

Seminar

Rabies and other lyssavirus diseases

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The full scale of the global burden of human rabies is unknown, owing to inadequate surveillance of this fatal disease. However, the terror of hydrophobia, a cardinal symptom of rabies encephalitis, is suffered by tens of thousands of people each year. The recent discovery of enzootic European bat lyssavirus infection in the UK is indicative of our expanding awareness of the Lyssavirus genus. The main mammalian vector species vary geographically, so the health problems created by the lyssaviruses and their management differ throughout the world. The methods by which these neurotropic viruses hijack neurophysiological mechanisms while evading immune surveillance is beginning to be unravelled by, for example, studies of molecular motor transport systems. Meanwhile, enormous challenges remain in the control of animal rabies and the provision of accessible, appropriate human prophylaxis worldwide.

For more than three millennia, rabies has been one of the best known and most feared human diseases. Each year, more than ten million people, many of whom are unvaccinated, endure protracted anxiety after exposure to an animal with suspected rabies.¹ Although human rabies encephalitis remains untreatable, the infection is eminently preventable. However, the genus *Lyssavirus* can still cause some surprises. In 1996 and 1998, two women died in Queensland, Australia, from infections with a newly discovered rabies-related virus (Australian bat lyssavirus [ABLV]).^{2,3} In 2002, a man died in Scotland after contracting European bat lyssavirus (EBLV) rabies, signalling the fact that, after a century of apparent freedom from rabies, the disease was once more enzootic in the UK. The aim of this seminar is to discuss developments, especially the importance of rabies-related viruses in Europe; recent hypotheses on the mechanisms of neural invasion; viral effects on neuronal function; clinical issues; and, most important, prevention of this fatal disease throughout its global distribution (figure 1).

Genus lyssavirus

Rabies, a single-stranded RNA virus, was the first of the seven lyssavirus genotypes to be identified. Of the other six rabies-related viruses,⁴⁻⁹ all but Lagos bat virus have caused fatal encephalitis in people, clinically indistinguishable from classic rabies, with the exception of Mokola virus (table 1). New lyssaviruses have lately been reported, Aravan from a single bat in Kyrgyzstan and Khujand from a bat in Tajikistan.¹⁰

EBLV in the UK

The UK was apparently free of indigenous rabies for a century, but in 1996 a Daubenton's bat (*Myotis daubentonii*) infected with EBLV was found at Newhaven on the south coast of England.¹¹ The animal was thought to have been imported from the continent; carriage of bats on the wind or in ships is well documented.¹² However, the isolation of EBLV from a juvenile Daubenton's bat in an established colony near the Lancashire canal in the

northwest of England in 2002¹³ and the death of a man in Scotland¹⁴ confirmed that the virus was indigenous. All three UK isolates were EBLV type 2a. This virus could have originated in continental Europe and have been enzootic in the British Isles for years. Changes in the prevalence of the virus, in human behaviour, or bat ecology could have created the opportunity for increased human contact.

EBLV in human beings

Bat rabies has been documented in continental Europe for about 50 years, but only four human infections are known. All the patients presented with clinical features of classic rabies: a girl in the Ukraine and another in the Russian Federation,¹⁵ one infected by EBLV 1a, the other by an untyped lyssavirus; a Swiss bat zoologist who died in Finland from infection with EBLV type 2b;¹⁶ and a Scottish bat conservationist who died of EBLV type 2a infection.¹⁴

Importance of EBLV

European insectivorous bats are protected. Epidemiological data depend on the examination of bats submitted for testing. The scale of this surveillance varies greatly. In the Netherlands in the 1980s hundreds of bats were examined, and about 7% were rabid.¹⁷ By contrast, in Belgium only 77 bats have been tested in 15 years, and none has been reported rabid (F Costy, personal communication). The reason for the poor surveillance of bat infection may be that the importance of EBLV infection in Europe has been overshadowed by the more obvious danger of fox rabies. Oral vaccination of foxes has controlled this epizootic in western Europe, but no means of vaccinating bats has emerged, and population control of protected species would be inappropriate. The control

Search strategy

We searched PubMed, Current Contents including "Agriculture, Biology and Environmental Sciences", "Life Sciences", and "Focus on Veterinary Science", and our own literature accumulated over many years. The restricted number of references and text permitted have constrained the citation of original papers. These should be sought in the reviews supplied.

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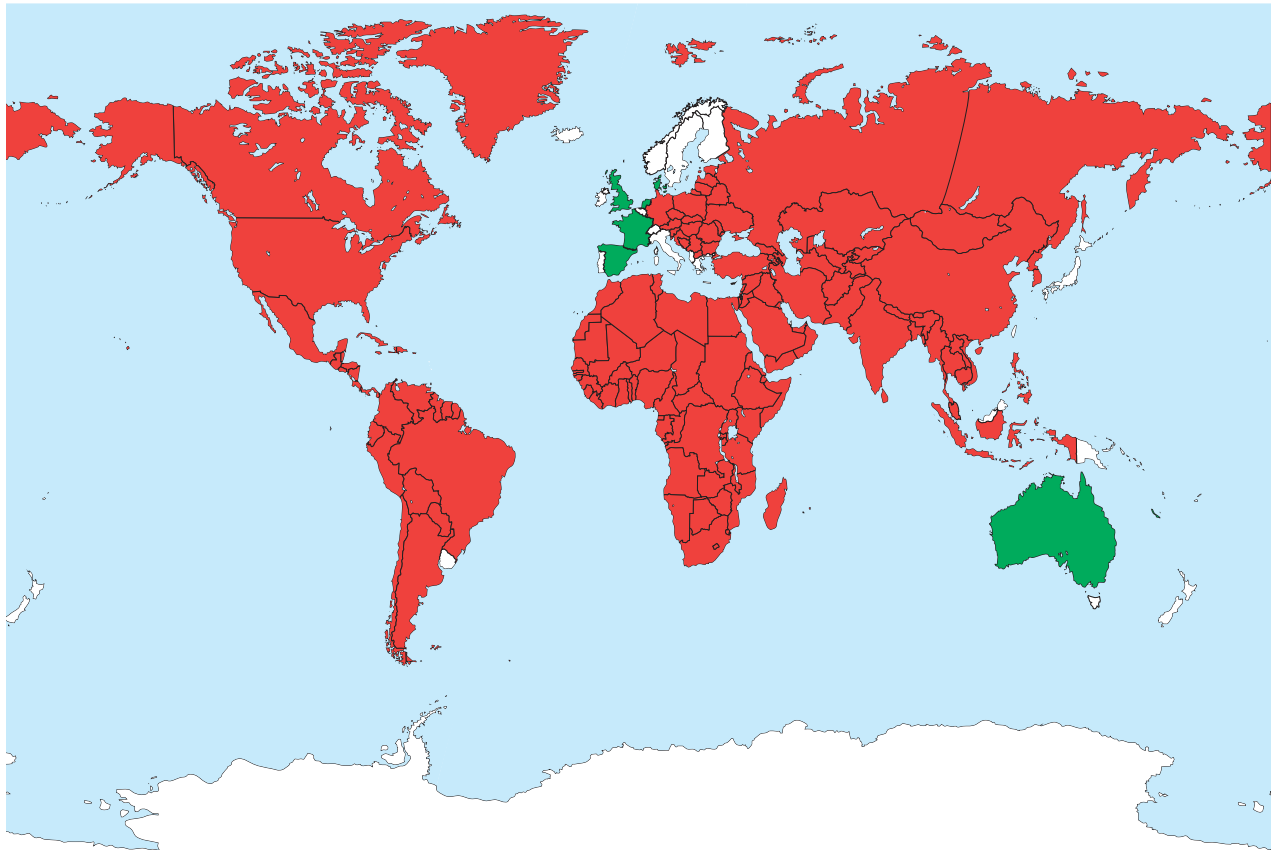


