Interleukin-12 Exhibits Potent Antiviral Activity in Experimental Herpesvirus Infections

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The prophylactic and therapeutic efficacy of interleukin-12 was studied by using murine models of herpes simplex virus infection. Prophylactic administration consisted of two intraperitoneal doses of interleukin-12 given 48 and 24 h prior to infection. Therapeutic intraperitoneal administration of interleukin-12 commenced 6 h after the mice were infected with herpes simplex virus and was continued daily for a total of 5 days. Interleukin-12 therapy improved the survival rates of mice with systemic herpes simplex virus infection compared with those of placebo-treated infected mice. Subcutaneous administration of interleukin-12 also improved the rate of survival of mice after systemic herpes simplex virus infection, although higher doses were required to give comparable effects. Combined prophylactic and therapeutic administration of interleukin-12 produced the greatest effect on survival after an otherwise lethal systemic infection. Intraperitoneal administration of interleukin-12 for 2 days before and 3 days after systemic infection with herpes simplex virus resulted in survival of 80% of the mice. These surviving mice were resistant to subsequent reinfection with herpes simplex virus. Such resistance was apparently specific for herpes simplex virus infection, since a second group of survivors succumbed to a lethal infection with murine cytomegalovirus. Infectious virus was recovered from lumbar ganglia explants dissected from survivors of prophylactic interleukin-12 therapy and cultured for 5 days in vitro, suggesting that interleukin-12 treatment did not prevent the establishment of latent herpes simplex virus infection. One action of interleukin-12 may be to enhance natural killer cell-mediated clearance of the virus. However, interleukin-12 therapy was also effective in mice carrying the *beige* mutation, which reduces natural killer cell lytic activity, suggesting that interleukin-12 has additional activities in vivo.

Interleukin-12 (IL-12) is a recently described cytokine (12, 28), which is produced by antigen-presenting cells such as B lymphocytes, macrophages and dendritic cells and has multiple activities (29). It promotes natural killer (NK) cell activity (5), reduces immunoglobulin E production, and enhances cytotoxic T-lymphocyte (CTL) maturation (3). In addition, it appears to play a critical role in the production of gamma interferon (IFN-γ) in vivo (12), and in inducing the maturation of type 1 T-helper (T_{H1}) cells from uncommitted type 0 T-helper (T_{H0}) cells (8). In view of this spectrum of activity, IL-12 can be considered a potent stimulator of cell-mediated immunity (CMI), and since CMI is important in the clearance of many viruses from the host, IL-12 has the potential to act as a therapy for this class of infection.

Several studies have demonstrated the role of CMI in the control of herpesvirus infections (17, 18). Reoccurrence of latent herpes simplex virus (HSV) infection has been linked to decreased antiviral CTL responses in guinea pigs (10). The lethal effect of HSV infection in mice can be prevented by the prior treatment of the animals with viral specific $CD4^+$ T lymphocytes (27), while depletion of the $CD8^+$ T-cell subset from HSV-resistant mice reduces viral clearance from the central nervous system (CNS). In addition, antiviral antibodies, while found in high levels in humans and mice after HSV infection, do not appear to be sufficient to prevent CNS infection (14), suggesting that a strong CMI response is required to effectively control HSV infections. Similar results have been obtained with a second herpesvirus, cytomegalovirus (CMV), although for this virus the initial control of viral spread appears to require good NK cell responses, with subsequent CTL responses serving to control and eventually terminate the primary infection (9). However, neither humoral nor cell-mediated responses appear to be able to prevent the establishment of latent herpesvirus infections (11).

We have investigated the antiviral efficacy of IL-12 in a murine model of HSV infections.

MATERIALS AND METHODS

Animals. Specific-pathogen-free female BALB/c, C57BL/6, and C57BL/6 *beige* mice were obtained from Harlan-Olac and used at 6 to 8 weeks of age.

Virus preparation and infection procedure. HSV-1 (SC16) and HSV-2 (333) were grown in cell culture in monolayers of Vero cells. Virus was harvested from trypsinized cell monolayers by sonication and clarified by centrifugation. Virus titers were determined by standard plaque assays on Vero cell monolayers. For in vivo infection, the animals were given a single intraperitoneal (i.p.) injection of 10^4 PFU of HSV-1 (SC16) or HSV-2 (333) and monitored for 20 days postinfection (p.i.).

