Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

CHAPTER 5

Lyssaviruses—Current Trends

Susan A. Nadin-Davis and **Christine Fehlner-Gardiner**

Contents

Centre of Expertise for Rabies, Ottawa Laboratory (Fallowfield), Canadian Food Inspection Agency, Ottawa, ON, Canada

Advances in Virus Research, Volume 71
ISSN 0065-3527, DOI: 10.1016/S0065-3527(08)00005-5

Abstract

Various technological developments have revitalized the approaches employed to study the disease of rabies. In particular, reverse genetics has facilitated the generation of novel viruses used to improve our understanding of the fundamental aspects of rabies virus (RABV) biology and pathogenicity and yielded novel constructs potentially useful as vaccines against rabies and other diseases. Other techniques such as high throughput methods to examine the impact of rabies virus infection on host cell gene expression and two hybrid systems to explore detailed protein–protein interactions also contribute substantially to our understanding of virus–host interactions. This review summarizes much of the increased knowledge about rabies that has resulted from such studies but acknowledges that this is still insufficient to allow rational attempts at curing those who present with clinical disease.

I. INTRODUCTION

The disease of rabies, known to mankind since the 23rd century BC (see Baer, 2007), remains the best known and most feared of all zoonotic diseases. Most rabies cases reported world-wide are caused by rabies virus (RABV), the prototype of the *Lyssavirus* genus of the family *Rhabdoviridae*, although all lyssaviruses can elicit the disease. Control of human rabies is achieved most effectively by elimination of the virus from animal reservoirs that maintain and transmit it to other species. In addition, highly efficacious regimens for human disease prevention exist (Rupprecht *et al.*, 2006). However, rabies still accounts for an estimated 55,000 human deaths world-wide each year (WHO, 2005). Dogs, which have long been associated with the disease, form the major rabies reservoir in the developing world where most human exposures and fatalities occur. Failure to prevent disease in many developing countries is due to several factors: poor control and limited vaccination of dogs, lack of public education, poor public health infrastructure, and the high cost and limited availability of antirabies biologicals. In contrast, developed countries have eliminated rabies from their dog populations and postexposure prophylaxis (PEP) is available to those exposed to the disease via contact with wildlife reservoirs.

This situation has driven many of the recent initiatives in rabies research that are reviewed in this chapter. At the more practical level, development of new reagents such as human neutralizing antirabies glycoprotein (G) monoclonal antibodies (Mabs) are being developed to replace the rabies immune globulin preparations that are in short supply. Efforts to develop improved and cost-effective rabies vaccines continue,

primarily to support countrywide pet vaccination programs in the developing world and oral wildlife vaccination for elimination of sylvatic rabies in much of the developed world. Control programs, which require ongoing and timely disease surveillance, are driving the need for field-based diagnostic methods to assist in sylvatic rabies eradication, as well as a comprehensive knowledge of rabies epidemiology and evolution now being revealed through molecular epidemiological techniques. The occasional case of human rabies in the developed world provides continued impetus for development of a therapeutic regimen. However, despite the recent advances in our understanding of virus–host interactions summarized here, successful treatment of clinical rabies requires yet deeper appreciation of the pathogenic mechanisms that result in patient death. The ability to manipulate the RABV genome through reverse genetics permits detailed analysis of the viral features responsible for disease and facilitates some novel applications, including the use of RABV as a neurological tracer to provide unique insights into neuronal pathways and development of novel vaccines directed against rabies and other diseases.

II. DEVELOPMENTS IN DIAGNOSTIC AND SURVEILLANCE TOOLS

A. Diagnosis

While the direct fluorescent antibody (DFA) test applied to fresh brain smears or impressions remains the gold standard method for animal rabies diagnosis, its two main drawbacks are the need for sample shipping to central laboratories and the costs involved in acquiring and maintaining a fluorescence microscope. These requirements often restrict sample submissions, particularly from remote areas, for example, arctic regions, and in many parts of the tropics where sample preservation is problematic. These limitations in turn result in rabies underreporting and underappreciation of its significance. A direct rapid immunohistochemical test (DRIT) for rabies, developed by the Rabies Section of the Centers for Disease Control and Prevention (CDC), may help to address this problem. DRIT employs a light microscope to detect RABV antigen in brain impressions using a cocktail of biotinylated antinucleocapsid Mabs with visualization using a streptavidin–peroxidase complex. In a field evaluation in Tanzania, DRIT performed comparably to the DFA (performed at the CDC) with regards to sensitivity and specificity and thus may, subject to mandated biocontainment requirements, be a viable means of testing animal specimens under field conditions (Lembo *et al.*, 2006). This could facilitate greatly improved surveillance in tropical

regions but it might also be useful in developed countries where increased local surveillance is needed to support wildlife oral vaccination campaigns. Another interesting possibility for rapid screening in the field is the use of lateral flow immunochromatography (Kang *et al.*, 2007), although comprehensive validation against established methods is required.

The need for antemortem human testing has driven development of alternate diagnostic tools, for example, reverse transcriptase polymerase chain reaction (RT-PCR) testing for the presence of viral RNA in saliva (reviewed in Trimarchi and Nadin-Davis, 2007). RT-PCR has also been applied to oral swab screening of wild bat populations for active lyssavirus infection in Europe where most bat species have protected status (Echevarría *et al.*, 2001). Although, real-time RT-PCR (RRT-PCR) would have additional advantages for human testing in terms of rapidity and sensitivity, only a few reports describe its application for detection of specific lyssavirus genotypes (Foord *et al.*, 2006; Wakeley *et al.*, 2005). The complexity of designing broadly cross-reactive reagents for a TaqMan-based approach is challenging due to the extent of lyssavirus sequence divergence (Hughes *et al.*, 2004b). A SYBR green-based RRT-PCR, that was more sensitive than conventional RT-PCR for the detection of virus in human saliva samples, may be of value providing its specificity can be assured (Nagaraj *et al.*, 2006). Further evaluation of a nucleic-acid sequence based amplification (NASBA) method, applied to human saliva and cerebrospinal fluid samples is needed (Wacharapluesadee and Hemachudha, 2001). The issue of testing solid organ transplant tissues has been broached in response to rabies transmission via organ transplantation (Srinivasan *et al.*, 2005), but such testing will remain impractical unless faster and more readily accessible tests become available (Jackson, 2004).

B. Viral typing

The existence of multiple RABV strains, each of which is maintained by a specific host within a geographically defined area, drives the need for strain discrimination tools that provide critical information in support of control programs. Both antigenic and molecular-based typing techniques have been increasingly applied over the last 15 years (reviewed in Nadin-Davis, 2007). Extensive phylogenetic analyses of RABV (genotype 1) isolates recovered world-wide have identified their detailed phylogeography and revealed the existence of a limited number of major lineages: the heterogeneous ''American indigenous'' lineage, found only in the Americas, comprised mostly of bat-associated strains and a few species of *Carnivora*; at least three distinct canid lineages circulating in South-East Asia; an ''Arctic'' lineage that includes viruses from northern circumpolar

regions as well as viruses from central and southern Asia; a canid lineage in western Africa; a lineage associated with herpestid and viverrid species in southern Africa; and the cosmopolitan lineage (Nadin-Davis and Bingham, 2004), probably widely dispersed as a result of human colonial activities in the 16th–18th centuries, that includes canid-associated viruses of Europe, the Middle East, Asia, and much of Africa, mongoose, skunk and canid associated viruses of the Americas and the Caribbean, as well as most vaccine strains. Ongoing genetic characterization of RABV isolates continues to extend our knowledge of RABV diversity and identifies newly emerging virus–host associations. For example, in the USA alone, a distinct viral variant associated with western pipistrelle bats (Franka *et al.*, 2006) and sustained intraspecific transmission of a bat variant within skunk populations in Arizona (Leslie *et al.*, 2006) were both recently recognized. In Latin America, additional bat-associated variants continue to be identified (Kobayashi *et al.*, 2005).

Consistent with current taxonomic trends in virology, phylogenetic analysis has been used to investigate membership in the *Lyssavirus* genus (Kuzmin *et al.*, 2006a) and to delineate the seven species (genotypes or GTs) currently recognized within the genus (Tordo *et al.*, 2004). However, as new viruses emerge (Kuzmin *et al.*, 2005, 2006b), additional species may be identified.

C. Evolutionary time frames

Recently, methods to estimate nucleotide substitution rates have helped to develop time-scaled phylogenetic trees and thereby explore the time frame of lyssavirus evolution. Regardless of gene region or variant type, RABV coding regions exhibit quite similar rates of nucleotide substitution, ranging from 1.2×10^{-4} to 5.3×10^{-4} nucleotide substitutions/site/year, and have a high synonymous to nonsynonymous mutation ratio (Badrane and Tordo, 2001; Davis *et al.*, 2006; Holmes *et al.*, 2002; Hughes *et al.*, 2005; Kuzmin *et al.*, 2007). The rate at the noncoding G–L region appears to be somewhat higher, around 1×10^{-3} nucleotide substitutions/site/year (Davis *et al.*, 2007; Hughes *et al.*, 2004a). Evaluation of the growth properties of RABV populations by Bayesian methods generally indicate an exponential growth profile with variable-rate relaxed molecular clocks providing for the best fit to the data.

The role of the *Chiroptera* as hosts for most current lyssavirus genotypes has suggested that the primordial lyssavirus was bat-associated and a host-switching event, between 888 and 1459 years ago, was postulated to have resulted in the emergence of carnivoran rabies (Badrane and Tordo, 2001). A rather shorter time frame of GT 1 lyssavirus diversity of approximately 500 years has also been proposed (Holmes *et al.*, 2002). Although all lyssavirus genes appear to be equally useful for phylogenetic

predictions (Wu *et al.*, 2007), Davis *et al.* (2006) found a lack of congruence in GT 1 evolutionary history, predicted using different genes and were unable to establish whether bat-associated variants or viruses associated with other terrestrial species comprised the most basal evolutionary group. By two different approaches, the most recent common ancestor (MRCA) of North American bat RABVs was dated to the mid-1600s (combined range of 1254–1782) (Hughes *et al.*, 2005); this phylogeny suggested that the parental virus initially branched to yield variants currently associated with free-tailed and vampire bats and this was followed by the emergence of distinct variants associated with solitary and colonial bats of North America. If this prediction is correct, genetic heterogeneity in bat RABVs must depend more on the migratory life-style of the host rather than on the age of the viral lineage.

The ''Arctic'' lineage is estimated to have emerged in Asia sometime between 1255 and 1786 and then spread northward into all circumpolar regions (Kuzmin *et al.*, 2008), as had previously been proposed (Nadin-Davis *et al.*, 2007). A study of two distinct southern African biotypes concluded that the canid and mongoose variants exhibit quite different evolutionary dynamics probably reflecting the different ecological niches of their hosts (Davis *et al.*, 2007). Moreover, the mean age for the MRCA of the mongoose variant was 73 years, placing its emergence around 1930 and not in the 1800s as had previously been supposed (Nel *et al.*, 2005). In general, the time frames estimated by Bayesian methods have wide ranges and predict much shorter time scales than suggested from anecdotal information. Unless larger datasets generate different conclusions, it appears that some of the viruses referred to in past outbreaks belonged to now extinct lineages (Badrane and Tordo, 2001). Coalescent methods generate more coherent time frames when applied to more recent outbreaks (Biek *et al.*, 2007; Hughes *et al.*, 2004a).

The European bat lyssavirus type-1 (EBLV-1) group (GT 5), harbored by serotine bats of several European countries, is estimated to have emerged between 500 and 750 years ago and has since diverged into two distinct populations (Davis *et al.*, 2005). The nucleotide substitution rate (5×10^{-5} substitutions/site/year) exhibited by these viruses is one of the lowest values recorded for an RNA virus (Jenkins *et al.*, 2002). The very high constraints against nonsynonymous substitution observed in EBLV-1 were explained by supposing that the virus had reached a fitness peak so that most amino acid changes reduce viral fitness.

D. Modeling applications

Combination of viral phylogenetic analysis with various mathematical models has provided a valuable toolset for furthering our understanding of the factors contributing to viral disease dynamics. The rapid and

well-documented spread of the mid-Atlantic raccoon rabies variant along the eastern seaboard of the USA over the last 30 years has provided extensive surveillance data that permit the development and assessment of such models (Real *et al.*, 2005b). A model that reflects the initial 4–5 year epizootic period of raccoon rabies, followed by smaller epizootics of decreasing size and periodicity, was developed (Childs *et al.*, 2000) followed by a stochastic spatial model of rabies spread in the state of Connecticut that identified rivers as key barriers to local dispersion (Smith *et al.*, 2002) and demonstrated the role of long-distance animal translocation in the emergence of new foci of disease (Smith *et al.*, 2005). This spatial model was also used to predict westward expansion of raccoon rabies across Ohio, following an outbreak that breached an oral vaccination zone intended to contain this epizootic, and to assist in the design of supplementary control activities (Russell *et al.*, 2005). More recently, other models explored how to most effectively use natural barriers such as waterways during wildlife oral vaccination campaigns to prevent rabies spread (Russell *et al.*, 2006). Coalescent-based estimates using raccoon RABV genetic data were in good agreement with the known spatial and demographic dynamics of disease spread with time and showed the importance of the initial wave of infection in determining spatial genetic structuring of the epizootic (Biek *et al.*, 2007). In studies of Arctic fox strain rabies, the ecogeographic patterns observed in southern Ontario (Tinline and MacInnes, 2004) were explained by a model that unified the spatial population dynamics and molecular evolution of the RABV (Real *et al.*, 2005a). Insights into patterns of viral spread may also be gained through raccoon genotyping to identify host subpopulation structure (Cullingham *et al.*, 2005). Further gains in our understanding of rabies dynamics and evolution will undoubtedly follow future application of such methods.

III. FUNDAMENTAL ASPECTS OF VIRUS–HOST INTERACTIONS

Despite its small size (about 12 kb) and limited coding capacity (five open reading frames (ORFs), in the order 3′-N-P-M-G-L-5′), RABV exhibits remarkable neurotropic and neuroinvasive properties that are central to its transmission cycle. Following deposition of virus in peripheral tissues, via the bite of an infected animal, the virus enters peripheral nerves and is transmitted via nerve connections into the central nervous system (CNS), where extensive viral propagation occurs prior to secretion of large amounts of virus in salivary glands in readiness for the next cycle (Jackson, 2007). While viral transcription and replication proceeds in a manner highly typical of the *Rhabdoviridae* (Wunner, 2007), many aspects of lyssavirus biology are unique to this genus. Detailed structural aspects

of RABV replication have recently been reviewed (Albertini *et al.*, 2008) so will not be discussed further, but many new insights into the molecular basis of virus–host cell interactions and RABV pathogenesis will be described.

A. What is the basis for RABV pathogenicity?

Despite the fatal outcome of rabies, limited histological lesions are seen in brains from clinical human cases, suggesting that rabies infection does not cause substantial neuronal death but rather neuronal dysfunction, mediated perhaps by impairment of neurotransmitter release and function and/or ion homeostasis (Fu and Jackson, 2005; Jackson, 2007). Some degeneration of neuronal processes was noted after infection with pathogenic but not attenuated viruses (Li *et al.*, 2005). RABV-infected mouse brain exhibits a number of ultrastructural changes in neurons including degradation of neuronal axons and dendrites, disruption of cytoskeletal integrity, vacuolation and mitochondrial swelling that might explain neuronal dysfunction (Scott *et al.*, 2008). The attenuated lab-adapted CVS-B2c strain, derived from the challenge virus standard (CVS) strain, causes much more severe histological damage to the murine CNS than the pathogenic silver-haired bat RABV (SHBRV) field isolate, (Wang *et al.*, 2005) and such differences need to be borne in mind when inferring mechanisms of pathogenesis from studies using lab-adapted strains.

B. Role of viral proteins

1. Glycoprotein

The G protein forms trimeric transmembrane spike structures that allow the virus to attach to neurons and then enter the cell via a fusion event; G protein is thus a key determinant of RABV's neurotropism and neuroinvasiveness (Wunner, 2007). While the inability of a G-deficient RABV to transfer between neurons clearly demonstrated the importance of G protein to viral spread (Etessami *et al.*, 2000), establishing the nature of the receptor(s) employed has proven challenging. RABV G binds to the neuronal nicotinic acetylcholine receptor (Gastka *et al.*, 1996) and the murine neural cell adhesion molecule (NCAM) (Thoulouze *et al.*, 1998), but neither of these receptors is absolutely required for RABV spread in mice.

