimental tumors and intracellular parasite infections through a mechanism that is most probably mediated by IFN-γ (for a review, see reference 1). The in vivo activity of IL-12 against viral infections appears to be correlated with the enhancement of the CTL response against virus-infected cells (11). However, its capacity to protect cells from a viral cytopathic effect cannot be excluded. In this case, IL-12 may be exerting a direct effect on cells or inducing antiviral resistance by stimulating NK release of IFN-γ. In this report, we describe a model of viral infection in which the mice are protected by IFN treatment. We show that mice infected with a lethal dose of encephalomyocarditis (EMC) virus can be rescued by a single injection of IL-12 prior to infection and that IL-12 has no intrinsic antiviral activity but exerts its protective effect in mice through the induction of IFN-γ.

IL-12 protects C57BL/6 mice from lethal EMC virus infection. Recombinant IFN-γ (1.2×10^7 U/mg of protein), IFN-αA/D (9×10^7 U/mg of protein), and IL-12 (6.3×10^6 U/mg of protein) were produced by Hoffmann La-Roche. IFN-αA/D is a recombinant molecule with residues 1 to 62 from human IFN-αA and residues 64 to 166 from human IFN-αD which is active on both human and mouse cells (15).

C57BL/6 mice infected with a lethal dosage of EMC virus (ATCC VR-129B) die within 15 days (12). When pretreated 18 h before the infection with 10^3 U of either IFN-αA/D or IFN-γ, 90% of the mice survive (not shown). Pretreatment of the mice with IL-12 before the lethal viral infection also induced a protective effect with 100, 57, and 28% survival observed in groups of mice injected with 20, 10, and 1 ng of IL-12, respectively (Fig. 1). The antiviral activity of IFNs can be measured in vitro in a bioassay based on reduction of EMC virus cytopathic effect (6) on L929 cell monolayers (ATCC CCL 1). Since IL-12 receptors have been described only for T and NK cells (5), the lack of IL-12 antiviral activity we observed in this test (data not shown) is most probably due to the absence of IL-12 receptors on fibroblasts.

Splenocytes stimulated in vitro with IL-12 produce an antiviral activity that is neutralized by anti-IFN-γ. In order to determine whether we could induce the IL-12 antiviral activity that we observed in vivo also in an in vitro assay, C57BL/6 splenocytes were distributed in 48-well Costar plates (5×10^5 cells per well) in RPMI medium (RPMI 1640, 5% fetal calf serum) with or without IL-12. The plates were incubated at 37°C, and supernatants were collected from days 1 to 7. The antiviral activity produced in the supernatants was measured by using L929 fibroblasts infected with EMC virus and calculated by taking into account the dilution giving 50% protection against the virus and referring to an internal IFN-γ standard curve. In the presence of 0.1 ng of IL-12 per ml, the splenocyte supernatants protected fibroblasts from viral cytopathic effect (Fig. 2). This activity was detected after 2 days of culture and steadily increased until days 4 to 7, reaching levels comparable to 50 to 100 U of IFN-γ per ml. The antiviral activity of the supernatants did not increase upon elevation of the concentration of IL-12 to 10 ng/ml (not shown). In the absence of IL-12, no antiviral activity could be detected in the supernatants (not shown). In addition, when splenocytes were cocultured with IL-12 and C15.6.7, a monoclonal antibody (MAb) neutralizing IL-12 activity (13), a dose-dependent decrease of antiviral activity was measured in the supernatants after 7 days of culture (Fig. 3A). Under the same conditions, 100 μg of irrelevant rat immunoglobulin G (Sigma, St. Louis, Mo.) did not impair the production of antiviral activity. If the antiviral activity measured is an intrinsic property of IL-12, MAb

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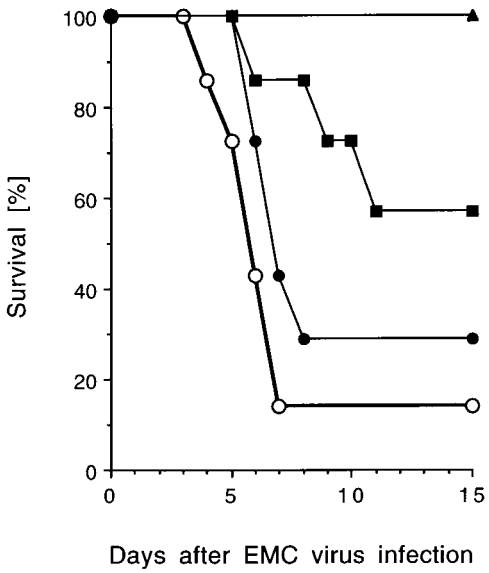

