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Current and future approaches to the therapy of human rabies

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ABSTRACT

Human rabies has traditionally been considered a uniformly fatal disease. However, recent decades have seen several instances in which individuals have developed clinical signs of rabies, but survived, usually with permanent neurologic sequelae. Most of these patients had received prophylactic rabies vaccine before the onset of illness. The best outcomes have been seen in patients infected with bat viruses, which appear to be less virulent for humans than strains associated with other rabies vectors. In 2003, an article by rabies experts suggested that survival might be improved through a combination of vaccine, anti-rabies immunoglobulin, antiviral drugs and the anesthetic ketamine, which had shown benefit in an animal model. One year later, a girl in Milwaukee who developed rabies after bat exposure was treated with some of these measures, plus a drug-induced (therapeutic) coma, and survived her illness with mild neurologic sequelae. Although the positive outcome in this case has been attributed to the treatment regimen, it more likely reflects the patient's own brisk immune response, as anti-rabies virus antibodies were detected at the time of hospital admission, even though she had not been vaccinated. This conclusion is supported by the failure of the "Milwaukee Protocol" to prevent death in numerous subsequent cases. Use of this protocol should therefore be discontinued. Future research should focus on the use of animal models to improve understanding of the pathogenesis of rabies and for the development of new therapeutic approaches.

1. Introduction

Rabies is the most severe acute viral infection of humans, with a case fatality rate of almost 100%. Although the prompt administration of rabies vaccine and rabies immune globulin after a dog bite or other recognized exposure can reliably prevent the disease, no effective measures have been identified to rescue a patient who has developed signs of illness. The past decade has seen intense interest in the treatment of rabies, in large part because of the survival of a young patient who was treated with a combination of drugs, including the induction of "therapeutic coma" (Willoughby et al., 2005). Unfortunately, numerous subsequent applications of this approach have failed to achieve success. This paper reviews the current status of rabies therapy and identifies promising directions for future research.

2. Rabies virus and the disease

Rabies is usually caused by infection with rabies virus, a single-stranded, negative-sense RNA virus in the genus *Lyssavirus*, family *Rhabdoviridae*; only very rarely is rabies caused by other non-rabies virus lyssaviruses (e.g., Duvenhage virus). Rabies is an acute viral infection of the central nervous system (CNS) that is transmitted by biting animals. Worldwide, most cases of human rabies occur in Africa and Asia as a result of exposure to dogs in rabies-endemic areas. In contrast, most cases in North America are caused by bat rabies virus variants, even though in many cases no bat exposure is recognized.

The incubation period of rabies may last 20–90 days or longer. During most of this period, there is a delay in progression of infection from the site of inoculation (Fig. 1). The virus subsequently spreads in peripheral nerves to the CNS and then within the CNS by fast axonal transport along neuroanatomical connections. After the development of CNS infection, the virus spreads centrifugally along sensory and autonomic nerves to multiple organs.

The prodromal symptoms of rabies are non-specific. Early localized symptoms include paresthesias, pruritus and pain at the site of entry, which are thought to result from infection and inflammation in local sensory ganglia. Eighty percent of patients then progress to encephalitic rabies, which is characterized by episodes of generalized arousal or hyperexcitability separated by lucid periods, autonomic dysfunction, and hydrophobia. The remainder develop paralytic rabies, with quadriparesis and sphincter dysfunction. Both forms of rabies are virtually always fatal. Patients who are