Figure 1: **Global distribution of lyssaviruses**

Distribution of rabies virus (red). Areas where rabies-related viruses only, not classic rabies genotype 1, are documented are classified by the WHO as "rabies-free" (green). White=rabies-free.

of EBLV infection is therefore regarded as an intractable problem, incurring only the costs of postexposure treatment, the protracted anxiety of exposed patients, and very rare deaths. The direct immunofluorescent screening test with genotype 1 conjugate has been unreliable for ABLV, genotype 7.¹⁸ Whether the use of a specific EBLV antibody conjugate would increase the sensitivity of the test is not known.

Cryptic bat rabies in the USA

In the Americas, all rabies viruses, including bat strains, are of genotype 1. Of 35 cases of indigenous human rabies in the USA reported between 1958 and 2000, 32 were caused by insectivorous-bat strains of rabies virus. 26 patients had no history of a bat bite, although 12 had had physical contact with a bat. 28 patients were infected by a virus strain associated with the solitary tree-roosting silver-haired bat (*Lasiurus noctivagans*) or the eastern pipistrelle bat (*Pipistrellus subflavus*).¹⁹ Since 1990, 27 people have died of bat variant lyssavirus encephalitis, but only two of them reported a bat bite. Rabies may be underdiagnosed in the USA.¹⁹

Doubt has been cast on the assumption that the two human cases of bat rabies virus infection in Texas in the 1950s were due to inhalation of virus in caves densely populated by Mexican free-tailed bats (*Tadarida brasiliensis mexicana*).²⁰ Although infection by the olfactory route has been shown experimentally in caves²⁰ and in human beings (two laboratory accidents involved the inhalation of modified virus during vaccine preparation),²¹⁻²³ no other patient who died of bat rabies had been in bat-infested caves. Percutaneous infection is thought more likely to have occurred by unnoticed skin

contact perhaps resulting in a minute bite.¹⁹ The virus associated with the silver-haired bat might be more infectious when inoculated superficially into the epidermis, since it replicates more readily in non-neuronal cells and at lower temperatures than dog rabies viruses.²⁴ The route of viral entry into epithelial nerves and eventually the central nervous system is unknown.

Is rabies a chronic or latent infection in animals?

Animal recovery from rabies

Natural rabies infection in all species generally causes an acute fatal illness, but rabies antibody has been detected in apparently healthy vector species including mongooses, skunks, raccoons, foxes, hyenas, jackals, fruit bats, vampire bats, insectivorous bats, and domestic dogs in Ethiopia.²⁵⁻³⁶

Transmission of rabies by asymptomatic animals is an intriguing possibility. In India a dog that had no detectable antibody was found to excrete rabies virus intermittently in its saliva over 30 months,³⁷ but the validity of this unusual finding has been questioned.³⁸ However, virus was isolated repeatedly from 0.5% of asymptomatic, naturally infected Ethiopian dogs,³⁹ and street rabies virus was isolated once from saliva of 0.3% of healthy dogs in Nigeria.⁴⁰

Seropositive vampire bats might have recovered from infection and they were thought to be asymptomatic rabies carriers,⁴¹ but the evidence was incomplete.^{33,38} Attempts to induce chronic infection experimentally have failed.⁴² Apparently healthy animals might be infectious during their prodromal illness.

Some wild-caught bats have antibody to rabies-related

Genotype	Virus	Source	Distribution
1	Rabies virus	Dog, fox, raccoon, bat, and others	Widespread
2	Lagos bat virus*	Bats, cats; has not been detected in human beings	Africa (rare)
3	Mokola*	Shrews, cats	Africa
4	Duvenhage	Insectivorous bat	Africa (rare)
5	EBLV type 1 ⁹		
	Type 1a	Insectivorous bat	Netherlands, † Denmark, Germany, Poland, Hungary, Russian Federation, France
	Type 1b	Insectivorous bat	Netherlands, † France, Spain
6	EBLV type 2 ^{9,11}		
	Type 2a	Insectivorous bat	Netherlands, † UK, Germany, ‡ Ukraine ‡
	Type 2b	Insectivorous bat	Switzerland (and Swiss man who died in Finland)
7	Australian bat lyssavirus	Frugivorous bat (or flying fox); insectivorous bat	Australia, ?Philippines §

*The genus has been divided into two phylogroups.⁴ Phylogroup II is Mokola and Lagos bat viruses. All other genotypes are in phylogroup I, and they cause fatal rabies-like encephalitis in human beings. Phylogroup II viruses are less pathogenic, but Mokola virus has probably caused three known human infections, including one fatal encephalitis without typical features of rabies.^{5,7} †The Netherlands is unusual in having three types of EBLV, including type 2a isolates from pond bats, *Myotis dasycneme*, which are not found in the UK. ‡Single isolate. §Serological evidence of its presence in the Philippines.⁸

Table 1: **Lyssavirus genus of the rhabdovirus family**

viruses: ABLV (genotype 7)^{8,43} and EBLV.⁴⁴⁻⁴⁶ Seropositive bats can survive for at least 3 years.⁴⁶ Viral RNA has been detected by RT-PCR in the saliva of apparently healthy Spanish bats, repeatedly in one,⁴⁷ but no virus was isolated from saliva. Rabies antibody might have been induced by viral contact without infection, but the presence of viral RNA in saliva indicates infection. Chronic infection involves continued viral replication, whereas carrier status implies transmissibility, perhaps by reactivation from a dormant or latent state. In both these settings, viral excretion could occur intermittently, but there are as yet no data on lyssaviruses to support this notion. Nevertheless, re-emergence of infectious EBLV in previously infected bats remains a possibility.