The low affinity nerve growth factor receptor (P75NTR) can also confer RABV binding to a nonpermissive cell line (Tuffereau *et al.*, 1998). P75NTR is a member of the tumor necrosis factor (TNF) receptor superfamily that binds to several neurotrophins and is targeted by several viruses of many different orders (Kinkade and Ware, 2006), although the viruses and the neurotrophins often target different

domains. The observation that RABV G binds to mammalian P75NTR but not to the avian equivalent was proposed to explain the virus' restriction to mammalian hosts (Langevin *et al.*, 2002). Moreover, RABV G binding to P75NTR requires Lys330 and Arg333 of antigenic site III, residues previously linked to RABV pathogenicity (see below) while mutations at residues flanking this antigenic site interfered with P75NTR binding (Langevin and Tuffereau, 2002). However, other observations argue against a universal role of P75NTR as a lyssavirus receptor. Only lyssaviruses of GT 1 (RABV) and GT 6, EBLV-2, interact with P75NTR, so lyssaviruses of all other GTs must gain cell entry via alternate receptor(s) (Tuffereau *et al.*, 2001). Furthermore, viral spread occurs throughout the CNS in P75NTR-deficient mice (Jackson and Park, 1999) and viral infection of primary neurons can occur in the absence of RABV G/P75NTR binding (Tuffereau *et al.*, 2007). While another receptor yet to be identified might function as a universal lyssavirus receptor, it remains possible that these viruses can use a number of different nerve cell receptors to gain entry into the host nervous system. Further study on nerve cell entry by field isolates may help to resolve this issue.

The specific role of G protein in determining viral spread within the CNS, and hence the extent of pathogenicity, was demonstrated in a study that examined the distribution over time of several viral constructs after stereotaxic inoculation into the rat hippocampus (Yan *et al.*, 2002). Structure–function studies involving the production of large numbers of chimeric viruses, each differing at just one amino acid within the G protein, reveal an increasing number of residues important to pathogenicity. In addition to an absolute requirement for Arg or Lys at residue 333 (Takayama-Ito *et al.*, 2006a; Tuffereau *et al.*, 1989), amino acids Ala242, Asp255 and Ile268, especially when present together, confer the highly pathogenic phenotype of the Nishigahara strain (Takayama-Ito *et al.*, 2006b). An Asn to Lys mutation at residue 194 restored pathogenicity to a highly attenuated RABV strain by increasing viral spread, eliciting faster cellular internalization and shifting the pH threshold for membrane fusion (Faber *et al.*, 2005b). However, in addition to the protein's primary structure, the level of G protein expression also has important pathogenic consequences; in general, more pathogenic strains produce lower amounts of G protein that in turn may limit the host's apoptotic (Yan *et al.*, 2001) and innate immune system responses (Wang *et al.*, 2005). Interestingly, nonpathogenic G genes are dominant over pathogenic versions (Faber *et al.*, 2007).

RABV virulence is increasingly being recognized as a multigenic trait. Regions of the RABV genome, including N, P, M and L genes, as well as noncoding elements, such as the trailer sequence and the G–L intergenic region, have all been reported to contribute to virulence and/or neuroinvasiveness (Faber *et al.*, 2004; Pulmanausahakul *et al.*, 2008; Shimizu *et al.*, 2007;

Yamada *et al.*, 2006) probably through interaction with intracellular factors necessary for efficient viral propagation.

2. Phosphoprotein

The P protein, with its highly modular organization that includes both conserved and variable domains (Nadin-Davis *et al.*, 2002), is emerging as a factor that contributes to viral virulence at many levels. In addition to full-length P protein (P1), the P gene ORF can generate up to four N-terminally truncated P products (P2–P5) (Chenik *et al.*, 1995). P proteins are targeted by two distinct cellular kinases (Gupta *et al.*, 2000) that phosphorylate several variably conserved sites (Nadin-Davis *et al.*, 2002). The cellular distribution of these various forms of P is determined by multiple localization signals present at different locations along its primary sequence. An N-terminal nuclear export signal (NES), present only in P1 and P2, normally directs these products into the cytoplasm while P3–P5 are localized to the nucleus; additionally the C-terminal P sequence contains both a nuclear localization signal (NLS) and a NES, functionalities of which appear to be determined by phosphorylation of a neighboring residue (Moseley *et al.*, 2007a; Pasdeloup *et al.*, 2005). The variable length, phosphorylation patterns, and cellular distribution of the P protein provide it significant potential versatility in its interactions with host cell factors.

a. Dynein LC8 Interaction One intriguing interaction of the lyssavirus P protein is its association with the dynein light chain LC8 (Jacob *et al.*, 2000; Raux *et al.*, 2000), a 10 kDa component of both the cytoplasmic dynein motor and Myosin V, which is involved in minus end-directed movement of organelles along microtubules and in actin-mediated vesicle transport in axons. Although functional studies on this interaction have been performed only on GT 1 and GT 3 representatives (Jacob *et al.*, 2000), the genus-wide importance of this association is suggested by the absolute conservation of the LC8 binding domain (BD) motif D(K/R)XTQT at P protein residues 143–148 (Nadin-Davis *et al.*, 2002; Poisson *et al.*, 2001). This finding fueled the hypothesis that P/LC8 interaction facilitated microtubule-directed minus-sense axonal transport of RABV ribonucleoprotein to the cell body and thus played a pivotal role in the spread of the virus within the host's nervous system. However, doubt on this model was cast by the fact that viruses lacking the LC8 BD, although slowed in their rate of spread, could still gain entry into the CNS from the periphery (Mebatsion, 2001; Rasalingam *et al.*, 2005). Tan *et al.* (2007) showed that loss of the LC8 BD from P reduced transcription levels in neuronal cells and thus proposed that P protein/LC8 association was important for efficient viral RNA polymerase activity, a role consistent with

the P protein's function as an L protein cofactor. Interestingly, the LC8 BD motif can facilitate nuclear protein importation, suggesting that this motif facilitates some nuclear function of P (Moseley *et al.*, 2007b).

b. Abrogation of the Interferon Pathway An early response to RABV infection of both neuronal cells (Préhaud *et al.*, 2005) and the mouse brain (Johnson *et al.*, 2006; Prosniak *et al.*, 2001; Saha and Rangarajan, 2003; Ubol *et al.*, 2006; Wang *et al.*, 2005) is an increase in many of the key components of the type-1 interferon (IFN) pathway that acts to protect the host from viral infections. Many viruses have adopted strategies to circumvent this process (reviewed by Haller *et al.*, 2006). The P protein determines RABV sensitivity to type-1 IFN (Shimizu *et al.*, 2006) and abrogates the host's innate immune system by interacting with the IFN pathway at multiple levels (Chelbi-Alix *et al.*, 2006).

Brzózka *et al.* (2005) demonstrated that full-length P protein inhibits phosphorylation of cytoplasmic interferon regulatory factor 3 (IRF3) by TANK-binding kinase 1 (TBK-1) thereby curtailing IFN induction by preventing IRF3-dependent transcription of the β-IFN gene in RABV-infected cells.

P protein also interferes with the IFN-effector pathway through an interaction of its C-terminus with STAT1, a critical mediator of the Janus kinase-signal transducer and activator of transcription (JAK/STAT) signal transduction pathway responsible for the expression of many IFN-effector genes. P protein does not interfere with STAT1 phosphorylation and activation but cytoplasmic forms of P prevent its nuclear accumulation in response to IFN induction (Brzózka *et al.*, 2006; Vidy *et al.*, 2005), while the nuclear-localized P3 binds directly to the DNA binding domain of activated STAT1, thereby suppressing transcription of IFN-inducible genes (Vidy *et al.*, 2007). The higher nuclear translocation of phosphorylated STATs in the presence of the attenuated CVS-B2c strain compared to the virulent SHBRV strain suggests the importance of this P function to virulence (Wang *et al.*, 2005).

A third target for the RABV P protein is the product of the promyelocytic leukaemia (PML) gene, now recognized as a primary target gene of the IFN pathway (Blondel *et al.*, 2002). PML exists in the nucleus as both a diffuse nucleoplasmic form and as discrete nuclear bodies in which it associates with several other nuclear proteins; this distribution is often disrupted in virus-infected cells. Through a motif present in the 125 C-terminal residues of P protein, P1 binds to PML and retains it in the cytoplasm while P3 binds to nuclear PML, reorganizes the nuclear bodies and increases their size. High levels of RABV growth in mouse embryo fibroblasts lacking PML suggest an antiviral effect of this product that is inhibited by P protein (Blondel *et al.*, 2002), but better understanding of PML's normal function is needed to fully appreciate this effect.

3. Other viral proteins

The multi-functional matrix protein may also contribute to viral virulence through many different mechanisms. It inhibits translation in RABV-infected cells via an interaction with the eIF3h subunit, part of the eIF3 complex involved in ribosomal dissociation that is critical to the cellular translation machinery (Komarova *et al.*, 2007). M protein, in concert with G protein, also regulates viral replication and facilitates cell-to-cell spread (Pulmanausahakul *et al.*, 2008). In addition, M protein mediates at least some of the virus' apoptotic effects. In a model of RABV-induced neuronal apoptosis, Kassis *et al.* (2004) found that cells transfected with M protein alone could induce caspase activation and apoptosis via a pathway involving the binding of TNF-related apoptosis-inducing ligand (TRAIL) to its receptors; M mediated release of a soluble, active form of TRAIL important to the early induction of this apoptotic pathway.

The L protein almost certainly contributes to RABV pathogenicity through interactions with various host cell components needed to support its role in viral transcription and replication but the details of such interactions remain to be explored.

C. Role of host cell pathways

The increasing availability of methods to explore large-scale changes in cellular expression patterns have facilitated studies to understand how experimental RABV infection changes host transcript levels. Using various methods, infection of mice with lab-adapted RABVs resulted in sequential changes in host cell transcription patterns (Johnson *et al.*, 2006; Prosniak *et al.*, 2001; Saha and Rangarajan, 2003; Ubol *et al.*, 2006; Wang *et al.*, 2005). Whereas the majority of genes were down-regulated as viral propagation progressed, select groups of genes were up-regulated. A relatively early response was the increased level of many mRNAs encoding products involved in innate immunity, including type-1 IFNs, toll-like receptors (TLRs) which play a critical role in initiating innate immunity (see McCoy and O'Neill, 2008), many IFN-effectors, as well as chemokines, cytokines and complement proteins involved in the inflammatory response. Indeed, of all genes up-regulated by RABV infection of a human neuron-derivative cell line, 24% were involved in immunity; moreover these neurons were found to express TLR3 which recognizes dsRNA, and could mount a corresponding innate immune response, a novel finding given the immunologically privileged nature of the CNS (Préhaud *et al.*, 2005). A comprehensive study of TLR induction in the murine CNS in response to RABV infection also identified an early up-regulation of TLR3 which would permit increased virus sensing mechanisms in the local vicinity of virally infected cells (McKimmie *et al.*, 2005).

Rabies-infected patients also exhibited enhanced TLR3 expression in neurons and occasionally in glial cells of the cerebellar cortex, a phenomenon that appeared to require induction by soluble factors rather than direct viral infection (Jackson *et al.*, 2006).

The innate immune response to RABV infection is followed by an increase in transcript levels for several products that may be significant to clinical disease and viral spread. These include growth factors, which may facilitate viral spread by promoting new axonal connections or prolonging infected neuron survival (Prosniak *et al.*, 2003b), certain metabolic enzymes, particularly those involved in nucleotide metabolism, selected receptors and transporters, notably dopamine transporter/receptor and serotonin receptor, consistent with the concept of disruptions in neurotransmission, as well as significant increases in ion transporters that could be responsible for reductions of intracellular sodium and calcium (Dhingra *et al.*, 2007; Prosniak *et al.*, 2001; Saha and Rangarajan, 2003; Ubol *et al.*, 2006; Wang *et al.*, 2005). Moreover, down-regulation of proteins involved in docking and fusion of synaptic vesicles at the presynaptic membrane resulted in synaptic vesicle accumulation (Dhingra *et al.*, 2007).

In later stages of disease, there are increases in yet other transcripts that encode products which may support RABV replication and spread, for example, heat shock protein 90 (Hsp-90) and CDC10; proteins involved in axonal guidance and cell repair, for example, neuroleukin (NLK) and apolipoprotein D (ApoD) important for lipid recycling. Finally, there is an up-regulation of products associated with programmed cell death (Prosniak *et al.*, 2001; Ubol *et al.*, 2006; Wang *et al.*, 2005).

1. Apoptosis

Apoptosis is now recognized as one critical means by which multicellular organisms try to protect themselves against pathogen invasion (Adams and Cory, 1998) and its role in experimental rabies pathogenesis was suggested when changes consistent with apoptosis, including DNA fragmentation and increased expression of the apoptotic enhancer Bax, were observed in neurons of RABV-infected mice (Jackson and Rossiter, 1997). Apoptosis was especially prominent in the pyramidal neurons of the hippocampus and cerebral cortex, prime targets for rabies infection, and suckling mice, which are more susceptible to the disease, exhibited higher levels of apoptosis than adults (Jackson and Park, 1998; Theerasurakarn and Ubol, 1998). Although elevated levels of Bax were observed in murine neuroblastoma cells within 24 h of infection, followed by up-regulation of caspase 1 (Ubol *et al.*, 1998), *bax*-deficient mice still exhibited significant apoptosis thereby implicating additional apoptotic modulators (Jackson, 1999). Using CVS strains that differ in their pathogenicity, Morimoto *et al.* (1999) observed a good correlation between high levels of apoptosis and high RABV G accumulation in mouse primary neuronal cell cultures.

Similar conclusions were made using recombinant viruses differing in G protein expression levels (Faber *et al.*, 2002); high expression of RABV G was accompanied by increases in caspase 3 activity and other apoptosis markers as well as higher anti-G antibody titers in mice. The speed or extent of apoptosis may directly determine the magnitude of the antibody response as suggested by experiments employing a recombinant virus expressing the pro-apoptotic cytochrome *c* gene (Pulmanausahakul *et al.*, 2001).

RABV pathogenesis may be determined to a large extent by the cell type undergoing apoptosis and the mechanisms inducing cell death. In a series of experiments on apoptosis in lymphocytes, the highly attenuated vaccine strain Evelyn Rokitnicki Abelseth (ERA) could induce both caspase-dependent and caspase-independent apoptotic pathways after infection of a Jurkat T cell line *in vitro*, unlike the neurovirulent CVS strain (Thoulouze *et al.*, 2003). This phenomenon was prevented by constitutive expression of the antiapoptotic Bcl-2 product. These studies also showed that ERA could up-regulate Bcl-2 levels in the Jurkat cell line, a phenomenon that resulted in long-term persistently infected cultures. This observation suggested a mechanism by which live attenuated RABVs such as ERA may, through increased Bcl-2 synthesis, persist *in vivo* in vaccinated animals; this effect could contribute to their efficacy as vaccines, but could also potentially result in a ''carrier'' state.

In an exploration of the role of the death-promoting factor Fas Ligand (FasL) and its receptor Fas in initiating apoptosis of activated lymphocytes *in vivo*, the virulent CVS strain, but not the attenuated PV strain, induced early production of FasL, primarily by infected neurons, which was associated with high levels of T cell apoptosis. Thus, CVS infection of mice induced only a transient migration of lymphocytes into the CNS in contrast to PV infection that permitted sustained T cell migration into the CNS and more limited CD3+ T cell apoptosis (Baloul *et al.*, 2004). Up-regulation of FasL by the neurovirulent CVS strain may limit CD3+ T cell mediated apoptosis of neuronal cells and thereby preserve the integrity of the neuronal network critical to virus spread.

The role of apoptosis in natural RABV infections is less evident. Compared to the CVS strain, infection of mice with SHBRV resulted in a lower overall level of neuronal apoptotic cell death (Yan *et al.*, 2001). If apoptosis is important for pathogenesis of street viruses, it may depend on the triggering of cell death by selected cell types.

2. The macrophage–monocyte lineage and inflammatory factors

Nitric oxide (NO) is a free radical produced by the enzyme nitric oxide synthase (NOS) that exists in both constitutive (cNOS) and inducible (iNOS) forms. High levels of NO are cytotoxic and activation of iNOS in macrophages is regarded as an inflammatory mediator (Aktan, 2004).

Up-regulation of iNOS in the RABV-infected rat brain was proposed to contribute to neuropathogenesis (Akaike *et al.*, 1995) and indeed treatment of RABV-infected mice with a selective inhibitor of iNOS delayed viral replication, apoptotic cell death and death of the host significantly (Ubol *et al.*, 2001). RABV virions can enter macrophages via endocytosis, and, despite limited virion replication, activate expression of iNOS as well as the CXC chemokine ligand 10 (CXCL10), a T helper cell type-1 chemoattractant (Nakamichi *et al.*, 2004); thus increases in NO levels contribute to macrophage activation in response to RABV infection. The significance of these observations was further extended to microglia cells, the functional equivalents of macrophages in the CNS. RABV entry into microglia was followed, even in the absence of significant replication, by strong induction of the chemokines CXCL10 and CCL5 through activation of several signaling pathways (Nakamichi *et al.*, 2005).

Both an attenuated vaccine strain and a pathogenic dog strain induced maturation of immature dendritic cells and monocytes, apparently through the induction of IFN-α1 mRNA (Li *et al.*, 2008). Consistent with other reports, the vaccine strain strongly up-regulated 26 genes involved in the NF- κB signaling pathway, including TLR3, TLR7 and STAT1, while the pathogenic strain elicited a much reduced response. Dendritic cells of the lymphoid tonsillar tissue, which comes into direct contact with oral vaccines, probably play an important role in eliciting immunity and attenuated vaccines may elicit protection, at least in part, by strong induction of the innate immune response at this site.

