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Regulation by Recombinant Interleukin-2 of Protective Immunity against Recurrent Herpes Simplex Virus Type 2 Genital Infection in Guinea Pigs

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The goal of our study was to determine whether recombinant interleukin-2 (rIL-2) could modify the recurrence pattern of chronic herpes simplex virus type 2 (HSV-2) genital infection in guinea pigs. Animals that developed symptomatic acute HSV-2 infection were distributed at 14 days after viral inoculation into several treatment groups, which were similar with respect to the severity of acute disease. Three rIL-2 dosages administered for 4 weeks in daily subcutaneous injections were tested in this study: 5×10^3, 5×10^4, and 2.5×10^5 U. Daily observations of the animals showed a significant decrease of the incidence of new recurrent lesions with the use of 5×10^4 U of rIL-2 (rate of recurrence, 0.08, compared with 0.21 in untreated controls), whereas the other rIL-2 regimens did not affect the overall rate of recurrence. Weekly analysis of recurrences showed that treatment with 5×10^4 U of rIL-2 was effective only during the first 3 weeks of use and that 2.5×10^5 U of rIL-2 markedly decreased the rate of recurrence in the first week of treatment but not in subsequent weeks. The loss of clinical protection in both groups coincided with the production of neutralizing antibodies to rIL-2. The immune mechanisms possibly involved in the protective effect of rIL-2 in chronic HSV-2 disease were further investigated. Production of gamma interferon correlated well with clinical protection, and circulating levels dropped at the time when neutralizing antibodies to rIL-2 developed. Nonspecific cytotoxicity represented by natural killer cell and lymphokine-activated killer cell activities was also increased in the treated guinea pigs. Antibody titers and lymphocyte proliferation to herpes simplex antigen were similar in rIL-2 and placebo recipients. Finally, we found that the rIL-2-induced immune stimulation was as protective against recurrent HSV-2 disease in guinea pigs as the viral suppression achieved with acyclovir. However, the biological activity of both drugs was not additive when they were coadministered.

Interleukin-2 (IL-2) is a glycoprotein secreted by T lymphocytes upon stimulation with antigens or mitogens (10, 12), which activates several different immune mechanisms. It has been shown to induce lymphokine-activated killer cells (LAK) (1, 13, 42), enhance natural killer cell (NK) activity (5, 14, 15, 54, 59), elicit gamma interferon (IFN-γ) production (11, 17, 19, 35), and stimulate B lymphocytes to generate both immunoglobulin M and immunoglobulin G (18, 32, 33, 55, 58, 62). When administered alone or with LAK, recombinant interleukin-2 (rIL-2) has a potent antitumor effect both in mice and humans (22, 24, 29, 30, 43, 44). It also restores immune functions, in particular those mediated by T helper cells in animals with naturally acquired or drug-induced immune deficiencies (26, 40, 45, 48, 50, 57).

In our previous studies, we have shown that human rIL-2 has a protective effect against acute herpes simplex virus type 2 (HSV-2) genital infection in guinea pigs, manifested by a decreased rate of infection, less severe acute disease, and lower mortality (61). Protection appears to be mediated principally by NK activation (60). Moreover, LAK production, early specific immunoglobulin G anti-HSV, and circulating IFN-γ may contribute to this effect. Short-term rIL-2 administration in the acute HSV-2 infection did not seem to induce specific immune memory against HSV-2 since animals escaping acute disease failed to develop both antibodies and specific HSV-stimulated lymphocyte transformation. In addition, recurrent disease in the animals that developed acute disease was not affected by the initial treatment with rIL-2.

Severity of recurrent HSV disease has been inversely correlated with production of IFN-γ (3, 37, 53) and immune-specific T-cell cytoxicity (36, 38). Since IL-2 has been shown to regulate and augment these functions, we wanted to determine whether prolonged administration of rIL-2 to clinically infected guinea pigs could protect against recurrences. The predictable pattern of virus recurrence in the guinea pig HSV-2 genital infection (47, 49, 61) makes this model particularly useful for studying immune modulation.

MATERIALS AND METHODS

Cells. Vero cells were propagated in minimal essential medium (MEM) (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% fetal calf serum (Hyclone, Provo, Utah) and 1% (vol/vol) antibiotic antimycotic mixtures (GIBCO) that will be further referred to as CMEM. Primary guinea pig embryo tissue cultures and human foreskin fibroblasts (HFF) were obtained as previously described (60) and propagated in CMEM. P815, a murine mastocytoma cell line, was cultivated in RPMI 1640 (GIBCO) containing 10% fetal calf serum and 1% antibiotic antimycotic mixtures (referred to as RPMI).