Similar findings have been reported by East and colleagues²⁹ in apparently healthy spotted hyenas in the Serengeti, Tanzania. As with European bats, some animals were rabies seropositive and viral RNA was detected by PCR in saliva, but no virus was cultured. Specific RNA was occasionally detected in the brains of hyenas that died incidentally, but no virus could be isolated. These markers of infection were not associated with symptomatic rabies or decreased survival. The genotype-1 rabies virus strain infecting the hyenas differed

from other local rabies isolates. The researchers inferred that infection was caused by a virus strain of low pathogenicity peculiar to this host species.

This concept of a rabies virus of low pathogenicity is reminiscent of “oulou fato” a type of dog rabies identified across sub-Saharan Africa in the early twentieth century.⁴⁸ The virus seemed to be less transmissible to people than other rabies viruses and was associated with predominantly paralytic disease. The finding of antibody, and rarely virus, in healthy African dogs^{30,35,39,40} suggests that oulou fato may still exist in Africa.

Pathogenesis

Bites by rabid animals generally inoculate virus-laden saliva through the skin into muscle and subcutaneous tissues. Other routes of infection are rare.⁴⁹ During the incubation period the virus can replicate locally in muscle cells or attach directly to nerve endings. Having gained access to peripheral nerves, it travels in a retrograde direction within the axoplasm. When the virus reaches the central nervous system, there is massive replication on membranes within neurons. Direct transmission of virus occurs from cell to cell across synaptic junctions. At the onset of illness when evidence of neuronal dysfunction appears, there is little or no apparent histopathological change. Centrifugal spread of virus from the central nervous system in somatic and autonomic nerves deposits virus in many tissues, including skeletal and cardiac muscle, adrenal glands, kidney, retina, cornea, pancreas, and nerves around hair follicles.⁵⁰ Productive viral replication with budding from plasma membranes takes place predominantly in the salivary glands, excreting virus that is transmissible to other mammals.

Viral invasion of cells

Local replication of virus (figure 2) in striated muscle at the bite site, before any contact with nervous tissue, could account for long incubation periods. In skunks, antigen was detectable in muscle for 2 months after inoculation.⁵¹ Virus is soon detectable experimentally at local motor or sensory nerve endings and, after superficial inoculation, in epithelial layers.⁵²

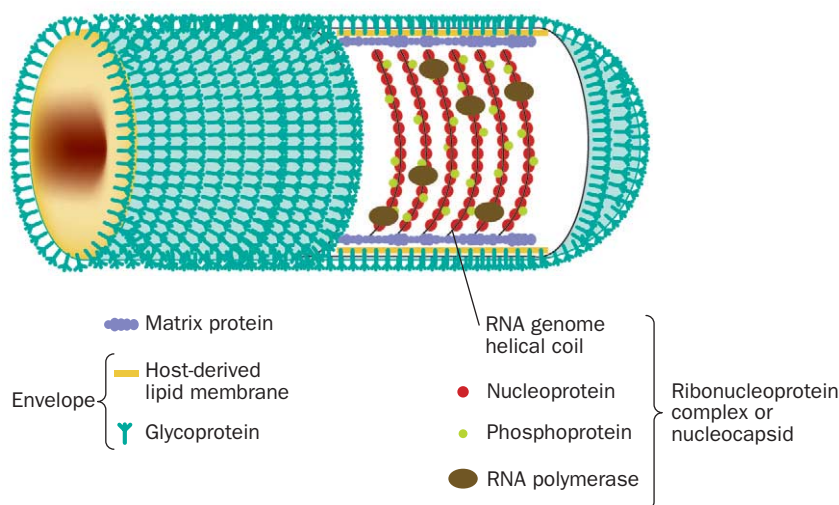


Figure 2: **Rabies virion**

Rabies virions measure 180×75 nm. The genome, a single non-segmented strand of negative-sense RNA of 11.9 kb, a nucleoprotein, a phosphoprotein, and an RNA-dependent RNA polymerase form the helical coil of the ribonucleoprotein complex core. A layer of matrix protein covers this cylindrical structure. The lipoprotein envelope is a host-derived lipid bilayer studded with rabies glycoprotein bearing trimeric spikes.

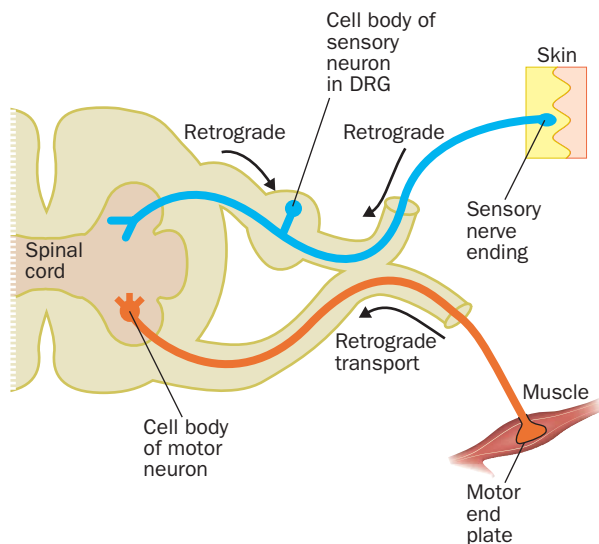


Figure 3: Viral pathway to the spinal cord

The cell bodies of the peripheral sensory pseudobipolar (unipolar) neurons in the dorsal root ganglion (DRG) have two axons and no dendrites. Retrograde transport is from the axon terminal towards the cell body.

