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GUEST COMMENTARY

Culture of Protozoan Parasites

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There are a number of issues involved in the culture of protozoan parasites that make these procedures highly complex and subject to many variables, some of which are known and some of which are still undefined. Certainly protozoan parasites have complex life cycles. They may have different morphological stages within the life cycle and may have both cold-blooded and warm-blooded animals as intermediate or definitive hosts within the life cycle. In vitro culture of organisms at any one of these stages within the life cycle involves a tremendous number of variables, including parasite stage, host site, host temperature, host immune responses, parasite species and/or strain, and parasite-protective mechanisms. To simulate the host environment in an in vitro culture system can be extremely demanding, assuming one can actually determine all the relevant variables (2, 3, 10).

Often the organisms are very fastidious in their growth requirements, and different lots of media and/or medium components may be toxic. Something as simple as the type of glass used for the culture container can have tremendous influence over the success or failure of culture trials. Many culture medium components require filter sterilization and have relatively short shelf lives. Another requirement for many medium formulations includes the addition of human or animal sera, which are expensive and highly variable and may contain factors that are detrimental to parasite growth. Much research has been devoted to the development of defined medium formulations, although, even with the elimination of serum, various other components may not have been totally defined. In some cases, growth factors have been identified and substituted for serum or serum components.

At the present time, are there any advantages in the in vitro culture of protozoan parasites, especially when revolutionary developments are going on in the field of molecular biology? One can argue that many types of research, including the diagnosis of etiologic agents, can be accomplished even when very small numbers of organisms are available. However, many areas of research can be undertaken only when large numbers of parasites with no contaminating bacteria or host materials are available. One of the great advantages of in vitro culture, especially axenic culture, is that obtaining a continuous supply of pure organisms without any bacterial and/or fungal contamination is possible (4, 9).

In vitro cultivation of parasitic protozoa that cause human disease is invaluable, as it provides not only information on the development of the parasite but also avenues for new approaches to the containment and/or eradication of the parasite. In vitro cultivation is important for a number of reasons, as follows: (i) as an important adjunct to diagnosis; (ii) to produce antigens used to prepare monoclonal and polyclonal antibodies against the organisms for use in immunologic tests; (iii) to identify specific proteins that may enhance the invasive properties of the parasite and in turn the development of monoclonal antibodies that will help neutralize parasitic invasion; (iv) to assess functional antibodies and cell-mediated protective systems against the parasites, assessments that can only be made in a cost-effective manner using in vitro culture; (v) to screen drugs, in vitro, in order to identify potential therapeutic agents; (vi) to differentiate susceptible from resistant isolates so that advances in chemotherapy can be made; (vii) to differentiate clinical isolates using techniques such as isoenzyme electrophoresis, monoclonal antibody techniques, and/or DNA probe techniques; (viii) to elucidate isolate and strain differences which will be a useful tool for molecular epidemiology; and (ix) to produce vaccines, as relatively large numbers of parasites at specific stages can be produced in culture. (x) In addition, continuous culture over long periods of time may cause attenuation of strains, and therefore, attenuated strains have potential in the development of suitable vaccines. (xi) In vitro cultivation also provides a system to assess vaccine efficacy, since it can only be done by using intact parasites that can be obtained in large quantities and without the contaminating influences of host components. Finally, culture can be used (xii) to provide the parasite inoculum used for experimental animal disease models; (xiii) to study the biochemistry, physiology, and metabolism of the parasites as well as determine their nutritional requirements; (xiv) to understand the ultrastructural organization of the parasite; and (xv) to provide a system suitable for the assay of lymphokines and other cytokines that may block invasion of the parasite.

Individuals working in this field have indicated that "black magic" may be required for culture success; therefore, clinical laboratories have been somewhat hesitant to undertake these types of diagnostic procedures on a routine basis. However, with proper culture controls in addition to patient specimen cultures, clinically relevant information can be obtained from these cultures when other diagnostic methods may be unsuccessful (1, 5, 6, 8). This is particularly true for cultures of some of the free-living amebae and the hemoflagellates (1, 7, 10). Another potential problem is associated with earlier research publications in which various media are mentioned but not

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thoroughly described; the same medium is often referred to by different names.

In vitro cultivation of the various parasites discussed in this issue will continue to be a challenging diagnostic option but, nevertheless, a fruitful area of research. It is anticipated that further research on the emerging and reemerging opportunistic parasites will likely include surface antigens that may be involved in the invasion of the host cell.

Following is an excellent, comprehensive collection of works on the current status of protozoan culture systems. This information should be helpful to all microbiologists in understanding the role that in vitro culture plays, not only in the research setting but also in the clinical arena.

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