For some experiments, animals surviving infection with HSV-2 (333) were reinfected on day 21 p.i. with an identical titer of HSV-1 (SC16) or HSV-2 (333) or with 10^5 PFU of murine CMV (mCMV) (derived from the salivary glands of mCMV-infected mice), and monitored for a further 20 days.

Antiviral therapy. IL-12 was diluted in phosphate-buffered saline (PBS)–1% (wt/vol) bovine serum albumin (BSA) and given at 5 ml per kg of body weight to groups of 15 mice by i.p. or subcutaneous injection once daily. For prophylactic administration, IL-12 was given on days 2 and 1 before infection, while for therapeutic administration, IL-12 was given on the day of infection (6 h p.i.) and on days 1, 2, 3, and 4 p.i. In some studies, prophylactic and therapeutic administration was combined by giving IL-12 on days 2 and 1 before infection, on the day of infection (6 h p.i.), and on days 1 and 2 p.i.

Acyclovir (ACV) was administered as a suspension in 20% polyethylene glycol 400 in arachis oil, given i.p. once daily, at a dose equivalent to 10 mg/kg of body weight. ACV administration commenced 6 h p.i. and continued to day 4 p.i.

Statistical analysis. Survival rates were recorded daily and compared across groups by using log rank analysis (23), which allows the shapes of the survival curves to be compared directly. In addition, survival rates on day 10 or 20 p.i. were compared between replicate experiments by Student's *t* test.
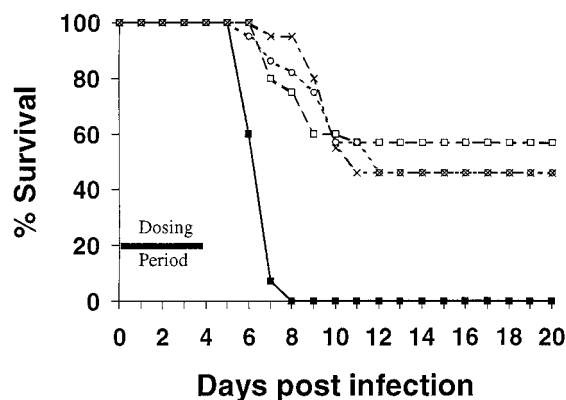
FIG. 1. Effect of therapeutic administration of IL-12 in HSV-1 (SC16) systemic infection. Groups of 15 female BALB/c mice were infected with 10^4 PFU of HSV-1 (SC16) by i.p. injection. At 6 h later, and daily thereafter for a further 4 days, the animals were treated with IL-12 by i.p. injection, at 5 ml per kg of body weight, of a solution of the cytokine in PBS–1% (wt/vol) BSA. Death rates were monitored for 20 days p.i., and survival curves were compared by log rank analysis. Symbols: ■, control; □, IL-12 at 50 ng/day ($P < 0.001$); ○, IL-12 at 100 ng/day ($P < 0.001$); ×, IL-12 at 300 ng/day ($P < 0.001$).

RESULTS

Treatment with a range of IL-12 concentrations (from 5 to 500 ng/day) delayed the onset of HSV-1- and HSV-2-induced mortality (Fig. 1 and 2) and also altered the extent of mortality. In a series of repeated experiments, IL-12 consistently improved the survival seen on day 20 p.i. from a mean ± standard error of the mean of 3.5% ± 5% in vehicle-dosed animals ($n = 11$) to 23% ± 17% in animals given 50 ng of IL-12 per day ($n = 11$; Student's P value, <0.001) and 30% ± 14% in animals given 300 ng of IL-12 per day ($n = 2$; Student's P value, <0.001). No clear dose-related response was seen, although 500 ng of IL-12 per day appeared to be less efficacious in preventing the mortality on day 20 p.i. Uninfected animals treated with IL-12 at a range of doses, from 50 up to 1,000 ng/day for 5 days, showed no evidence of ill effects (data not shown), suggesting that the reduced survival seen in the groups
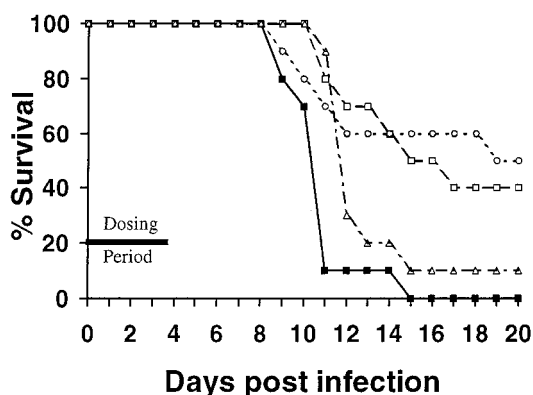
TABLE 1. Effect of prophylactic administration of IL-12 in HSV-2 (333) systemic infection in repeated trials[a]

