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Review Article

RABIES VIRUS PROTEINS AND THEIR MECHANISM OF PATHOGENECITY

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ABSTRACT

Rabies is a zoonotic viral disease. Rabies is transmited by bites of a rabid animal. The rabies virus encoded five proteins, namely glycoprotein, nucleoprotein, phosphoprotein, matrixprotein and RNA dependnt RNA transcriptase protein. These proteins play an important role in inducing immune against rabies infection, regulating and transcription, interaction with the cytoplasmic domain of the glycoprotein and the RNP during virus assembly and budding, RNA synthesis and capping respectively. The pathogenesis of rabies virus is unclear, but recent progresses have been made to elucidate these phenomenon. Centripetal spread of the virus to the central nervous system and spread within the central nervous system occur by fast axonal transport. Antibodies induced by vaccination, particularly those with neutralizing activity, play prominent role in immune defense against infection.

KEY WORDS: Encoded protein, Pathogenesis, Rabies virus.

INTRODUCTION

Rabies is one of the oldest known and most feared human diseases recognized since the early period of civilization (Laouini *et al*., 1998). Despite extensive investigation in the past 100 years, rabies remains a public health threat around the globe. Although there are some rabies-free countries and islands, such as Japan, New Zealand, Greece, and Portugal. Rabies is prevalent in all the continental regions of Asia, Africa, Europe and America. Among human infections, rabies is believed to be the tenth most common cause of death. Once clinical symptoms occur, the disease is almost invariably fatal. It is estimated that rabies causes 50,000 deaths each year. Asia accounts for more than 90% of all rabies fatalities with India alone reporting 30,000 deaths per year (Plotkin, 2000). Rabies, or hydrophobia (fear of water) is known as a disease that affects all warmblooded animals including domestic animals such as dogs and cats, wild animals such as raccoons, skunks, bats, foxes and also ruminants such as cows and deer (Moran, 2002). Rabies virus is usually spread in the saliva, when an infected animal bites another. Less often, an animal or person is infected by contact with infectious saliva or neurological tissues, through mucous membranes or breaks in the skin. The rabies virus is not transmitted through intact skin. Rabies is a classic zoonosis, which means that it is an illness that is passed directly from animal to animal and from animal to human. Rabies virus, the prototype of the Lyssa virus genus of the

family Rhabdoviridae, is an enveloped, non-segmented, negative- stranded ribonucloprotein virus. Rabies virus has a simple genome encoding five proteins: The nucleoprotein, the phosphoprotein, the matrixprotein, the glycoprotein and the RNA-dependent RNA polymerase protein (Anilionis *et al*., 1981). The viral ribonucleic acid, which is always encapsidated by Nucleoprotein, forms the ribonucleoprotein, which is the template for viral replication and transcription. The ribo nucleoprotein together with phosphoprotein and RNA-dependent RNA polymerase protein forms the viral replication complex, which is surrounded by the host cell-derived membrane that also contains glycoprotein and matrixprotien has been proposed to bridge the RNP and the cytoplasmic domain of rabies virus glycoprotein to form the bullet-shaped virion. The genomic ribonucleic acid of rabies virus, like other non-segmented negative-strand RNA viruses, is tightly encapsidated by the nucleoprotein and this nucleoprotein RNA complex, together with the phosphoprotein and the RNA-dependent RNA polymerase , forms the rib nucleoprotein complex (De Mattos *et al*., 2001).

Therefore, the objectives of this review are:

- To high light structure and function of proteins encoded by rabies virus.
- To describe the pathogenesis of rabies virus.

