Rabies

Thiravat Hemachudha, MD, Supaporn Wacharapluesadee, PhD, Jiraporn Laothamatas, MD, and Henry Wilde, MD

Corresponding author
Thiravat Hemachudha, MD
Neurology Division, Department of Medicine, Chulalongkorn University Hospital, Rama 4 Road, Bangkok 10330, Thailand.
E-mail: th-cu@usa.net

Current Neurology and Neuroscience Reports 2006, **6:**460–468
Current Science Inc. ISSN 1528-4042

Despite increases in our understanding of rabies pathogenesis, it remains an inevitably fatal disease. Lack of awareness, low level of political commitment to rabies control, and failure to recognize and correlate clinical, laboratory, and neuroimaging features contribute to continuing deaths. Clinical symptomatology, once believed to be unique, may be variable, even in patients associated with lyssaviruses of the same genotype. This article discusses virus transport, the role of virus and host response mechanisms in relation to protean clinical manifestations, and mechanisms responsible for relative intactness of consciousness in human rabies. Differential involvement of the anterior horn cell in furious rabies and the peripheral nerve in paralytic rabies is summarized. Escape mechanisms from host defenses explain why a fatal outcome is unavoidable regardless of therapy. Neuroprotective treatment, using a coma-induction regimen, proves not to be beneficial. Survival of patients with excellent recovery relies on early innate and adaptive immunity plus adequate intensive care support.

Introduction

Rabies kills at least 55,000 people each year but it receives little attention [1••]. Dogs are the major reservoirs and play a pivotal role in rabies transmission, particularly in countries where rabies is endemic [2,3•]. Financial expenditures in Africa ($653 million dollars), Asia ($20.5 million dollars), and the United States ($300 million dollars) in 2003 were mainly for rabies post-exposure prophylaxis (PEP) and for prevention and control of rabies in wildlife [1••].

Genetic analysis of the virus defines variants associated with particular hosts and determines geographic localization and transmission dynamics [3•,4]. It also helps discriminate between renewed activity and reincursion from other regions. The spread of rabies virus from infected dogs to wildlife is common in canine rabies–endemic areas [3•]. The opposite is true in developed countries, where wildlife, including bats, causes interspecies transmission to terrestrial animals [5,6]. Without continued surveillance and canine control in rabies-endemic areas, increasing deaths are unavoidable. China saw a reemergence of rabies, recording 2651 rabies deaths in 2004 [2]. The United Kingdom has reported only 25 cases since 1902. Twenty-four of them were related to dog or cat exposures abroad and one was caused by infection with a European lyssavirus type 2 variant [7]. Tissue transplantation and vascular graft transmitted rabies to 15 individuals [7,8•,9•]. Cryptic cases, without bite history, were associated with bat exposures. Bats are the most common rabies transmitters to humans in the United States [5,10,11]. Rabies virus variants associated with four insectivorous bat species (*Lasionycteris noctivagans*, *Pipistrellus subflavus*, *Tadarida brasiliensis*, and *Myotis californicus*) account for 32 of the 35 indigenous rabies cases that were reported in the United States from 1958 to 2000 [5]. The first two variants were responsible for 19 of 26 cryptic cases. This may suggest an adaptation of virus to these rare and infrequently encountered species [5]. Vampire bats remain an important threat in parts of Central and South America. There were 62 hematophagous and eight nonhematophagous bat-related cases from 1993 to 2002 [11].

Rabies virus belongs to the Mononegavirales order (nonsegmented negative strand RNA viruses), Rhabdoviridae family, and *Lyssavirus* genus [1••]. Studies of pathogenicity, induction of apoptosis, cell receptor recognition, and genetic characterization of viral nucleoprotein (N), phosphoprotein (P), and glycoprotein (G) delineated lyssaviruses into seven genotypes. Current vaccine strains are effective against phylogroup 1 viruses (genotypes 1 [rabies virus], 4 [Duvenhage virus], 5 and 6 [European bat lyssavirus types 1 and 2], and 7 [Australian bat lyssavirus]) but not to phylogroup II (genotypes 2 [Lagos bat virus] and 3 [Mokola virus]). Aravan, Khujand, Irkut, and West Caucasian bat lyssavirus are other tentative genotypes. These isolates were from Central Asia, East Siberia, and the Caucasian region [1••].

G protein is a determinant of virus entry and interaction with cell receptors, promoting virus and cell membrane fusion, axonal or transynaptic transport, and stimulation of neutralizing antibody [12–14]. L, pseudo-, G, and P genes

Table 1. Summary of patients who acquired rabies by tissue transplantation

Year / place	History of exposure	Rabies type	Initial diagnosis	Pitfalls	Donated organ (recipients, *n*)
1978 / USA	None	Classic paralytic form	Guillain-Barré syndrome	Accompanied by seizures/coma; unexplained by Guillain-Barré syndrome	Cornea (1)
1979 / France	Yes (in Egypt)	Classic paralytic form with delirium since onset	Lower motor neuron weakness, encephalitis, myocarditis	Inadequate history taking; relied on negative results of virus investigations	Cornea (1)
1981 / Thailand	?	Classic furious form; patient refused to take food and water and had mental confusion and right leg pain	None; coma and died on admission	Inadequate history taking; no review of history of illness	Cornea (2)
1987 / India	No detail	No detail	No detail	Should exclude any with unknown detail	Cornea (2)
1994 / Iran	Injury by hunting knife on left fingers	Classic furious form with hydrophobia and aggression; local pain at left arm	No diagnosis; coma on admission	Inadequate history taking; no review of clinical illness	Cornea (2)
2004 / USA	Reported being bitten by a bat	4 days before coma developed fever, nausea vomiting, difficulty swallowing, and fluctuating blood pressure (all autonomic dysfunctions); typical of classic rabies	Cocaine/marijuana effect; subarachnoid hemorrhage	History incomplete; progressive neurologic deterioration incompatible with intoxication; extent and location of hemorrhage correlate with presentations?	Iliac artery, liver, kidney (4)
2004 / Germany	Visited India, bitten by dog	Severe headache, mental change, aggressive behavior followed by coma; typical of nonclassic rabies	Cocaine, toxic psychosis	History incomplete; progressive neurologic deterioration incompatible with intoxications; no investigation to explain neurologic deficits	Lung, kidneys, pancreas (3 died); liver, corneas (3 survived)