Rabies virus can infect a great variety of neuronal and non-neuronal cells *in vitro*. Non-specific viral attachment to several types of cell-surface receptors including carbohydrates, phospholipids, and sialylated gangliosides has been demonstrated. Specific binding occurs at neuromuscular junctions, where virus colocalises with the nicotinic acetylcholine receptor.^{53,54} Binding at this postsynaptic site is competitive with cholinergic ligands, including the snake venom neurotoxin α -bungarotoxin, which shows sequence homology with the envelope glycoprotein of rabies virus. Concentration of virus at this site increases its chances of entering the axon terminal across the synaptic cleft.

Rabies virus attaches specifically to two other receptors on neuronal cell membranes: the neural cell adhesion molecule⁵⁵ and the p75 neurotrophin receptor (p75NTR).⁵⁶ Two neurotransmitter receptors in the central nervous system, for N-methyl-D-aspartate subtype R1 and GABA, have been suggested as possible receptors for rabies virus.⁵⁷

Rabies genotype 1 and EBLV type 2 (genotype 6) bind avidly to p75NTR, but EBLV type 1 (genotype 5) and the other lyssaviruses do not.⁵⁸ Rabies virus binds to mammalian but not to avian p75NTR, which is consistent with the lack of rabies pathogenicity in birds. Mazarakis and colleagues^{59,60} have suggested that binding to this receptor might not only enable entry into a cell but also facilitate fast axonal transport.

Rabies virus enters cells by adsorptive endocytosis into endosomes. Soon after infection, virus may be associated with synaptic vesicles, since it colocalises with synapsin I, or with early acidic endosomes,⁵⁴ in which case viral glycoprotein could fuse with the endosomal membrane releasing the core ribonucleoprotein complex (figure 2) into the cytosol. The rhabdovirus glycoprotein has a unique reversible fusion inactive state at low pH, so it can resist fusion⁶¹ and could remain intact in vesicles.

Transport of virus to the brain

Rabies virus migrates along peripheral nerves towards the central nervous system at about 50–100 mm per day⁶² via the fast axonal transport system. Because this movement is strictly retrograde, it is used experimentally to track

neural pathways.⁶³ Infection is thought to be via sensory as well as motor nerves, because antigen was detected in sensory nerve endings and dorsal root ganglia soon after peripheral inoculation in several studies.^{52,62} However, Mazarakis and colleagues have shown that rabies glycoprotein invasion of the central nervous system apparently does not occur via this sensory nerve pathway (figure 3). Using a rabies-glycoprotein-pseudotyped lentivirus vector,⁵⁹ which is effectively transported in a single neuron but cannot cross synapses, they showed that injection of muscle with the lentivector resulted in transgene expression in the ventral horn of the spinal cord. Injection of skin resulted in transgene expression in dorsal root ganglia but not further into the dorsal horn of the spinal cord. Injection into the dorsal horn of the spinal cord, however, produced transgene expression in the relevant dorsal root ganglia but not in the skin (Mazarakis ND, personal communication). This finding shows that rabies-glycoprotein-pseudotyped lentivirus is retrogradely transported in neurons, and thus it can pass to the spinal cord through the motor nerves, but not via this pathway in peripheral sensory nerves. If any lentivirus entered sympathetic motor nerves supplying the skin, it might reach the sympathetic ganglia, but not the spinal cord in this experiment. Further studies showed the abolition of this retrograde transport by substitution of the arginine-333 residue on the glycoprotein.⁶⁰

Two groups of researchers found independently that, like some other virus proteins, rabies phosphoprotein can interact with the light-chain LC8 protein, a highly conserved cytoplasmic component of dynein cargo-binding complex and myosin V, and an inhibitor of neuronal nitric oxide synthase.^{64,65} LC8 also colocalises with viral ribonucleoprotein *in vitro*.⁶⁵ The myosin V actin-based motor complex drives cytoplasmic vesicular transport in the endoplasmic reticulum, so LC8 binding to rabies proteins indicates its possible involvement in viral pathogenesis early in the cycle of neuronal infection.⁶⁵ The association of rabies phosphoprotein with the LC8 component of dynein would enable axonal retrograde transport of the viral ribonucleoprotein complex, as predicted by Murphy.⁵² However, retrograde transport of virus can still occur if the phosphoprotein binding site is deleted, hence binding of viral proteins to the LC8 molecule is not essential for pathogenesis.⁶⁶

Figure 4^{67,68} shows two methods by which rabies proteins might be transported. In theory, if on entry to a neuron the virus in an acidic endosome fused with the vesicle membrane, the liberated naked nucleocapsid (including the phosphoprotein) could be attached to dynein via LC8; if the virus remained intact, the envelope glycoprotein could be the ligand for vesicular transport through the p75NTR as suggested by Mazarakis and colleagues.⁶⁰ Free nucleocapsids and whole virions in vesicles within axons have been shown in electron micrographs by Gosztonyi.⁶⁹ This process is found with herpes simplex virus, the components of which are transported separately along microtubules, as either nucleocapsids or glycoprotein within vesicles.⁷⁰ Whether either mechanism is used by rabies virus remains to be proved.

Spread of virus within the CNS

Viral replication is intraneuronal, but the mechanism of interneuronal spread is unknown. The fact that budding of virus is very rarely seen at synapses by electron microscopy suggests that infectious naked nucleocapsids are transferred across synapses.⁷¹ However, interneuronal infection is dependent on the presence of viral

