Augmentation of Natural Immune Defence Mechanisms and Therapeutic Potential of a Mismatched Double-stranded Polynucleotide in Cutaneous Herpes Simplex Virus Type 2 Infection

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SUMMARY

We studied the effect of an analogue of polyinosinic acid:polycytidylic acid, the mismatched poly(rI).poly(rC12U), on herpes simplex virus type 2 (HSV-2)-induced cutaneous disease in the guinea-pig. Recurrence patterns and HSV-2-induced immune responses were also defined. Intranasal administration (1.5 µg/g body weight, five doses at 48 h intervals) of poly(rI).poly(rC12U) during initial HSV-2 infection caused a significant (P < 0.05) reduction in virus titres in the skin and decreased (P < 0.01) the duration and severity of the primary cutaneous lesions. The incidence and frequency of subsequent recurrent episodes were also significantly (P < 0.01) reduced. Titres of serum neutralizing antibody were identical in treated and untreated animals. Interferon (IFN) activity was detectable in the sera from poly(rI).poly(rC12U)-treated animals. Peripheral blood mononuclear (PBL) and spleen cells from treated animals had enhanced cytotoxic activity for HSV-2-infected and uninfected target cells. The cytotoxic activity of the PBL was enhanced by treatment in vitro with poly(rI).poly-(rC12U) or IFN.

INTRODUCTION

Herpes simplex virus type 2 (HSV-2) is a common sexually transmitted virus. It causes a broad spectrum of diseases ranging from encephalitis to mucocutaneous lesions. Cutaneous disease is characterized by sporadic recurrent episodes caused by virus reactivated from persistently infected dorsal root ganglia. Oral acyclovir therapy of initial genital herpes reduces the severity of the disease (Bryson et al., 1983). However, this treatment alters HSV-specific antibody responses, and treated patients may develop earlier and more severe recurrences than placebo-treated groups (Ashley & Corey, 1984; Bernstein et al., 1984). More recently, we (Sheridan et al., 1985, 1987) and others (Shillitoe et al., 1977; Cunningham & Merigan, 1983) reported that recurrent HSV is associated with reduction of lymphokine activity, including interferon (IFN) and/or interleukin-2 both of which mediate natural killer (NK) cell enhancement.

We therefore initiated an evaluation of the effect of a biological response modifier (BRM), an analogue of polyinosinic acid:polycytidylic acid, the mismatched polynucleotide duplex poly-(rI).poly(rC12U), on HSV-2 cutaneous disease. We chose this particular BRM as it appears to be less toxic than poly(rI).poly(rC) (Ts'o et al., 1976; Brodsky et al., 1985) while retaining its ability to induce IFN synthesis and stimulate NK activity (Ts'o et al., 1976; Nolibe et al., 1985). The guinea-pig model of recurrent cutaneous HSV-2 was selected because of its similarity to the natural history of infection in humans (Scriba & Tatzber, 1981; Donnenberg et al., 1980).

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Cells and virus. Human newborn foreskin (MRHF) fibroblasts and rhesus monkey MA104 cells (Whittaker, M.A. Bioproducts, Walkersville, Md., U.S.A.) were grown in Eagle's MEM with 10% foetal calf serum (FCS) and 25 mM-HEPES buffer. Guinea-pig lung fibroblasts (JH4 clone 1, American Type Culture Collection) were grown in medium F12 (M.A. Bioproducts) with 10% FCS. Vesicular stomatitis virus (VSV) Indiana strain was grown in mouse L cells and used in IFN assays. A single stock of HSV-2 (strain G) was used throughout these studies.

