

Evaluating Viral Agents in Marmoset Colitis

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The evidence presented at this meeting suggests that knowing the cause of the acute bouts of diarrhea and of the chronic colitis in marmosets is important for the successful breeding and survival of these animals in captivity. In addition, this information could further our general understanding of the causes of chronic colitis in man. Clearly, comprehensive studies on the etiology of the colitis are needed to determine if it is caused by bacteria, parasites, viruses, dietary deficiencies, or by a combination of these factors. The current evidence is consistent with the hypothesis that viral agents may cause some of the diarrhea of marmosets.

Drs. Russell and Brian presented preliminary data on the possible etiologic role of the coronaviruses in marmoset colitis. While their studies do not yet establish such a role, the coronaviruses were a good choice for initial investigations because these viruses can infect the colon (unlike most other enteric viruses), and they can establish persistent infections which could allow them to cause chronic disease. Russell and Brian's data, suggesting that marmoset sera contain antibodies that react with at least some of the proteins of the bovine coronaviruses, represent an important first step for their studies. If confirmed, well-characterized reagents (antibodies or cloned genes) and viral antigens prepared from the cultivatable bovine viruses will be able to be applied in a number of experimental approaches to rapidly assess whether these agents cause the colitis.

Additional systematic work is required to directly demonstrate whether other known gastroenteritis viruses (rotaviruses, atypical rotaviruses, Norwalk-like viruses, astroviruses, caliciviruses, enteric ade-

noviruses, or the newly discovered Breda viruses) also play a role in this colitis. It is appropriate to consider the properties of gastroenteritis viruses that may contribute to the success of these studies and to highlight new noninvasive methods for detecting gastroenteritis viruses that should be considered for these studies.

Most gastroenteritis viruses are noncultivable and, therefore, methods other than direct cultivation must be used to identify them. Electron microscopy remains the primary method for virus identification, and the ability to detect virus in stools is often dependent upon the timing of collection of the stool samples. Virus is most frequently detected immediately prior to, or at the onset of, the diarrhea. If direct visualization of virus particles is not successful, immune electron microscopy (IEM) is an alternative method worth trying in case virus concentrations are low. For IEM, sera collected from animals who have recovered from the colitis could be used to screen stools from other animals with colitis or stools from newborns with diarrhea that are threatened with high mortality rates.

New molecular biology techniques are also noteworthy because they are noninvasive and they can be used in both prospective and retrospective studies. For example, immunocytochemistry can be performed on serial sections from formalin-fixed tissues that have already been examined for pathology, providing a good hyperimmune antibody that cross-reacts with the suspected viral antigen is available. Since viral antigens are stable to the fixation procedures required for making routine pathology sections, this can be a very powerful tool to examine the prevalence of a putative etiologic agent in retrospective studies.

Nucleic acid hybridization techniques are also becoming increasingly effective tools to evaluate the etiologic role of agents in a particular disease.

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EVALUATING VIRAL AGENTS IN COLITIS

This technique does not require virus cultivation, providing nucleic acid probes (DNA, RNA, or cloned genes) are available that will cross-react with the putative agents. Nucleic acid probes are rapidly becoming available to many of the known gastroenteritis viruses, and these cloned genes can be used in "dot-blot assays" to detect virus in stool or tissue samples. The major advantage of this method is its sensitivity, which appears to be greater than electron microscopy. Therefore, dot-blot assays are useful to detect noncultivable agents that are excreted at low concentrations.

While performing these studies, it is important to remember the criteria for establishing an etiologic role for noncultivable agents. The specific virus must be regularly associated (rather than isolated) with the disease. The disease must be transmissible to susceptible hosts by materials known to be free of nonviral agents, and proper controls and immunologic studies must be done to exclude the virus from being fortuitously present or from being

picked up from experimental animals or cell lines used in the studies. Serologic studies are important where viruses cannot be cultivated, and it should be possible to show that specific antibody is absent before infection and that antibody is produced or at least enhanced after infection. In addition, the absence of antibody should correlate with susceptibility and its presence should correlate with protection from infection.

In summary, very specific and sensitive methods are now available to perform noninvasive studies on the viral etiology of diarrhea in animals and man. It will be surprising if a well-planned, systematic study does not reveal viral agents as the cause of some of the diarrhea in these marmosets. These studies are important because they can be performed without further trauma to the animals, and the results may increase the marmoset's breeding capacity and survival in captivity. Without significant progress in this area, the continued use of marmosets in research cannot be recommended.