A review of current scientific advances in the fight against Hendra virus infection

Hendra (HeV) and Nipah viruses (NiV) comprise the genus Henipavirus of the family Paramyxoviridae. Both cause fatal encephalitis and respiratory disease in humans as well as high morbidity and fatal disease in animals. They have continued to cause sporadic outbreaks since they were identified in Australia in1994, and Malaysia and Singapore in 1998/9 respectively.

HeV and NiV have a negative-sense, single stranded RNA genome, with a lipid envelope derived from the host cell membrane. They are classified as Biosafety Level 4 (BSL-4) pathogens with broad tissue and species tropism, which is unusual because most Paramyxoviruses are single species restricted and do not have other reservoirs in nature. Current evidence indicates that Megachiropteran bats from the Pteropodidae family, commonly known as flying foxes or fruit bats, are the natural reservoir of HeV in Australia and NiV overseas. Evidence of Henipavirus infection has also now been reported in a wide range of both frugivorous and insectivorous bats in Asia and West Africa (Bishop et al 2008, Hayman et al 2008, Li et al 2008).

As well as spillover of infection occurring from bats to amplifying vertebrate animal hosts and then to humans, direct human-to-human transmission of NiV from patients with respiratory disease is possible (Gurley et al 2007). Direct bat-to-human transmission of NiV has also occurred by people drinking date palm juice contaminated with fruit bat saliva, urine and faeces (Luby et al 2006). Therefore, apart from well documented horse-to-human transmission of HeV, the possibility of human-to-human and bat-to-human infection of HeV cannot be excluded. In addition to acute lethal infection with high mortality rates, these viruses may lead to late-onset or relapse of encephalitis years after initial infection, as well as persistent or delayed neurological sequelae (Playford et al 2010, Wong 2010). They are therefore of concern from human health and national livestock perspectives, being classified by the National Institute of Allergy and Infectious Diseases (NIAID) and the Centers for Disease Control (CDC) in the USA as “Critical Biological Agents Category C”. These are agents that are a risk to national security because they may be amplified in cell culture or embryonated chicken eggs, and could be used as bio-terror weapons capable of targeting humans, as well as livestock which could act as virus amplifiers.

Since the pivotal discoveries in 2005 of host cell proteins Ephrin B2 ligand and then Ephrin B3 ligand as being the functional cellular receptors of Henipaviruses (Bonaparte et al 2005, Bishop et al 2007, Negrete et al 2005, Negrete et al 2006), there has been focussed research to identify agents capable of being used as vaccine candidates or therapeutics. Much of this work has been funded in the USA by the NIAID, a component of the National Institutes of Health (NIH), Department of Health and Human Services under a United States Presidential Directive subsequent to 9/11.

Recent advances in vaccine development and therapies

There are currently no registered human or veterinary therapies or vaccines for the treatment or prevention of HeV or NiV infection.

The International Henipavirus Workshop held in Queensland in October 2009 was co-sponsored by the World Health Organisation (WHO) and the Food and Agriculture Organisation of the United Nations. Major clinical recommendations made to WHO as a result of that Workshop were that “Vaccination for horses should be prioritised for Hendra control” and “Further clinical studies were needed to evaluate the safety and effectiveness of therapies for treatment and post exposure prophylaxis”.

Because of the lethality and broad tropism of the viruses, particularly the affinity for the central nervous system, the window of opportunity for successful treatment after exposure or infection is currently unknown and is likely to be very narrow. In the unfortunate circumstance that a person does become exposed or infected then we need to know the best treatment options available, based on the latest cutting-edge research.

HeV horse vaccine development

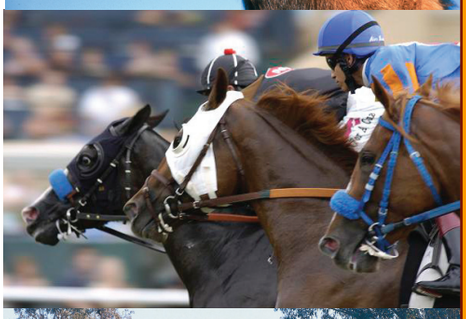
The possibility of preventing HeV infection in horses by effective immunisation will be a far better option than trying to treat human infection from horse-to-human transmission. Two potential vaccine candidates have been identified with the soluble G glycoprotein (sG), which contains no RNA genetic material, and was identified in 2006 as a potent immunogen in vaccine trials using cats as the experimental model (Mungall et al 2006).

