Paralysis of Street Rabies Virus-Infected Mice Is Dependent on T Lymphocytes

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Street rabies virus (SRV)-infected T-lymphocyte-deficient (nude) mice, in contrast to euthymic mice, did not develop hindlimb paralysis prior to death. To document the role of T lymphocytes in rabies virus-associated paralysis, 10⁶ spleen cells from normal immunocompetent euthymic mice were transferred to nude mice and the recipient mice were challenged with SRV. One hundred percent of the reconstituted mice developed paralysis and died. Depletion of T cells from the donor spleen suspension prior to transfer abrogated the development of paralysis but did not prevent the deaths of the recipient animals. Mice receiving 10⁶ rabies virus-immune spleen cells did not become paralyzed and did not die. Nude mice inoculated with either rabies virus-immune or normal mouse serum prior to and following SRV inoculation did not develop paralysis. Immune serum protected the mice, whereas animals inoculated with normal serum died. Central nervous system inflammatory responses in nude mice immunologically reconstituted with normal spleen cells were characterized by diffuse cellular infiltrates in the parenchyma and extensive perivascular cuffing. Perivascular infiltrates included CD8⁺ and CD4⁺ T lymphocytes and Mac-1⁺ macrophage-microglial cells. Inflammatory cells in the pia-arachnoid were limited to CD8⁺ lymphocytes and Mac-1⁺ cells. These observations indicate that paralysis of SRV-infected mice is dependent on T lymphocytes. Whether injury leading to paralysis is mediated by T lymphocytes or by an influence of T lymphocytes on macrophage-microglial cells or other cells remains to be determined.

Immunocompetent animals infected with rabies viruses exhibit a variety of pathological changes in the central nervous system (CNS), including neurophagia, necrosis, perivascular cuffing with mononuclear cells, focal and regional gliosis, and microglial reactions (5, 9, 14, 28, 48). Perivascular cuffs contain lymphocytes, plasma cells, macrophages, and occasionally erythrocytes (14, 28). In cases of severe encephalitis, some of the perivascular cuffs are widened by loosely arranged macrophages and glial cells extending into the parenchyma. Extensive regional accumulations of macrophages in the cerebellar molecular layer and in the cerebral cortex also have been observed (5). The intensity of the inflammatory lesions in rabies virus encephalitis tends to be influenced by the host species infected, the virus strain, and the course of the infection (28).

Hindlimb paralysis is a major neurological sign of rabies or rabies-related virus infections in immunocompetent mice and hamsters (3, 14, 22, 43). In contrast, paralysis seldom occurs during similar infections of T-cell-deficient nude mice (11, 14, 22, 37) or immunosuppressed immunocompetent mice or hamsters (14, 37, 38, 43). There is, however, one single report showing that athymic nude mice inoculated with the highly virulent challenge virus standard fixed rabies virus develop paralysis of the inoculated limb 6 days after infection (12). Nonetheless, the presence of inflammatory cells in the CNSs of rabies virus-infected immunocompetent animals suggests that the occurrence of hindlimb paralysis is dependent on the host immune response to the virus. More specifically, the lack of paralysis in nude mice infected with street rabies viruses (SRV) suggests that T cells play a major role in the induction of paralysis.

To determine the importance of T cells in paralysis, spleen cells were passively transferred from immunocompetent donors to nude mice which were then inoculated with an SRV. The HSD (Harlan Sprague Dawley) nul/+ and HSD nul−/− mice were obtained from Harlan Sprague Dawley, Inc., Indianapolis, Ind., and have been maintained as a closed colony at the Rocky Mountain Laboratories since 1979. Nude mice were maintained in a laminar flow barrier system from reproduction through experimentation. All mice were infected at 8 to 12 weeks of age with a wild-type SRV isolated from an adult bat (Eptesicus fuscus) (17). The 10% brain suspension titer was 10⁶.⁵ mouse intracranial (i.c.) 50% lethal doses (LD₅₀/0.03 ml).