also contribute to pathogenicity [14–16]. Preservation of neuronal integrity by avoiding apoptosis and escape strategies from the host immune response are crucial for survival [17•,18]. However, such in vitro and in vivo experimental models using gene-manipulated viruses may not mimic the natural disease in humans and dogs, which typically manifests as furious or paralytic rabies [18].

Clinical Screening in Rabies Diagnosis

The clinical presentation of rabies can be divided into classical or nonclassical types. The classic forms of encephalitic (furious) and paralytic (dumb) rabies are almost always attributed to canine rabies. Classic rabies can be divided into five stages: the incubation period, the prodrome, the acute neurologic phase, coma, and death.

The diagnosis of furious rabies should be obvious with a history of exposure and presence of cardinal manifestations (fluctuating consciousness between lucid calm and agitation, phobic spasms, and autonomic stimulation) [18,19]. Unfortunately, many confirmed rabies patients presented in coma or did not manifest such features. One third of cases present with paralysis or other atypical features (eg, gait ataxia, hemiparesis, paraparesis, bulbar muscle dysfunction, myoclonus) [10,18,19]. Atypical or nonclassical rabies is common in bat-related cases. There also have been at least 15 recorded cases of transmission of rabies by tissue transplantation and vascular conduit graft from seven rabies-infected donors from 1978 to 2004 [4,7,9•]. Inadequate history taking or failure to recognize symptoms and signs were responsible for these tragedies (Table 1). Phobic spasms and abnormal behavior may be intermittent [18,19].

Despite similarities between paralytic rabies and Guillain-Barre syndrome (GBS), progression to coma, myoedema, and bladder incontinence clearly differentiate these two conditions [18–20]. Other etiologies, such as metabolic syndromes, intoxication, or the use of illicit drugs, should match the clinical setting. Substance abuse may cause cerebral hemorrhage or infarction, but the extent and location of these must be consistent with the clinical findings.

Neuroimaging and Molecular Techniques in Antemortem Diagnosis

Classic and nonclassic human rabies associated with dog or bat variants result in similar MRI abnormalities in the form of ill-defined hyperintense T2-weighted signals at brainstem, thalamus, hypothalamus, hippocampus and basal ganglia, and subcortical and deep white matter [21•]. MRI details must be analyzed jointly with clinical findings, such as behavioral changes with or without motor deficits, the presence or absence of brainstem signs, the pattern of spinal cord/root involvement, and rapidity of progression from onset to coma [18]. Rabies patients rarely develop coma within the first 3 days after onset. Gadolinium contrast–enhanced lesions appear only when rabies patients become comatose. This suggests that the blood-brain barrier remains intact until preterminal stage. This was supported by the absence of cerebrospinal fluid (CSF) rabies antibody in a rabies patient who had been given a large intravenous dose of human rabies immune globulin (RIG) [22].

CT has no value in diagnosing rabies, but may suggest other "midline" encephalitides from flaviviruses in which edema and minute hemorrhages may be present [18,19].

Rabies serum antibodies were detected in only 25% of dog-related human cases and none were found in the CSF [18]. Rabies virus antigen at neck hair follicles demonstrated by fluorescent antibody technique (FAT) or immunohistochemistry have rarely been of clinical value. Corneal FAT for rabies antigen is unreliable. Isolation of rabies virus in neuroblastoma cells from saliva is sensitive and reliable in rabies antibody–negative patients but requires at least 48 hours for the result. All samples must be maintained frozen with no preservative.

Molecular techniques, such as rapid test–polymerase chain reaction and nucleic acid sequence–based amplification (NASBA), are useful [4,23]. Samples can be kept at 4°C for 24 hours before examination. We diagnosed rabies using NASBA during life in 30 of 32 patients (27 furious, 5 paralytic) by demonstration of rabies RNA in saliva, CSF, urine, and hair follicles (Hemachudha, Unpublished data) [23]. Samples from all sources should be tested simultaneously owing to intermittency of virus shedding. Negative results require repeat testing. Saliva is the best source during the first week. CSF provided more positive results than urine during days 1 to 3, and both became comparable during days 4 to 6. Hair follicles were tested in three furious cases and all were positive. Negative results (two of 32) were seen in repeated tests performed on two paralytic patients. Postmortem examination or needle aspiration of brain via the orbit should be performed in all patients with encephalitis.

Hematogenous Spread

Transmission of rabies virus by solid organ and vascular structure transplantation raises the question of whether viremia is involved [24]. MRI abnormalities in such recipients were significantly different as compared with naturally infected cases in terms of location, extent, and intensity of T2-weighted signal changes [8•,21•]. Rabies virus has been reported to replicate in macrophages, and rabies RNA was detected in blood of clinically ill rabies-infected mice [25].

The presence of rabies antigen within nerve fibers in transplanted organs and blood vessels [9•], and the fact that the virus can be spilled in the peritoneal cavity supplied with free nerve endings, argues against the role of hematogenous spread [24]. Immunosuppression in transplanted recipients would allow more effective replication, thus resulting in more extensive MRI abnormalities.

