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Experimental rabies vaccines for humans

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Abstract

Rabies remains a global public health threat that kills more than 55,000 people per year. Rabies disproportionately affects children and, therefore, is ranked the seventh most important infectious disease due to years lost. Prevention of human rabies is accomplished by controlling rabies in domestic and wild animals, including the use of vaccination programs. The usefulness of human rabies vaccines is hampered by high cost, complicated vaccination regimens and lack of compliance, especially in areas of Africa and Asia where human rabies infections are endemic. A single-dose vaccine would greatly benefit efforts to combat this global health threat. However, a single-dose vaccine based on current inactivated vaccines does not appear feasible and other approaches are needed. Technology has advanced since modern human rabies vaccines were developed over 40 years ago. In addition, our understanding of immunological principles that influence the outcome of vaccination has increased. This article describes the current status of inactivated rabies virus vaccines and recent developments arising from the use of reverse genetics technologies designed to develop replication-deficient or single-cycle live rabies virus-based vectors for use as a single-dose rabies vaccine for humans.

Keywords

attenuated rabies vaccines; post-exposure vaccines; pre-exposure vaccines; rabies vaccines; replication-deficient vectors; reverse genetics; single-cycle vectors

Rabies remains a global health threat

The development of a rabies vaccine for humans has a distinguished history that dates back more than 120 years (Figure 1). Since then, countless lives have been saved and human suffering reduced. However, despite the availability of effective rabies vaccines for humans and intensive efforts to control rabies in wildlife and domestic animals worldwide, more than 55,000 people die from rabies virus (RV) infections every year [101]. Up to 60% of these cases are in children [101], making rabies the seventh most important infectious disease in terms of years lost [1]. In China, rabies became the leading cause of infectious disease mortality in May 2006, which is a more than 27% increase since 2005 [2]. Worldwide, over 14 million people received post-exposure treatment after being bitten by a potentially infected animal, typically dogs [101]. In the USA, efforts to control rabies in dogs and wildlife populations have been largely successful; furthermore, resources are

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available to administer postexposure prophylaxis (PEP) appropriately. Nonetheless, the overall number of rabid animals detected has increased by 5 and 17% in wildlife and domestic animals, respectively [3], exemplifying the difficulty in containing zoonotic viral infections and highlighting the need to have animal bites evaluated in terms of potential exposure to rabies. Taken together, the development of more effective human rabies vaccines for use in both developing and industrialized countries is critically needed.

Correlates of vaccine-induced protection against RV infection

Correlates of vaccine-induced protection against RV infection are clearly defined for human rabies vaccines. Virus-neutralizing IgG (but not IgM) antibodies (VNAs) against RV glycoprotein (G) are considered protective. The WHO considers a VNA titer of 0.5 international units (IU)/ml to be demonstrative of an adequate immune response to rabies vaccination [4,5,102].

Since the correlate for protection against RV has been defined by VNAs only, little information is available on the potential benefits resulting from modulating the quality of vaccine-induced immunity in the context of novel human rabies vaccines. Although most currently approved vaccines against other infectious diseases rely on VNAs for assessing respective vaccine formulation efficacy, it is clear that cell-mediated immunity also plays a role in protection [4]. In the case of RV infections, there is little indication that CD8⁺ T cells play a role in vaccine-induced viral clearance [6]. However, CD4⁺ T cells are required for the development of anti-RV antibodies [6–9]. In humans, CD4⁺ T-cell responses are the predominant T-cell subset induced by inactivated RV vaccines [10] and elevated VNA titers are associated with higher percentages of proliferating CD4⁺ T cells [10]. Furthermore, after a single primary immunization with inactivated RV vaccine, antibody-secreting plasma cells can be detected in peripheral blood between 7 and 14 days (peaking at day 10) [11] and memory B cells can be detected for approximately 1 month postvaccination starting at day 10 [11].