The role of TNF-α, another pro-inflammatory cytokine produced by cells of the monocyte/macrophage lineage, was investigated using a recombinant RABV expressing a soluble form of TNF-α (Faber *et al.*, 2005a). High levels of soluble TNF-α reduced RABV replication in neuroblastoma cells without inducing significant levels of apoptosis. Furthermore, unlike animals challenged with a wildtype (wt) virus, mice survived challenge with the TNF-α-expressing recombinant virus due to significant inflammatory mechanisms as well as a direct antivirus effect. Consistent with such observations, a prior study had suggested the protective effect of the p55 TNF-α receptor, a mediator of T cell protection, in RABV ocular disease (Camelo *et al.*, 2001). In human cases, RABV-infected neurons induced expression of both TNF-α and iNOS in adjacent astrocytes or microglial cells but not in the neurons themselves (Nuovo *et al.*, 2005).

3. Role of noncoding RNAs?

Saha *et al.* (2006) observed that neurotropic viruses including RABV induce production of a novel noncoding (nc) RNA, designated VINC (virus inducible ncRNA). While the function of this nuclear-localized RNA remains obscure, this observation sets the precedent that RABV

infection can alter the ncRNA complement of infected cells. Given the recent recognition of the role of microRNAs (miRNAs) in control of gene expression and the recent discovery of viral-encoded miRNAs that affect gene expression patterns in virus-infected cells (Qi *et al.*, 2006), this suggests novel means by which RABV could control host cell expression.

D. Considerations for future studies on rabies pathogenesis

While various experimental models of rabies, including the extensive use of laboratory strains of mice, provide important insights into the disease, many of the host's transcriptional changes observed upon RABV infection likely represent common host pathways for countering infection by neurotrophic viruses (Saha and Rangarajan, 2003). Dissecting out those changes that are especially relevant to RABV pathogenesis will prove difficult although, based on the information now being collected, transgenic mice might yet play an important role in this regard. Furthermore, there are significant differences in the extent of gene activation and the cell types affected in mice depending on the RABV strain employed (Prosniak *et al.*, 2003b). Street strains limit the response of type-1 IFN and inflammatory pathways far more effectively than avirulent strains, perhaps due in part to a lower level of G expression that limits TLR3-associated activation of the innate immune system (Wang *et al.*, 2005). An accurate appreciation of the pathogenic mechanisms operating in natural infections will require further studies employing street isolates inoculated into their normal reservoir hosts. Presently the lack of tools and reagents to study many of the processes described here in natural rabies hosts (e.g., dog, fox, skunk, raccoon, and bat species) impedes such work. Furthermore, it is evident that a full appreciation of RABV propagation and virulence requires not only an understanding of which host genes play pivotal roles but in which cell types these genes function. Studies that do not consider the heterogeneous cell population of the CNS may miss effects that are restricted to a particular cell type. Ultimately, a combination of immunohistochemical methods and *in situ* RT-PCR (see Nuovo *et al.*, 2005) may prove invaluable for identifying the cell types and molecular mechanisms most critical to RABV pathogenicity in natural infections.

IV. REVERSE GENETICS—METHODOLOGY AND APPLICATIONS

RABV was the first member of the *Mononegavirales* order to be successfully manipulated by reverse genetics to generate infectious, replication-competent virus (Schnell *et al.*, 1994). Subsequent observations, that a

foreign gene could be inserted within an intergenic region of the RABV genome without significant effect on *in vitro* replication of the virus, that this insert was translated into functional protein and that the recombinant RABV (rRABV) was genetically stable (Mebatsion *et al.*, 1996), established the broad utility of this technology. Indeed the fact that the RABV genome can accommodate large increases in size (at least 55% more than wt RABV) (McGettigan *et al.*, 2003b) and that reporter gene expression levels are dependent on insert orientation (Finke and Conzelmann, 1997, 1999), genomic position (Wu and Rupprecht, 2008) or the nature of the intergenic sequences (Finke *et al.*, 2000) provides substantial flexibility in engineering novel and useful recombinants. Reverse genetics is now used for various applications, particularly to investigate RABV pathogenesis (see previous section) and to generate novel RV vaccines (Schnell *et al.*, 2005).

The RABV reverse genetics system was initially developed based on the SAD B19 genome sequence, but systems for other lab-adapted RABV strains, including RC-HL (Ito *et al.*, 2001, 2003), HEP-Flury (Inoue *et al.*, 2003), and ERA (Wu and Rupprecht, 2008), as well as the SHBRV-18 wildlife isolate (Faber *et al.*, 2004), have now been described. As the technology has become more widely used, many methodological improvements have been developed. The original system employed host cells infected with a recombinant vaccinia virus expressing T7 polymerase to drive the transcription of the helper plasmids and the genomic cDNA. Use of host cell lines stably expressing T7 polymerase eliminated the need for the vaccinia construct and circumvented vaccinia virus-induced cytotoxicity and the requirement for removal of contaminating virus from the rRABV cultures (Buchholz *et al.*, 1999; Ito *et al.*, 2003). One such cell line facilitated the recovery of an RC-HL-derived rRABV containing two copies of the G gene (Hosokawa-Muto *et al.*, 2006).

Other improvements that increase rRABV/minigenome RNA yields have included construction of new helper plasmids that render the T7 RNA polymerase-transcripts cap-independent for translation (Ito *et al.*, 2003) and the inclusion of ribozyme sequences at the termini of the cloned viral genome to permit generation of RNA with precise 5′ and 3′ genomic ends (Inoue *et al.*, 2003; Le Mercier *et al.*, 2002). A recovery system using expression plasmids bearing the cytomegalovirus (CMV) immediate early promoter in place of the T7 promoter, and which is transcribed by the host cell RNA polymerase II, yielded higher virus recoveries from multiple cell types compared to a comparable T7 promoter-driven system (Inoue *et al.*, 2003). Use of both CMV and T7 promoters enhanced transcription efficiency and, using a T7 polymerase engineered to localize to the nucleus (Wu and Rupprecht, 2008), facilitated recovery of a recombinant ERA virus in which the order of the M and G genes was reversed; such a construct could not be recovered previously.

A. RABV vaccines

One application of this technology is to generate novel rabies vaccines. While the use of inactivated rabies vaccines that are safe and efficacious following parenteral administration in humans and animals is well established, logistical considerations dictate that oral vaccination will remain the primary means of controlling sylvatic rabies (Rupprecht *et al.*, 2006). Only live attenuated vaccines have to date been proven effective via the oral route. There is continued interest in developing live attenuated RABV vaccines that are safer and more efficacious than the ERA/SAD B19 strains currently used for oral vaccination of foxes in Europe and North America and, in particular, vaccines that are efficacious in other wildlife reservoirs. A common approach is to engineer viruses that express higher levels of G protein that in turn elicits production of protective virus neutralizing antibody (VNA). A recombinant ERA, in which the order of the M and G genes was rearranged so as to increase G protein expression, was less virulent in mice than parental ERA (Wu and Rupprecht, 2008). A rRABV derived from the RC-HL strain carrying two copies of the wt G gene had increased levels of G protein in virions and in infected cells; this double G virus remained pathogenic in mice but after inactivation it was significantly more immunogenic than the parent virus and may be useful as an inactivated rabies vaccine (Hosokawa-Muto *et al.*, 2006). SAD B19-derived constructs, in which the G gene was mutated to encode either a Glu or Gln at position 333 instead of Arg, had greatly reduced pathogenicity in mice and elicited VNA upon intramuscular (i.m.) and oral inoculation that afforded protection from lethal challenge (Faber *et al.*, 2002; Morimoto *et al.*, 2001). A rRABV containing two copies of the Glu333-mutated G gene (SPBNGA-GA) resulted in significantly increased G protein expression and hence elicited significantly higher VNA titers in mice and improved survivorship after challenge over the construct (SPBN-GA) with one G gene copy (Faber *et al.*, 2002).

Pulmanausahakul *et al.* (2001) found that a rRABV encoding the pro-apoptotic protein cytochrome *c* (SPBN-Cyto *c*(+)) had markedly reduced pathogenicity and increased immunogenicity in mice following intranasal administration when compared to a rRABV carrying an inactive cytochrome *c* gene. It was subsequently shown that SPBNGA, SPBNGA-GA, and SPBN-Cyto *c*(+) viruses could be grown to high titer and were genetically stable at position 333, important considerations for candidate vaccines (Dietzschold *et al.*, 2004). However, serial passage through neonatal mice resulted in a secondary mutation, Asn to Lys at position 194, in some GA genes which led to an increased pathogenicity. Interestingly, only one GA gene copy of SPBNGA-GA acquired this mutation and this was insufficient to increase pathogenicity (Dietzschold *et al.*, 2004).

The utility of these constructs as oral vaccines has been examined in species other than laboratory rodents. SPBN-GA, SPBNGA-GA, and SPBN-Cyto *c*(+) administered *per os* caused no adverse effects in beagles; the majority of vaccinates seroconverted within 1–2 weeks postvaccination and all vaccinates survived i.m. challenge with street virus of canine origin (Rupprecht *et al.*, 2005). The safety and efficacy of rRABV with one or two copies of the G gene carrying the Glu333 mutation and an additional Asn to Ser mutation at position 194 (SPBN-GAS and SPBN-GASGAS) following oral vaccination in African mongooses and raccoons was explored. No adverse effects were observed in either species. SPBN-GAS induced VNA and protected African mongooses from lethal challenge (Blanton *et al.*, 2006). Only two out of five raccoons vaccinated with SPBN-GAS developed VNA and survived challenge; all five raccoons immunized with SPBN-GASGAS survived challenge despite detection of VNA in only two vaccinates (Blanton *et al.*, 2007), consistent with the observation that rRABV overexpressing G protein had increased immunogenicity in mice (Faber *et al.*, 2002).

In a different approach to vaccine development, rRABV completely apathogenic for neonatal mice following intracranial inoculation were produced by deletion of either the P gene (Shoji *et al.*, 2004) or the M gene (Ito *et al.*, 2005). Progeny virions could be recovered from cells providing the missing proteins in *trans* and were able to infect cells normally susceptible to RABV; however, infection did not spread beyond the initially infected cell. When given to mice by either intraperitoneal (i.p.) or i.m. inoculation, the P-deficient rRABV elicited VNA in a dose-dependent manner and protected against lethal intracranial challenge with CVS (Shoji *et al.*, 2004). Similarly, i.m. and intranasal inoculation with the M-deficient rRABV elicited VNA production, but protection against challenge was not determined (Ito *et al.*, 2005).

While these preliminary studies in various animal models have examined rRABV immunogenicity and, in some cases, efficacy, evaluation of the optimal dose, duration of immunity, safety and efficacy of these candidate vaccines administered in a bait format appropriate for wildlife vaccination is needed before field application can be considered.

B. Vaccines for other diseases

The use of rRABV vectors for development of vaccines against other diseases, especially for acquired immunodeficiency syndrome (AIDS), has also been explored. A chimeric protein comprising the envelope (Env) protein's extracellular domain of human immunodeficiency virus type-1 (HIV-1) and the RABV G protein's transmembrane and cytoplasmic domains was expressed by a rRABV construct, incorporated into

virions, and exhibited many of the biological properties of wt Env (Foley *et al.*, 2002; Schnell *et al.*, 2000). The resulting viruses exhibited a cellular tropism similar to that of HIV-1. Similarly, replication-competent rRABVs containing the HIV-1 *gag* gene within the G/L intergenic region (McGettigan *et al.*, 2001b) or the N/P intergenic region (McGettigan *et al.*, 2003a) were recovered and expressed functional Gag product following infection of human cells. These results suggested that rRABV might be a suitable vaccine vector for HIV-1.

A strong neutralizing antibody response to the Env-derived gp120 subunit was elicited if mice received an i.m. immunization with rRABV expressing HIV-1 Env protein and were subsequently boosted with recombinant gp41 and gp120, indicating that the HIV-1 Env-pseudotyped rRABV could prime the immune system for production of Env-specific neutralizing antibody (Schnell *et al.*, 2000). After a single i.p. inoculation with the same rRABV, a long-lasting CD8+ cell mediated cytotoxic T lymphocyte (CTL) response directed against multiple HIV-1 Env epitopes was induced (McGettigan *et al.*, 2001a). Similarly, rRABV expressing HIV-1 Gag could elicit CD8+ cell-dependent Gag-specific CTL responses (McGettigan *et al.*, 2001b, 2003a). A rRABV expressing both HIV-1 Gag-Pol and an HIV-1 Env/RABV G chimera was generated (McGettigan *et al.*, 2003b) and produced correctly-processed functional proteins.

The demonstration, that development of an immune response to the vesicular stomatitis virus (VSV) glycoprotein ectodomain was not impaired by prior immunization with an identical RABV vector expressing a wt RABV glycoprotein, suggested that a heterologous prime/boost approach might be a useful strategy for foreign antigens presented in rRABV vectors (Foley *et al.*, 2000). Priming with an RABV/HIV-1 Env recombinant followed by boosting with a heterologous rhabdovirus vector (VSV/HIV-1 Env) resulted in robust humoral and CTL responses in mice (Tan *et al.*, 2005). A robust cellular response against the HIV antigen was achieved in macaques given a primary immunization with RABV/HIV-1 Env and secondary immunization with a rRABV expressing the HIV-1 Env as well as a chimeric VSV G with the RABV G cytoplasmic domain in place of the wt G protein (McKenna *et al.*, 2007). Furthermore, this prime/boost approach was able to protect macaques against challenge with a pathogenic simian–HIV strain.

In a different approach to modifying immune responses to HIV-1 antigens, McGettigan *et al.* (2006) produced RABV vectors co-expressing HIV proteins and murine cytokines. Compared to the parental RABV vector expressing HIV-1 Gag or Env, co-expression of IL-4 decreased the cellular immune response and abrogated the serological response to Gag and Env, whereas co-expression of IL-2 induced strong cellular responses and induced seroconversion against Env after two inoculations (McGettigan *et al.*, 2006).

Inactivated RABV particles can serve as immunostimulatory carrier molecules for display of B cell antigens. Green fluorescent protein (GFP) presented as a fusion protein with the RABV nucleoprotein induced a strong antibody response against GFP in mice, whereas recombinant GFP or a combination of wt RNP with recombinant GFP did not (Koser *et al.*, 2004). Mice inoculated i.m. with either killed or live rRABV expressing a fusion protein of RABV G protein and the PA domain-4 of *Bacillus anthracis* developed anti-PA antibodies after a single inoculation and robust anamnestic responses to boosts with the same rRABV (Smith *et al.*, 2006).

Immunization of mice with live rRABV expressing the hepatitis C virus E2 envelope protein as a fusion with the RABV G cytoplasmic domain did not induce specific antibody; however, animals boosted with killed recombinant virus as a source of recombinant E2 did mount a potent humoral immune response (Siler *et al.*, 2002). A single injection with RABV-E1E2, induced a long-lasting antigen-specific cellular immune response. Faber *et al.* (2005c) showed that a single i.m. inoculation of mice with rRABV expressing the SARS-CoV virus envelope spike protein (S) gene induced a strong SARS-CoV-neutralizing antibody response while the same RABV vector expressing the SARS-CoV nucleocapsid protein (N) did not elicit production of any N-specific antibodies, suggesting that the nature of the immune response to antigen introduced via RABV-derived vectors is antigen-dependent. While these preliminary studies show that antigen presented in the context of RABV can stimulate both humoral and cellular immune responses, further studies will be required to determine the quality of these responses vis-à-vis protection from virulent challenge.

V. OTHER STRATEGIES FOR RABIES VACCINE DEVELOPMENT

Further vaccine development is currently targeting two main rabies reservoir groups: wildlife species not readily immunized using current oral vaccines and dogs in the developing world, which require stable, safe, and inexpensive products given either by the oral or parenteral route. Two prominent strategies which have sought to address these deficiencies are described.

A. Adenovirus recombinants

While the vaccinia-rabies glycoprotein recombinant V-RG was, until recently, the only heterologous recombinant vaccine employed for rabies control in the field (Brochier *et al.*, 1991), the potential utility of various adenovirus recombinants has been explored. Constructs which have been generated include those based on human adenovirus type-5 (HAd5),

using either E3-deleted (Yarosh *et al.*, 1996) or E1-deleted vector backbones (Vos *et al.*, 2001; Xiang *et al.*, 1996), chimpanzee adenovirus serotype 68 (AdC68) (Xiang *et al.*, 2002) or canine adenovirus type-2 (CAV-2) (Hu *et al.*, 2006; Li *et al.*, 2006). The immunogenicity of many of these constructs was established in the murine model as well as in a number of other target species. An AdRG1.3 construct, originally described by Yarosh *et al.* (1996), and shown to be highly efficacious in skunks via the oral route (Knowles *et al.*, manuscript in preparation) is now being assessed in a bait formulation in limited field trials for rabies control in skunks in Ontario, Canada (see http://www.mnr.gov.on.ca/MNR/csb/news/2006/aug18nr2_06.html). CAV-2 recombinant vaccines, based upon a live vaccine already employed in dogs, were immunogenic in mice (Li *et al.*, 2006) and in dogs after subcutaneous inoculation (Hu *et al.*, 2006). Oral administration in dogs elicited strong long-term serological responses and protected against challenge (Zhang *et al.*, 2008) while protective serological responses in cats required i.m. administration (Hu *et al.*, 2007). Further evaluation of some of these constructs for licensing purposes appears likely. One concern with use of adenoviral-based vaccines is that prior adenovirus infection might generate immunity to the vector and thereby limit the vaccine's efficacy. Indeed, the anti-RABV G antibody response of mice given a HAd5RABG recombinant can be compromised by pre-exposure to the homologous adenovirus (Xiang *et al.*, 2002). The use of vaccines based on several different adenovirus vectors that elicit limited serological cross-reactivity may be one means of overcoming such a limitation (Xiang *et al.*, 2002).

