



Scientists Confirm Discovery of New Virus Responsible For Deaths of Three Transplant Recipients From Single Donor in Victoria, Australia

In the first application of high throughput DNA sequencing technology to investigate an infectious disease outbreak, scientists from the Mailman School, the Victorian Infectious Diseases Reference Laboratory (VIRDL) in Melbourne, Australia, the Centers for Disease Control, and 454 Life Sciences link the discovery of a new arenavirus to the deaths of three transplant recipients who received organs from a single donor in Victoria, Australia in April 2007. The full findings (<http://content.nejm.org/cgi/content/full/NEJMoa073785>) are published in the March 2008 issue of the *New England Journal of Medicine* and are now online.

After failing to implicate an agent using other methods including culture, PCR, and viral microarrays, RNA from the transplanted liver and kidneys was analyzed using rapid sequencing technology established by 454 Life Sciences and bioinformatics algorithms developed at the Mailman School. Examination of tens or thousands of sequences yielded 14 that resembled arenaviruses at the protein level. Thereafter, the team cultured the virus, characterized it by electron microscopy and developed specific molecular and antibody assays for infection. The presence of virus in multiple organs, IgM antibodies in the organ donor, and increasing titer of antibody in a recipient were used to implicate the virus as the cause of disease. The arenavirus lymphocytic choriomeningitis virus (LCMV) has been implicated in a small number of cases of disease transmission by organ transplantation, however, the newly discovered virus, which may be a new strain of LCMV, is sufficiently different that it could not be detected using existing screening methods.

"High throughput sequencing and methods for cloning nucleic acids of microbial agents directly from clinical samples offer powerful tools for pathogen surveillance and discovery," stated W. Ian Lipkin, MD, John Snow Professor of Epidemiology and professor of Neurology and Pathology at Columbia University and director of the Center for Infection and Immunity at the Mailman School of Public Health. He added, "As globalization of travel and trade brings new infectious agents into new contexts, speed and accuracy of pathogen identification are increasingly important when it can alter treatment, assist in containment of an outbreak, or, as in this case, enable improvements in screening that will enhance the safety of transplantation."

Last spring, scientists from the Victorian Infectious Disease Reference Laboratory contacted Dr. Lipkin after their initial state-of-the-art investigation into the cause of the transplant patient deaths failed to turn up leads. Dr. Lipkin and his team built on their work, utilizing tools for pathogen surveillance and discovery developed at Columbia and 454 Life Sciences.

"The small pieces of viral genetic material recovered through this powerful high throughput sequencing method were used to design specific tests for detecting the virus in clinical samples and enabling detailed characterization." said Gustavo Palacios, PhD, first author of the paper and assistant professor in the Center for Infection and Immunity at the Mailman School. Surveys at Columbia and the VIRDL revealed that viral RNA was present in a total of 22 out of 30 samples of tissue, blood, or cerebrospinal fluid from all three recipients, and the sequencing was identical in all samples, which is consistent with the introduction of a single virus into all transplant recipients. PCR surveys of other stored plasma specimens from solid organ transplant recipients in the same city and timeframe not linked to the cluster, revealed no evidence of infection with this pathogen. Sherif Zaki and colleagues at the CDC demonstrated the presence of the viral proteins in organs of recipients using antibodies to LCMV, and provided the first pictures of the virus by electron microscopy.

Dr. Lipkin and his team have demonstrated that this technology can be employed to address a wide variety of suspected infectious disease outbreaks. Examples of the successful application of molecular technologies in infectious diseases include the identification of Borna disease virus, Hepatitis C virus, West Nile virus, and SARS coronavirus, among others.

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