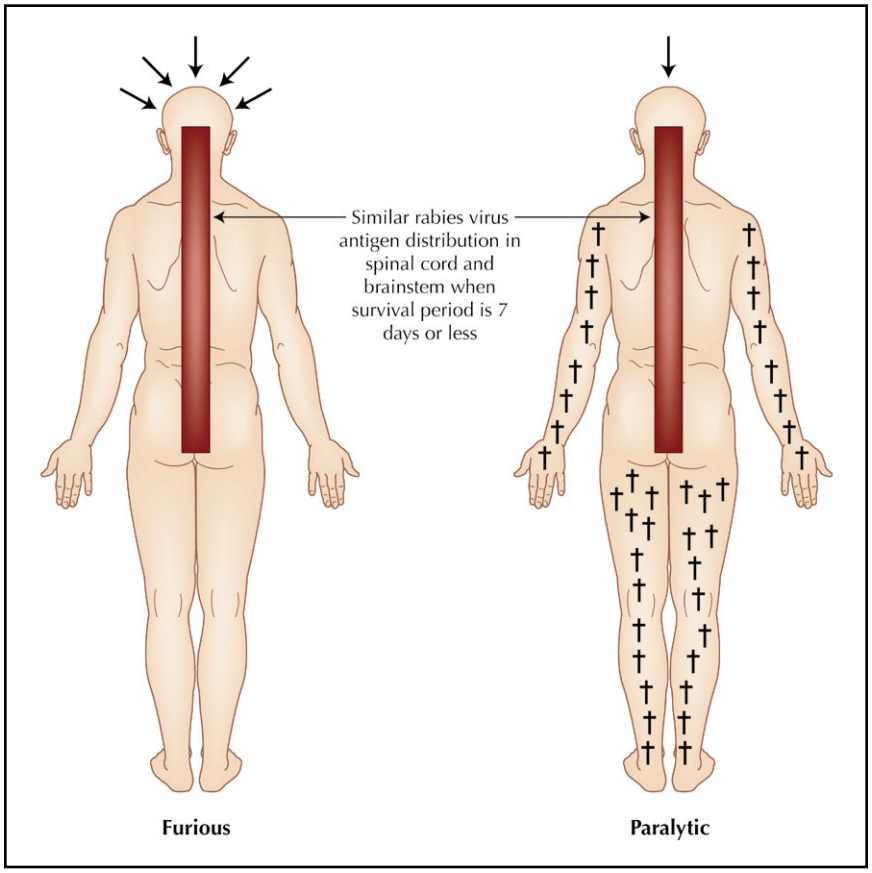

Figure 1. An illustration of furious and paralytic rabies. Similar rabies virus antigen distribution (*bar*) is seen at spinal cord, brainstem, and thalamus in both clinical forms when survival time is 7 days or less. Furious rabies patients exhibit signs indicative of limbic dysfunction (*arrow*) without clinical weakness. Relative sparing of consciousness is seen in most paralytic rabies patients. Pure motor weakness of limb muscles (†) and absence of deep tendon reflexes are hallmarks.

Rabies virus and RNA were not found from blood collected at the time of natural death in four rabid dogs [26]. RNA was found in urine, bladder tissue, trigone, urethral sphincter, and nerves. It was less frequently detected in ureter, renal pelvis, renal medulla, and cortex [26]. Rabies virus antigen was not found in samples of kidney obtained from three rabies patients [4].

Mechanisms of Clinical Diversities in Furious and Paralytic Human Rabies

Central nervous system (CNS) rabies antigen distribution and MRI findings are similar in both furious and paralytic rabies when the survival period is 7 days or less (Fig. 1) [18,19,21•]. Spinal cord, brainstem, thalamus, hypothalamus, and basal ganglia are predominantly involved. Although hippocampus is involved in MRI, it contains less rabies antigen during the early phase.

Specific virus variants in furious and paralytic rabies

Clinical diversities may not be fully explained by virus variants. Only minor nucleotide differences with no specific pattern were found in 1432, 1575, and 894 nucleotide regions from the rabies N, G, and P genes, respectively, of samples obtained from two furious and two paralytic dog-related rabies patients [27]. All differences in the amino acid of G protein were not in an interactive region with receptors known to be responsible for virus pathogenicity. They did not lie in an immunodominant G domain. Likewise, none of the amino acid differences of P protein were within the putative interactive site with dynein. Amino acid patterns of N protein were identical among both human and canine samples from the same geographic location regardless of clinical forms. Analysis of genetic diversity of rabies G genes, isolated from furious and paralytic dogs and from within a single infected dog, revealed that the ectodomain of the glycoprotein was highly conserved among the virus isolates [28]. Comparisons of the cloned sequences of the G gene in the virus population within an intra-host revealed closely related heterogeneous populations with minor substitutions at nucleotide (0.19%) and amino acid levels, negating the role of quasispecies. A single infected dog in Thailand transmitted the furious form of rabies to one patient and paralytic rabies to another [18,19]. Nevertheless, virus variants may play a role in determining paralytic and atypical manifestations associated with vampire and other bat variants [27].

Is it the virus or immune response that determines manifestation of furious rabies?

Studies using experimental models and cell culture–adapted or genetically manipulated viruses confirmed that immunity can contribute to pathogenesis and to accelerated death in animals [29]. This and the direct effect from virus alter neurotransmitter and electrophysiologic functions [19].