B. DNA vaccines

The stability and low cost of DNA vaccines makes them ideal candidates for use in the developing world. Proof of principle for this approach, which involves inoculation of the animal with DNA of a plasmid that directs expression of the RABV G protein under a strong viral promoter, was demonstrated in the mouse model some years ago (Xiang *et al.*, 1994). Difficulties in transferring this technology to larger mammals were encountered, though its feasibility for eliciting strong immune responses in cats and dogs was reported by Osorio *et al.* (1999) and protection of beagles from challenge using a two-dose immunization schedule administered i.m. was reported shortly thereafter (Perrin *et al.*, 2000). Evaluation of different methods of immunizing dogs using a single DNA vaccine dose suggested that intradermal (i.d.) application into the ear pinnae was the method of choice (Lodmell *et al.*, 2003) and could protect dogs challenged 1 year later (Lodmell *et al.*, 2006). Experimental and field trials undertaken in Tunisia, suggested that DNA immunization, administered by jet injector into the inner ear of local dogs, was more effective in

eliciting long-lasting VNA and protection than a cell culture-derived vaccine (Bahloul *et al.*, 2006). Intranasal application of a two-dose regimen of DNA vaccine has also been reported to induce a good immune response in dogs (Tesoro Cruz *et al.*, 2006). An evaluation of DNA vaccines to immunize horses against rabies reported that formulation of the DNA with a cationic lipid, given as a two-dose regimen, elicited a good serological response (Fischer *et al.*, 2003); the application of this technology in equines could potentially be very important in Latin America where transmission of vampire bat rabies to livestock continues to cause significant losses. Bahloul *et al.* (2003) also proposed a potential role for DNA immunization in PEP based on experiments in the mouse model where a single dose of DNA vaccine given the same day as a RABV challenge was more effective than five doses of cell culture-derived vaccine given over 28 days, though it must be pointed out that overall protection rates (53% and 40%, respectively) were low.

The potential application of DNA immunization to humans was suggested by a study showing that a DNA vaccine appeared to be comparable to cell culture-derived vaccine in eliciting VNA and protection from challenge in macaque monkeys, although multiple applications of vaccine were necessary (Lodmell *et al.*, 1998). A later study showed some protection from challenge in macaques 1 year after administration of a single DNA vaccine dose given either via gene gun or i.m., though protection was not complete and did not appear to correlate well with presence of VNA (Lodmell *et al.*, 2002a). Another study suggested the superiority of i.m. over i.d. administration of DNA vaccines in nonhuman primates and demonstrated the need for a booster dose 6 months after the initial immunization to maintain adequate VNA levels (Biswas *et al.*, 2001). Use of DNA immunization for PEP in monkeys was less effective, with a 50% protection rate compared to 75% using the human diploid cell vaccine, despite attempts to accelerate the VNA induction using gene gun vaccination into specific tissues (Lodmell *et al.*, 2002b). Based on these data, the further development of DNA vaccines for human disease prevention, especially for PEP, is not currently advocated (Ertl, 2003).

DNA vaccines have attempted to address the issue of vaccine coverage. Present vaccines, which are based on selected strains of GT 1, do not elicit production of antibodies that effectively neutralize members of the more divergent lyssaviruses, especially those of GTs 2 and 3. However, Jallet *et al.* (1999) described various DNA vaccines, comprising chimeric glycoprotein constructs generated from the G genes of the PV (GT 1), Mokola (GT 3), and EBLV-1 (GT 5) lyssaviruses, which broaden the spectrum of protection compared to the homologous sequences. Moreover, attempts to generate a DNA vaccine against Mokola virus using the Mokola G gene inserted into several different plasmid vectors showed some promise in mouse models (Nel *et al.*, 2003). Since very few reported

rabies cases are due to lyssaviruses other than those of GT 1, a significant rise in the number of such cases would be needed to drive further commercial development of such vaccines; the recent emergence of several divergent lyssaviruses (see Section II.B) may result in greater attention to this issue in the future.

Although there are still some safety concerns with DNA vaccines, for example, genome integration, overall these vaccines are considered generally safe and free of adverse reactions. New approaches for vaccine delivery (e.g. electroporation) appear to be overcoming the limited potency observed for first generation products, especially in larger mammals and humans (Ulmer *et al.*, 2006). Thus, future licensing of some of these products for veterinary applications may proceed, especially if efficiency gains through the use of multi-cistronic DNA vaccines (see Patial *et al.*, 2007) can be realized.

VI. THE CHALLENGE OF RABIES BIOLOGICS FOR PASSIVE IMMUNITY

Effective rabies PEP of humans requires not only an efficacious vaccine but passive immunity applied to the wound area that can limit virus propagation and spread until an adaptive immune response develops. Passive immunity is normally supplied as human (HRIG) or equine rabies immunoglobulin (ERIG) preparations (Rupprecht *et al.*, 2005) but the limited supply of these reagents often results in incomplete and ineffective PEP. Consequently, the development of alternative preparations comprising human Mabs has become a priority. Since a recent review has summarized most activities in this area (Nagarajan *et al.*, 2008), only a few salient points are presented here.

The anti-G Mabs chosen for this application should be virus neutralizing and should bind to well conserved G protein epitopes to ensure as broad a range of cross-reactivity as possible (Champion *et al.*, 2000; Sloan *et al.*, 2007). The divergent nature of many of the nonGT 1 lyssaviruses should be taken into consideration in this regard (Hanlon *et al.*, 2005). Moreover, to prevent inadvertent selection of RABV escape mutants by such preparations, more than one G protein epitope should be targeted and hence, a Mab cocktail containing two or more Mabs is desirable (Bakker *et al.*, 2005; Marissen *et al.*, 2005; Prosniak *et al.*, 2003a) and safety and efficacy similar to that achieved with HRIG should be demonstrated (Goudsmit *et al.*, 2006). While such reagents can be produced in cell culture systems, production of functional Mabs in plant based systems has been proposed as a cost-effective alternative (Ko *et al.*, 2003).

VII. NOVEL APPLICATIONS OF RABV

A. Use as a neuronal tracer

The mammalian brain is composed of a series of neural networks and pathways, and knowledge of the interconnectedness of these networks is essential to an understanding of how the brain functions. Fine mapping of CNS neuronal pathways at the cellular level requires the use of neuronal tracers and although a variety of chemical compounds have been employed for such purposes (reviewed in Köbbert *et al.*, 2000), these are limited due to nonspecific cell uptake and dilution effects that restrict their usefulness to cells immediately adjacent to those of the injection site. To overcome such limitations, neurotropic viruses such as herpes simplex virus-1 (HSV-1), pseudorabies virus (reviewed in Loewy, 1998), and more recently, RABV were investigated for their utility in mapping neuronal pathways. Viral replication in infected cells naturally amplifies its signal and allows mapping of higher-order neural connections. Despite their advantages, tracer studies using α-herpesviruses can be confounded due to virus-induced cytotoxicity and the generation, under certain conditions, of spurious labeling by local and nonspecific spread between cells (reviewed in Norgren and Lehman, 1998).

RABV is particularly useful for studying motor networks as it exclusively infects motoneurons, rather than sensory or sympathetic neurons (Graf *et al.*, 2002; Kelly and Strick, 2000; Tang *et al.*, 1999; Ugolini, 1995) and it propagates by transneuronal transfer (Astic *et al.*, 1993; Coulon *et al.*, 1989; Kucera *et al.*, 1985; Lafay *et al.*, 1991). Following stereotactic injection of virus into specific nerves, muscles or brain areas of the test subject and a defined incubation time, the animal tissue is perfused with fixative, sectioned, and finally stained to determine the distribution of infected cells. In theory, by adjusting the survival time following RABV inoculation, an unlimited number of serially connected neurons can be visualized and precise mapping of neuronal networks and connections is possible. Model systems thus employed include primates, rats, guinea pigs, and adult and neonatal mice. Since the rate of RABV transport can vary depending on the virus preparation—variant, passage history, size of inoculum—as well as on the animal species (Kelly and Strick, 2000; Ugolini, 1995), the time course of labeling must be established for each model system to allow determination of the sequential order of neurons in a pathway. While the CVS variants (CVS-11, -26, -N2c and -B2c) employed as neuronal tracers are fixed viruses, with various levels of pathogenicity, tracing studies are usually sufficiently short such that the test subjects do not develop clinical rabies.

The potential use of RABV as a neuronal tracer was established using a well-characterized motoneuron network, the hypoglossal system, which

showed that RABV could label all main groups of second-order neurons and higher order hypoglossal-related neurons without cytotoxicity (Ugolini, 1995). There was no lateral spread of RABV at the site of inoculation, either in muscle (Morcuende *et al.*, 2002) or in the brain (Astic *et al.*, 1993; Ugolini, 1995) and spread was strictly transneuronal occurring exclusively at synaptic connections (Kelly and Strick, 2003). RABV was transported solely in the retrograde direction following intracortical and i.m. injection in primates (Kelly and Strick, 2000) and inoculation into the hypoglossal nerve (Ugolini, 1995) or the bulbospongiosus muscle (Tang *et al.*, 1999) of rats.

RABV has been used in two main types of investigation. First, inoculation of RABV into muscle cells has been used to identify the motoneurons that innervate the tissue, as well as second- and higher-order neurons that relay signals to the CNS; this includes studies of pathways controlling digit movements (Rathelot and Strick, 2006), the oculomotor system (Graf *et al.*, 2002; Morcuende *et al.*, 2002), the olfactory system (Astic *et al.*, 1993), and respiratory networks (Gaytan *et al.*, 2002; Viemari *et al.*, 2004). Secondly, RABV has been injected into specific function-related areas of the brain to identify cells that are pre-synaptic to those initially infected, thereby revealing the neurons that project into these sites. A number of such studies have examined the organization of neuronal pathways and the connections between the primary motor cortex and the cerebellum or the basal ganglia (Clower *et al.*, 2005; Kelly and Strick, 2004; Lu *et al.*, 2007), and within the visual cortex (Nassi and Callaway, 2006). Use of RABV as a neuronal tracer in these systems has delineated connections to a level that was not previously possible using conventional tracers.

These studies commonly indicate a higher level of complexity than anticipated. For example, in a guinea pig model considerable cross-talk was demonstrated between neuronal systems controlling horizontal and vertical eye movement with an unexpectedly large number of labeled interneurons in and around the oculomotor and trochlear nuclei proper and surprisingly, labeling of structures known to be involved in directional heading during navigation (Graf *et al.*, 2002). Morcuende *et al.* (2002) mapped the neuronal premotor networks involved in eyelid responses in a rat model and developed a comprehensive picture of the pre-motor networks mediating reflex, voluntary, and limbic-related eyelid responses. This work also highlighted the potential sites for motor learning involved in classical eyelid conditioning.

Using combined studies with a retrograde tracer (RABV) and an anterograde tracer (HSV-1) Kelly and Strick (2003) demonstrated that multiple closed loop circuits characterize cerebral–cerebellar interactions in the primate brain in contradiction to the traditional view that connections between these regions comprise a massive open loop system;

rather the data suggested the existence of separate circuits for cognitive and motor operations.

Another study challenged the traditional view of connections between cortex, basal ganglia, and cerebellum. By exploring outputs to the anterior intraparietal area (AIP), which responds to sight of objects as well as to the act of grasping them, from both the cerebellum and basal ganglia, Clower *et al.* (2005) were the first to demonstrate an anatomical pathway linking basal ganglia output with the parietal lobe in primates. Basal ganglia input into the AIP may provide an anatomical explanation for poorly understood nonmotor (visuomotor and visuospatial) deficits observed in Parkinson's disease patients.

Distinct, closed-loop circuits between cortex and sub-cortical areas were also demonstrated in RABV tracing experiments that examined the basal ganglia and cerebellar inputs to the supplementary motor area (SMA) and the pre-supplementary motor area (pre-SMA) (Akkal *et al.*, 2007). Together with previous studies demonstrating that the SMA and pre-SMA neurons project to separate regions of the basal ganglia (Inase *et al.*, 1999), these observations suggest that the SMA and the pre-SMA are nodes in distinct neural systems that form separated closed-loop circuits with the basal ganglia. The observation that the basal ganglia, rather than the cerebellum, provided the dominant input to these two nodes may provide an anatomical explanation for why cerebellar hyperactivity in Parkinson's disease does not normalize SMA activity. Hoshi *et al.* (2005) demonstrated an anatomical pathway that directly links the output stage of cerebellar processing to the input stage of basal ganglia processing. This novel observation implies that the cerebellum may be able to adjust basal ganglia activity and suggests a role for the cerebellum in motor and cognitive functions normally associated with basal ganglia dysfunction.

In many cases, the maps generated by RABV retrograde tracing have provided an anatomical explanation to support pathways or functional models established using other methodologies. Gaytán *et al.* (2002) used RABV tracing to characterize the neuronal networks projecting to the main respiratory motoneurons in adult mice, work which provided a basis for the neural network that integrates respiratory-related activities in complex behavioral responses. RABV tracing confirmed the involvement of A6 neurons in development of a normal respiratory rhythm in mice (Viemari *et al.*, 2004) and also resolved an ongoing controversy by showing that cortico-motoneuronal (CM) cells affecting digit muscles are found in only one area of the primary motor cortex but those for each muscle have a widespread distribution and overlap with the area known to contain shoulder representation, a feature that may allow a wide range of muscle synergies to occur (Rathelot and Strick, 2006).

Traditionally, immunohistochemical or immunofluorescence methods were used to trace RABV progression through neuronal networks while

infected cells were identified to type either by morphology or by double-staining for cell-specific markers (Tang *et al.*, 1999). Reverse genetic techniques have permitted the development of novel RABV tracers labeled with endogenously-expressed fluorescent proteins. RABV particles expressing a P protein/enhanced GFP (eGFP) fusion product were generated and used to track binding of virus to cells and virion internalization *in vitro* (Finke *et al.*, 2004). More recently, double-labeled virions bearing two distinct fluorescent protein tags fused to P and G proteins were generated (Klingen *et al.*, 2008) thereby permitting distinction of naked RNP from intact, enveloped viruses. Using neuronal cell cultures, these viruses demonstrated a role for RABV glycoprotein in virus particle transport within the neuron. While their utility for *in vivo* studies has yet to be tested, such viruses may allow real-time tracking of RABV infection.

Virus particles, in which the G gene was replaced by that encoding eGFP, could be generated by complementation with a G protein-expressing plasmid; these virions infected neurons and replicated their core components, but could not spread beyond the initially infected cells (Wickersham *et al.*, 2007a). Such infected neurons expressed high levels of eGFP thus permitting a detailed study of the morphology and physiology of neurons at injection sites in the brain. RABV-eGFP was found to be superior to another monosynaptic retrograde tracer, HIV-1 pseudotyped with RABV G protein. Further refinements have been made to this system, in which RABV-eGFP was pseudotyped with the envelope protein EnvA from the subgroup A avian sarcoma and leucosis virus. RABV-eGFP/EnvA can infect only those cells expressing the EnvA receptor, the gene for which is introduced by electroporation, and can spread monosynaptically to projecting cells if the RABV G is also provided in *trans* (Wickersham *et al.*, 2007b). In this way, neurons directly pre-synaptic to the EnvA receptor-expressing cells can be unambiguously identified. This system is novel and powerful in that for the first time, it provides a tool for identifying neurons that are monosynaptically connected to a cell group, or even a single cell, and can distinguish weak, direct connections from strong, indirect ones, features which sometimes can confound interpretation of results using conventional RABV *trans*-synaptic tracers. While this system presently remains at the proof of principle stage, its implementation *in vivo* appears possible.

While the expression of fluorescent proteins by RABVs will clearly extend their utility in physical mapping of neuronal pathways, expression of markers other than eGFP, such as sensors of neural activity or photosensitive ion channels, presents the possibility of using these recombinant viruses for physiological studies of neuronal function, thereby opening up new avenues of investigation.

