Rabies pathogenesis update

Atualização sobre a patogênese da raiva

Actualización sobre la patogénesis de la rabia

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ABSTRACT

Human rabies continues to be an important public health problem. Our understanding of the disease has been acquired from studies in experimental animal models. There are many unanswered questions in rabies pathogenesis, although there has been recent progress. Rabies virus-infected neurons may not function normally due to degenerative changes involving neuronal processes, including both dendrites and axons. Street rabies virus infection may not be cleared from the central nervous system because immune effectors cannot be delivered into brain tissues as a result of poor blood-brain barrier permeability. No effective therapy is available for human rabies. Therapeutic (induced) coma has failed repeatedly. An improved understanding of rabies pathogenesis may offer new insights for the development of novel therapies for human rabies.

Keywords: Virulence Factors; Rabies; Encephalitis, Viral.

INTRODUCTION

Human rabies remains an important public health problem, with at least 55 thousand fatal cases per year[51]. Human rabies can be effectively prevented, but it remains an almost invariably fatal disease despite aggressive approaches to therapy[14]. Our basic understanding of rabies pathogenesis leaves many important questions unanswered. A better understanding of rabies pathogenesis may be helpful in making future advances in the therapy of human rabies. In this review, selected topics in the pathogenesis of rabies will be discussed with an emphasis on recent developments.

OVERVIEW OF RABIES PATHOGENESIS

Much of what is known about the pathogenesis of rabies has been learned from studies performed in animal models, usually in rodents infected with strains of fixed (laboratory adapted) rabies virus. There is a long and variable incubation period in human and animal rabies usually lasting 20 to 90 days, but in humans it may rarely last longer than one year[44]. After a bite from a rabid animal, saliva containing infectious rabies virus is inoculated into subcutaneous tissues and muscles. The best experimental animal studies to date examining the events that take place during the incubation period were performed in striped skunks using a Canadian isolate of street rabies virus obtained from skunk salivary glands[4]. Studies performed using reverse transcriptase-polymerase chain reaction (RT-PCR) amplification showed that when skunks were sacrificed 62 to 64 days post-inoculation, viral genomic RNA was frequently present in the inoculated muscle (found in four of nine skunks), but not in either spinal ganglia or the spinal cord. Immunohistochemical studies performed prior to the development of clinical disease showed evidence of infection of extrafusal muscle fibers and occasional fibrocytes at the site of inoculation. We can conclude that rabies virus is present at or near the site of the bite during most of the incubation period. The infection of muscle fibers may be a critical pathogenetic step for the virus to gain access to the peripheral nervous system.

Detailed pathogenesis studies of the early events in "natural" rabies models have not yet been performed in bats. Bats usually produce much more superficial bites than terrestrial vectors. There is a rich sensory and autonomic innervation of skin and subcutaneous tissues that becomes involved in a similar time course as with infections due to deeper-biting vectors. In North America most victims of rabies do not have a history of an animal bite, likely because they were not aware of the bite. In part, this is related to the small size of the bats, for example, silver-haired bats. The bat rabies virus variant most commonly

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responsible for human rabies in North America is associated with silver-haired/eastern pipistrelle bats[37]. Bat bites may produce lesions that appear quite trivial[18]. Experimental studies on the silver-haired bat virus indicate that the virus replicates well at lower than normal body temperatures (34° C) and has higher infectivity than coyote street virus in cell types present in the dermis, including fibroblasts and epithelial cells[36]. Hence, the silver-haired bat virus is likely well adapted for efficient local replication in the dermis, which could explain the success of this variant.

Rabies virus binds to nicotinic acetylcholine receptors at the neuromuscular junction[32]. The virus travels towards the central nervous system (CNS) within motor and sensory axons by retrograde fast axonal transport at a rate of 12-100 mm per day[29,34,47]. Rabies virus has been used as a neuroanatomical tracer in order to define circuits of synaptically-linked neurons in rodents and primates, and these studies have shown that axonal transport of rabies virus occurs exclusively in the retrograde direction[45,27]. When neurons are infected in the spinal cord, there is a subsequent spread from neuron to neuron within axons in the CNS by fast axonal transport along neuroanatomical connections. Many neuronal cell types are infected in a widespread distribution in the CNS; infection of non-neuronal cells occurs much less commonly. Brain infection results in behavioral changes, likely due to infection of neurons in limbic areas, and this facilitates transmission by biting in rabies vectors. There is a spread of rabies virus away from the CNS (centrifugal spread) along neuronal pathways, particularly involving the parasympathetic nervous system, which is responsible for infection of the salivary glands, skin (skin biopsy is a useful diagnostic test), heart, and a variety of other organs[14,29]. Infectious rabies virus is secreted into the saliva of rabies vectors, which is important for transmission to other hosts.