Guinea-pig model of recurrent disease. Hartley strain guinea-pigs (300 g; Charles River Breeding Laboratories, Wilmington, Mass., U.S.A.) were injected subcutaneously in the right hind footpad with 1 x 10^6 p.f.u. of HSV-2. They were monitored daily for cutaneous lesions scored by two independent observers. Two criteria of disease severity were used. The first was a lesion score: 0, negative; 1, slight to moderate erythema without swelling; 2, severe erythema, swelling and small vesicles; 3, severe erythema, swelling and large vesicles. The uninfected footpads (score, 0) served as controls. Mean lesion scores are the average of the total of these daily scores over the specified time (Bernstein et al., 1986). The second criterion of disease severity was the difference between the sizes of infected and uninfected footpads, measured with calipers (Mitutsoyo, Tokyo, Japan) (Donnenberg et al., 1980). Results are expressed as mean values ± standard error about the mean (s.e.m.). Animals were followed for 3 months for the development of clinical recurrences reported as the number of episodes/animal. A separate recurrence was scored when a new lesion appeared (Donnenberg et al., 1980).

Toxicity. In one series of experiments poly(rI),poly(rC12U) in 0.3 ml saline was injected intravenously (single dose) into groups of four guinea-pigs. In the second series, poly(rI).poly(rC12U) in 50 μl was given as intranasal (i.n.) drops to groups of three guinea-pigs. Animals were exposed to multiple doses at 48 h intervals for 19 days. They were followed for morbidity and/or mortality for 21 days. Saline was used as control.

Virus isolation. Swabs of cutaneous lesions were collected in 1 ml of MEM on day 6 post-infection (p.i.). Tenfold dilutions were inoculated onto MRHF cell cultures in 16 mm wells (Costar). Following 2 h at 37 °C, the cells were overlaid with a 2% methyl cellulose (4000 centipoise) overlay and scored for virus plaques. Ganglia corresponding to the injection site were collected from latently infected animals at 28 days p.i. They were trypsinized (0-25%, 20 min) and co-cultured (6 weeks) on confluent monolayers of MHRF cells as described (Scriba & Tatzber, 1981).

Interferon assays. Antiviral activity in cell culture supernatants was assayed on VSV-infected JH4 cells using a colorimetric assay (Finter, 1969). The microtitre procedure of Havell & Vlček (1972) was used to assay for IFN activity in guinea-pig serum. Each plate contained an uninfected and a VSV-infected cell control. Antiviral titres are expressed as the reciprocal of the highest dilution that protected 50% of the cells.

Virus neutralization. HSV antibody was assayed by the plaque reduction multiplicity analysis previously described, and the extent of virus neutralization was expressed as K values (Aurelian et al., 1970). For comparative purposes, assays were performed simultaneously with the same virus preparations.

Lymphocyte proliferation. Spleen cells (SC) and peripheral blood lymphocytes (PBL) were prepared as described by Donnenberg et al. (1980). They were cultured (2 x 10^6/ml) with 25 μg/ml of u.v.-inactivated HSV-2 or 'mock' (uninfected cell) antigen for 6 days and assayed for [3H]thymidine incorporation in a 4 h pulse. Results are expressed as net c.p.m. (i.e. mean c.p.m. experimental - mean c.p.m. phosphate-buffered saline control). Based on previous findings (Donnenberg et al., 1980) with 'mock' antigen, response was considered positive if it registered >200 net c.p.m.

NK assay. MA104 cells 'mock'-infected with phosphate-buffered saline (PBS), or infected with HSV-2 for 4 h (target cells) were labelled for 1 h with 100 μCi of 51Cr (as NaCrO4; Amersham) at 37 °C, washed three times, resuspended in RPMI 1640 with 50 μM-2-mercaptoethanol, 25 mM-HEPES pH 7.2 and 10% heat-inactivated FCS and dispersed (1 x 10^4 cells/50 μl) in conical bottom microtitre plates (Dynatech Laboratories, Alexandria, Va., U.S.A.) together with PBL (effector cells). Microtitre plates were centrifuged for 5 min at 75 g and incubated for 18 h at 37 °C in a 5% CO2 atmosphere. The supernatants were harvested using the Titertek Supernatant Collection System (Flow Laboratories) and the radioactivity was determined with a Beckman 5500 gamma counter. The 51Cr release was calculated according to the formula: percent specific lysis = ([experimental c.p.m. - spontaneous c.p.m.]/[maximum c.p.m. - spontaneous c.p.m.]) x 100, where spontaneous release was obtained from target cells incubated in medium and maximum release was obtained from target cells incubated (37 °C, 4 h) with 1% Triton X-100. Spontaneous release values for infected or uninfected MA104 cells were always less than 18%. A similar assay was recently described by Weinberg et al. (1986).