Human rabies patients with intact T-cell immunity to rabies and a high concentration of serum interleukin (IL)-2 receptor and IL-6 die earlier (average of 5.7 days) and present with furious rabies. Those lacking such responses survive longer (average 11 days) and present with paralytic rabies [19,20]. The initiating event may start at the brainstem, where rabies antigen is preferentially localized. Production of cytokine-chemokine-nitric oxide by rabies-infected cells leads to functional modification of the limbic system and stimulation of the hypothalamic-pituitary-adrenal axis. The p55 kD tumor necrosis factor-α receptors may be activated in furious rabies. Rabies virus antigen is thus recognized. This is followed by recruitment of immune cells and intensification of limbic symptoms. Vβ8 T cells are stimulated by rabies virus nucleocapsid superantigen, resulting in reamplification of the cytokine cascade and exaggerating disturbances of the limbic and sympathetic nervous system [19].

The immune hypothesis is based on preferential localization of rabies antigen or protein at similar location in both forms. This may not be the case when distribution of rabies gene, not the antigen, is examined. The first MRIs with almost identical patterns were done in five patients 48 hours or later after clinical onset [21•]. It remains possible that each clinical entity may have its own particular MRI pattern when examination is done earlier.

Two different patterns of MRI abnormalities as well as distribution of rabies virus RNA and cytokine mRNA responses were found in naturally infected furious and paralytic dogs at early stage (Hemachudah, Unpublished data). A more widespread distribution of rabies gene and MRI abnormalities, involving all brain regions in furious rabies, may be an initial event followed by immune responses.

Pathogenesis of human paralytic rabies

Peripheral nerve issue

Paralytic rabies was initially recorded in 1887 [20], and this term should be used instead of "dumb rabies" because "calm" clinical features can be seen during the precomatose stage in both forms. It is the pure motor weakness of proximal musculature and longer survival time that characterize this entity. Whether weakness is caused by anterior horn cell or peripheral nerve dysfunction has been debated since the Trinidad paralytic rabies outbreaks (1929 to 1939), which were associated with vampire bats. Acute autoimmune motor axonal neuropathy and polio-like weakness caused by anterior horn cell dysfunction in flavivirus infection share similar clinical features with paralytic rabies [19,20].

We demonstrated differential involvement of peripheral nerve (most likely demyelination) in three paralytic patients, with multifocal demyelination and length-dependent sensory neuropathy in one, and severe reduction in conduction velocities and marked prolongation of distal latencies in another. There was progressive loss of motor and sensory amplitudes without accompanied denervation potentials during sequential examinations on days 3, 4, 6, and 8 after onset in a third patient who also had early abnormalities in late response, indicative of proximal nerve segment involvement [30••]. Anterior horn cell was involved in furious rabies, even without demonstrable clinical weakness (Fig. 2). Neuropathic pain, a local prodrome, was due to dorsal root ganglionopathy based on evidence of absence or progressive decline in sensory nerve action potential amplitudes in the bitten segment [30••].

Rabies virus as ribonucleoprotein or whole virion can be transported via retrograde axoplasmic flow from the peripheral inoculation site via peripheral nerves to the CNS [13,14]. An interaction between P and dynein motor protein via the LC8 binding motif and G protein (in case of whole virion) is required for the transport mechanism. Which mechanism dominates is controversial. The precise steps for G-mediated transport are not known, but neurotrophin p75 NTR may be a factor [31]. G protein can specifically bind to p75 NTR, and ligand p75 NTR complexes are internalized via clathrin-coated pits into early endosomes. They then can move in a retrograde manner [13,14].

Retrograde axonal transport may not be the sole mechanism. The challenge virus standard (CVS) and not the street viruses were used in nonhuman primate models [4,32]. A superficial wound or scratch is sufficient to cause infection in bat-related cases [10]. More effective local propagation in dermis and fibroblasts than in muscle cells at neuromuscular junctions has been demonstrated in some bat variants [10,18,19]. Local neuropathic pain at the bitten area or extremity is more common (70% vs 30% in dog-related cases) [19]. Anterograde transport along sensory pathways may also play a role.

Mechanisms responsible for peripheral nerve injury in paralytic rabies

There may be more than one mechanism involved in mediating peripheral nerve damage in paralytic rabies [20]. Both axonopathy and demyelination were described in paralytic rabies patients based on electrophysiologic and pathologic examinations. Sheik et al. [33] demonstrated scanty inflammation in the spinal cord and nerve roots in a Chinese paralytic patient who presented with acute motor sensory axonal neuropathy [33]. Activated macrophages that were HLA-DR positive and with Wallerian-like degeneration were found more abundantly in ventral than dorsal nerve roots. Peripheral nerves appeared almost normal. Co-localization of human IgG and rabies N protein and human IgG and C3d were found on axons from the ventral roots. This suggests an antibody-mediated complement attack at rabies virus–containing axons [33].

