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Containing the *Contagion*: Treating the Virus That Inspired the Film

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Abstract

Postexposure passive immunotherapy protects African green monkeys from lethal challenge with *Contagion*-related Hendra virus.

“Somewhere, somehow, the wrong bat met up with the wrong pig,” says Dr. Ally Hextall to Dr. Ellis Cheever of the Centers for Disease Control in Steven Soderbergh's recent film *Contagion*. Dr. Hextall has just figured out that the version of the meningoencephalitic virus (MEV)-1 that is causing an incipient pandemic contains a mix of genetic material from bat and pig viruses. Up there, on the big screen, if you pay careful attention, you can actually see the words “Nipah virus” with what looks like GenBank accession numbers as Hextall lines up viral sequences on her computer screen. Now, in an example of life imitating art, Bossart *et al.* (1) report the efficacy of a new immunotherapy in a nonhuman primate model after exposure to an infectious agent that is closely related to the Nipah virus (NiV).

NiV shares a high degree of sequence similarity with the Hendra virus (HeV). NiV and HeV infections cause respiratory and brain inflammation, and with mortality rates in people that can exceed 70%, these two notorious assassins are the deadliest paramyxoviruses known to infect humans. In fact, NiV and HeV are the only paramyxoviruses that are classified as biosafety level 4 (BSL4) pathogens owing to their extreme virulence and bioterrorism potential (2). The genetic distinctiveness of NiV and HeV warranted designation as a new genus (*Henipavirus*) within the *Paramyxoviridae* family, which includes more familiar human pathogens such as the measles, mumps, parainfluenza, and respiratory syncytial viruses. Although these latter paramyxoviruses cause significant morbidity in susceptible populations, the infections themselves are relatively self-limiting, likely because most of these viruses have had time to adapt and coevolve with their human hosts; a virus that kills its host too rapidly may compromise its own chances for maximal spread. Viruses that cause acute morbidity and have high mortality rates are usually, although not always, an indication of recent zoonotic transmission from their natural animal reservoirs.

NiV and HeV were first isolated in the 1990s during fatal outbreaks among pigs (NiV), thoroughbred horses (HeV), and humans who came in contact with the infected animals (2). The natural reservoir appears to be species of fruit bats—called flying foxes—found in Southeast Asia and Australia, although cross-reactive sera have also been found in bat species as far west as Madagascar and Ghana. Horses and pigs serve as amplifying reservoirs for HeV and NiV, respectively, but direct bat-to-human and human-to-human transmission can occur (Fig. 1) (3). Since 2001, outbreaks of NiV in Bangladesh and HeV in

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Queensland, Australia, have occurred almost annually, including the most recent outbreaks of NiV and HeV in March and June–July 2011, respectively.

As with all viruses in the *Paramyxovirinae* subfamily, henipavirus entry into permissive mammalian cells requires coordinated action of the viral fusion protein (F) and the attachment viral envelope glycoprotein (G). Binding of NiV-G or HeV-G to cell surface receptors induces the allosteric triggering of F, which results in a conformational cascade that leads to viral–cell membrane fusion (4). Thus, blocking of receptor interactions with G necessarily inhibits membrane fusion and viral entry. Henipaviruses exploit highly conserved cellular receptors—ephrinB2 and ephrinB3, two membrane-associated proteins involved in axon path finding and endothelial cell guidance during angiogenesis—a feature that accounts for the viruses' unusually broad species tropism.

The clear correlation between tissue-specific receptor usage and cellular tropism of the viruses and the end-organ pathology that is associated with the morbidity and mortality of henipavirus infections makes the study of henipavirus entry particularly illuminative. For example, ephrinB2 is highly expressed in microvascular endothelial cells and neurons, and ephrinB2 usage by the virus accounts for the endothelial syncytia and multiorgan vasculitis seen in autopsy samples from patients who died from henipavirus infections. Furthermore, ephrinB3 (but not ephrinB2) is expressed in the brainstem, and thus, ephrinB3-mediated entry likely contributes to the ultimate cause of death, brainstem neuronal dysfunction (5). The broad species tropism also allows for the development of multiple animal models that reflect various pathogenic aspects of NiV and HeV infections seen in humans. These animal models are particularly important for satisfying the U.S. Food and Drug Administration's two-animal rule when evaluating the efficacy of therapeutics against highly lethal infectious agents for which human testing is precluded.