NK enhancing activity assay. Triplicate samples of PBL (NK effector cells) were incubated (4 x 10^5 cells/microtitre well) for 2 h at 37 °C in 50 μl of RPMI containing 125 μg of poly(rI).poly(rC12U) and 25 to 50 units of human IFN-α (Lee Biomolecular Research Laboratories, San Diego, Ca., U.S.A.) or in 50 μl of guinea-pig IFN [induced in JH4 cells with poly(rI).poly(rC12U)]. 51Cr-labelled MA104 targets (10^6/well) were added directly to the wells, to give an effector:target (E:T) ratio of 80:1. The plates were incubated at 37 °C for 18 h and the supernatants were harvested and counted. 51Cr-labelled MA104 targets incubated with poly(rI).poly(rC12U) or IFN in the absence of effector cells served as negative control. Radioactivity values in the negative controls were...
similar to the spontaneous release values. The enhancement of NK activity was calculated as described (Sheridan et al., 1985) according to the formula: percent NK enhancement = \[1 - \frac{(~\text{specific lysis effector cells})}{(~\text{specific effector cells} + \text{supernatant})}\] \times 100. In this assay the percent specific lysis by effector cells averaged 24.2 ± 3.

RESULTS

Poly(rI).poly(rC_{12}U) induces IFN activity

The ability of the polynucleotide duplex to induce antiviral activity in guinea-pig cells was studied in JH4 cells treated with 2.5 mg/ml of poly(rI).poly(rC_{12}U) for 1 h at 37 °C. Supernatant fluids collected 24 h later were exposed to 70 °C for 30 min in order to inactivate residual poly-(rI).poly(rC_{12}U) (Ts'o et al., 1976) and assayed for antiviral activity on VSV-infected JH4 cells. Antiviral activity (2000 units/ml) was considered to be due to IFN as it was resistant to pH 2.0, sensitive to trypsin and was removed by adsorption of the supernatant fluids with rabbit antibody to IFN-α (1 × 10^5 neutralizing units/ml; Interferon Sciences, New Brunswick, N.J., U.S.A.) followed by agarose beads coated with goat antibody to rabbit IgG (Sigma) (data not shown).

The ability of poly(rI).poly(rC_{12}U) to induce antiviral activity in vivo was tested in groups of three guinea-pigs each, respectively exposed to five i.n. doses (1.5 μg/g body weight) of poly-(rI).poly(rC_{12}U) or saline (at 48 h intervals). Sera were collected before treatment and 12 h after the last polynucleotide dose. Antiviral activity (80 units/ml) was detected only in the sera from the poly(rI).poly(rC_{12}U)-treated animals after therapy. Sera collected prior to therapy and from the saline-treated guinea-pigs did not have antiviral activity.

Effect of poly(rI).poly(rC_{12}U) on HSV-2 growth in culture

The effect of poly(rI).poly(rC_{12}U) on HSV-2 growth in vitro was tested in MRHF cells under conditions similar to those that induce antiviral (IFN) activity. Briefly, the cells were exposed (1 h, 37 °C) to increasing concentrations (0-0025 to 2.5 mg/ml) of poly(rI).poly(rC_{12}U) or saline followed by virus challenge. Virus titres were determined 48 h later. Extensive (80 to 100%) reduction in virus titres was observed in cells treated with 0.25 to 2.5 mg/ml of poly(rI).poly(rC_{12}U) for 1 h at 24 h before infection (Fig. 1a) or as late as 1 h p.i. (Fig. 1b).