B. Use of RABV proteins for molecular targeting

RABV proteins and modular functional domains derived from them have been used to target the delivery of molecules to specific cells or sites within a cell. Replacement of endogenous envelope proteins of retrovirus-derived vectors with RABV glycoprotein (''pseudotyping'') alters their cellular tropism, permitting the infection of neuronal cells *in vitro* and *in vivo* (Azzouz *et al.*, 2004; Mazarakis *et al.*, 2001; Mochizuki *et al.*, 1998; Parveen *et al.*, 2003; Teng *et al.*, 2005). The utility of these pseudotyped virus vectors for transgene delivery to neurons *in vivo* has been investigated by incorporating genes encoding fluorescent proteins or β-galactosidase into the vectors, and determining the number and distribution of labeled cells (Mentis *et al.*, 2006; Parveen *et al.*, 2003). Transduction of neurons was observed following injection of the vectors into the CNS of rats and mice, as well as into muscles, indicating that the RABV glycoprotein facilitated retrograde transneuronal transport of the pseudotyped viruses. In addition, neurotrophic factors such as insulin-like growth factor I and the *survival motor neuron* gene product were efficiently delivered to and expressed in motor neurons *in vitro* (Teng *et al.*, 2005) and *in vivo* (Azzouz *et al.*, 2004). Targeting of genes to specific cell types will be critical to the development of gene therapies for treatment of degenerative diseases of the CNS and other neurological disorders.

While full-length RABV glycoprotein targets pseudotyped viruses to neurons, delivery of small interfering RNA (siRNA) to neurons could be achieved using a synthetic peptide corresponding to the acetylcholine receptor binding site (ARBS) of the RABV glycoprotein extracellular domain (Kumar *et al.*, 2007). Delivery of siRNA was achieved following both intracranial and intravenous administration of the ARBS-siRNA complexes, indicating that the complexes could be transported across the blood brain barrier. Furthermore, the siRNA was functional; expression of GFP in GFP transgenic mice, and endogenously expressed Cu–Zn superoxide dismutase, were both silenced by appropriate siRNAs and intravenous administration of an ARBS-siRNA targeting Japanese encephalitis virus was protective against fatal encephalitis in a mouse model of infection. While the mechanisms of ARBS peptide transport across the brain endothelium and detachment of the siRNA from the peptide in the cytoplasm remain to be delineated, use of the ARBS peptide for delivery of siRNA, and other small molecular weight therapeutics, to the CNS in a minimally invasive fashion appears most promising.

Other functional domains of RABV proteins have been used to change the cellular distribution of recombinant proteins. Expression cassettes, in which cDNA for a protein of interest is fused in-frame between cDNA

encoding the signal sequence and the transmembrane and cytoplasmic domains of the RABV G protein, direct production of recombinant proteins that are targeted to and remain anchored within the cellular membrane (Gupta *et al.*, 1998; Klingen *et al.*, 2008). This approach produced a membrane-anchored form of the β subunit of human chorionic gonadotropin (β (h)CG) that retained the antigenic epitopes of its secreted form (Gupta *et al.*, 1998). Similarly, a red fluorescent protein (RFP) was efficiently targeted to the plasma membrane in cells transfected with an RFP-glycoprotein construct (Klingen *et al.*, 2008). Nuclear accumulation of various chimeric proteins, mediated by a NLS from RABV or other viruses, was enhanced by the presence of the RABV P protein LC8 BD (Moseley *et al.*, 2007b) a finding that may provide for improved nuclear targeting of therapeutics.

VIII. CONCLUDING REMARKS

Despite the many advances in our understanding of rabies–host interactions, and the ability to engineer RABV to produce novel constructs useful for many applications, clinical rabies in humans remains virtually 100% fatal. Even if current approaches to eliminate rabies from known carnivore reservoirs were eventually successful, the occasional cryptic infection of humans from chiropteran reservoirs (Messenger *et al.*, 2002), which are generally not targeted by any comprehensive control strategies at present, will continue to drive the need for effective therapy. Indeed, much excitement surrounded the recovery of a patient from Wisconsin who survived clinical rabies without ever receiving antirabies biologics. The therapy applied in this case involved induction of coma and provision of antiviral drugs and agents to combat neuronal damage while the patient developed an adaptive immune response (Willoughby *et al.*, 2005). However, several attempts to replicate this success failed (Christenson *et al.*, 2007; Hemachudha *et al.*, 2006; Schmiedel *et al.*, 2007), perhaps either due to differences in pathogenicity of the RABV strains involved or variations in patient care, particularly prior to the diagnosis of rabies. To establish the merit of this approach, further experimentation using various animal and nonhuman primate models may be required. In a mouse model opening of the blood–brain barrier to allow infiltration of immune effectors appears to be a critical feature for preventing a lethal rabies outcome (Roy and Hooper, 2007; Roy *et al.*, 2007). As well, future development of novel antiviral therapies (Real *et al.*, 2004) may greatly improve the odds of surviving this most lethal of diseases.

REFERENCES

Adams, J. M., and Cory, S. (1998). The Bcl-2 protein family: Arbiters of cell survival. *Science* **281:**1322–1326.

Akaike, T., Weihe, E., Schaefer, M., Fu, Z. F., Zheng, Y. M., Vogel, W., Schmidt, H., Koprowski, H., and Dietzschold, B. (1995). Effect of neurotropic virus infection on neuronal and inducible nitric oxide synthase activity in rat brain. *J. Neurovirol.* **1:**118–125.

Akkal, D., Dum, R. P., and Strick, P. L. (2007). Supplementary motor area and presupplementary motor area: Targets of basal ganglia and cerebellar output. *J. Neurosci.* **27:**10659–10673.

Aktan, F. (2004). INOS-mediated nitric oxide production and its regulation. *Life Sci.* **75:**639–653.

Albertini, A. A. V., Schoehn, G., Weissenhorn, W., and Ruigrok, R. W. H. (2008). Structural aspects of rabies virus replication. *Cell Mol. Life Sci.* **65:**282–294.

Astic, L., Saucier, D., Coulon, P., Lafay, F., and Flamand, A. (1993). The CVS strain of rabies virus as transneuronal tracer in the olfactory system of mice. *Brain Res.* **619:**146–156.

Azzouz, M., Le, T., Ralph, G. S., Walmsley, L., Monani, U. R., Lee, D. C. P., Wilkes, F., Mitrophanous, K. A., Kingsman, S. M., Burghes, A. H. M., and Masarakis, N. D. (2004). Lentivector-mediated *SMN* replacement in a mouse model of spinal muscular atrophy. *J. Clin. Invest.* **114:**1726–1731.

Badrane, H., and Tordo, N. (2001). Host switching in Lyssavirus history from the chiroptera to the carnivora orders. *J. Virol.* **75:**8096–8104.

Baer, G. M. (2007). The history of rabies. *In* ''Rabies'' (A. C. Jackson and W. H. Wunner, eds.), 2nd Ed. pp. 1–22. Academic Press, San Diego.

Bahloul, C., Ahmed, S. B. H., B'chir, B. I., Kharmachi, H., Hayouni, E. A., and Dellagi, K. (2003). Post-exposure therapy in mice against experimental rabies: A single injection of DNA vaccine is as effective as five injections of cell culture-derived vaccine. *Vaccine* **22:**177–184.

Bahloul, C., Taieb, D., Diouani, M. F., Ahmed, S. B. H., Chtourou, Y., B'chir, B. I., Kharmachi, H., and Dellagi, K. (2006). Field trials of a very potent rabies DNA vaccine which induced long lasting virus neutralizing antibodies and protection in dogs in experimental conditions. *Vaccine* **24:**1063–1072.

Bakker, A. B. H., Marissen, W. E., Kramer, R. A., Rice, A. B., Weldon, W. C., Niezgoda, M., Hanlon, C. A., Thijsse, S., Backus, H. H. J., de Kruif, J., Dietzschold, B., Rupprecht, C. E., *et al.* (2005). Novel human monoclonal antibody combination effectively neutralizing natural rabies virus variants and individual *in vitro* escape mutants. *J. Virol.* **79:**9062–9068.

Baloul, L., Camelo, S., and Lafon, M. (2004). Up-regulation of Fas ligand (FasL) in the central nervous system: A mechanism of immune evasion by rabies virus. *J. Neurovirol.* **10:**372–382.

Biek, R., Henderson, J. C., Waller, L. A., Rupprecht, C. E., and Real, L. A. (2007). A high-resolution genetic signature of demographic and spatial expansion in epizootic rabies virus. *Proc. Natl. Acad. Sci. USA* **104:**7993–7998.

Biswas, S., Kalanidhi, A. P., Ashok, M. S., Reddy, G. S., Srinivasan, V. A., and Rangarajan, P. N. (2001). Evaluation of rabies virus neutralizing antibody titres induced by intramuscular inoculation of rabies DNA vaccine in mice and Bonnet monkeys (Macaca radiata). *Indian J. Exp. Biol.* **39:**533–536.

Blanton, J. D., Meadows, A., Murphy, S. M., Manangan, J., Hanlon, C. A., Faber, M.-L., Dietzschold, B., and Rupprecht, C. E. (2006). Vaccination of small Asian mongoose (*Herpestes javanicus*) against rabies. *J. Wildl. Dis.* **42:**663–666.

Blanton, J. D., Self, J., Niezgoda, M., Faber, M.-L., Dietzschold, B., and Rupprecht, C. (2007). Oral vaccination of raccoons (*Procyon lotor*) with genetically modified rabies virus vaccines. *Vaccine* **25:**7296–7300.

Blondel, D., Regad, T., Poisson, N., Pavie, B., Harper, F., Pandolfi, P. P., de Thé, H., and Chelbi-Alix, M. K. (2002). Rabies virus P and small P products interact directly with PML and reorganize PML nuclear bodies. *Oncogene* **21:**7957–7970.

Brochier, B., Kieny, M. P., Costy, F., Coppens, P., Bauduin, B., Lecocq, J. P., Languet, B., Chappuis, G., Desmettre, P., Afiademanyo, K., Libois, R., and Pastoret, P.-P. (1991). Large scale eradication of rabies using recombinant vaccinia-rabies vaccine. *Nature* **354:**520–522.

Brzózka, K., Finke, S., and Conzelmann, K.-K. (2005). Identification of the rabies virus alpha/beta interferon antagonist: Phosphoprotein P interferes with phosphorylation of interferon regulatory factor 3. *J. Virol.* **79:**7673–7681.

Brzózka, K., Finke, S., and Conzelmann, K.-K. (2006). Inhibition of interferon signaling by rabies virus phosphoprotein P: Activation-dependent binding of STAT1 and STAT2. *J. Virol.* **80:**2675–2683.

Buchholz, U. J., Finke, S., and Conzelmann, K.-K. (1999). Generation of bovine respiratory syncytial virus (BRSV) from cDNA: BRSV NS2 is not essential for virus replication in tissue culture, and the human RSV leader region acts as a functional BRSV genome promoter. *J. Virol.* **73:**251–259.

Camelo, S., Castellanos, J., Lafage, M., and Lafon, M. (2001). Rabies virus ocular disease: T-cell–dependent protection is under the control of signaling by the p55 tumor necrosis factor alpha receptor, p55TNFR. *J. Virol.* **75:**3427–3434.

Champion, J. M., Kean, R. B., Rupprecht, C. E., Notkins, A. L., Koprowski, H., Dietzschold, B., and Hooper, D. C. (2000). The development of monoclonal human rabies virus-neutralizing antibodies as a substitute for pooled human immune globulin in the prophylactic treatment of rabies virus exposure. *J. Immunol. Methods* **235:**81–90.

Chelbi-Alix, M. K., Vidy, A., Bougrini, J. E., and Blondel, D. (2006). Rabies viral mechanisms to escape the IFN system: The viral protein P interferes with the IRF-3, Stat1 and PML nuclear bodies. *J. Interferon Cytokine Res.* **26:**271–280.

Chenik, M., Chebli, K., and Blondel, D. (1995). Translation initiation at alternate in-frame AUG codons in the rabies virus phosphoprotein mRNA is mediated by a ribosomal leaky scanning mechanism. *J. Virol.* **69:**707–712.

Childs, J. E., Curns, A. T., Dey, M. E., Real, L. A., Feinstein, L., Bjørnstad, O. N., and Krebs, J. W. (2000). Predicting the local dynamics of epizootic rabies among raccoons in the United States. *Proc. Natl. Acad. Sci. USA* **97:**13666–13671.

Christenson, J. C., Holm, B. M., Lechlitner, S., Howell, J. F., Wenger, M., Roy-Burman, A., Hsieh, C. J., LaBar, L., Petru, A., Davis, S., Simons, N., Apolinario, P., *et al.* (2007). Human rabies-Indiana and California, 2006. *Morb. Mortal. Wkly. Rep.* **56:**361–365.

Clower, D. M., Dum, R. P., and Strick, P. L. (2005). Basal ganglia and cerbellar inputs to 'AIP'. *Cereb. Cortex* **15:**913–920.

Coulon, P., Derbin, C., Kucera, P., Lafay, F., Prehaud, C., and Flamand, A. (1989). Invasion of the peripheral nervous systems of adult mice by the CVS strain of rabies virus and its avirulent derivative AvO1. *J. Virol.* **63:**3550–3554.

Cullingham, C. I., Kyle, C. J., and White, B. N. (2005). An investigation of raccoon subspecies designations and their association with differences in population response to the rabies virus. ''The XVI International Conference on Rabies in the Americas'', Ottawa, Canada, p. 69.

Davis, P. L., Holmes, E. C., Larrous, F., Van der Poel, W. H. M., Tjørnehøj, K., Alonso, W. J., and Bourhy, H. (2005). Phylogeography, population dynamics, and molecular evolution of European bat lyssaviruses. *J. Virol.* **79:**10487–10497.

Davis, P. L., Bourhy, H., and Holmes, E. C. (2006). The evolutionary history and dynamics of bat rabies virus. *Infect. Genet. Evol.* **6:**464–473.

Davis, P. L., Rambaut, A., Bourhy, H., and Holmes, E. C. (2007). The evolutionary dynamics of canid and mongoose rabies virus in southern Africa. *Arch. Virol.* **152:**1251–1258.

Dhingra, V., Li, X., Liu, Y., and Fu, Z. F. (2007). Proteomic profiling reveals that rabies virus infection results in differential expression of host proteins involved in ion homeostasis and synaptic physiology in the central nervous system. *J. Neurovirol.* **13:**107–117.

Dietzschold, M.-L., Faber, M., Mattis, J. A., Pak, K. Y., Schnell, M. J., and Dietzschold, B. (2004). *In vitro* growth and stability of recombinant rabies viruses designed for vaccination of wildlife. *Vaccine* **23:**518–524.

Echevarría, J. E., Avellón, A., Juste, J., Vera, M., and Ibáñez, C. (2001). Screening of active lyssavirus infection in wild bat populations by viral RNA detection on oropharyngeal swabs. *J. Clin. Microbiol.* **39:**3678–3683.

Ertl, H. C. (2003). Rabies DNA vaccines for protection and therapeutic treatment. *Expert Opin. Biol. Ther.* **3:**639–644.

Etessami, R., Conzelmann, K.-K., Fadai-Ghotbi, B., Natelson, B., Tsiang, H., and Ceccaldi, P.-E. (2000). Spread and pathogenic characteristics of a G-deficient rabies virus recombinant: An *in vitro* and *in vivo* study. *J. Gen. Virol.* **81:**2147–2153.

Faber, M., Pulmanausahakul, R., Hodawadekar, S. S., Spitsin, S., McGettigan, J. P., Schnell, M. J., and Dietzschold, B. (2002). Overexpression of the rabies virus glycoprotein results in enhancement of apoptosis and antiviral immune response. *J. Virol.* **76:**3374–3381.

Faber, M., Pulmanausahakul, R., Nagao, K., Prosniak, M., Rice, A. B., Koprowski, H., Schnell, M. J., and Dietzschold, B. (2004). Identification of viral genomic elements responsible for rabies virus neuroinvasiveness. *Proc. Natl. Acad. Sci. USA* **101:**16328–16332.

Faber, M., Bette, M., Preuss, M. A. R., Pulmanausahakul, R., Rehnelt, J., Schnell, M. J., Dietzschold, B., and Weihe, E. (2005a). Overexpression of tumor necrosis factor alpha by a recombinant rabies virus attenuates replication in neurons and prevents lethal infection in mice. *J. Virol.* **79:**15405–15416.

Faber, M., Faber, M.-L., Papaneri, A., Bette, M., Weihe, E., Dietzschold, B., and Schnell, M. J. (2005b). A single amino acid change in rabies virus glycoprotein increases virus spread and enhances virus pathogenicity. *J. Virol.* **79:**14141–14148.

Faber, M., Lamirande, E. W., Roberts, A., Rice, A. B., Koprowski, H., Dietzschold, B., and Schnell, M. J. (2005c). A single immunization with a rhabdovirus-based vector expressing severe acute respiratory syndrome coronavirus (SARS-CoV) S protein results in the production of high levels of SARS-CoV-neutralizing antibodies. *J. Gen. Virol.* **86:**1435–1440.

Faber, M., Faber, M.-L., Li, J., Preuss, M. A. R., Schnell, M. J., and Dietzschold, B. (2007). Dominance of a nonpathogenic glycoprotein gene over a pathogenic glycoprotein gene in rabies virus. *J. Virol.* **81:**7041–7047.

Finke, S., and Conzelmann, K. K. (1997). Ambisense gene expression from recombinant rabies virus: Random packaging of positive- and negative-strand ribonucleoprotein complexes into rabies virions. *J. Virol.* **71:**7281–7288.