IL-12 dose (ng/day)	No. of repeats	% Survival[b] on:	
		Day 10 p.i.	Day 20 p.i.
0	3	42 ± 40	13 ± 13
50[d]	3	70 ± 9	61 ± 9[c]
100[d]	1	73	60
300[d]	1	64	40

[a] Groups of 15 mice were dosed with IL-12 by i.p. injection of 5 ml, per kg of body weight, of a solution of cytokine in PBS–1% (wt/vol) BSA at 48 h and again at 24 h prior to infection with 10^4 PFU of HSV-2 (333).
[b] The numbers are mean percent survival values ± standard error of the mean, on day 10 or 20 p.i., of the indicated number of repeats, compared by Student's t test.
[c] $P < 0.01$ by Student's t test.
[d] Significant protection by log rank analysis of survival curves ($P < 0.005$).

given higher doses was not simply a reflection of IL-12-induced toxicity.

Extended therapy for 10 days after infection did not further improve the ability of IL-12 to prevent HSV-2-induced mortality in this model (data not shown). In the HSV-2 infection model, lumbar ganglia from 12 of 56 IL-12-treated, infected mice were found to contain latent HSV-2 on day 35 p.i., as shown by in vitro culture of these tissues and viral plaque assay (data not shown). It therefore appeared that at least in some animals, IL-12 treatment did not prevent the establishment of viral latency despite protecting these animals from the lethal effects of HSV-2 infection. Since too few control animals survive for comparison, it is not possible to ascertain if the establishment of latency following i.p. infection with the titers of HSV-2 used in this system is common.

IL-12 could also protect animals from lethal HSV-2 infection when given only before infection (Table 1), and in this case the efficacy appeared to be improved over that seen with therapeutic administration. However, IL-12 showed the greatest efficacy when administered both before and after infection with HSV-2 (Fig. 3). Thus, treatment with 50 ng of IL-12 per

FIG. 2. Effect of therapeutic administration of IL-12 in HSV-2 (333) systemic infection. Groups of 15 female BALB/c mice were infected with 10^4 PFU of HSV-2 (333) by i.p. injection. At 6 h later, and daily thereafter for a further 4 days, the animals were treated with IL-12 by i.p. injection, at 5 ml per kg of body weight, of a solution of the cytokine in PBS–1% (wt/vol) BSA. Death rates were monitored for 20 days p.i., and survival curves were compared by log rank analysis. Symbols: ■, control; □, IL-12 at 50 ng/day ($P < 0.005$); ○, IL-12 at 100 ng/day ($P < 0.01$); △, IL-12 at 500 ng/day ($P < 0.05$).
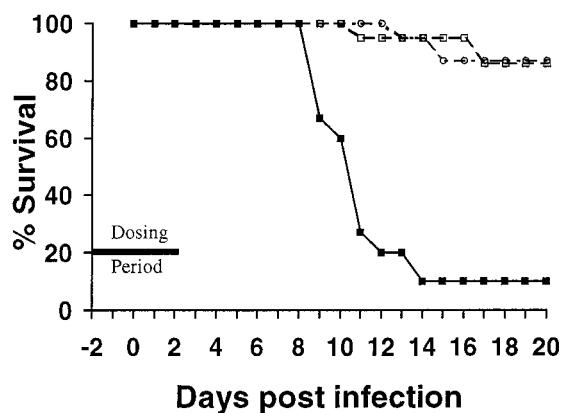