Immunocompetent and athymic nude mice were inoculated i.c., intraperitoneally (i.p.), or in the footpad (FP) with SRV to determine whether the clinical signs of rabies in immunodeficient and immunocompetent mice were influenced by the route of inoculation. Mice were weighed and observed daily for body weight loss and paralysis. The onset of weight loss was defined as the first day on which a steady negative weight change was greater than the range of daily variation in body weight established for uninfected animals (29). This weight loss was always ≥10% and was regarded as the initial clinical sign of the onset of illness. In both groups, the loss of body weight was the initial clinical sign of infection. Paralysis did not occur in the immunodeficient nude mice. In immunocompetent mice, the loss of weight was followed by uneven leg movement and weakness of the hindlegs, which were the initial signs of paralysis. The
development of extensor spasticity was an indication of severe paralysis. In FP-inoculated mice, an initial ascending paralysis usually appeared in the inoculated limb, with eventual development of bilateral paralysis. Paralysis occurred in either or both legs following i.p. inoculation (data not shown). The data in Table 1 show that 90 to 100% of the i.c. or FP-inoculated immunocompetent mice developed paralysis, whereas paralysis occurred in only 20% of the i.p. inoculated immunocompetent mice and in none of the immunodeficient nude mice. Furthermore, none of the nude mice or i.c. inoculated immunocompetent mice survived, whereas survival occurred in 29 and 100%, respectively, of the FP- and i.p. inoculated immunocompetent mice.

To determine whether paralysis developed in immunologically reconstituted SRV-infected animals, 10⁸ normal spleen cells from immunocompetent HSD-nu/+ mice were transferred intravenously to athymic HSD-nu/nu mice. The spleen cells were suspended in phosphate-buffered balanced salt solution supplemented with 2% heat-inactivated fetal calf serum (PBBS-2). Erythrocytes were lysed with 0.16 M NH₄Cl Tris-HCl buffer (pH 7.65). The data in Table 2 show that following i.p. SRV inoculation, 100% of the reconstituted nude mice became paralyzed. The normal spleen cells also were treated with monoclonal anti-mouse Thy 1.2 antibody (Sigma Chemical Co., St. Louis, Mo., or ICN Immunobiologicals, Lisle, Ill.) plus rabbit complement (Cedarlane Laboratories, Ltd., Hornby, Ontario, Canada) as previously reported (41). Following treatment, a sample of the treated cells was stained directly with fluorescein isothiocyanate (conjugated anti-mouse Thy 1.2 monoclonal antibody (Becton-Dickinson Immunocytometry Systems, Mountain View, Calif.) and analyzed for membrane fluorescence with a Becton-Dickinson FACStar flow cytometer. Thy 1.2⁺ cells were not detected in cell suspensions treated with anti-Thy 1.2⁺ plus complement. Furthermore, paralysis never occurred when the donor spleen cells were treated with anti-Thy 1.2 and complement prior to their transfer (Table 2). Interestingly, all nude mice reconstituted with cells from normal euthymic mice died of rabies. Similar transfer experiments were then done with spleen cells from rabies virus-immune mice. In these studies, the Evelyn-Rokitnicki-Abelseth strain of rabies virus was used to initially immunize the HSD nul/+ mice; 0.3 ml of an undiluted Evelyn-Rokitnicki-Abelseth virus pool containing 10⁵.8 mouse i.c. LD₅₀/0.03 ml was inoculated i.p. and followed 7 days later by an i.p. inoculation of 0.3 ml of undiluted SRV. Spleen cells from the immunized HSD nul/+ mice were collected 14 days later. It was determined that nude mice reconstituted with 10⁸ rabies virus-immune spleen cells did not develop paralysis and did not die. However, if the mice were reconstituted with only 10⁶ immune cells, paralysis did develop and the mice died (data not shown). Thus, high, but not low, numbers of rabies virus-immune cells protected nude mice from paralysis and death, whereas all nude mice reconstituted with similar high concentrations of normal cells developed paralysis and died.

Next, we asked whether nude mice reconstituted with rabies virus-immune serum developed paralysis following SRV infection. Mice were inoculated i.p. 1 day before and 2, 4, 7, 10, and 15 days after SRV challenge with a pool of undiluted anti-SRV serum that had an anti-rabies virus neutralization titer of 1:5,120 (39). None of the mice became paralyzed, and all survived the lethal challenge of SRV. Similarly, all control nude mice that received SRV only or a similar regimen of normal mouse serum prior to SRV inoculation also failed to develop paralysis, but these mice died (data not shown). Thus, neither rabies virus-immune nor normal serum could transfer paralysis-inducing activity to SRV-infected nude mice. The immune serum did, however, protect the animals.