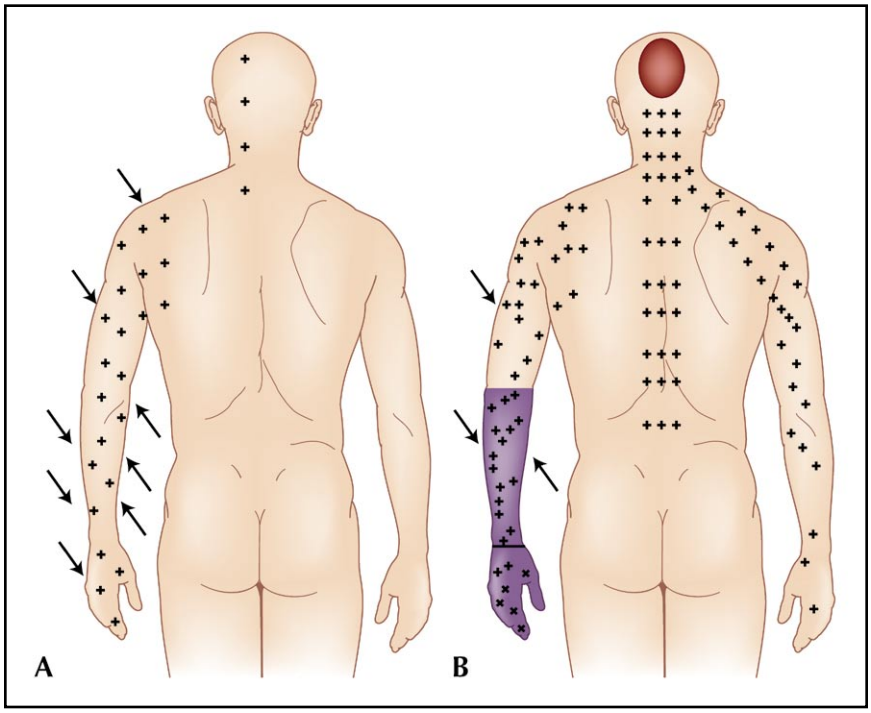

Figure 2. Anterior horn cell involvement in a furious rabies patient who was bitten on the left hand and had local neuropathic prodrome (as seen from the back of the patient). Electrodiagnostic studies were performed on three occasions. **A,** On day 3 after onset, the mental state was clear. Pain was present in the left arm (*arrows*). The only abnormality was the presence of abundant fibrillations and positive sharp waves in left C5–C8 limb and cervical paraspinal muscles (+). Motor and sensory functions were intact. Diminished to absent deep tendon reflexes were noted on the left arm. On day 5 (not shown in figure), there was approximately 50% reduction of sensory nerve action potential (SNAP) amplitudes in the left upper limb nerves compared with those obtained on the right side. Diminished pinprick sensation up to elbow was noted (*shaded area*). Pain became intense. **B,** By day 6, there was a further reduction of SNAP amplitudes on the left upper extremity along with progression of fibrillations and positive sharp waves involving bilateral C5–C7 limb and paraspinal muscles. Motor conduction studies, including F-waves, remained normal. Mild weakness of left hand and wrist muscles was detected. Pain was less severe and tolerable. There was a slight progression of diminished sensation on the area above the left elbow (*shaded area*) along with absence of deep tendon reflexes and joint position sense of the left arm. He was confused and disoriented (*circle*), and he died on day 8.

Pathologic findings of three paralytic rabies patients showed moderate to severe inflammatory infiltration by T cells at dorsal and ventral spinal nerve roots and dorsal root ganglia [21•,30••]. The spinal gray matter was minimally inflamed, with perivascular cuffing and some degree of microglial proliferation. Anterior horn cells were intact. Luxol fast blue stain also showed peripheral demyelination. Inflammation was found predominantly in spinal cord and much less in spinal nerve roots in two furious rabies patients. However, inflammation of the spinal cord is not a constant finding in furious rabies, whereas absence of spinal cord inflammation is almost universal in paralytic rabies [18]. Findings of spinal root and peripheral nerve demyelination and inflammation were in accord with a previous histopathologic report performed on 11 paralytic patients [20]. None of our paralytic rabies patients had anti-GM1, -GD1a, -GalNAc-GD1a, -GD1b, -GT1a, and -GQ1b ganglioside antibodies nor did they have rabies antibody in the CSF [20,30••]. Coexisting inflammation of spinal nerve roots and demyelination suggests a T-cell–mediated immune attack on peripheral nerve antigen. None of our paralytic rabies patients had cellular immunity against rabies antigen [19,20].

Escape Phenomenon of Spinal Cord and Brainstem

Both forms of patients usually remain alert until the preterminal stage [19]. Brainstem functions remain almost throughout the whole course. An electroencephalogram (EEG) mimicking brain death was observed in a rabies patient who had intact multimodality evoked potentials [4]. Anterior horn cell dysfunction with no demonstrable clinical weakness is evident from onset in furious rabies [20,30••] (Fig. 2).

Preservation of the integrity of infected neurons is essential for the virus to propagate from periphery to the CNS. Intact spinal cord and brainstem pathways are, therefore, crucial. The pathogenicity of a particular strain correlates inversely with its ability to induce apoptosis. Apoptosis-inducing potentials have been attributed to G, M, and possibly P proteins [12,14]. Expression of

G levels must be kept at a minimum to prevent functional impairment of infected neurons. Attenuated strains or recombinant viruses, with G protein derived from the attenuated ERA rabies strain, can trigger cell death via caspase-dependent and caspase-independent pathways [34,35]. M protein also induces tumor necrosis factor–related apoptosis-inducing ligand (TRAIL)-mediated apoptosis, which involves caspase-8 [36]. Massive infection eventually leads to neuronal death by inhibition of protein synthesis, which is required in maintaining neuronal function [12].

Although apoptosis was evident by terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) staining in most of the 15 regions of the brain, brainstem, and spinal cord of five furious and five paralytic rabies patients, we found that neuronal cells, particularly in the brainstem and spinal cord, had delay in apoptosis, especially that mediated by cytochrome c of the mitochondrial pathway, despite the abundant presence of rabies antigen [17•]. This phenomenon may be unique to rabies. Site-specific mechanisms of neuronal death pathway have been demonstrated in weanling mice infected with a neurovirulent strain of Sindbis virus (SV) [37]. Anterior horn cells in SV were degenerated or necrotic but not apoptotic, whereas hippocampal neurons could be either apoptotic or necrotic. AMPA receptor–mediated anterior horn cell death is the responsible mechanism because paralysis can be delayed or prevented by treatment with glutamate receptor antagonists and not by Bcl2 and Bax [37]. All brain and spinal cord neurons in rabies were apoptotic with only one exception (in a total of 15 cases), which was a patient who had chromatolysis of anterior horn cells at the bitten segment [17•,21•,30••].