In addition to the role of CD4⁺ T and B cells in vaccine-induced protection, recent studies indicate that other antibody-related attributes can contribute to vaccine-induced efficacy against other infectious diseases, suggesting these parameters may also apply to immune responses elicited by future RV vaccines. For example, lower titers of high-avidity neutralizing antibodies to influenza virus were more effective than high titers of low-avidity neutralizing antibodies [12] and an intraperitoneally administered measles virus vaccine elicited the production of protective, high-affinity IgG2a antibodies compared with intranasal inoculation, which induced a mixed, lower affinity IgG1/IgG2a response that was less effective [13]. Taken together the type and quality of the induced CD4⁺ T- and B-cell responses may help to increase the efficacy of a single-dose rabies vaccine. Additional research will be needed to define the role of immune parameters that can influence the magnitude and/or quality of the antibody response leading to more effective vaccines [14].

Inactivated RV-based vaccines for humans

As noted previously, human rabies is preventable by controlling rabies in both wildlife and domestic animal populations and by carrying out PEP. Typically, pre-exposure vaccination is reserved for people at risk for infection, including individuals working in rabies diagnostic or research laboratories, veterinarians, animal handlers (including bat handlers), and animal rehabilitators and wildlife officers. Since children under the age of 15 years are disproportionately affected by RV infections, the WHO recommends that children in rabies-endemic areas also receive pre-exposure vaccinations [15].

WHO-recommended PEP for areas of the world that can afford this treatment consists of adequate wound cleaning and a complex immunotherapy strategy consisting of a single passive immunization dose of rabies immune globulin (RIG) and five active immunizations with an inactivated RV-based vaccine (Figure 2) [102,103]. Several developments in the use of inactivated RV-based vaccines have increased their utility or hold the potential for improvements. The Advisory Committee on Immunization Practices (ACIP) provisionally approved a recommendation to reduce the number of inactivated RV-based vaccine doses from five to four during human rabies PEP treatment in the USA for previously unvaccinated individuals [16]. Individuals with underlying immunosuppression should still receive five full doses of inactivated vaccine. The US CDC supports this decision and formal publication of the recommendations is available [16]. Current WHO recommendations remain unchanged [103]. One advantage of the modified immunization protocol is the reduction in the amount of vaccine used and the number of visits to a medical facility required to complete the vaccination schedule, thereby increasing compliance. Although this change in the vaccination regimen will reduce the cost of PEP, a regimen based on four vaccine doses plus RIG remains complex and costly, and the impact of this modification on the global effectiveness of human rabies vaccination and prevention is unknown.

Years of research aimed at developing vaccination strategies that use less inactivated vaccine to decrease the cost of rabies PEP in developing countries have produced viable vaccination alternatives. As a result, the WHO now recommends two vaccine schedules based on an intramuscular route and two regimens based on an intradermal route (Figure 2). The intradermal route is used in tropical countries in an effort to reduce rabies vaccination costs [15,17–19]. Recently, the WHO modified the two-site intradermal regimen by deleting the requirement for a vaccine dose on day 90 and doubling a dose on day 28 [104]. This effectively reduces the number of visits to a healthcare facility from five to four visits. In a further development for the use of intradermal regimens, Warrell *et al.* recently reported on an effective four-site intradermal regimen that requires only three visits to a healthcare clinic and uses only half the amount of vaccine [20]. The four-site intradermal regimen has not yet been incorporated into WHO recommendations [103]; however, this regimen will help to increase compliance and reduce the cost of human rabies prevention worldwide. Despite the progress in developing alternative immunization schedules, they remain complex, require expensive RIG, multiple visits to medical facilities and skilled practitioners capable of administering the vaccine intradermally. These obstacles contribute to decreasing the widespread use and thus reducing the effectiveness of these vaccination regimens.

