Coronaviruses in the Limelight

Kenneth McIntosh

Division of Infectious Diseases, Children's Hospital Boston, and Department of Pediatrics, Harvard Medical School, Boston, Massachusetts

(See the article and brief report by Esper et al., on pages 492–8 and 499–502, respectively.)

For ~35 years after their first description by Tyrrell and Byneo in 1965 [1], the field of human coronaviruses (HCoVs) was pretty dull. There were classic early descriptions of their respiratory pathogenicity in volunteer studies [2, 3], and there were seroepidemiologic studies of the 2 most easily studied strains, HCoV-229E and HCoV-OC43 [4–6]. Efforts to implicate HCoVs in diseases of the gastrointestinal tract were largely unsuccessful, with the possible exception of a postulated role in necrotizing enterocolitis of newborns [7]. During this time, the fields of animal CoVs and of the molecular biology of CoVs were, in contrast, buzzing. CoVs were discovered in large numbers and were implicated in a rich variety of animal diseases in multiple species. Diseases as widely varying as progressive peritonitis, nephritis, acute and chronic hepatitis, and subacute encephalitis were described, along with the more traditional respiratory and gastrointestinal syndromes, and pathogenesis was explained through broad mixtures of viral cytopathogenicity, immunologic damage, and genetic susceptibilities. The CoV genome proved to be the largest of all of the RNA viruses and

to have a unique strategy of replication, with transcription and protein production occurring through a nested set of mRNA molecules [8].

Then, in 2003, the appearance of severe acute respiratory syndrome (SARS) suddenly brought the field of HCoVs back into the limelight. It seemed clear that this disease, unique in its clinical spectrum, resulted from the movement of an animal CoV across species lines, and it seemed possible that the virus spread in the human population through a process of adaptation by deletion and mutation [9, 10]. The rapid recognition of the etiology of SARS depended heavily on genomic sequence data assembled from the study of multiple animal CoVs, allowing the SARS agent to be quickly identified and classified and leading to the development of detection methods that would guide the containment of the epidemic.

It is in this context that the article and brief report by Esper et al. that appear in this issue of the *Journal of Infectious Diseases* should be read [11, 12]. In the first of these papers, Esper et al. use the accumulated knowledge of the coronaviral genomic sequence to search for new HCoVs in children with respiratory disease [11]. The authors' discovery of a previously undescribed HCoV was accomplished through the design of a polymerase chain reaction assay that was based on the common region of the polymerase gene. This method was logical, intelligent, and highly original—and a new vi-

rus, designated "New Haven coronavirus" (HCoV-NH), did appear.

In fact, Esper et al.'s finding was not surprising. The reason for this is not that a very similar HCoV was being described by 2 independent groups of virologists in The Netherlands [13, 14] at the same time (that virus was not known when Esper et al. started their work), but rather that, in some of the earliest work on CoVs during the 1960s, viruses were reported that were then forgotten—viruses that came from adults with respiratory illness, that grew only in human embryonic tracheal organ culture, that caused illness in volunteers, and that were not, or were only distantly, antigenically related to the 2 HCoV species that were subsequently the best studied, HCoV-229E and HCoV-OC43. One of these forgotten viruses, B814, was the first HCoV to be described [1]. The others— HCoV-OC16, HCoV-OC37, and HCoV-OC48—were 3 of the 6 strains recovered from organ culture in my laboratory [15]. All 4 of these strains produced colds in volunteers [2, 3], but none grew in tissue culture, and none could be adapted to grow in animal models. Thus, the subsequent neglect of these potentially important viruses stemmed from the fact that, essentially, no methods were available to study them at the time.

Esper et al.'s findings on the clinical impact of HCoV-NH infection, although limited, are consistent both with those from Europe on the novel HCoV reported in The Netherlands and with the available

information on HCoV-229E and HCoV-OC43 infection in children hospitalized with acute respiratory disease [5, 13, 14]. However, the details from Esper et al.'s study—the seasonal distribution, the percentage of positive samples, the associated respiratory syndromes, and the numbers of infected children at various ages, for example—were heavily influenced by both the particular population that was investigated and the clinical setting, so it is essentially impossible to draw conclusions on the epidemiology, pathogenicity, and relative importance of HCoV-NH in relation to other respiratory viruses. On the other hand, it seems to be likely that this is not the last chapter in the story of respiratory HCoVs and that additional studies will clarify the picture. Moreover, it seems quite possible that other strains will be found, by use of similar methods.

In contrast, the findings reported in Esper et al.'s brief report were, to this reader, quite surprising [12]. Esper et al. have shown a temporal association with quite strong statistical significance between infection with HCoV-NH and Kawasaki disease, establishing this association by means of a case-control study in hospitalized children. Until there are corroborating studies, it would seem wise to retain a healthy level of skepticism with regard to the significance of this association. Nevertheless, the findings are extremely interesting and set off in me a series of thoughts as to what might be going on.

