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Ocular Pathogens for the Twenty-First Century

RUSSELL N. VAN GELDER

PROGRESS IN MICROBIOLOGY HAS ALWAYS BEEN driven by technological advances. van Leeuwenhoek, the great seventeenth-century pioneer of microscopy, was the first to observe bacteria and protozoa in the 1670s, but it would be nearly 200 years until Pasteur, using nineteenth-century technologies of sterilization and culture media, definitively established the independent nature of microbes and linked specific bacteria to disease. The next 50 years were the first golden era of microbiology, with discovery of most of the organisms now linked to human disease. These included *Staphylococcus* by Ogston in 1880, *Pseudomonas* by Gessard in 1882, *Mycobacterium tuberculosis* by Koch the same year, *Streptococcus* by Fehleisen and Pasteur in 1883, and *Haemophilus* by Pfeiffer in 1892. *Treponema pallidum*, the cause of syphilis, was discovered using the newer technique of dark field microscopy in 1905 by Schaudinn, and *Toxoplasma gondii* was isolated by Nicolle and Manceaux in 1908. Because viral isolation and characterization required yet more advanced technology (including cell culture, centrifugation techniques, and electron microscopy), viral discovery lagged that of the microbes, with isolation of herpes simplex in 1925, varicella zoster in 1953, cytomegalovirus in 1957, and Epstein-Barr virus (EBV) in 1963.

Over the past half century, however, very few new pathogens with relevance to ocular disease have been discovered. Human immunodeficiency virus is the significant exception, with its identification as the causative virus of acquired immunodeficiency syndrome in 1983. Human herpes virus 6 (HHV6) was discovered in 1986, and HHV8 was discovered in 1995, both from patients with human immunodeficiency virus. The former has been associated rarely with uveitis, and the latter is associated primarily with Kaposi sarcoma in immunocompromised patients. The etiologic agent of Whipple disease, *Tropherema whippelii*, was long known to be bacterial, but was unculturable and was characterized molecularly by Relman and associates in 1992.

Does this mean that science has identified all the major pathogens associated with ocular disease? This is highly doubtful. The yields for microbial culture for 2 relatively common, clearly infectious entities—corneal ulcer and postoperative endophthalmitis—remain relatively low, at approximately 55% and 70%, respectively. Through ribosomal DNA-polymerase chain reaction (PCR)-based molecular biology techniques for the identification of bacteria and fungi, researchers have shown that microbial DNA can be detected in nearly every case of endophthalmitis and corneal ulcer. In the case of bacterial endophthalmitis, Okhravi and associates examined 37 cases of endophthalmitis, of which 15 had negative culture results.[1] PCR results matched culture results for the 22 culture-positive cases. In each culture-negative case, bacterial PCR was detected (compared with the 5% false-positive rate in control samples). Of these 15 culture-negative samples, 8 were found to be from previously unidentified bacteria. Similarly, Kim and associates studied 16S bacterial and 18S fungal PCR results of corneal ulcers from a large cohort in Aravind, India.[2] This group found that approximately 55% of corneal ulcers showed positive culture results, with the large majority of PCR results in agreement with culture results. Of the 52 culture-negative ulcers studied, 48 yielded positive PCR results. Of these, 13 were either novel bacteria, novel fungi, or very unusual fungi (such as *Pythium*, *Cladosporium*, and *Botryodiplodia* species). These results suggest that we have not identified all the microbes responsible for these common, clearly infectious ocular inflammatory conditions and that perhaps 10% of infectious endophthalmitis and corneal ulcer cases are associated with novel or very unusual organisms.

With the application of even more sophisticated molecular biologic techniques, we are now aware of the great biodiversity of the natural world and of our own bodies. Venter and associates' sampling of deep ocean life by exhaustive, high-throughput DNA sequencing has shown that less than 1% of the DNA-based life forms in the ocean have been identified to date.[3] Within our own bodies, the human microbiome project has revealed remarkable variability in the core microbiome in different sites. For example, a survey of skin bacteria by 16S DNA sequencing technology revealed more than 200 genera identified among 10 individuals, many of them very unusual and unique to a single individual or single skin site.[4]

In this issue of The Journal, De Groot-Mijnes and associates apply molecular pathogen detection techniques to the study of idiopathic uveitis.[5] This group has been collecting ocular fluid samples assiduously from patients with uveitis and had amassed 629 biopsy samples from patients with uveitis sampled over a 5-year period. All were deemed idiopathic on the basis of a laboratory work-up showing negative results. All samples were screened by PCR and intraocular antibody production for the herpes family viruses herpes simplex virus, varicella

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From the Departments of Ophthalmology and Biological Structure, University of Washington School of Medicine, Seattle, Washington.

