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REVIEW

A Systematic Approach to Novel Virus Discovery in Emerging Infectious Disease Outbreaks

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The discovery of novel viruses is of great importance to human health—both in the setting of emerging infectious disease outbreaks and in disease syndromes of unknown etiology. Despite the recent proliferation of many efficient virus discovery methods, careful selection of a combination of methods is important to demonstrate a novel virus, its clinical associations, and its relevance in a timely manner. The identification of a patient or an outbreak with distinctive clinical features and negative routine microbiological workup is often the starting point for virus hunting. This review appraises the roles of culture, electron microscopy, and nucleic acid detection–based methods in optimizing virus discovery. Cell culture is generally slow but may yield viable virus. Although the choice of cell line often involves trial and error, it may be guided by the clinical syndrome. Electron microscopy is insensitive but fast, and may provide morphological clues to choice of cell line or consensus primers for nucleic acid detection. Consensus primer PCR can be used to detect viruses that are closely related to known virus families. Random primer amplification and high-throughput sequencing can catch any virus genome but cannot yield an infectious virion for testing Koch postulates. A systematic approach that incorporates carefully chosen combinations of virus detection techniques is required for successful virus discovery. *(J Mol Diagn 2015, 17: 230–241; http://dx.doi.org/10.1016/j.jmoldx.2014.12.002)*

The Importance of Virus Discovery

Novel viruses are important causes of emerging infectious diseases, as illustrated by multinational outbreaks of severe respiratory illness due to two novel coronaviruses in recent times: severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus. Such outbreaks entail considerable public health concern, requiring investigators to discover the infectious agent in a timely manner by efficiently using virus diagnostic tools.

Even outside outbreak settings, many clinical syndromes encountered by clinicians on a daily basis, such as coryza, pneumonia, diarrhea, meningoencephalitis, hepatitis, epididymo-orchitis, and sialadenitis, have no identifiable infectious etiology, raising the possibility of infections by as-yet undiscovered pathogens. At the present time, >200 viruses are known to cause human disease; extrapolation of recent trends anticipates that pathogenic virus discovery is likely to continue unabated

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in the near future.[1] Given the importance of microbial stimuli in determining short- and long-term immunological stimulation, it is conceivable that some of these undiscovered viruses might even contribute to autoimmune, degenerative, and neoplastic conditions of currently unknown etiology.[2] Novel viruses may also play a role in the etiology of common bacterial infections by impairing local and systemic immune mechanisms—a phenomenon that is frequently noted in viral infections, such as measles.[3]

The importance of discovering viruses that play an etiological role in infectious disease outbreaks and clinical syndromes transcends academic interest. If direct causality between the novel virus and the disease syndrome is established by clinical associations and animal models, then further study of the novel virus is indicated for defining specific diagnostic, therapeutic, and preventative measures. This was illustrated by the discovery of the SARS-CoV, which paved the way to an understanding of the origins of the outbreak, specific control measures, diagnostics, and therapeutic initiatives. Discovery of novel viruses may also carry important implications for blood product, organ transplant, bioterrorism, and laboratory safety. Furthermore, the experience with smallpox has shown that viruses and the diseases they cause may be eradicated by global health initiatives. Therefore, rigorous investigation for novel viral agents is of considerable importance to human health.

The Steps in Virus Discovery

Case Identification and Selection

The process of virus discovery begins with case selection (namely, the identification of a patient or a group of patients with distinctive clinical features that could represent infection by a novel virus in a population with little herd immunity) (Figure 1). Such distinctive features may include unusually severe clinical presentations, an increased incidence of the disease syndrome above the baseline, uncommonly seen radiological features, and/or idiosyncratic laboratory test results. Patients may report unusual dietary, environmental, zoonotic, sexual, or travel-related exposures that alert the clinician to the possibility of a rare or new pathogen. The emerging novel virus may also manifest as an outbreak of illness with no identifiable microbial cause. A thorough history, physical examination, and basic laboratory results will enable the formulation of a precise case definition, and facilitate case finding and outbreak investigation. Looking for potentially affected cases among close contacts (household, occupational, nosocomial, and sexual contacts) must always be considered because it provides evidence for transmissibility of the disease, indicating an infectious etiology. In addition to infectious disease syndromes, viruses play a direct carcinogenic role in many neoplasias; infectious etiologies should be considered in neoplasias occurring in immunocompromised patients[4] (eg, HIV-positive or post-organ transplant patients) or in defined geographical areas.

Scenario 1: Discovery of SARS-CoV

In 2002, rumors of an outbreak of fatal pneumonia of unknown cause were reported in southern China by the mass media and later confirmed by government officials. Affected individuals were often linked in time and nosocomial transmission chains, with some early cases reporting an occupational exposure to wild caged animals in markets and restaurants. Case definitions and outbreak information widely disseminated over the World Wide Web enabled the efficient identification of SARS patients by health care workers. Research efforts culminated in the cell culture isolation (discussed in detail later) of the novel SARS-CoV from two such patients presenting with severe pneumonia.[5]

Scenario 2: Discovery of Hepatitis E Virus

A major epidemic of waterborne icteric illness occurred in the Kashmir valley of India from 1978 to 1979. In a resource-poor setting with minimal access to serological confirmation, the epidemic was initially attributed to hepatitis A. However, the unusual severity of the illness in pregnant women, a feature not associated with hepatitis A infection, prompted investigators to perform extensive clinical and serological surveillance of affected patients. This led to the recognition that patients in the epidemic did not have any serological evidence of recent hepatitis A infection. Case definitions and clinical description of the novel non-A, non-B hepatitis entity enabled the recognition of similar patients globally, leading to the discovery of the hepatitis E virus from the stool of a human volunteer (discussed in detail later) through immunoelectron microscopy.[6]

Specimen Collection and Processing

Careful specimen collection from patients suspected to harbor novel viruses is crucial to their successful recovery. Clinical specimens must be of good quality and obtained serially throughout the course of illness to capture the novel virus at the time of peak viral load. Viral load dynamics vary, but in general, specimens that are collected early in the course of the illness just before the illness nadir are likely to contain a high viral load. The clinical syndrome often dictates the sites of specimen collection. For example, lower respiratory tract specimens are particularly valuable in patients presenting with pneumonia. However, specimen collection must not be limited to these sites alone because the detection of the novel virus at body sites distant from the focus of infection may illustrate important points regarding tissue tropism and routes of transmission of the new virus, as was illustrated by the high fecal viral loads of SARS-CoV. In general, respiratory tract aspirates, throat swabs, urine, feces, buffy coat, plasma, tissue biopsy specimens, vesicle fluids, and cerebrospinal fluid may all be considered for virus detection in appropriate clinical settings. In addition, acute and convalescent sera should always be stored to retrospectively identify an antibody response to the newly detected virus. Discovery of a novel virus in specimens does