First, the skepticism. The linking of an agent to Kawasaki disease follows a long trail of previously failed or still-struggling attempts to identify the etiologic agent of this important syndrome, ranging from an unidentified retrovirus [16], to parvovirus B19 [17], to Epstein-Barr virus [18], to *Chlamydia pneumoniae* [19], and to toxin-producing *Staphylococcus aureus* or *Streptococcus pyogenes* [20], with a scattering of others along the way. Although the association shown by Esper et al. was statistically compelling, previous initial find-

ings were equally so (the first descriptions of both parvovirus B19 and the toxin-producing bacteria included similarly significant associations) and have been difficult to confirm.

There are, however, some tantalizing facts about both CoVs and Kawasaki disease that might allow for cautious optimism with regard to Esper et al.'s reported association. First, there was early epidemiologic evidence [21], subsequently confirmed [22], that a respiratory syndrome preceded the onset of Kawasaki disease. (Incidentally, the interval between the onset of the respiratory syndrome and the onset of Kawasaki disease appeared to be ∼2 weeks [21], which seems a long time for the shedding of a respiratory HCoV, although pertinent data on infants are lacking [23–25].) Second, Kawasaki disease is frequently seasonal, with peaks during the winter and spring; its seasonality is roughly similar to that of infection with respiratory HCoVs [5, 6]. Third, and more recently, there has been evidence from molecular immunopathologic studies by Rowley et al. indicating that, during Kawasaki disease, some external agent triggers a powerful IgA response in the respiratory tract as well as in other organs (including medium and large muscular arteries), suggesting that the target of the extensive immunologic reaction during Kawasaki disease is a specific microbe (rather than an nonspecific stimulus, such as a superantigen) and that this microbe enters the body through the respiratory tract [26–28]. If this is, in fact, the case, then a respiratory HCoV might be the inciting agent.

Fourth, the SARS story reminds us of what veterinarian virologists have known for many years: that CoVs, with their huge genome, are capable of enormously varied pathogenicity, causing diseases that affect multiple organs through a variety of pathogenetic mechanisms. Also, SARS-CoV crossed species lines and was genetically quite distant from the 3 known CoV

groups, whereas HCoV-NH (along with its companion novel virus reported in The Netherlands) appears to be a member of the group 1 CoVs and has other features that make it appear to be closer to HCoV-229E and HCoV-OC43 in its pathogenicity [13]. If HCoV-NH is, in fact, the agent responsible for Kawasaki disease and is acting alone, then we have to postulate that it has acquired a pathogenicity that is quite different from that of its close relatives and of other respiratory viruses.

Clearly, a lot more work needs to be done. Because Esper et al.'s study of Kawasaki disease was epidemiologic, confirmation in broader epidemiologic terms (other places, other times, other detection methods, other populations) is required, as is nonepidemiologic confirmation through the demonstration of an immunologic response to HCoV-NH and of its presence in biopsy specimens. If the association is confirmed, then the pathophysiologic mechanism will need to be further worked out. Kawasaki disease has a complex pathogenesis and has been the subject of much study by microbiologists, immunologists, rheumatologists, cardiologists, and molecular biologists [29]. Much is known about many of the mechanisms of Kawasaki disease, and in some way these must be linked to the presumed microbial etiology. A broad question would be: Is the pathogenesis of Kawasaki disease the product of HCoV-NH infection by itself? There are several animal CoV diseases that are models of complex pathophysiologic mechanisms—the multiple sclerosis–like disease of mice caused by certain neurogenic strains of mouse hepatitis virus [30] and the complex, immunologically mediated, progressive feline peritonitis caused by the CoV of that name [31], for example. In these diseases, genetics, the immune system, and the complex CoV genome all interact. Alternatively, might there be another microbial pathogen involved in the pathogenesis of Kawasaki disease, such that it is a

2-hit disease requiring both a virus and a toxin?

We will watch this field with great interest.