Such a phenomenon was also documented by in vitro and in vivo experiments, thus explaining the intrinsic properties of spinal motoneuron [38]. In theory, such phenomenon can also be modulated. Nerve growth factor (NGF) signaling via tyrosine kinase A (TrkA) inhibits apoptosis otherwise induced by neurotrophin binding to p75 NTR [39]. NGF binding to p75 NTR in the absence of TrkA leads to apoptosis. Whether rabies virus binds to this receptor, which is absent in spinal cord [31] and intensifies the apoptotic process only at particular regions, remains to be determined. Neurotrophin does not support transcription and replication of rabies virus [40]. Because less virus protein is found in the brain than in brainstem and spinal cord, it could be that neurotrophin also affects the balance between transcription and replication in the brain where it contains p75 NTR receptor, resulting in a more rapid neuronal death. Immune-inflammatory cells in rabies-infected spinal cord and brainstem [17•,21•,30••] may be more protective than destructive. Infiltrating immune cells can produce brain-derived neurotrophic factor, thus protecting neurons [39]. Apoptotic cascades and the effect on up- or downregulation of cellular genes at different regions may not be identical [14, 41]. Microglia can also be infected, releasing nitric oxide and chemokine [42]. Susceptibility to infection of microglia at different CNS regions may be variable.

How Does Rabies Virus Evade the Immune Response?

Neither innate nor adaptive immunity effectively operates in human rabies [18,19]. The presence of T-cell immunity to rabies virus and elevated levels of serum cytokines are detrimental because death is accelerated, particularly in furious rabies patients. Significantly elevated levels of cortisol during the first 3 days of clinical illness do not affect the T-cell immunity response to rabies virus and survival period, nor do they predict clinical manifestations [18]. This indicates intense stimulation to and intactness of the hypothalamic-pituitary-adrenal axis. It argues against reports in rabies-infected animal models that cortisol and corticotrophin-releasing factor production, in response to IL-1 in the brain, suppresses T-cell and natural killer cell functions [18]. Serum neutralizing antibody could be detected in 25% of dog-related cases, and time of appearance was not dependent on the length of survival [18,19]. Circulating B cell numbers are diminished. Defective N protein recognition has also been found in rabies patients. Natural killer cells were defective, although their numbers were normal. Attempts to restore immune functions by vaccination or by administering high-dose intravenous human RIG or interferon (IFN) failed or induced complications [1••,22]. Examination of the brain did not reveal any significant changes. Only mild perivascular cuffing was detected in patients who died within 5 to 7 days [18,21•,30••]. Marked microglial proliferation and nodules with minimal inflammation, scattering throughout the brain, and liquefaction necrosis could be demonstrated only when the survival time was longer than 7 days [18,22].

In experimental murine models, rabies virus can upregulate Toll-like receptor (TLR)-2 (dependent on virus load), -3, and -9 (on type-1 interferon) in the CNS [43]. Studies in infected human postmitotic neuron-derivative cell lines (in the absence of glia) confirmed that TLR-3–positive human neurons become strong producers of β IFN once infected [44]. Nevertheless, rabies P protein can mediate inhibition of the IFN system by inhibition of production or impairment of IFN regulatory factor-3 (IRF-3) phosphorylation and IFN signaling via blocking nuclear transport of signal transducer and activator of transcription (STAT)-1 and by altering promyelocytic leukemia (PML) nuclear bodies by retaining PML in the cytoplasm [45]. α and β IFN genes and those involved in signal, activation pathways, and effectors of IFN, as well as TLR, chemokines, cytokines, and complement, were not upregulated

in mice brains infected with pathogenic virus [41]. HLA-G1 (a nonclassic HLA class 1 molecule that provides escape from adaptive immunity) expression on cultured rabies–infected neurons, may also be regulated by rabies [46]. Early triggering of Fas ligand on infected brain neurons, which then induces apoptosis of invading T cells, was also found [47].

Is There a Magic Bullet for Treatment of Rabies?

There have been only two rabies survivors with excellent recovery [24,48••]. Both experienced bat bites. The recent patient was treated with coma-induction therapy and the N-methyl-D-aspartate antagonist ketamine, aimed at reducing brain excitotoxicity and autonomic reactivity [48••]. Coma-induction therapy failed to save one of our patients.

We treated a 33-year-old male, dog-related furious rabies patient on March 30, 2006. He had an incubation period of 2 months and did not receive PEP. He was fully conscious at start of coma-induction treatment on day 5 after onset. Diagnosis was confirmed by demonstration of rabies RNA in hair follicles. He received ketamine, diazepam, and ribavirin with supplemental thiopental to achieve EEG burst suppression. He had myocarditis and neurogenic pulmonary edema and later developed cardiac and renal failure. He survived 12 days. Rabies RNA remained detectable in hair follicles and in saliva from day 2 to 8. Rabies antibody was not found in sera and CSF by virus neutralization and indirect FAT during his entire clinical course, but virus could be isolated from brain and spinal cord.

Neuronal injury mediated by excitotoxicity has not been demonstrated in rabies as previously shown in neuroadapted SV [37]. Two survivors with near complete recovery had neutralizing antibody present in the first available samples on the hospital days 10 and 1, respectively [49]. Early appearance of native immune response must have superseded neuronal death. Intensive care with ventilatory support should be reserved for a patient who remains alert and has demonstrable serum and CSF rabies antibody; otherwise, comfort care with adequate sedation should remain the main modalities in managing rabies patients.

