Pathogenesis of Herpes Simplex Virus in Congenitally Athymic Mice: the Relative Roles of Cell-mediated and Humoral Immunity


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SUMMARY

Athymic nude (nu/nu) mice were inoculated in the ear pinna with 10⁴ p.f.u. herpes simplex virus type 1 (strain SC16). Initially, the virus was observed to replicate in the pinna, spreading via a neurological route to the dorsal root ganglia, spinal cord, brain and adrenal glands. Following the transfer of lymphoid cells from day 7 herpesvirus-infected hairy immunocompetent donors into infected nude mice, virus was not isolated from the pinna and nervous system of the majority of the mice. The passive transfer of neutralizing polyclonal anti-herpesvirus serum or neutralizing monoclonal anti-gp D serum did not reduce infectivity in the pinna, but markedly reduced the amount of virus in the ganglia and spinal cord. These data suggest that neutralizing antibodies play an important role in restricting the movement of virus to the nervous system, whereas cell-mediated immune (CMI) mechanisms are essential for eliminating virus from the pinna.

INTRODUCTION

Herpes simplex virus is characterized by an acute mild, local infection in humans. Recovery is the rule but the virus commonly becomes latent in related sensory ganglia. No infective virus can be demonstrated but, when sensory ganglia are explanted, virus may regularly be reactivated (Baringer & Swoveland, 1973). In man, the latent virus may from time to time reactivate in vivo either simultaneously or in response to various stimuli. As a result of centrifugal spread the virus may establish a recurrent infection at or near the original site of infection and may even give rise to a recrudescent lesion there (for review, see Wildy et al., 1981). Various laboratory models have been used to investigate these phenomena. Among the best is the mouse-ear model in which all the phases of infection (acute, latent, reactivation, recurrence and recrudescence) have been demonstrated (Hill et al., 1975; Hill & Blyth, 1976).

It is likely that the control of latency will be modulated immunologically; for example, reactivation and recrudescence seem to be associated with defects in the effector arm of immunity (Wilton et al., 1972; Kapoor & Basu, 1979; Donnenberg et al., 1980). We have therefore embarked on a systematic immunological study of the mouse-ear model. To date, we have accumulated a body of data mainly on the cell-mediated aspects of herpesvirus infections (Nash & Gell, 1980; Nash et al., 1980a, b, 1981a, b).

In this paper we make use of the nude (nu/nu) mouse which provides the opportunity to study the role of immunological factors in pathogenesis of herpesvirus infection. It will become apparent that the progress of infection is affected by cell-mediated immune (CMI) mechanisms and also to an unexpected extent by antibody.

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METHODS

Mice. Inbred Balb/c mice and congenitally athymic nude (nu/nu) mice (4 to 8 weeks old) on a Balb/c background were bred in the department. MF1/olac athymic nude (nu/nu) mice were obtained from Olac, Bicester, Oxford, U.K.

Virus strains. SC16, a clone of herpes simplex virus type 1 (HSV-1) oral isolate (Hill et al., 1975) was used for subcutaneous (s.c.) injections in the pinna. CI(101)TK−, a bromodeoxyuridine-resistant TK− deficient mutant of strain CI(101) (Dubbs & Kit, 1964) was used for intravenous (i.v.) injections. All stocks were prepared in BHK21 cells and stored at -70 °C until required.

Inoculation of virus and measurement of ear swelling. Strain SC16 (10⁴ p.f.u. in 20 μl Eagle’s Glasgow-modified medium) was inoculated s.c. into the pinna of the left ear of anaesthetized mice. The thickness of ears on successive days was measured using a Mitutoyo Engineer’s screw gauge micrometer. The results were expressed as the difference between infected and uninfected pinnae (Nash et al., 1980a).

Infectivity assays. The left ear pinna, cervical dorsal root ganglia (C₂, C₃ and C₄), coeliac ganglia, spinal cord (2 to 3 mm² portion of cervical segment adjacent to C₂, C₃ and C₄ ganglia), brain, adrenal glands, heart, kidneys, eyes, lungs, liver, spleen and draining lymph nodes (LN) were removed from nude mice and stored at -70 °C until required. Organs were homogenized separately in 1 ml Eagle’s medium supplemented with 10% (v/v) tryptose-phosphate broth and 10% (v/v) calf serum (ETC). The infectious virus was assayed using BHK21 cells (Russell, 1962).