Toxicity

To determine the toxicity of poly(rI).poly(rC_{12}U) for guinea-pigs, groups of four animals were given a single high (400 μg) dose by intravenous injection, or a multiple of five high (400 μg) doses (at 48 h intervals) by the i.n. route. Animals were followed for 21 days, during which time they remained free of any untoward symptoms including fever, weight loss, nose bleeds, upper respiratory symptoms, ulceration and local inflammation.

Effect of poly(rI).poly(rC_{12}U) on HSV-2-induced immunity

Groups of five HSV-2-infected guinea-pigs were given five i.n. doses of poly(rI).poly(rC_{12}U) (1.5 μg/g body weight) or saline beginning 6 h p.i. and at 48 h intervals thereafter. Sera were obtained at 13 weeks p.i. and assayed for virus-specific neutralizing antibody. Antibody titres were similar in both the poly(rI).poly(rC_{12}U)- and saline-treated groups (K = 0.23 ± 0.027 and 0.2 ± 0.05 respectively). Similarly, PBL obtained from the two groups at 40 days p.i. did not differ in their ability to mount a virus-specific proliferative response (net c.p.m. = 30467 ± 8061 and 26280 ± 10364 for the poly(rI).poly(rC_{12}U) and saline groups respectively). On the other hand, the NK activity of PBL and SC from poly(rI).poly(rC_{12}U)-treated animals was higher than that of the lymphoid cells from animals treated with saline, both against ‘mock’-infected and HSV-2-infected targets (Table 1). Furthermore, NK activity (measured against ‘mock’-infected targets) was enhanced by exposure of the PBL in vitro to poly(rI).poly(rC_{12}U) or guinea-pig IFN (produced in JH4 cells). Maximal enhancement (56% ± 8.8% for poly(rI).poly(rC_{12}U) and 68.9% ± 8.2% for guinea-pig IFN) was evidenced by PBL from animals treated with poly(rI).poly(rC_{12}U) in vivo. The NK activity of the PBL from the poly(rI).poly(rC_{12}U) but not the saline-treated animals was also enhanced by exposure in vitro to human IFN-α (Fig. 2).
Fig. 1. Effect of poly(rI), poly(rC12U) on HSV-2 growth in culture. MRHF cells were exposed (1 h, 37 °C) to various concentrations of poly(rI), poly(rC12U) 24 h before HSV-2 infection (a), or to 0.25 (■) or 2.5 (○) mg/ml before infection with HSV-2 (−24 h and −1 h) or at 0 to 4 h p.i. (b) (m.o.i. of 5 p.f.u./cell).

Fig. 2. PBL obtained from HSV-2-infected guinea-pigs treated with saline (□) or with poly(rI), poly(rC12U) (five i.n. doses, 1.5 μg/g body weight) (■) were assayed for NK activity against uninfected MA104 targets following exposure in vitro (2 h, 37 °C) to poly(rI), poly(rC12U) (1, 125 μg), guinea-pig IFN (2, 50 μl) or human IFN-α (3, 25 units; 4, 50 units).

Table 1. Effect of poly(rI), poly(rC12U) on NK activity

<table>
<thead>
<tr>
<th>Effectors from treatment groups* (n = 5)</th>
<th>Effector:target ratio</th>
<th>NK Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Uninfected target</td>
</tr>
<tr>
<td>SC</td>
<td>E:T†</td>
<td></td>
</tr>
<tr>
<td>Poly(rI), poly(rC12U)</td>
<td>80:1</td>
<td>92:16 ± 3:04‡</td>
</tr>
<tr>
<td>Saline</td>
<td>80:1</td>
<td>48:65 ± 1:66</td>
</tr>
<tr>
<td>Poly(rI), poly(rC12U)</td>
<td>40:1</td>
<td>ND§</td>
</tr>
<tr>
<td>Saline</td>
<td>40:1</td>
<td>ND</td>
</tr>
<tr>
<td>PBL (expt. 1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(rI), poly(rC12U)</td>
<td>80:1</td>
<td>10:31 ± 1:34</td>
</tr>
<tr>
<td>Saline</td>
<td>80:1</td>
<td>4:90 ± 4:90</td>
</tr>
<tr>
<td>Poly(rI), poly(rC12U)</td>
<td>40:1</td>
<td>ND</td>
</tr>
<tr>
<td>Saline</td>
<td>40:1</td>
<td>ND</td>
</tr>
<tr>
<td>PBL (expt. 2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(rI), poly(rC12U)</td>
<td>80:1</td>
<td>27:70 ± 7:0</td>
</tr>
<tr>
<td>Saline</td>
<td>80:1</td>
<td>13:80 ± 3:7</td>
</tr>
</tbody>
</table>