FIG. 3. Effect of combined prophylactic and therapeutic administration of IL-12 in HSV-2 (333) systemic infection. Groups of 15 female BALB/c mice were treated with IL-12 by i.p. injection of 5 ml, per kg of body weight, of a solution of cytokine in PBS–1% (wt/vol) BSA at 48 h and again at 24 h prior to i.p. infection with 10^4 PFU HSV-2 (333). IL-12 treatment was given 6 h later and daily thereafter for a further 2 days. Death rates were monitored for 20 days p.i., and survival curves were compared by log rank analysis. Symbols: ■, control; □, IL-12 at 50 ng/day ($P < 0.001$); ○, IL-12 at 100 ng/day ($P < 0.001$).
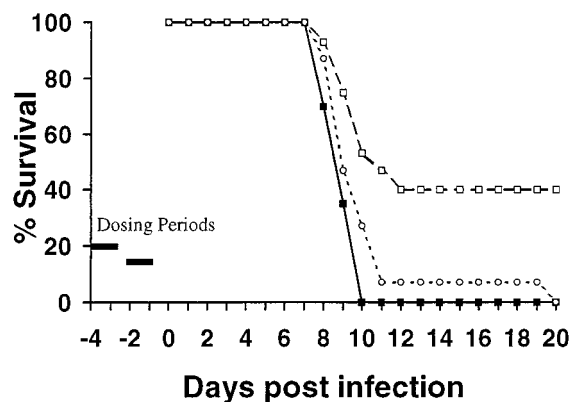
FIG. 4. Effect of delaying infection on the efficacy of prophylactic IL-12 administration in HSV-2 (333) systemic infection. Groups of 15 female BALB/c mice were treated with two daily treatments of IL-12 by i.p. injection of 5 ml, per kg of body weight of a solution of cytokine in PBS–1% (wt/vol) BSA prior to i.p. infection with 10^4 PFU of HSV-2 (333). Infection was carried out at either 24 or 72 h after the final dose of IL-12, and the survival rates were monitored for 20 days p.i. Survival curves were compared by log rank analysis. Symbols: ■, control; □, days −2 and −1 ($P < 0.001$); ○, days −4 and −3 (P = not significant).
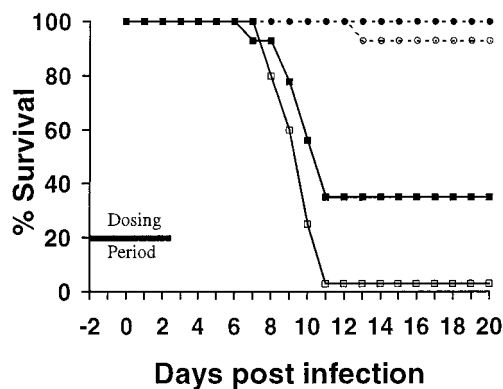

FIG. 5. Effect of IL-12 in HSV-2 (333) systemic infection of wild-type and *beige* C57BL/6 mice. Groups of 15 female wild-type or *beige* mutant C57BL/6 mice were treated with 50 ng of IL-12 per day by i.p. injection of 5 ml, per kg of body weight, of a solution of the cytokine in PBS–1% (wt/vol) BSA for 2 days prior to i.p. infection with 10^4 PFU HSV-2 (333). IL-12 treatment was given 6 h later and daily thereafter for a further 2 days. Death rates were monitored for 20 days p.i., and survival curves were compared by log rank analysis. Symbols: ■, wild-type control; ●, wild type given IL-12 at 50 ng/day ($P < 0.001$); □, *beige* control; ○, *beige* mutant given IL-12 at 50 ng/day ($P < 0.001$).

day only after infection resulted in 23% ± 17% survival on day 20 p.i., treatment with this dose of IL-12 only before infection gave 61% ± 9% survival ($n = 3$; Student's P value, <0.005), and treatment both before and after infection resulted in 80% ± 5% survival ($n = 4$; Student's P value, <0.001 versus therapeutic administration and <0.02 versus prophylactic administration).

The beneficial effect of IL-12 predosing was lost if infection was delayed relative to the IL-12 therapy (Fig. 4). Animals treated with 50 ng of IL-12 per day for 2 days and then left for a further 2 days before infection showed no resistance to a lethal HSV-2 infection, in contrast to animals that were infected immediately after the IL-12 therapy (0 versus 40% survival on day 20 p.i.; $P < 0.05$). These data indicate that prophylactic IL-12 therapy induced short-term resistance. To investigate if this resistance was a consequence of enhanced NK cell cytotoxic activity, HSV-2 infections were carried out in mice carrying the *beige* mutation, which reduces NK lytic activity. The lack of availability of this mutation on the BALB/c background in the United Kingdom led us to investigate the effects of HSV infection and of IL-12 therapy in C57BL/6 mice (Fig. 5). Wild-type C57BL/6 mice appeared more resistant to HSV-2 infection than did age-matched BALB/c mice, with 33% of the C57BL/6 animals surviving an infection which is normally fully lethal in BALB/c mice. In contrast, the *beige* mutant C57BL/6 mice were fully susceptible, suggesting that NK lytic activity may be important in C57BL/6 mice for resistance to HSV-2 infection. Nevertheless, IL-12 was equally effective in both wild-type and mutant C57BL/6 mice, indicating that NK lytic activity is not crucial for the antiviral activity of IL-12.

