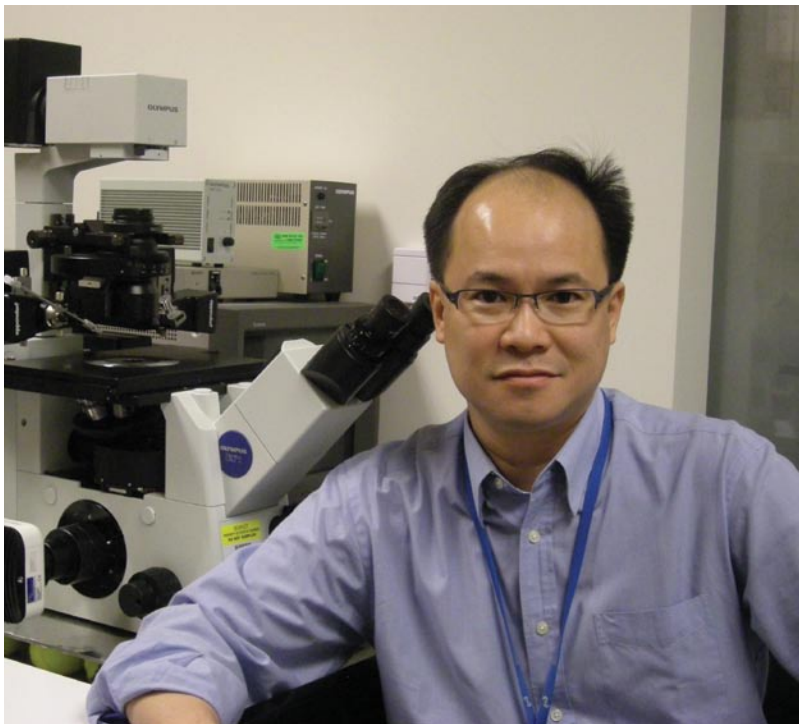
New Opportunity to Better Understand Huntington's Disease

New primate model may help scientists to develop more effective therapies for Huntington's disease and create similar primate models for other genetic disorders. **BY KAREN EDDLEMAN**

Although strides have been made in understanding neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's diseases, a lack of useful animal models has stood in the way of advancing research that might lead to effective treatments or cures. Now — for the first time ever — a team of researchers has successfully introduced a gene for a human disease into a primate, creating an animal model that shows disease progression and symptoms characteristic of human Huntington's disease, an incurable and inherited genetic disorder affecting the brain. This advance was achieved by a team of scientists led by Anthony W.S. Chan, principal investigator, at the NCRR-supported Yerkes National Primate Research Center (NPRC), Emory University, one of eight primate research centers supported by NCRR. Chan and his colleagues hope the new animal model will herald a new age in Huntington's disease research and ultimately help lead to a cure.

As reported in the journal *Nature* (453:921–924, 2008), Chan's team introduced a gene that can cause Huntington's disease in humans (an altered form of the gene *HTT*) into the germline DNA of a rhesus macaque monkey (*Macaca mulatta*), resulting in what is termed a transgenic animal model. Although scientists identified the precise location of the *HTT* gene 15 years ago and have engineered rodents and other animals to carry the altered *HTT* gene, research has been hampered because these models do not experience the same brain and behavioral changes as observed in humans.



■ Anthony Chan heads the team at the NCRR-supported Yerkes National Primate Research Center that developed the first monkey model genetically modified to have a human disease.

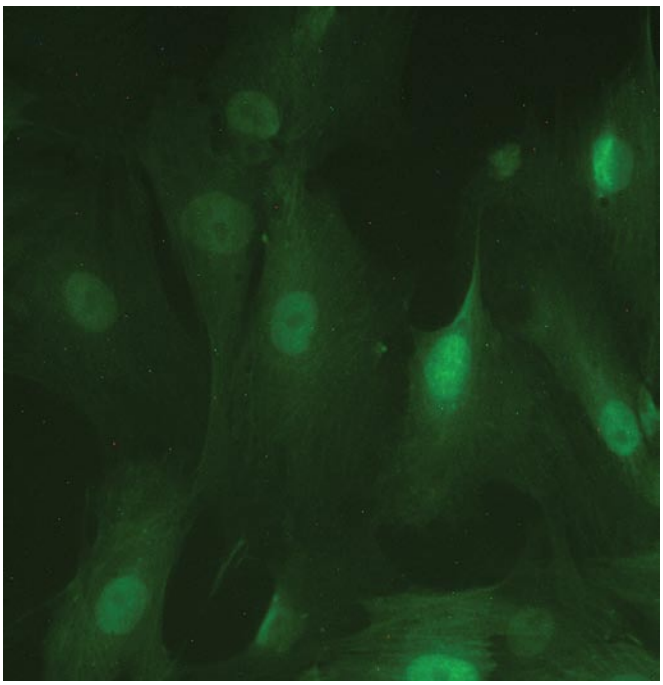
Huntington's disease occurs in one of every 10,000 persons — nearly 30,000 in the United States — and about 150,000 more are at risk of inheriting the disease from a parent. It causes uncontrolled movements and stumbling, short-term memory loss, depression, mood changes, and sometimes aggressive or