Analyses of the inflammatory cells present in brains of nude mice that had been reconstituted with 10⁸ normal spleen cells and then inoculated i.p. with SRV were done at various times postinfection. Three control groups included brains from nude mice that were (i) not treated, (ii) reconstituted with cells only, or (iii) only inoculated with SRV. Brain sections were prepared by the methods of McLean and Nakane (24) and Mori et al. (25). Briefly, 2-μm-thick longitudinal sections of the left hemispheres of the brains were fixed in a 2% paraformaldehyde solution containing 0.075 M lysine and 0.01 M sodium periodate for 6 h at 4°C. The specimens were then embedded in OCT compound (Miles Scientific, Elkhart, Ind.) and quick-frozen in a mixture of acetone and dry ice. Four-micrometer frozen sections were cut in a cryostat, placed on poly-l-lysine-coated glass slides, air dried at room temperature for 30 min, and treated with 4°C acetone for 10 min. Specimens from the right hemispheres of the brains were fixed with 10% buffered formalin, embedded in paraffin, and cut at 4 μm.

CD4⁺, CD8⁺, and Mac-1⁺ cells were detected by using culture supernatant fluids of rat hybridomas producing monoclonal antibodies to CD4 (GK1.5) (7), CD8 (2.43) (35)
and Mac-1 (M1/70.15.11.5, HL) (40) as primary antibodies. All hybridomas were obtained from the American Type Culture Collection. Biotinylated anti-rat immunoglobulin G (heavy plus light chains) (Vector Laboratories, Burlingame, Calif.) was used as the second antibody, and sections were stained by the avidin-biotin-peroxidase complex technique according to the method of Hsu et al. (13), with some modifications. Briefly, before being stained with a primary antibody, the sections were incubated with 5% normal rabbit serum in phosphate-buffered saline (PBS) for 30 min at room temperature to block nonspecific binding sites, washed with PBS, and then incubated with primary antibodies at 4°C overnight. The sections were washed and incubated with the secondary antibody, washed again, and incubated with avidin-biotin-peroxidase complex (ABC kit PK-4000; Vector Laboratories) for 30 min at room temperature. After being washed with PBS, sections were incubated with 3,3′-diaminobenzidine (20 mg of 3,3′-diaminobenzidine and 65 mg of sodium azide in 100 ml of 0.05 M Tris-HCl buffer, pH 7.6, containing 0.005% hydrogen peroxide) and counterstained with methyl green. PBS was used as a negative control for the primary antibodies.

Perivascular cuffing or infiltration of cells into the meninges was seldom observed in the brains of the three control groups or in reconstituted mice during the preclinical stages of infection (days 1, 3, and 5 after SRV inoculations) (data not shown). In contrast, inflammatory reactions were consistently observed in the thalamus, hypothalamus, midbrain, and pons at day 7 (initial day of body weight loss) day 10 (initial paralytic stage) and day 13 (severe paralytic stage) postinfection of reconstituted SRV-infected animals. An example of the perivascular cuffing consistently observed in the reconstituted SRV-infected nude mice is shown in Fig. 1. No CD4+ T lymphocytes were detected in brains of nude mice that received SRV only (Fig. 2a), but they were consistently found in the brains of reconstituted SRV-infected mice within or adjacent to blood vessels in the parenchyma (Fig. 2b) and in the meninges (Fig. 2c). CD8+ T lymphocytes were present in similar areas of the brains of reconstituted mice but were also widely distributed throughout the parenchyma, in which they did not appear to be associated with blood vessels (Fig. 3b).

The data in Fig. 4a illustrate that Mac-1+ cells were not detected in the parenchyma of uninfected nude mice that had been inoculated with normal spleen cells but were present in the parenchyma of nude mice that received only an SRV inoculation (Fig. 4b). Interestingly, a marked increase in the number of Mac-1+ cells was observed in the parenchyma of the reconstituted SRV-infected nude mice compared with the numbers that were detected in nude mice that received only SRV (Fig. 4c). Similar cells also were observed in Virchow-Robin spaces in the parenchyma (Fig. 4d) and in the meninges (Fig. 4e). Thus, a marked inflammatory response occurred, as shown by the presence of CD4+ and CD8+ T lymphocytes and Mac-1+ cells, in the brains of reconstituted SRV-infected nude mice. Furthermore, the inflammatory response correlated with the onset of paralytic symptoms. The lack of a T-lymphocyte inflammatory response in nude mice that were infected, but not reconstituted or reconstituted with cells that did not contain T lymphocytes, correlated with their failure to develop paralysis during infection.

