Correspondence

Detection of Epstein-Barr virus in archival Hodgkin's disease specimens

The Epstein-Barr virus (EBV) is associated with several malignancies, including endemic Burkitt's lymphoma, undifferentiated nasopharyngeal carcinoma, post-transplant lymphoproliferative disease, and Hodgkin's disease. The "gold standard" for the detection of EBV infection in clinical tissues is RNA in situ hybridisation that targets the abundantly produced Epstein-Barr virus early RNAs (EBERs).[1] This approach is effective in the detection of latent EBV infection in routinely processed, paraffin wax embedded histological material, and has been widely used to analyse the association of EBV with a variety of malignant and non-malignant diseases. In addition, a range of monoclonal antibodies directed against latent EBV proteins has enabled viral gene expression to be investigated in EBV associated diseases. For example, in Hodgkin's disease, the latent membrane protein 1 (LMP1) is consistently expressed in EBV associated cases and can be detected using the CS1-4 monoclonal antibody reagent.[2]

Although these approaches are effective on optimally processed histological material, little is known about their ability to detect EBV in tissues stored for many years, or those that may have been processed before the introduction of automated paraffin wax embedding. Therefore, we wished to investigate whether these methods could be used to detect EBV in Hodgkin's disease specimens stored for 50 years or more. In addition, these specimens were processed before the advent of improved tissue embedding procedures.

Paraffin wax embedded tissue blocks were retrieved from 14 patients diagnosed with Hodgkin's disease between 1943 and 1952.

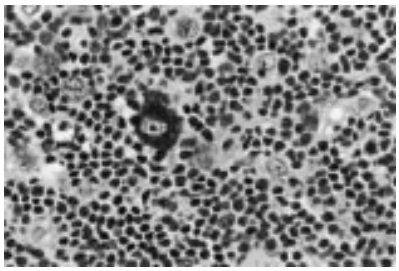

Figure 1 Immunohistochemical staining for latent membrane protein 1 in an archival Hodgkin's disease specimen, with staining confined to the cytoplasm of Hodgkin-Reed-Sternberg cells.

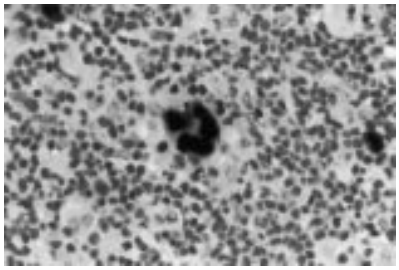

Figure 2 In situ hybridisation for Epstein-Barr virus early RNAs. Positive result seen in an archival Hodgkin's disease specimen.

Specimens were reviewed by haematoxylin and eosin staining in all cases. All histologically confirmed Hodgkin's disease specimens were subjected to EBER in situ hybridisation and immunohistochemistry for LMP1, as described previously.[3 4]

LMP1 was detected in five of 14 Hodgkin's disease specimens, where staining was confined to the cytoplasm of Hodgkin-Reed-Sternberg cells (fig 1). Four of these five cases were also positive for EBER in situ hybridisation (fig 2).

These results indicate that archival specimens stored for periods in excess of 50 years, or those that were subject to less than optimal tissue processing, are still viable for the detection of EBV. These approaches will allow the collection of valuable comparative information about whether EBV prevalence in certain tumour types, such as Hodgkin's disease, has altered over time.[5]

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Book reviews

Cytomegalovirus Protocols. Sinclair J, ed. ($69.50.) Humana Press, 1999. ISBN 0 896 03749 5.

The more we learn about human cytomegalovirus (HCMV) infections, the more it becomes apparent that this herpesvirus can cause important problems in humans. As a clinical virologist, I looked forward to reading this book, which was written by a panel of expert, international researchers. Their remit to provide comprehensive protocols to cover cellular and molecular techniques for HCMV was ambitious, but is something that is needed.

The layout of each chapter is logical, with a brief introduction and then the protocol under three headings (materials, methods, and notes). Unfortunately, this is my first criticism. If the intention is to provide a protocol manual for use in the laboratory, it is inconvenient to have to jump pages to follow one technique. It would have made much more sense to have the complete protocol described in one section rather than in three separate sections.

The protocols are excellent, with considerable detail. The materials sections are useful in referring to commercial manufacturers of key products. The notes section is a great practical idea and gives valuable additional information on the techniques. My second criticism is that, at times, a considerable degree of prior knowledge and experience is assumed, especially in the more complex molecular biology techniques. Some guidance on the interpretation of the results of each protocol would also have been useful. As with any book on protocols, occasionally, crucial information seems to be missing—for example, how to determine a cut off point for a serological assay.

For a virology diagnostic laboratory in the UK, only a few protocols would be relevant. This book is primarily aimed at those doing virology research, but is an indicator of the speed of development of molecular biological techniques. Amazingly, for a book of protocols, it was an enjoyable read.

DARREL HO-YEN

Molecular Techniques in Medicine. Hildebrandt F, Igarashi P, eds. (£49.50.) Springer-Verlag, 1999. ISBN 3 540 57129 9.