Finke, S., and Conzelmann, K. K. (1999). Virus promoters determine interference by defective RNAs: Selective amplification of mini-RNA vectors and rescue from cDNA by a 3′ copy-back ambisense rabies virus. *J. Virol.* **73:**3818–3825.

Finke, S., Cox, J. H., and Conzelmann, K. K. (2000). Differential transcription attenuation of rabies virus genes by intergenic regions: Generation of recombinant viruses overexpressing the polymerase gene. *J. Virol.* **74:**7261–7269.

Finke, S., Brzózka, K., and Conzelmann, K. K. (2004). Tracking fluorescence-labeled rabies virus: Enhanced green fluorescent protein-tagged phosphoprotein P supports virus gene expression and formation of infectious particles. *J. Virol.* **78:**12333–12343.

Fischer, L., Minke, J., Dufay, N., Baudu, P., and Audonnet, J.-C. (2003). Rabies DNA vaccine in the horse: Strategies to improve serological responses. *Vaccine* **21:**4593–4596.

Foley, H. D., McGettigan, J. P., Siler, C. A., Dietzschold, B., and Schnell, M. J. (2000). A recombinant rabies virus expressing vesicular stomatitis virus glycoprotein fails to protect against rabies virus infection. *Proc. Natl. Acad. Sci. USA* **97:**14680–14685.

Foley, H. D., Otero, M., Orenstein, J. M., Pomerantz, R. J., and Schnell, M. J. (2002). Rhabdovirus-based vectors with human immunodeficiency virus type 1 (HIV-1) envelopes display HIV-1-like tropism and target human dendritic cells. *J. Virol.* **76:**19–31.

Foord, A. J., Heine, H. G., Pritchard, L. I., Lunt, R. A., Newberry, K. M., Rootes, C. L., and Boyle, D. B. (2006). Molecular diagnosis of lyssaviruses and sequence comparison of Australian bat lyssavirus samples. *Aust. Vet. J.* **84:**225–230.

Franka, R., Constantine, D. G., Kuzmin, I., Velasco-Villa, A., Reeder, S. A., Streicker, D., Orciari, L. A., Wong, A. J., Blanton, J. D., and Rupprecht, C. E. (2006). A new phylogenetic lineage of rabies virus associated with western pipistrelle bats (*Pipistrellus hesperus*). *J. Gen. Virol.* **87:**2309–2321.

Fu, Z. F., and Jackson, A. C. (2005). Neuronal dysfunction and death in rabies virus infection. *J. Neurovirol.* **11:**101–106.

Gastka, M., Horvath, J., and Lentz, T. L. (1996). Rabies virus binding to the nicotinic acetylcholine receptor α subunit demonstrated by virus overlay protein binding assay. *J. Gen. Virol.* **77:**2437–2440.

Gaytán, S. P., Pásaro, R., Coulon, P., Bevengut, M., and Hilaire, G. (2002). Identification of central nervous system neurons innervating the respiratory muscles of the mouse: A transneuronal tracing study. *Brain Res. Bull.* **57:**335–339.

Goudsmit, J., Marissen, W. E., Weldon, W. C., Niezgoda, M., Hanlon, C. A., Rice, A. B., de Kruif, J., Dietzschold, B., Bakker, A. B. H., and Rupprecht, C. E. (2006). Comparison of an anti-rabies human monoclonal antibody combination with human polyclonal anti-rabies immune globulin. *J. Infect. Dis.* **193:**796–801.

Graf, W., Gerrits, N., Yatim-Dhiba, N., and Ugolini, G. (2002). Mapping the oculomotor system: The power of transneuronal labelling with rabies virus. *Eur. J. Neurosci.* **15:**1557–1562.

Gupta, A., Chandrasekhar, S., Pal, R., Talwar, G. P., and Singh, O. M. (1998). Identification of novel transmembrane gene sequence and its use for cell-surface targeting of beta subunit of human chorionic gonadotropin. *DNA Cell Biol.* **17:**573–581.

Gupta, A. K., Blondel, D., Choudhary, S., and Banerjee, A. K. (2000). The phosphoprotein of rabies virus is phosphorylated by a unique cellular protein kinase and specific isomers of protein kinase C. *J. Virol.* **74:**91–98.

Haller, O., Kochs, G., and Weber, F. (2006). The interferon response circuit: Induction and suppression by pathogenic viruses. *Virology* **344:**119–130.

Hanlon, C. A., Kuzmin, I. V., Blanton, J. D., Weldon, W. C., Manangan, J. S., and Rupprecht, C. E. (2005). Efficacy of rabies biologics against new lyssaviruses from Eurasia. *Virus Res.* **111:**44–54.

Hemachudha, T., Sunsaneewitayakul, B., Desudchit, T., Suankratay, C., Sittipunt, C., Wacharapluesadee, S., Khawplod, P, Wilde, H, and Jackson, A. C. (2006). Failure of therapeutic coma and ketamine for therapy of human rabies. *J. Neurovirol.* **12:**407–409.

Holmes, E. C., Woelk, C. H., Kassis, R., and Bourhy, H. (2002). Genetic constraints and the adaptive evolution of rabies virus in nature. *Virology* **292:**247–257.

Hoshi, E., Tremblay, L., Féger, J., Carras, P. L., and Strick, P. L. (2005). The cerebellum communicates with the basal ganglia. *Nat. Neurosci.* **8:**1491–1493.

Hosokawa-Muto, J., Ito, N., Yamada, K., Shimizu, K., Sugiyama, M., and Minamoto, N. (2006). Characterization of recombinant rabies virus carrying double glycoprotein genes. *Microbiol. Immunol.* **50:**187–196.

Hu, R., Zhang, S., Fooks, A. R., Yuan, H., Liu, Y., Li, H., Tu, C., Xia, X., and Xiao, Y. (2006). Prevention of rabies virus infection in dogs by a recombinant canine adenovirus type-2 encoding the rabies virus glycoprotein. *Microbes Infect.* **8:**1090–1097.

Hu, R. L., Liu, Y., Zhang, S. F., Zhang, F., and Fooks, A. R. (2007). Experimental immunization of cats with a recombinant rabies-canine adenovirus vaccine elicits a long-lasting neutralizing antibody response against rabies. *Vaccine* **25:**5301–5307.

Hughes, G. J., Páez, A., Bóshell, J., and Rupprecht, C. E. (2004a). A phylogenetic reconstruction of the epidemiological history of canine rabies virus variants in Colombia. *Infect. Genet. Evol.* **4:**45–51.

Hughes, G. J., Smith, J. S., Hanlon, C. A., and Rupprecht, C. E. (2004b). Evaluation of a TaqMan PCR assay to detect rabies virus RNA: Influence of sequence variation and application to quantification of viral loads. *J. Clin. Microbiol.* **42:**299–306.

Hughes, G. J., Orciari, L. A., and Rupprecht, C. E. (2005). Evolutionary timescale of rabies virus adaptation to North American bats inferred from the substitution rate of the nucleoprotein gene. *J. Gen. Virol.* **86:**1467–1474.

Inase, M., Tokuno, H., Nambu, A., Akazawa, T., and Takada, M. (1999). Corticostriatal and corticosubthalamic input zones from the presupplementary motor area in the macaque monkey: Comparison with the input zones from the supplementary motor area. *Brain Res.* **833:**191–201.

Inoue, K., Shoji, Y., Kurane, I., Iijima, T., Sakai, T., and Morimoto, K. (2003). An improved method for recovering rabies virus from cloned cDNA. *J. Virol. Methods* **107:**229–236.

Ito, N., Takayama, M., Yamada, K., Sugiyama, M., and Minamoto, N. (2001). Rescue of rabies virus from cloned cDNA and identification of the pathogenicity-related gene: Glycoprotein gene is associated with virulence for adult mice. *J. Virol.* **75:**9121–9128.

Ito, N., Takayama-Ito, M., Yamada, K., Hosokawa, J., Sugiyama, M., and Minamoto, N. (2003). Improved recovery of rabies virus from cloned cDNA using a vaccinia virus-free reverse genetics system. *Microbiol. Immunol.* **47:**613–617.

Ito, N., Sugiyama, M., Yamada, K., Shimizu, K., Takayama-Ito, M., Hosokawa, J., and Minamoto, N. (2005). Characterization of M gene-deficient rabies virus with advantages of effective immunization and safety as a vaccine strain. *Microbiol. Immunol.* **49:**971–979.

Jackson, A. C. (1999). Apoptosis in experimental rabies in *bax*-deficient mice. *Acta Neuropathol.* **98:**288–294.

Jackson, A. C. (2004). Screening of organ and tissue donors for rabies. *Lancet* **364:**2094–2095.

Jackson, A. C. (2007). Pathogenesis. *In* ''Rabies'' (A. C. Jackson and W. H. Wunner, eds.), 2nd Ed. pp. 341–381. Academic Press, San Diego.

Jackson, A. C., and Park, H. (1998). Apoptotic cell death in experimental rabies in suckling mice. *Acta Neuropathol.* **95:**159–164.

Jackson, A. C., and Park, H. (1999). Experimental rabies virus infection of p75 neurotrophin receptor-deficient mice. *Acta Neuropathol.* **98:**641–644.

Jackson, A. C., and Rossiter, J. P. (1997). Apoptosis plays an important role in experimental rabies virus infection. *J. Virol.* **71:**5603–5607.

Jackson, A. C., Rossiter, J. P., and Lafon, M. (2006). Expression of Toll-like receptor 3 in the human cerebellar cortex in rabies, herpes simplex encephalitis, and other neurological diseases. *J. Neurovirol.* **12:**229–234.

Jacob, Y., Badrane, H., Ceccaldi, P.-E., and Tordo, N. (2000). Cytoplasmic dynein LC8 interacts with lyssavirus phosphoprotein. *J. Virol.* **74:**10217–10222.

Jallet, C., Jacob, Y., Bahloul, C., Drings, A., Desmezieres, E., Tordo, N., and Perrin, P. (1999). Chimeric lyssavirus glycoproteins with increased immunological potential. *J. Virol.* **73:**225–233.

Jenkins, G. M., Rambaut, A., Pybus, O. G., and Holmes, E. C. (2002). Rates of molecular evolution in RNA viruses: A quantitative phylogenetic analysis. *J. Mol. Evol.* **54:**156–165.

Johnson, N., McKimmie, C. S., Mansfield, K. L., Wakeley, P. R., Brookes, S. M., Fazakerley, J. K., and Fooks, A. R. (2006). Lyssavirus infection activates interferon gene expression in the brain. *J. Gen. Virol.* **87:**2663–2667.

Kang, B., Oh, J., Lee, C., Park, B.-K., Park, Y., Hong, K., Lee, K., Cho, B., and Song, D. (2007). Evaluation of a rapid immunodiagnostic test kit for rabies virus. *J. Virol. Methods* **145:**30–36.

Kassis, R., Larrous, F., Estaquier, J., and Bourhy, H. (2004). Lyssavirus matrix protein induces apoptosis by a TRAIL-dependent mechanism involving caspase-8 activation. *J. Virol.* **78:**6543–6555.

Kelly, R. M., and Strick, P. L. (2000). Rabies as a transneuronal tracer of circuits in the central nervous system. *J. Neurosci. Methods* **103:**63–71.

Kelly, R. M., and Strick, P. L. (2003). Cerebellar loops with motor cortex and prefrontal cortex of a nonhuman primate. *J. Neurosci.* **23:**8432–8444.

Kelly, R. M., and Strick, P. L. (2004). Macro-architecture of basal ganglia loops with the cerebral cortex: Use of rabies virus to reveal multisynaptic circuits. *Prog. Brain Res.* **143:**449–459.

Kinkade, A., and Ware, C. F. (2006). The DARC conspiracy—virus invasion tactics. *Trends Immunol.* **27:**362–367.

Klingen, Y., Conzelmann, K. K., and Finke, S. (2008). Double-labeled rabies virus—live tracking of enveloped virus transport. *J. Virol.* **82:**237–245.

Ko, K., Tekoah, Y., Rudd, P. M., Harvey, D. J., Dwek, R. A., Spitsin, S., Hanlon, C. A., Rupprecht, C. E., Dietzschold, B., Golovkin, M., and Koprowski, H. (2003). Function and glycosylation of plant-derived antiviral monoclonal antibody. *Proc. Natl. Acad. Sci. USA* **100:**8013–8018.

Kobayashi, Y., Sato, G., Shoji, Y., Sato, T., Itou, T., Cunha, E. M. S., Samara, S. I., Carvalho, A. A. B., Nociti, D. P., Ito, F. H., and Sakai, T. (2005). Molecular epidemiological analysis of bat rabies viruses in Brazil. *J. Vet. Med. Sci.* **67:**647–652.

Köbbert, C., Apps, R., Bechmann, I., Lanciego, J. L., Mey, J., and Thanos, S. (2000). Current concepts in neuroanatomical tracing. *Prog. Neurobiol.* **62:**327–351.

Komarova, A. V., Real, E., Borman, A. M., Brocard, M., England, P., Tordo, N., Hershey, J. W. B., Kean, K. M., and Jacob, Y. (2007). Rabies virus matrix protein interplay with eIF3, new insights into rabies virus pathogenesis. *Nucleic Acids Res.* **35:**1522–1532.

Koser, M. L., McGettigan, J. P., Tan, G. S., Smith, M. E., Koprowski, H., Dietzschold, B., and Schnell, M. J. (2004). Rabies virus nucleoprotein as a carrier for foreign antigens. *Proc. Natl. Acad. Sci. USA* **101:**9405–9410.

Kucera, P., Dolivo, M., Coulon, P., and Flamand, A. (1985). Pathways of the early propagation of virulent and avirulent rabies strains from the eye to the brain. *J. Virol.* **55:**158–162.

Kumar, P., Wu, H., McBride, J. L., Jung, K.-E., Kim, M. H., Davidson, B. L., Lee, S. K., Shankar, P., and Manjunath, N. (2007). Transvascular delivery of small interfering RNA to the central nervous system. *Nature* **448:**39–43.

Kuzmin, I. V., Hughes, G. J., Botvinkin, A. D., Orciari, L. A., and Rupprecht, C. E. (2005). Phylogenetic relationships of Irkut and West Caucasian bat viruses within the *Lyssavirus* genus and suggested quantitative criteria based on the N gene sequence for lyssavirus genotype definition. *Virus Res.* **111:**28–43.

Kuzmin, I. V., Hughes, G. J., and Rupprecht, C. E. (2006a). Phylogenetic relationships of seven previously unclassified viruses within the family *Rhabdoviridae* using partial nucleoprotein gene sequences. *J. Gen. Virol.* **87:**2323–2331.

Kuzmin, I. V., Niezgoda, M., Carroll, D. S., Keeler, N., Hossain, M. J., Breiman, R. F., Ksiazek, T. G., and Rupprecht, C. E. (2006b). Lyssavirus surveillance in bats, Bangladesh. *Emerg. Infect. Dis.* **12:**486–488.

Kuzmin, I. V., Hughes, G. J., Botvinkin, A. D., Gribencha, S. G., and Rupprecht, C. E. (2008). Arctic and arctic-like rabies viruses: Distribution, phylogeny and evolutionary history. *Epidemiol. Infect.* **136:**509–519.

Lafay, F., Coulon, P., Astic, L., Saucier, D., Riche, D., Holley, A., and Flamand, A. (1991). Spread of the CVS strain of rabies virus and of the avirulent mutant AvO1 along the olfactory pathways of the mouse after intranasal inoculation. *Virology* **183:**320–330.

Langevin, C., and Tuffereau, C. (2002). Mutations conferring resistance to neutralization by a soluble form of the neurotrophin receptor (p75NTR) map outside of the known antigenic sites of the rabies virus glycoprotein. *J. Virol.* **76:**10756–10765.

Langevin, C., Jaaro, H., Bressanelli, S., Fainzilber, M., and Tuffereau, C. (2002). Rabies virus glycoprotein (RVG) is a trimeric ligand for the N-terminal cysteine-rich domain of the mammalian p75 neurotrophin receptor. *J. Biol. Chem.* **277:**37655–37662.

Lembo, T., Niezgoda, M., Velasco-Villa, A., Cleaveland, S., Ernest, E., and Rupprecht, C. E. (2006). Evaluation of a direct, rapid immunohistochemical test for rabies diagnosis. *Emerg. Infect. Dis.* **12:**310–313.

Le Mercier, P., Jacob, Y., Tanner, K., and Tordo, N. (2002). A novel expression cassette of lyssavirus shows that the distantly related Mokola virus can rescue a defective rabies virus genome. *J. Virol.* **76:**2024–2027.

Leslie, M. J., Messenger, S., Rohde, R. E., Smith, J., Cheshier, R., Hanlon, C., and Rupprecht, C. E. (2006). Bat-associated rabies virus in skunks. *Emerg. Infect. Dis.* **12:**1274–1277.

Li, X.-Q., Sarmento, L., and Fu, Z. F. (2005). Degeneration of neuronal processes after infection with pathogenic, but not attenuated, rabies viruses. *J. Virol.* **79:**10063–10068.

Li, J., Faber, M., Papaneri, A., Faber, M.-L., McGettigan, J. P., Schnell, M. J., and Dietzschold, B. (2006). A single immunization with a recombinant canine adenovirus expressing the rabies virus G protein confers protective immunity against rabies in mice. *Virology* **356:**147–154.