2. CHARACTERISTICS OF RABIES VIRUS

2.1. Morphology

The rabies virus is grouped under order Mononegavirales, family Rhabdoviridae and genus Lyssa virus. The Lyssa virus encompasses a fascinating variety of agents infecting a great number of hosts. The family Rhabdoviridae consists of more than 175 different viruses isolated from vertebrate, invertebrate and plants. From those the lyssa virus is one among the 175 viruses. The lyssa virus is enveloped, enlongated rod like shape encompassing single stranded RNA as a primary unifying feature of the group, approximately less than 180 nm in length and 45 to 100 nm in diameter. The virion appears either bullet shaped particle with one round and one flatted end or as bacilliform particle that appears hemiserphical at both ends when matured ((David *et al*., 2001). The rabies virus from the surface in wards consists of surface projection, membrane envelope and helical ribonucleic protein capsid. The fine fringe of the surface projection is 8 nm thick and usually does not cover the planar end of particles and individual projection is placed at 4.9nm intervals. The surface projections are glycosylated protein and these glycoproteins are involved in the attachment of the virus to some cell surfaces. There are different amino acids, which are pathogenic and non pathogenic at different position of glycoprotiens, for instance a single amino acids substitution replacing arginine at position 333 of the glycol protein molecule renders of the virus non pathogenic (Dietzschold *et al*., 1992). The center of the rabies virion is made of the single-stranded, genomic RNA, which is tightly bound by the nucleocapsid (N) protein. Together, the N protein/RNA combination makes up the ribonucleprotein complex (RNP), which assumes a helical shape inside the virus particle. Also associated with the RNP are two other proteins, the large (L) protein and the phosophoprotein (P). Surrounding the RNP is an envelope made up of host-cell lipids with in which resides the membrane glycoprotein (G) (De Mattos *et al*., 2001).

Generally, Lyssa virus encodes five protiens designated as G (Glycoprotiens), N (Nucleo protein), P or (Phosphoprotien), M (Matrix protein) and L (RNA-dependent RNA transcriptase) (Anilionis *et al*., 1981).

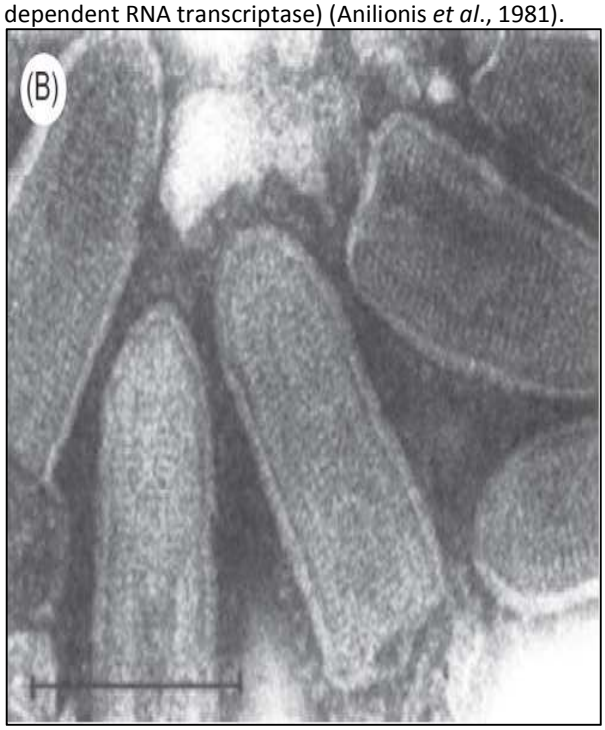

Figure 1: Virus showing characteristic bullet-shaped virions (Fauquet *et al.,* 2005).

2.2. Transmission

In the usual circumstance the only risk of rabies virus transmission is by bite or scratch of rabid animal and contamination of skin wound by fresh saliva. Transmission may also occur via aerosol and this is referred as inhalation route of infection come under suspicion. Ingestion of the virus can also lead to infection if the dose is large enough. Organ transplantation have also been incriminated as means of rabies virus transmission for instance, human rabies had been founded to be transmitted through corneal transplantation. There is a possibility of human to human transmission through bit of rabid patient (WHO, 1992).

