

Scientists Create New Animal Model for Parainfluenza Virus-5 Infection

Alan Cocchetto, NCF Medical Director

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A scientific team, from Northwestern University, has just published new research in the prestigious medical journal *Virology* that may ultimately prove to be the first animal model for CFIDS/ME, a disease that affects millions of people here in the U.S. The group's publication was formally titled "Enabled interferon signaling evasion in an immune-competent transgenic mouse model of Parainfluenza Virus-5 infection."

Background:

In March of 2006, the National CFIDS Foundation contacted Robert Lamb, Ph.D., Sc.D., at Northwestern University, to potentially assist the Foundation with its ongoing research efforts that revolved around Parainfluenza Virus-5 (PIV-5) infection and its direct effect on STAT1, a key protein responsible for host innate immunity. Dr. Lamb, who is the John Evans Professor of Molecular and Cellular Biology and Investigator at the Howard Hughes Medical Institute, is one of this country's preeminent research scientists in the field of virology. He is a past president of the American Society for Virology, a member of the National Academy of Sciences and a Fellow of the American Academy of Arts and Sciences. In addition, he is a co-author of *Field's Virology*, a book considered to be the 'bible' for virologists. The NCF approached Dr. Lamb primarily because his research laboratory investigates the molecular structure and the mechanism of replication of PIV-5.

According to Dr. Lamb, parainfluenza viruses "cause many biologically and economically important diseases of humans and lower animals." As such, "most viruses induce interferons, cytokines that defend the cell from a virus infection. Thus, most viruses encode proteins that defeat either induction of interferon synthesis or prevent the induction of transcription of cellular genes caused by interferons binding to their receptors." In the case of PIV-5, its "V protein mediates the proteasome-mediated degradation of the transcription factor STAT1 (signal transducer and activator of transcription). We are examining the pathway by which the V protein mediates STAT1 destabilization." To the NCF, Dr. Lamb's research was critically important due to the fact that acquired STAT1 deficiencies had been previously identified in CFIDS/ME patients by three research groups worldwide. These included Kenny DeMeirleir, M.D., Ph.D., who is Chief Scientist with R.E.D. Laboratories in Belgium and Co-editor of the *Journal of Chronic Fatigue Syndrome* as well as a member of the Board of Directors for the International Association for Chronic Fatigue Syndrome (IACFS); Robert Suhadolnik, Ph.D., who is Professor of Biochemistry at Temple University School of Medicine in Philadelphia; and Donald Carrigan, Ph.D. and Konstance Knox, Ph.D., who are founding scientists of the Institute for Viral Pathogenesis in Milwaukee, whose STAT1/PIV-5 research was directly funded by the NCF and whose STAT1 research was formally presented at an American Association for Chronic Fatigue Syndrome (AACFS) conference in 2004.

Meetings between Dr. Lamb and another Northwestern University scientist, Dr. Curt Horvath, had taken place shortly after the NCF's research data had been thoroughly reviewed. It was then determined that a postdoc would be assigned as a liaison to the NCF and to pursue some initial research studies that would be completed by the Horvath Laboratory at Northwestern. The postdoc assigned to the NCF's efforts was Thomas Kraus, Ph.D. whose research for this work was funded by a pilot grant from the Evanston Northwestern Healthcare Medical Group.

Curt Horvath, Ph.D., has his own laboratory at Northwestern that focuses on signal transduction and gene expression in mammalian cells. The NCF had prior communications with Dr. Horvath when he held a position at Mount Sinai School of Medicine in New York. Since the formation of his new lab, his research group now explores the specific biology of STAT proteins. Because STAT proteins have a tight relationship with interferons, there is an important interplay between an infection by a pathogen and the subsequent cellular defensive response by the host's immune system. Simply stated, Dr. Horvath's group explores how viruses evade the host interferon (IFN) response. His group does this through the use of parainfluenza viruses. More specifically, these scientists utilize one member of the parainfluenza virus family, PIV-5, to learn how this rubulavirus directly targets and degrades STAT1 to evade the immune system of the host. This is worth noting since preliminary research studies by the NCF and others had identified PIV-5 infections in CFIDS/ME patients.

Newest Research:

According to this latest paper, "The co-evolution of viruses with their hosts has resulted in diverse mechanisms to subvert the anti-viral immune system. Understanding how these virulence factors affect viral pathogenesis is a critical first step in identifying targets for pharmaceutical intervention or vaccine development. The paramyxovirus PIV-5 has been implicated in upper respiratory tract disease and has been isolated from the cerebral spinal fluid of a dog with posterior paralysis. In humans, PIV-5 has been linked to both multiple sclerosis and chronic fatigue and immune dysfunction syndrome, although direct causative associations are poorly understood. PIV-5 can persistently infect cells and has been isolated from several cultured cell lines. The observation that humans are one of the favored hosts for PIV-5 replication is linked to the virus's efficacy in blocking type I and type II interferon signaling as a result of the specific degradation of the interferon signaling molecule, STAT1. PIV-5 induced STAT1 degradation requires the actions of a single viral protein known as V."