PHOTO BY KAREN W.Y. CHONG

> ... Chan's team introduced a gene that can cause Huntington's disease in humans ... into the germline DNA of a rhesus macaque monkey ... resulting in what is termed a transgenic animal model.

antisocial behavior. The disease inevitably leads to death 15 to 20 years after symptoms appear, usually in middle age. It is one of several diseases caused by abnormal repetition within the DNA of the *HTT* gene, which codes for a protein called huntingtin. The modified form of the huntingtin protein contains a section with extra glutamine amino acids; it causes cells to die in certain areas of the brain, affecting neurological functions. Currently, there is no treatment to delay or prevent Huntington's disease.

Now, research into Huntington's disease has been jumpstarted by Chan's work, which was supported by NCRR and the National Institute of Neurological Disorders and Stroke. Because the transgenic macaque model developed at the Yerkes NPRC shows many of the symptoms and the disease progression seen in humans with Huntington's disease, it offers the possibility of testing innovative therapies intended to ameliorate disabling symptoms and perhaps extend the lives of Huntington's patients.



■ A tissue culture established with the bone marrow stem/stromal cells from a transgenic Huntington's monkey emits a greenish light when viewed under a fluorescent microscope. The cells glow because they express a jellyfish gene encoding for green fluorescent protein, which is a marker for incorporation of the *HTT* gene into the macaque DNA.

In 2001, Chan, while working at the NCRR-funded Oregon NPRC, successfully introduced a jellyfish gene for green fluorescent protein (GFP), creating the world's first transgenic nonhuman primate. According to Chan, "The next step was to try to insert a human gene that causes disease."

In 2002, Chan joined the team at the Yerkes NPRC, where studies of neurodegenerative disorders, including Alzheimer's and Parkinson's diseases, are a main focus. "Dr. Chan is one of the very few investigators who could do this work. He has experience in manipulating monkey embryos," says John Harding, director of primate research at NCRR. Chan's research is critical, in his view, "because rodent models cannot give the answers we need; we have to rely on nonhuman primates, which are physiologically very similar to humans."

The study team developed this transgenic monkey model by introducing a genetically altered human *HTT* gene as well as the jellyfish *GFP* gene (used as a marker to show that gene transfer has been achieved) into monkey eggs using a viral vector. The eggs were fertilized, and the resulting embryos were introduced into surrogate mothers, resulting in five live births. All of the monkeys were shown to have the modified human *HTT* gene incorporated into the DNA of all of their cells, and three expressed very high levels of the mutant huntingtin protein.

The Yerkes NPRC was the ideal setting for this work. NCRR's eight NPRCs together have more than 26,000 animals representing more than 20 species of nonhuman primates, mostly macaques. Research studies at these facilities are tackling questions about human health and disease that cannot be assessed ethically in humans or answered in other species. "The Yerkes NPRC is one of the few places in the world where scientists can find expertise in neurobiology and nonhuman primate transgenesis, noninvasive imaging technology, husbandry, and behavioral and cognitive assessment tools in one place," says

Continued on page 15

PHOTO BY ANTHONY CHAN

Mass Producing Antibodies

Supported by an IDeA grant, researchers find a way to rapidly generate human monoclonal antibodies to potentially treat the flu and other infectious diseases. **BY LAMONT WILLIAMS**

The human immune system is a formidable wall of defense against bacteria and viruses, but it is not impenetrable and is always a work in progress. Recently, at the Oklahoma Medical Research Foundation (OMRF), a team of researchers supported by NCRR's Institutional Development Award (IDeA) program found a way to help fortify the immune system like never before. "Scientists have been searching for technology like this for 25 years," says immunologist J. Donald Capra, president emeritus of OMRF and former principal investigator of the IDeA grant that funded this work.

First, the research team, led by Patrick Wilson at OMRF in collaboration with Rafi Ahmed of Emory University, made the important discovery that there is a rapid production of antibody-secreting plasma cells in patients following vaccination for influenza in which the antibodies produced have a strong affinity for the vaccine. Armed with this knowledge, they devised a way to isolate a substantial population of these immune cells, which can be used to quickly produce what are called human monoclonal antibodies. Monoclonal antibody therapy has been shown to be useful in the treatment of a variety of diseases. "Vaccines may not give full protection and may cause adverse side effects in some patients," Wilson says. "Human monoclonal antibody therapy will likely be more effective and has a low risk of being rejected by a patient's immune system."