References

1. Tyrrell DA, Bynoe ML. Cultivation of a novel type of common-cold virus in organ cultures. Br Med J **1965**; 5448:1467–70.
2. Bradburne AF, Bynoe ML, Tyrrell DAJ. Effects of a "new" human respiratory virus in volunteers. Br Med J **1967**; 3:767–9.
3. Bradburne AF, Somerset BA. Coronative antibody titres in sera of healthy adults and experimentally infected volunteers. J Hyg (Lond) **1972**; 70:235–44.
4. Hamre D, Beem M. Virologic studies of acute respiratory disease in young adults. V. Coronavirus 229E infections during six years of surveillance. Am J Epidemiol **1972**; 96:94–106.
5. McIntosh K, Kapikian AZ, Turner HC, Hartley JW, Parrott RH, Chanock RM. Seroepidemiologic studies of coronavirus infection in adults and children. Am J Epidemiol **1970**; 91:585–92.
6. Monto AS, Lim SK. The Tecumseh study of respiratory illness. VI. Frequency of and relationship between outbreaks of coronavirus infection. J Infect Dis **1974**; 129:271–6.
7. Chany C, Moscovici O, Lebon P, Rousset S. Association of coronavirus infection with neonatal necrotizing enterocolitis. Pediatrics **1982**; 69:209–14.
8. Lai MMC, Holmes KV. Coronaviridae: the viruses and their replication. In: Knipe D, Howley P, eds. Fields Virology. 4th ed. London: Lippincott Williams & Wilkins, 2001:1163–86.
9. The Chinese SARS Molecular Epidemiology Consortium. Molecular evolution of the SARS coronavirus during the course of the SARS epidemic in China. Science **2004**; 303:1666–9.
10. Peiris JS, Yuen KY, Osterhaus AD, Stohr K. The severe acute respiratory syndrome. N Engl J Med **2003**; 349:2431–41.
11. Esper F, Weibel C, Ferguson D, Landry ML, Kahn JS. Evidence of a novel human coronavirus that is associated with respiratory tract disease in infants and young children. J Infect Dis **2005**; 191:492–8 (in this issue).
12. Esper F, Shapiro ED, Weibel C, Ferguson D, Landry ML, Kahn JS. Association between a novel human coronavirus and Kawasaki disease. J Infect Dis **2005**; 191:499–502 (in this issue).
13. van der Hoek L, Pyrc K, Jebbink MF, et al. Identification of a new human coronavirus. Nat Med **2004**; 10:368–73.
14. Fouchier RA, Hartwig NG, Bestebroer TM, et al. A previously undescribed coronavirus associated with respiratory disease in humans. Proc Natl Acad Sci USA **2004**; 101:6212–6.
15. McIntosh K, Dees JH, Becker WB, Kapikian AZ, Chanock RM. Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. Proc Natl Acad Sci USA **1967**; 57:933–40.
16. Burns JC, Geha RS, Schneeberger EE, et al. Polymerase activity in lymphocyte culture supernatants from patients with Kawasaki disease. Nature **1986**; 323:814–6.
17. Nigro G, Zerbini M, Krzysztofiak A, et al. Active or recent parvovirus B19 infection in children with Kawasaki disease. Lancet **1994**; 343:1260–1.
18. Kikuta H, Sakiyama Y, Matsumoto S, et al. Detection of Epstein-Barr virus DNA in cardiac and aortic tissues from chronic, active Epstein-Barr virus infection associated with Kawasaki disease–like coronary artery aneurysms. J Pediatr **1993**; 123:90–2.
19. Normann E, Naas J, Gnarpe J, Backman H, Gnarpe H. Demonstration of *Chlamydia pneumoniae* in cardiovascular tissues from children with Kawasaki disease. Pediatr Infect Dis J **1999**; 18:72–3.
20. Leung DY, Meissner HC, Fulton DR, Murray DL, Kotzin BL, Schlievert PM. Toxic shock syndrome toxin–secreting *Staphylococcus aureus* in Kawasaki syndrome. Lancet **1993**; 342:1385–8.
21. Bell DM, Brink EW, Nitzkin JL, et al. Kawasaki syndrome: description of two outbreaks in the United States. N Engl J Med **1981**; 304:1568–75.
22. Treadwell TA, Maddox RA, Holman RC, et al. Investigation of Kawasaki syndrome risk factors in Colorado. Pediatr Infect Dis J **2002**; 21:976–8.
23. Callow KA. Effect of specific humoral immunity and some non-specific factors on resistance of volunteers to respiratory coronavirus infection. J Hyg (Lond) **1985**; 95:173–89.
24. Myint S, Siddell S, Tyrrell D. Detection of human coronavirus 229E in nasal washings using RNA:RNA hybridisation. J Med Virol **1989**; 29:70–3.
25. Callow KA, Parry HF, Sergeant M, Tyrrell DA. The time course of the immune response to experimental coronavirus infection of man. Epidemiol Infect **1990**; 105:435–46.
26. Rowley AH, Shulman ST, Mask CA, et al. IgA plasma cell infiltration of proximal respiratory tract, pancreas, kidney, and coronary artery in acute Kawasaki disease. J Infect Dis **2000**; 182:1183–91.
27. Rowley AH, Shulman ST, Spike BT, Mask CA, Baker SC. Oligoclonal IgA response in the vascular wall in acute Kawasaki disease. J Immunol **2001**; 166:1334–43.
28. Rowley AH, Baker SC, Shulman ST, et al. Detection of antigen in bronchial epithelium and macrophages in acute Kawasaki disease by use of synthetic antibody. J Infect Dis **2004**; 190:856–65.
29. Burns JC, Glode MP. Kawasaki syndrome. Lancet **2004**; 364:533–44.
30. Pewe L, Perlman S. Cutting edge: CD8 T cell–mediated demyelination is IFN-gamma dependent in mice infected with a neurotropic coronavirus. J Immunol **2002**; 168:1547–51.
31. de Groot-Mijnes JD, van Dun JM, van der Most RG, de Groot RJ. Natural history of a recurrent feline coronavirus infection and the role of cellular immunity in survival and disease. J Virol **2005**; 79:1036–44.