Finding the Cause of Kawasaki Disease: A Pediatric Infectious Diseases Research Priority

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(See the article by Dominguez et al., on pages 1697–1701.)

Approximately 60 times per year at Children’s Memorial Hospital in Chicago, we diagnose acute Kawasaki disease (KD) in a previously healthy infant or child. As a pediatric infectious diseases specialist, I find this diagnosis to be one of the most difficult to discuss with a family. I can explain that their child has KD, but I must tell them that the cause of the illness is unknown, although it is highly likely to be the result of infection with an unknown microbe. I must inform them that their child could develop serious lifelong heart disease after the illness. I tell them that although intravenous gammaglobulin (IVIG) with aspirin is effective therapy for most children with acute KD, its mechanism of action is unknown, and some children do not respond. In the present era of sophisticated medicine, this conversation, with all its unknowns, is very unsettling both to me and to the family. In addition, when I diagnose acute KD in a child during the first week of fever and promptly administer IVIG and aspirin therapy but the child fails to respond and coronary aneurysms develop and worsen despite additional therapies, I ponder the sobering reality that even those of us who have devoted a great deal of our careers to the study of KD cannot always stop this process. All of this leads directly to the question: what is the cause of this disease?

Finding the cause of KD is a pediatric infectious diseases research priority. Identification of the causative agent(s) would be the most promising step toward developing a diagnostic test and specific therapy and, ultimately, preventing the disease. Therefore, a study by Esper et al. [1] from Yale, published in the Journal of Infectious Diseases in February 2005, that implicated an association between the new human coronavirus (HCoV) NL-63 and KD, was met with great interest. Those investigators reported detection of the virus in respiratory samples from 8 of 11 patients with KD and 1 of 22 control subjects by reverse transcription–polymerase chain reaction (RT-PCR). As a result of that article, laboratories around the world performed additional studies to determine whether an association between KD and HCoV NL-63 could be confirmed. This year, the National Institutes of Health (NIH) awarded an R21 grant proposal to the world, the epidemiology and public health implications of KD.

In November 2005, the results of an international, multicenter, collaborative study [5] showed that HCoV NL-63 could be detected in only 1 of 48 respiratory samples from children with acute KD from 3 different geographic areas; the single patient in whom the virus was detected had a diagnosis of a coexisting upper-respiratory tract infection. In January 2006, Chang et al. [6], from Taiwan, reported that HCoV NL-63 was not detected in respiratory samples from any of 53 patients with acute KD. In this issue of the Journal of Infectious Diseases, Dominguez et al. [7], from Denver, report results of a blinded, case-control, retrospective study. They determined that HCoV NL-63 was detected in nasopharyngeal washes from 2 (7.7%) of 26 children with KD and 4 (7.7%) of 52 matched control subjects. Therefore, in a community in which HCoV NL-63 clearly was circulating among children, the virus was detected in control subjects and patients with KD at the same prevalence,
which indicates the lack of an association between KD and HCoV NL-63. All 5 studies published after the original report by Esper et al. are in agreement; it is now quite clear that the elusive etiologic agent of KD is not HCoV NL-63.

Why has it been so difficult to find the causative agent(s) of KD? Occasionally, landmark discoveries such as the identification of the causative agents of specific infectious diseases have been made serendipitously. However, in most cases, success has been contingent on a great deal of hard work in a funded research program. Historically, funding for KD research has been low. At present, there are only 2 NIH-funded R01 projects focusing on KD, compared with ~120 focusing on congenital heart disease [2]. There are many factors that make KD a difficult disease to study: because it is an illness of small children, obtaining biopsy specimens for research from lymph nodes or other tissues is very problematic; the target tissue of the disease—the coronary artery—is unavailable to the researcher for study; because it is an illness of small children, obtaining biopsy specimens for research from lymph nodes or other tissues is very problematic; the target tissue of the disease—the coronary artery—is unavailable to the researcher for study; and, to date, there has not been success in developing an animal model by injection of tissue or fluid samples from patients with KD into any animal species. Deaths from KD are fortunately uncommon, but, unfortunately, research is seldom considered when a death occurs. Tissue samples obtained at autopsy are routinely fixed in formalin and embedded in paraffin. Fresh tissue is generally not saved for molecular analysis or electron microscopic studies. These difficulties have discouraged many basic scientists from studying the disease. Most KD researchers are physician-scientists who care for patients with KD in clinical practice and are struck by the need to better understand this potentially devastating illness.

