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VIROLOGY - CHAPTER TWENTY

RABIES

Dr Richard Hunt

Rabies virus belongs to the family: Rhabdoviridae. (Greek: Rhabdos: rod). Rhabdoviridae can infect a variety of animals and plants.

The most important rhabdovirus, as far as human disease is concerned, is rabies virus. Worldwide, it is estimated that approximately 55,000 persons die of rabies each year. According to CDC, most (more than 90%) of all animal cases of rabies reported occur in wild animals; before 1960, most were in domestic animals. The principal rabies hosts today are wild carnivores and bats. The number of human deaths from rabies in the United States has declined from more than 100 annually in 1900 to one or two per year in the by the end of the century. These deaths are usually due to exposure to indigenous rabid bats, skunks, or raccoons, or to exposure to rabid dogs while traveling overseas.

Modern prophylaxis is nearly 100% successful.

TABLE 1
Rhabdoviruses

Type	Virus	Distribution	Species infected	Disease
Vesiculovirus	Vesicular stomatitis virus (VSV)	Caribbean	Cattle, pigs horse	Acute, self limiting
Lyssavirus	Rabies virus	Worldwide	Many mammals including humans	Slow, progressive
Plant rhabdoviruses Cytorhabdovirus	Lettuce necrotic yellows virus			
Nucleorhabdovirus	potato yellow dwarf virus			
Other animal rhabdoviruses			Mammals, fish, birds, arthropods	

TEACHING OBJECTIVES

To know the different types of rhabdoviruses

To learn about the structure and replication of these negative strand RNA viruses

To understand the pathology of rabies

TABLE 2 Other (non-rabies) lyssaviruses reported to infect humans		
Virus	**Country and year of infection**	**Animal vector**
Australian Bat Lyssavirus	Australia - 1996/97	Bats
European bat lyssavirus-1	Russia - 1985	Bats
European bat lyssavirus-2	Finland - 1985 Scotland - 2002	Bats
Duvenhage	South Africa - 1970/2006 Kenya - 2007	Bats
Mokola	Nigeria - 1968/71	No identified
Others that were untyped	Ukraine - 1977 China - 2002 Ukraine - 2002	Bats

STRUCTURE OF RHABDOVIRUSES (figure 1)

Rhabdoviruses are negative strand RNA viruses; that is they have a single strand of RNA that is anti-sense to the messenger RNA needed to code for viral proteins. This means that the RNA cannot code directly for protein synthesis and must be copied to positive strand mRNA. As a result, the virus must carry its own RNA-dependent RNA polymerase.

As their name suggests these viruses are rod shaped. They have one end that is rounded and are often referred to as bullet-shaped. Each virus particle is up to 100nm diameter and 400 nm long but this is very variable. They have an envelope derived from the host cell plasma membrane. The virus has only five proteins.

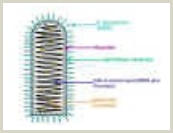
Figure 1A - Rhabdovirus structure General structure of a rhabdovirus

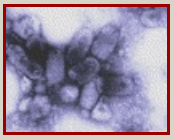
Figure 1B - Negative stain electron micrograph of rabies virus Wadsworth Center, NY Dept of Health


Figure 2 - Replication of rabies virus The cycle of rabies infection and replication CDC

G (Surface) Protein

This is the surface glycoprotein spike and exists as trimers. There are about 1200 G proteins (400 trimers) per virus particle. It is a transmembrane protein with an N-terminal signal sequence. The G protein binds to cellular receptors and is the target of neutralizing antibodies. There are three sugar chains that are N-glycosidically attached. Penetration of the virus into the cytoplasm takes place in the endocytic pathway and not at the plasma membrane. This is because the G protein trimer undergoes a change in conformation at pH 6.1 which stabilizes the trimer and probably allows a hydrophobic region of the molecule to become exposed and to embed in the membrane of the cell to be infected.