A recent study by Strady *et al.* showed that three inoculations of currently used inactivated RV-based vaccine in a pre-exposure setting was needed to reduce the percentage of poor responders (<0.5 IU/ml) to just 3% [21], indicating that a reduction in the number of inoculations of inactivated vaccines is not a viable alternative. Furthermore, vaccines comprised of inactivated viral particles are generally poor immunogens since they lack the capacity to elicit potent inflammatory responses required for effective CD4⁺ T-cell help. In addition, inactivated RV vaccines generate a Th2-biased antibody response characterized by IgG1 antibodies in mice [22], rather than the more potent antiviral IgG2a antibodies (Figure 3). Further modifications to the formulation would be needed to increase the utility of current inactivated RV-based vaccines for humans. The use of adjuvants can improve antigen delivery or augment vaccine-induced immunity in the context of various vaccines now being tested in human clinical trials [23]. For human rabies vaccines, development of effective adjuvants is still lacking. Only a few recent studies describing preclinical data using CpG oligodeoxynucleotides (ODNs) [24,25] or poly(lactide-co-glycolide) microspheres [26] as adjuvants have suggested that the efficacy of inactivated RV-based vaccines can be improved through the use of proper adjuvants. The reason for the lack of

interest in testing novel adjuvants in inactivated RV-based vaccines for humans is unclear, but may relate to safety concerns (or the perception that these formulations are unsafe), lack of appropriate animal models that adequately predict the effects of adjuvants in humans [23], the cost of developing and producing new adjuvant vaccines, or the unsuccessful development of alternative technologies that show more promise than adjuvanted, inactivated RV-based vaccines for humans. Regardless, the safety of newer adjuvant formulations is improving and our understanding of the immune parameters affected by adjuvants is increasing. It is likely that as alternative adjuvants are developed and tested in the context of vaccines for other infectious agents, they can be developed for use with inactivated RV-based vaccines.

The absence of necessary improvements to inactivated RV-based vaccines (or lack of attention to the development of alternative vaccine designs) now or in the future makes implementation of effective rabies prevention programs that rely solely on PEP increasingly difficult for human rabies prevention in rabies-endemic countries. This is especially true for the children in rabies-endemic areas in the absence of animal rabies control and pre-exposure prophylaxis. Pre-exposure vaccination requires three inoculations of inactivated vaccine and the WHO recommends children in areas where rabies is endemic to receive pre-exposure immunizations [15]. Nonetheless, the cost of childhood immunizations against RV is likely to be prohibitive in all but a very few dog-enzootic countries where PEP is already available. To address this concern, several studies characterized the safety, immunogenicity and/or cost-effectiveness of pre-exposure vaccination in children using inactivated RV-based vaccines [19,27–33]. When the incidence of dog bites is between 2 and 30%, a cost comparison between rabies pre- and post-exposure vaccine regimens in Thai children is estimated to be the same [27,34]. It is generally agreed that the most effective means of preventing human rabies is to eliminate rabies in dogs [15]. Until such efforts are successful, however, protecting children through pre-exposure vaccination in areas of the world most affected by rabies needs to be considered.

Live replication-deficient RV-based vaccines for humans

As discussed previously, compared with live viral vaccines, inactivated RV-based vaccines are generally poor immunogens since they do not induce potent inflammatory responses needed for effective T- and B-cell responses [35]. Therefore, other vaccine technologies being formulated and tested to replace inactivated RV-based vaccines for humans in both pre- and post-exposure applications. Many of these vaccines exploit the potent and effective immunogenic properties uniquely conferred by live virus vaccines to elicit both effective innate and adaptive immunity. Other vaccine-specific technologies, such as poxvirus- and adenovirus-based vaccines and DNA vectors have been reviewed elsewhere [24,36,37]. The efficacy of these vaccines remains to be demonstrated in humans. In the rabies vaccine field, recent advances have allowed for the development of safe and immunogenic replication-deficient or single-cycle RV-based vaccines that have been characterized and tested in several relevant small-animal and in nonhuman primate models of disease. Such novel vaccines provide the ability to induce potent and protective anti-RV immunity, potentially requiring a single RV vaccine dose in humans.

