IL-12 Stimulates an Antiviral Type 1 Cytokine Response but Lacks Adjuvant Activity in IFN-γ-Receptor-Deficient Mice

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Cytokines can be used as adjuvants to enhance and direct protective immune responses induced by vaccines. IL-12, a cytokine that favors the maturation of Th1-type cells and stimulates associated cell-mediated responses was evaluated as immunologic adjuvant for a viral vaccine in a mouse challenge model. When it was administered together with inactivated pseudorabies virus, a herpes simplex virus related α-herpesvirus, increased production of IFN-γ by ex vivo-stimulated splenocytes was observed as well as augmented production of antiviral serum IgG2a. This was associated with increased protection against a lethal challenge infection. Injection of IFN-γ-neutralizing Ab reduced the increased antiviral resistance in IL-12-treated mice. Also, in mice bearing an inactivated IFN-γ-receptor gene IL-12 failed to stimulate protection against challenge and the synthesis of antiviral IgG2a. However, in these IFN-γ-receptor knockout mice, increased antiviral IgG2b levels and enhanced IFN-γ secretion, with minimal IL-4 production, by ex vivo-stimulated splenocytes was observed. In wild-type mice administration of recombinant IFN-γ but not IL-2 mimicked the immune-stimulating activity of IL-12; it is therefore likely that the IL-12 adjuvant activity is largely mediated by physiologic IFN-γ. *The Journal of Immunology*, 1995, 155: 2525–2532.

Interleukin-12 is a cytokine produced by activated macrophages and B cells; it stimulates NK and T cells to produce IFN-γ, to proliferate and become cytolytic (see review in Ref. 1). This recently discovered cytokine is therefore promising as an adjuvant for vaccines against pathogens that require cell-mediated immune responses for protection, such as most intracellular parasites and viruses (2). Indeed, IL-12 can substitute for the bacterial adjuvant *Corynebacterium parvum* in a *Leishmania major* vaccine and incite protection-associated Th1 responses (3). In mice stimulated with goat anti-mouse IgD Ab, simultaneous injection of IL-12 increased Th1-type immune responses as evidenced by increased IFN-γ, inhibited IL-4 gene expression, and reduced production of IgG1 and IgE (4).

In the experiments reported here, we show that IL-12 injected at the time of vaccination increases immunity against a neurotropic herpesvirus (pseudorabies virus (PRV)[2]) infection of mice. PRV (synonyms are Aujeszky's disease virus and suid herpesvirus type 1) is an α-herpesvirus related to herpes simplex virus. It has a broad host range, including most domestic and wild animals. In the mouse, virulent PRV is highly neurotropic and produces lytic infections of cells in the central nervous system leading to fatal encephalitis. Immunization with inactivated PRV leads to protective immunity in mice (5); however, the exact mechanism of protection is unknown. In the present study we demonstrate that antiviral resistance augmented by IL-12 coincides with increased virus-specific IgG2a production; it is absent after anti-IFN-γ treatment and in IFN-γ-receptor-deficient mice but can be replaced by recombinant IFN-γ.

Materials and Methods

Mice

The mutant (129/Sv/Ev) mouse strain deficient in expression of the intact IFN-γ receptor (IFN-γ-$R^{-/-}$), generated by gene targeting in murine embryonic stem cells (5), was kindly provided by Dr. M. Aguet (Genentech Inc., San Francisco, CA). These mice develop a normal immune response, possess IFN-γ-independent macrophage and NK cell activity, and constitutively express MHC Ag. However, they lack a functional

Received for publication April 25, 1995. Accepted for publication June 20, 1995.

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[2] Abbreviations used in this paper: PRV, pseudorabies virus; IFN-γ-$R^{-/-}$, IFN-γ-receptor deficient; $TCID_{50}$, 50% tissue culture infective dose; pfu, plaque-forming unit.

0022-1767/95/$02.00

IFN-γ system (6, 5, 7). In the experiments we used IFN-γ-$R^{-/-}$, wild-type 129/Sv/Ev mice, or C57BL/6 mice of both sexes (purchased from the breeding facilities of the Central Animal Laboratory, Utrecht). All animals were housed in filter top cages and immunized at 4 to 5 wk of age. The animal experiments had been approved by the Institutional Animal Welfare Committee.

Virus

Virulent wild-type PRV (strain NIA-3), used for challenge infections, was obtained from the Central Veterinary Institute (Lelystad, The Netherlands). A PRV preparation inactivated by binary ethyleneimine (antigenic mass corresponding to $10^{8.8}$ 50% tissue culture infective dose ($TCID_{50}$)/ml) was kindly provided by Dr. N. Visser (Intervet International, Boxmeer, The Netherlands). Inactivation was confirmed by the absence of cytopathic effect and of viral Ag expression (tested by indirect immunofluorescence) in BHK cells, and by inoculation of the vaccine preparation into rabbits.