All books are idiosyncratic, reflecting their authors in some way or other. This is a multi-author book, so its idiosyncracies mirror that fact. It is intended to be a working book, not bedtime reading, and it does not fail the latter criterion. It's not something for the wee small hours. Whether it's something for the daily round, the common task, is another matter. It all depends. Depends on the task, and upon you. So what is it? It starts from a narrow view of molecular biology. Molecular biology is claimed to be nothing more than the science of DNA and RNA, which would probably come as quite a surprise to the founders of the *Journal of Molecular Biology*. In other words, proteins are largely ignored. However, that a working book is focused is no bad thing. Too wide a range and it would be unmanageable in a laboratory. So what is it? It's a collection of recipes and tips on how to manipulate DNA and RNA with brief introductions to the technique being considered. It covers all the basic methods that a "molecular biologist" considers important: how to extract nucleic acids, how to modify and analyse them, how to clone DNA fragments, how to make and use various sorts of DNA libraries. In addition, it covers some more specialist areas, such as chromosome analysis by fluorescent hybridisation and transgenic animals. So is it useful? Well, yes and no! I haven't tried the recipes as such in detail, but they are along the same lines as other similar books, and I'm sure they work. So is it useful? Well, yes and no! It depends on what you want to do and on you. If you want to do everything from scratch, much of this book would be useful. But in my experience not many people want to do that, and the commercial companies provide so many kits for so many methods with such good instructions that one hardly bothers to do it any other way. The easy ways have enormous attraction. When you can buy a gene walking kit, why screen a λ gt library? For the more specialist topics, I would hate to think that this was all I had.

So why did I mention idiosyncracies? Well, there are omissions; quantitative polymerase

chain reaction, gel shift and supershift assays for transcription factors to name but a few. And on the side of superfluity, does one really need two chapters on pulsed field electrophoresis? In 15 years of working in and visiting molecular biology laboratories, I have only ever come across one group using the technique, and when I showed some interest, they gave me the equipment because they needed the space for something else. It's still somewhere about, unused of course, but that's my deficiency probably. Would I buy this book for my research group? Probably not! But I might buy it to use some of the basic recipes for setting up undergraduate practical sessions.

DAVID BOYER RAMSDEN

Molecular and Cellular Pediatric Endocrinology. Handwerger S, ed. ($125.00.) The Humana Press, 1999. ISBN 0 896 03406 2

This book forms part of the Contemporary Endocrinology series published by Humana with an amazing 12 other titles appearing in 1999. As the editor indicates, the aim of this book is to concentrate on those topics that have shown the most rapid change, and the book itself is therefore not comprehensive, although individual chapters are. As an approximation, growth receives the greatest attention, followed by steroid hormones and then a miscellany of topics—diabetes (mellitus and insipidus), thyroid cancer, and hypophosphataemic rickets. In no sense is the term "miscellany" intended to be derogatory; I particularly enjoyed the chapters on diabetes insipidus, thyroid cancer, and aspects of pituitary development, reflecting personal areas of ignorance and/or interest. The emphasis for most chapters is on molecular rather than cellular aspects, and most cover the clinical implications. An odd chapter here or there is rather dense, with no illustrations, making it heavy reading for someone new to the field, but on the whole all are well written and well referenced. An enjoyable and informative read and, although one might not read it cover to cover, it will be of interest to researchers new to the field and to clinicians and scientists providing a clinical service.

P M CLARK

Antiviral Methods and Protocols. Methods in Molecular Medicine, Volume 24. Kinchington D, Schinazi RF, eds. ($99.00.) Humana Press, 2000. ISBN 0 896 03561 1.

This is an interesting collection of techniques used to search for compounds active against a variety of different viruses, and also to identify mutations in viral genes conveying resistance to antivirals. Although antiviral compounds were discovered and described in research laboratories more than 60 years ago, their widespread clinical application has only come of age during the past 20–30 years. Over the past decade, a rational application of antivirals has started—that is, they are used after the viruses to be attacked have been investigated and found to be susceptible to particular compounds.

This volume deals with a variety of assays used to determine the antiviral activity of compounds against several viruses of clinical importance (hepatitis viruses, herpesviruses, human immunodeficiency viruses (HIV), influenza viruses, and papilloma viruses). The introductory chapter on antiviral compounds is very comprehensive but could have been slightly longer. Very interesting is the chapter on laboratory safety considerations, also outlining differences in hazards and risk evaluations carried out for various microorganisms in the USA, the EC, and the UK.