Animals infected with HSV-2 and given a 5-day course of IL-12 therapy showed a reduced susceptibility to reinfection with HSV-1 or HSV-2 infection but not to mCMV infection (Table 2) compared to age-matched mice not given IL-12. The resistance to reinfection seen in the IL-12-protected animals is similar to that seen in mice protected from HSV-2 by treatment with the antiherpesvirus chemotherapeutic agent ACV. This suggests that IL-12 may be acting primarily to prevent the lethal consequence of acute HSV infection, allowing antiviral immune responses to develop in surviving animals. Further

studies to characterize the processes involved in this protection are under way.

When IL-12 was given at 50 ng/day by subcutaneous (s.c.) rather than by i.p. injection, the efficacy against HSV-2 infection was somewhat reduced (Table 3), with only 12% of treated animals surviving infection (compared to 23% of animals treated with 50 ng of IL-12 per day i.p.), although this difference was not statistically significant. ACV at the suboptimal dose of 10 mg/kg/day i.p. similarly gave only moderate protection against HSV-2 (333) infection. However, combination therapy with 50 ng of IL-12 per day s.c. and 10 mg of ACV per kg per day i.p. provided enhanced protection against lethal HSV-2 infection compared to that seen with either agent alone, with 71% of codosed animals surviving to day 20 p.i. ($P < 0.01$ for combination therapy compared to either treatment alone by Student's t test). This enhancement was also seen in animals given daily doses of ACV and intermittent doses of IL-12 on either days 0, 2, and 4 p.i. or on days 1 and 3 p.i. only. A single dose of IL-12 on day 0 combined with daily ACV was not sufficient to improve survival in this study. We are currently investigating other combinations of ACV and IL-12 therapy.

TABLE 2. IL-12 promotes resistance to reinfection with alphaherpesviruses but not with betaherpesviruses[a]

Infection on day 0	Treatment on days 0 to 4	Reinfection on day 20	% Survival on day 40
Mock	PBS	HSV-2	10
Mock	IL-12	HSV-2	10
HSV-2[b]	IL-12	HSV-2	71
HSV-2[b]	IL-12	HSV-1	78
HSV-2	IL-12	mCMV	0

[a] A group of 100 female BALB/c mice were treated with IL-12 by i.p. injection of 5 ml per kg of body weight of a solution of cytokine in PBS–1% (wt/vol) BSA for 2 days prior to i.p. infection with 10^4 PFU of HSV-2 (333). IL-12 treatment was given 6 h later and daily thereafter for a further 2 days. Death rates were monitored for 20 days p.i. Surviving animals were randomly allocated into groups of 15 and reinfected with HSV-2 or with an identical titer of HSV-1 (SC16) or with 10^5 PFU of mCMV (Smith) by i.p. injection and monitored for a further 20 days. Survival curves were compared by log rank analysis.
[b] Significant protection by log rank analysis of survival curves ($P < 0.005$).

TABLE 3. IL-12 and ACV combination therapy in HSV-2
systemic infection[a]

Group	No. of expt	Time of administration of:		% Survival on day 20 p.i.[b]
		ACV (10 mg/kg/day i.p.)	IL-12 (50 ng/day s.c.)	
1	4	None	None	2 ± 3
2	4	Days 0–4	None	11 ± 12
3	4	None	Days 0–4	12 ± 14
4[c]	4	Days 0–4	Days 0–4	71 ± 18[d]
5[c]	1	Days 0–4	Days 0, 2, and 4	60
6[c]	1	Days 0–4	Days 1 and 3	100
7[c]	1	Days 0–4	Day 0 only	0

[a] Groups of 15 female BALB/c mice were infected with 10^4 PFU of HSV-2 (333) by i.p. injection. At 6 h later, and daily thereafter for a further 4 days, the animals were treated by (i) i.p. injection of 10 mg, per kg of body weight, of ACV suspended in 20% PEG 400–80% arachis oil, (ii) 50 ng of IL-12 per day by s.c. injection at 5 ml, per kg body weight, of a solution of the cytokine in PBS–1% (wt/vol) BSA, or (iii) both therapies as indicated. Death rates were monitored for 20 days p.i., and survival rates were compared by log rank analysis.