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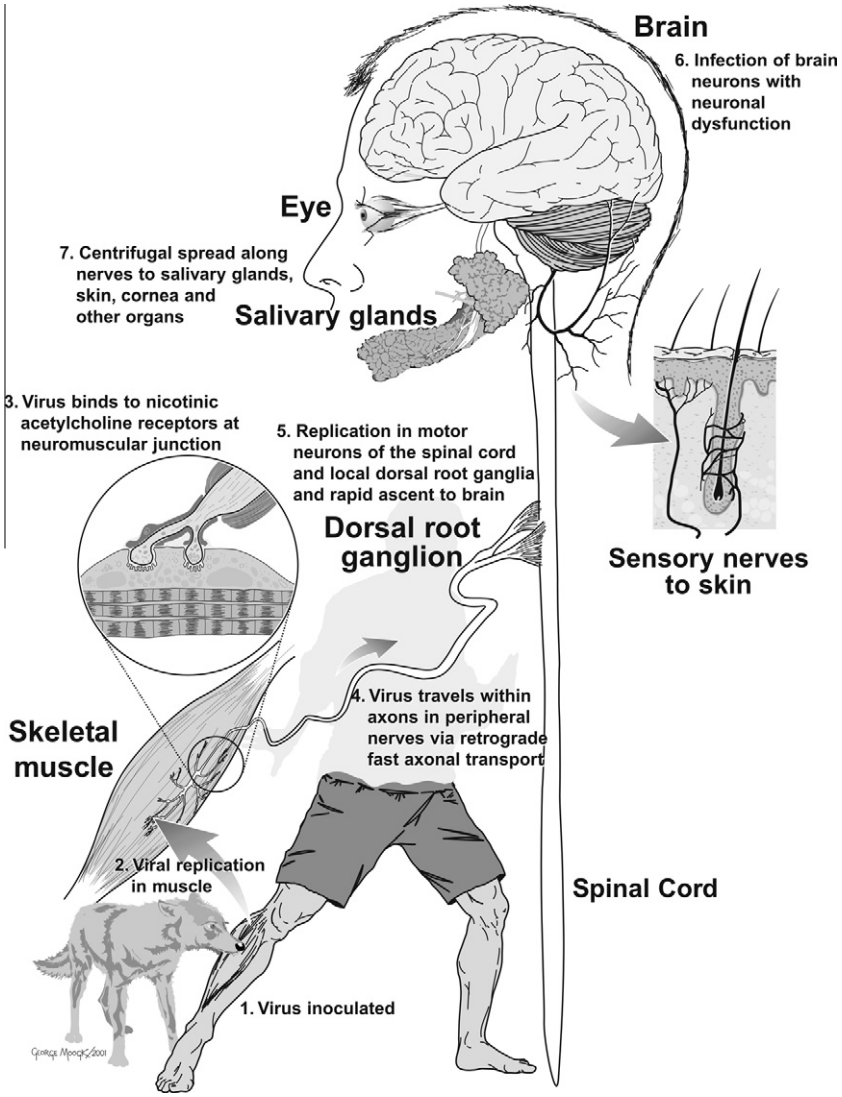

Fig. 1. Schematic diagram showing the steps in the pathogenesis of rabies after an animal bite. From Jackson, A.C., 2007. Pathogenesis. In: Jackson, A.C., Wunner, W.H. (Eds.), Rabies, second ed. Elsevier Academic Press, London, pp. 341–381; Copyright Elsevier.

managed aggressively in critical care units frequently develop cardiopulmonary and other complications, including multiple organ failure.

3. How does postexposure prophylaxis prevent rabies?

Rabies can be effectively prevented after a recognized exposure through postexposure prophylaxis (PEP), providing current recommendations are followed closely (Manning et al., 2008; World Health Organization, 2005). PEP consists of immediate wound cleansing, active immunization with multiple doses of rabies vaccine, and passive immunization with human rabies immune globulin, injected into and around the wound and intramuscularly. The objective of PEP is to prevent rabies virus from gaining access to the nervous system. It is of no proven value after clinical signs of rabies develop.

Infection with rabies virus induces a neutralizing antibody response, but patients may die before antibodies become detectable in the serum or cerebrospinal fluid (CSF). Individuals have occasionally been found to have anti-rabies antibodies in their serum, without a history of neurological illness (Black and Wiktor, 1986; Gilbert et al., 2012; Orr et al., 1988; Ruegsegger et al., 1961). These cases are thought to represent unrecognized natural exposures to rabies virus, leading to immunization without CNS involvement.