Virus. HSV-2 MS was propagated in Vero cells at 32°C and quantitated by a plaque assay as previously described (61). Infectivity titers were approximately 10^7 PFU/ml. Bovine vesicular stomatitis virus Indiana strain was propagated and quantitated by a plaque assay on guinea pig embryo cultures. Titers were 10^8 PFU/ml.

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IL-2. Human rIL-2 (lot LP 315; specific activity, 3×10^6 to 4×10^6 U/mg, which corresponds to 7 to 9 biological response modifier program U/mg) was provided by Cetus Corp. (Emeryville, Calif.). The lyophilized rIL-2 was suspended in sterile water and used immediately or after storage for 48 h or less at 4°C.

Animals, experimental infection, and treatment. Female Hartley strain guinea pigs (250 to 350 g) were supplied by EZH Caviary (Williams, Calif.). Viral inoculation was done with 10^4 PFU of HSV-2 MS in 0.1 ml of solution, delivered intravaginally through a plastic catheter attached to a syringe and followed by insertion of a plug of Gellfoam (The Upjohn Co., Kalamazoo, Mich.). The animals were monitored daily for clinical disease, and lesions were scored as previously described (61). At the end of the 14-day acute phase, guinea pigs similar with respect to severity of acute infection were distributed among the several groups of treatment. During the chronic phase of the disease, the animals were observed daily for 15 or 28 days. New lesions consisting of papules, vesicles, or ulcerations in the perineal area and hind legs were scored as positive recurrences. Reactivation in old scars or significant extension of preexisting lesions were also scored as recurrent disease. The duration of the recurrent lesions ranged from 1 to 4 days and reflected the severity of the injury. However, since many lesions ulcerated, making it difficult to distinguish between an active ulcer and a healing one, each recurrence was scored only once, independent of its duration. The guinea pigs were enrolled into several groups of treatment which consisted of daily rIL-2 doses of 5×10^3, 5×10^4, or 2.5×10^5 U, administered subcutaneously for 15 or 28 days. A control group which received excipient buffer was also included in each experiment. Acyclovir (ACV) (Burroughs Wellcome Co., Research Triangle Park, N.C.) was administered in the drinking water at a concentration of 5 mg/ml on the basis of previous kinetic studies (8). Considering the amount of water that was consumed daily by the guinea pigs, each animal probably received 15 to 20 mg of ACV per day. Clinical observations of recurrent disease were done by an investigator who was not aware of the identities of the different treatment groups. For immunologic studies, animals were bled by intracardiac puncture under general anesthesia induced with 5 mg of xylazine per kg of body weight and 44 mg of ketamine per kg of body weight.

Lymphocyte transformation assay. Guinea pig peripheral blood mononuclear cells (PBML) were obtained by Ficoll-Hypaque gradient (specific gravity, 1.09) separation as previously described (61). The cells were resuspended in RPMI supplemented with 2.2 mg of $NaHCO_3$ per ml, 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer–0.05 mM 2-mercaptoethanol at a concentration of 3.5×10^6 cells per ml. The PBML were incubated for 6 days in U-bottom microtiter plates, 5×10^5 cells per well, in the presence of 2.5% phytohemagglutinin P (Difco Laboratories, Detroit, Mich.), UV-irradiated HSV-2, or UV-irradiated Vero cell control at the preestablished optimal dilution of 1:40 (vol/vol). A 6-h pulse of [^{3}H]thymidine (2 μCi/ml) was given on day 6. Incorporated radioactivity is expressed as a stimulation index, which represents the ratios of counts per minute in antigen-stimulated triplicate wells to counts per minute in control triplicate wells.

[^{3}H]leucine release cytolytic assays. NK targets were prepared by infecting HFF monolayers with HSV-2 MS at a multiplicity of infection of 20 PFU per cell (60). After 48 h of growth in MEM containing 1% fetal calf serum, 1% antibiotic antimycotic mixtures, and 50 μCi of [^{3}H]leucine (Amersham Corp., Amersham, United Kingdom), the infected targets (HSV-2/HFF) were washed and suspended at 2×10^5 cells per ml in RPMI. LAK targets, P815, were also cultivated with 50 μCi of [^{3}H]leucine in RPMI for 48 h, washed, and suspended at 2×10^5 cells per ml. Labeled cells ($n = 10^4$) were incubated in triplicate U-bottom wells of microtiter plates with 5×10^5 guinea pig PBML in 0.2 ml of total volume per well for 18 h at 37°C. Spontaneous release was determined by incubating the target cells in RPMI, and maximum release was elicited by adding 1% Triton X-100. The plates were centrifuged at $200 \times g$ for 10 min, and 100 μl of the supernatant was diluted in 1.5 ml of Aquasol (Du Pont Co., Wilmington, Del.) for measurement of radioactivity in a Packard scintillation counter, model 760 (Packard Instrument Co., Inc., Rockville, Md.). Specific release was calculated as [(mean counts per minute in triplicate wells − mean spontaneous release)/(mean maximum release − mean spontaneous release)] × 100. Spontaneous release was always approximately 10%.