EFFICACY MODEL

In this issue of *Science Translational Medicine*, Bossart *et al.* (1) report on impressive in vivo efficacy, in HeV-infected African green monkeys (AGMs), of postexposure immunotherapy with a recombinant human monoclonal antibody (mAb), m102.4, directed against HeV-G. This nonhuman primate (NHP) model recapitulates many of the pathogenic events associated with HeV infection in human patients. The m102.4 version of the therapeutic mAb is an in vitro affinity-matured subclone of version m102, which was isolated by screening a nonimmune human antibody phage display library against soluble HeV-G. m102.4 was generated by a combination of light-chain variable domain (VL) shuffling and random mutagenesis of the heavy-chain variable domain (VH) of m102 and selected for better cross-reactivity, relative to m102, with soluble NiV-G (6). The VH and VL domains of m102.4 were then grafted onto a human IgG1 scaffold.

The postexposure efficacy of m102.4 and the extent of its therapeutic window were tested in three separate groups of AGMs that had been infused with 100 mg (~20 mg/kg) of m102.4 at 10, 24, and 72 hours after intratracheal administration of HeV (4×10^5 TCID₅₀; 50% tissue culture infective dose, the amount of virus required to produce a cytopathic effect in 50% of inoculated tissue culture cells). In each of the treatment groups, a second infusion of m102.4 was given ~2 days after the first dose. Control animals were inoculated identically and left untreated.

Remarkably, infusion of m102.4, even when administered 72 hours postinfection, resulted in protection of the animals from HeV-mediated mortality. The same research group had previously demonstrated the postexposure efficacy of m102.4 passive immunotherapy using the ferret model (7) challenged with NiV. Together, these studies should make excellent candidates for meeting FDA's two-animal rule. That m102.4 is highly cross-reactive against

NiV—the other member of this lethal group of emerging paramyxoviruses—broadens the impact of this new research and suggests its efficacy against henipaviruses as a group.

Bossart *et al.* (1) should be commended for a well-designed efficacy study that provides results that are relevant to a human therapeutic situation. The pharmacokinetic studies and the careful histological, viro-logical, and serological analyses of control and antibody-treated animals all contribute to a convincing study that demonstrates the *in vivo* efficacy of passive immunotherapy using m102.4. The authors' data clearly illustrate the dramatic reduction in viral-antigen load in the brain and lungs of monkeys treated with m102.4 on days 3 and 5 after HeV infection [fig. S2 in (1)]. It is also entirely satisfying to learn that the elimination half-life of m102.4, a human IgG1 mAb, is much longer in nonhuman primates than in ferrets (~11 days in NHP versus ~2 days or fewer in ferrets). Not surprisingly, the longer elimination half-life of m102.4 in AGMs compared to ferrets translated into an extended therapeutic window. Thus, m102.4 protected the monkeys from HeV-mediated mortality even when given 3 days after virus exposure, while m102.4 protects ferrets only when given within 10 hours of virus (NiV) exposure (1, 7).

Inspection of the data also revealed that sterilizing immunity might not be a necessary goal of passive immunotherapy, at least for HeV infections, as long as the mAb therapy provides the host with a window of opportunity to mount its own protective adaptive immune response. Thus, even though animals treated at the latest time points (days 3 and 5 postinfection) began to show neurological signs of infection starting at day 7 and appeared to deteriorate over the next 10 days, they eventually recovered in coincidence with a sharp rise in anti-F antibody titers around day 20. This observation also raises the question of how HeV replication is controlled in the central nervous system if, as one presumes, IgG molecules don't normally distribute efficiently across the blood-brain barrier.

SOCIETAL IMPLICATIONS

The current study raises a host of scientific, economic, and public policy questions regarding the development, distribution, and use of mAb-based therapies for severe viral illnesses, especially those caused by “orphan” viruses; these are usually emerging viruses such as NiV and HeV that cause a disease prevalence too low for mAb-based therapies to be economically viable. As such, orphan virus-directed mAb therapies tend not to be adopted for development by traditional pharmaceutical or large biotechnology companies. Ironically, the high specificity of mAb-based therapies also precludes their individual applicability to a broader spectrum of viruses, further constraining the market potential of any particular mAb-based therapeutic. An interesting exception is bavituximab, which targets externalized phosphatidylserine on a variety of virion membranes and virus-infected cells and currently is in clinical trials for the adjunctive treatment of hepatitis C virus infections.

mAb therapy has undergone a renaissance since the turn of the century, with more than 20 FDA-approved therapies (in a market of \$20 billion in 2010), mostly for treating cancer and autoimmune conditions (8). Interestingly, the only FDA-approved mAb treatment for an infectious disease is palivizumab, a humanized mAb against the F protein of respiratory syncytial virus (RSV)—a distantly related paramyxovirus from a subfamily (*Pneumovirinae*) different from henipavirus.