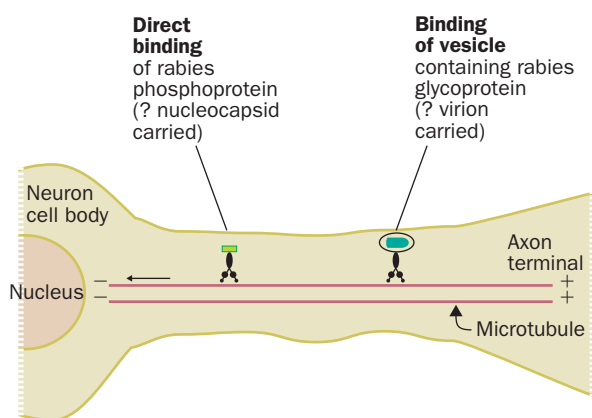


Figure 4: **Hypotheses on viral retrograde axonal transport**

Two ways in which rabies proteins might be carried on microtubular motor molecules: by direct attachment to the dynein motor or by encapsulation in vesicles attached to the dynein motor. The retrograde molecular motor, dynein, carries cargo along the tracks of axonal microtubules. For direct binding, rabies phosphoprotein could attach to the dynein LC8 molecule enabling axonal retrograde transport of viral proteins, and possibly of the whole viral ribonucleoprotein complex. An alternative mechanism has been shown for the retrograde transport of tetanus toxin. Vesicles containing the toxin colocalise in axons with the p75NTR, a membrane marker of this retrograde endocytic pathway.⁶⁷ The idea^{59,60} that rabies glycoprotein, which binds to p75NTR, might share this protected means of axonal transport is supported by the fact that Mokola-virus-glycoprotein-pseudotyped lentivirus is not transported efficiently in a retrograde direction.⁶⁸ Mokola virus does not bind to p75NTR.⁶⁸ Theoretically, during rabies infection, the glycoprotein in the vesicular cargo could be either a whole virion or a subviral fragment bearing a glycoprotein molecule.

glycoprotein,⁷² which suggests that intact virus can cross the synapse. The mechanism of uptake is unknown, but one possibility is through the synaptic vesicle recycling system.⁵⁴ Among street viruses, the dynamics and effects of infection can be influenced by the particular strain, since dog rabies viruses are specific for neurons, whereas bat strains can also infect astrocytes.⁷³

Effect of virus on neuronal function

In human beings, the symptoms of encephalitis and even death can occur with only minor histopathological changes. Rabies virus must have some profound effects on the functions of infected and some uninfected neurons. Few abnormalities of organelle structure are seen on electron microscopy in neurons infected with street virus.⁵² Minor electroencephalographic changes during animal infection indicate neuronal dysfunction.⁶² Although MRI shows a range of abnormalities as the human encephalitis progresses, no consistent pattern has yet emerged.

Abnormalities of neurotransmitter functions affecting serotonin, opioid, GABA, and muscarinic acetylcholine transmission have been found experimentally, in some cases in specific brain areas, and not always associated with the presence of virus.^{38,62,74} Results show no clear explanation of the limbic-system dysfunction suggested by the classic clinical features. Changes in neurotransmitter functions could lead to failure of brain networking and regulation of responses.

The involvement of excitatory aminoacids in neuronal toxicity is a possibility. Many non-competitive antagonists of N-methyl-D-aspartate have an antiviral effect on viral replication.⁶² Surprisingly, one of these, ketamine, specifically inhibits transcription of the rabies virus genome.⁷⁵

The effect of infection on the function of neuronal membrane ion channels⁷⁶ could be reduction of normal inhibitory events. Apoptosis could contribute to

pathogenesis since fatal infection of mice is associated with apoptosis of T cells invading the brain and neuronal preservation. By contrast, neuronal apoptosis occurs in non-lethal infection, with development of an immune response.^{38,77} The role of nitric oxide toxicity in neuronal dysfunction in rabies is not clear,³⁸ but it could be related to the LC8 inhibition of neuronal nitric oxide synthase, through interaction with the viral phosphoprotein.⁶⁵

Although rabies infection progressively decreases host gene expression overall, a few genes are upregulated, some associated with the interferon response, host-cell protein synthesis, synaptic vesicle function, and neuron growth and spread, even in some uninfected or non-neuronal cells.⁷³ One hypothesis on the cause of death is therefore that short-circuiting of normal neural pathways results from the formation of new interneuronal connections.⁷⁸ Another hypothesis is that disruption of neuronal metabolism ends in the exhaustion of metabolic pools.⁷⁹

Viral virulence and the immunology of recovery

Rabies virus virulence is influenced by its glycoprotein envelope. Factors associated with increased virulence experimentally are the presence of the surface aminoacid residue arginine-333,⁸⁰ the very low external expression of viral glycoprotein on infected cells, and the absence of apoptosis until a terminal stage.^{77,81}

Recovery from predictably fatal rabies encephalitis has been achieved only in rats treated with one monoclonal antibody.⁸² Animal experiments show that early induction of neutralising antibody is essential for recovery, associated with the inhibition of intercellular viral spread, reduction of viral gene expression, and the early induction of inflammation.^{82,83} Dietzschold and colleagues suggest that viral glycoprotein on the cell surface acts as a signal-transducing receptor. The external trigger of antibody binding to the protein could initiate changes probably ending in apoptosis. This idea might explain why administration of antibody at a late stage results in acceleration of the disease.⁷⁹ The important consequence in human infection is that recovery is inevitably accompanied by neuronal loss.⁷⁷ Although a cell-mediated immune response and interferons (predominantly interferon γ) contribute to recovery from attenuated virus infection, they are not essential. CD8-positive T cells might have a minor role.^{83,84}

Expression of MHC class I mRNA is slightly upregulated in the central nervous system in rabies infection but this change is unrelated to the outcome. MHC class II mRNA is greatly upregulated in avirulent virus infection, but there is very little expression in lethal disease, indicating the importance of a T-helper-cell response.⁸⁵ In mice, the immune response, not the direct effect of the virus, is the cause of paralysis.^{77,86} Species variation in response to rabies makes interpretation of animal data of uncertain relevance to human disease.

Clinical issues

In its classic furious form with hydrophobia or aerophobia, human rabies encephalitis is unmistakable. However, clinical descriptions over the past two centuries have shown the protean manifestations of this disease.^{49,87,88} Local paraesthesia at the site of the bite (most commonly itching) is the only reasonably suggestive prodromal symptom. Paralytic forms of rabies and rare presentations with subtle seizures or with psychiatric disturbances are especially likely to be misdiagnosed. In a patient with an acute neuropsychiatric illness, a history of travel to a rabies-endemic area during the previous

	Aim	Test
Ante mortem		
Skin punch biopsy; repeat until a diagnosis is made	Antigen detection Viral RNA	IFA test on frozen section RT-PCR
Saliva, tears, CSF; repeat until a diagnosis is made	Virus isolation	Tissue culture Suckling mouse inoculation
Serum	Viral RNA Antibody detection	RT-PCR Unvaccinated, test immediately* Vaccinated, save and compare a few days later
CSF	Antibody detection	Test immediately with serum*
Post mortem (any of the above tests, but most important)		
Brain Needle necropsy† or two or more samples—eg, brainstem and cerebellum	Antigen detection Viral RNA Virus isolation	IFA test on impression smear RT-PCR Tissue culture Suckling mouse inoculation
Retrospective diagnosis	Antigen detection in formalin-fixed tissue	Enzyme methods

IFA=immunofluorescent antibody; CSF=cerebrospinal fluid. *In unvaccinated patients, rabies antibody generally appears in the second week of illness. Rabies-specific IgM has been detected in serum and in some cases in CSF in low concentrations, but no earlier than IgG. IgM has also been found in postvaccinal encephalitis after nervous-tissue vaccination.⁹¹ High concentrations of antibody in the CSF have been considered diagnostic despite vaccination.⁹⁴ †Needle necropsy: with a Vim-Silverman or other long biopsy needle.⁴⁹ Corneal smear test not recommended: unreliable results, mostly false-negative but false-positive results occur.