M (matrix) protein

This is a peripheral membrane protein (originally M stood for membrane) that appears to line the inner surface of the viral membrane, though this remains somewhat controversial. It may act as a bridge between the membrane or G protein and the nucleocapsid.

Nucleocapsid

This is the infectious ribonucleoprotein core of the virus. It is a helical structure that lies within the membrane. In negative stain electron micrographs, such as seen in figure 1, the nucleocapsid has a striated appearance.

N (Nucleoprotein) protein. This is the major structural protein and covers the RNA genome. It protects the genome from nucleases and holds it in a conformation that allows transcription

L (Large) protein and **NS (nonstructural, otherwise known as P (phospho))** protein together form the RNA-dependent RNA polymerase or transcriptase. The L protein has a molecular weight of 240 kiloDaltons and its gene takes up 60% of the genome (figure 3).

REPLICATION (figure 2)

Binding

The receptors for rhabdoviruses have yet to be definitively identified but some experiments point to phospholipids, particularly phosphatidyl serine, as the cell surface receptor molecule.

Penetration

After endocytosis, pH-dependent fusion with the membrane of the endocytic vesicle occurs. The nucleocapsid enters the cytoplasm. All subsequent stages take place here with no involvement of the nucleus of the cell.

Transcription

First, the polymerase, which is carried in the entering virus, makes five individual mRNAs, one for each viral protein. Note, the RNA must be made before any viral protein synthesis and so the infecting virus must supply the polymerase enzyme. (As might be expected, this primary transcription process takes place in the presence of protein synthesis inhibitors). The mRNAs are capped, methylated and polyadenylated. The sequence of transcription is N, NS(P), M, G and L with synthesis of the mRNAs being attenuated at each gene junction (figure 3). This means that less of the L mRNA is made than any of the others.

Figure 3 - Rhabdovirus genome The rhabdovirus genome CDC

Replication

In addition, the polymerase transcribes the negative-sense genomic RNA into a positive sense strand. This serves as a template for the transcriptase to transcribe new negative sense genomic RNA molecules. This replicative phase does require protein synthesis and the same polymerase is involved. In the replicative phase, this enzyme must ignore signals that define the individual mRNA species and make one single RNA molecule. The switch between transcription of mRNAs and replication of genomic RNAs seems to be controlled by the level of N protein

Assembly

The G protein mRNA is translated in association with the endoplasmic reticulum and transported via the Golgi body to the cell surface. Here, it forms patches with which the M protein associates. The genomic length negative strand RNA molecules associate with N, L and NS (P) proteins forming the core nucleocapsids. This, in turn, associates with the M protein at the inner surface of the plasma membrane or perhaps in the cytoplasm. The interaction between nucleocapsid and M protein causes the former to change configuration so that it appears more condensed. The nucleocapsid then buds through the membrane.

PATHOGENESIS

VESICULAR STOMATITIS VIRUS (VSV)

VSV infects cattle in Caribbean and occasionally in US. It is also found in horses and pigs but rarely humans

RABIES

Transmission

Rabid animals become aggressive and harbor the virus in saliva and thus transmission is frequently via animal bites. In rare cases, rabies has been transmitted by corneal transplant or transplant of other tissues, or through contact of infected saliva with mucosal membranes or an open wound in the absence of a bite. The CDC states: “Inhalation of aerosolized rabies virus is also a potential non-bite route of exposure, but other than laboratory workers, most people are unlikely to encounter an aerosol of rabies virus”. It has been suggested that people in infected bat caves may be exposed to aerosolized virus. Most bats are not infected.

Disease

The virus binds to nerve or muscle cells at the site of the inoculation via nicotinic acetylcholine receptors. Here the virus can remain for a prolonged period of time (up to several months). The virus can replicate in muscle cells at the site of the bite with no obvious symptoms. This is the incubation phase.