Experimental protocol

On day 0 groups of 6 to 10 mice were immunized i.p. with inactivated PRV in conjunction with either IL-12 or IFN-γ, with PBS serving as a control. At day 7 the spleens were removed and splenocyte single cell suspensions were tested for cytokine production. Other groups of mice were bled from the retro-orbital plexus at day 27 and their sera analyzed for virus-specific IgG1 and IgG2a. At day 28 all mice were challenged with 250 plaque-forming units (corresponding to 250 LD_{100} units) of virulent PRV via the i.p. route. This challenge infection produces infections of cells of the central nervous system, leading to fatal encephalitis within 4 to 5 days.

Cytokines

Murine rIL-12 (lot MRB 021893-3.2) was kindly provided by Dr. S. Wolf (Genetics Institute, Inc., Cambridge, MA). It was expressed from cloned cDNAs and had a specific activity of 5.5×10^6 U/mg. Endotoxin contamination, as measured in the *Limulus* amebocyte assay, was 11.4 to 22.7 EU/mg IL-12. Rat rIFN-γ (4×10^6 U/mg), which displays activity in both rats and mice (8), was kindly provided by Dr. P. H. Van der Meide (ITRI-TNO, Rijswijk, The Netherlands). Per mouse either 1 μg (i.e., 5.5×10^3 U) IL-12 or 0.25 μg (i.e., 10^3 U) IFN-γ as a dilution in 250 μl PBS was injected 15 to 90 min before immunization. The choice of these cytokine doses was based on previous studies reporting in vivo adjuvant activity of IL-12 (3) and IFN-γ (9) in other model systems.

PRV-specific serum Ab isotypes

Levels of virus-specific IgG1 and IgG2a, which are normally associated with IL-4 and IFN-γ production, respectively (10), were determined in an ELISA; 96-well flat-bottom plates were incubated overnight at 4°C with inactivated PRV in $NaHCO_3$ (0.05 M, pH 9.6), washed with tap water, and saturated with 1% BSA (Sigma Chemical Co., St. Louis, MA) in PBS. Twofold serum dilutions (in PBS containing 0.05% Tween-20 and 0.1% BSA; 100 μl/well) were added and incubated for 1 h at 37°C. After washing with tap water, a 1:6400 dilution of isotype-specific peroxidase-conjugated goat anti-mouse Ig (Southern Biotechnology Associates Inc., Birmingham, AL) was added. After incubation for 1 h at 37°C and another wash, the substrate was developed with tetramethylbenzidine for 10 min at room temperature. The reaction was stopped with 2 M sulphuric acid and read at 450 nm in a Titertek Multiskan MC. The titer was defined as the reciprocal of the highest dilution with an absorbance twice that of the background value.

In vitro analysis of cytokine production

For the analysis of cytokine production, splenocytes (1.5×10^6 cells/ml) were cultured in 24-well plates (Nunc, Breda, The Netherlands) and stimulated with PRV Ag (5–50 μg/ml) or Con A (5 μg/ml). Supernatants of stimulated cultures were harvested at 48 h and stored at −20°C until use. IFN-γ and IL-4 were measured by two-site ELISA (Holland Biotechnology, Leiden, The Netherlands and PharMingen, San Diego, CA) using standard curves established with known amounts of murine rIFN-γ (kindly provided by Dr. H. Heremans, Leuven, Belgium) and rIL-4 (Genzyme Corp., Cambridge, MA).

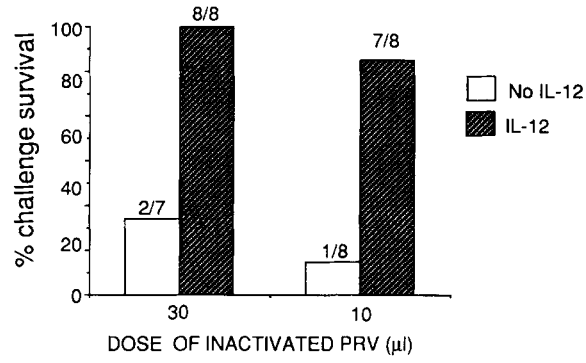

FIGURE 1. Effect of IL-12 on protection by inactivated PRV. C57BL/6 mice (7–8 per group) were immunized i.p. with 30 or 10 μl inactivated PRV (antigenic mass equivalent to $10^{8.8}$ $TCID_{50}$/ml); 60 to 90 min before immunization the animals had been injected i.p. with 1 μg (5.5×10^3 U) IL-12/mouse or PBS. The animals were challenged i.p. with 250 pfu of virulent PRV at day 28. Columns indicate the percentage of challenge survivors (actual numbers on top).