Lymphoid cell suspensions. For adoptive transfer studies donor Balb/c lymphoid cells were obtained from three sources. (i) Immune LN cells: these cells were obtained from the draining LN of Balb/c mice infected in the pinna with 10⁴ p.f.u. SC16 7 days previously (Nash et al., 1980a, b). (ii) Immune LN and spleen cells: these cells were obtained from Balb/c mice which had been infected 7 days beforehand with 10⁴ p.f.u. CI(101)TK− intravenously (i.v.) and 10⁴ p.f.u. SC16 s.c. in the pinna (Nash et al., 1981a). The draining LN and spleens were collected; cell suspensions were made separately and equal numbers of lymphoid cells were mixed. (iii) Normal LN cells: Balb/c lymph nodes were also collected from normal unimmunized mice. After collection, the lymph nodes were gently homogenized, the resulting cell suspensions filtered through muslin gauze and washed twice in HEPES-buffered Eagle’s medium. Spleen cells were prepared separately using the same procedure (Nash et al., 1980a). Immune and normal lymphoid cells were transferred i.v. to infected nude mice of the same genetic background.

Antibodies

Rabbit anti-HSV-1 serum. Hyperimmune serum (50% neutralization titre 10⁻¹) was obtained from the departmental stock. The antiserum had been raised by several intramuscular injections of freeze-dried extracts of RK13 cells infected with HSV-1 (strain HFEM) emulsified in Freund’s adjuvant (Watson et al., 1966).

Monoclonal antibodies. Monoclonal antibodies (LP₁, LP₂ and LP₃) were prepared by fusing spleen cells from Balb/c mice, hyperimmunized with HSV-2-infected Balb/3T3 cells, with the myeloma cell line NS₁. A detailed description of the preparation and characterization of these antibodies will appear elsewhere (C. S. McLean et al., unpublished results). All the antibodies are IgG and cross-react with HSV-1-infected cells in radioimmune assay and immunofluorescence tests. LP₂ neutralizes both HSV-1 and HSV-2, while LP₁ and LP₃ neutralize neither. Antibody LP₁ reacts with a 65 000 mol. wt. phosphorylated polypeptide from lysates of HSV-1- or HSV-2-infected cells and from lysates of purified virions. This polypeptide has the characteristics of VP16 of HSV-1. LP₂ and LP₃ precipitate a heterogeneous group of glycosylated polypeptides in the 58000 to 63000 mol. wt. range. The
Herpes simplex virus infection in nude mice

Two groups of mice (i) congenitally athymic nude and (ii) hairy Balb/c mice were infected with 10^4 p.f.u. of SC16 in the left pinna and on successive days the ear thickness response was measured. Hairy mice produce a much increased ear swelling but nude mice showed little inflammation. Presumably, the presence of T cells in hairy mice contributed to the response (Fig. 1).

Three mice from each group were killed on days 1, 3, 5, 8, 14 and 19 after infection and virus infectivity was assayed in different organs. In nude mice the virus appeared to spread sequentially from the left ear to left dorsal root ganglia, spinal cord, brain and left adrenal

RESULTS

Primary ear swelling response and spread of virus in congenitally athymic mice

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Neutralizing antibody determinations. Neutralizing antibody activity was determined using flat-bottom (Linbro) microculture plates. Dilutions of antiserum were mixed with an equal volume of a fixed amount of virus and held overnight at 4 °C in small bottles. Virus infectivity was later assayed using BHK21 cells. Each serum was assayed in triplicate. Neutralization of virus infectivity by 50% plaque reduction was considered the endpoint. Normal mouse serum was always set up in parallel for a control. Antibody titres were expressed as the reciprocal of serum dilution.

Determination of total anti-herpesvirus binding activity. Total anti-herpesvirus binding activity was measured using 125I-labelled staphylococcal protein A. (C. S. Mclean, unpublished results). BHK21 monolayers were grown in flat-bottom microculture wells and infected at high multiplicity (10 p.f.u./cell). After 7.5 h the cells were fixed in 2% glutaraldehyde. Dilutions of antiserum were then added to the wells. Forty-five min later the cell monolayers were washed with phosphate-buffered saline, and bound 125I was estimated using a gamma counter (Philips). Antibody titres were expressed as the reciprocal of serum dilution.