The authors continue, "To validate these observations in an intact animal, and as a means to establish a model system to better understand how the ability to block IFN signaling affects viral pathogenesis in vivo, a transgenic mouse...was created. These mice are normal by all criteria, but unlike WT (wild type) mice, infection with PIV-5 induces loss of STAT1 and inhibition of IFN signaling, recapitulating the phenotype observed in cultured cells. Furthermore, results indicate that the enabled IFN signaling inhibition is advantageous to virus replication in vivo, as the lungs of PIV-5 infected transgenic mice contain more virus than wild-type mice. The increased viral load resulted in a coordinate increase in the expression of inflammatory signaling proteins....This confirms that the ability to limit PIV-5 replication is impaired in the transgenic mouse."

One of the conclusions that these authors reach is that these results "support the conclusion that PIV-5 mediated STAT1 degradation in the lungs of the transgenic mice can dampen the innate antiviral response and allow for unrestricted viral replication....these experiments show that viral IFN evasion of PIV-5 results in an immediate increase in viral replication at the site of primary infection followed by an increase in a local inflammatory response."

Discussion:

The authors state, "PIV-5 is a zoonotic virus found in humans whose pathogenesis is uncertain. In immuno-competent mice, PIV-5 appears to be non-pathogenic. However, because its normal IFN evasion strategies are compromised, efficient replication of the virus is restricted from mice....In the...transgenic mice described here, PIV-5 is better able to recapitulate the human infection due to the enabled block in IFN signaling, a critical parameter for viral pathogenesis."

Continuing their discussion, "In this unique animal infection model, IFN signaling and antiviral responses remain intact, providing the ability to examine virus replication in a more natural context of a fully immune-competent host. This situation differs greatly from strategies used previously to investigate PIV-5 immune responses and pathogenesis. In previous reports, immune compromised mice were used to study the immune response to and pathogenesis of PIV-5 infections. When SCID (severe combined immune deficiency) mice were used, PIV-5 infection resulted in a short-term weight loss and efficient recovery from infection, leading to the conclusion that the adaptive immune system is not vital to the antiviral immune response to PIV-5. Indeed these studies were the first to demonstrate the importance of IFN responses in controlling PIV-5 infection. Later studies took advantage of mice harboring a deficiency in the STAT1 gene. In this situation, the host is systemically deficient in responses to both type I and type II IFN's, causing dramatic consequences on innate and adaptive immunity. Infection of STAT1 deficient mice with PIV-5 results in 100% mortality, dramatically different from the outcome of any natural PIV-5 infection reported. These data prove that the virus can replicate efficiently in mice, and that the innate immune response is critical. However, a drawback to these immune-compromised mouse experiments is that it is not possible to determine the natural progression of pathogenesis during infection or evaluate the contributions of host responses to viral pathogenesis. Based on our analysis...during a natural PIV-5 infection, we expect that STAT1 would be degraded in the infected cells, leaving the STAT1-dependent immune response of non-infected cells intact."

Conclusion:

The authors comment that "Although PIV-5 does not robustly infect murine (rat/mouse) cells for reasons that may include differences in receptor binding and membrane fusion as well as immune effects, low level persistent infections can be established in cultured mouse cells....It is quite interesting to note that in infected transgenic mice, where IFN evasion has led to an early increase in viral load, there is a parallel increase in inflammatory response. Based on these

findings, it is tempting to speculate that PIV-5 mediated IFN evasion offers only a short-lived advantage to the virus, and that clearance by the host immune system occurs efficiently with or without IFN evasion. In other words, the consequence of IFN evasion for PIV-5 is an increase in the viral load at the primary site of infection, providing a short-term increase in viral load that also may heighten the chance of horizontal transmission. Further experimentation is required to test this concept. PIV-5 has long served as a prototypic member of the larger Paramyxovirus family. This family of viruses includes re-emerging viruses like measles and mumps, as well as newly emerging deadly viruses Hendra and Nipah virus. All of these pathogenic paramyxoviruses express V proteins which inhibit IFN responses. In all cases, the consequence of IFN evasion has only been examined in vitro. The...transgenic model described here provides an excellent experimental system to probe the consequences of innate immune evasion during infection of an intact host organism. The data reported here lay the foundation to study the role that IFN evasion has on adaptive immune responses, viral pathogenesis, disease progression, virus clearance and virus transmission."

Summary:

- * PIV-5 is a zoonotic virus (communicable from animals to humans under natural conditions)
- * PIV-5 has been linked to CFIDS and MS
- * New mouse model created for PIV-5 infection
- * PIV-5 induces the loss of STAT1 in infected cells in-vivo
- * PIV-5 appears to be capable of producing persistent infection
- * PIV-5 causes an increase in inflammation
- * PIV-5 infection may increase horizontal transmission
- * Mouse model provides new platform to study PIV-5 pathogenesis including pharmaceutical targets (antivirals, disease intervention/antagonism, etc.)

In closing, the National CFIDS Foundation wishes to express their utmost appreciation to Dr. Robert Lamb for his kind discussions and suggestions; to Dr. Thomas Kraus for his patience and enthusiasm regarding NCF research; and to Dr. Curt Horvath who has offered guidance and expertise regarding the Foundation's ongoing efforts. This research truly reinforces what an old Chinese proverb says: "It is possible to move a mountain by carrying small stones." This animal model should prove to greatly assist the medical community at large with their understanding of the CFIDS/ME disease process and hopefully will also provide patients with the validation that a persistent viral infection with PIV-5 may ultimately form the fundamental basis for this disease.

Reference:

Enabled interferon signaling evasion in an immune-competent transgenic mouse model of parainfluenza virus 5 infection; Kraus TA, Garza L, Horvath CM; *Virology*, 2007 Oct 25 [Epub ahead of print]