FIG. 1. Protection of C57BL/6 mice from lethal EMC virus infection with IL-12. Groups of seven to nine mice were injected with IL-12 18 h before the inoculation of the virus on day 0. The survival of mice treated with 1 (●), 10 (■), and 20 (▲) ng of IL-12 was compared with the survival of untreated mice infected with the virus only (○). The results of a representative experiment are expressed as percent survival. Three individual experiments were performed.

C15.6.7 should also neutralize the antiviral activity present in the supernatants of IL-12-stimulated splenocytes when assayed on fibroblast monolayers infected with EMC virus. To test this hypothesis, supernatants from IL-12-stimulated splenocytes were diluted to a final concentration of 5 antiviral units/ml, mixed with different concentrations of C15.6.7 MAb, and incubated with EMC virus-infected fibroblasts. Figure 3B shows that the anti-IL-12 MAb (up to 100 μg/ml tested) was unable to neutralize the antiviral activity, suggesting that a secondary mediator is responsible for the antiviral activity. Since IL-12 stimulates T and NK cells to produce IFN-γ, we tried to neutralize the IL-12-induced antiviral activity by using XMG1,2, a MAb directed against mouse IFN-γ (3). Supernatants of splenocytes stimulated with IL-12 in the presence of 100 ng of XMG1,2 MAb per ml for 7 days did not contain any detectable antiviral activity (Fig. 3A). Moreover, the anti-IFN-γ MAb (50% inhibitory concentration, 30 ng/ml) showed a 20-fold-higher inhibitory activity than the anti-IL-12 MAb (50% inhibitory concentration, 700 ng/ml) in this assay. Finally, the addition of 50 ng of XMG1,2 per ml to IL-12-stimulated supernatants in the antiviral assay completely abrogated the antiviral activity present in the supernatants (Fig. 3B). This inhibition was specific, as irrelevant rat immunoglobulin G did not neutralize this antiviral activity. These experiments show that IFN-γ is the factor responsible for the antiviral activity released by splenocytes after stimulation with IL-12.

IL-12 does not protect mice that lack IFN-γR from EMC virus infection. To demonstrate that the antiviral activity induced in vivo by the injection of IL-12 is mediated by the endogenous production of IFN-γ, we measured the capacity of IL-12 to protect 129 Sv/Ev IFN-γR$^{0/0}$ mice (9) from EMC virus infection. When infected with EMC virus only, 129 Sv/Ev IFN-γR$^{0/0}$ and 129 Sv/Ev wt (wild-type) control mice showed similar mortality curves. Figure 4 shows the results of a pool of two or three experiments in which mice were treated with 2,000 U of IFN-αA/D, 5,000 U of IFN-γ, or 50 ng of IL-12 18 h before infection with EMC virus. The 129 Sv/Ev IFN-γR$^{0/0}$ mice pretreated with IL-12 showed the same mortality curve as the untreated 129 Sv/Ev IFN-γR$^{0/0}$ mice infected with the virus. As expected, treatment with IFN-γ did not protect 129 Sv/Ev IFN-γR$^{0/0}$ mice, whereas IFN-αA/D was able to protect most of them from a lethal infection. In contrast, the 129 Sv/Ev wt mice were protected when treated with either IFN-αA/D, IFN-γ, or IL-12. The inability of IL-12 to protect 129 Sv/Ev IFN-γR$^{0/0}$ mice from viral infection may be due either to the absence of a response to IFN-γ or to an impaired response to IL-12. However, 129 Sv/Ev IFN-γR$^{0/0}$ and 129 Sv/Ev wt splenocytes produce similar levels of antiviral activity when stimulated in vitro with 0.1 ng of IL-12 per ml (Fig. 2), and both