Conclusions

Rabies remains an enigmatic and nontreatable disease. Our effort to control human rabies should focus on public education and participation and awareness of physicians of its diverse manifestations. There must be political commitment to control rabies vectors. Appropriate rabies PEP must be made available. The scarce and unaffordable human and equine rabies immunoglobulin, in the future, should be replaced by effective monoclonal antibodies [50].

Acknowledgment

Supported in part by grants from National Genetic Engineering and Technology.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1.•• WHO: *WHO Expert Consultation on Rabies. WHO technical report series 931*. 2005.

This is a synopsis of rabies in humans and animals. It contains the most current information on rabies prevention and control including guidelines for pre- and postexposure prophylaxis.

2. Zhang YZ, Xiong CL, Xiao DL, et al.: **Human rabies in China.** *Emerg Infect Dis* 2005, **11:**1983–1984.

3.• Denduangboripant J, Wacharapluesadee S, Lumlertdacha B, et al.: **Transmission dynamics of rabies virus in Thailand: implications for disease control.** *BMC Infect Dis* 2005, **5:**52.

This article presents the theory of compartmentalization and provides evidence for the spread of canine rabies by human-facilitated translocation. The presence of more than one virus group in a region might indicate continuous gene flow, reflecting an active trafficking of the canine vector.

4. Jackson AC, Wunner WH: *Rabies*. Amsterdam: Academic Presss; 2002.

5. Messenger SL, Smith JS, Orciari LA, et al.: **Emerging pattern of rabies deaths and increased viral infectivity.** *Emerg Infect Dis* 2003, **9:**151–154.

6. Davis PL, Bourhy H, Holmes EC: **The evolutionary history and dynamics of bat rabies virus.** *Infect Genet Evol* 2006, In press.

7. Johnson N, Brookes SM, Fooks AR, Ross RS: **Review of human rabies cases in the UK and in Germany.** *Vet Rec* 2005, **157:**715.

8.• Burton EC, Burns DK, Opatowsky MJ, et al.: **Rabies encephalomyelitis: clinical, neuroradiological, and pathological findings in 4 transplant recipients.** *Arch Neurol* 2005, **62:**873–882.

This article reviews the pitfalls in diagnosing rabies and details uncharacteristic neuroimaging features in rabies-transplanted individuals compared with naturally infected cases.

9.• Srinivasan A, Burton EC, Kuehnert MJ, et al.: **Transmission of rabies virus from an organ donor to four transplant recipients.** *N Engl J Med* 2005, **352:**1103–1111.

This article documents the transmission of rabies through solid organ transplantation and vascular graft.

10. Rupprecht CE, Hemachudha T: **Rabies.** In *Infections of the Central Nervous System,* edn 3. Edited by Scheld M, Whitley RJ, Marra C. Philadelphia: Lippincott, Williams & Wilkins; 2004:243–259.

11. Belotto A, Leanes LF, Schneider MC, et al.: **Overview of rabies in the Americas.** *Virus Res* 2005, **111:**5–12.

12. Dietzschold B, Schnell M, Koprowski H: **Pathogenesis of rabies.** *Curr Top Microbiol Immunol* 2005, **292:**45–56.

13. Schnell MJ, Tan GS, Dietzschold B: **The application of reverse genetics technology in the study of rabies virus (RV) pathogenesis and for the development of novel RV vaccines.** *J Neurovirol* 2005, **11:**76–81.

14. Finke S, Conzelmann KK: **Replication strategies of rabies virus.** *Virus Res* 2005, **111:**120–131.

15. Faber M, Pulmanausahakul R, Nagao K, et al.: **Identification of viral genomic elements responsible for rabies virus neuroinvasiveness.** *Proc Natl Acad Sci U S A* 2004, **101:**16328–16332.