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A. K. Kapoor and Others

Table 1. Infectious herpes simplex virus titres in various tissues of hairy and athymic nude mice at different days post-infection

<table>
<thead>
<tr>
<th>Day</th>
<th>HSV titre (log_{10} p.f.u./organ)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contralateral adrenal gland</td>
</tr>
<tr>
<td></td>
<td>Hairy</td>
</tr>
<tr>
<td>1</td>
<td>3.7</td>
</tr>
<tr>
<td>(3.5-3.9)</td>
<td>(3.3-3.5)</td>
</tr>
<tr>
<td>3</td>
<td>3.9</td>
</tr>
<tr>
<td>(3.7-4.1)</td>
<td>(3.5-4.1)</td>
</tr>
<tr>
<td>5</td>
<td>3.9</td>
</tr>
<tr>
<td>(3.8-4.0)</td>
<td>(4.4-4.7)</td>
</tr>
<tr>
<td>8</td>
<td>0.9</td>
</tr>
<tr>
<td>(0-2.9)</td>
<td>(5.3-5.5)</td>
</tr>
<tr>
<td>14</td>
<td>&lt;0</td>
</tr>
<tr>
<td>(5.4-6.1)</td>
<td>(3.8-4.3)</td>
</tr>
<tr>
<td>19</td>
<td>&lt;0</td>
</tr>
<tr>
<td>(5.4-5.6)</td>
<td>(3.3-4.3)</td>
</tr>
</tbody>
</table>

* Mice were infected in the left ear pinna with 10^4 p.f.u. SC16.
† Geometric mean is shown (range given in parentheses).
‡ The second, third and fourth dorsal root ganglia were dissected from each mouse and pooled.
§ Ipsilateral adrenal glands contained infectious virus on day 14 after infection.

Infectious virus in the contralateral adrenal gland (Table 1). Two of three remaining nude mice that were sick on the 19th day after infection were found to have infectious virus in the contralateral adrenal gland. Furthermore, in infected nude mice virus was isolated simultaneously from the left coeliac ganglion and left adrenal gland, suggesting spread of virus through the coeliac plexus. Occasionally, virus was also isolated from the left eye, left kidney and heart on the 14th day. Virus was not isolated from blood, lymph nodes, spleen, Liver, lungs and testes of nude mice.

Effect on the course of herpesvirus infection of transferring immune lymphoid cells or antibodies

It was clear that virus remained localized in left ears of nude mice for 3 days after infection and apparently virus-spread from the left ear to the left dorsal root ganglion occurred between the third and fifth days after infection. In view of these findings, nude mice were infected for 3 days before receiving immune lymphoid cells (4 x 10^7/mouse) or anti-herpesvirus antibodies. The mice were killed 11 days after transfer; at this time, infectious virus could be isolated from several tissues of unprotected mice (Table 1). In one experiment the mice were killed 6 days after transfer.

In a set of four independent experiments (Table 2), control groups of infected mice which received no cell or antibody transfers behaved very much alike and in accordance with previous results (Table 1). When immune LN cells were transferred after 3 days, considerable protection was observed but this was variable, for example almost complete clearance in the ear (experiment 2) against much reduced virus titres (experiment 1). However, virus was not isolated from the nervous system (experiments 1, 2 and 3), except in certain mice where no protection was observed. Greater protection was observed when immune LN and spleen cells were given together (experiment 2). By contrast, transfer of LN cells from non-immune mice had little protective effect either on acute local infection or on centripetal spread of virus (experiments 3 and 4).

In all the above experiments it was difficult to rule out a protective effect of antibodies, because neutralizing antibodies were detected in mice 11 days after normal or immune cell
transfers (experiments 1 to 4). Therefore, hyperimmune rabbit anti-herpesvirus serum was given (100 μl/mouse) to one group of infected nude mice (experiment 4). The hyperimmune serum failed to clear the virus in the left ears but infectious virus was not isolated from the central nervous system. In this experiment the group receiving normal LN cells produced neutralizing antibodies (titre = 20) and infectious virus was not detected in the ganglia of two out of four mice; moreover, virus could not be isolated from the brain and adrenal glands 14 days after infection.