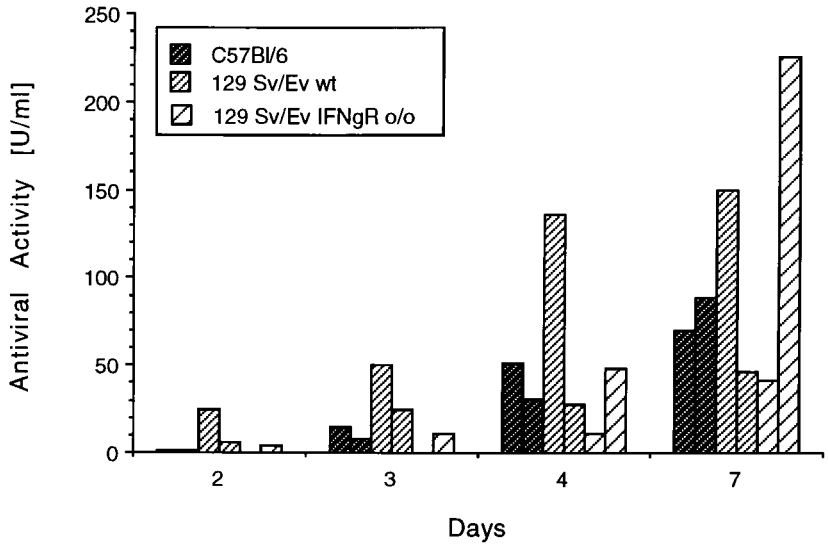

FIG. 2. Production of antiviral activity in the supernatant of C57BL/6, 129Sv/Ev wt, and 129 Sv/Ev IFN-γR$^{0/0}$ splenocytes activated in vitro with IL-12. Splenocytes (two mice per strain, tested separately) were cultured in the presence of IL-12 for up to 7 days. The supernatants of these cultures were tested for their capacity to protect mouse L929 fibroblasts from EMC virus cytopathic effect. The results are expressed in units of antiviral activity per milliliter of culture supernatant, with 1 U defined as the activity giving 50% protection from viral cytopathic effect.

