Microbe hunting in the 21st century

W. Ian Lipkin[1]

Center for Infection and Immunity, Mailman School of Public Health of Columbia University and World Health Organization Collaborating Centre on Diagnostics, Surveillance and Immunotherapeutics for Emerging Infectious and Zoonotic Diseases, New York, NY 10032

A paper from Delwart and colleagues a recent issue of PNAS (1) affords an opportunity not only to discuss a new virus potentially linked to flaccid paralysis but also to review global efforts to eradicate poliovirus, the major cause of flaccid paralysis; recent advances in microbe hunting; and the potential complexity of proving causation when discovering novel microbes.

An Egyptian stone carving of a young man with a withered leg, dated 1300 BC, depicts what is considered to represent the first description of flaccid paralysis attributable to infection (Fig. 1). Although Von Heine recognized clinical poliomyelitis as an entity distinct from other forms of paralysis in 1840 (2), and several outbreaks of acute disease followed in Europe and the United States in the late 1800s (2, 3), an infectious basis was only first proposed by Wickman in 1905 (4) but not established until 1908, when Landsteiner and Popper demonstrated transmission in animal models (2–5). Poliovirus propagation in tissue culture by Enders, Robbins, and Weller, in 1948 (2), set the stage for vaccine development in independent efforts by Koprowski, Sabin (live attenuated virus vaccine), and Salk (inactivated virus vaccine) (6). Since its launch in 1988, the World Heath Organization (WHO) Global Polio Eradication Initiative has dispensed more than 10 billion doses of live attenuated vaccine, reducing the global incidence of poliomyelitis by >99% (7). Despite recent setbacks in Nigeria and India (8), there is cautious optimism that wild-type poliovirus will be eradicated. However, a new challenge has arisen with the emergence of neurovirulent vaccine-derived polioviruses: realization that the promise of poliovirus eradication will at minimum require a period of consolidation, during which vaccination using inactivated poliovirus continues (7).

Polioviruses are not unique in their potential to cause flaccid paralysis. Another enterovirus, enterovirus 71 (EV71), can too, albeit at lower frequency (9). First identified in the United States in 1969, EV71 is associated with neurological disease worldwide but most commonly in South Asia, where it has been implicated in epidemics of diarrhea, hand-foot-mouth disease, and paralytic illness (10). EV71 infection can also be asymptomatic. Indeed, a study of normal Norwegian infants found the prevalence of EV71 in stool to be ≈7% (11). There is no EV71 vaccine.

Fig. 1. Earliest known representation of limb atrophy presumed due to poliomyelitis. Egypt, 1403–1365 BC. Reprinted with permission from Ny Carlsberg Glyptotek.

In this issue Delwart and colleagues (1) describe the identification of a new virus in stool samples obtained from children in Pakistan under the auspices of the WHO Global Polio Eradication Initiative. The agent, a picornavirus like polioviruses and EV71, is provisionally named by using the acronym cosavirus (common stool-associated virus), designating the source of its discovery. Unlike polioviruses and EV71, cosaviruses have not yet been cultured. Cosaviruses were discovered by using purely molecular methods designed to reveal any nucleic acid in stool protected by the presence of a capsid. Although they were initially found in children with flaccid paralysis, subsequent analyses indicated that cosaviruses are found with similar frequency in stool of healthy children. Although this later work eliminates a simple relationship between infection and flaccid paralysis, it by no means excludes a causal correlation. Asymptomatic infections are also observed with poliovirus. Only 1% of poliovirus infections result in poliomyelitis (3).

The discovery of a microbe in association with disease is only the first step in establishing a causal relationship or understanding the mechanisms by which it causes disease. Pasteur, Koch, and Loeffler proposed criteria to define a causative relationship between agent and disease that evolved into what are now known as Koch's postulates. These included the presence of the agent in every case of a disease, specificity for that disease, and the capacity to cause the same disease in naïve hosts after propagation in culture. The criteria were modified by Rivers (12) for viruses, chiefly through consideration of adaptive immunity as evidence of infection, and by Fredericks and Relman (13) to reflect the introduction of culture-independent molecular methods. Although the original Koch's postulates remain the most compelling proof of causation, there are instances wherein neither they nor the modified criteria adequately address the needs of microbe hunters. Some agents cannot be cultured. There may be no animal model with which to test the potential of a candidate agent to reproduce the original disease. Specificity in clinical presentation is the exception rather than the rule. Infections with different microbes may result in similar signs and symptoms. As noted above, infections with both polioviruses and EV71 (and perhaps cosaviruses) can culminate in flaccid paralysis. Single-nucleotide mutations can have a profound impact on transmission and virulence. Manifestations of infection may also vary with genetic susceptibility, age, nutrition, and previous exposure to similar agents. Proving causation is particularly challenging in instances where microbes have effects that are remote in time or space, or require cofactors such as coinfection for expression. In many acute infectious diseases, the responsible agent is readily implicated because it replicates at high levels in the affected tissue at the time the disease is manifest, mor-

Author contributions: W.I.L. wrote the paper.

The author declares no conflict of interest.

[1]E-mail: wil2001@columbia.edu.

See companion article on page 20482 in issue 51 of volume 105.