To determine whether there was a difference in the localization or the amount of virus present in brains of nude mice that received SRV only or were reconstituted with cells prior to infection, mice were examined at day 10 (initial paralytic stage) and day 13 postinoculation (severe paralytic stage). The right hemisphere of the brain was prepared as a 10% suspension in minimal essential medium containing 2% bovine serum albumin and was assayed for infectious virus by i.c. inoculation of mice. The LD₅₀/0.03 ml of mouse brain suspension was calculated by the method of Reed and Muench (32) by using 10-fold dilutions inoculated into six mice for each dilution. Direct staining for SRV antigen on sodium periodate-fixed brain sections was performed with fluorescein isothiocyanate-conjugated equine or bovine antirabies virus globulin (Becton-Dickinson Microbiology Systems, Cockeysville, Md.). Infectious virus and SRV fluorescent antigen were detected at both intervals in mice that received SRV alone. In contrast, neither infectious virus nor SRV antigen was detected on day 10 postinoculation in reconstituted mice (data not shown). Although both assays in reconstituted mice were positive on day 13, the infectious virus titers in the reconstituted mice were 100-fold lower than in mice that received SRV alone. At this time, SRV antigen was detected in neurons throughout the brains of mice that received SRV alone or mice that were reconstituted with cells (data not shown). Thus, even though passively transferred normal cells delayed viral invasion of the CNSs and suppressed viral replication in brains of reconstituted mice, these cells were associated with the induction of paralysis and failed to protect the mice from a lethal infection. Furthermore, virus injection of neurons, though probably necessary, appeared not to be sufficient for the induction of paralysis.

Occasionally, rabies virus infection produces an illness in which paralysis is the outstanding clinical feature in the absence of the classic symptoms of hydrophobia and periods of excited behavior (28). The most striking pathological feature of paralytic rabies is a severe inflammation of the spinal cord and brain stem characterized by dense perivascular infiltration by lymphocytes and proliferation of microglial cells in both diffuse and modular fashion (6). The most severe inflammation is associated with marked loss of nerve cells and advanced degeneration of the remaining cells. In the brain, the most consistent inflammatory change consists of perivascular lymphocytic infiltration in the medulla oblongata. The lesions in animals with paralytic rabies, however, are not always confined to the brain stem and spinal cord (28). At the present time, there is a paucity of information concerning the reasons for humans or animals developing paralytic, or “dumb,” rabies instead of classical “furious” rabies. It has been suggested by Iwasaki et al. (14) and Smith (37) that the host immune response is responsible for the local ascending paralysis that develops in immunocompetent mice. Furthermore, it has been observed that the onset of paralysis in rabies virus-infected immunosuppressed mice occurs in parallel with a return of immune responsiveness (38). Our data strongly support the suggestions of Iwasaki et al. and Smith since we have shown that T lymphocytes were required for the development of paralysis in mice experimentally infected with SRV.

It also was shown in our studies that all immunodeficient nude mice died following the development of paralysis if they had been reconstituted with normal spleen cells or low numbers of rabies virus-immune spleen cells prior to SRV infection. In contrast, 100% of the nude mice were protected against paralysis and death when they had been reconstituted with high numbers of rabies virus-immune spleen cells. These data suggest that there is a subtle balance between survival and death during rabies virus infections and that the immune system can be either detrimental or beneficial to
rabies virus-infected animals. Enhancement of rabies virus infections by the host's immune response is not that uncommon and has been previously documented (2, 4, 36, 38). In those studies, it was determined that inadequately immunized monkeys, hamsters, or mice with low levels of anti-rabies virus antibody die more quickly after a rabies virus challenge than do nonvaccinated control animals which receive an identical lethal challenge of rabies virus. This occurrence was termed the early death phenomenon, and it has been shown that both rabies virus-immune B lymphocytes and rabies virus-specific antibody can induce early death (30, 31). This dual role of the humoral immune response to rabies virus remains an enigma, and the mechanism causing early death is unresolved. Nonetheless, even though early death was not apparent in our inadequately reconstituted immunodeficient animals, the mice did develop immunopathological lesions in the CNSs and became paralyzed. Our data illustrating that immunopathological lesions and paralysis did not occur in mice that had been reconstituted with T-lymphocyte-free spleen cells or immune serum emphasize that T cells and not rabies virus-specific antibody or B cells are the component of the immune system causing paralysis.