Interferon assays. Guinea pig plasma samples were stored at −20°C until interferon titers were measured in a plaque reduction assay as previously described (60). Briefly, guinea pig embryo fibroblasts at 90% confluency in six-well Costar tissue culture dishes (Costar, Cambridge, Mass.) were incubated overnight at 37°C with a 10-fold dilution of guinea pig plasma in CMEM starting at 1:10. Thereafter, the monolayers were allowed to absorb 40 PFU of bovine vesicular stomatitis virus and were subsequently incubated for 48 h under a 1% agarose overlay. Plaques were revealed with neutral red (Sigma Chemical Co., St. Louis, Mo.), 0.3% in saline. The interferon titer was calculated as previously described (25) and represents the reciprocal of the dilution that reduces 50% of the cytopathic effect of bovine vesicular stomatitis virus. Control wells with medium or recombinant interferon α A/D bgl (Hoffmann-La Roche Inc., Nutley, N.J.) were included in each assay. The serum IFN-γ was characterized by ultracentrifugation at 100,000 × *g* for 90 min, trypsin digestion (200 μg/ml) for 4 h at 37°C, cross-species lack of activity on HFF monolayers, and lability to pH 2 for 4 h at 4°C, as previously described (3, 25, 60).

ELISA for HSV-2 antibody. The plasma obtained from the guinea pigs was stored at −20°C until tested for antibodies as previously described (60) by an enzyme-linked immunosorbent assay (ELISA). Briefly, viral and mock antigens were obtained from infected and uninfected Vero cells, respectively, sonicated, and solubilized with 0.1% Triton X-100. Immulon 2 microtiter plates (96 wells; Dynatech Laboratories, Inc., Alexandria, Va.) were incubated overnight with 50 μl of antigen diluted in carbonate buffer (pH 9.6). Untreated sites were blocked with 10% horse serum in phosphate-buffered saline, and fourfold dilutions of the plasma samples were added to duplicate wells, starting with a 1:10 solution of plasma in phosphate-buffered saline with 1% horse serum. Bound antibodies were revealed by using a Vectastain kit for guinea pig immunoglobulin G (Vector Laboratories, Inc., Burlingame, Calif.) and ABTS substrate (Zymed Laboratories, South San Francisco, Calif.). The reaction was stopped with sodium azide (0.1 M), and the absorbance was measured in a microplate reader (model MA 308; Whitaker Bioproducts, Walkerville, Calif.). The antibody titer was considered the last dilution at which the difference of the mean absorbance in duplicate wells between viral and mock antigens was higher than 0.1. To ensure homogeneous results, each assay included a standard serum of known antibody titer.

ELISA for antibody to rIL-2. A standard indirect ELISA

was also used to quantitate levels of antibodies specific for IL-2. Immulon 1 microtiter plates (96 wells; Dynatech) were coated with antigen by an overnight incubation at 25°C with each well containing 1 μg of rIL-2 in 0.05 M carbonate buffer, pH 9.6. Assays were done on 3-fold serial dilutions of an initial 10-fold dilution of plasma (by using a dilution buffer of 0.05% Tween 20 and 0.5% bovine serum albumin in phosphate-buffered saline). After incubation in antigen-coated wells for 2 h at 25°C, unbound immunoglobulin was removed by five washes with 0.05% Tween 20 in 0.9% NaCl. Incubation was continued for an additional 2 h with a 1,000-fold dilution of horseradish peroxidase-labeled rabbit anti-guinea pig immunoglobulin (Accurate Chemical Scientific Corp., Westbury, N.Y.). After washing and incubation for 20 min with the chromogenic substrate *o*-phenylenediamine (Sigma), the reaction was stopped with 0.05 M H_2SO_4 and quantitated at 490 nm by using a microplate reader (Titertek Multiscan; Flow Laboratories, Inc., McLean, Va.). The ELISA titer was defined as the product of absorbance and dilution factor, when the absorbance was in the range 0.1 to 0.5.