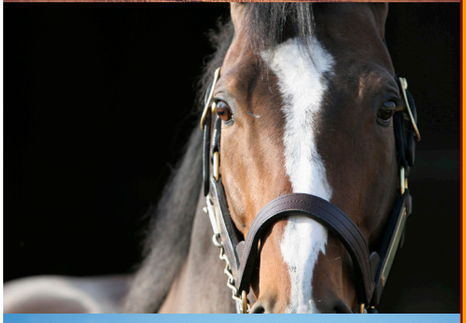
In mid-2010, approval of $600,000 was received from the Queensland and Australian Governments for the conduct of HeV horse vaccine trials at the Australian Animal Health Laboratory (AAHL) under the leadership of Dr Deborah Middleton.

After rigorous safety testing and a concerted collaborative effort between Professor Christopher Broder from the Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA, a USA pharmaceutical company and AAHL, an adjuvanted HeV soluble G (HeV sG) vaccine was eventually made available to AAHL’s scientific team and the first phase of the horse vaccination trials commenced in mid-October 2010. This was a monumental event in the fight against HeV which first emerged 16 years previously.

Dr Deborah Middleton, Theme Leader, Transforming Animal Biosecurity, CSIRO, AAHL, Victoria

A primary vaccine dose followed by a booster after 3 weeks resulted in increasing titres of serum neutralising anti-Hendra antibodies. These vaccinated animals were fully protected and survived a lethal oronasal live virus challenge 3 weeks later. In subsequent testing there was no evidence of viral replication in any of the tissues examined in the vaccinated animals. (D Middleton pers. comm.) The control (unvaccinated) animal developed fulminating lethal





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disease identical to that seen in a previous 2008 live virus challenge study (Middleton 2008).

HeV sG horse vaccination trials commence in October 2010 at the Australian Animal Health Laboratory- a monumental event.

The other vaccine candidate, the ALVAC canarypox vectored recombinant subunit coding for Hendra G and Hendra F glycoproteins has also been identified as a potential vaccine candidate against HeV based on the effectiveness of this platform of delivery in immunisation trials against NiV using pigs (Weingartl et al 2006).

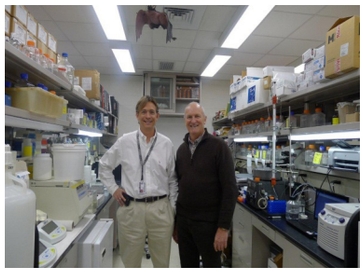
Canarypox virus (ALVAC) vaccine vectors induce antibody and cytoxic T-cell responses and were used in Australia in 2007 as the vaccine viral vector for the highly successful control and eradication of equine influenza virus in Australia.

Significant steps are being made towards making HeV horse vaccine a commercial reality but full funding and evaluation of vaccine candidates for efficacy, safety, immunogenicity, duration of immunity and subsequent field trial testing is crucial. The Queensland Horse Council www.qldhorsecouncil.com has established a special Hendra Virus Horse Vaccine Development Fund where all donations are forwarded to AAHL for this purpose.

Human monoclonal antibody

Professor Christopher Broder from the Department of Immunology and Microbiology, Uniformed Services University, Bethesda, Maryland, USA and his team, in conjunction with scientists at AAHL, were responsible for identifying and testing a fully human monoclonal antibody, m102.4 (mAb), as a prophylactic or post-exposure therapeutic (Bossart et al 2009). Professor Broder was also co-author of a 2005 paper identifying Ephrin B2 as a functional receptor of the Henipaviruses (Bonaparte et al 2005).

The mechanism of mAb action involves binding a single epitope within the receptor binding domain of the Henipavirus G glycoprotein and effectively blocking receptor engagement and infection, completely protecting ferrets from disease when given 10 hours after a lethal NiV challenge.



Professor Chris Broder, USU, Bethesda, Maryland USA with Dr Reid

The mAb also demonstrated neutralising activity against a range of HeV and NiV isolates, supporting its potential broad applicability as a post exposure therapeutic for Henipavirus-infected individuals. This study was the first successful and viable post-exposure antibody therapy for NiV using a human monoclonal antibody (Bossart et al 2009). mAb is cross reactive against HeV and NiV attachment G glycoproteins, potently neutralises both viruses in vitro and maintains its biological activity in vivo suggesting its possible utility as a passive therapeutic modality following Henipavirus infection (Zhu et al 2008).