4. Why is the prognosis so poor in human rabies?

In contrast to rabies, acute encephalomyelitis caused by West Nile virus, Japanese encephalitis virus and other arboviruses has a lower case fatality rate, though survivors often have severe neurological sequelae (Jackson, 2013b). Because viral clearance from the CNS is essential for recovery, immunocompromised patients tend to develop more severe disease. Neutralizing anti-rabies virus antibodies are thought to be the critical mediator of the immune response in rabies, and there is evidence that antibodies can actually help clear rabies virus infection from infected neurons (Dietzschold et al., 1992). The poor prognosis in rabies may reflect the fact that infection induces immune unresponsiveness, characterized by impaired T-cell function, with altered cytokine patterns, inhibition of T-cell proliferation, and the destruction of immune cells (Lafon, 2013). Recent studies in laboratory animals infected with wild-type (“street”) rabies virus indicate that even

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Table 1
Seven cases of human rabies with recovery. Adapted from Jackson, A.C., 2007. Human disease. In: Jackson, A.C., Wunner, W.H. (Eds.), Rabies, second ed. Elsevier Academic Press, London, pp. 309–340; Copyright Elsevier.

Location	Year	Age of patient	Source of infection	Immunization prior to onset	Neurologic sequelae	References
United States	1970	6	Bat bite	Duck embryo vaccine	None	Hattwick et al. (1972)
Argentina	1972	45	Dog bites	Suckling mouse brain vaccine	Mild	Porras et al. (1976)
United States	1977	32	Laboratory (vaccine strain)	Pre-exposure vaccination	Severe	Tillotson et al. (1977a,b)
Mexico	1992	9	Dog bites	Postexposure vaccination (combination)	Severe[a]	Alvarez et al. (1994)
India	2000	6	Dog bites	Postexposure vaccination (combination)	Severe[b]	Madhusudana et al. (2002)
United States	2004	15	Bat bite	None	Mild	Hu et al. (2007); Willoughby et al. (2005)
Brazil	2008	15	Vampire bat bite	Postexposure vaccination	Severe	Ministerio da Saude in Brazil (2008)

[a] Patient died less than four years after developing rabies with marked neurological sequelae (Dr. L. Alvarez, personal communication).
[b] Patient died about two years after developing rabies with marked neurological sequelae (Dr. S. Mahusudana, personal communication).

in situations in which a robust immune response develops in the periphery, immune effectors are unable to penetrate the blood–brain barrier and clear CNS infection (Roy et al., 2007).

A review of the literature identifies only seven well-documented cases in which humans have survived rabies (Table 1). This excludes two cases which were reported as rabies, but in which the individuals were probably not infected with the virus, since neither developed neutralizing anti-rabies virus antibodies, and one had a highly atypical clinical course and did not require intensive care (Holzmann-Pazgal et al., 2010; Wiedeman et al., 2012). Six of the seven survivors were given rabies vaccine before the onset of illness, suggesting that vaccination played a role in reducing disease severity. Interestingly, the two patients who survived with few or no neurologic sequelae, a 15-year girl from Wisconsin (Hu et al., 2007; Willoughby et al., 2005) and a 6-year old boy from Ohio (Hattwick et al., 1972), were infected with bat rabies viruses. This suggests the possibility that rabies virus variants that circulate in bats may be less virulent for humans than those transmitted by dogs (Lafon, 2005), especially in light of the fact that the number of cases caused by canine rabies virus variants has been many orders of magnitude larger than those due to bat rabies virus variants. Further comparative studies should be performed to confirm if this is really true.

5. Approaches to the therapy of rabies: the "Milwaukee protocol"

In 2003, a group of physicians and researchers with expertise in rabies published an article describing a variety of potential therapies, including rabies vaccination, rabies immune globulin, ribavirin, interferon-α and ketamine (Jackson et al., 2003). Because combination therapies have shown success in the treatment of cancer and a variety of infectious diseases, including human immunodeficiency virus infection and chronic hepatitis C, the authors suggested a similar approach to rabies. The inclusion of ketamine as part of combination therapy was based on animal studies performed nearly two decades earlier at the Institut Pasteur (Lockhart et al., 1991).

In the following year, a combination approach was used to treat a 15-year-old girl in Wisconsin, who had been bitten by a bat on her left hand about a month before admission, and had not received PEP (Willoughby et al., 2005). Neutralizing anti-rabies virus antibodies were demonstrated in her serum and CSF shortly after presentation. She was treated with ketamine (48 mg/kg/day as a continuous intravenous infusion) and given antiviral therapy with intravenous ribavirin and amantadine (200 mg/day given enterally). She also underwent induced therapeutic coma with intravenous midazolam and supplemental phenobarbital, to maintain a burst-suppression pattern on her electroencephalogram. This therapeutic approach has subsequently been dubbed the "Milwaukee Protocol."