Table 2: **Diagnosis of human rabies**

months or even years, and a history of a bite by a domestic or wild mammal, especially a carnivore or bat, will raise the possibility of rabies. However, rabies has developed in people in America who have had such trivial contacts with bats that they passed unnoticed. So far, no distinctive clinical features have been associated with infections by any of the rabies-related viruses. Furious, paralytic, and atypical manifestations have been reported in these patients.^{2,3,14,88}

Diagnosis of human rabies encephalitis

The laboratory diagnosis of rabies is rarely attempted in less developed countries, but confirmation of infection during life will guide management of the patient, relatives, and staff; prevent unnecessary investigations; and allow characterisation of the virus. Routine tests might show plasma neutrophil leucocytosis. Mild pleiocytosis is seen in only 60% of patients in the first week.⁸⁹ The diagnosis can be made by early identification of antigen or viral RNA or by virus isolation, and in unvaccinated people, antibody detection (table 2).^{49,89-94}

Survivors of rabies

Since 1970, there have been reports of five patients said to have survived rabies encephalitis (table 3).^{21,22,94-97} All these patients had received some rabies vaccine before the onset of symptoms, but none had had rabies immune globulin (RIG). Neither rabies virus nor viral antigen was detected, but samples were taken when neutralising antibody was present. All the diagnoses were based on high antibody concentrations in the cerebrospinal fluid. In the future, the diagnosis of rabies in such cases might be confirmed by RNA detection by RT-PCR. The diagnosis remains in doubt, however, in the patients who were given vaccines of nervous-tissue origin, because postvaccinal encephalitis can produce similar signs and symptoms.^{98,99} The term "limited survival" is more appropriate than "recovery" in the three patients given tissue-culture vaccines, since all had profound residual neurological deficits. Severe impairment of nervous function was irreversible despite control of the infection, presumably by the immune response.

Patient and reference	Exposure	Treatment	Incubation period and disease	Diagnosis	Outcome
9 years, boy; Ohio USA; 1970 ⁹⁴	Thumb bite by proven rabid big brown bat (<i>Eptesicus fuscus</i>)	Duck-embryo vaccine next day	20 days: encephalitis, weakness of bitten arm, focal seizures, paralysis, cerebral oedema, coma, atrial arrhythmia	High concentrations of antibody in CSF and serum	Intensive care, complete recovery in 6 months
45 years, woman; Argentina; 1972 ⁹⁵	Bite by clinically rabid dog, which died 4 days later	Suckling-mouse-brain rabies vaccine 10 days later	21 days: signs of cerebellar dysfunction: tremors, myoclonic spasms, ataxia hypertonia, dysphonia; quadriplegia, dysphagia, varying levels of consciousness; cardiac conduction defect	High concentrations of antibody in CSF and serum	Recovered but relapsed twice after vaccine boosters, slow resolution over 1 year
32 years, man; New York, USA; 1977 ^{21,22}	Inhaled aerosol of fixed rabies virus, SAD strain, in laboratory	Only pre-exposure duck-embryo rabies vaccine*	21 days: fever, encephalitis, spastic hemiparesis, myoclonus, impaired consciousness, respiratory arrest	High concentrations of antibody in CSF and serum	Gradual improvement, personality disorder, dementia
9 years, boy; Mexico; 1992 ⁹⁶	Head bite by proven-rabid dog	Vero-cell vaccine next day	19 days: encephalitis, fever, convulsions, intracranial hypertension, deep coma, quadriplegia	High concentrations of antibody in CSF and serum	Improved slightly, reacted to painful stimuli, blind and deaf; died at 34 months
6 years, girl; India; 2000 ⁹⁷	Face and hand bites by stray dog, which died 4 days later	No wound cleaning, chick-embryo rabies vaccine same day	16 days: would not drink, fever, hallucinations, coma, excessive salivation, focal seizures	High concentrations of antibody in CSF and serum	3 months of coma slow improvement; at 18 months spasticity, tremors, involuntary movements

*Neutralising antibody titre 1:32, 6 months before exposure.

Table 3: **Human recovery from rabies encephalitis**

Management

The mortality from rabies is 100% in unvaccinated patients. Despite many attempts at intensive-care treatment over the past 30 years, no vaccinated patient has recovered without severe sequelae. Life can be prolonged, but many complications arise. Heavy sedation and analgesia should be given to relieve the agonising symptoms. Ketamine is an appropriate anaesthetic, although the concentrations reached in the central nervous system are probably insufficient for an antiviral effect as discussed above.¹⁰⁰ Immunosuppressive and antiviral drugs have not proved useful.

Until a new specific therapy is available, palliative care is recommended. Patients and their relatives should be advised that although intensive-care therapy may prolong life, there can be no expectation of survival in unvaccinated patients. Previously immunised patients will have severe permanent neurological disabilities.¹⁰⁰

Prevention

Since rabies is untreatable, prevention of infection is paramount. The most efficient way to control human rabies is to eliminate infection in animal vectors. Domestic-dog strains of rabies virus account for more than 90% of human disease worldwide. Rabies in stray dogs can be reduced by parenteral vaccination, fertility control, and clearing rubbish to reduce the food supply.¹⁰¹ Vaccination of wildlife vectors with oral live attenuated rabies virus or vaccinia-recombinant vaccines has virtually eliminated fox rabies in western Europe, and vaccinia-recombinant vaccines have been used against coyote, raccoon, and fox rabies in North America. Other similar vaccines are being developed. A single human infection by vaccinia-recombinant animal rabies vaccine has been reported in a pregnant woman with a chronic skin condition, epidermolytic hyperkeratosis.¹⁰² Despite much effort, DNA vaccination against rabies has not proved practicable.¹⁰³