Neutralization assay for rIL-2 antibodies. The neutralizing capacity of rIL-2 antibodies was measured as their ability to inhibit LAK generation from PBML. An rIL-2 solution of 10^5 U/ml was mixed with fivefold dilutions of guinea pig serum samples containing rIL-2 antibodies at an ELISA titer of 250. The starting dilution was 1:2. A twofold serum dilution from an animal that had not received rIL-2 was also included as a control. After 2 h of incubation at 37°C, 50 μl of each solution was added to 5×10^5 guinea pig PBML, which were subsequently tested for LAK activity in triplicate wells in an 18-h [^{3}H]leucine release lytic assay of P815 cells (effector-to-target cell ratio, 50:1). Spontaneous release of P815 cells in these assays was also tested in wells that contained 50 μl of control negative guinea pig serum and did not differ from standard spontaneous release wells.

RESULTS

rIL-2 decreases the rate of recurrence in chronic genital HSV-2 infection in guinea pigs. To determine the effect of rIL-2 in the recurrent phase of HSV-2 disease in guinea pigs, 24 animals that had developed symptomatic acute HSV-2 infection were distributed into two groups, which were similar with respect to severity of their acute disease. Each group of animals received daily injections of 5×10^4 U of rIL-2, the dose that proved to be most effective against acute HSV-2 disease (61), or excipient buffer for 28 days. During that time, the animals were examined daily under blind conditions and new lesions or reactivations of old ones were scored as positive recurrences. The incidence of recurrences among the guinea pigs that received rIL-2 was significantly lower than that in the control, with an overall rate of recurrence of 0.08, compared with 0.21 in the control ($P < 0.025$ by Student's t test for two means) (Fig. 1). When animals were paired according to the severity of acute infection, the difference in recurrence rate became significant to $P < 0.005$ by the paired t test. During the study period, three animals died, two in the control group and one in the rIL-2 treatment group. They were excluded from the analysis of the data. The weekly incidence or recurrence in the two groups showed that the guinea pigs that received a placebo had a high recurrence rate in the first week of observation, followed by a progressive decrease in recurrence rate over the next 3 weeks from 0.38 to 0.13. In contrast, rIL-2-treated animals had a significantly lower rate

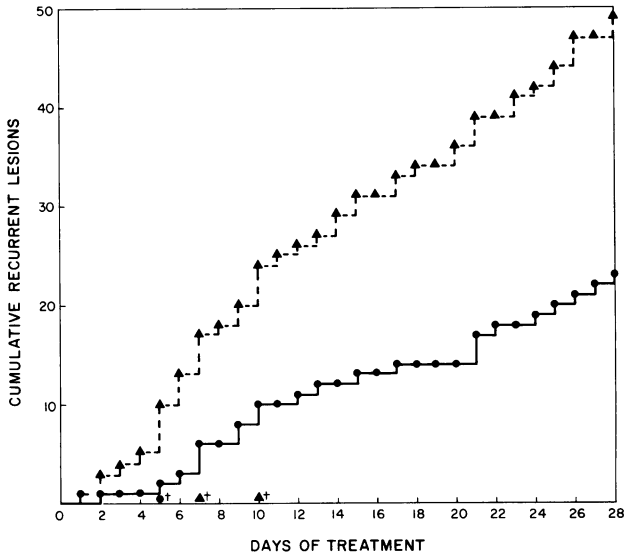

FIG. 1. Protective effect of rIL-2 against recurrent HSV-2 infection in guinea pigs. Data were derived from 24 guinea pigs that developed symptomatic acute HSV-2 genital infection and at 14 days postinoculation were distributed into two treatment groups similar with respect to severity of acute disease. The groups were randomly assigned to doses of 5×10^4 U of rIL-2 per day (●) or placebo (▲) for 28 days. Animals were observed daily for recurrences (defined as new papules, vesicles, or ulcers) as well as reactivation of old scars or extension of preexistent lesions on the perineal area or hind legs. The three guinea pigs that died (†) during the study period were excluded from the final analysis. The rIL-2 treatment group had an incidence of recurrence lower than that of the control group ($P < 0.025$ by Student's t test for two means).

of recurrence in the first week of treatment, which remained stable over the next 21 days, ranging from 0.13 to 0.09. In consequence, during the last week of observation, there was no significant difference between the two treatment groups.

Immunologic observations. Humoral and cellular immune parameters were assessed at 3, 10, and 21 days of treatment in an equal number of randomly selected animals that received 5×10^4 U of rIL-2 or placebo.

HSV-2 antibodies in rIL-2-treated and control animals. The antibody titers measured by an ELISA did not differ between the two groups (Table 1).