The young patient survived with mild neurological deficits (Hu et al., 2007), but as stated in an editorial accompanying the case report (Jackson, 2005), it is unclear why she survived. Good medical treatment in a critical care unit likely played an important role in the favorable outcome, but there is much less certainty about the benefit of any specific therapy. In particular, therapeutic coma was the most dubious and controversial component of the protocol, and the one most likely to cause harm (Jackson, 2005). Therapeutic coma is effective for status epilepticus (Claassen et al., 2012), but there is no clear scientific rationale or other evidence supporting its use for rabies or other CNS infections. The further evaluation of ketamine, including *in vitro* studies of virus-infected primary neurons and experimental studies in mice, has also cast doubt on its therapeutic value (Weli et al., 2006).

Since the "Milwaukee Protocol" was first used in 2004, there have been at least 26 reports of the failure of similar approaches to therapy (Table 2) (Jackson, 2013a), and there have likely been additional instances of treatment failure that have not been published. Notably, the online clinical reference UpToDate® does not recommend use of the Milwaukee Protocol, pending further data (Rupprecht, 2012). Important potential adverse effects of the Milwaukee Protocol include immunosuppression from barbiturates (particularly the short-acting barbiturate thiopental) (Neuwelt et al., 1982), midazolam (Freire-Garabal et al., 1992), ketamine (Wilson et al., 1971), and ribavirin (Powers et al., 1982), and cessation of the therapy may even potentially lead to the immune reconstitution inflammatory syndrome (Reinke et al., 2013). Continued repetition of the Milwaukee Protocol has made it more difficult to move forward with the development of new therapies. In particular, assessment of the protocol's true efficacy has been obscured by claims of survival in two cases in Colombia and Peru that were actually fatal and by the inclusion of a patient who received rabies vaccine before the onset of illness (Ministerio da Saude in Brazil, 2008) and of a young patient in California who never developed neutralizing anti-rabies virus antibodies in the serum or CSF and recovered quickly from the illness (Wiedeman et al., 2012), and likely did not have rabies. Unfortunately, reviews of the protocol's efficacy have not provided literature citations or basic information about the ages, dates and geographical locations of

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Table 2
Fatal cases of human rabies in which patients were treated with the main components of the "Milwaukee Protocol." (Updated from Jackson, A.C., 2011. Therapy in human rabies, in Research Advances in Rabies, in: Jackson, Alan C. (ed.), Advances in Virus Research 79, 365–375; Copyright Elsevier.)

Case no.	Year of death	Age and sex of patient	Source of infection	Country	Reference
1	2005	47 Male	Kidney and pancreas transplant (dog)	Germany	Maier et al. (2010)
2	2005	46 Female	Lung transplant (dog)	Germany	Maier et al. (2010)
3	2005	72 Male	Kidney transplant (dog)	Germany	Maier et al. (2010)
4	2005	Unknown	Dog	India	Bagchi (2005)
5	2005	7 Male	Vampire bat	Brazil	[a]
6	2005	20–30 Female	Vampire bat	Brazil	[a]
7	2006	33 Male	Dog	Thailand	Hemachudha et al. (2006)
8	2006	16 Male	Bat	USA (Texas)	Houston Chronicle (2006)
9	2006	10 Female	Bat	USA (Indiana)	Christenson et al. (2007)
10	2006	11 Male	Dog (Philippines)	USA (California)	Aramburo et al. (2011); Christenson et al. (2007)
11	2007	73 Male	Bat	Canada (Alberta)	McDermid et al. (2008)
12	2007	55 Male	Dog (Morocco)	Germany	Drosten (2007)
13	2007	34 Female	Bat (Kenya)	The Netherlands	van Thiel et al. (2009)
14	2008	5 Male	Dog	Equatorial Guinea	Rubin et al., (2009)
15	2008	55 Male	Bat	USA (Missouri)	Pue et al. (2009); Turabelidze et al. (2009)
16	2008	8 Female	Cat	Colombia	Juncosa, (2008)
17	2008	15 Male	Vampire bat	Colombia	Badillo et al. (2009)
18	2009	37 Female	Dog (South Africa)	Northern Ireland	Hunter et al., (2010)
19	2009	42 Male	Dog (India)	USA (Virginia)	Blanton et al., (2010)
20	2010	11 Female	Cat	Romania	Luminos et al. (2011)
21	2011	41 Female	Dog (Guinea-Bissau)	Portugal	Santos et al. (2012)
22	2011	25 Male	Dog (Afghanistan)	USA (Massachusetts)	Javaid et al. (2012)
23	2012	63 Male	Brown bat	USA (Massachusetts)	Greer et al. (2013)
24	2012	9 Male	Marmoset	Brazil	NE 10 (2012)
25	2012	41 Male	Dog (Dominican Republic)	Canada (Ontario)	Branswell (2012)
26	2012	29 Male	Dog (Mozambique)	South Africa	IAfrica.com (2012); Times Live (2012)