Li, J., McGettigan, J. P., Faber, M., Schnell, M. J., and Dietzschold, B. (2008). Infection of monocytes or immature dendritic cells (DCs) with an attenuated rabies virus results in DC maturation and a strong activation of the NFκB signaling pathway. *Vaccine* **26:**419–426.

Lodmell, D. L., Ray, N. B., Parnell, M. J., Ewalt, L. C., Hanlon, C. A., Shaddock, J. H., Sanderlin, D. S., and Rupprecht, C. E. (1998). DNA immunization protects nonhuman primates against rabies virus. *Nat. Med.* **4:**949–952.

Lodmell, D. L., Parnell, M. J., Bailey, J. R., Ewalt, L. C., and Hanlon, C. A. (2002a). One-time gene gun or intramuscular rabies DNA vaccination of non-human primates: Comparison of neutralizing antibody responses and protection against rabies virus 1 year after vaccination. *Vaccine* **20:**838–844.

Lodmell, D. L., Parnell, M. J., Bailey, J. R., Ewalt, L. C., and Hanlon, C. A. (2002b). Rabies DNA vaccination of non-human primates: Post-exposure studies using gene gun methodology that accelerates induction of neutralizing antibody and enhances neutralizing antibody titers. *Vaccine* **20:**2221–2228.

Lodmell, D. L., Parnell, M. J., Weyhrich, J. T., and Ewalt, L. C. (2003). Canine rabies DNA vaccination: A single-dose intradermal injection into ear pinnae elicits elevated and persistent levels of neutralizing antibody. *Vaccine* **21:**3998–4002.

Lodmell, D. L., Ewalt, L. C., Parnell, M. J., Rupprecht, C. E., and Hanlon, C. A. (2006). One-time intradermal DNA vaccination in ear pinnae one year prior to infection protects dogs against rabies virus. *Vaccine* **24:**412–416.

Loewy, A. D. (1998). Viruses as transneuronal tracers for defining neural circuits. *Neurosci. Biobehav. Rev.* **22:**679–684.

Lu, X., Miyachi, S., Ito, Y., Nambu, A., and Takada, M. (2007). Topographic distribution of output neurons in cerebellar nuclei and cortex to somatotropic map of primary motor cortex. *Eur. J. Neurosci.* **25:**2374–2382.

Marissen, W. E., Kramer, R. A., Rice, A., Weldon, W. C., Niezgoda, M., Faber, M., Slootstra, J. W., Meloen, R. H., Clijsters-van der Horst, M., Visser, T. J., Jongeneelen, M., Thijsse, S., *et al.* (2005). Novel rabies virus-neutralizing epitope recognized by human monoclonal antibody: Fine mapping and escape mutant analysis. *J. Virol.* **79:**4672–4678.

Mazarakis, N. D., Azzouz, M., Rohll, J. B., Ellard, F. M., Wilkes, F. J., Olsen, A. L., Carter, E. E., Barber, R. D., Baban, D. F., Kingsman, S. M., Kingsman, A. J., O'Malley, K., *et al.* (2001). Rabies virus glycoprotein pseudotyping on lentiviral vectors enables retrograde axonal transport and access to the nervous system after peripheral delivery. *Hum. Mol. Genet.* **10:**2109–2121.

McCoy, C. E., and O'Neill, L. A. J. (2008). The role of toll-like receptors in macrophages. *Front. Biosci.* **13:**62–70.

McGettigan, J. P., Foley, H. D., Belyakov, I. M., Berzofsky, J. A., Pomerantz, R. J., and Schnell, M. J. (2001a). Rabies virus based vectors expressing human immunodeficiency virus Type 1 (HIV-1) envelope protein induce a strong, cross-reactive cytotoxic T-lymphocyte response against envelope proteins from different HIV-1 isolates. *J. Virol.* **75:**4430–4434.

McGettigan, J. P., Sarma, S., Orenstein, J. M., Pomerantz, R. J., and Schnell, M. J. (2001b). Expression and immunogenicity of human immunodeficiency virus type 1 gag expressed by a replication-competent rhabdovirus-based vaccine vector. *J. Virol.* **75:**8724–8732.

McGettigan, J. P., Pomerantz, R. J., Siler, C. A., McKenna, P. M., Foley, H. D., Dietzschold, B., and Schnell, M. J. (2003a). Second-generation rabies virus-based vaccine vectors expressing human immunodeficiency virus Type 1 Gag have greatly reduced pathogenicity but are highly immunogenic. *J. Virol.* **77:**237–244.

McGettigan, J. P., Naper, K., Orenstein, J., Koser, M., McKenna, P. M., and Schnell, M. J. (2003b). Functional human immunodeficiency virus type 1(HIV-1) Gag-Pol or HIV-1 Gag-Pol and Env expressed from a single Rhabdovirus-based vaccine vector genome. *J. Virol.* **77:**10889–10899.

McGettigan, J. P., Koser, M. L., McKenna, P. M., Smith, M. E., Marvin, J. M., Eisenlohr, L. C., Dietzschold, B., and Schnell, M. J. (2006). Enhanced humoral HIV-1-specific immune responses generated from recombinant rhabdoviral-based vaccine vectors co-expressing HIV-1 proteins and IL-2. *Virology* **344:**363–377.

McKenna, P. M., Koser, M. L., Carlson, K. R., Montefiori, D. C., Letvin, N. L., Papaneri, A. B., Pomerantz, R. J., Dietzschold, B., Silvera, P., McGettigan, J. P., and Schnell, M. J. (2007). Highly attenuated rabies virus-based vaccine vectors expressing simian-human immunodeficiency virus (89.6P) Env and simian immunodeficiency virus (mac239) Gag are safe in rhesus macaques and protect from an AIDS-like disease. *J. Infect. Dis.* **195:**980–988.

McKimmie, C. S., Johnson, N., Fooks, A. R., and Fazakerley, J. K. (2005). Viruses selectively upregulate Toll-like receptors in the central nervous system. *Biochem. Biophys. Res. Commun.* **336:**925–933.

Mebatsion, T. (2001). Extensive attenuation of rabies virus by simultaneously modifying the dynein light chain binding site in the P protein and replacing Arg333 in the G protein. *J. Virol.* **75:**11496–11502.

Mebatsion, T., Schnell, M. J., Cox, J. H., Finke, S., and Conzelmann, K. K. (1996). Highly stable expression of a foreign gene from rabies virus vectors. *Proc. Natl. Acad. Sci. USA* **93:**7310–7314.

Mentis, G. Z., Gravell, M., Hamilton, R., Shneider, N. A., O'Donovan, M. J., and Schubert, M. (2006). Transduction of motor neurons and muscle fibers by intramuscular injection of HIV-1-based vectors pseudotyped with select rabies virus glycoproteins. *J. Neurosci. Methods* **157:**208–217.

Messenger, S. L., Smith, J. S., and Rupprecht, C. E. (2002). Emerging epidemiology of bat-associated cryptic cases of rabies in humans in the United States. *Clin. Infect. Dis.* **35:**738–747.

Mochizuki, H., Schwartz, J. P., Tanaka, K., Brady, R. O., and Reiser, J. (1998). High-titer human immunodeficiency virus type 1-based vector systems for gene delivery into nondividing cells. *J. Virol.* **72:**8873–8883.

Morcuende, S., Delgado-Garcia, J. M., and Ugolini, G. (2002). Neuronal premotor networks involved in eyelid responses: Retrograde transneuronal tracing with rabies virus from the orbicularis oculi muscle in the rat. *J. Neurosci.* **22:**8808–8818.

Morimoto, K., Hooper, D. C., Spitsin, S., Koprowski, H., and Dietzschold, B. (1999). Pathogenicity of different rabies virus variants inversely correlates with apoptosis and rabies virus glycoprotein expression in infected primary neuron cultures. *J. Virol.* **73:**510–518.

Morimoto, K., McGettigan, J. P., Foley, H. D., Hooper, D. C., Dietzschold, B., and Schnell, M. J. (2001). Genetic engineering of live rabies vaccines. *Vaccine* **19:**3543–3551.

Moseley, G. W., Filmer, R. P., DeJesus, M. A., and Jans, D. A. (2007a). Nucleocytoplasmic distribution of rabies virus P-protein is regulated by phosphorylation adjacent to C-terminal nuclear import and export signals. *Biochem.* **46:**12053–12061.

Moseley, G. W., Roth, D. M., Dejesus, M. A., Leyton, D. L., Filmer, R. P., Pouton, C. W., and Jans, D. A. (2007b). Dynein light chain association sequences can facilitate nuclear protein import. *Mol. Biol. Cell* **18:**3204–3213.

Nadin-Davis, S. A. (2007). Molecular epidemiology. *In* ''Rabies'' (A. C. Jackson and W. H. Wunner, eds.), 2nd Ed. pp. 69–122. Academic Press, San Diego.

Nadin-Davis, S. A., and Bingham, J. (2004). Europe as a source of rabies for the rest of the world. *In* ''Historical Perspective of Rabies in Europe and the Mediterranean Basin'' (A. A. King, A. R. Fooks, M. Aubert, and A. I. Wandeler, eds.), pp. 259–280. OIE, Paris.

Nadin-Davis, S. A., Abdel-Malik, M., Armstrong, J., and Wandeler, A. I. (2002). Lyssavirus P gene characterization provides insights into the phylogeny of the genus and identifies structural similarities and diversity within the encoded phosphoprotein. *Virology* **298:**286–305.

Nadin-Davis, S. A., Turner, G., Paul, J. P. V., Madhusudana, S. N., and Wandeler, A. I. (2007). Emergence of arctic-like rabies lineage in India. *Emerg. Infect. Dis.* **13:**111–116.

Nagaraj, T., Vasanth, J. P., Desai, A., Kamat, A., Madhusudana, S. N., and Ravi, V. (2006). Ante-mortem diagnosis of human rabies using saliva samples: Comparison of real time and conventional RT-PCR techniques. *J. Clin. Virol.* **36:**17–23.

Nagarajan, T., Rupprecht, C. E., Dessain, S. K., Rangarajan, P. N., Thiagarajan, D., and Srinivasan, V. A. (2008). Human monoclonal antibody and vaccine approaches to prevent human rabies. *Curr. Top. Microbiol. Immunol.* **317:**67–101.

Nakamichi, K., Inoue, S., Takasaki, T., Morimoto, K., and Kurane, I. (2004). Rabies virus stimulates nitric oxide production and CXC chemokine ligand 10 expression in macrophages through activation of extracellular signal-regulated kinases 1 and 2. *J. Virol.* **78:**9376–9388.

Nakamichi, K., Saiki, M., Sawada, M., Takayama-Ito, M., Yamamuro, Y., Morimoto, K., and Kurane, I. (2005). Rabies virus-induced activation of mitogen-activated protein kinase and NF- κB signaling pathways regulates expression of CXC and CC chemokine ligands in microglia. *J. Virol.* **79:**11801–11812.

Nassi, J. J., and Callaway, E. M. (2006). Multiple circuits relaying primate parallel visual pathways to the middle temporal area. *J. Neurosci.* **26:**12789–12798.

Nel, L. H., Niezgoda, M., Hanlon, C. A., Morril, P. A., Yager, P. A., and Rupprecht, C. E. (2003). A comparison of DNA vaccines for the rabies-related virus, Mokola. *Vaccine* **21:**2598–2606.

Nel, L. H., Sabeta, C. T., von Teichman, B., Jaftha, J. B., Rupprecht, C. E., and Bingham, J. (2005). Mongoose rabies in southern Africa: A re-evaluation based on molecular epidemiology. *Virus Res.* **109:**165–173.

Norgren, R. B. Jr., and Lehman, M. N. (1998). Herpes simplex virus as a transneuronal tracer. *Neurosci. Biobehav. Rev.* **22:**695–708.

Nuovo, G. J., DeFaria, D. L., Chanona-Vilchi, J. G., and Zhang, Y. (2005). Molecular detection of rabies encephalitis and correlation with cytokine expression. *Mod. Pathol.* **18:**62–67.

Osorio, J. E., Tomlinson, C. C., Frank, R. S., Haanes, E. J., Rushlow, K., Haynes, J. R., and Stinchcomb, D. T. (1999). Immunization of dogs and cats with a DNA vaccine against rabies virus. *Vaccine* **17:**1109–1116.

Parveen, Z., Mukhtar, M., Rafi, M., Wenger, D. A., Siddiqui, K. M., Siler, C. A., Dietzschold, B., Pomerantz, R. J., Schnell, M. J., and Dornburg, R. (2003). Cell-type-specific gene delivery into neuronal cells *in vitro* and *in vivo*. *Virology* **314:**74–83.

Pasdeloup, D., Poisson, N., Raux, H., Gaudin, Y., Ruigrok, R. W. H., and Blondel, D. (2005). Nucleocytoplasmic shuttling of the rabies virus P protein requires a nuclear localization signal and a CRM1-dependent nuclear export signal. *Virology* **334:**284–293.

Patial, S., Chaturvedi, V. K., Rai, A., Saini, M., Chandra, R., Saini, Y., and Gupta, P. K. (2007). Virus neutralizing antibody response in mice and dogs with a bicistronic DNA vaccine encoding rabies virus glycoprotein and canine parvovirus VP2. *Vaccine* **25:**4020–4028.

Perrin, P., Jacob, Y., Aguilar-Sétien, A., Loza-Rubio, E., Jallet, C., Desmézières, E., Aubert, M., Cliquet, F., and Tordo, N. (2000). Immunization of dogs with a DNA vaccine induces protection against rabies virus. *Vaccine* **18:**479–486.

Poisson, N., Real, E., Gaudin, Y., Vaney, M.-C., King, S., Jacob, Y., Tordo, N., and Blondel, D. (2001). Molecular basis for the interaction between rabies virus phosphoprotein P and the dynein light chain LC8: Dissociation of dynein-binding properties and transcriptional functionality of P. *J. Gen. Virol.* **82:**2691–2696.

Préhaud, C., Mégret, F., Lafage, M., and Lafon, M. (2005). Virus infection switches TLR-3-positive human neurons to become strong producers of beta interferon. *J. Virol.* **79:**12893–12904.

Prosniak, M., Hooper, D. C., Dietzschold, B., and Koprowski, H. (2001). Effect of rabies virus infection on gene expression in mouse brain. *Proc. Natl. Acad. Sci. USA* **98:**2758–2763.

Prosniak, M., Faber, M., Hanlon, C. A., Rupprecht, C. E., Hooper, D. C., and Dietzschold, B. (2003a). Development of a cocktail of recombinant-expressed human rabies virus-neutralizing monoclonal antibodies for postexposure prophylaxis of rabies. *J. Infect. Dis.* **188:**53–56.

Prosniak, M., Zborek, A., Scott, G. S., Roy, A., Phares, T. W., Koprowski, H., and Hooper, D. C. (2003b). Differential expression of growth factors at the cellular level in virus-infected brain. *Proc. Natl. Acad. Sci. USA* **100:**6765–6770.

Pulmanausahakul, R., Faber, M., Morimoto, K., Spitsin, S., Weihe, E., Hooper, D. C., Schnell, M. J., and Dietzschold, B. (2001). Overexpression of cytochrome c by a recombinant rabies virus attenuates pathogenicity and enhances antiviral immunity. *J. Virol.* **75:**10800–10807.

Pulmanausahakul, R., Li, J., Schnell, M. J., and Dietzschold, B. (2008). The glycoprotein and matrix protein of rabies virus affect pathogenicity by regulating viral replication and facilitating cell-to-cell spread. *J. Virol.* **82:**2330–2338.

Qi, P., Han, J. X., Lu, Y. Q., Wang, C., and Bu, F. F. (2006). Virus-encoded microRNAs: Future therapeutic targets? *Cell. Mol. Immunol.* **3:**411–419.

Rasalingam, P., Rossiter, J. P., Mebatsion, T., and Jackson, A. C. (2005). Comparative pathogenesis of the SAD-L16 strain of rabies virus and a mutant modifying the dynein light chain binding site of the rabies virus phosphoprotein in young mice. *Virus Res.* **111:**55–60.

Rathelot, J. A., and Strick, P. L. (2006). Muscle representation in the macaque motor cortex: An anatomical perspective. *Proc. Natl. Acad. Sci. USA* **103:**8257–8262.

Raux, H., Flamand, A., and Blondel, D. (2000). Interaction of the rabies virus P protein with the LC8 dynein light chain. *J. Virol.* **74:**10212–10216.

Real, E., Rain, J.-C., Battaglia, V., Jallet, C., Perrin, P., Tordo, N., Chrisment, P., D'Alayer, J., Legrain, P., and Jacob, Y. (2004). Antiviral drug discovery strategy using combinatorial libraries of structurally constrained peptides. *J. Virol.* **78:**7410–7417.

Real, L. A., Henderson, J. C., Biek, R., Snaman, J., Jack, T. L., Childs, J. E., Stahl, E., Waller, L., Tinline, R., and Nadin-Davis, S. (2005a). Unifying the spatial population dynamics and molecular evolution of epidemic rabies virus. *Proc. Natl. Acad. Sci. USA* **102:**12107–12111.