Specific cellular immunity to HSV-2 in rIL-2-treated and control animals. PBML from 14 animals equally distributed between placebo and rIL-2 (5×10^4 U) treatment groups were cultured in the presence of viral and cell control antigens for 6 days and assayed for the incorporation of [^{3}H]thymidine (Table 1). There was no significant difference in lymphocyte transformation by HSV-2 antigens between the treated and untreated animals.

Effect of rIL-2 administration on production of circulating IFN-γ. Table 1 summarizes the levels of interferon measured in the plasma of 14 randomly selected guinea pigs from both control and rIL-2-treated groups at 3, 10, and 21 days of treatment. Circulating IFN-γ was detected only in the animals that received rIL-2. It is of interest that at 21 days of treatment the mean titer of IFN-γ was 3 ± 2 U/ml, significantly lower than the levels detected at 3 and 10 days (40 ± 10 and 38 ± 14 U/ml, respectively). The decrease in the circulating IFN-γ coincided with the rise in rIL-2 antibodies and the loss of clinical protective effect of the drug against recurrent disease.

TABLE 1. Immune functions of rIL-2-treated and control guinea pigs with chronic HSV-2 infection[a]

Day	HSV-2 antibodies[b]		Lymphocyte stimulation[c]		NK activity[d]		LAK activity[d]		Circulating IFN-γ[e]	
	0[f]	5 × 10⁴[f]	0	5 × 10⁴	0	5 × 10⁴	0	5 × 10⁴	0	5 × 10⁴
3	2.5 ± 0.6	2.8 ± 0.7	83 ± 37	29 ± 13	8 ± 3	12 ± 4	1 ± 2	13 ± 6[g]	0	40 ± 10[g]
10	3.0 ± 0.8	3.0 ± 0.7	90 ± 49	77 ± 31	10 ± 5	16 ± 3	1 ± 5	15 ± 6[g]	0 ± 1	38 ± 14[g]
21	2.5 ± 0.8	3.2 ± 1.1	64 ± 28	17 ± 7	18 ± 2	22 ± 9	4 ± 5	16 ± 6[g]	0	3 ± 2

[a] Data were derived from seven animals in each treatment group; values represent mean ± standard error.
[b] Antibody titers were measured in an ELISA and expressed as $\log_{10}$ highest positive dilution.
[c] Values represent stimulation indices measured by [^{3}H]thymidine incorporation of PBML cultured in the presence of HSV-2 antigen for 6 days.
[d] Values represent specific lysis of HSV-2/HFF (NK target) and P815 (LAK target) effected by PBML, effector-to-target cell ratio = 50:1, measured in an 18-h [^{3}H]leucine release assay.
[e] IFN-γ titers of plasma samples were measured in a plaque reduction assay.
[f] Treatment regimen (units of rIL-2 per dose).
[g] $P < 0.05$, Student's t test for two means.

Effect of rIL-2 administration on cellular cytotoxicity. PBML of 14 guinea pigs equally distributed among placebo and rIL-2 (5×10^4 U) recipients were assayed for lytic capacity against HSV-2/HFF and P815 by using an 18-h [^{3}H]leucine release assay. On the basis of our previous study (60), HSV-2/HFF was selected for measuring NK activity and P815, an NK-resistant target, was used for measuring LAK activity. Table 1 summarizes the results of the cytolytic assays at days 3, 10, and 21 of treatment. NK activity was present in both the control and treatment groups. HSV-2/HFF lysis by PBML from animals that received rIL-2 was consistently higher, between 25 and 60% above that of the control. However, statistical significance was not reached. In contrast, LAK-mediated lysis of P815 was detected only in PBML from rIL-2-treated guinea pigs, which may have contributed to the enhanced antiviral defenses observed in this treatment group.

Relation between rIL-2 dose and duration of its protective effect against recurrent HSV-2 disease in guinea pigs. We also compared the clinical effect of 5×10^4 U of rIL-2 with two other treatment doses: 5×10^3 and 2.5×10^5 U (Fig. 2). The best level of protection was achieved with the intermediate dose of 5×10^4 U of rIL-2 per kg, which resulted in a recurrence rate of 0.07. The overall rate of recurrence with the lowest and highest doses of rIL-2 that we tested was not different from that in the control (0.15 and 0.19, respectively, versus 0.20). Table 2 displays weekly analyses of the recurrence rate in the four treatment groups. Control animals and recipients of 5×10^3 U of rIL-2 showed a high rate of recurrence during week 1 of treatment (0.41 and 0.32, respectively) and a three- to fourfold decrease over the next 3 weeks. Guinea pigs that received daily doses of 5×10^4 U of rIL-2 showed a significant decrease in incidence of recurrent lesions during the first 3 weeks, four to six times lower than the placebo group. However, the protective effect was lost during week 4 of treatment. In addition, the highest dose of rIL-2 significantly decreased the incidence of lesions in week 1 of treatment but did not affect the subsequent course of the disease. These data suggest that chronic administration of rIL-2 has a beneficial effect which is limited in duration and that higher doses of rIL-2 give shorter protection.