Where do we go from here to determine the etiology of KD? Several years ago, my colleagues and I discovered that, surprisingly, IgA plasma cells infiltrate tissues during acute KD, including the respiratory tract and inflamed arterial tissue [8, 9]; analysis of the IgA genes present in arterial tissue during KD showed that a restricted number of IgA antibodies were being made in the arterial wall, which is consistent with a response to specific antigen(s) [10]. To identify a KD-associated antigen targeted by these antibodies, we made synthetic versions of the prevalent IgA antibodies and used them in immunohistochemical experiments on acute KD and control tissues; several of the most prevalent IgA antibodies detected antigen in bronchial epithelium from patients with acute KD, but not that from control subjects, and in macrophages in other inflamed tissues from patients with acute KD [11–13]. Light and electron microscopic studies of the antigen detected in formalin-fixed ciliated bronchial epithelium from patients with acute KD indicated that the antigen resides in cytoplasmic inclusion bodies that are consistent with aggregates of viral protein and nucleic acid [12]. There is virtually no precedent for inclusion bodies containing human antigen(s) to be formed in tissues during an acute illness such as KD, but there are many examples of inclusion body formation by infectious agents in tissues during acute infectious diseases. This makes it highly likely that the inclusion bodies in ciliated bronchial epithelium during KD contain components or products of the KD infectious agent. Of note, our KD synthetic antibodies do not bind to HCoV NL-63–infected cells. We have identified inclusion bodies by immunohistochemical analysis in patients with KD from Japan and the United States (in Asian and non-Asian patients) using a single monoclonal antibody. Thus, the probability that KD is caused by a single infectious agent or a group of closely related agents appears to be very high.

Better visualization of the inclusion bodies by transmission electron microscopic examination of glutaraldehyde-fixed medium-sized ciliated bronchial epithelial cells from persons who have died from acute KD is a high priority for the research of the etiology of KD at present. Ultrastructural examination of other optimally fixed tissues, such as coronary arteries, is also critical. Fatalities attributable to KD continue to occur in the United States and worldwide, and it should be possible to place coronary artery and lung samples that contain medium-sized bronchial into glutaraldehyde, formalin, and optimal cutting-temperature compound for storage at −70°C (for advice regarding tissue processing, contact the author at a-rowley@northwestern.edu or Children’s Memorial Hospital, paging 773-880-4000). Ultrastructural analysis of cytoplasmic inclusion bodies and the identification of viral particles or other microbial elements in glutaraldehyde-fixed tissue could provide much-needed direction for future studies of the etiology of KD.

If a new microbial agent is identified from these or other studies as a possible etiology of KD, I strongly believe that a multicenter study incorporating several different laboratories should be organized rapidly to test the possible association, such as the one we put together to test the HCoV NL-63 hypothesis [5]. The list of KD investigators who at one time or another reported that a specific agent causes KD is indeed lengthy. Most discarded hypotheses of the etiology of KD originated from studies performed at a single geographic location in a single laboratory. The benefits of a multicenter approach include the opportunity to include centers with large numbers of patients with KD, the avoidance of potential individual laboratory misadventures that might result in falsely positive or falsely negative results, and, most important, the opportunity to be fairly certain that the agent is actually related to KD before the publication of an association. I believe that this type of study would best serve the interests of patients with KD, their families, KD researchers, and the pediatric and infectious diseases communities.

References

1. Esper F, Shapiro ED, Weibel C, Ferguson D, Landry ML, Kahn JS. Association between a