Statistical analysis

Evaluation of statistical differences between data obtained from mutant and wild-type mice was performed by using the Wilcoxon-Mann-Whitney test.

Results

IL-12 stimulates vaccination-induced protection against PRV infection

Virulent PRV is highly neurotropic and produces lytic, often fatal infections; however, a single immunization with inactivated PRV can protect mice in a dose-dependent way against a lethal challenge infection (9). To study the effect of IL-12, groups of mice were immunized with low protective doses of inactivated PRV and additionally injected with either IL-12 or PBS. The mice that had received IL-12 showed a distinct increase in survival upon challenge infection (Fig. 1): rates between 87 and 100% were observed, whereas 12 to 28% percent was noted among the control mice, receiving 10 or 30 μl vaccine respectively. IL-12 injection without immunization did not protect against PRV challenge (results not shown).

Effect of IL-12 on antiviral IgG responses

Resistance against PRV correlates with the level of circulating virus-specific Ab (5). We therefore measured antiviral IgG-isotype responses in the sera of IL-12 treated mice at day 27 after immunization, a point in time when peak Ab titers are found (Schijns, unpublished data). Figure 2 shows a 13- to 96-fold increase of antiviral IgG2a ($p = 0.001 - 0.0008$), and to a lesser extent (4- to 6-fold)

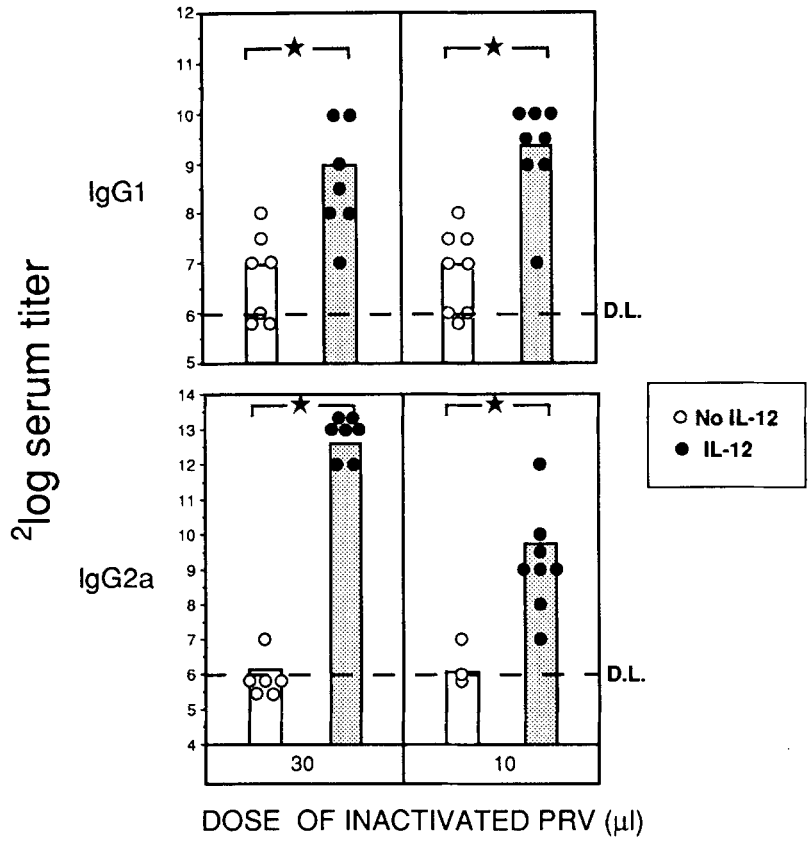

FIGURE 2. PRV-specific serum IgG1 and IgG2a titers in C57BL/6 mice injected with IL-12 or PBS. The animals were immunized with either 30 or 10 µl inactivated PRV (antigenic mass equivalent to $10^{8.8}$ $TCID_{50}$/ml); 60 to 90 min before immunization the animals had been injected i.p. with 1 µg (5.5×10^3 U) IL-12/mouse (●) or PBS (○). ELISA titers on day 27 are shown for individual mice and have been determined by twofold serial dilutions. Mean values per group are represented by the hatched (IL-12 treated) and open (PBS injected) columns. Asterisks indicate significant differences (for p values see text).

of IgG1 ($p = 0.003 - 0.008$). The dominance of virus-specific IgG2a production indicates a stimulatory effect of IL-12 on endogenous IFN-γ activity (10).

Effect of IL-12 on virus-specific lymphokine production

To determine the effect of IL-12 on endogenous antigen-specific cytokine production, we isolated spleen cells (at day 7 after immunization) from mice that had received the vaccine together with IL-12. The splenocytes were stimulated with PRV Ag (50 µg/ml), and 48 h later their supernatants were harvested for analysis of IFN-γ and IL-4 production. Cultures derived from mice that had been vaccinated without IL-12 secreted marginal amounts (≤1 U/ml) of IFN-γ while those from the IL-12-treated animals produced up to 11.5 U/ml. Ag stimulated cultures derived from mice treated with IL-12 alone, and naive animals produced nondetectable amounts of IFN-γ (<1 U/ml).

