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Book review

***Rotaviruses: Methods and Protocols*, Edited by Ulrich Desselberger and James Gray, The Humana Press, NJ, USA, 1999.**

This is volume 34 in the series, Methods in Molecular Medicine. This volume focuses on methods and protocols for rotavirus and was edited by Ulrich Desselberger and Jim Gray from the Public Health Laboratory in Cambridge, UK. The intent of the volume is to collect modern molecular techniques that are applied to rotavirus research, with sufficient background information and overview material to put those methods into context.

Contents include a basic chapter on rotaviruses by Ulrich Desselberger and a chapter by B.V.V. Prasad and Mary Estes on electron cryomicroscopy and computer image processing techniques and how they have unveiled important information on structure and function of rotavirus particles. The third chapter is on virus replication by John Patton, Vladimir Chizhikov, Zenobia Taraporewala, and Dayue Chen. The fourth chapter is on rotavirus entry to tissue culture cells by Joanna Gilbert and Harry Greenberg. The next chapter, on mixed infections with rotaviruses, by Robert Ramig, focuses on unique biologic and genetic features of rotaviruses that derive from the segmentation of the gene. A chapter on pathogenesis and animal models by Linda Saif and Lucy Ward focuses on the gnotobiotic piglet model developed by Dr Saif. Next is a chapter on immunologic methods and correlates of protection by Christine McCartney and Paul Offit. In vivo studies of immunity to rotaviruses, described by Manuel Franco and Harry Greenberg, focuses primarily on the mouse model. Next is an evaluation of rotavirus vaccines and small animal models by Max Ciarlet and Margaret Conner, which focuses on the rabbit model. Methods that permit rotavirus detection, serotyping, genotyping, sequencing and phylogenetic analysis are described by Miren Gomara, Jon Green, and Jim Gray. Next is a chapter on the epidemiology of group A rotaviruses by Mary Ramsey and David Brown and a chapter on future rotavirus research by Ulrich Desselberger and Mary Estes.

The strength of this volume is its comparatively narrow focus on methods, especially those methods needed at this time to further our understanding of rotavirus biology. The authors are all strong investigators in the methods they review and the material has benefited from good organization and editorial review. The volume also benefits from being current, with most chapters having references through 1998 and a couple with references into 1999.

Knowledge of the molecular biology of rotaviruses has advanced considerably with delineation of the products of each of the 11 gene segments and being tantalizingly close to being able to rescue laboratory-induced modified genes. For this, the structural studies, especially those indicating that viral proteins may organize the rotavirus genes in the infectious virion, might be just the clue needed to accomplish this long-sought goal. The animal models have been extremely useful in understanding components of the immune system that are involved in protecting from and clearing rotaviruses infections, however, two important issues of protection in the human

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remain unclear. First is the importance of the inoculum dose, for which the animal models have not been able to provide a clear understanding. It is apparent humans are exposed to a wide range of inocula and it is unclear how the animal models explain the variability of outcome of infection, even from first infections. The second issue is protective correlates, as described by Drs McCartney and Offit and his colleague. In my opinion, the literature has unnecessarily confused this issue because of inadequate analyses of laboratory data, inadequate laboratory methods for measuring antibody correlates, and improper or poorly understood study designs for evaluating protective correlates. This applies especially to seeking antibody correlates in vaccine trials, where all of these issues have influenced published results. The issues I have summarized here are made clear in this volume and are examples of the quality of background material provided in what would otherwise be understood to be a purely 'methods' volume.

When one reviews the methods themselves, several strengths are apparent. First, the methods are far more detailed than usually available in reviews of rotavirus molecular biology and, especially, more detailed than one finds in most publications describing new methods. The frequent use of explanatory notes that provide key insights into each method is laudatory. The persons writing these methods are intimately familiar with them and it would be surprisingly if a trained virologist would not be able to make prompt headway with the application of these methods should he/she seek to begin rotavirus studies. Although books of this kind do not lend themselves well to close and careful reading at a single sitting, a few issues were noted, as follows:

1. An oversight occurs on page 1, where the non-structural proteins are designated as NSP1 to NSP5. Table 1 on page 2, giving gene protein assignments, uses the nomenclature 'NS # #' and the correspondence between 'NSP' and 'NS # #' is not explained.
2. On page 2, VP6 is indicated as the protein that carries group-specific determinants. Group determinants also are present on other rotavirus proteins, although VP6 is the major group antigen.
3. On page 5 the suggestion is that the diarrhea of rotavirus infection is caused primarily by necrosis of apical villi and resulting primary malabsorption. This understanding is a subject of considerable investigation and evidence suggests multiple pathways for inducing diarrhea may exist, as raised in other chapters.
4. On page 6, the concept of so-called 'nursery' strains is introduced. Such strains are suggested to cause asymptomatic infection. As is clear in later chapters, this concept is questionable.
5. A few typographical errors occur from time to time, but fortunately these are decidingly few. On the other hand, most of them could have caught by the use of a spellchecker.
6. On page 48, in the methods describing purification of triple-layered virions, the variable stability of human rotavirus strains in the conditions described should have been noted. The method is appropriate for several well-characterized stable animal strains that have been commonly used in molecular studies, however, clinical strains are variably stable in these conditions.
7. The grammar is pleasantly accurate with a few exceptions, an example being on page 61, where the phrase, 'CPEs are usually complete', occurs.
8. Some inconsistencies become apparent only to someone who reads chapters back-to-back. These have to do primarily with the level of detail in the description of supplies and reagents for different methods. For example, MA104 cells are generically cited in a number of chapters, whereas in other chapters the supplier and passage number of the cell line is specified. Another example is the description of media, with simple details on minimum essential medium (MEM) being provided in one chapter and a greater level of detail in other chapters. This variability could have been avoided by editorial imposition of a standard level of detail from protocol to protocol or by having a central listing of common reagents that would have been referred to in multiple chapters. The latter approach would have shortened the volume.