[b] The numbers are mean percent survival ± standard error of the mean, on day 20 p.i., of the indicated number of repeats, compared by Student's t test.

[c] Significant protection by log rank analysis of survival curves ($P < 0.005$).

[c] $P < 0.01$.

[d] $P < 0.005$.

DISCUSSION

We have shown in the studies described here that exogenous IL-12 can protect mice from an otherwise lethal infection with HSV-1 or HSV-2, that this protection may be independent of NK lytic activity, and that protected mice resist further infection with alphaherpesviruses but not with the betaherpesvirus mCMV. The degree of IL-12-induced protection was relatively independent of the dose of IL-12 used, but the timing of the treatment in relation to the infection was important. When IL-12 was given only before infection, the protection afforded appeared to be short-lived, suggesting that it may involve non-specific responses. Treatment only after infection was able to provide protection in a proportion of animals but did not prevent the establishment of latent CNS infection. The optimal efficacy was seen when IL-12 was given for 2 days before infection and continued for 2 days after infection. Animals so treated were resistant to a normally lethal titer of HSV, and preliminary studies indicate that this protection cannot be overcome by increasing the viral titer used in the infection (data not shown). In addition, protected animals appear to resist further infection with the closely related virus HSV-1 (SC16) but not with mCMV.

IL-12 has been shown to protect thermally injured mice from lethal HSV-1 infection in a model of burn-related infection (13) and to reduce the severity of CNS disease following infection with encephalomyocarditis virus (22) in an IFN-γ-dependent manner. In addition, IL-12 treatment enhances the protection afforded by immunization against respiratory syncytial virus infection (24) and enhances the induction of T_{H1}-type immune responses. A similar boost to T_{H1} responses has been reported in transgenic mice expressing hepatitis B e antigen following therapy with IL-12 (15), and IL-12 can reduce the replication of hepatitis B virus in transgenic mice expressing the full viral genome (2). Others have also shown that prophylactic IL-12 therapy can reduce vesicular stomatitis virus infection in the CNS (1) and mCMV and lymphocytic choriomeningitis virus (LCMV) infection (20). However, the latter group also report differences in the responses to IL-12 depending on the virus used and suggest that high-dose IL-12

therapy may be detrimental in infections where there is already a strong induction of immune reactivity. Overactivation of immune responses leading to excessive tumor necrosis factor alpha release may be involved in the reported high-dose IL-12-induced toxicity in the models of LCMV infection (21), although we have shown that similar doses of IL-12 do not appear to be detrimental in mCMV or HSV infections. In the present study, doses of IL-12 higher than 300 ng/day were less beneficial than lower doses, but the animals did not show noticeable signs of treatment-induced toxicities and IL-12 was not obviously toxic when administered to noninfected mice at doses as high as 1,000 ng/day. Any "immunotoxic" effect of IL-12 may therefore be dependent on the virus model used and the degree (and possibly the nature) of immune system activation.

Optimal antiviral therapy may require a combination approach, involving both chemotherapeutic agents and immune system stimulants. Previous workers have used combinations of ACV and IFN-α in vitro to reduce HSV-1 replication in cell cultures (25). Here we have demonstrated that cotherapy with low-dose ACV and IL-12 in vivo is more beneficial than therapy with either agent alone, allowing lower doses of reagent to be used and potentially reducing drug-induced toxicity. Such combination therapy may also be beneficial in reducing the emergence of viral resistance to chemotherapeutic agents by increasing the immune pressure on the virus. We are currently investigating the use of combinations of IL-12 and antiviral agents in other viral infection models.

Previous workers have shown beneficial effects of IL-12 therapy on animal models of intracellular infection with *Leishmania major* (16) and have shown that these effects may require both IFN-γ-dependent and IFN-γ-independent events. In contrast, the antiviral effect of IL-12 may involve primarily IFN-γ (22). An important source of IFN-γ in primary immune responses may be the NK cell, and we are investigating the efficacy of IL-12 in animals depleted of IFN-γ activity and of NK activity.