NEURONAL DYSFUNCTION AND DEATH IN RABIES VIRUS INFECTION

Natural rabies is normally characterized by severe neurologic signs and a fatal outcome. However, neuropathologic changes in the CNS are relatively mild, consisting of mild inflammation with little neuronal degeneration, supporting the concept that neuronal dysfunction, rather than neuronal cell death, plays an important role in producing the disease[15,25]. A variety of experimental studies of rabies virus infection have investigated potential abnormalities in neurotransmission involving acetylcholine[13,48,6], serotonin[3,2], and -amino- -butyric acid (GABA)[30]. Abnormalities of uncertain significance were found, but no fundamental defect was demonstrated that explains the neuronal dysfunction in rabies.

Dysfunction of ion channels has been shown in rabies virus – infected cultured mouse neuroblastoma NA cells with the whole-cell patch clamp technique[12]. The infection reduced the functional expression of voltage-dependent sodium channels and inward rectifier potassium channels, and there was a lower resting membrane potential reflecting membrane depolarization. There was no change in the expression of delayed rectifier potassium channels, indicating that nonselective dysfunction of ion channels had not occurred. The reduction in sodium channels and inward rectifier potassium channels could prevent infected neurons from firing action potentials and generating synaptic potentials, resulting in functional impairment.

Koprowski and co-workers[28] have hypothesized that nitric oxide neurotoxicity may mediate neuronal dysfunction in rabies. Induction of inducible nitric oxide synthase mRNA[28] and increased brain levels of nitric oxide[10] have been demonstrated in rabies virus-infected rodents, but the significance of these findings remains uncertain. The role of nitric oxide in rabies pathogenesis needs further study. Excitoxicity has been studied in rabies virus-infected primary neuron cultures and in an experimental mouse model of rabies, but no importante role for excitoxicity in neuronal dysfunction or death was found[49].

Using subtraction hybridization, Prosniak et al[38] showed that infection of mice with fixed rabies virus results in down-regulation of about 90% of genes in the normal brain by more than 4-fold. Only about 1.4% of genes became up-regulated, including genes involved in regulation of cell metabolism, protein synthesis, growth and differentiation. Using proteomic profiling of brain homogenates from mice infected with street (wild-type) rabies virus, Dhingra et al[5] found that the levels of proteins involved in ion homeostasis were altered (H^+ ATPase and Na^+/K^+ ATPase were up-regulated and Ca^{2+} ATPase was down-regulated). Also, they found down-regulation of proteins involved in docking and fusion of synaptic vesicles to the presynaptic membrane, a process relevant to synaptic physiology. These abnormalities could lead to neuronal dysfunction.

Neurotropic viruses may cause cell death by either apoptosis or necrosis[9,1,7]. Apoptosis depends on synthesis of macromolecules and requires energy, while necrosis is associated with energy failure. Each of these forms of cell death is associated with typical morphologic features. The challenge virus strain (CVS) of fixed rabies virus has been observed to induce apoptotic cell death in rat prostatic adenocarcinoma cells[23], mouse neuroblastoma cells[46], and mouse embryonic hippocampal neurons[35]. Prominent apoptotic death of neurons has been observed in the brains of mice of various ages inoculated intracerebrally with the CVS strain of fixed rabies virus[23,46,19]. However, neuronal death is not prominent after peripheral inoculation of adult animals[43,15,39]. Attenuated viruses are stronger inducers of neuronal apoptosis in cultured primary neurons and also in mice after peripheral inoculation than more virulent strains[35,21]. Contrary to previous reports[26], we have recently provided strong evidence that neuronal apoptosis does not play an important role in human rabies[20]. Hence, in rabies virus infection there are complex mechanisms involved in cell death versus survival of neurons both *in vitro* and in animal models using different viral strains and routes of inoculation, but neuronal cell death is not prominent in natural rabies. In general, apoptosis is a host mechanism employed to limit viral spread and does not serve a fundamental role in the pathogenesis of rabies[15].