16. Lafon M: **Subversive neuroinvasive strategy of rabies virus.** *Arch Virol Suppl* 2004, **18:**149–159.

17.• Juntrakul S, Ruangvejvorachai P, Shuangshoti S, et al.: **Mechanisms of escape phenomenon of spinal cord and brainstem in human rabies.** *BMC Infect Dis* 2005, **5:**104.
Article provides an explanation of the mechanism responsible for the relative intactness of brainstem and spinal cord despite abundance of virus antigen.
18. Hemachudha T, Rupprecht C: **Rabies.** In *Principle of Neurological Infectious Diseases*. Edited by Roos K. New York: McGraw Hill; 2004:151–174.
19. Hemachudha T, Laothamatas J, Rupprecht CE: **Human rabies: a disease of complex neuropathogenetic mechanisms and diagnostic challenges.** *Lancet Neurol* 2002, **1:**101–109.
20. Hemachudha T, Wacharapluesadee S, Mitrabhakdi E, et al.: **Pathophysiology of human paralytic rabies.** *J Neurovirol* 2005, **11:**93–100.
21.• Laothamatas J, Hemachudha T, Mitrabhakdi E, et al.: **MR imaging in human rabies.** *Am J Neuroradiol* 2003, **24:**1102–1109.
This article presents MRI details in furious and paralytic human rabies at different clinical stages.
22. Hemachudha T, Sunsaneewitayakul B, Mitrabhakdi E, et al.: **Paralytic complications following intravenous rabies immune globulin treatment in a patient with furious rabies.** *Int J Infect Dis* 2003, **7:**76–77.
23. Hemachudha T, Wacharapluesadee S: **Antemortem diagnosis of human rabies.** *Clin Infect Dis* 2004, **39:**1085–1086.
24. Jackson AC: **Rabies: new insights into pathogenesis and treatment.** *Curr Opin Neurol* 2006, **19:**267–270.
25. Lodmell DL, Dimcheff DE, Ewalt LC: **Viral RNA in the bloodstream suggests viremia occurs in clinically ill rabies-infected mice.** *Virus Res* 2006, **116:**114–118.
26. Sitprija V, Sriaroon C, Lumlertdaecha B, et al.: **Does contact with urine and blood from a rabid dog represent a rabies risk?** *Clin Infect Dis* 2003, **37:**1399–1400.
27. Hemachudha T, Wacharapluesadee S, Lumlertdaecha B, et al.: **Sequence analysis of rabies virus in humans exhibiting encephalitic or paralytic rabies.** *J Infect Dis* 2003, **188:**960–966.
28. Khawplod P, Shoji Y, Ubol S, et al.: **Genetic analysis of dog rabies viruses circulating in Bangkok.** *Infect Genet Evol* 2006, **6:**235–240.
29. Hooper DC: **The role of immune responses in the pathogenesis of rabies.** *J Neurovirol* 2005, **11:**88–92.
30.•• Mitrabhakdi E, Shuangshoti S, Wannakrairot P, et al.: **Difference in neuropathogenetic mechanisms in human furious and paralytic rabies.** *J Neurol Sci* 2005, **238:**3–10.
This article documents differential involvement of peripheral nerves and anterior horn cells in human paralytic and furious rabies and shows that dorsal sensory ganglionopathy as responsible for local prodrome.
31. Lafon M: **Rabies virus receptors.** *J Neurovirol* 2005, **11:**82–87.
32. Kelly RM, Strick PL: **Rabies as a transneuronal tracer of circuits in the central nervous system.** *J Neurosci Methods* 2000, **103:**63–71.
33. Sheikh KA, Ramos-Alvarez M, Jackson AC, et al.: **Overlap of pathology in paralytic rabies and axonal Guillain-Barre syndrome.** *Ann Neurol* 2005, **57:**768–772.
34. Prehaud C, Lay S, Dietzschold B, Lafon M: **Glycoprotein of nonpathogenic rabies viruses is a key determinant of human cell apoptosis.** *J Virol* 2003, **77:**10537–10547.
35. Thoulouze MI, Lafage M, Yuste VJ, et al.: **Apoptosis inversely correlates with rabies virus neurotropism.** *Ann N Y Acad Sci* 2003, **1010:**598–603.
36. Kassis R, Larrous F, Estaquier J, Bourhy H: **Lyssavirus matrix protein induces apoptosis by a TRAIL-dependent mechanism involving caspase-8 activation.** *J Virol* 2004, **78:**6543–6555.
37. Griffin DE: **Neuronal cell death in alphavirus encephalomyelitis.** *Curr Top Microbiol Immunol* 2005, **289:**57–77.
38. Guigoni C, Coulon P: **Rabies virus is not cytolytic for rat spinal motoneurons in vitro.** *J Neurovirol* 2002, **8:**306–317.
39. Kerschensteiner M, Stadelmann C, Dechant G, et al.: **Neurotrophic cross-talk between the nervous and immune systems: implications for neurological diseases.** *Ann Neurol* 2003, **53:**292–304.
40. Castellanos JE, Martinez-Gutierrez M, Hurtado H, et al.: **Studying neurotrophin antiviral effect on rabies-infected dorsal root ganglia cultures.** *J Neurovirol* 2005, **11:**403–410.
41. Wang ZW, Sarmento L, Wang Y, et al.: **Attenuated rabies virus activates, while pathogenic rabies virus evades, the host innate immune responses in the central nervous system.** *J Virol* 2005, **79:**12554–12565.
42. Nakamichi K, Saiki M, Sawada M, et al.: **Rabies virus-induced activation of mitogen-activated protein kinase and NF-kappaB signaling pathways regulates expression of CXC and CC chemokine ligands in microglia.** *J Virol* 2005, **79:**11801–11812.
43. McKimmie CS, Johnson N, Fooks AR, Fazakerley JK: **Viruses selectively upregulate Toll-like receptors in the central nervous system.** *Biochem Biophys Res Commun* 2005, **336:**925–933.
44. Prehaud C, Megret F, Lafage M, Lafon M: **Virus infection switches TLR-3-positive human neurons to become strong producers of beta interferon.** *J Virol* 2005, **79:**12893–12904.
45. Chelbi-Alix MK, Vidy A, Bougrini JE, Blondel D: **Rabies viral mechanisms to escape the IFN system: the viral protein P interferes with IRF-3, Stat1, and PML nuclear bodies.** *J Interferon Cytokine Res* 2006, **26:**271–280.
46. Lafon M, Prehaud C, Megret F, et al.: **Modulation of HLA-G expression in human neural cells after neurotropic viral infections.** *J Virol* 2005, **79:**15226–15237.
47. Baloul L, Camelo S, Lafon M: **Up-regulation of Fas ligand (FasL) in the central nervous system: a mechanism of immune evasion by rabies virus.** *J Neurovirol* 2004, **10:**372–382.
48.•• Willoughby RE Jr, Tieves KS, Hoffman GM, et al.: **Survival after treatment of rabies with induction of coma.** *N Engl J Med* 2005, **352:**2508–2514.
There have been only two survivors of rabies with near full recovery. Both were associated with bat exposures and had early native immune response. It is doubtful whether successful outcome relies on coma-induction treatment, which aims to reduce brain excitotoxicity.
49. Hemachudha T, Wilde H: **Survival after treatment of rabies.** *N Engl J Med* 2005, **353:**1068–1069; author reply 1068–1069.
50. Goudsmit J, Marissen WE, Weldon WC, et al.: **Comparison of an anti-rabies human monoclonal antibody combination with human polyclonal anti-rabies immune globulin.** *J Infect Dis* 2006, **193:**796–801.