Rabies virus possesses a relatively simple, modular genome organization, encoding five structural proteins designated N (nucleoprotein), P (phosphoprotein), M (matrix protein), G (glycoprotein), and L (RNA-dependent RNA polymerase) (reviewed in [38,39]). This modular genomic architecture facilitates manipulation using reverse genetics approaches [40] and several highly attenuated replication-competent RV-based vectors have been developed and shown to be safe and immunogenic in mice [41] and nonhuman primates [42,43], suggesting their potential use as next-generation human rabies vaccines. However,

as with any live replication-competent viral vector, residual vector-associated pathogenicity is a concern for their use in humans, particularly when an effective and safe, albeit complex and costly, vaccine already exists. One of the latest applications of reverse genetics was the development of replication-deficient or single-cycle vectors where one of the five essential RV genes was deleted. The respective gene product of the deleted gene is supplied for generating progeny virions by complementing cell lines *in trans* during vaccine production; however, the virus cannot effectively complete its lifecycle in cells that do not express the complementary gene product. These manipulations attenuate the virus while maintaining its capacity to induce inflammatory responses that in turn elicit effective adaptive immune responses. To date, replication-deficient RV vectors have been generated in which the RV *M*, *P*, or *G* genes have been deleted, and preclinical studies in mice and nonhuman primates have demonstrated the significant promise of these vaccine vectors to replace current RV-based vaccines for humans.

Phosphoprotein gene-deleted RV-based vaccines

The RV *P* serves as a nonenzymatic cofactor and regulator protein of the RV polymerase (*L*) by interacting with viral and cellular proteins involved in viral replication [39,44,45]. RV *P* has also been shown to be an IFN- α/β antagonist [46] and prevent IFN- α/β stimulated JAK–STAT signaling in RV-infected cells [47]. Owing to the critical requirement for RV *P* in the lifecycle of RV, viral gene transcription but not genome replication is observed in cells infected with a *P* gene-deleted RV [48]. This makes these vectors harmless (safe) for suckling mice [48] and T- and B-cell-deficient *Rag2*^{-/-} mice [22]. Importantly, *P* gene-deleted vectors do not spread from the periphery to the CNS even in *Rag2*^{-/-} mice, further demonstrating their safety due to their inability to invade neuronal tissue. Consistent with the critical role for RV *P* in viral transcription and genome replication, the major antigenic determinant for RV, the RV *G*, is expressed at low levels and for only a short period of time postinfection [22]; within 48 h RV *G* expression *in vitro* is reduced to background levels. This might have been a concern for a human rabies vaccines where presentation of RV *G* to the immune system is critical. Yet, *P* gene-deleted RV induced a tenfold increase in protective efficiency compared with an inactivated RV-based vaccine, which demonstrates the potential use of a *P* gene-deleted RV in pre-exposure settings [22]. In addition, the *P* gene-deleted vector induced a more balanced IgG2a/IgG1 antibody response that more closely mimicked the response observed following immunization with a live RV vaccine, compared with the IgG1-dominated response elicited by inactivated RV-based vaccine immunizations. The induction of higher IgG2a antibodies (or a mixture of IgG2a and IgG1 antibodies) might be in part responsible for the effectiveness of the live *P* gene-deleted RV compared with inactivated RV-based vaccines [22]. However, while *P* gene-deleted RV vaccines show promise in pre-exposure settings, their primary drawback is the relatively slow induction of anti-RV immunity. This caveat most likely precludes their use in post-exposure settings where a rapid response is critical for preventing virus spread to the CNS [22]. Taken together, a *P* gene-deleted RV is safe and induces an immune response that provides the benefits of a live virus vaccine, making it suitable for pre-exposure scenarios but probably not for PEP due to the slow induction of immunity following administration.