* Guinea-pigs infected with HSV-2 (1 × 10⁵ p.f.u.) in the footpad were given five i.n. doses of poly(rI), poly(rC12U) (1.5 μg/g body weight) or saline beginning 6 h p.i. and at 48 h intervals thereafter. PBL and SC were obtained 9 days p.i. n, Number of animals/group.
† E:T, Effector:target ratio.
‡ Results are expressed as mean values ± s.e.m.
§ ND, Not done.

Effect of poly(rI), poly(rC12U) administration on HSV-2-induced cutaneous disease

Groups of 20 guinea-pigs infected with HSV-2 (1 × 10⁵ p.f.u.) in the footpad were given five i.n. doses of poly(rI), poly(rC12U) (1.5 μg/g body weight) or saline starting 6 h p.i. and at 48 h intervals thereafter. They were examined daily for cutaneous disease, and the results are summarized in Fig. 3. In the saline group, severe lesions (score 2.0 ± 0.1) were first seen at 2 days p.i. Maximal severity (lesion score 3.0) was observed on days 5 to 9 and the lesions lasted for at least 14 days. At this time the average lesion score was still elevated (2.0 ± 0.3), for a mean total
HSV-2 therapy with a non-toxic IFN inducer

Fig. 3. Effect of i.n. administration of poly(rI), poly(rC12U) on cutaneous HSV-2 lesions expressed as lesion scores (a) or footpad size (b). Guinea-pigs infected with $1 \times 10^5$ p.f.u. of HSV-2 in the footpad received five doses (1.5 μg/g body weight) of poly(rI), poly(rC12U) or saline beginning 6 h p.i. and at 48 h intervals thereafter. Lesion scores expressed as group averages ± S.E.M. are significantly ($P < 0.01$) lower in poly(rI),poly(rC12U)- (■) as compared to saline- (○) treated animals by unpaired Student’s t-test. Footpad sizes, expressed as group averages ± S.E.M. are significantly lower in the poly(rI),poly(rC12U)- (■) than in the saline- (○) treated animals by unpaired Student’s t-tests (*, $P < 0.05$; **, $P < 0.01$). Uninfected footpads (○) are shown for comparison.

Fig. 4. Groups (n = 5) of guinea-pigs were infected with HSV-2 and treated with poly(rI).poly(rC12U) (ialect) or saline (ialect). Swabs of the skin lesions were collected in 1 ml MEM on day 6 p.i. Ganglia corresponding to the infected sites (lumbosacral) were obtained 28 days later. Results are expressed as average p.f.u. ± S.E.M. 1, ganglionic virus; 2, cutaneous virus.

lesion score of 33.9 (from days 2 to 14). The poly(rI).poly(rC12U)-treated animals remained essentially free of symptoms except for relatively mild lesions (score 1.0 ± 0.5) on days 5 to 8 p.i. for a mean total lesion score of 3.4 ($P < 0.001$ by unpaired Student’s t-test) (Fig. 3a). Similar differences between the two study groups were observed using the footpad size as a criterion of disease severity (Fig. 3b).