During the prodromal period of rabies patients may experience pain, paresthesias, or itching at the site of the wound (often healed), which likely reflects involvement of local sensory (e.g., dorsal root) ganglia[14]. There is neuronal infection with inflammation and neuronal degeneration in dorsal root ganglia in human rabies and in most experimental models of rabies[40]. This neuronal degeneration is out of proportion with what is seen in the CNS of the mouse model after footpad inoculation with CVS[40], and it is highly inflammatory, which suggests an immune-mediated process. The neuronal degeneration has neither the morphologic features of necrosis nor the morphologic or biochemical features of apoptosis. In gangliocytes there is an early 'axotomy response' and later the gangliocytes contain numerous autophagic compartments. At advanced stages of degeneration there are partially membrane-bound empty vacuoles in gangliocytes. Autophagy is an important mechanism involved in this degenerative neuronal process.

Li and colleagues[33] have suggested that degeneration of neuronal processes and disruption of synaptic structures may form the basis for neuronal dysfunction in rabies virus infection. They showed severe destruction and disorganization of neuronal processes in silver stained hippocampal sections from mice infected intracerebrally with the pathogenic N2C strain of rabies virus. Our laboratory has recently examined morphological changes in neurons (with an emphasis on the structural integrity of neuronal processes) following hindlimb footpad inoculation of transgenic mice expressing yellow fluorescent protein (YFP) in a subpopulation of neurons, which facilitates visualization of the morphological details of dendrites, axons, and presynaptic nerve terminals[43,8]. In these mice, YFP expression is driven in a subpopulation of neurons using the *thy1* vector lacking exon 3 and its flanking introns, and there are strong fluorescent signals in dendrites, axons, and presynaptic nerve terminals[8]. In this model, conventional histopathology showed mild inflammatory changes without significant degenerative neuronal changes. However, at late clinical time points concomitant with the development of severe clinical neurological disease, fluorescence microscopy showed marked abnormalities, especially beading and/or swelling involving dendrites and axons of layer V cortical pyramidal neurons, severe involvement of axons in the brainstem and the inferior cerebellar peduncle, and severe abnormalities affecting axons of cerebellar mossy fibers. The involvement of hippocampal pyramidal neurons was much less severe, perhaps because these neurons become infected much later after peripheral inoculation in this model[22]. The structural changes may take a period of time to develop perhaps because they are mediated by abnormal axoplasmic structural protein function. Toluidine blue-stained resin sections and electron microscopy showed vacuolation in cortical neurons that corresponded to swollen mitochondria, and vacuolation in the neuropil of the cerebral cortex. Axonal swellings were observed. Vacuolation was also observed ultrastructurally in axons and in pre-synaptic nerve endings. These morphological changes are sufficient to explain the severe clinical disease and fatal outcome, and they provide strong evidence that the fundamental defect in rabies virus-infected neurons may involve neuronal processes, which is not apparent in routine histopathological studies.

BLOOD-BRAIN BARRIER IN RABIES

Hooper and colleagues[10] compared mice infected with a lethal silver-haired bat rabies variant (SHBRV) with mice infected with an attenuated laboratory adapted strain (CVS-F3) in which there is viral clearance without sequelae. They found that in both viral infections there was a strong virus-specific immune response in the periphery of the host. However, invasion of the CNS tissues by immune cells was reduced with the SHBRV due to an inability to enhance blood-brain barrier permeability, resulting in failure of viral clearance[42]. Mice with an SJL background are less susceptible to lethal infection with rabies virus and have a greater capacity to mediate CNS inflammatory responses. SHBRV infection in these mice is associated with the induction of greater blood-brain barrier permeability and CNS inflammation, resulting in greater viral clearance and improved survival[41]. Administration of a steroid hormone in these mice resulted in reduced blood-brain barrier changes and resulted in greater mortality[41]. Hence, blood-brain barrier permeability is likely of paramount importance for viral clearance in rabies and therapeutic approaches should be taken to enhance this permeability. Therapies that promote closure the blood-brain barrier, such as the administration of corticosteroids, should be carefully avoided in the management of human rabies patients.