WEB RESOURCES

CDC Rabies Page

Figure 4 - Rabies pathogenesis

CDC

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1. Raccoon is bitten by a rabid animal
2. Virus enters wound via saliva
3. Virus spreads through nerves to spinal cord and brain
4. Incubation period of 3-12 weeks with no symptoms
5. In brain the virus replicates and spreads to other tissues including the salivary glands. Signs of disease occur
6. The animal dies within a week

The virus then moves along the nerve axons to the central nervous system using retrograde transport. The virus arrives at the dorsal root ganglia and the spinal cord. From here, spread to the brain occurs. A variety of cells in the brain can be infected including in the cerebellum, the Purkinjes cells and also cells of the hippocampus and pontine nuclei. This is the prodromal phase. Infection of the brain leads to encephalitis and neural degeneration although elsewhere the virus seems to cause little in the way of a cytopathic effect. Involvement of the brain leads to coma and death. This is the neurological phase and during this period, the virus can spread from the central nervous system, via neurons, to the skin, eye and various other sites (adrenals, kidneys, pancreatic acinar cells) and the salivary glands (figure 4).

There are various factors that determine the timing of the onset of symptomatic rabies but most important are the number of virus particles in the infection and how close the bite is to the brain. The immunological status of the patient is also important. It should be noted that the immune response to naturally acquired virus is slow and a good neutralizing response is not seen until the virus has reached the brain which is too late for survival. Cell-mediated immunity plays little role in a rabies infection. Rabies is almost always fatal and only three survivors of symptomatic rabies have been documented. Nevertheless, a good immune response that eliminates the infection, can be achieved using a vaccine even after infection because of the long incubation phase.

Epidemiology

Rabies is usually transmitted by an animal bite. Worldwide most cases arise from a dog bite. Canine rabies is prevalent in Latin America, Asia and Africa.

In recent years (1990 - 2004), in the US the majority of cases (35 out of 47) have been associated with bat rabies; of the remaining cases, two were acquired in the US (one dog/coyote like-strain and one raccoon strain) and 10 were acquired outside the US (all dog/coyote like strains).

Many animals in the US are infected with rabies viruses, including raccoons (especially along the eastern seaboard states), skunks, coyotes, and foxes. Small rodents are rarely infected, but there have been cases reported, especially in woodchucks. Dogs, cats and cattle are potential vectors - in the US immunization of pets has lessened the risk of pets acquiring rabies from wild animals. Bats also carry rabies, although most bats are not infected. Bats have very small, sharp teeth, and people who are bitten may not be aware of the bite, or do not bother to do anything about it. With most bites from other rabid animals, the victim normally seeks treatment because the bite is more serious and also because the animal appeared to behave in a suspicious fashion; the level of awareness seems to be lower for suspiciously behaving bats. Immunization of pets and prompt response for bites from most suspicious animals may explain why bat-transmission of rabies has been the predominant mode of transmission in recent years.

In many cases of bat-associated rabies, there is no record of a bite. In some cases, the victim or their family may be aware that they handled a bat or that an oddly behaving bat was found (e.g. a bat which is active by day, is easily approached, is unable to fly, is in a room in a house or on a lawn). However, if the victim is not able to answer questions it may be difficult to obtain a history of bat contact since they may not have found the incident worth mentioning to anyone.

Human to human transmission has occurred in a few cases of corneal transplants (when it was not realized that the encephalitis was due to rabies). This has led to stricter criteria in screening of potential donors for encephalitis so that those who might have rabies (or Creutzfeld-Jakob disease) are not accepted. Recently (2004) an organ donor who died of a brain hemorrhage also had rabies and it was transmitted to 4 recipients. Apart from transplant cases, no human-human spread of the disease has ever been documented.

Cases of human rabies in US 1995-2011

Table 2 - Major animal reservoirs of rabies	
North America	Skunks, raccoons, bats, foxes
South America	Rabid dogs, vampire bats
Europe	Badgers, foxes

In many western countries where rabies is endemic, vaccination of animals has reduced the rate of human disease and in the United States there is approximately one case of human rabies per year. In countries such as the United Kingdom, where there is no rabies in the wild animal population, vaccination is not used. In some other countries, rabies is much more of a problem. For example, India records about 25,000 cases of human rabies per year, mainly from dog bites. In South America, rabies transmission by vampire bats is a major problem for the cattle industry (table 2).