So far there is no means of controlling rabies in some inaccessible vector species, such as insectivorous bats, despite their potential for infecting people. Avoidance of contact with bats, pre-exposure vaccination, and prompt postexposure treatment for people who may have been exposed are the only means of preventing human infection.¹⁰⁴

Human prophylaxis

Rabies vaccines

Two rabies vaccines are now licensed for use in the UK and USA: human-diploid-cell vaccine (HDCV; Imovax Rabies, Aventis Pasteur, Lyon, France) and purified chick-embryo-cell vaccine (PCECV; Rabipur, RabAvert, Chiron Behring). Both are sold in single-dose 1 mL vials. Elsewhere, purified vero-cell vaccine (PVRV; Verorab, Aventis Pasteur) is widely available in a single-dose 0.5 mL vial. Rabies vaccine adsorbed (BioPort, Lansing, MI, USA) is also licensed in the USA.

Pre-exposure treatment

The most successful form of rabies prevention is pre-exposure vaccination. No rabies deaths have been reported in anyone who has had pre-exposure treatment followed by a booster dose after exposure. For people given pre-exposure vaccine, postexposure treatment is simplified—only two doses of vaccine (days 0 and 3) and no RIG. Prophylaxis is recommended for people at occupational risk and for travellers to areas where dog rabies is endemic, mainly in Asia and Africa.^{104,105}

The standard pre-exposure regimen is three doses of a tissue-culture vaccine intramuscularly (deltoid) on days

0, 7, and 28 (or day 21).¹⁰⁶ A booster dose after 1 year increases and prolongs the antibody response.¹⁰⁷ The frequency of vaccine booster doses varies according to the risk of exposure to rabies, and treatment can be avoided if adequate neutralising antibody is shown. In the USA, antibody testing or booster vaccination is recommended every 6 months for people at high risk (eg, laboratory staff handling the virus) and every 2 years for those at frequent risk (eg, some wildlife officers). At the other extreme, no booster doses are deemed necessary for travellers and others at low risk.¹⁰⁴ The secondary immune response to emergency booster doses of rabies vaccine is predictably prompt,¹⁰⁸ and the rapidity is likely to be the important component of postexposure treatment, rather than the actual antibody concentration achieved. Evidence of immunity persists for 5–10 years, although in one study, the antibody concentration declined in 3.5% of vaccinees but responded to boosting, albeit at a lower concentration than the rest.¹⁰⁷ This finding accords with Kuwert and colleagues' identification of "poor responders" with lower later antibody induction.¹⁰⁹ Repeated vaccine treatment can be avoided if the presence of neutralising antibody is proven.

An economical pre-exposure regimen, recommended by WHO¹⁰⁶ and approved and previously used in the USA, is to give 0.1 mL vaccine intradermally on the same 3 days as for intramuscular vaccination. The pharmaceutical companies cannot now sanction this approach because only 1 mL ampoules are produced, which do not comply with regulations for multidose vials.

Postexposure treatment^{104,106}

Rabies virus in an animal's saliva can infect mucous membranes or tissues through broken skin. Intact skin is protective, but minute lesions caused by bats may result in infection. Other routes of infection are rare.⁴⁹ Rabies mortality after untreated bites by proven rabid dogs was 35–57% in India.^{110,111} The risk of infection is increased in severe exposure: if bites are on the head, neck, or hands, or are multiple or deep. Modern postexposure treatment is highly successful; failures of optimum treatment are exceptional.¹¹² However, in many cases complete treatment starting on the day of the bite is not given. Wound care with passive and active rabies immunisation are essential especially after severe exposure. Postexposure treatment is assumed to neutralise or inactivate virus while it is still in the wounds, before it gains access to the nervous system where it is protected from immune attack. Therefore, treatment after exposure to rabies virus is very urgent, even if the patient was bitten months before. The decision to give postexposure treatment depends on the assessment of risk of infection in each patient, influenced by the circumstances of the exposure, the mammal concerned, and results of tests.⁴⁹

Experiments have shown that thorough washing of rabies-infected wounds with soap and water can increase survival by 50%.¹¹³ However, this cheap, available treatment is omitted in most cases. Virucidal agents based on iodine or alcohol are advised, and antibacterial treatment should be considered.^{49,106}

The standard vaccine regimen is five doses intramuscularly into the deltoid on days 0, 3, 7, 14, and 28. Injections can be given into the anterolateral thigh for children, but should not be given into the buttock. Non-specific side-effects have been attributed to tissue-culture rabies vaccines, but they are very rarely associated with neurological features.⁹⁹

Passive immunisation with human RIG lowers mortality after severe exposure,¹¹⁴ but many years of

clinical experience suggest that it is not as important for milder rabies exposure (eg, single bites on limbs) as for more severe exposures. A single dose is infiltrated locally around the bite wound as soon as possible after the incident.¹⁰⁶ RIG is expensive and increasingly scarce, so other products are under investigation including a mixture of specific monoclonal antibodies.¹¹⁵

The efficacy of postexposure treatment depends on the efficient, immediate, accurate delivery of all three components of recommended treatment; the competence of the host immune response, and the susceptibility of the infecting virus to the immunity induced by the vaccine. Although delay and errors in implementing treatment can in theory be overcome, immunosuppression by drugs or chronic diseases such as cirrhosis could prove fatal. The antibody response to rabies vaccine is greatly impaired or absent in HIV-infected patients who have low counts of CD4-positive lymphocytes.¹¹⁶ Use of a double dose of vaccine has been suggested in these circumstances, but in the absence of good data, use of the most immunogenic vaccine regimen (with multiple-site intradermal injections on the first day) might be the most appropriate approach.¹¹⁷

There is no evidence of a lack of efficacy of rabies vaccines against genotype 1 rabies strains, but experiments on their protective effect against EBLV have given varying results, especially with antigens of different viral origins.¹¹⁸ The Pitman-Moore strain is used in HDCV and PVRV manufacture, and the Flury-LEP strain for PCECV, but some studies have used another strain, Pasteur virus. Although some human serology and animal experiments have shown poor results with viruses of vaccines for human use against EBLV type 1 virus,¹¹⁹⁻¹²² HDCV afforded protection against challenge with EBLV type 1 and partial protection against EBLV type 2b in mice.¹²³ Furthermore, human vaccinees had neutralising antibody to EBLV type 1,¹²⁴ and there is some evidence of EBLV-specific cell-mediated immunity.¹²⁵