It is of interest that the route of SRV inoculation did not influence the development of paralysis or death in immunodeficient nude mice. Significant differences in these parameters were observed, however, when different routes of SRV inoculation were used with immunocompetent animals. The majority of the immunocompetent animals developed paralysis following FP or i.c. inoculation; however, paralysis occurred in only 20% of those mice that had been inoculated i.p. Furthermore, all i.c. and 71% of the FP-inoculated mice died, whereas none of the i.p. inoculated animals died. The minimal development of paralysis and lack of mortality

FIG. 1. Hematoxylin and eosin stain showing perivascular cuffing 10 days after SRV inoculation in the brain of a paralyzed nude mouse that received normal euthymic mouse spleen cells 1 day prior to virus inoculation. Magnification, ×1,200.
following the i.p. inoculation of SRV have been previously observed in other euthymic strains of mice (17–19). It was determined in those studies that the failure to develop paralysis and the resistance of SJL/J and CBA/J mice to SRV infection was associated with the high concentrations of neutralizing antibody in serum that quickly developed following the i.p. inoculation of SRV. Similar antibody responses were detected in the i.p. inoculated euthymic HSD nu/+ mice used in this study, whereas low and delayed antibody responses were detected in the nude mice that were reconstituted with spleen cells of unimmunized mice or $10^6$ immune spleen cells (data not shown).

Perivascular cuffing or cell infiltration into the meninges was seldom observed during this study in SRV-infected nude mice that had not been reconstituted with spleen cells. Similar results have been reported by Iwasaki et al., who failed to detect perivascular or subarachnoid cell infiltration in nude mice infected with the challenge virus standard ts 2 strain of fixed rabies virus (14). A CNS inflammatory response did occur, however, if the nude mice used in our studies had been reconstituted with normal spleen cells prior to SRV infection. Furthermore, paralysis occurred coincident with the development of inflammatory lesions in the brain, in which CD4⁺ and CD8⁺ T lymphocytes were consistently present in the meninges and within or adjacent to blood vessels in the parenchyma. CD8⁺ cells, but not CD4⁺ cells, were also detected widely distributed throughout the parenchyma in areas apparently devoid of blood vessels. Interestingly, 14-day-old rats infected with a nonfatal dose of measles virus also exhibit a marked infiltration of T lymphocytes of cytotoxic suppressor phenotype in the brain parenchyma, whereas T-helper-cell phenotypes mainly were located perivascularly (27). The CD8⁺ T lymphocytes diffusely distributed in the parenchyma may be involved in cytotoxic elimination of SRV-infected cells, thereby killing motor neurons and directly impairing CNS motor functions and inducing hind leg paralysis. It has been shown, however, that cells in the CNS express a minimal number of, if any, class I major histocompatibility complex (MHC) molecules (16, 21, 27, 46), and it is known that cytotoxic CD8⁺ T cells kill virus-infected cells when viral antigens are expressed on the cell surface in context with class I MHC molecules (8). Nonetheless, killing could possibly be occurring since cells in the CNS, such as astrocytes, oligodendrocytes, and endothelial cells, can be induced to express class I MHC molecules during certain viral infections (23, 34, 42). Furthermore, it is known that gamma interferon (47) and, to a lesser extent, alpha/beta interferon (26, 47) induce and increase expression of class I MHC molecules on neurons, oligodendrocytes, astrocytes, and microglia. In support of the possibility that interferon may be similarly active here, our earlier studies have shown that alpha/beta interferon is present in high concentrations in rabies virus-infected brains (20). Thus, stimulation of expression of class I MHC molecules may occur during rabies virus infections and permit recognition and cell lysis by cytotoxic CD8⁺ cells.