Symptoms

Vaccination, even after exposure, is extremely effective at preventing disease. Without such treatment, rabies is almost invariably fatal (although, see the case report at left). During the incubation/prodromal period, symptoms include: pain or itching at the site of the wound, fever, headache and gastrointestinal problems. After this period (usually of up to two weeks), CNS infection is apparent. In up to half of patients, hydrophobia is seen. This fear of water is the result of the pain associated with drinking. There are also seizures and hallucinations. In some patients paralysis is the only symptom and this may lead to respiratory failure. Following the neurological phase, the patient becomes comatose. Because of the neurological problems including respiratory paralysis, death ensues.

WEB RESOURCES

Bats and Rabies (CDC)
Rabies: Question and Answer (CDC)

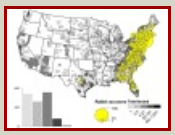
CASE REPORT
Recovery of a Patient from Clinical Rabies --- Wisconsin, 2004

Investigation of Rabies Infections in Organ Donor and Transplant Recipients

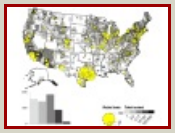
Map of terrestrial rabies reservoirs in the United States during 2010. Raccoon rabies virus variant is present in the eastern United States, Skunk rabies in the Central United States and California, Fox rabies in Texas, Arizona, and Alaska, and Mongoose rabies in Puerto Rico.

CDC

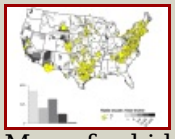
Graph of rabid wild animals reported in the United States from 1960-2010



Map of rabid raccoons reported in the United States during 2010. Majority of the cases occur in the eastern United States.



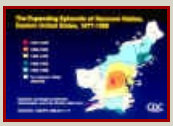
Map of rabid bats reported in the United States during 2010. Cases are broadly distributed throughout United States.



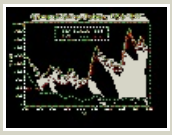
Map of rabid skunks reported in the United States during 2010. Majority of the cases occur in central and eastern United States

Map of rabid foxes reported in the United States during 2010. Cases primarily distributed in eastern United States.

Map of rabid dogs and cats reported in the United States during 2010.



The Expanding Epizootic of Raccoon Rabies, Eastern United States, 1977-1996



Cases of animal rabies in the United States, 1955-1999

Figure 5 (All images from CDC)

Diagnosis

Overt symptoms clearly define symptomatic rabies in people who suffer animal bites but by this time, therapeutic intervention is too late. After a bite, laboratory tests can determine whether an animal is indeed rabid. The presence of rabies virus in an animal or an infected person is determined by multiple tests:

- Serology (neutralizing serum or cerebrospinal fluid antibodies in an unvaccinated person are diagnostic but usually are only detectable late in disease).
- Immunofluorescence antigen determination using biopsy skin, brain or corneal specimens (figure 8). A full thickness nuchal skin biopsy (skin biopsy from the nape of the neck in which the observer looks at the nerves at the base of the hair follicles) or brain biopsy can be examined for rabies antigen using a direct fluorescent antibody test.
- Saliva may be tested for rabies virus RNA by RT-PCR (reverse transcription-polymerase chain reaction) or by isolation of the virus.
- Histologically very characteristic is the presence of Negri bodies. These are eosinophilic intracytoplasmic inclusions formed by aggregates of nucleocapsids in neurons of about 50 to 80% of infected humans (table 3 and figure 7). They are typical of rabies, but the results need to be read by someone experienced with rabies and there can be false positives - so all such results need to be confirmed by another method.
- Other tests include the growing of virus in the brains of mice or in culture, after which antigen tests are used to determine the presence of virus. Also anti-rabies antibodies can be detected BUT only very late in the disease. Polymerase chain reaction (PCR) can also be used to detect virus (figure 6).