Specifically stimulated splenocytes isolated from naive animals and mice immunized without IL-12 produced no detectable IL-4 (<0.5 U/ml). Splenocytes derived from IL-12-treated immunized mice produced minimal amounts of IL-4 (0.9 ± 0.1 U/ml; not shown).

Adjuvant activity of IL-12 is absent in anti-IFN-γ-treated and IFN-γ-R-deficient mice

To examine the significance of endogenous IFN-γ for the observed enhancement of protective immunity, we tested IL-12 adjuvant activity in mice receiving the well-characterized (11, 12) IFN-γ-neutralizing mAb DB-1 (5×10^3 neutralizing U/mouse shortly before immunization). A single injection of DB-1 abrogated the increased resistance against PRV in IL-12-treated mice (Fig. 3). IL-12 adjuvant activity was also absent in IFN-γ-$R^{-/-}$ mice, which have a nonfunctional IFN-γ system (5); normal 129/Sv/Ev mice served as a control (Fig. 4). A dose of inactivated PRV, which protected 3 of 7 wild-type mice (42%), protected 5 of 6 mice (83.6%) when IL-12 was coadministered. However, the same vaccination regime protected only 2 of 8 (25%) mutant IFN-γ-$R^{-/-}$ mice. Similarly, in a second experiment a vaccine dose protecting 5 of 10 wild-type mice and 4 of 10 IFN-γ-$R^{-/-}$ mice protected in conjunction with IL-12 9 of 10 wild-type but only 5 of 10 mutant mice (see Table I). Together, these data indicate that the IL-12 effect is largely IFN-γ dependent.

The impaired antiviral resistance in IFN-γ-$R^{-/-}$ mice could be explained by the requirement of IFN-γ for effector mechanisms operative after challenge infection. However, injection of 1 mg/mouse of IFN-γ-neutralizing mAb

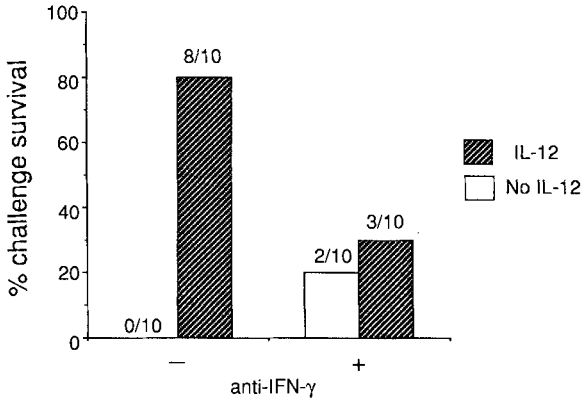

FIGURE 3. Effect of IL-12 on protection by inactivated PRV in mice treated with IFN-γ-neutralizing Ab. C57BL/6 mice (10 per group) were i.p. immunized with 10 μl inactivated PRV (antigenic mass equivalent to $10^{8.8}$ $TCID_{50}$/ml); 90 min before immunization the animals had been i.p. injected with 1 μg (5.5×10^3 U) IL-12/mouse or PBS injected. Immediately before IL-12 injection some mice were treated with IFN-γ-neutralizing mAb DB-1 (5000 neutralizing U/mouse). The animals were i.p. challenged with 250 pfu of virulent PRV at day 28. Columns indicate the percentage of challenge survivors (actual numbers on top).

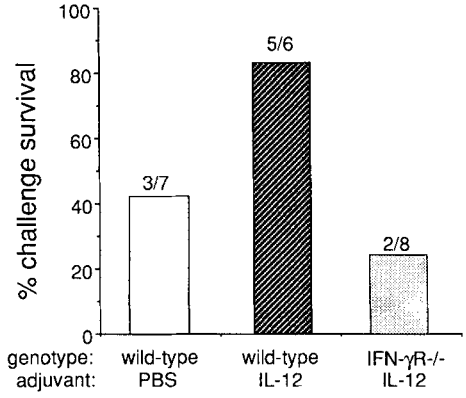

FIGURE 4. Effect of IL-12 on protection by inactivated PRV in IFN-γ-$R^{-/-}$ mice. Wild-type 129/Sv/Ev or mutant IFN-γ-$R^{-/-}$ mice (6–8 per group) were i.p. immunized with 10 μl inactivated PRV (antigenic mass equivalent to $10^{8.8}$ $TCID_{50}$/ml); 90 min before immunization the animals had been i.p. injected with 1 μg (5.5×10^3 U) IL-12/mouse or PBS injected. The animals were i.p. challenged with 250 pfu of virulent PRV at day 28. Columns indicate the percentage of challenge survivors (actual numbers on top).