© 2008 by The National Academy of Sciences of the USA

phological changes consistent with infection are evident, the agent is readily identified with classical or molecular methods, and there is evidence of an adaptive immune response. However, implication of agents may be difficult when classical hallmarks of infection are absent or mechanisms of pathogenesis are indirect or subtle. In these instances investigators must resort to statistical assessments of the strength of the epidemiological association, based on the presence of the agent or its footprints (nucleic acid, antigen, and preferably, an immune response) in hosts that are ill, and the biological plausibility indicated by analogy to diseases with related organisms, for which linkage is persuasive. Formal, definitive proof of causation may not be obtained until a specific intervention such as a drug or a vaccine is shown to prevent disease (14).

Cultivation-independent molecular methods for pathogen discovery have transformed microbiology and medicine. Whereas the discovery of bornavirus nucleic acids in the 1980s (15) demanded years of tedious and complex subtractive cloning, subsequent technical advances, including PCR, microarrays, and high-throughput pyrosequencing, have dramatically reduced the interval and expertise required for pathogen surveillance and discovery (14). Although exciting in its promise, this revolution is also daunting. 16S ribosomal RNA analyses of external and internal surfaces of humans are revealing a staggering diversity of prokaryotic microflora. We do not have comparable data for human viral microflora because there is no functional equivalent of an rRNA survey. Nonetheless, a conservative estimate of 50,000 vertebrate species, each harboring 20 endogenous viruses, predicts the presence of 1 million different viruses, >99.9% of them currently unknown. Many have the potential to cross species and cause disease in humans, livestock, or wildlife. How then should we proceed? A practical approach would be to focus our sights first on the low-hanging fruit, i.e., acute diseases for which the probability of identification of causative agents is high, and success would likely enable new strategies for intervention (e.g., pneumonia, meningitis, encephalitis, and infectious diarrheas). The efficiency and throughput of DNA sequencing and synthesis are increasing at a rate even faster than that projected by Moore's law for computing power (50% every 24 months) (16). Thus, it will not be long before the costs for sequencing genomes of hosts and pathogens drop to tractable levels, allowing us to capture and understand more complex nature–nurture interactions, including those that may pertain to cosaviruses and EV71. Perhaps the most difficult challenge will be that of obtaining and integrating population and exposure data before the onset of disease so that we can examine the effects of host and environmental factors over the lifespan. Efforts are underway to do this through establishment of prospective birth cohorts comprising populations of 100,000 or more children and their parents. Such cohorts, joined with improvements in pathogen surveillance and discovery, toxicology, genetics, proteomics, and systems biology, will allow us to view the world of microbial pathogenesis in three dimensions—(genes) × (environment) × (timing)—yielding insights not only into acute diseases but also into chronic ones.

Unlike polioviruses and EV71, cosaviruses have not yet been cultured.

ACKNOWLEDGMENTS. This work was supported by National Institutes of Health Grants AI070411, AI57158 (Northeast Biodefense Center-Lipkin), HL083850, and EI017404 and by Google.org.

1. Kapoor A, *et al.* (2008) A highly prevalent and genetically diversified *Picornaviridae* genus in South Asian children. *Proc Natl Acad Sci USA* 105:20482–20487.
2. Shulman ST (2004) The history of pediatric infectious diseases. *Pediatr Res* 55:163–176.
3. Racaniello VR (2006) One hundred years of poliovirus pathogenesis. *Virol* 344:9–16.
4. Kristensson K (1999) The discovery of the poliovirus. *Brain Res Bull* 50:461.
5. Landsteiner K, Popper E (1908) Mikroscopische Präparate von einem menschlichen und zwei Affenrückenmarken. *Wien Klin Wochenschr* 21:1830.
6. Norrby E, Prusiner SB (2007) Polio and Nobel prizes: Looking back 50 years. *Ann Neurol* 61:385–395.
7. Technical Consultative Group to the World Health Organization on the Global Eradication of Poliomyelitis (2002) ''Endgame'' issues for the Global Polio Eradication Initiative. *Clin Infect Dis* 34:72–77.
8. World Health Organization (2008) Progress towards interrupting wild poliovirus transmission worldwide, January 2007–April 2008. *Weekly Epidemiol Rec* 19:170–176.
9. Solomon T, Willison H (2003) Infectious causes of acute flaccid paralysis. *Curr Opin Infect Dis* 16:375–381.
10. McMinn PC (2002) An overview of the evolution of enterovirus 71 and its clinical and public health significance. *FEMS Microbiol Rev* 26:91–107.
11. Witsø E, *et al.* (2007) Asymptomatic circulation of HEV71 in Norway. *Virus Res* 123:19–29.
12. Rivers TM (1937) Viruses and Koch's postulates. *J Bacteriol* 33:1–12.
13. Fredericks DN, Relman DA (1996) Sequence-based identification of microbial pathogens: A reconsideration of Koch's postulates. *Clin Microbiol Rev* 9:18–33.
14. Lipkin WI (2008) Pathogen discovery. *PLoS Pathog* 4:e1000002.
15. Lipkin WI, Travis GH, Carbone KM, Wilson MC (1990) Isolation and characterization of Borna disease agent cDNA clones. *Proc Natl Acad Sci USA* 87:4184–4188.
16. Livstone MS, van Noort D, Landweber LF (2003) Molecular computing revisited: A Moore's Law? *Trends Biotechnol* 21:98–101.