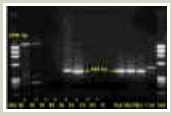
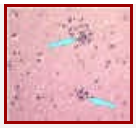


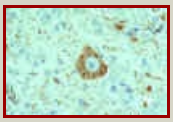
Figure 6 PCR test results for the presence of rabies virus. The arrows indicate positions of positive bands CDC

Table 3	
Histopathologic evidence of rabies encephalomyelitis (inflammation) in brain tissue and meninges	
Mononuclear infiltration	 Perivascular cuffing of lymphocytes or polymorphonuclear cells or inflammation around a blood vessel CDC
Lymphocytic foci	

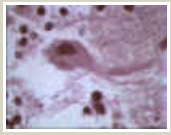
 Babes nodules consisting of glial cells Image: CDC

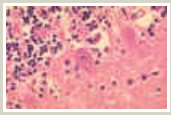
Negri bodies (see below)

 Figure 7 Neuron without Negri bodies CDC

 Rabies virus-infected neuronal cell with intracytoplasmic inclusions (Negri bodies). The red stain indicates areas of rabies viral antigen by using IHC or avidin-biotin complex CDC

 Negri body in infected neuron CDC

 Negri body in brain cell © Bristol Biomedical Image Archive. Used with permission

 Histopathology of rabies, brain. Characteristic Negri bodies are present within a Purkinje cell of the cerebellum in this patient who died of rabies. CDC/Dr. Makonnen Fekadu maf1@cdc.gov

 Rabies virus budding from an inclusion (Negri body) into the endoplasmic reticulum in a nerve cell. A. Negri body.
B. Notice the abundant RNP in the inclusion.
C. Budding rabies virus. CDC

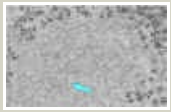
 Ribonucleoprotein. Notice the abundant strands of coiled RNP (almost everything in the image is RNP). CDC

Figure 8 Direct fluorescent antibody test (dFA)
The dFA test is based on the principle that an animal infected by rabies virus will have rabies virus protein (antigen) present in its tissue. Because rabies is present in nervous tissue (and not blood like many other viruses), the ideal tissue to test for the presence of rabies antigen is brain. The most important part of a dFA test is fluoresecently-labeled anti-rabies antibody. When labeled antibody is added to rabies-suspect brain tissue, it will bind to rabies antigen if it is present. Unbound antibody can be washed away and the areas where the antigen has bound antibody will appear as a bright fluorescent green color when viewed with a fluorescence microscope. If rabies virus is absent there will be no staining. The rabies antibody in the dFA test is primarily directed

PREVENTION AND TREATMENT OF A PERSON WHO MAY HAVE BEEN EXPOSED

The wound should be immediately and thoroughly washed with soap and water, then treated with 40-70% ethyl alcohol or an antiseptic such as benzyl ammonium chloride. The State Health authorities should be promptly informed. The risk of exposure to rabies and whether prophylactic treatment should be given are determined in consultation with the State Health Department. If the animal is available, the brain should be examined for rabies virus antigen by fluorescent antibody. (In some cases, if the bite was from a domesticated cat or dog, the animal may be kept under close observation).

Post-exposure prophylaxis

Rabies vaccine
This is an inactivated vaccine and is strongly immunogenic. It is grown in human diploid cells or rhesus monkey lung cells and is more potent and has fewer side effects than the vaccine used in the early 1980's. A purified chick embryo cell grown vaccine is also available. The vaccine is administered as a series of injections over a 4-week period. HRIG (human rabies immunoglobulin) is also given.

Human rabies immunoglobulin (HRIG)
HRIG is prepared from the plasma of hyperimmune donors. Up to half of the recommended dose is infiltrated into the wound area if possible. The remainder is given as an intramuscular injection. A separate syringe and a separate site are used for the HRIG and the vaccine so that the HRIG does not neutralize the vaccine.