3. STRUCTURE AND FUNCTION OF RABIES VIRUS PROTEINS

3.1. Glycoprotein

The Glycoprotein is the primary surface antigen capable of inducing and reacting with virus-neutralizing antibodies (VNAs), stimulation of specific T cells which express helper, suppressor or cytotoxic activities, it is associated with receptor activities and determination of virulence. Almost all major human and veterinary vaccines are based on the functional aspects of the G protein of RABV (Anilionis *et al.,* 1981). The G protein gene has been cloned and sequenced from a number of lyssa viruses (Anilionis et *al.,* 1981). Sequences contain a single open reading frame encoding a 522 - 524 aminoacid protein in lyssa viruses, with approximately 90% homology at the amino acid level. Mature G protein has 505 amino acids. A soluble form of G protein (Gs) is secreted from virus-infected cells; it lacks 58 amino acids from the C-terminus, which may result from proteolytic cleavage of newly synthesized protein. The Gs protein is antigenically identical to full-length protein, but does not confer protection against lethal challenge. This difference may be related to the ability of G protein to aggregate or to form a multimeric structure more immunogenic than Gs monomers (Dietzschold *et al*., 1992). With intracellular transport, G protein produced in the rough endoplasmic reticulum may exist in an inactive conformational state during transit through the Golgi apparatus, perhaps to avoid nonspecific membrane fusion (Gaudin *et al*., 1995). Antigenic determinants of G protein to either B or T cells have been identified by using cyanogen bromide (CNBr)-cleaved peptide fragments or escape mutants resistant to neutralization. The CNBr fragments can be mapped and tested for antigenicity and immunogenicity (Dietzschold *et al*., 1992). When administered to laboratory rodents, CNBr fragments induced antibody that bound to virus and G protein, but with low neutralizing activity, suggesting that antigenic structure of neutralizing epitopes was conformational. Rabies virus G is also of major importance immunologically for the induction of the host immune response against virus infection (Chenik *et al*., 1995). Mutations in the rabies virus G play a critical role in viral pathogenesis. It is remarkable that this single amino acid substitution can affect the rate of virus spread from cell to cell as well as the neuronal pathway that the virus takes to reach the CNS (Kawai *et al*., 1999).

3.2. Nucleoprotein

The Nucleoprotein is the major component of the viral RNP. Amino acid sequences of RABV nucleoprotein have been deduced from the primary nucleotide sequence of several strains. Nucleoprotein plays important roles in regulating viral transcription and replication via encapsidation of genomic RNA. The N protein is a major target antigen for T-helper cells, which cross-react among lyssa viruses. Thus, viral RNP can prime T cells, elicit N-protein-specific antibodies, and serve to enhance immune responses (Hooper *et al*., 1998). Glycoprotein and nucleoprotein are the major antigens capable of inducing immune against rabies infection both should be included in the development of genticaly engineered vaccines (WHO, 1992). Rabies virus nucleocapsid protein serves the critical function of tightly packaging the RNA genome into an RNase-resistant core that is the template for both transcription and replication (Blumberg *et al*., 1983).

3.3. Matrix protein

Matrixprotein is believed to link the viral envelope with the RNP, the M of rabies virus is the smallest and most abundant protein in the virion and it contains 202 amino acids. Comparison of the amino acid sequences among different RABV strains showed 91% to 94% homology (Hiramatsu *et al*., 1993). One primary function of M protein appears to involve the interaction with the cytoplasmic domain of the G protein and the RNP during virus assembly and budding, condensation of helical nucleocapsid cores into tight coils, association with membrane bilayers, and involvement in the cytopathogenesis of virus-infected cells (Flamand *et al*., 1993).

3.4. RNA dependent RNA transcriptase protein

The RNA Dependent RNA Transcriptase protein is the largest lyssa virus protein, containing 2,127 to 2,142 amino acids. Its functions include RNA synthesis and capping (Wagner, 1990). It is one of the least well studied lyssa virus antigens. Sequence homology among Rhabdoviruses and other members of the Mononegavirales (Poch *et al*., 1990). These regions may be functional domains responsible for the multiple catalytic activities of the L protein. A 400 to 450 nucleotide area, located between the beginning of the L gene and the end of the G gene, is bounded by transcription termination and polyadenylation-like signals, but it is a noncoding region, without apparent pseudogene functions (Ravkov *et al*., 1995).

3.5. Phospoprotein

The Phospoprotein constitutes a part of the viral RNP and contains 297 to 303 amino acids. In contrast to divergence in the vesiculo viruses, sequence comparisons of RABV Phospoprotein show a high degree of homology, from 92% to 98%. The P is phosphorylated, but sites of phosphorylation have not been precisely determined (Larson and Wunner, 1990). A unique cellular protein kinase has been discovered that phosphorylates. P suggesting an important role in protein structure or function. A B-cell antigenic determinant has been mapped between residues 75 and 90 of the P. The P expressed in vaccinia virus stimulates

strong CTLs and weak T-helper cells in H-2 k mice (Gupta *et al*., 2000).