A quantity of the mAb was sent to Brisbane in late 2009, and again last year in May 2010 it was imported under emergency conditions on compassionate grounds, after urgent and close personal co-operation with Professor Broder, for use in human patients who were exposed to, or infected with HeV. There were apparently no significant untoward reactions involved with its use.

Following $300,000 funding approval in 2010 from the Queensland Government towards the project, the Australian Institute for Bioengineering and Nanotechnology (AIBN) at the University of Queensland was contracted by Qld Health to produce mAb 102.4 under a license agreement between the Henry M Jackson Foundation in the USA and Queensland Health.

During my visit to the AIBN in December, team leader Dr Trent Munro advised that excellent progress had been made in the scaled-up production process using their expression system and over 30 gms had been produced and purified under “close to” GMP conditions in the high-tech facility. This will be available for emergency human use if the need arises, however there is currently no published data on its therapeutic value in humans, particularly whether the mAb can cross the blood brain barrier. The production process is very expensive and additional funding will be required to replenish this stock in 12 months when its shelf life is scheduled to expire. Some of the stock may be used in future experimental non-human primate animal studies overseas.

Monoclonal antibody production at AIBN, University of Queensland

Chloroquine and ribavirin

The antimalarial drug chloroquine has been previously identified as effective against NiV in vitro (Porotto et al 2009) and both chloroquine and the antiviral ribavirin has been used to treat acutely ill and convalescent HeV patients. Ribavirin has also been used for the treatment of acute Nipah encephalitis patients where it only slightly decreased mortality (Chong et al 2001). This may be explained by its failure to cross the blood brain barrier. In support of this notion (Porotto et al 2010), hamsters with measles encephalitis died with ribavirin treatment if the route of injection was intraperitoneal, while intracranial injection of ribavirin resulted in complete protection (Reuter et al 2010).

Recent experimental work confirmed the strong antiviral activity of both drugs against HeV and NiV in inhibiting viral spread in vitro, however no efficacy of their use in preventing death either alone or in combination using ferrets and golden hamsters as experimental animal models could be demonstrated (Pallister et al 2009, Freiberg et al 2010).

Another study of the effectiveness of ribavirin as a pre or post exposure therapy against HeV infection in African green monkeys demonstrated that its use as a stand-alone therapy against HeV is questionable (Rockx et al 2010).

These findings appear to raise serious questions about their efficacy in humans. Nonetheless, because of their lower cost, physician familiarity with usage

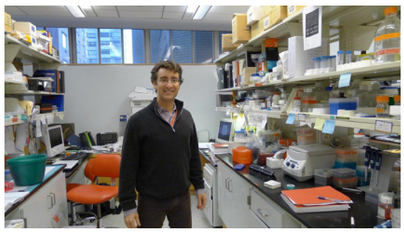
and ready availability compared with novel therapeutics such as monoclonal antibodies, they may offer some benefit depending on timing, dose, route of administration and level of infectious viral challenge in countries where other more expensive treatments are unavailable. There are plans in place for them to be used in humans in countries such as Bangladesh and India if there is another outbreak of NiV.

Potential new therapeutic agents

Internationally significant advances in identifying and trialling new therapies have recently been made. On a visit to the USA in December 2010, I met with leading research scientists from medical laboratories in New York and Los Angeles to discuss these recent developments. The visits were important to gain understanding of the significance of these advances and to provide a personal perspective to the researchers of the threat of HeV on veterinarians and horse industry stakeholders here in Australia. The interaction provided a frank two way exchange of new information and perspectives and valuable international networks were established.

Cholesterol tagged peptides

Professor Matteo Porotto and Professor Anne Moscona, Professor of Paediatrics and Immunology from Weill Cornell Medical College in New York have recently published work (Porotto et al 2010) on cholesterol tagged peptides which have demonstrated effectiveness in preventing NiV infection in trials using golden hamsters, as an established small animal model. The tagged peptides interact with the F glycoprotein before the fusion process with the host cell membrane thus preventing viral entry. The in vivo efficacy of cholesterol tagged peptides, and in particular their ability to penetrate the CNS, suggests that they are promising candidates for the prevention or therapy of infection by NiV and HeV and other lethal Paramyxoviruses.