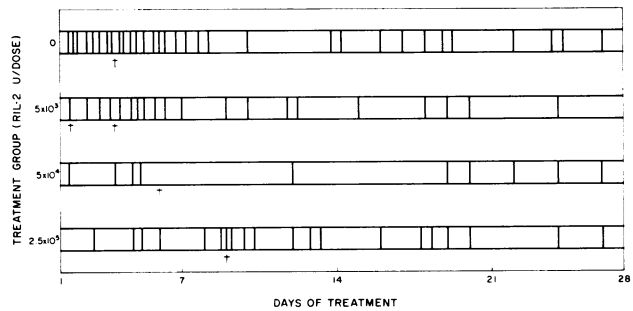

FIG. 2. Effect of dose variation of rIL-2 on protection against recurrent HSV-2 genital disease in guinea pigs. Data were obtained from 24 animals that developed symptomatic acute HSV-2 disease, were distributed at 14 days postinoculation into four groups similar with respect to severity of acute disease, and received one of the following treatments daily for 28 days: 5×10^3 U of rIL-2; 5×10^4 U of rIL-2; 2.5×10^5 U of rIL-2; and excipient buffer (0 U of rIL-2). Symbols: vertical line, new recurrent lesions defined as described in the legend to Fig. 1; †, death.

Immunogenicity of rIL-2 after chronic administration in guinea pigs. Three animals that received daily doses of 5×10^4 U of rIL-2 were tested for production of antibody to rIL-2 at 0, 3, 10, and 21 days after the initiation of treatment. The antibody titers at 3 and 10 days were 0.6 ± 0.1 (mean ± standard deviation) and 0.8 ± 0.2, similar to levels observed before treatment (0.15 ± 0.1). However, at 21 days there was a significant production of rIL-2 antibodies, with a mean titer of 160 ± 70. Guinea pigs that received 2.5×10^5 U of rIL-2 already had elevated antibody titers after 10 days of treatment, with an average titer of 32 ± 28.

To determine whether the rIL-2 antibodies that were detected in high titers by an ELISA had neutralizing activity, fivefold dilutions of serum samples, starting at 1:2, were tested for the ability to inhibit rIL-2-generated LAK activity. A control serum from an animal that had not received rIL-2 and was negative for rIL-2 antibodies in an ELISA was also

TABLE 2. rIL-2 dosage variation in recurrent HSV-2 genital infection in guinea pigs

Week of treatment	Recurrence rate with rIL-2 dose (U of rIL-2/dose)[a]:			
	0	5 × 10³	5 × 10⁴	2.5 × 10⁵
1	0.41 ± 0.14[b]	0.32 ± 0.24	0.09 ± 0.07[b]	0.07 ± 0.08[b]
2	0.12 ± 0.16	0.11 ± 0.15	0.02 ± 0.06[b]	0.21 ± 0.20
3	0.12 ± 0.11	0.08 ± 0.08	0.02 ± 0.06[b]	0.12 ± 0.06
4	0.10 ± 0.17	0.06 ± 0.08	0.09 ± 0.11	0.05 ± 0.11
Avg	0.20 ± 0.17	0.15 ± 0.11	0.07 ± 0.07[c]	0.12 ± 0.09

[a] Each dose group contained six animals. Values represent mean ± standard deviation of rate of recurrence. Rates for individual animals were calculated as number of new lesions per number of days of observation.
[b] $P < 0.005$, compared with control group.
[c] $P < 0.0125$, compared with control group.

included. The experimental conditions used for testing rIL-2-induced LAK activity were established in our previous study on LAK generation from guinea pig splenocytes (60). The results (Fig. 3) show that LAK activity can be generated in these conditions from PBML. Also, positive sera inhibited LAK generation in a dose-dependent manner with a neutralizing titer of 1:50. The serum derived from an animal that had not received rIL-2 but that was otherwise similar with respect to age and stage of disease to the donors of the positive sera failed to inhibit LAK generation (specific P815 lysis of 62% at an effector-to-target cell ratio of 50:1). This demonstrates that rIL-2 neutralization occurred only in the presence of rIL-2 antibodies and that nonspecific serum factors were not involved in the inhibition of LAK activity.