APPROACHES TO THE THERAPY OF HUMAN RABIES

There is no established therapy for human patients with rabies. An approach to the management of human rabies was recently published[24]. This article recommended using a combination of therapies and discussed the pros and cons of using a variety of specific agents. In 2004, a 15-year-old patient survived rabies who had not received rabies vaccine prior to the onset of clinical disease[50]. She was bitten by a bat on a finger and did not seek medical attention or receive treatment at that time. About one month after the bite, she developed typical clinical features of rabies, and neutralizing anti-rabies virus antibodies were detected in her sera and cerebrospinal fluid. Her therapy included therapeutic (induced) coma using intravenous midazolam for seven days; a burst-suppression pattern on her electroencephalogram was maintained and supplemental phenobarbital was given. She also received therapy with ketamine and antiviral therapy, including ribavirin and amantadine. She improved and was discharged from the hospital with neurologic deficits, but she subsequently had progressive neurologic improvement[11]. This is the first documented survivor who had not received rabies vaccine prior to the onset of clinical rabies. As discussed in the accompanying editorial, it is unknown if therapy with one or more specific agents played an important role in the favorable outcome of this patient[16]. However, since that time, there have been at least 13 known cases in which the main components of this approach (the "Milwaukee" protocol) have been used, and fatal outcomes have

resulted[17]. The induction of coma *per se* has not been shown to be useful in the management of infectious diseases of the nervous system, and there is no evidence supporting this approach in rabies or other viral encephalitises. Hence, therapeutic coma should not become a routine therapy for the management of rabies.

The development of neutralizing anti-rabies virus antibodies early in the patient's clinical course, which probably occurs in less than 20% of all patients with rabies, likely contributed to a favorable outcome. Bat rabies viruses may be less neurovirulent than canine or other variants that are responsible for most human cases of rabies[31], and rabies due to canine rabies virus variants may have a worse outcome than cases caused by bat rabies variants. Finally, most survivors of rabies have shown neutralizing anti-rabies virus antibodies in sera and cerebrospinal fluid, but other diagnostic laboratory tests are usually negative for rabies virus antigen and RNA in fluids and tissues (brain tissues are not tested). This may be due to effective viral clearance.

CONCLUSIONS

The bases for neuronal dysfunction in rabies are complex, but they may involve degenerative changes involving neuronal processes such as dendrites and axons. For reasons that are unclear, neuronal injury is greater in dorsal root ganglia than in CNS neurons. Permeability of the blood-brain barrier to immune effectors is important for viral clearance and recovery from rabies. No effective therapy for human rabies is available. Hopefully, an improved understanding of rabies pathogenesis will lead to the development of novel therapies for human rabies.

Atualização sobre a patogênese da raiva

RESUMO

A raiva humana continua sendo um problema de saúde pública. O nosso conhecimento acerca da doença tem sido construído por meio de estudos em modelos animais experimentais. Há muitas perguntas não respondidas envolvendo a patogênese da raiva, a despeito de termos observado um avanço nos últimos tempos. Os neurônios infectados pelo vírus da raiva podem não operar normalmente devido às mudanças degenerativas envolvendo processos neurais, incluindo dendrites e axônios. As infecções por vírus de rua não podem ser extirpadas do sistema nervoso central, uma vez que os efetores imunes não podem ser transportados aos tecidos do cérebro devido à pequena permeabilidade da barreira hematoencefálica. Não existe uma terapia eficaz contra a raiva humana. O coma terapêutico (induzido) tem falhado sistematicamente. Uma melhor compreensão da patogênese da raiva pode oferecer novas ideias para o desenvolvimento de modernas terapias contra a raiva humana.

Palavras-chave: Fatores de Virulência; Raiva; Encefalite Viral.

Actualización sobre la patogénesis de la rabia

RESUMEN

La rabia humana sigue siendo un problema de salud pública. Nuestro conocimiento sobre esa enfermedad se ha construido a través de estudios en animales de experimentación. Hay muchas preguntas sin respuesta que rodean la patogénesis de la rabia, a pesar del avance observado en los últimos tiempos. Las neuronas infectadas con rabia no pueden funcionar normalmente debido a cambios degenerativos que afectan a procesos neurales, incluyendo a dendritas y axones. Las infecciones causadas por el virus natural de la rabia no pueden eliminarse del sistema nervioso central, ya que los efectores inmunológicos no pueden ser transportados a los tejidos del cerebro, debido a la permeabilidad de la barrera hematoencefálica. No existe una terapia eficaz contra la rabia humana. El estado de coma terapéutico (inducido) ha fracasado sistemáticamente. Una mejor comprensión de la patogénesis de la rabia puede aportar nuevas ideas para el desarrollo de terapias modernas contra la rabia humana.

Palabras clave: Factores de Virulencia; Rabia; Encefalitis Viral.

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