So far there has never been a case of someone who received appropriate post-exposure

against the nucleoprotein of the virus. Rabies virus replicates in the cytoplasm of cells, and infected cells may contain large round or oval inclusions containing collections of nucleoprotein (N) or smaller collections of antigen that appear as dust-like fluorescent particles if stained by the dFA procedure CDC

CASE REPORTS

Recovery of a Patient from Clinical Rabies --- Wisconsin, 2004

Investigation of Rabies Infections in Organ Donor and Transplant Recipients

Presumptive Abortive Human Rabies --- Texas, 2009

Recovery of a Patient from Clinical Rabies — California, 2011

prophylaxis in the US developing rabies. (About 40,000 people per year are treated in the US).

Pre-exposure prophylaxis

People at risk for rabies infection may be vaccinated as a preventive measure. Such individuals include

- rabies-laboratory workers
- certain people in areas with enzootic rabies who are at risk for exposure to rabid animals: veterinarians and their staff, wildlife control workers, spelunkers (mainly those cave explorers who go into undeveloped caves with bat colonies); travelers who will be spending more than a month in areas with enzootic rabies.

People at high risk for exposure to rabid animals should have regular serologic testing and booster vaccinations when necessary.

If a vaccinated person is exposed to rabies, they still need to get post-exposure prophylaxis, but the number of post-exposure vaccination shots is reduced and HRIG is not used.

Treatment

If symptoms are localized to the site of the bite, aggressive antiviral therapy (vaccine, HRIG, ribavirin, interferon, monoclonal antibodies, etc) may be tried. There is no specific anti-viral treatment once CNS symptoms develop. Intensive supportive care is given. Five of the six known survivors of rabies infection received prophylaxis prior to developing clinical symptoms. There have been several documented cases of a non-vaccinated survivors of rabies. (See case report at left and section below).

In Texas in 2009, an adolescent girl developed encephalitis after exposure to bats, two months before illness. Anti-rabies virus antibodies were detected in her serum and cerebrospinal fluid using an indirect fluorescent antibody test. However, the presence of rabies virus-neutralizing antibodies was not detected until after she had received single doses of rabies vaccine and human rabies immune globulin. She required multiple hospitalizations and follow-up visits for recurrent neurologic symptoms but survived without intensive care.

Treatment of rabies using induced coma

The Milwaukee Protocol

While rapid post-exposure treatment of a rabies-infected patient before neurological symptoms have developed is usually successful, once these symptoms develop the disease was considered fatal as a result of temporary brain dysfunction.

There are now six humans who are known to have been infected with the rabies virus who have survived without post-exposure vaccination before the onset of symptoms. The procedure used to treat these individuals involves giving anti-viral drugs while the patient is in a chemically-induced coma. It was used first on a Wisconsin teenager, Jeanna Giese, by Dr Rodney Willoughby in Milwaukee, Wisconsin and is variously referred to as the Wisconsin protocol, the Willoughby protocol or, most frequently, the Milwaukee protocol. Details from CDC of this and other cases are found at links on the left.

As in most cases in the United States, Ms Giese was infected by a rabid bat which she had picked up and the infection was transmitted by a bite from the bat. The bite was treated with hydrogen peroxide but the family then ignored the potential for infection. The patient subsequently (about five weeks) developed a fever with neurological symptoms that included a jerking of her arm, slurring of her speech and diplopia (double vision) and was diagnosed with rabies. No live virus was isolated but anti-rabies antibodies had been induced.

The Milwaukee protocol involves putting the patient into a coma (to

protect the brain) for long enough to develop anti-viral neutralizing antibodies. The coma was induced with ketamine, a drug used in general anesthesia, and midazolam which is a benzodiazepine sedative. In addition, the patient was administered two anti-viral drugs: ribavirin and amantadine. In a revised version of the protocol, ribavirin is not used. The patient remained comatose until an immune response to fight off the virus was apparent. Ms Giese did have some neurological symptoms as a result of brain damage caused by the virus and needed further therapy.

 Return to the Virology section of Microbiology and Immunology On-line

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