Comparison of antiviral effect of rIL-2 and ACV on recurrent HSV-2 infection. Groups of six guinea pigs which underwent comparable acute HSV-2 genital disease were randomly assigned to one of the four following treatment regimens: ACV in the drinking water at 5 mg/ml accompanied by daily injections of 5×10^4 U of rIL-2, same ACV regimen and daily injections of excipient buffer, injections of rIL-2 alone, and injections of excipient buffer alone. The animals were monitored under blind conditions for 15 days. The incidence of new recurrences in these groups of guinea pigs is represented in Fig. 4. The control group had an overall rate of recurrence of 0.24, significantly higher than ACV with rIL-2, ACV alone, and rIL-2 alone, which showed recurrence rates of 0.08, 0.10 and 0.12, respectively. There was no significant difference among the treatment groups that received one or two active drugs.

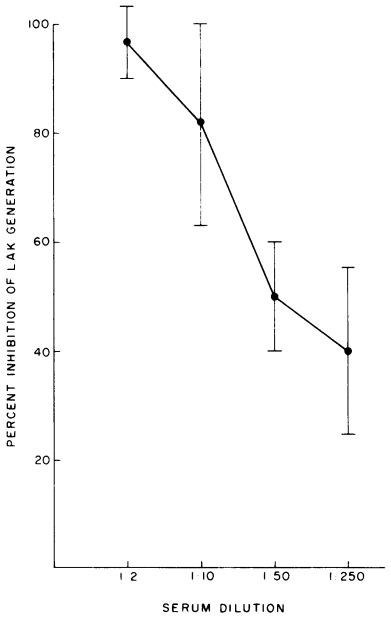

FIG. 3. Production of neutralizing antibodies to human rIL-2 in guinea pigs chronically treated with rIL-2. PBML from three guinea pigs were cultured in medium containing 10^4 U of rIL-2 per ml which was previously exposed to the indicated concentrations of positive guinea pig serum (ELISA titer of rIL-2 antibodies = 250). The LAK activity of the stimulated PBML was measured in an 18-h [^{3}H]leucine release assay of P815, effector-to-target cell ratio = 50:1. The control was LAK activity of PBML stimulated with rIL-2 that was exposed to guinea pig serum negative in antibodies to rIL-2. Data represent mean ± standard deviation of percent inhibition of LAK activity compared with that of the control.

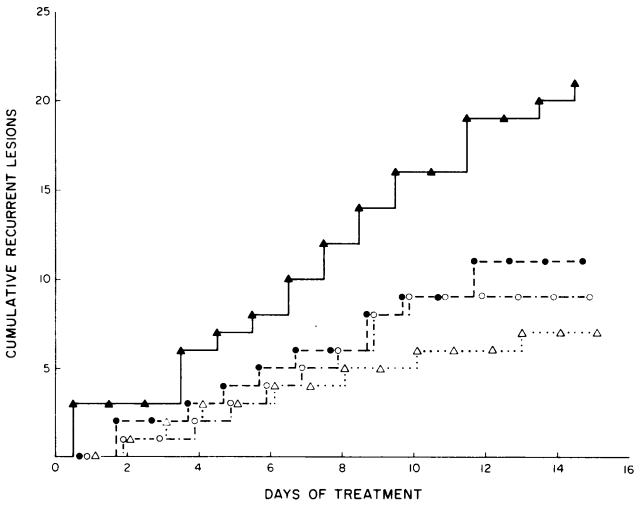

FIG. 4. Comparative effect of rIL-2 and ACV administration in recurrent HSV-2 infection in guinea pigs. Data represent observations on 24 guinea pigs that in the end of acute HSV-2 disease were enrolled in the following treatment groups: 5 mg of ACV per ml in drinking water associated with daily injections of 5×10^4 U of rIL-2 for 15 days (○); ACV, same dose as above, with placebo injections (△); rIL-2 alone, same schedule as above (●); placebo alone (▲). The incidence of recurrent lesions was significantly higher in untreated animals when compared with any of the drug-treated groups ($P < 0.05$ by Student's t test for two means), whereas no difference was observed among the treatment groups.