The authors of the current study must find it comforting that m102.4 compares well with palivizumab in terms of dosage and efficacy. For example, serum concentrations of palivizumab of 40 µg/ml reduce pulmonary viral load 100-fold in the cotton rat model. Viral load reduction of a similar magnitude (1 to 2 days postinfusion) was also seen in tracheal aspirates of RSV-infected pediatric patients treated intravenously (IV) with 15 mg/kg of palivizumab (9). Similarly, m102.4 was used at ~20 mg/kg IV in the NHP model and

maintained serum concentrations of at least 500-fold above its in vitro IC₅₀ (50% inhibitory concentration; 0.6 µg/ml) for no fewer than 5 days after the final dose in the various treatment groups (1). Tissue viral load was negligible in m102.4-treated animals compared to the widespread dissemination seen in untreated control animals. Thus, m102.4 appears to be at least as effective against HeV as palivizumab is against RSV in reducing tissue viral load. m102.4 likely will do equally well against NiV in the NHP model, as it binds to NiV-G with a higher affinity and neutralizes NiV at a lower IC₅₀ (0.04 µg/ml) relative to HeV (6).

Despite the impressive efficacy of mAb therapy for acute HeV (and likely NiV) disease in the NHP model, the development of mAb therapies for other severe viral illnesses will in all likelihood remain a highly empirical endeavor. For example, when tested on Vero cells, the KZ52 human mAb against the Ebola virus glycoprotein (GP) has an in vitro IC₅₀ (0.05 to 0.3 µg/ml) comparable to that of m102.4 for NiV and HeV (0.04 to 0.6 µg/ml) (6). KZ52 even showed efficacy in the guinea pig model when administered (25 mg/kg) up to 1 hour postchallenge with guinea pig-adapted Ebola virus. However, in the NHP model, the KZ52 mAb given at 50 mg/kg IV showed no protection and had no effect on viral load despite a confirmed serum concentration of KZ52—at the time of viral challenge—that was 100 times the mAb's in vitro IC₉₀ (10).

This puzzling dichotomy highlights the importance of understanding the host-specific nature of viral pathogenic events and of clearly identifying that neutralizing antibodies are a bona fide immune correlate of protection. In the case of Ebola virus infections, the protective efficacy of antibodies elicited by prior vaccination may differ qualitatively from those that are transferred by passive immunization at the time of viral challenge. The former is integrated with other aspects of the adaptive immune system already primed to deal with an Ebola virus challenge, whereas the latter functions passively in relative isolation. In addition, the effector functions of the antibody Fc region may play unexpected roles in vivo that are not apparent in vitro. Moreover, because field isolates of Ebola virus only establish productive infections in primates, small-animal challenge studies are usually performed with extensively adapted viruses, further confounding the translation of efficacy studies between small animal and NHP models. In the context of passive mAb-based immunotherapy, our understanding of viral pathogenesis and immunology currently is insufficient to predict which antibodies would be most efficacious against which pathogen targets. The success of m102.4 for postexposure immunotherapy treatment of HeV disease versus the dichotomous results from the Ebola virus experience underscores the importance of validating small-animal efficacy studies in NHP models.

Now, the obvious question is who will bear the cost of actually bringing m102.4 to the small market that henipavirus disease constitutes. The total number of confirmed human cases of HeV infections is no more than a dozen since the discovery of HeV in 1994, while the total number of NiV cases may come close to 500 (with the original NiV outbreak in Malaysia and Singapore in 1999 accounting for slightly more than half of that number). Into this calculus come the following questions: What is the target population that will benefit from m102.4's efficacy as a post- or even preexposure prophylactic therapeutic? Is producing and stockpiling a biologic for a lethal but rare emerging viral disease economically tenable? Will Bangladeshi villagers and exposed veterinary personnel in Australia have equal access to mAb therapy? How does the apparent success of mAb-based therapy against henipaviruses (category C “priority pathogens”) affect the therapeutic strategies being developed for other viral priority pathogens? Indeed, how does a society prioritize among the long list of category A, B, and C priority pathogens?

Difficult questions, yes. But in a world with increasingly limited resources and exponentially increasing demands, we must come to an agreement on these issues as a

society. The excellent science reported by Bossart *et al.* can at least help us get started by providing reliable information with which to fuel policy discussions.

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Fig. 1. Bat-Man

Proposed zoonotic transmission events that led to outbreaks of Nipah (Bangladesh and Malaysia) and Hendra virus (Australia) infections. Fruit-eating bats belonging to the *Pteropus* genus (known as flying foxes) are the natural reservoir for Nipah and Hendra viruses. Pigs and horses are the intermediate-amplifying hosts for Nipah and Hendra viruses in Malaysia and Australia, respectively. In Bangladesh, direct bat-to-human transmission and human-to-human transmission account for the almost annual outbreaks of Nipah virus.