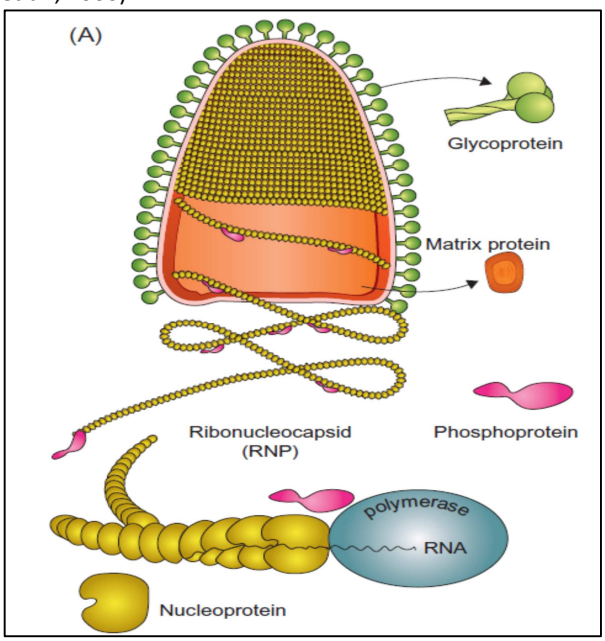

Figure 2: Diagram illustrating a Rhabdovirus virion and the nucleocapsid (Fauquet *et al.,* 2005).

4. PATHOGENESIS

The spread of the rabies virus from the peripheral nerves of infected animals to the central nervous system (CNS) was documented at an early date, however, neural dysfunctions due to rabies infection is not well understood (Kristensso *et al*., 1996). Almost all cases of human rabies are caused by a bite from rabid animals. Efficient transmission depends on the severity of the bite and on the amount of virus in the saliva. Particularly deep bites in to the muscular tissues where nicotinic acetylcholine receptors are densely packed, enhances transmission. Although non bite exposures are extremely unusual cause of human rabies, contamination of open wounds and skin abrasion with the saliva of rabid animals carries risk of the disease. Deposition of virus laden saliva into the conjunctiva, oral mucus membranes or genitalia is also incriminated as a mode of transmission (Hemachudha *et al*., 2002).

4.1. Deposition of virus in to tissues

After inoculation of infectious saliva by bite, virus may either persist and replicate at the inoculation site for hours to weeks or follow a relatively rapid centripetal course directly to the CNS with replication and dissemination prior to the development of a significant immune response. Several studies collectively suggest that RABV can infect muscle cells and replicate before invasion of the peripheral and CNS (Charlton *et al*., 1997). While others demonstrate that virus can enter the nervous system without prior local replication (De Mattos *et al.,* 2001). Thus, during the incubation period, virus may reside in the periphery, it may remain sequestered within neurons, or perhaps it can persist in macrophages. The initial infection event may depend on the genotype of the virus. In case of canine Lyssa virus, the viral glycoprotein may bind to nicotinic acetycholine receptor on the muscle (Lewis *et al*., 2005), where in case of some bat lyssavirus, the virus may bind to unknown receptor in the epidermis or dermis (Hemachudha *et al*., 2002). The incubation period of rabies may range from two weeks to several months and rarely even to years. The characteristic of long incubation period or eclipse phase may be explained partly by localization of the virus within the muscle or other cells at the neuromuscular junction (Charlton *et al*., 1997). It is also considered that bite around the head and neck of human is more likely to result in clinical disease with shorter incubation periods than those following bites to the extremities. In certain circumstances, this persistence may provide an opportunity for host immune clearance and for post-exposure treatment (WHO, 1992). With high dose of the virus, there is a greater probability that there will be uptake of virions by both muscle cells and axon terminals at the inoculation site. In this case, muscle cells infection is predominantly incidental; the main course of infection and progression is via retrograde axoplasmic flow to the CNS or to the spinal ganglia. This may result in a fairly short incubation periods.When low dose of the virus, there is a greater probability that virions will enter either to the axon terminals or muscle fibers (or in some cases to neither). This could result in an early transit to the CNS via axons of the peripheral nerves or retention of the virus in the muscle for varying periods. This may contribute for wider ranges of rabies incubation periods (Charlton *et al*., 1997).