Poly(rI).poly(rC12U) treatment (five i.n. doses; 1.5 μg/g body weight at 48 h intervals) was also effective when therapy was initiated before infection (−4 h) or as late as 20 h p.i. Under both regimes, lesions were first seen 5 days p.i. as compared to 2 days p.i. in the respective saline groups. They lasted 2 to 3 days (days 5 to 8 p.i.) as compared to 10 to 12 days (days 2 to 14 p.i.) in the respective saline groups. On the days of maximal severity for the poly(rI).poly(rC12U)-treated group (day 6 p.i.) the average lesion scores in the treated groups were significantly ($P < 0.01$ by two-tailed t-test) lower (1.0 ± 0.6 and 0.6 ± 0.3 for the −4 and 20 h p.i. respectively) than those from the respective saline groups (2.6 ± 0.6). There was no difference between the saline and poly(rI).poly(rC12U) groups when therapy was initiated at 48 h p.i.

Effect of poly(rI).poly(rC12U) treatment on cutaneous and ganglionic virus

The effect of poly(rI).poly(rC12U) treatment on virus replication at the site of the cutaneous lesion was studied in two groups of 10 guinea-pigs each treated respectively with the polynucleotide duplex (1.5 μg/g body weight, five i.n. doses at 48 h intervals) or saline. Lesion swabs were collected at the time (6 days p.i.) of maximal disease severity in both study groups (Fig. 3). Ganglia corresponding to the HSV-2 injection site (lumbosacral) were obtained at 28 days p.i. As shown in Fig. 4, virus titres were significantly ($P < 0.05$) lower in the cutaneous lesions from the poly(rI).poly(rC12U)-treated (24 ± 8.5 p.f.u.) than the saline-treated (73 ± 19 p.f.u.) animals. At 2 and 10 days p.i. virus was isolated only from the saline-treated, but not from
Table 2. Effect of poly(rI).poly(rC12U) treatment of primary HSV-2 cutaneous lesions on the development of recurrent disease*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. animals/total (%)</th>
<th>Duration† (days ± S.E.M.)</th>
<th>No. episodes/animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(rI).poly(rC12U)</td>
<td>11/20 (55)</td>
<td>4.0 ± 1.0</td>
<td>1-5</td>
</tr>
<tr>
<td>Saline</td>
<td>17/18 (94)</td>
<td>6.7 ± 2.4</td>
<td>2-7</td>
</tr>
</tbody>
</table>

* Guinea-pigs infected with HSV-2 (1 × 10^5 p.f.u.) in the footpad were given five i.n. doses of poly(rI).poly(rC12U) (1.5 µg/g body weight) or saline during primary infection (beginning 6 h p.i.) and followed for 3 months after resolution of the primary lesions.
† Starting on the day of onset of clinically evident recurrent symptoms.
‡ Statistical analysis by two-tailed t-test.

The poly(rI).poly(rC12U)-treated animals (data not shown). The titres of ganglionic virus were also reduced in the poly(rI).poly(rC12U)-treated animals but the difference was not significant (Fig. 4).

Poly(rI).poly(rC12U) treatment of the primary infection reduces subsequent recurrent episodes

Guinea-pigs given five i.n. doses of poly(rI).poly(rC12U) (1.5 µg/g body weight at 48 h intervals beginning at 6 h p.i.) or saline were followed for 3 months for the development of recurrent lesions. Only 55% of the poly(rI).poly(rC12U)-treated animals sustained recurrent symptoms as compared to 94% of those in the saline group (P < 0.01), and the number of recurrent episodes per animal was reduced (Table 2). The duration and severity of the individual episodes were similar in both study groups.

Poly(rI).poly(rC12U) treatment of HSV-2-infected animals at the time of recurrent disease did not significantly alter subsequent episodes. Thus, five of 17 (29%) and one of six (17%) animals respectively treated with five i.n. doses (1.5 µg/g body weight) of poly(rI).poly(rC12U) or saline beginning on day 1 of a clinically evident recurrent episode remained free of subsequent recurrences during a 5 month follow-up period. The two groups were also similar with respect to the number of subsequent episodes (1.3 and 1.8 for treated and saline respectively), their duration (9.1 ± 1.7 and 9.0 ± 2.8 days for treated and saline respectively) and their severity (lesion scores 2.7 ± 0.3 and 2.2 ± 0.1 for treated and saline respectively).