Table I. *Effect of IL-12 on antiviral IgG isotype responses and challenge resistance in IFN-γ$^{-/-}$ mice*[a]

Genotype	Adjuvant IL-12	Serum Anti-PRV Titer[b] IgG1	IgG2a	IgG2b	IgG3	No. Survivors/No. Infected Mice
IFN-γ-$R^{+/+}$	−	142 ± 27	486 ± 66	1718 ± 250	770 ± 177	5/10[c]
IFN-γ-$R^{+/+}$	+	86 ± 19	1910 ± 372*	2635 ± 569	1164 ± 264	9/10
IFN-γ-$R^{-/-}$	−	752 ± 137*[d]	137 ± 42*	2503 ± 341	136 ± 35*	4/10
IFN-γ-$R^{-/-}$	+	917 ± 250*	157 ± 27*	5847 ± 304*	235 ± 117*	5/10

[a] Wild-type 129/Sv/Ev or mutant IFN-γ-$R^{-/-}$ mice (10 per group) were i.p. immunized with 10 μl inactivated PRV (antigenic mass equivalent to $10^{8.8}$ $TCID_{50}$/ml); 90 min before immunization the animals had been i.p. injected with 1 μg (5.5×10^3 U) IL-12/mouse or PBS injected. The animals were i.p. challenged with 250 pfu of virulent PRV at day 28.

[b] ELISA titers of day 27 serum anti-PRV IgG isotypes were determined for individual mice. Mean values ± SEM per group are given.

[c] The animals were i.p. challenge infected with 250 pfu of virulent PRV at day 28 after immunization.

[d] An asterisk indicates a p value of <0.05 when compared with responses obtained in untreated wild-type groups given PRV vaccine only.

DB-1 at 30 min before challenge infection did not affect protection elicited by a high dose of inactivated PRV (5).

IL-12 fails to increase antiviral IgG2a responses in IFN-γ-$R^{-/-}$ mice

The involvement of endogenous IFN-γ in IL-12-modulated antiviral IgG responses was determined by analysis of day 27 sera of immunized IFN-γ-$R^{-/-}$ mice. In these animals, IL-12 stimulated IgG2b production (Table I) but had no stimulatory effect ($p = 0.002$) on IgG2a production (Fig. 5 and Table I). As compared with wild-type mice, IgG1 responses were increased ($p = 0.001$) in IFN-γ-$R^{-/-}$ mice. However, in both wild-type and mutant mice there was no difference in the amounts of IgG1 produced by animals that had received IL-12 vs those that had not (Table I).

Administration of IL-12 to IFN-γ-$R^{-/-}$ mice enhances IFN-γ production in vitro by bulk stimulated splenocytes

Mice lacking the IFN-γ receptor generate a Th1 characteristic cytokine profile after infection with PRV (5) or *L. major* (7). We determined the effect of IL-12 on endogenous IFN-γ and IL-4 production in IFN-γ-$R^{-/-}$ mice. Figure 6 shows that specific stimulation of IFN-γ-$R^{-/-}$ splenocytes from IL-12-treated animals resulted in a 30-fold increase of IFN-γ production (30.5 U/ml) as compared with cultures derived from untreated immunized mice (1.2 U/ml). Thus, IL-12 is able to enhance type 1 immune responses independent of IFN-γ. No IL-4 (<0.5 U/ml) was detected in the supernatants of specifically stimulated splenocytes isolated from IFN-γ-$R^{-/-}$ mice immunized without IL-12 or naive animals. Similarly,