Professor Matteo Porotto, Weill Cornell Medical College, New York, USA

Professor Moscona and Professor Porotto outlined plans to validate their findings in non-human primate African Green Monkey and Squirrel Monkey trials with plans for these primate trials under BSL4 laboratory conditions probably at the NIAID Rocky Mountain Laboratory in Montana after receipt of NIH funding. They indicated that their in vivo trial work had been significantly frustrated by being unable to access BSL4 laboratory space in laboratories around the world.

Their laboratory has stock of this peptide and has also identified and holds stock of other exciting therapeutic freeze dried peptide candidates which are more effective in preventing fusion and indicated that they could be produced under GMP in approximately 7 weeks if needed, and are happy to co-operate with public health authorities if required.

Professor Anne Moscona, Professor of Paediatrics and Immunology, Weill Cornell Medical College, New York, USA and Dr Reid

It should be noted that the peptides described in the current work inhibit the virus at a post attachment stage, and are therefore expected to act synergistically with agents such as neutralising monoclonal antibodies that block viral attachment. Combination therapy is now the mainstay for HIV and other viral diseases where multiple therapeutic options are available.

This team has also developed very specific respiratory tract and CNS cell lines for disease pathogenesis and pharmacological studies and are working on a subcutaneous injection with sustained release that could be used as a complimentary therapeutic.

Proteasome inhibitors

In Los Angeles, the team led by Professor Benhur Lee from the Dept. of Microbiology, Immunology and Molecular Genetics at UCLA, has identified the proteasome inhibitor bortezomib which has demonstrated in vitro effectiveness in preventing Nipah viral assembly and budding during viral replication (Wang et al 2010). Professor Lee was also co-author of a 2005 paper in *Nature* identifying Ephrin B2 as the receptor for NiV (Negrete et al 2005). This drug is already USA FDA approved for the treatment of multiple myeloma and mantle cell lymphoma in humans and an oral form is expected to soon be available. Professor Lee explained that his team discovered quite unexpectedly that the matrix protein of NiV needs to transit through the nucleus before gaining the functional ability to localise and bud from the cellular plasma membrane.

Ubiquitination of a conserved lysine residue in NiV-M is critical for nuclear export, subsequent membrane localisation and viral budding. Proteasome inhibitors such as bortezomib, which deplete cellular pools of ubiquitin, potently reduce viral titres during live NiV infection at a concentration of the drug which is 100-fold less than the peak plasma concentration that can be achieved in humans. This opens up the possibility of using an “off-the-shelf” therapeutic agent against Henipavirus infections and newer proteasome inhibitors are also under investigation.

In unpublished trials with guinea pigs, the drug was given by the intraperitoneal route because of the difficulty of giving i/v injections under BSL4 conditions. Sufficiently high protective serum levels were not achieved and the drug was not therapeutically effective. Further small animal trials are planned with an oral form of the drug which expected to soon receive FDA approval.

Human antibody production from cloned reactive B-cells

With technological advances in recent years, viral immunologists can now isolate monoclonal antibodies from virus-specific B cell clones from patient survivors of various infections. In fact, monoclonal antibodies against the 1918 Spanish Flu virus have been isolated from survivors of the actual pandemic.

Once these B-cell clones have been isolated, they can be grown up and potentially used to produce quantities of monoclonal antibodies for characterisation of the immune response, and potent ones can potentially be developed into therapeutics (e.g. infusion of potent monoclonal antibodies many hours post-infection have shown to be protective for many animal models of virus infections, including Ebola and Human Immunodeficiency Virus). Potent antibodies probably bind to more than one epitope within the receptor and attachment binding domains of the virus.

Although the process of isolating and

characterising specific monoclonal antibodies is technically challenging, from the patient's perspective it only involves a one-time donation of 50-60 ml of blood, and currently I am collaborating with Professor Lee with a view to progressing this study with his team at UCLA in California and other leading University Medical Centres in the USA.

Conclusion

Practitioners should remain focused on the risks that Hendra virus represents to personal safety and should never be lulled into a false sense of security even if an outbreak does not occur for several months. Based on the known pathogenesis and lethality of the virus, there is likely to be only a narrow window of opportunity for prophylaxis after exposure or treatment after confirmed infection; and the effectiveness of current modalities is still uncertain. For this reason, prevention is better than cure, and a safe, effective horse vaccine will provide additional protection in addition to the adoption of good personal biosecurity practices.

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