Modified *P* gene-deleted RV-based vaccines

To increase the speed and magnitude of *P* gene-deleted RV vaccine-mediated immunity, a strategy was used that effectively increased anti-RV immunity induced by a replication-competent RV [49]. Mice immunized with a recombinant replication-competent RV expressing two RV *G* genes showed an eightfold higher protection compared with protection elicited by a single RV *G* gene-expressing vaccine. The potential exists that the increase RV *G* expression level by two RV *G* genes was the cause of the higher VNA and protection detected; however, similar differences were seen in anti-RV *N* antibody titers, indicating an

overall enhancement in vaccine-induced immunity. It was speculated that overexpression of RV G induces apoptosis [49], which generates apoptotic bodies that may serve as effective antigen-presenting structures [50], and might explain the enhancing effect against RV N. The approach to express two copies of the RV G gene by a *P* gene-deleted RV proved to be beneficial in increasing the speed and magnitude of the anti-RV G and -RV N responses [22]. The observation that VNA titers greater than 0.5 IU/ml were detectable within 5 days postinoculation demonstrated the value of this vaccine construct as a candidate vaccine for PEP. However, RV G expression did not exceed that of the replication-competent RV, even with the additional RV G gene. This observation indicated that it is unlikely that expression levels alone play a role in elevating the magnitude of the immune response and that other factors are likely involved, meaning that the underlying mechanism remains to be elucidated. Nonetheless, this strategy significantly increased the efficacy of *P*-deleted vectors for use in both pre- and post-exposure settings.

Matrix protein gene-deleted RV-based vaccines

The generation of the first *M* gene-deleted RV using reverse genetics technology established a critical role for RV M in virus assembly and budding [51]. *M* gene-deleted RV lost its characteristic bullet shape and cell-free infectious virus was reduced by as much as 500,000-fold when compared with the parental strain due to its budding defect [51]. *M* gene-deleted RV contains all of the necessary viral machinery to complete viral gene transcription and genome replication; therefore, *M* gene-deleted RV is able to express antigen over a longer period of time compared with *P* gene-deleted RV that is only capable of completing primary transcription [52]. As observed for the *P* gene-deleted RV, *M* gene-deleted RV is safe in suckling mice [53] and *Rag2*^{-/-} mice [52]. In addition, this mutant RV did not disseminate from the periphery to the CNS in *Rag2*^{-/-} mice as was described for the *P* gene-deleted RV. *M* gene-deleted RV was also more immunogenic and provided superior protection when compared with protection elicited by inactivated vaccines [52,53]. However, in parallel experiments, the *M* gene-deleted RV induced anti-RV antibody responses and provided protection against pathogenic RV challenge more effectively than the *P* gene-deleted RV [52]. Importantly, VNA titers were detectable 5 days postinoculation with only minimal doses (10⁵ focus forming units [ffu] per mouse) and only 10³ ffu per mouse was needed to induce protective immunity within 14 days postimmunization. The rapid and potent immune responses observed in mice was confirmed in nonhuman primates [52]. The results obtained in two different relevant animal models indicated the potential use of *M* gene-deleted RV as a human RV vaccine.

In addition to the rapid induction of immune responses, there was a significant shift in the nature of the antibody response observed in mice and nonhuman primates immunized with a *M*-deleted RV compared with the response elicited by inactivated or live RV vaccines [52]. The dominant response detected was a Th1-type response, as determined by IgG2a antibodies, replacing a Th2- or mixed Th2/Th1-type response seen with inactivated or live virus vaccines, respectively. In nonhuman primates, IgG1 antibodies, which are the functional equivalent to IgG2a in mice [54], were detected [52]. In addition, vaccination with the *M* gene-deleted RV induced the production of antibodies with higher avidity compared with antibodies produced following immunization with human diploid cell vaccine. Since vaccine efficacy has been based on the induction of high VNA titers alone, the significance of a shift in the antibody quality to higher affinity IgG2a antibodies is not known. IgG2a antibodies possess the ability to clear both free and cell-associated viruses via a wide variety of effector mechanisms, including neutralization, Fc-mediated effector systems and cell signaling [55]. In addition, cells secreting IFN- γ (a Th1-type cytokine) that targets ICAM-1 on neurovascular endothelial cells, might help to clear RV from the CNS [56]. Taken together, a Th1-type response might prove useful when PEP is delayed and cells

in the periphery and/or CNS are already infected. Additional studies will be needed to evaluate whether, in addition to neutralization, the nature of the immune response elicited following immunization with the *M* gene-deleted RV improves vaccine-induced immunity.