FIG. 2. Detection of CD4⁺ T lymphocytes in the brains of nude mice. (a) Nonparalyzed control nude mouse that received SRV but was not reconstituted with spleen cells. (b) Densely stained CD4⁺ T lymphocytes within Virchow-Robin space around blood vessels in the parenchyma and (c) in the meninges of paralyzed nude mice that received normal spleen cells prior to inoculation with SRV. Magnification, ×800.
FIG. 3. Detection of CD8\(^+\) T lymphocytes in the brains of nude mice. (a) Nonparalyzed control nude mouse that received SRV but was not reconstituted with spleen cells. (b) Densely stained CD8\(^+\) T lymphocytes not associated with blood vessels but distributed throughout the parenchyma of a paralyzed nude mouse that received normal spleen cells prior to infection with SRV. Magnification, \(\times 1,200\).

In contrast to CD4\(^+\) and CD8\(^+\) cells, Mac-1\(^+\) cells were detected in the parenchyma of unreconstituted nude mice that received only SRV. A similar microglial proliferation in the absence of perivascular and subarachnoid cell infiltration in the CNSs of rabies virus-infected nude mice has been previously noted (14). Interestingly, we observed an increased number of Mac-1\(^+\) cells in the parenchyma, and Mac-1\(^+\) cells were also detected in inflammatory cuffs and meninges of SRV-infected nude mice that had been reconstituted with normal spleen cells. The increase in the number of Mac-1\(^+\) cells was detected prior to the increase in the number of CD8\(^+\) T lymphocytes in the parenchyma. This observation suggests that macrophage-derived cytokines (10) could be inducing T-cell proliferation, enhancing inter-
FIG. 4. Detection of Mac-1+ cells in the brains of nude mice. (a) Parenchyma of a nonparalyzed nude mouse that received only normal spleen cells. (b) Densely stained Mac-1+ cells in the parenchyma of a nonparalyzed nude mouse 10 days following inoculation of SRV. Other panels show Mac-1+ cells 10 days after SRV inoculation in the parenchyma (c), perivascular spaces of the parenchyma (d), and meninges (e) of paralyzed nude mice that received normal spleen cells prior to infection with SRV. Magnification, ×800.

leukin-1 and gamma interferon production, and stimulating an infiltration of T lymphocytes into the parenchyma. At this time we are uncertain whether the microglial-monocyte Mac-1+ cells were infected with SRV. We also do not know whether the increase in the number (activation) of these cells following reconstitution with normal spleen cells was their response to the SRV invasion of the CNS, whether the activation was due to a secondary stimulatory effect caused by T-lymphocyte lymphokines, or both. We did notice, however, that ramified microglial cells were positive for rabies virus antigen following fluorescent staining (data not shown). Because it has been shown that rabies virus does not replicate in vitro in macrophages (45), we assume that the positive staining for rabies virus antigen in the microglial cells was due to the phagocytosis of degenerating infected neurons. Furthermore, it is known that degenerating neu-
rons are frequently surrounded by macrophages and occasionally other inflammatory cells in rabies virus-infected brains (5). Our studies also showed that SRV fluorescent antigen was distributed in similar areas of the brains of nonparalyzed and paralyzed nude mice. Similar results have been shown in studies of human encephalitic (furious) and paralytic (dumb) rabies in that neither the distribution of rabies virus antigen nor inflammation paralleled clinical presentation (44). Thus, the essence of our results and those of Tirawatpong and associates (44) suggests that the localization of virus in the brain does not account for the two different clinical presentations of rabies virus infection.

Although the present experiments do not delineate the relative role of Mac-1+ cells or the role of CD4+ and CD8+ T lymphocytes in the induction of paralysis, the failure to induce paralysis in SRV-infected nude mice following the passive transfer of rabies virus-immune serum or spleen cells that had been treated with anti-Thy 1.2+ plus complement suggests a T-lymphocyte dependency. Furthermore, preliminary experiments with T-cell subsets suggest that paralysis can be induced solely with CD8+ lymphocytes but not with CD4+ lymphocytes (20a). The cytotoxic T-cell subset also has been shown to be the most important T cell involved in the immunopathology of lymphocytic choriomeningitis virus infections of mice (1, 15). In contrast, Borna disease virus-induced meningoencephalomyelitis is caused by a virus-specific CD4+ T-cell-mediated immune reaction (33). Experiments are in progress to fully define the roles of CD8+ and CD4+ lymphocytes and Mac-1+ cells, the possible interactions between these cells, and the role of cytokines present in the CNS in the onset of paralysis of rabies virus-infected mice.

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