Passive transfer of monoclonal antibodies to infected nude mice

From the above results it appeared that antibodies could protect nude mice from infection of the nervous system. This observation was investigated further in MF1/olac nude mice injected with various monoclonal antibodies against herpesvirus. The course of infection in MF1/olac nude mice was found to be very similar to that observed in inbred nude mice on the Balb/c background. Therefore, the same schedule of infection and passive transfer of antibodies was followed. MF1/olac nude mice were infected with 10^4 p.f.u. SC16 s.c. in the left pinna and 3 days later monoclonal antibodies (50 μl/mouse) were transferred i.v. All the nude mice were killed on day 14 after infection unless otherwise specified.

In Table 3, three experiments are shown, one of which was done in Balb/c nude mice. Once again, control infected mice which received no antibody behaved much as expected (see Tables 1 and 2). Hyperimmune rabbit anti-herpesvirus serum had little effect on the titre of virus in the pinna but markedly reduced the amount of virus in the spinal cord. Of the three monoclonal antibodies, LP1 (anti-65K protein, experiment A) had no marked effect, while the neutralizing antibody LP2 (anti-gp D, experiments A, B and C) behaved like hyperimmune serum in reducing the amount of virus isolated from the nervous system without much affecting virus titres in the ear. LP3, on the other hand, which is non-neutralizing but reacts with the same glycoprotein had no protective effect in experiment A (in experiment B the data are difficult to interpret).
Table 3. Effect of transferring polyclonal antiserum and monoclonal antibodies to nude mice 3 days after infection with herpes simplex virus type 1

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Nature of antibody transfer†</th>
<th>Ear</th>
<th>Ganglia</th>
<th>Spinal cord</th>
<th>Brain</th>
<th>Adrenal</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>LP₁ (NN)</td>
<td>4.6, 5.1, 5.2, 5.7</td>
<td>1.6, 1.6, 2.0, 3.9</td>
<td>&lt;0, &lt;0, 1.8, 4.5</td>
<td>&lt;0, &lt;0, &lt;0, 5.5</td>
<td>&lt;0, &lt;0, &lt;0, 5.8</td>
</tr>
<tr>
<td></td>
<td>LP₁ (N)</td>
<td>3.6, 4.9, 5.4, 5.6</td>
<td>&lt;0, &lt;0, 1.6, 1.7</td>
<td>&lt;0, &lt;0, &lt;0, &lt;0</td>
<td>&lt;0, &lt;0, &lt;0, &lt;0</td>
<td>&lt;0, &lt;0, &lt;0, &lt;0</td>
</tr>
<tr>
<td></td>
<td>LP₁ (NN)</td>
<td>5.0, 5.4, 5.9, 5.9</td>
<td>2.3, 3.3, 3.3, 4.5</td>
<td>1.6, 4.7, 4.8, 4.9</td>
<td>&lt;0, 4.4, 4.4, 5.2</td>
<td>&lt;0, 4.4, 4.4, 5.2</td>
</tr>
<tr>
<td></td>
<td>No antibody</td>
<td>4.9, 5.7, 6.0</td>
<td>2.6, 2.7, 4.8</td>
<td>&lt;0, 5.0, 3.4</td>
<td>&lt;0, &lt;0, 4.1</td>
<td>&lt;0, &lt;0, 4.1</td>
</tr>
<tr>
<td>B</td>
<td>Rb anti-HSV-1</td>
<td>4.5, 5.0, 5.1</td>
<td>&lt;0, 1.0, 1.0</td>
<td>&lt;0, &lt;0, &lt;0</td>
<td>&lt;0, &lt;0, &lt;0</td>
<td>&lt;0, &lt;0, &lt;0</td>
</tr>
<tr>
<td></td>
<td>LP₁ (N)</td>
<td>4.3, 3.3, 3.5</td>
<td>&lt;0, 1.0, 1.1</td>
<td>&lt;0, &lt;0, &lt;0</td>
<td>&lt;0, &lt;0, &lt;0</td>
<td>&lt;0, &lt;0, &lt;0</td>
</tr>
<tr>
<td></td>
<td>LP₁ (NN)</td>
<td>4.1, 4.0, 5.3</td>
<td>&lt;0, &lt;0, 1.2</td>
<td>&lt;0, &lt;0, 1.4</td>
<td>&lt;0, &lt;0, &lt;0</td>
<td>&lt;0, &lt;0, &lt;0</td>
</tr>
<tr>
<td></td>
<td>No antibody</td>
<td>3.0, 4.2, 5.2</td>
<td>&lt;0, 2.0, 2.3</td>
<td>&lt;0, 2.0, 3.4</td>
<td>&lt;0, &lt;0, &lt;0</td>
<td>&lt;0, &lt;0, &lt;0</td>
</tr>
<tr>
<td>C</td>
<td>LP₂ (N)</td>
<td>4.6, 5.1, 5.5, 5.8</td>
<td>1.2, 2.1, 2.1, 2.3</td>
<td>ND</td>
<td>&lt;0, &lt;0, 1.1, 2.0</td>
<td>&lt;0, &lt;0, &lt;0, &lt;0</td>
</tr>
<tr>
<td></td>
<td>No antibody</td>
<td>6.1, 6.1, 6.3, 7.1</td>
<td>2.1, 3.1, 4.3, 4.5</td>
<td>ND</td>
<td>1.0, 2.1, 4.7, 5.1</td>
<td>&lt;0, &lt;0, 5.7, 5.8</td>
</tr>
</tbody>
</table>