4.2. Migration of the virus from peripheral to central nervous system

After budding from the plasma membrane of muscle cell, virus is taking up in to unmyelinated nerve ending at the neuromuscular or at the muscle spindles (Tsiang, 1988). The final release of virions from infected fibers with ensuing uptake by axon terminals probably is due to budding on the sarcolemma or disintegration of individual muscle fibers subjected to long term massive infection (Charlton *et al*., 1997). The virus is transported to the CNS via retrograde axoplasmic flow, which is evidenced by inhibition of the flow by colchicine or vinblastine injection (Tsiang, 1988). The form of virus during axoplasmic transport, either as intact virus or as a nucleoprotein complex, remains unclear (De Mattos *et al.,* 2001). At the dorsal root ganglia, viral replication may be recognized, and attacked by immune effectors, resulting in ganglioneuronitis and clinical prodrome of neuropathic pain at the bite. At this local prodromal stage, prophylaxis with standard tissue culture vaccine

and rabies immunoglobulin has not been able to avert death (Hemachudha *et al*., 2002). Virus travel from the peripheral nerves to the CNS occurs at a fairly constant rate of 8-20mm/day, and the time taken depends on the distance of the inoculation site from the CNS. Movement may facilitate virus spread across cell to cell junction, supported by observation of budding on axonal membranes and viral inclusions at nodes of ranvier. Viral distribution via cerebrospinal fluid (CFS) may also contribute to virus spread within the CNS (De Mattos *et al*., 2001). Studies have also showed that the neuromuscular junction is the major site of entry in to neurons. Rabies viruses can enter directly in to nerves without previous replication in muscle or skin (Lewis *et al*., 2005). The nicotinic acetylcholine receptor is unlikely to be the only receptor that mediates viral entry in to neurons, since it is not present in all types of neurons susceptible to lyssa virus. The virus may also use carbohydrates, phospholipids, gangliosides, neural cell adhesion molecule (CD56), and low affinity nerve growth factor receptor to gain entry in to the neurons in both *in vitro* and *in vivo* models (Hemachudha *et al*., 2002). Although, recent evidence has show that the virus phosphoprotein interacts with the cytoplasmic dynein light chain, which is an important component of the microtubule based transport system, it is not clear whether this alone accounts for the viral transport mechanism. Once the virus is in a neuron, rapid amplification takes place. Virus disseminates via plasma membrane budding and direct cell to cell transmission or by trans-synaptic propagation. The glycoprotein is required for attachment to neuronal receptors as well as for trans-synaptic spread (Jackson, 2002).

4.3. Spread throughout the central nervous system

Once in the CNS, the exact pathway of virus propagation is not known (Mitrabhakdi *et al*., 2005). After infection develops in spinal cord or brainstem neurons, the virus disseminates rapidly throughout the CNS by fast axonal transport along neuroanatomical connection (Tirwatnpong *et al*., 1989). It has been reported that the viral antigens preferentially localize in the spinal cord, brainstem, thalamus and basal ganglia have also reported such predilection site and relation to the pathway by which the virus enters the brain, to the occurrence of virus receptors and to the metabolic properties of the neurons. Such sites include neurons in certain brainstem nuclei, purkinje cells in the cerebellum, pyramidal cells in the hippocampus and cortical neurons in the temporal lobes of the brain (Kristensson *et al*., 1996). Under natural condition, rabies virus infection of the CNS causes relatively mild neuronal pathology without prominent evidence of neuronal death. Together, these observations have led to the concept that the neurological disease in rabies must result from neuronal dysfunction cell death (Jackson, 2002). Both *in vitro* and animal model observation in rabies agree to the conclusion that necrotic process is usually lacking with apoptosis becomes dominant findings (Juntrakul *et al*., 2005). The down regulation of glycoprotein expression by some viruses mays contribute to viral pathogenesis by preventing apoptosis (De Mattos *et al*., 2001). In paralytic rabies, the medulla and the spinal cord are mainly involved by extensive neuronal damage and inflammation. In the encephalitic form, it is the brainstem and the cerebrum, particularly the limbic system that are heavily involved. Involvement of the basal ganglia and the thalamus is usually seen late in the course of the disease. The early localization of the virus in the limbic system with cortical sparing correlates clinically with behavioural and emotional changes seen in an alert and congintively intact patient. Rabies virus reaches the CNS early in the disease and returns to the periphery late in the process by intra axonal transport (Awasthi *et al*., 2001). Clinical illness begins up on arrival of the virus in to the CNS. Human rabies may present in one of two forms: encephalitic (furious form) and paralytic (dump form) (Tirawatnpong *et al*., 1989). Limbic symptoms dominate the clinical picture in the former, whereas paralysis of lower motor neuron type dominates in the later. The site of neuronal involvement responsible for clinical weakness in paralytic rabies remains unknown (Mitrabhakdi *et al*., 2005).