It has been suggested that there may be a transient induction of IL-12 p40 expression very early after viral infection (7) and that this may play a role in directing the subsequent immune response to the virus. The transient nature of this endogenous IL-12 response might reflect the known ability of many viruses to induce immunosuppression, suggesting that exogenous IL-12 might be beneficial in the treatment of such infections. Certain viruses (e.g., LCMV) appear to induce poor IL-12 responses (19), possibly by active suppression of the production of this cytokine. There are also several reports of an early failure in the induction of IL-12 responses in cells taken from individuals with human immunodeficiency virus infection, which may contribute to the failure of the host to control the infection (4, 6, 26, 30). The data presented here suggest a potential role for IL-12 in the treatment of chronic viral infection, either as a monotherapy or in combination with chemotherapeutic antiviral agents.

REFERENCES

1. **Bi, Z., P. Quandt, T. Komatsu, M. Barna, and C. S. Reiss.** 1995. IL-12 promotes enhanced recovery from vesicular stomatitis virus infection of the central nervous system. J. Immunol. **155:**5684–5689.
2. **Cavanaugh, V. J., L. G. Guidotti, and F. V. Chisari.** 1997. Interleukin-12 inhibits hepatitis B virus replication in transgenic mice. J. Virol. **71:**3236–3243.
3. **Chehimi, J., S. E. Star, I. Frank, M. Rengaraju, S. J. Jackson, C. Llanes, M. Kobayashi, B. Perussia, D. Young, E. Nickbarg, S. F. Wolf, and G. Trinchieri.** 1992. Natural killer (NK) cell stimulatory factor increases the cytotoxic activity of NK cells from both healthy donors and human immunodeficiency virus-infected patients. J. Exp. Med. **175:**789–796.

4. **Chehimi, J., S. E. Starr, I. Frank, A. D'Andrea, X. Ma, R. R. MacGregor, J. Sennelier, and G. Trinchieri.** 1994. Impaired interleukin 12 production in human immunodeficiency virus infected patients. J. Exp. Med. **179:**1361–1366.

5. **Chehimi, J., N. M. Valiante, A. D'Andrea, Z. Rosado, M. Kobayashi, B. Perussia, S. F. Wolf, S. E. Star, and G. Trinchieri.** 1993. Enhancing effect of natural killer cell stimulatory factor (NKSF/interleukin-12) on cell-mediated cytotoxicity against tumor-derived and virus-infected cells. Eur. J. Immunol. **23:**1826–1830.

6. **Chougnet, C., T. A. Wynn, M. Clerici, A. L. Landay, H. A. Kessler, J. Rusnak, G. P. Melcher, A. Sher, and G. M. Shearer.** 1996. Molecular analysis of decreased interleukin 12 production in persons infected with human immunodeficiency virus. J. Infect. Dis. **174:**46–53.

7. **Coutelier, J.-P., J. Van Broeck, and S. F. Wolf.** 1995. Interleukin-12 gene expression after viral infection in the mouse. J. Virol. **69:**1955–1958.

8. **Germann, T., M. K. Gately, D. S. Schoenhaut, M. Lohoff, F. Mattner, S. Fischer, S.-C. Jin, E. Schmitt, and E. Rude.** 1993. Interleukin-12/T cell stimulating factor, a cytokine with multiple effects on T helper type 1 (T_h1) but not on T_h2 cells. Eur. J. Immunol. **23:**1762–1770.

9. **Ho, M.** 1991. Murine cytomegalovirus, p. 327–353. *In* Cytomegalovirus: biology and infection, 2nd ed. Plenum Medical Book Co., New York, N.Y.

10. **Iwasaka, T., J. F. Sheridan, and L. Aurelian.** 1983. Immunity to herpes simplex virus type 2: recurrent lesions are associated with the induction of suppressor cells and soluble suppressor factors. Infect. Immun. **42:**955–964.

11. **Jonjic, S., I. Pavic, B. Polic, I. Crnkovic, P. Lucin, and U. H. Koszinowski.** 1994. Antibodies are not essential for the resolution of primary cytomegalovirus infection but limit dissemination of recurrent virus. J. Exp. Med. **179:**1713–1717.

12. **Kobayashi, M., L. Fitz, M. Ryan, R. M. Hewick, S. C. Clark, S. Chan, R. Loudon, F. Sherman, B. Perussia, and G. Trinchieri.** 1989. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. J. Exp. Med. **170:**827–845.

13. **Matsuo, R., M. Kobayashi, D. N. Herndon, R. B. Pollard, and F. Suzuki.** 1996. Interleukin 12 protects thermally injured mice from herpes simplex virus type 1 infection. J. Leukocyte Biol. **59:**623–630.