* Experiments A and B were performed in MF1 nude mice; experiment C was performed in Balb/c nude mice. In experiment B, all animals were killed on day 9 after infection. In experiments A and C, animals were killed on day 14 after infection.
† NN, Non-neutralizing antibody; N, neutralizing antibody.
‡ ND, Not determined.

Table 4. Effect of transfer of neutralizing antibody on different days after infection of nude mice

<table>
<thead>
<tr>
<th>Nature of antibody transfer</th>
<th>Time after infection (days)</th>
<th>Ear</th>
<th>Ganglia</th>
<th>Spinal cord</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP₁ (N)†</td>
<td>1</td>
<td>1-4, 4-4, 4-4, 5-1</td>
<td>&lt;0, &lt;0, &lt;0, 1-3</td>
<td>ND</td>
</tr>
<tr>
<td>LP₁ (N)</td>
<td>3</td>
<td>3-3, 4-3, 5-2, 5-3</td>
<td>&lt;0, &lt;0, &lt;0, &lt;0</td>
<td>160</td>
</tr>
<tr>
<td>LP₁ (N)</td>
<td>7</td>
<td>5-2, 5-5, 5-7</td>
<td>1-3, 1-3, 1-6</td>
<td>&gt;160</td>
</tr>
<tr>
<td>LP₁ (N)</td>
<td>10</td>
<td>4-6, 5-9, 5-6, 6-0</td>
<td>1-7, 1-7, 2-2, 3-9</td>
<td>&gt;160</td>
</tr>
<tr>
<td>No antibody</td>
<td>10</td>
<td>4-5, 5-6, 6-7, 6-7</td>
<td>1-6, 3-2, 3-7, 4-2</td>
<td>&gt;160</td>
</tr>
</tbody>
</table>

* All the animals were killed on day 14 after infection.
† N, Neutralizing antibody.

ND, Not determined.
Herpes simplex virus infection in nude mice

Transfer of LP₂ antibody even on day 7 and 10 after infection appeared to reduce the amount of infectious virus in the central nervous system (Table 4). However, in all cases it was evident that antibody (neutralizing or non-neutralizing) did not clear infectious herpesvirus from the pinna by day 14 after infection (Table 3).

Effect of transfer of lymphoid cells and antibodies on primary ear thickness response in nude mice

In all the above experiments (Tables 2 and 3) the primary ear thickness response was also measured every day for 4 days following transfer of cells or antibodies by the method described earlier. Nude mice failed to develop a significant increase in ear thickness even after transfer of immune cells or antibodies.