Real, L. A., Russell, C., Waller, L., Smith, D., and Childs, J. (2005b). Spatial dynamics and molecular ecology of North American rabies. *J. Hered.* **96:**1–8.

Roy, A., and Hooper, D. C. (2007). Lethal silver-haired bat rabies virus infection can be prevented by opening the blood-brain barrier. *J. Virol.* **81:**7993–7998.

Roy, A., Phares, T. W., Koprowski, H., and Hooper, D. C. (2007). Failure to open the blood-brain barrier and deliver immune effectors to central nervous system tissues leads to the lethal outcome of silver-haired bat rabies virus infection. *J. Virol.* **81:**1110–1118.

Rupprecht, C. E., Hanlon, C. A., Blanton, J., Manangan, J., Morrill, P., Murphy, S., Niezgoda, M., Orciari, L. A., Schumacher, C. L., and Dietzschold, B. (2005). Oral vaccination of dogs with recombinant rabies virus vaccines. *Virus Res.* **111:**101–105.

Rupprecht, C. E., Willoughby, R., and Slate, D. (2006). Current and future trends in the prevention, treatment and control of rabies. *Expert Rev. Anti Infect. Ther.* **4:**1021–1038.

Russell, C. A., Smith, D. L., Childs, J. E., and Real, L. A. (2005). Predictive spatial dynamics and strategic planning for raccoon rabies emergence in Ohio. *PLoS Biol.* **3:**382–388.

Russell, C. A., Real, L. A., and Smith, D. L. (2006). Spatial control of rabies on heterogeneous landscapes. *PLoS ONE* **1:**e27. doi:10.1371/journal.pone.0000027.

Saha, S., and Rangarajan, P. N. (2003). Common host genes are activated in mouse brain by Japanese encephalitis and rabies viruses. *J. Gen. Virol.* **84:**1729–1735.

Saha, S., Murthy, S., and Rangarajan, P. N. (2006). Identification and characterization of a virus-inducible non-coding RNA in mouse brain. *J. Gen. Virol.* **87:**1991–1995.

Schmiedel, S., Panning, M., Lohse, A., Kreymann, K. G., Gerloff, C., Burchard, G., and Drosten, C (2007). Case report on fatal human rabies infection in Hamburg, Germany, March 2007. *Euro Surveill.* **12:**E070531.5.

Schnell, M. J., Mebatsion, T., and Conzelmann, K. K. (1994). Infectious rabies viruses from cloned cDNA. *EMBO J.* **13:**4195–4203.

Schnell, M. J., Foley, H. D., Siler, C. A., McGettigan, J. P., Dietzschold, B., and Pomerantz, R. J. (2000). Recombinant rabies virus as potential live-viral vaccines for HIV-1. *Proc. Natl. Acad. Sci. USA* **97:**3544–3549.

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

Scott, C. A., Rossiter, J. P., Andrew, R. D., and Jackson, A. C. (2008). Structural abnormalities in neurons are sufficient to explain the clinical disease and fatal outcome of experimental rabies in yellow fluorescent protein-expressing transgenic mice. *J. Virol.* **82:**513–521.

Shimizu, K., Ito, N., Sugiyama, M., and Minamoto, N. (2006). Sensitivity of rabies virus to type 1 interferon is determined by the phosphoprotein gene. *Microbiol. Immunol.* **50:**975–978.

Shimizu, K., Ito, N., Mita, T., Yamada, K., Hosokawa-Muto, J., Sugiyama, M., and Minamoto, N. (2007). Involvement of nucleoprotein, phosphoprotein, and matrix protein genes of rabies virus in virulence for adult mice. *Virus Res.* **123:**154–160.

Shoji, Y., Inoue, S., Nakamichi, K., Kurane, I., Sakai, T., and Morimoto, K. (2004). Generation and characterization of P gene-deficient rabies virus. *Virology* **318:**295–305.

Siler, C. A., McGettigan, J. P., Dietzschold, B., Herrine, S. K., Dubuisson, J., Pomerantz, R. J., and Schnell, M. J. (2002). Live and killed rhabdovirus-based vectors as potential hepatitis C vaccines. *Virology* **292:**24–34.

Sloan, S. E., Hanlon, C., Weldon, W., Niezgoda, M., Blanton, J., Self, J., Rowley, K. J., Mandell, R. B., Babcock, G. J., Thomas, W. D., Jr., Rupprecht, C. E., and Ambrosino, D. M. (2007). Identification and characterization of a human monoclonal antibody that potently neutralizes a broad panel of rabies virus isolates. *Vaccine* **25:**2800–2810.

Smith, D. L., Lucey, B., Waller, L. A., Childs, J. E., and Real, L. A. (2002). Predicting the spatial dynamics of rabies epidemics on heterogeneous landscapes. *Proc. Natl. Acad. Sci. USA* **99:**3668–3672.

Smith, D. L., Waller, L. A., Russell, C. A., Childs, J. E., and Real, L. A. (2005). Assessing the role of long-distance translocation and spatial heterogeneity in the raccoon rabies epidemic in Connecticut. *Prev. Vet. Med.* **71:**225–240.

Smith, M. E., Koser, M., Xiao, S., Siler, C., McGettigan, J. P., Calkins, C., Pomerantz, R. J., Dietzschold, B., and Schnell, M. J. (2006). Rabies virus glycoprotein as a carrier for anthrax protective antigen. *Virology* **353:**344–356.

Srinivasan, A., Burton, E. C., Kuehnert, M. J., Rupprecht, C., Sutker, W. L., Ksiazek, T. G., Paddock, C. D., Guarner, J., Shieh, W.-J., Goldsmith, C., Hanlon, C. A., Zoretic, J., *et al.* (2005). Transmission of rabies virus from an organ donor to four transplant recipients. *N. Engl. J. Med.* **352:**1103–1111.

Takayama-Ito, M., Inoue, K.-I., Shoji, Y., Inoue, S., Iijima, T., Sakai, T., Kurane, I., and Morimoto, K. (2006a). A highly attenuated rabies virus HEP-Flury strain reverts to virulent by single amino acid substitution to arginine at position 333 in glycoprotein. *Virus Res.* **119:**208–215.

Takayama-Ito, M., Ito, N., Yamada, K., Sugiyama, M., and Minamoto, N. (2006b). Multiple amino acids in the glycoprotein of rabies virus are responsible for pathogenicity in adult mice. *Virus Res.* **115:**169–175.

Tan, G. S., McKenna, P. M., Koser, M. L., McLinden, R., Kim, J. H., McGettigan, J. P., and Schnell, M. J. (2005). Strong cellular and humoral anti-HIV Env immune responses induced by a heterologous rhabdoviral prime-boost approach. *Virology* **331:**82–93.

Tan, G. S., Preuss, M. A. R., Williams, J. C., and Schnell, M. A. (2007). The dynein light chain 8 binding motif of rabies virus phosphoprotein promotes efficient viral replication. *Proc. Natl. Acad. Sci. USA* **104:**7229–7234.

Tang, Y., Rampin, O., Giuliano, F., and Ugolini, G. (1999). Spinal and brain circuits to motoneurons of the bulbospongiosus muscle: Retrograde transneuronal tracing with rabies virus. *J. Comp. Neurol.* **414:**167–192.

Teng, Q., Garrity-Moses, M., Federici, T., Tanase, D., Liu, J. K., Mazarakis, N. D., Azzouz, M., Walmsley, L. E., Carlton, E., and Boulis, N. M. (2005). Trophic activity of rabies G protein-pseudotyped equine infectious anemia viral vector mediated IGF-I motor neuron gene transfer in vitro. *Neurobiol. Dis.* **20:**694–700.

Tesoro Cruz, E., Hernández González, R., Alonso Morales, R., and Aguilar-Setién, A. (2006). Rabies DNA vaccination by the intranasal route in dogs. *Dev. Biol. (Basel)* **125:**221–231.

Theerasurakarn, S., and Ubol, S. (1998). Apoptosis induction in brain during the fixed strain of rabies virus infection correlates with onset and severity of illness. *J. Neurovirol.* **4:**407–414.

Thoulouze, M.-I., Lafage, M., Schachner, M., Hartmann, U., Cremer, H., and Lafon, M. (1998). The neural cell adhesion molecule is a receptor for rabies virus. *J. Virol.* **72:**7181–7190.

Thoulouze, M.-I., Lafage, M., Yuste, V. J., Baloul, L., Edelman, L., Kroemer, G., Israel, N., Susin, S. A., and Lafon, M. (2003). High level of Bcl-2 counteracts apoptosis mediated by a live rabies virus vaccine strain and induces long-term infection. *Virology* **314:**549–561.

Tinline, R. R., and MacInnes, C. D. (2004). Ecogeographic patterns of rabies in southern Ontario based on time series analysis. *J. Wildl. Dis.* **40:**212–221.

Tordo, N., Benmansour, A., Calisher, C., Dietzgen, R. G., Fang, R.-X., Jackson, A. O., Kurath, G., Nadin-Davis, S., Tesh, R. B., and Walker, P. J. (2004). Rhabdoviridae. *In* ''Virus Taxonomy, VIIIth Report of the ICTV'' (C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, and L. A. Ball, eds.), pp. 623–644. Academic Press, London.

Trimarchi, C. V., and Nadin-Davis, S. A. (2007). Diagnostic evaluation. *In* ''Rabies'' (A. C. Jackson and W. H. Wunner, eds.), 2nd Ed. pp. 411–469. Academic Press, San Diego.

Tuffereau, C., Leblois, H., Bénéjean, J., Coulon, P., Lafay, F., and Flamand, A. (1989). Arginine or lysine in position 333 of ERA and CVS glycoprotein is necessary for rabies virulence in adult mice. *Virology* **172:**206–212.

Tuffereau, C., Bénéjean, J., Blondel, D., Kieffer, B., and Flamand, A. (1998). Low-affinity nerve-growth factor receptor (P75NTR) can serve as a receptor for rabies virus. *EMBO J.* **17:**7250–7259.

Tuffereau, C., Desmézières, E., Bénéjean, J., Jallet, C., Flamand, A., Tordo, N., and Perrin, P. (2001). Interaction of lyssaviruses with the low-affinity nerve-growth factor receptor $p75^{NTR}$. *J. Gen. Virol.* **82:**2861–2867.

Tuffereau, C., Schmidt, K., Langevin, C., Lafay, F., Dechant, G., and Koltzenburg, M. (2007). The rabies virus glycoprotein receptor p75NTR is not essential for rabies virus infection. *J. Virol.* **81:**13622–13630.

Ubol, S., Sukwattanapan, C., and Utaisincharoen, P. (1998). Rabies virus replication induces Bax-related, caspase dependent apoptosis in mouse neuroblastoma cells. *Virus Res.* **56:**207–215.

Ubol, S., Sukwattanapan, C., and Maneerat, Y. (2001). Inducible nitric oxide synthase inhibition delays death of rabies virus-infected mice. *J. Med. Microbiol.* **50:**238–242.

Ubol, S., Kasisith, J., Mitmoonpitak, C., and Pitidhamabhorn, D. (2006). Screening of upregulated genes in suckling mouse central nervous system during the disease stage of rabies virus infection. *Microbiol. Immunol.* **50:**951–959.

Ugolini, G. (1995). Specificity of rabies virus as a transneuronal tracer of motor networks: Transfer from hypoglossal motoneurons to connected second-order and higher order central nervous system cell groups. *J. Comp. Neurol.* **356:**457–480.

Ulmer, J. B., Wahren, B., and Liu, M. A. (2006). Gene-based vaccines: Recent technical and clinical advances. *Trends Mol. Med.* **12:**216–222.

Vidy, A., Chelbi-Alix, M., and Blondel, D. (2005). Rabies virus P protein interacts with STAT1 and inhibits interferon signal transduction pathways. *J. Virol.* **79:**14411–14420.

Vidy, A., Bougrini, J. E., Chelbi-Alix, M. K., and Blondel, D. (2007). The nucleocytoplasmic rabies virus P protein counteracts interferon signaling by inhibiting both nuclear accumulation and DNA binding of STAT1. *J. Virol.* **81:**4255–4263.

Viemari, J. C., Bévengut, M., Burnet, H., Coulon, P., Pequignot, J. M., Tiveron, M. C., and Hilaire, G. (2004). Phox2a gene, A6 neurons, and noradrenaline are essential for development of normal respiratory rhythm in mice. *J. Neurosci.* **24:**928–937.

Vos, A., Neubert, A., Pommerening, E., Müller, T., Döhner, L., Neubert, L., and Hughes, K. (2001). Immunogenicity of an E1-deleted recombinant human adenovirus against rabies by different routes of administration. *J. Gen. Virol.* **82:**2191–2197.

Wacharapluesadee, S., and Hemachudha, T. (2001). Nucleic-acid sequence based amplification in the rapid diagnosis of rabies. *Lancet* **358:**892–893.

Wakeley, P. R., Johnson, N., McElhinney, L. M., Marston, D., Sawyer, J., and Fooks, A. R. (2005). Development of a real-time, TaqMan reverse transcription-PCR assay for detection and differentiation of lyssavirus genotypes 1, 5, and 6. *J. Clin. Microbiol.* **43:**2786–2792.

Wang, Z. W., Sarmento, L., Wang, Y., Li, X.-Q., Dhingra, V., Tseggai, T., Jiang, B., and Fu, Z. F. (2005). Attenuated rabies virus activates, while pathogenic rabies virus evades, the host innate immune responses in the central nervous system. *J. Virol.* **79:**12554–12565.

Wickersham, I. R., Finke, S., Conzelmann, K. K., and Callaway, E. M. (2007a). Retrograde neuronal tracing with a deletion-mutant rabies virus. *Nat. Methods* **4:**47–49.

Wickersham, I. R., Lyon, D. C., Barnard, R. J. O., Mori, T., Finke, S., Conzelmann, K. K., Young, J. A. T., and Callaway, E. M. (2007b). Monosynaptic restriction of transsynaptic tracing from single, genetically targeted neurons. *Neuron* **53:**639–647.

Willoughby, R. E., Tieves, K. S., Hoffman, G. M., Ghanayem, N. S., Amlie-Lefond, C. M., Schwabe, M. J., Chusid, M. J., and Rupprecht, C. E. (2005). Survival after treatment of rabies with induction of coma. *N. Engl. J. Med.* **352:**2508–2514.

World Health Organization (2005). WHO expert consultation on rabies, 2004. First Report: WHO technical report series #931. World Health Organization, Geneva, Switzerland.

Wu, X., and Rupprecht, C. E. (2008). Glycoprotein gene relocation in rabies virus. *Virus Res.* **131:**95–99.

Wu, X., Franka, R., Velasco-Villa, A., and Rupprecht, C. E. (2007). Are all lyssavirus genes equal for phylogenetic analyses? *Virus Res.* **129:**91–103.

Wunner, W. H. (2007). Rabies virus. *In* ''Rabies'' (A. C. Jackson and W. H. Wunner, eds.), 2nd Ed. pp. 23–68. Academic Press, San Diego.

Xiang, Z. Q., Spitalnik, S., Tran, M., Wunner, W. H., Cheng, J., and Ertl, H. C. J. (1994). Vaccination with a plasmid vector carrying the rabies virus glycoprotein gene induces protective immunity against rabies virus. *Virology* **199:**132–140.

Xiang, Z. Q., Yang, Y., Wilson, J. M., and Ertl, H. C. J. (1996). A replication defective recombinant adenovirus serves as a highly efficient vaccine carrier for the rabies virus glycoprotein. *Virology* **219:**220–227.

Xiang, Z., Gao, G., Reyes-Sandoval, A., Cohen, C. J., Li, Y., Bergelson, J. M., Wilson, J. M., and Ertl, H. C. J. (2002). Novel, chimpanzee serotype 68-based adenoviral vaccine carrier for induction of antibodies to a transgene product. *J. Virol.* **76:**2667–2675.

Yamada, K., Ito, N., Takayama-Ito, M., Sugiyama, M., and Minamoto, N. (2006). Multigenic relation to the attenuation of rabies virus. *Microbiol. Immunol.* **50:**25–32.

Yan, X., Prosniak, M., Curtis, M. T., Weiss, M. L., Faber, M., Dietzschold, B., and Fu, Z. F. (2001). Silver-haired bat rabies virus variant does not induce apoptosis in the brain of experimentally infected mice. *J. Neurovirol.* **7:**518–527.

Yan, X., Mohankumar, P. S., Dietzschold, B., Schnell, M. J., and Fu, Z. F. (2002). The rabies virus glycoprotein determines the distribution of different rabies virus strains in the brain. *J. Neurovirol.* **8:**345–352.

Yarosh, O. K., Wandeler, A. I., Graham, F. L., Campbell, J. B., and Prevec, L. (1996). Human adenovirus type 5 vectors expressing rabies glycoprotein. *Vaccine* **14:**1257–1264.

Zhang, S., Liu, Y., Fooks, A. R., Zhang, F., and Hu, R. (2008). Oral vaccination of dogs (Canis familiaris) with baits containing the recombinant rabies-canine adenovirus type-2 vaccine confers long-lasting immunity against rabies. *Vaccine* **26:**345–350.