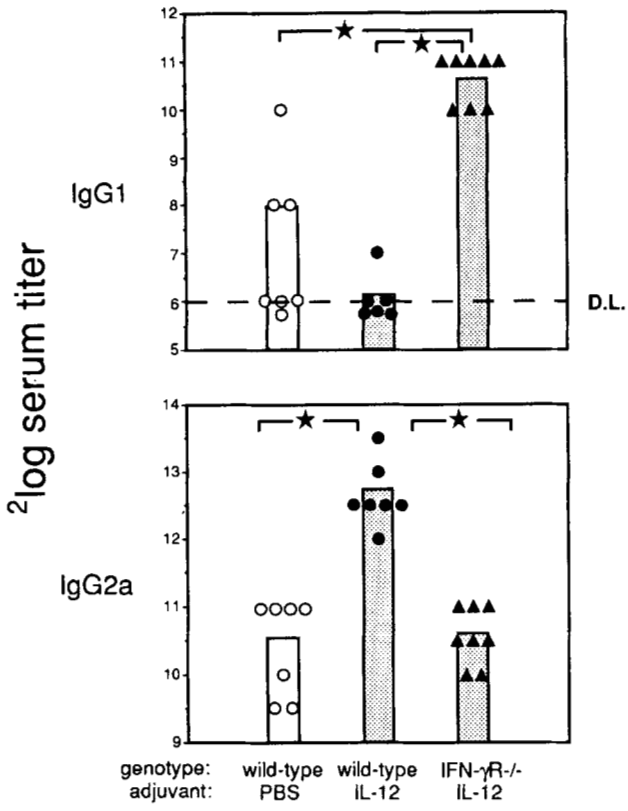

FIGURE 5. PRV-specific serum IgG1 and IgG2a titers in IL-12-injected wild-type and IFN-γ-R$^{-/-}$ mice. The animals were immunized with 10 μl inactivated PRV (antigenic mass equivalent to $10^{8.8}$ $TCID_{50}$/ml); 60 to 90 min before immunization the animals had been i.p. injected with 1 μg (5.5×10^3 U) IL-12/mouse (● and ▲) or PBS injected (○). ELISA titers on day 27 are shown for individual mice and have been determined by twofold serial dilutions. Mean values per group are represented by the hatched (IL-12 treated) and open (PBS injected) columns. Asterisks indicate significant differences (for *p* values see text).

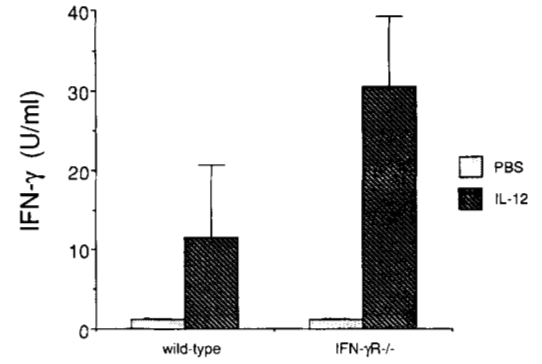

FIGURE 6. In vitro IFN-γ production by splenocytes derived from IL-12-treated wild-type and IFN-γ-R$^{-/-}$ mice. Splenocytes were isolated from mice immunized 7 days before with $0.2 \times 10^{8.8}$ $TCID_{50}$ inactivated PRV together with PBS or 1 μg (5.5×10^3 U) IL-12/mouse. They were cultured for 48 h in the presence of 50 μg/ml of PRV Ag and their supernatants harvested for IFN-γ detection. Mean cytokine concentrations ± SEM in duplicate cultures of 3 to 4 mice per group are shown. Similar responses were noted after stimulation with 5.0 μg/ml of PRV Ag.

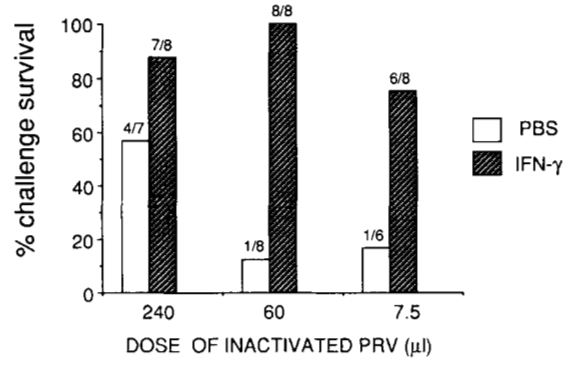

FIGURE 7. Effect of IFN-γ on protection by inactivated PRV. C57BL/6 mice (6–8 per group) were i.p. immunized at day 0 with the indicated volumes of inactivated PRV (antigenic mass equivalent to $10^{8.8}$ $TCID_{50}$/ml); 30 min before immunization the animals had been i.p. injected with 0.25 μg (10^3 U) IFN-γ/mouse or PBS injected. The animals were i.p. challenged with 250 pfu of virulent PRV at day 28. Columns indicate the percentage of challenge survivors (actual numbers on top).

there was no increase in the amounts of IL-4 produced by splenocytes derived from IL-12-treated immunized wild-type (0.9 ± 0.1 U/ml) or IFN-γ-R$^{-/-}$ (0.8 ± 0.1 U/ml) mice (not shown). Upon Con A stimulation (5 μg/ml), similar levels of IL-4 (4–5 U/ml) and IFN-γ (50–70 U/ml) were present in culture supernatants from both immune and naive mice of either genotype isolated from either IL-12 or control-treated animals (data not shown). These observations suggest that exogenous IL-12 stimulates Th1-type responses both in wild-type and IFN-γ-R$^{-/-}$ mice.