Although commercially produced RIG did not protect mice against challenge with EBLV type 1,¹²⁶ it has neutralised this virus.¹²⁴ In another study, RIG did not cross-neutralise EBLV type 1, but it did neutralise EBLV type 2.¹²⁷ Antibody to an experimental DNA rabies glycoprotein antigen of the Pasteur virus strain cross-neutralised fairly well with EBLV type 2a, but poorly with type 1.⁴ All three studies of the efficacy of vaccines against both EBLV types 1 and 2 showed different results for the two subtypes.^{4,123,127} Rabies vaccines should be tested against appropriate wild-type lyssaviruses.²⁴

Meanwhile, hundreds of people bitten by bats, some proven EBLV positive, have received postexposure vaccination. None has developed EBLV encephalitis. Although the protection from current rabies vaccines and RIG is likely to be less efficient against EBLV than against genotype 1 rabies infection, there is currently no other treatment. This uncertainty increases the importance of pre-exposure vaccination and the urgency of postexposure treatment in anyone exposed to rabies-related viruses.

Rabies prophylaxis in less developed countries

The recommendations for prophylaxis outlined above are applicable worldwide, but implementation is impossible where medications are unaffordable or unobtainable and health facilities remote. In South Africa in 2001, 26% of rabies-vaccine treatment facilities had no vaccine in stock, and 53% had no RIG.¹²⁸ Postexposure treatment is given to more than 7 million patients each year in Asia alone, with the highest rate of 766 per 100 000 population per year in Vietnam.¹²⁹ These numbers indicate a potential

source of widespread long-lasting anxiety suffered by bite victims and their families, in many cases in addition to the financial burden of paying for vaccine. Although in China, Thailand, Sri Lanka, and the Philippines tissue-culture vaccines are now used exclusively, rabies vaccines of nervous-tissue origin are still widely used elsewhere. Semple (sheep-brain) vaccine is produced in India, Pakistan, Bangladesh, and Nepal; suckling-mouse-brain vaccine in Vietnam and some countries in Africa and South America; and Fermi vaccine containing partially inactivated virus in Ethiopia.¹³⁰ These vaccines are weak antigens. In Karachi, Pakistan, 40% of patients with rabies encephalitis had received a full course of Semple vaccine;¹³¹ and in Delhi, India, 10.6% had been fully vaccinated and others had had incomplete vaccine courses.¹³² Neurological complications to nervous-tissue vaccines still occur, with a frequency estimated at 1 in 200 for Semple vaccine.¹³³

In anticipation of the cessation of production of Semple vaccine in India, tissue-culture vaccines are already produced or are being developed locally. Through technology transfer from Chiron, PCECV (Rabipur, Hoechst, Mumbai, India) is now successfully produced in, and exported from, India.

In an attempt to overcome the very high cost of tissue-culture vaccines, two economical multisite intradermal regimens for postexposure treatment have been developed and are now recommended by WHO.¹⁰⁶ They use only 40% of the amount of vaccine needed for the standard intramuscular course.

For the eight-site intradermal regimen, on day 0 a whole 1 mL vial is injected into the skin, divided between eight sites, about 0.1 mL PCECV or HDCV per site; doses of 0.1 mL are injected intradermally at four sites on day 7; and 0.1 mL is injected intradermally at one site on days 28 and 91.¹³⁴ There is no report of the use of PVRV, which contains 0.5 mL per ampoule, with this schedule. Although the equivalent dose would be 0.05 mL per site, in a recent study twice this volume of PVRV was used.¹³⁵

The two-site intradermal postexposure regimen has been used widely in parts of Asia where RIG is generally available. It was designed for use with PVRV,¹³⁶ with an intradermal dose of 0.1 mL per site. If other (1.0 mL) vaccines are used, each dose is 0.2 mL. On days 0, 3, and 7, one intradermal dose is given at two sites; on days 28 and 91, a single intradermal dose is given.

These two intradermal regimens use the same amount of vaccine, but a comparative study showed that the eight-site method consistently induced significantly higher concentrations of neutralising antibody from day 7 onwards than the two-site regimen, which is important when RIG is not available.¹³⁷

A further reduction in the cost of the two-site regimen has been proposed by administration of 0.1 mL instead of the standard 0.2 mL PCECV per intradermal site.¹³⁸ The new regimen was tested in two non-randomised trials in 50¹³⁹ and 120¹⁴⁰ confirmed rabies-exposed patients with transdermal (category III) lesions; some of the former and all the latter also received RIG. This method would be predicted to give better protection than the current Semple vaccine, but in comparison with the standard intradermal regimens, the margin of safety is likely to be lower, especially after severe exposure.

Human RIG is prohibitively expensive, but equine RIG may be available in less developed countries. However, less than 2% of all vaccine-treated patients also receive RIG.¹⁴¹ This low proportion and the high prevalence of HIV and AIDS in many areas combine to reduce the efficacy of postexposure treatment in some patients.

The future

The greatest challenge to rabies control worldwide is the extent of the dog rabies epizootic in Asia and Africa. Control is hindered by ignorance of the varied ecology of the disease. A current WHO initiative in Asia may yield data to direct implementation of potentially highly efficient methods to control dog rabies and also ensure safer, more appropriate human prophylaxis.

In Europe, moves to improve surveillance should reveal more detail of the distribution of EBLV infection. Assessment of the risk of human exposure requires better understanding of the infectivity of bats in which viral RNA, but no virus, is detectable.

Recent rapid progress in elucidation of the neurophysiology of neuronal transport and synaptic function might indicate how rabies virus steals its way into and through the brain. Further knowledge of the influence of the virus on host-cell gene expression could help unravel the mysteries of rabies pathogenesis in molecular detail. Any innovations in methods of prophylaxis and effective treatment depend on the results of such future research. Meanwhile, the excellent human rabies tissue-culture vaccines could be used more effectively in less developed countries to replace all nervous-tissue vaccines with highly immunogenic economical intradermal tissue-culture regimens. This change is strongly endorsed by WHO. New monoclonal-antibody products already being investigated could begin to address the crisis in the global supply of RIG.

Lyssaviruses cause unrecognised disease and suffering, but methods for radical amelioration exist, and should be implemented.

Conflict of interest statement

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