Inquiries to Russell N. Van Gelder, Department of Ophthalmology, University of Washington Medical School, Campus Box 359608, 325 9th Avenue, Seattle, WA 98104; e-mail: russvg@uw.edu

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zoster virus, and cytomegalovirus (as well as *Toxoplasma gondii*). Four hundred eighty-nine samples showed negative results for these agents. The authors ran a series of PCR reactions against 139 of these negative samples (those for which sufficient fluid was available for PCR analysis), looking for adenovirus, EBV, HHV6, *Mycoplasma pneumonia*, and *Chlamydia* by DNA analysis, and coronavirus, enterovirus, metapneumovirus, influenza virus, parainfluenza virus, human parechovirus, respiratory syncytial virus, and rubella virus by analysis of RNA through reverse-transcription PCR. Of these 139 samples, one each demonstrated positive results for EBV, rubella, and HHV6. Four samples showed positive results for human parechovirus.

The finding of approximately 5% of idiopathic uveitis being associated with a potential viral pathogen is consistent with another recent study, in which Drancourt and associates studied 1321 patients with idiopathic uveitis using culture, PCR-based diagnostics, and serologic approaches.[6] This group identified fastidious bacteria, herpes family viruses, or fungi in 11% of cases. Most of these cases were associated with fastidious bacteria such as *Bartonella*, *Borrelia*, *Chlamydia*, or *Coxiella* (which, with the exception of *Chlamydia*, were not tested for in De Groot-Mijnes and associates' study).

Although EBV, rubella, and HHV6 have been associated previously with uveitis, this is the first association of a parechovirus with uveitis. Parechovirus is a tiny, single-stranded RNA picornavirus whose genome consists of a single, 7.3-kb transcript. These viruses primarily have been associated with gastroenteritis, but have been found in association with encephalitis and flaccid paralysis in children, suggesting central nervous system involvement.[7] The patients in the present study showing positive results for this virus all had unilateral anterior uveitis.

Of course, being found at the scene of the crime does not prove that one is the perpetrator. Parechovirus infections are nearly universal, with more than 95% of the population being seropositive in adulthood for this virus. However, unlike the herpes family of viruses, it does not seem that the parechoviruses establish latent infection; thus, finding of their RNA in ocular samples is suggestive of ongoing infection. Koch's postulates[8,9]—that the causative organism is isolated from every case of disease, can cause disease in naïve hosts, and can be reisolated from the newly induced disease—have been met for very few organisms. When Koch promulgated these postulates in 1890, important aspects of infectious disease pathologic features, including immunity to organisms, host susceptibility factors, and the presence of viruses, had yet to be discovered. In the modern age, we must weigh a preponderance of evidence to establish causality of a particular microbe or virus in the pathogenesis of disease. Is the organism found in every case of disease? Or perhaps a number of diseases have a final common appearance (as for example, the nearly identical appearance of acute retinal necrosis syndrome caused by varicella zoster virus versus herpes simplex virus). Are the levels of the organism in disease cases very high (as detected by real-time PCR for example)? Such analysis has been critical to associating a potentially latent virus such as cytomegalovirus with Posner-Schlossman disease in the past few years,[10–14] and in establishing active infection versus latency in patients with EBV.[15] Are there multiple signs of the disease—for instance, positive PCR and positive serologic results from the eye? Intraocular antibody analysis was critical in linking rubella virus with Fuchs heterochromic iridocyclitis.[16–19] Does the disease respond to treatment for the presumed pathogen? Such analysis was compelling in linking HHV6 to panuveitis.[11,20]

In the current work, the finding of parechovirus RNA indicative of active infection provides for a compelling hypothesis for disease, but as the authors appropriately note, does not yet approach the level of evidence required to make a definitive, etiologic relationship. Demonstration of serologic response, generation of animal models of uveitis with this virus, or quantitative correlation of viral loads with disease severity all would help bolster the argument for a causal relationship. Because there are no antiviral agents targeted against parechoviridae currently, there is no opportunity for the ultimate test of response to treatment.

The genesis of a hypothesis, however, is a significant step forward. As the next wave of technologies advancing microbiology becomes available—including panmicrobial and panviral gene chips,[21–23] deep sequencing for pathogens, and characterization of short RNAs as signatures for infectious agents[24]—the importance of testing intraocular fluids will increase. These techniques also may find application in the resolving the role of infectious agents that may serve as triggers for other common ocular diseases, such as glaucoma[25] and macular degeneration.[26,27]

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