IFN-γ mimics the adjuvant effect of IL-12

The lack of effect in anti-IFN-γ-treated and IFN-γ-R$^{-/-}$ mice suggested that IL-12 exerts its potentiating activity via endogenous IFN-γ. To determine whether IFN-γ could replace IL-12, groups of mice were immunized with low doses of inactivated PRV and injected with IFN-γ (10^3 U/mouse) or PBS. In the controls receiving 7.5 or 60 μl vaccine, only few animals per group (1/6 to 1/8, respectively) survived, while immunization in conjunction with IFN-γ protected most (6/8) or all (8/8) mice, respectively, against lethal challenge without disease signs (Fig. 7). A dose of 10^4 U IFN-γ had similar effects (data not shown). Mice receiving IFN-γ without immunization all succumbed to the challenge infection (data not shown). Analysis of the Ab responses measured in sera taken at one day before challenge revealed significantly increased levels of

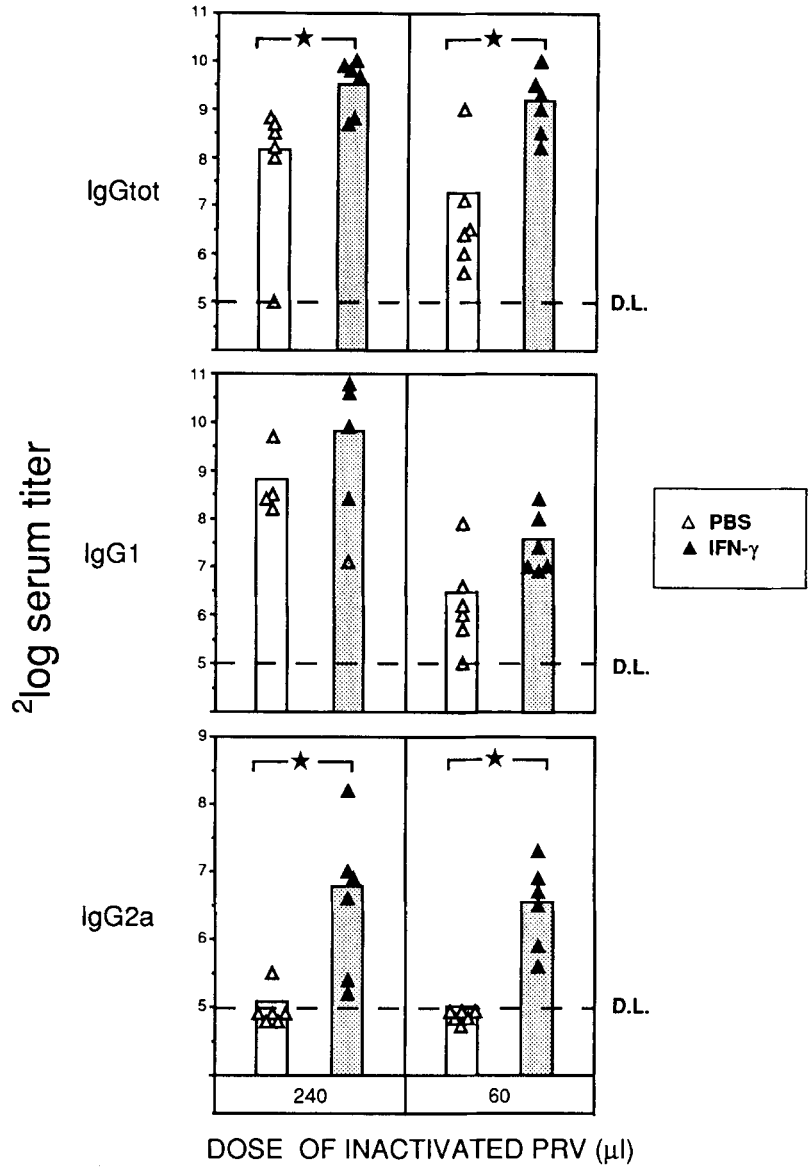

FIGURE 8. PRV-specific serum IgG (total), IgG1, and IgG2a titers in IFN-γ-injected or PBS-injected mice. The animals were immunized with either 240 or 60 μl inactivated PRV (antigenic mass equivalent to $10^{8.8}$ $TCID_{50}$/ml); 30 min before immunization the animals had been i.p. injected with 0.25 μg (10^3 U) IFN-γ/mouse (▲) or PBS injected (△). ELISA titers on day 27 are shown for individual mice and have been determined by twofold serial dilutions. Mean values per group are represented by the hatched (IFN-γ treated) and open (PBS injected) columns. Asterisks indicate significant differences (for p values see text).

total IgG (heavy and light) and IgG2a (p = 0.01 and 0.002, respectively) (Fig. 8), a pattern similar to that of the IL-12-treated mice (Fig. 2). Levels of IgG1, IgG2b, and IgG3 were not significantly increased (Fig. 8 and not shown). The IFN-γ effects were contrasted by those observed in mice receiving IL-2 (3×10^4 U/mouse daily on days 0–2) that showed no enhanced immunity to PRV infection and no increased antiviral Ab production (not shown).