[a] Personal communication from Dr. Rita Medeiros, University of Para, Belem, Brazil.

patients (Willoughby, 2009). The use of the "Milwaukee Protocol" should therefore be discontinued.

6. The way forward

There is an obvious need to re-assess clinical approaches to the treatment of rabies. First, it must be recognized that any aggressive approach to rabies therapy will require the full resources of a critical care unit, with access to medical specialists, and that it will have a high probability of failure. The following should be considered "favorable" factors for initiating aggressive therapy:

- administration of rabies vaccine prior to the onset of illness;
- young age, good baseline health and normal immune function;
- infection by a bat rabies virus variant, rather than a canine rabies virus variant;
- early presence of anti-rabies virus neutralizing antibodies in the serum and CSF; and
- mild neurological disease at the time of initiating therapy (Jackson, 2011).

In addition to supportive critical care, antiviral and neuroprotective approaches should be important components of therapy. There remains uncertainty whether rabies vaccine and/or rabies immune globulin should be included in the therapy (Jackson et al., 2003), but there is no clear evidence that administration of rabies vaccine to a patient with rabies leads to an unfavorable outcome or 'early death' phenomenon.

7. The role of antiviral therapy

Antiviral drugs, which aim to inhibit viral replication and spread, are a logical component of combination therapy for rabies. However, ribavirin and interferon-α are the main currently available agents with known activity against rabies virus, and studies of their efficacy have been very limited (Jackson et al., 2003). Ribavirin inhibited rabies virus *in vitro* (Bussereau et al., 1983; Bussereau and Ermine, 1983), but it was not effective in laboratory animals (Bussereau et al., 1988), and a patient given intrathecal and intravenous ribavirin did not survive (Warrell et al., 1989). In contrast, interferon-α was effective in rabies virus-infected monkeys (Weinmann et al., 1979), but no beneficial effect was seen in three patients given high doses of intrathecal and intravenous interferon-α at an early stage of clinical rabies (Warrell et al., 1989). Because penetration of the blood–brain barrier is essential for therapeutic efficacy in CNS infections without resorting to intrathecal administration, this is a potential limitation for both of these antiviral agents. An experimental study in rats showed that intranasal therapy with ribavirin could bypass the blood–brain barrier (Colombo et al., 2011). Molecular strategies to inhibit the replication of RNA viruses and the associated challenges have recently been reviewed (Bray, 2008; Leyssen et al., 2008). Viral enzymes, particularly polymerases, are potential targets of antiviral drugs (Oberg, 2006). New broad-spectrum RNA polymerase inhibitors, such as favipiravir (T-705) (Furuta et al., 2009), which has shown efficacy in a mouse model of western equine encephalitis (Julander et al., 2009), appears to avoid the toxicity of ribavirin and may be useful in rabies. Oligonucleotide antiviral therapeutics will also be a future area for development (Spurgers et al., 2008).

There is little evidence supporting therapy of rabies with amantadine, apart from one *in vitro* study (Superti et al., 1985). Ketamine was reported to inhibit the replication of rabies virus in cell culture at high concentrations (1–2 mM), by inhibiting genome transcription (Lockhart et al., 1992). After stereotaxic inoculation of a strain of fixed rabies virus into the neostriatum of rats, high-dose ketamine (60 mg/kg given intraperitoneally every 12 h) led to reduced infection in multiple brain regions, including the hippocampus, cerebral cortex, and thalamus (Lockhart et al., 1991). However, more recent evidence from studies in primary neuron cultures and in mice does not support this approach (Weli et al., 2006). Hence, there is no basis for the continued use of ketamine for the treatment of human rabies.