G gene-deleted RV-based vaccines

As with the case for *M* gene-deleted RV, a *G*-deleted RV was first constructed, recovered and studied for its role in the virus lifecycle [57]. However, given the critical role of RV *G*-specific VNA titers, it would seem counterintuitive to develop a *G* gene-deleted RV as a vaccine against RV infection. Nevertheless, a single-cycle RV-based vaccine vector was constructed in which the RV *G* gene was deleted and in its place, a HIV gene was inserted to express a HIV antigen [58]. RV *G* was supplied *in trans* from complementing cell lines and, therefore, the virus was capable of infecting target cells but was unable to spread [57,58]. The single-cycle *G* gene-deleted RV induced somewhat lower total anti-RV *G* IgG antibody titers compared with its replication-competent counterpart; however, the profile of potent anti-RV *G* IgG1/IgG2a antibodies detected was the same as the profile elicited by the replication-competent RV. It was suggested that viral spread between the replication competent and single-cycle vectors was similar at the injection site and, therefore, the replication competent viruses were actually phenotypically similar to the single-cycle vector [58]. Based on these observations, it was suggested that both vaccine constructs elicited similar 'danger signals', resulting in immune cell recruitment, thereby eliciting similar immune responses [58]. It is these danger signals that probably initiate the potent anti-RV *G* antibody responses observed, making the *G* gene-deleted RV a viable alternative for safe and effective human RV vaccines.

Special consideration for vaccines that target virus clearance from the CNS

Different parameters must be considered when developing treatments for RNA or DNA virus infections of the CNS (reviewed in [59]). In virtually all cases, exposure to viruses that ultimately infect the CNS occurs at the periphery (i.e., outside of the peripheral or central nervous systems). Therefore, systemic immunity is critical for vaccine-induced efficacy. Once these viruses invade the CNS, they can be cleared from neurons via a combination of innate immunity (initially IFN- β mediated), followed by resident virus-specific CD4⁺ T and B cells secreting IFN- γ and antibodies, respectively (Figure 3). Together, IFN- γ and antibodies are able to control long-term viral replication in the CNS [59]. Recent evidence suggested that this also applies to the clearance of pathogenic RV from the CNS [60]. During the course of a pathogenic RV infection, the blood-brain barrier (BBB) remains intact, preventing these effectors from entering the CNS and clearing the infection. However, attenuated RV strains may be unique in that they hold the potential to alter BBB permeability, allowing effectors to enter the CNS and prevent infection with pathogenic RV. Inactivated RV-based vaccines are not able to increase BBB permeability and are, therefore, not as effective as a live-attenuated vaccine in initiating virus clearance from the CNS [60]. This, in combination with potent peripheral inflammatory responses, might explain the potency of live RV vaccines and conversely limitations with current inactivated RV vaccines. It remains to be determined whether replication-deficient RVs can influence immunity once the virus enters the CNS, although a couple of studies indicate that this might be feasible. Baer *et al.* were able to show that a live attenuated RV strain was able to prevent death from symptomatic rabies encephalitis in dogs while an inactivated vaccine resulted in a complete lack of protection [61]. An intrathecal inoculation of the ERA strain of RV induced neutralizing antibodies in the cerebrospinal fluid, which might have been the mechanism of action that resulted in some dogs surviving, although that was not definitively shown. Recently, an experimental live attenuated RV-based vaccine was shown to increase BBB permeability [62], indicating that this might also occur after vaccination with replication-deficient RV that parallels the efficacy observed for live RV vaccines. However,