Discussion

In this report we use the athymic nude mouse as a model for studying the relative role of cell-mediated and antibody-mediated immunity to HSV infection. The nude mice infected s.c. in the pinna with 10⁴ p.f.u. of HSV-1 (SC16) produced a local infection which spread sequentially along the peripheral nerves to the central nervous system. This type of progression has been observed previously in juvenile conventional mice after foot-pad and nasal mucosal infections (Evans et al., 1946; Slavin & Berry, 1943). It has also been observed in more mature conventional mice inoculated with larger doses of virus (Hill et al., 1975) and in mice previously given 10% NaCl s.c. (Olitsky & Schlesinger, 1941; Wildy, 1967; Stevens & Cook, 1971). However, in this study no infectious virus was detected in the cervical ganglia and central nervous system in normal littermates infected with 10⁴ p.f.u. of SC16; probably a latent infection was established, but this was not investigated. These results are in agreement with earlier observations (Hill et al., 1975).

Another interesting finding in nude mice was the detection of virus in the homolateral coeliac ganglia and suprarenals at the same time (14 days after infection) suggesting virus spread via the autonomic nervous system. We believe that the occasional detection of virus in the homolateral kidney and in the heart also occurs via the autonomic nervous system as suggested earlier in pseudorabies virus infection (Field & Hill, 1974).

Evidently, the results of infectious virus assays in nude mice suggest that, in the absence of T cell immunity, virus grows freely after s.c. infection. In contrast to our findings, nude mice are as resistant as their normal littermates following intraperitoneal (i.p.) infection with herpesvirus, suggesting that the route of inoculation has an effect on host susceptibility. Protection of the host after i.p. infection is thought to be a property of natural killer (NK) cell responses, interferon and possibly activated macrophages (Lopez, 1980; Zawatzky et al., 1979). Clearly, such mechanisms are of less importance in mice infected s.c. since Balb/c, MF1 and C57BL10 nude mice are more susceptible than their normal immunocompetent littermates (this report; A. A. Nash & H. J. Field, unpublished results; Nagafuchi et al., 1979).

One of the mechanisms by which T cells aid in the protection of the host against viruses is by cooperating with B cells to produce neutralizing antibodies (Burns et al., 1975). In our model, the passive transfer of a polyclonal neutralizing antiserum resulted in a marked reduction of infectious virus in ganglia. Similar observations have been made on juvenile conventional mice (Evans et al., 1946). Our results differ from those of Openshaw et al. (1979); in their study, transfer of immune anti-herpesvirus serum did not prevent the development of acute ganglionic infection in nude mice. Neither did Wildy (1967) find any effect on the centripetal progression of virus by passively administered antibody in conventional mice. These discrepancies are probably due to differences in virus dose, strain of virus and in routes of inoculation of virus as well as antibody titre.
In order to probe further the mechanism whereby antibody mediates protection, monoclonal antibodies were administered. Only the neutralizing monoclonal antibody (LP₂) was effective in restricting the virus spread; again there was a failure to reduce the infectious virus in the pinna. LP₂ is known to have anti-gp D activity suggesting the presence of a functional epitope concerned with virulence of herpesvirus. However, another anti-gp D antiserum (LP₃) failed to neutralize the infectivity in vitro, which correlates with the failure to protect in vivo. Such data argue for neutralization per se as one of the important mechanisms, limiting the spread of virus to the central nervous system. Probably, this involves neutralization of free virus before it gains access to peripheral nerve endings, as well as at synaptic junctions.

The most effective procedure for reducing or eliminating virus from the pinna and the nervous system was by transferring immune cells. Immune LN cells are known to contain cytotoxic T cells and delayed hypersensitivity/helper T cells, both considered to be important in rapid clearance of herpesvirus from the pinna (Nash et al., 1981b). After transfer of immune LN cells, certain mice escape protection (such animals are being studied more closely to explain this phenomenon); however, the majority of nude mice are clearly protected. Moreover, the nude mice failed to develop increased primary ear thickness even after transfer of immune cells. These observations are being studied further.

Clearly, the immune mechanisms involved in the protection of mice are complex. The nude mouse model reported herein provides a way whereby the relative roles of cellular and humoral immunity can be studied in relation to local cutaneous and nervous system infection.

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