Discussion

When IL-12 was included in a vaccine against the neurotropic pseudorabies herpesvirus, this resulted in a strong enhancement of protection against challenge with virulent PRV. Since cytokines have pleiotropic effects, it is difficult to exactly define the activity in the cascade of cellular events leading to antiviral protection by IL-12. Although MHC class I-restricted presentation during the induction phase cannot be excluded, the Ags of the inactivated PRV vaccine are probably processed by the endocytotic pathway and presented to $CD4^+$ T cells by MHC class II molecules. The presence of IL-12 during Ag presentation has been shown to favor the expansion of Th1-type $CD4^+$ T cells, which are characterized by IFN-γ production (1, 3, 4). In line with these observations, IL-12 treatment stimulated PRV-specific IFN-γ production by splenocytes isolated from both IFN-γ-$R^{-/-}$ and wild-type mice. However, the requirement of IFN-γ for IL-12 to mediate its effects remains controversial (13). In mice injected with anti-IgD Ab the IL-12 induced inhibition of IL-4 production and reduced IgG1 and IgE secretion was reversed by anti-IFN-γ Ab (4). This observation is consistent with the

finding that anti-IFN-γ blocks the inhibitory effects of IL-12 on IgE secretion in mice infected with the nematode parasite *Nippostrongylus brasiliensis* (14). By contrast, IL-12 has been shown to inhibit IgE synthesis in vitro in the presence of neutralizing anti-IFN-γ mAb (15). Furthermore, anti-IFN-γ failed to inhibit the IL-12 enhanced priming for IFN-γ production in an accessory-cell dependent priming system using TCR transgenic $CD4^+$ T cells (16). Interestingly, Ag-stimulated immune splenocytes from IL-12-treated IFN-γ-$R^{-/-}$ mice produced high levels of IFN-γ but only marginal amounts of IL-4 similar to splenocytes isolated from IL-12-treated wild-type mice. This observation suggests that in IFN-γ-$R^{-/-}$ mice the shift towards a Th2 cytokine pattern does not occur after IL-12 treatment. This may be due to the Th1-type commitment of this mouse strain (5, 7) and/or to the dominance of IL-12 to maintain Th1 responses and suppress Th2 reactions, even in IFN-γ-system-deficient (IFN-γ-$R^{-/-}$) mice. Indeed, the in vivo induction of hapten-protein conjugate-specific Th1 cells by IL-12 has been shown not to be affected by anti-IFN-γ Abs (17). The IFN-γ dependence of IL-12 adjuvant activity in the present model is suggested by the increased virus-specific IgG2a levels and by the lack of enhanced protective immunity and reduced PRV-specific IgG2a in anti-IFN-γ mAb-treated and IFN-γ-R-deficient mice. Furthermore, exogenous IFN-γ was able to replace IL-12 to induce increased antiviral IgG2a and challenge resistance.

Although induced Th1 cells preferentially activate cell-mediated responses, we observed that resistance to challenge infection was associated with a specific Ab response. Also, passive immunization of naive mice provided complete protection against an otherwise lethal PRV infection (5). Furthermore, immunization of B cell deficient (μMT/μMT) mice, which are unable to generate virus-specific Abs (18) but which can develop virus-specific T cell responses (19), failed to protect them against PRV (manuscript in preparation). In C57BL/6 mice IL-12 induces a 13- to 96-fold increase of antiviral IgG2a and, to a lesser extend (4- to 6-fold), IgG1. Such an increase in IgG1 was not observed in 129/Sv/Ev mice, which can be explained by the genetic background differences between both strains. In particular, the IFN-γ-associated IgG2a isotype is likely involved in protection since in IFN-γ-$R^{-/-}$ mice high IgG1 and IgG2b levels in the presence of low IgG2a could not prevent neurologic disease and mortality. We therefore speculate that the cytokine-augmented protection includes Ab-mediated virus neutralization by complement- or cell-dependent cytotoxicity, functions predominantly mediated by IgG2a, the major complement- and Fc-receptor-binding Ig isotype.

Previous studies have demonstrated that IL-12 alone and IFN-γ in conjunction with *C. parvum* increase the protective capacity of soluble *Leishmania* Ag (3, 20) requiring cell-mediated effector mechanisms for protection. In the present study we show that both cytokines alone can act as adjuvants for an inactivated herpesvirus vaccine, which results in an Ab (IgG2a)-associated protection. A similar observation has been made when IFN-γ was included in an inactivated rabies vaccine (9). These findings emphasize the importance of the type-1 cytokines IL-12 and IFN-γ in the generation of Ab-dependent antiviral immunity. Further studies on the mechanism of action, efficacy, and toxicity of IL-12 in vivo will be important in evaluating its potential as an adjuvant for human and veterinary vaccines.

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