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8. The role of neuroprotective therapies

Treatments are needed to prevent neuronal damage in human rabies, but effective therapies to reduce neuronal injury for acute neurological diseases are currently very limited. A "trial and error" approach to finding an effective treatment is unlikely to succeed. In the case of acute stroke, numerous clinical trials have shown a lack of efficacy of candidate neuroprotective drugs, despite promising studies in animal models (Sutherland et al., 2012).

One approach that has proven effective in trials in Australia and Europe of patients who remained unconscious after witnessed cardiac arrest due to ventricular fibrillation is therapeutic hypothermia, in which body cooling is used to prevent neuronal injury and improve clinical outcomes (Bernard et al., 2002) (The Hypothermia After Cardiac Arrest Study Group, 2002). There is also interest in using hypothermia for traumatic brain injury (Christian et al., 2008) and for acute ischemic stroke (Watson, 2012), but its efficacy has not yet been established in clinical trials. Hypothermia reduces cerebral metabolism, production of reactive oxygen species, lipid peroxidation and inflammatory responses, at least partially explaining its benefit. There is evidence that similar effects may be helpful in rabies, based on recent insights into the role of oxidative stress in its pathogenesis obtained from studies in cultured neurons and laboratory animals (Jackson et al., 2010; Scott et al., 2008). Mitochondrial free radical production is thought to be an important target mechanism for therapeutic hypothermia in ischemia/reperfusion injury (Lampe and Becker, 2011).

In addition to the induction of generalized hypothermia, regional methods can be applied to the head and neck using a cooling helmet (Wang et al., 2004) or by intranasal administration of an inert coolant that rapidly evaporates after contact with the nasopharynx (Busch et al., 2010; Castren et al., 2010). Regional cooling is associated with less adverse systemic effects, and would be expected to produce less impairment of natural or vaccine-induced systemic immune responses, which are essential for viral clearance. Cooling could be maintained for a period of 24 to 72 h, which would provide some time for the development of a systemic immune response in addition to the desired neuroprotective effect. Although rabies virus replication is fairly efficient at lower-than-normal body temperatures (e.g., 34 °C), particularly for bat rabies virus variants (Morimoto et al., 1996), hypothermia might be expected to reduce viral spread due to the inhibition of fast axonal transport (Bisby and Jones, 1978) and trans-synaptic spread. Ideally, new therapeutic approaches should first be evaluated in good animal models of rabies before being used to treat patients.

9. Challenges of studying rabies therapy in laboratory animals and humans

The evaluation of potential therapies for human rabies in laboratory animals is expected to be very challenging. Even the best animal model cannot replicate the management of critically ill patients, which require a variety of resources, including the expertise of multiple specialists, readily available diagnostic investigations, therapies for a wide range of potential systemic complications, and around-the-clock care. A veterinary critical care setting would be the most appropriate setting for this approach, despite the difficult challenges involved.

Trials of experimental therapies in rabies patients are not appropriate at this time, because no known approach has a reasonable chance of demonstrating efficacy. Should such a therapy be developed, its evaluation in patients will be very challenging, because testing will have to be performed at sites with the necessary resources for critical care management, while recognizing that the financial costs will be high and the chance of success low. Most cases of human rabies occur in resource-poor and resource-limited areas of Africa and Asia, where canine rabies is endemic and appropriate facilities are often not available for intensive medical care. No government or non-governmental funding agency is likely to invest in a trial without a high probability of demonstrating efficacy. The potential market for anti-rabies therapeutics, which is mostly located in countries with limited resources, also would not justify significant investment by the pharmaceutical industry. Funding for the prevention of human rabies in developing countries, through canine vaccination and the rapid and reliable provision of PEP after recognized exposures, would provide a much better return on investment.

10. Conclusion

New approaches are needed for the treatment of rabies, which may combine hypothermia, antiviral drugs, and other therapeutic agents. Much work is needed to identify new therapies, which will require a better understanding of basic mechanisms involved in the pathogenesis of rabies.

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