it is important to note that the replication-competent RV-based vaccine used in this study was administered intracranially [62] and, therefore, it is not possible to determine whether BBB permeability was increased after peripheral inoculation. Regardless, increased BBB permeability is encouraging for the use of replication-deficient RVs that induce immunity that mimics live RV vaccines but intrinsically provide greater safety. However, altering the BBB permeability too much can exacerbate RV-induced disease as demonstrated by a study that showed that excessive infiltration and accumulation of inflammatory cells in the CNS and severe enhancement of BBB permeability was responsible for increased RV pathogenesis [63]. One caveat associated with these observations, however, is that this study utilized recombinant, attenuated RVs expressing specific inflammatory cytokines and therefore most likely did not reflect the same natural immune responses (or changes to BBB permeability) induced by attenuated RV strains. Nonetheless, it is important to consider potential adverse effects that might be associated with manipulating the BBB permeability for vaccination purposes. With that being said, the ability to clear RV from the CNS postinfection would increase the utility of human RV vaccines and warrants additional study.

Expert commentary

Countless lives have been saved since the development of nervous tissue-derived human rabies vaccines began approximately 120 years ago. Nonetheless, rabies remains a global health threat despite it being a vaccine-preventable disease. Prevention of human rabies is a complex process that includes controlling rabies in wildlife as well as feral and domesticated dogs through vaccination programs. Ideally, controlling rabies in animals, particularly the major reservoir worldwide (i.e., dogs), is the most effective means to prevent human rabies. Strategies aimed at controlling rabies in dogs have successfully reduced not only rabies in dogs but also in humans [64–66]. Nonetheless, this is proving difficult and highlights the problems associated with controlling and, where possible, eradicating rabies such that humans no longer need to worry about rabies as a zoonotic diseases. Until such time, vaccination of humans as well as animals will continue to be an important component of human rabies prevention programs. However, the utility of current inactivated RV-based vaccines is hampered by the high cost and complicated vaccination regimens that often result in a lack of compliance. Further improvements to current inactivated RV-based vaccines is questionable in the absence of specific changes to their formulations aimed at increasing their effectiveness, suggesting that alternative approaches to the development of novel human rabies vaccines are urgently needed. Live RV vaccines hold the greatest potential to induce strong anti-RV immunity through the activation of inflammatory responses needed for the induction of effective innate and adaptive immune responses. Nonetheless, there is a concern for the use of live virus vaccines in humans due to adverse effects of residual pathogenesis. The benefit of replication-deficient or single-cycle RV-based vaccines is that they circumvent these issues and have shown promise as being both efficacious and safe in relevant animal models, including mice and nonhuman primates. Other replication-deficient viral vectors such as adenovirus and poxviruses are being testing as potential human RV vaccines and have also shown promise in preclinical studies although their efficacy in humans remains to be determined. Taken together, the use of live replication-deficient viral vectors that could replace current inactivated human rabies vaccines warrants additional study.

Five-year view

Live replication-deficient viral vectors have shown promise as single-dose human rabies vaccines that could potentially replace current inactivated vaccines. Nonetheless, additional preclinical immunogenicity and safety studies are needed in animal models over the next 5

years before they can be used in humans. These additional studies include biodistribution, toxicity and histopathological testing, as well as genetic and thermal stability studies. Once these studies are completed, the potential exists for them to be tested in human clinical trials.

Postexposure prophylaxis will continue to be the primary strategy to prevent human infections, although the use of pre-exposure vaccination in target populations, such as in children in rabies endemic areas, should be implemented. Nonetheless, programs to control rabies in wildlife and dogs will be the most effective means to prevent rabies in humans worldwide. In addition, education through increased awareness of rabies and how it is transmitted from animals to humans will also play an important role in the prevention of human rabies cases in the next 5 years. The challenge for the next 5 years is to use available but scarce vaccine and RIG effectively. In addition to government agencies, groups such as the Alliance for Rabies Control and their partners will be instrumental in communicating and educating the public and health officials on the need for effective rabies control programs and implementing strategies to effectively use currently available resources.