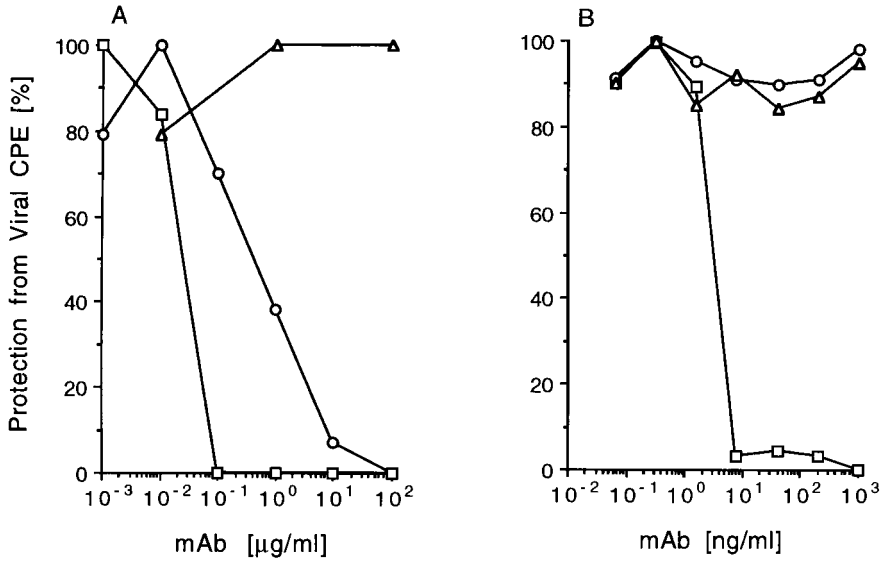

FIG. 3. Neutralization of the antiviral activity produced in vitro in the supernatants of IL-12-activated C57BL/6 splenocytes. (A) Splenocytes from C57BL/6 mice were stimulated for 7 days with 0.1 ng of IL-12-per ml in the presence of different concentrations of anti-IL-12 MAb (○), anti-IFN-γ MAb (□), or irrelevant rat immunoglobulin G (△). The supernatants of the cultures were tested for the presence of residual antiviral activity on mouse L929 fibroblasts infected with a lytic concentration of EMC virus. Results are expressed as percentages of the antiviral activity obtained in the absence of antibody. Both anti-IL-12 and anti-IFN-γ MAbs were able to inhibit the IL-12-induced antiviral activity. (B) Splenocytes from C57BL/6 mice were stimulated for 7 days with 0.1 ng of IL-12 per ml. The supernatants of the cultures were diluted to a final antiviral concentration of 5 U/ml and coincubated in the presence of different concentrations of MAbs on mouse L929 fibroblasts infected with a lytic concentration of EMC virus. Only anti-IFN-γ MAb (□), not anti-IL-12 MAb (○), neutralized the antiviral activity contained in the IL-12-stimulated supernatants. CPE, cytopathic effect.

antiviral activities can be neutralized by anti-IFN-γ MAb (not shown). Thus, the inability of 129 Sv/Ev IFN-γ$R^{0/0}$ mice to respond to IFN-γ determines why IL-12 cannot rescue these mice from lethal effects of EMC virus infection.

In conclusion, we have demonstrated that a single injection of IL-12 can protect mice from a lethal infection of EMC virus and that this antiviral activity is mediated by endogenous IFN-γ. We have also shown that IL-12 does not have any intrinsic antiviral activity in vitro but, rather, stimulates splenocytes to produce IFN-γ.

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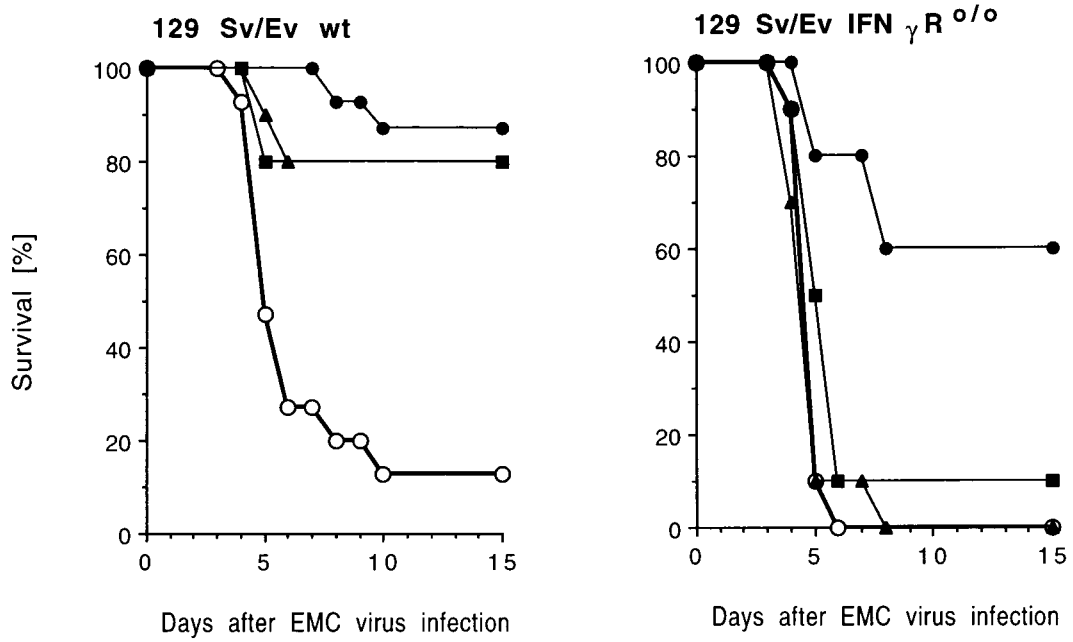

FIG. 4. Protection of 129 Sv/Ev IFN-γ$R^{0/0}$ and 129 Sv/Ev wt mice from lethal EMC virus infection. Mice were injected with 2,000 IU of IFN-αA/D (■), 5,000 IU of IFN-γ (●), or 50 ng of IL-12 (▲) 18 h before inoculation of the virus on day 0. The survival of mice treated with cytokine was compared with that of untreated mice infected with the virus (○). Data from two or three individual experiments for 129 Sv/Ev IFN-γ$R^{0/0}$ mice and 129 Sv/Ev wt mice, respectively, were pooled, with five mice per treatment group in each experiment. Results are expressed as percent survival.

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