4.4. Centrifugal spread from the central nervous system

Eventual centrifugal spread from the CNS along the neural pathways to the heart, skin and other organs, especially to the salivary and serous glands of the tougue, is an important component to complete the infection cycle (Hemachudha *et al*., 2002). The spread of the virus in to the salivary gland, which represents the final phase of infection, is important for transmission from animal to animal and from animal to human. Virus is found in the salivary glands in the majority of rabid foxes, raccoons, skunks and dogs. Much of the virus is produced in mucogenic acinar cells and is delivered into the saliva by normal secretary flow. In the nervous system most virus is formed by budding on intra cytoplasmic membranes; however, in the salivary glands, virion bud on plasma membranes at the apical (luminal) surface of mucous cells and are released in high concentration in to the saliva (Murphy *et al*., 1999). All major neural and non-neural organs, except the blood, may contain significant amounts of virus. Organs of patients with an unexplained neurological disease, if transplanted, may transmit rabies (Hemachudha *et al*., 2002). Other sites in which the virus can be found include sensory nerve end-organs in oral and nasal cavities, taste buds, adrenal glands, pancreas, kidney,

heart muscle, brown fat, hair follicles, retina and cornea (De Mattos *et al*., 2001).

5. IMMUNE RESPONSE

Antibodies induced by vaccination, particularly those with neutralizing activity and play a prominent role in immune defense against infection. Immunity may also be naturally acquired after multiple exposures to virus, on rare occasions (Follmann *et al*., 1994). The G protein represents the only antigen that induces VNA and is able to confer immunity against a lethal challenge infection. The ability to induce VNA depends on the intact secondary and tertiary structure of the G protein. Protective activity induced by Gs is reduced compared with that of the virion-associated G protein (Hooper *et al*., 1998). VNAs exert their protective effect by neutralization of extracellular virus, by complement-mediated lysis of virus-infected cells, and by antibody-dependent cytotoxicity. VNA can mediate viral clearance from the CNS without other immune effectors (Dietzschold *et al*., 1992). The RNP is a major antigenic complex that induces a virus-specific antibody response, and antibodies directed against RNP can contribute to protection against infection. Animals treated with anti-N sera can be protected against a subsequent challenge with RABV, and anti-N sera can exhibit an antiviral activity *in vitro* (Lodmell *et al*., 1993). Infection with RABV results in the generation of virus-specific CD8+ and CD4+ T cells. The G protein is one of the antigens that induce cytotoxic T-lymphocyte responses. Some mouse strains infected with virus also develop strong CTL responses to the phosphoprotein. However, the role of CD8+ T cells in immune defense is unclear. Some investigators report clearance of rabies virus after transfer of RABV-specific T cells and protection against rabies by a CTL clone, whereas other investigators showed that CTLs are insufficient to protect against challenge, and *in vivo* depletion of CD8+ T cells had no effect on host resistance to street virus infection (Larson and Wunner, 1990). In contrast, CTLs may actually be involved in the immunopathology and have been implicated in neuritic paralysis. By comparison, the induction of CD4+ T cells is an integral part of the protective immune response against rabies. Elimination of CD4+ cells abrogates the production of IgG neutralizing antibody in response to virus infection (Perry, 1991). The RNP contains major epitopes that induce CD4+ cell responses, and the majority of this T cells cross-react with other lyssa viruses. The RNP-specific T cells, which can augment the production of VNA through a mechanism known as intra structural antigen recognition, are believed to be the major factor that mediates the protective immune response induced by internal viral antigens (Dietzschold *et al*., 1992).

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