The method devised by Wilson and his colleagues allows them to identify and isolate specific antibody-secreting cells from people who have previously been administered an influenza vaccine. They can then clone the antibody genes from those cells and use those genes to quickly produce an abundance of antibodies to the particular strain of influenza virus the vaccine was designed to fight. The antibodies can potentially be used in patients to augment the treatment of the deadly illness; one that claims more than 25,000 Americans every year.

Patrick Wilson (pictured) and colleagues have discovered a method to create human monoclonal antibodies directly and within only a few weeks of vaccination.

Although researchers have known that human monoclonal antibody therapy can treat a multitude of diseases effectively, it has not been widely used because of the enormous time and expense needed to generate the antibodies. Monoclonal antibodies can be made by using mice, but these antibodies are often

PHOTO BY OKLAHOMA MEDICAL RESEARCH FOUNDATION

> "Scientists have been searching for technology like this for 25 years."
>
> —IMMUNOLOGIST J. DONALD CAPRA

not compatible with human physiology, causing illness in some patients. The antibodies produced by Wilson and colleagues are fully human, circumventing such problems, and they have demonstrated that they can produce them quickly. "We have shown that we can create human monoclonal antibodies from antibody-secreting cells directly and within only a few weeks of vaccination," Wilson says.

NIH laid the foundation for this success story more than 15 years ago through the establishment of the IDeA program. Administered by NCRR's Division of Research Infrastructure, the IDeA program is designed to foster health-related research at institutions in states where per capita NIH funding historically has been low. IDeA grants offer junior investigators research opportunities, support faculty development, enhance research infrastructure, and increase the number of competitive investigators in 23 states and Puerto Rico. "Efforts and success stories similar to that of Wilson and his team also are occurring in other IDeA states, with regard to other research challenges," says Fred Taylor, NCRR's IDeA program director.

One component of the IDeA program is the Centers of Biomedical Research Excellence (COBRE) initiative, which facilitates the development of new disease-specific research centers or augments the capability of existing centers. Wilson was supported by a COBRE grant entitled "Mentoring Immunology in Oklahoma: A Biomedical Program." Institutions in the IDeA network have very limited funds to establish research centers and, in particular, recruit new investigators. The COBRE program provides funds for these efforts. "Without the program, Wilson may not have gotten in the door," Capra says. "The COBRE provided funds for his recruitment and for pilot projects and, in general, gave him the freedom to operate in a way that he would likely not have if the COBRE did not exist."

Another contributing factor to Wilson's success, Capra says, was the core facilities at OMRF, many of which were largely established by COBRE funds. The program supports infrastructure, such as these core facilities, that most institutions in IDeA states cannot afford. As a COBRE investigator at OMRF, Wilson had access to these cores, where he could have such work as DNA sequencing, cell sorting, and microscopy done inexpensively and quickly. "The COBRE program helped me to hit the ground running," Wilson says. "The cores were a central element of my success." Capra notes that his successor, Steve Prescott, used institutional funds to establish a new core at OMRF to perpetuate Wilson's monoclonal technology. "Dozens of scientists at OMRF and surrounding institutions like the University of Oklahoma Health Sciences Center are using the technology in their own research areas," Capra says.

Although the COBRE is designed to establish biomedical research centers at institutions that historically have been at a funding disadvantage, the ultimate goal is to train young investigators at these institutions to become leaders in critical areas of research with independent funding support. "To a large extent," Capra says, "the investigators recruited through our COBRE have remained at the institution to stay close to the mentors they obtained by way of the program. This is another success of the program."

Wilson's findings have important implications for therapy, not only for influenza, but for a broad range of infections and even cancer. Currently, he and his colleagues are using the same approach to isolate human monoclonal antibodies to hepatitis C, pneumococcal pneumonia, anthrax toxin, and yellow fever. "There is a plethora of emerging infectious diseases to which this technology may be applied," Wilson notes. "In the case of a highly virulent virus, we now possess the technology to quickly and efficiently produce antibodies that might be useful in treatment." In addition to immunology, the COBRE is enabling critical research in heart disease, diabetes, and dozens of other health challenges. ■

The research described in this article is supported by the National Center for Research Resources (NCRR) and the National Institute of Allergy and Infectious Diseases. One investigator on the research team was supported by the Swedish Research Council. The NIH Institutional Development Award (IDeA) program and its Centers of Biomedical Research Excellence are supported by the Division of Research Infrastructure of NCRR. For more information about the IDeA program, see www.ncrr.nih.gov/idea.

ADDITIONAL READING: Wrammert, J., Smith, K., Miller, J., et al., Rapid cloning of high-affinity human monoclonal antibodies against influenza virus. *Nature* 453:667–671, 2008.