14. **McKendall, R. R.** 1983. Delayed IgG-mediated clearance of herpes simplex virus type 1 from the CNS but not the footpad during the early stages of infection: possible result of relative integrity of the blood-brain barrier. J. Gen. Virol. **64:**1965–1972.

15. **Milich, D. R., S. F. Wolf, J. L. Hughes, and J. E. Jones.** 1995. Interleukin 12 suppresses autoantibody production by reversing helper T-cell phenotype in hepatitis B e antigen transgenic mice. Proc. Natl. Acad. Sci. USA **92:**6847–6851.

16. **Murray, H. W., and J. Hariprashad.** 1995. Interleukin-12 is effective treatment for an established systemic intracellular infection: experimental visceral leishmaniasis. J. Exp. Med. **181:**387–391.

17. **Nash, A. A., H. J. Field, and R. Quartey-Papafio.** 1980. Cell-mediated immunity in herpes simplex virus-infected mice: induction, characterization and antiviral effects of delayed type hypersensitivity. J. Gen. Virol. **48:**351–357.

18. **Oakes, J. E.** 1975. Role for cell-mediated immunity in the resistance of mice to subcutaneous herpes simplex virus infection. Infect. Immun. **12:**166–172.

19. **Orange, J. S., and C. A. Biron.** 1996. An absolute and restricted requirement for IL-12 in natural killer cell IFN γ production and antiviral defense. Studies of natural killer and T cell responses in contrasting viral infections. J. Immunol. **156:**1138–1142.

20. **Orange, J. S., S. F. Wolf, and C. A. Biron.** 1994. Effects of IL-12 in the response and susceptibility to experimental viral infections. J. Immunol. **152:**1253–1264.

21. **Orange, J. S., T. P. Salazar-Mather, S. M. Opal, R. L. Spencer, A. H. Miller, B. S. McEwen, and C. A. Biron.** 1995. Mechanism of interleukin-12 mediated toxicities during experimental viral infections: role of tumor necrosis factor and glucocorticoids. J. Exp. Med. **181:**901–914.

22. **Ozmen, L., M. Aguet, G. Trinchieri, and G. Garotta.** 1995. The in vivo antiviral activity of interleukin-12 is mediated by gamma interferon. J. Virol. **69:**8147–8150.

23. **Peto, R., M. C. Pike, P. Armitage, N. E. Breslow, D. R. Cox, S. V. Howard, N. Mantel, K. McPherson, J. Peto, and P. G. Smith.** 1977. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. Br. J. Cancer **35:**1–39.

24. **Tang, Y. W., and B. S. Graham.** 1995. Interleukin 12 treatment during immunization elicits a T helper cell type 1 like immune response in mice challenged with respiratory syncytial virus and improves vaccine immunogenicity. J. Infect. Dis. **172:**734–738.

25. **Taylor, J. L., P. Tom, J. Guy, R. M. Selvarajan, and W. J. O'Brien.** 1994. Regulation of herpes simplex virus thymidine kinase in cells treated with a synergistic antiviral combination of alpha interferon and acyclovir. Antimicrob. Agents Chemother. **38:**853–856.

26. **Than, S., R. Hu, N. Oyaizu, J. Romano, X. Wang, S. Sheikh, and S. Pahwa.** 1997. Cytokine pattern in relation to disease progression in human immunodeficiency virus-infected children. J. Infect. Dis. **175:**47–56.

27. **Wachsman, M., J. H. Luo, L. Aurelian, and E. Paoletti.** 1992. Protection from herpes simplex virus type 2 is associated with T cells involved in delayed type hypersensitivity that recognize glycosylation-related epitopes on glycoprotein D. Vaccine **10:**447–454.

28. **Wolf, S. F., P. A. Temple, M. Kobayashi, D. Young, M. Dicig, L. Lowe, R. Dzialo, L. Fitz, C. Ferenz, R. M. Hewick, K. Kelleher, S. H. Herrmann, S. C. Clark, L. Azzoni, S. H. Chan, G. Trinchieri, and B. Perussia.** 1991. Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biologic effects on T and natural killer cells. J. Immunol. **146:**3074–3081.

29. **Wolf, S. F., D. Sieburth, and J. Sypek.** 1994. Interleukin 12: a key modulator of immune function. Stem Cells **12:**154–164.

30. **Yoo, J., H. Chen, T. Kraus, D. Hirsch, S. Polyak, I. George, and K. Sperber.** 1996. Altered cytokine production and accessory cell function after HIV-1 infection. J. Immunol. **157:**1313–1320.