DISCUSSION

An important feature of these studies is the observation that i.n. administration of poly(rI).poly(rC12U) during primary HSV-2 infection significantly decreases cutaneous virus titres, presumably thereby reducing the amount of virus available to establish latent ganglionic infection and cause recurrent disease. This decrease is associated with enhanced natural defence mechanisms.

The concept that BRMs may have therapeutic potential in HSV infections is based on the findings that natural immune defences play a decisive role in recovery from HSV disease (Kirchner et al., 1982; Habu et al., 1984; Fitzgerald et al., 1985) and recurrent HSV lesions are associated with immune suppression (Shillitoe et al., 1977; Sheridan et al., 1985, 1987; Cunningham & Merigan, 1983; Donnenberg et al., 1980). Intranasal administration was selected as it is non-invasive and the lung has the highest NK cell activity (Nolibe et al., 1981) and appears to be the most important IFN-producing organ (Ho & Ke, 1970). Intranasal delivery of poly(rI).poly(rC12U) was shown to enhance lung NK activity (Nolibe et al., 1985) and guinea-pigs were protected from systemic HSV infection by i.n. administration of MTP-PE, another BRM (Dietrich et al., 1986). However, to our knowledge, the effect of a BRM on cutaneous HSV-2 disease is still unknown.
Consistent with previous reports for our guinea-pig model (Scriba & Tatzber, 1981; Donnenberg et al., 1980), saline-treated animals first showed severe lesions 2 days p.i., with maximal severity (lesion score 3.0 ± 0.1) observed on days 5 to 8 p.i. Disease lasted until day 12 to 14 p.i. for a total of 10 to 12 days and a total lesion score of 33.4. Relatively mild symptoms (average lesion score 1.5 ± 0.3 detectable only on day 6 p.i.) and a total lesion score of 3-4 were found for the poly(rI). poly(rC12U)-treated animals. Furthermore, treatment significantly reduced the number of animals with recurrent disease and the frequency of recurrent episodes per animal. The significant (P < 0.05) decrease in cutaneous virus titres and the reduced titres of ganglionic virus observed in treated as compared to untreated animals, are consistent with the interpretation (Blyth et al., 1980; Klein, 1980) that treatment decreases the establishment of ganglionic infection and recurrent disease by reducing the titres of cutaneous virus. However, disease pathogenesis may affect therapeutic efficacy. Indeed, the frequency of recurrent episodes in our model and in humans is significantly lower (one episode per 4 to 8 weeks) than that described for the vaginal model in which acyclovir therapy had no beneficial effect on recurrent disease (Bernstein et al., 1986).

While our data are consistent with previous findings regarding the ability of poly(rI). poly(rC12U) to induce IFN production (Ts'o et al., 1976; Greene et al., 1978) and stimulate NK activity (Nolibe et al., 1985; Zarling et al., 1980), the exact mechanism whereby it exerts anti-HSV activity remains unclear. The relatively low titres (80 units/ml) of serum IFN may reflect low IFN induction potential, or suboptimal sampling time (Kern et al., 1975). However, IFN detection, and the significantly enhanced cytotoxicity of PBL and SC populations, suggest that poly(rI). poly(rC12U) and/or stimulated lung cytotoxic cells are systemically distributed following i.n. delivery. Presumably cytotoxicity is mediated by NK cells, as it is not H-2 restricted, it is enhanced by in vitro exposure to IFN or poly(rI). poly(rC12U) and HSV-2-infected targets are lysed preferentially (Weinberg et al., 1986). Furthermore, cytotoxic cells are non-adherent and their activity is prevented by complement-mediated lysis with anti-sialo GM1 antibody (L. Aurelian, unpublished results).

Clinically, the findings are potentially significant since i.n. delivery is non-invasive and there was no detectable toxicity. In conjunction with drugs, such as acyclovir, that are designed to inhibit virus replication directly, poly(rI). poly(rC12U)-mediated immunopotentiation may greatly improve the clinical management of the patient with cutaneous HSV-2 disease.

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