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• of interest

•• of considerable interest

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Figure 1. Timeline of human rabies virus vaccine development

Approximately 120 years ago, one of the first vaccines for humans was developed by Louis Pasteur and tested on a 9-year-old boy who sustained severe wounds after being bitten by a rabid dog (reviewed in [67]). The crude nervous tissue-derived preparation was used thereafter throughout the world to reduce suffering associated with rabies infections. Years later, alternative vaccine preparations were developed as chemically inactivated rabies virus strains that further increased their safety and utility. However, high toxicity and severe side effects associated with these crude formulations (including demyelination of peripheral and central nerves and occasionally death) had to be addressed. Almost 80 years later, Kissling developed systems to grow rabies virus in nonneuronal tissue culture, which resulted in the development of tissue culture-derived human rabies vaccines, which to date have largely replaced nervous tissue-derived vaccines.

Figure 2. WHO-recommended rabies virus vaccine regimens

The standard WHO intramuscular regimen is used in the USA and other developed countries and is effective if administered in a timely and appropriate manner. Of note, the Advisory Committee on Immunization Practices recently recommended a reduction in the number of vaccine doses, from five to four, in standard intramuscular vaccine regimens, which is supported by the US CDC, but not yet adopted by the WHO. However, WHO approved regimens designed to lower the cost of vaccination when resources are not available. For the eight-site id. regimen, the one dose on day 90 may be replaced by two id. injections on day 30. In addition, an alternative four-site id. immunization strategy was recently developed that requires only three visits to a health care clinic and half the amount of vaccine. This simplified regimen has yet to be included in WHO recommendations for rabies vaccinations. While reduced amounts of the vaccine are used in these alternative protocols, expensive RIG is still recommended in severe exposures and the vaccine dosing remains complex. Simplified vaccination protocols that can be universally applied are needed.

id.: Intradermal; im.: Intramuscular; RIG: Rabies immune globulin. Adapted from [102].

Figure 3. Immunity by current and potentially future rabies virus vaccines

As with other viruses that infect the CNS, exposure to RV usually occurs at peripheral sites. As such, peripheral vaccine-induced immunity, specifically IgG virus-neutralizing antibodies against RV G, is critical for protection against RV infections. There is little indication that CD8⁺ T cells significantly contribute to protection. Taken together, CD4⁺ T cells and antibody-secreting B cells are important for effective anti-RV immunity. **(A)** In the periphery, inactivated RV vaccines induce a predominant Th2-type response, characterized by IgG1 antibodies in mice. **(B)** Adjuvants, which have the capacity to modulate immune responses, are not widely being tested to increase the immunogenicity of inactivated RV vaccines; however, this will likely change as novel adjuvants are developed in the context of other infectious agents. Live RV vaccines, on the other hand, inherently induce proinflammatory responses and very potent anti-RV immunity. Typically, live RV strains induce a mixed antibody response characterized by IgG1 and IgG2a antibodies in mice and higher avidity antibodies in nonhuman primates compared with responses elicited by inactivated RV. Since protection against RV infection is defined by virus-neutralizing antibodies alone, the significance of modulating the quality of the immune response is still not known. However, as noted in the text, the quality of the antibody response can influence vaccination outcome. The potential exists that modulating immunity can increase the time between exposure and symptoms by clearing both free and cell-associated virus and by clearing virus from the CNS. **(C)** Effectors needed to clear RV from the CNS are being defined and are similar to those needed to clear other viruses from the CNS. Live attenuated but not inactivated vaccines have the ability to alter the blood–brain barrier permeability in a CD4⁺- and IFN- γ -dependent manner, allowing effectors, specifically antibody secreting β -cells, to enter the CNS and facilitate RV clearance from the CNS. Taken together, it is becoming apparent that vaccines that induce immunity to mimic a live RV vaccine strain and potent peripheral immunity, while clearing RV in the CNS, if needed, will greatly improve the utility of RV vaccines for humans.

RV: Rabies virus; TCR: T-cell receptor.