DISCUSSION

Our results demonstrate that rIL-2 has a protective effect against recurrent HSV-2 infection in guinea pigs. Specifically, there was a two- to threefold decrease in the recurrence rate in animals that received daily doses of 5×10^4 U of rIL-2 for 28 days, compared with untreated controls. Both lower and higher doses of rIL-2 appeared to be ineffective. This finding is in accordance with our previous observations on the protective effect of rIL-2 against acute HSV-2 infection in guinea pigs, in which a similar dose response was present, with peak activity at approximately 5×10^4 U of rIL-2 per dose (61). A biphasic dose response was also recently reported in acute HSV neonatal infection in mice treated with IL-2 (S. Kohl, D. C. Anderson, M. S. West, and L.-S. Loo, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother, abstr. no. 1173, 1986). The studies that documented the inability of high doses of rIL-2 to protect against acute HSV disease also showed that high doses of rIL-2 failed to stimulate cytotoxic defenses.

Other factors, such as drug immunogenicity, may also contribute to the lack of protection with high doses of rIL-2. Animals that received 5×10^4 U of rIL-2 developed neutralizing antibodies to rIL-2 after 3 weeks of treatment, whereas recipients of 2.5×10^5 U had significant antibody production after 1 week. In addition, there was a chronologic correlation between production of antibodies to rIL-2 and loss of clinical protection against HSV-2 recurrences. The immunogenicity of rIL-2 in our model may be related to the use of a human-derived protein in the guinea pigs and also to the recombinant nature of the drug. At this point, no data are available for comparison regarding antibody production from either the mice or the human studies that made use of human rIL-2.

We also observed that IFN-γ production paralleled the

clinical protection conferred by rIL-2 against chronic HSV-2 disease and may constitute an important parameter in rIL-2 treatment. It has been previously shown that high levels of IFN-γ induced by HSV antigen in lymphocyte cultures correspond to longer intervals between herpes labialis recurrences in humans (3). In addition, IFN production is decreased immediately before and during HSV recurrence (34). However, it is still unclear whether IFN-γ has a direct antiviral effect or acts by enhancing phagocytosis and intracellular killing of macrophages (4, 21), NK (16), and cytotoxic T cells (28, 56). In particular, cytotoxic T lymphocytes have been strongly implicated in host defenses against viral diseases (9, 36) and deserve further investigation in our model.

We also studied the role of nonspecific cytotoxic mechanisms in mediating the protective effect of rIL-2 against chronic HSV-2 infection in guinea pigs. Both NK and LAK activities were enhanced in rIL-2 recipients throughout the treatment course. LAK-mediated lysis was restricted to animals that received rIL-2, whereas NK activity was also present in untreated controls. In fact, IFN-α induced by the viral infection may also stimulate NK activity, diminishing the relative contribution of IL-2 to this immune function. In our previous study (60), we showed that guinea pig NK and in vitro-generated LAK are able to kill HSV-2-infected targets. Therefore, rIL-2 stimulation of these cytotoxic cells might contribute to the protective effect against clinical reactivation of HSV-2 genital infection in the guinea pigs. However, in our model, LAK generation did not seem to be the most important mediator of the rIL-2 protective effect, since LAK transfer could not reproduce rIL-2 protection against acute disease and there were also time and dose discrepancies between LAK activation and clinical protection (60). Furthermore, LAK activity was demonstrated in the chronically treated animals at a time when clinical protection of rIL-2 had already been lost. LAK-mediated lysis can extend up to 5 days after discontinuance of rIL-2 administration (data not shown), explaining the presence of LAK activity when neutralizing antibodies to rIL-2 were being produced.

Animals that were protected by rIL-2 against recurrent disease did not show any difference in antibody titers or lymphocyte stimulation when compared with controls. This finding is in accordance with previous studies that failed to establish any correlation between antibody titers to HSV and specific lymphocyte stimulation and frequency of HSV recurrences in humans (38, 39, 41, 63).

In the present study, we demonstrated that the protection conferred to the guinea pigs by rIL-2 immune stimulation is comparable to ACV viral suppression. However, the combination of the two drugs did not have an additive effect, which may indicate that a critical amount of viral antigen has to be presented to the host immune system for rIL-2 stimulation to be specifically directed against HSV-2 infection. On the other hand, we cannot exclude that this phenomenon may be unique to the guinea pig experimental model. Although ACV has been shown to possess a potent antiviral activity against HSV both in tissue culture (7, 46) and in human studies (2, 51), it was only moderately effective in guinea pig acute HSV-2 infection (20, 23, 31). Our data show that ACV partially suppressed HSV-2 recurrences in the guinea pigs, at a lower level when compared with the human trials (6, 27, 52). This suggests that rIL-2 also might have better protective activity in humans and that the immunogenicity of this lymphokine might be less important when it is used in the species from which it is derived.

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