9. On page 81, a number of terms are introduced, with elementary definitions such as 'MOI' for 'multiplicity of infection'. I suggest that the volume would have benefited by providing a central glossary of terms to avoid introducing such terms in the middle of a technical chapter.
10. On the top of page 82, Dr Ramig indicates that carefully defined reassortant gene combinations can be generated from mixed infections. I believe our ability to pre-select reassortant combinations in any particular experiment is depressingly weak, although a few specific selection methods, such as introduction of neutralizing monoclonal antibodies to select against gene 4 or gene 9 reassortants, are available.
11. Another strength of this volume is that some older methods that have been around for 20 or 25 years are provided. Some such methods have fallen into the arena of legend and recalled childhood memories for some virologists in the field, as our use of them has diminished. It is refreshing to be able to know that a current volume on methods has such material available for reference.
12. On page 120, Drs Offit and McCartney indicate how study design or the way that antibody studies have been performed have masked our ability to recognize antibody correlates of infection in vaccine studies. Another possibility not mentioned is simply improper data analysis.
13. On pages 120 and 123, the described ELISA for detecting human fecal rotavirus-specific total Ig and IgA has two limitations. One is that single-shelled rotavirus particles may not be the best antigen for certain antibody studies. One must consider whether the strain for the ELISA is matched to the strains of population exposure. Strain-to-strain variation in sensitivity of the antibody assay, as well as in measuring responses to particular antigenic types, has been this reviewer's experience.
14. On page 134, Drs Franco and Greenberg indicate that rotavirus replication is almost exclusively limited to mature enterocytes. Although this has been evaluated in some detail in animal models, the site(s) of viral site replication in natural human infections remains uncertain. Certainly, rotavirus replication in the intestine occurs, but how frequently and to what degree rotavirus replication in the liver, kidney, and nervous system might occur is yet undetermined. This issue is prompted by studies in patients with neurologic symptoms in whom rotavirus is present in cerebrospinal fluid. One study of immunocompromised children, rotavirus replication in the liver and kidney was demonstrated. This focus on rotavirus replication in the intestine is repeated on the first page of the chapter by Ciarlet and Conner, in which they state 'rotavirus is a localized enteric infection'. Again, this is an area of uncertainty in natural human infections.
15. On page 178, in the method for detecting antigens in stool, the dilution of a sample is specified as not needed. It is important to recognize that this finding is a result of experiments evaluating whether dilution is needed. In all such assays for antigen and antibody quantification it is important to determine whether a prozone effect exists at low dilutions of the samples being analyzed. This applies to tissue culture as well as clinical material.
16. On page 218, the paper by Velazquez describing the cohort study of rotavirus infection in Mexico, in which the efficacy of natural infection as a protective event was evaluated, is cited as reporting the majority of children had rotavirus infections by the age of 5 years. In that study, the duration of follow-up was the first 2 years of life and the majority of children had two infections in that period.
17. On page 220, there is a suggestion that rotavirus is not predominantly a zoonosis. In my opinion, care must be utilized in making judgments about of frequency cross-species transmission of rotaviruses at this time. It is clear that rotaviruses spread easily from human to human. This ease may mask frequent animal↔human transmission that will be revealed only when a vaccine is widely used in humans.

18. On page 233, the reference by Torok is a duplication.
19. On page 235, the authors of reference 94 are Grant AD and Eke B.
20. Missing from this book are methods for evaluating the anti-rotavirus glycoprotein lactadherin, which has been demonstrated to attenuate natural infection when present in higher concentration in the milk being consumed by suckling infants. This is an important area of research, which potentially affects study of cell binding and uptake of the virus, protection, and alternatives to vaccination for prevention of infection. Methods for the detection of lactadherin and its characterization have been published.
21. The final chapter by Desselberger and Estes contains some sharp insights on the future needs for and possibilities of rotavirus research. One of the most important and exciting possibilities is how studies of structure and function of the rotavirus particle may provide the final insight needed to develop procedures for rescue of viable, induced rotavirus variants.
22. Another observation that was not explored elsewhere in the volume is the induction of cytokines by rotavirus infection. Such cytokine induction may be a key issue in understanding how the Rotashield vaccine removed from the market might have caused intussusception.
23. On page 243, line 18, I believe 'fecal IgG' should be 'fecal IgA.'
24. The authors point out on page 244 that the immune responses to rotavirus vaccine(s) in the immunocompromised host have not been evaluated in detail. Thus, the safety of any vaccine in these populations is yet unsure.

This itemization points out a number of issues where differences in understanding exist in the literature. By providing this list, this reviewer is again able to point out the high quality of this volume on rotavirus methods, by noting again the breadth of review material, which in a 245-page volume does not detract from the primary purpose of the volume. This volume succeeds as a concise and useful summary of methods needed for rotavirus research in the laboratory.

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