For hepatitis B virus (HBV) several cell bound assays (Hep AD38 assay, and cell line 2.2.15 assay) are described, the importance of which cannot be overestimated. Systems have been found that allow limited replication of HBVs in vitro, in which compounds inhibiting different steps of the HBV replication cycle (be it at reverse transcription, DNA synthesis, or other steps) can be investigated. Some of the assays are still very research orientated, whereas others lend themselves to application in specially equipped clinical laboratories. A high throughput assay for hepatitis C virus (HCV) helicase activity is presented that might be of interest to test for anti-HCV compounds; however, no practical examples for antivirals used against HCV are given. For herpesviruses, conventional plaque reduction assays and other antiviral activity assays are presented. Of interest is an assay that allows the measurement of the inhibition of TPA stimulated EBV antigen synthesis in cells. Furthermore, assays are described for monitoring the drug resistance of herpes simplex viruses, particularly against aciclovir, but also derivatives thereof (for example, ganciclovir) and phosphonoformic acid (PFA). Strains resistant to these drugs emerge in immunocompromised patients and their detection and monitoring are of great clinical importance.

Cell culture based and biochemical assays (targeting the viral enzymes reverse transcriptase (RT), protease, and integrase) are presented for HIV. The detection of specific mutations conveying drug resistance against AZT, ddC, ddI, d4T, 3TC and others can be facilitated by a reverse hybridisation assay (line probe assay; LiPA), whereby polymerase chain reaction (PCR) amplicons of the HIV RT gene are hybridised against a panel of specific immobilised oligonucleotide probes. This assay, which is described in full, gives very good results for recognising known mutations, but is limited in its power to recognise mutations in general. Because HIV genes are highly diverse, laboratories have started to sequence the amplicons of RT and protease genes of clinical isolates to obtain all mutations of the genes that might be important for the development of drug resistance. Large numbers of mutations associated with drug resistance, which are not detectable by LiPA, have been identified in this way. The sequencing procedure is of particular relevance for the evaluation of HIV protease gene mutations. Unfortunately, a precise procedure to carry out these increasingly important tests, in a manner that can be adapted to specially equipped routine laboratories, have only been alluded to and not described in detail.

Although papillomaviruses (HPV) are important human pathogens and their replication inhibition is of great potential preventative value, the tests described for assaying for DNA binding and helicase activities of the E1 protein of HPV are not put in a firm context of testing for antivirals, and their clinical importance is not made clear. Similarly, the methods described to characterise influenza viruses, which are mutant in their sensitivity to neuraminidase inhibitors (for example, 4-guanidino-neu5ac2en), are not put into a strong clinical context. The principle and potential of so called antisense oligonucleotides as possible antiviral therapeutics is only mentioned briefly, but no tests to evaluate them are identified.

Overall, I found the methods described well and in detail, but the introductory paragraphs of chapters were short on background information. In contrast, the annotations of individual authors about possible pitfalls and problems of methods, as well as ways to overcome them, are very helpful. More examples on clinical applications for which, after all, these methods are important would have been beneficial. Most references are up to date up to about 1996. Given the increasing importance of antiviral testing in clinical settings this book is timely in addressing the topic.

U DESSELBERGER

Molecular Biology of the Lung: Volume I, Emphysema and Infection. Volume II, Asthma and Cancer. Stockley RA, ed. (£205.00.) Birkhauser Verlag, 1998. ISBN 3 7643 5969 2.

About 10 years ago the American Thoracic Society and the American Physiological Society each launched a new journal dedicated to cell and molecular biology of the lung. These actions reflected appreciation of the skyrocketing growth occurring in these areas of lung research. *Molecular Biology of the Lung, Volumes I and II,* is another indicator of how far the field of lung cell and molecular biology has progressed.

The subtitles for these volumes, "Emphysema and Infection" for Volume I, and "Asthma and Cancer" for Volume II, show that the study of lung cell and molecular biology has extended to lung diseases. Indeed, "Diseases" could have been added to the book's title after "Lung" to highlight that the overriding focus is on the cell and molecular biology of lung diseases rather than on the normal lung. However, pulmonary clinicians beware! Without considerable grounding in contemporary biology it would be a struggle to comprehend much of what is presented. These chapters are not watered down basic science of disease meant to be enrichment reading for physicians. One indication of the book's seriousness in covering information in depth is the density of text pages relative to figures, tables, and charts. Most chapters have only a few figures; some have none. Another indication is that the references are almost exclusively to original research papers in basic science journals, rather than to reviews or articles in journals typically read by clinicians.

Each volume leads with an excellent chapter not linked to a single lung disease. In Volume I, the introductory chapter presents principles of making genetically altered mice and in Volume II it covers principles of gene therapy and applicability to specific diseases, such as surfactant protein B deficiency. These themes are taken up in some of the subsequent chapters, for example in the use of transgenic mice to study lung infections, and the application of gene therapy for lung cancer.

Emphysema is particularly well suited for coverage in a book about lung cell and molecular biology because of the compelling amount of cellular and biochemical data

pointing to inflammation and proteinase imbalance as key features of its development. The editor's prominent role in this field clearly shows in the selection of six interesting and authoritative chapters about mouse models of emphysema, serine proteinases, elastase inhibitors, and connective tissue genes. I found the chapter about the regulation of neutrophil proteinases especially informative. Although at least three or four years have elapsed since the chapters in this section were written, judging from the reference lists, they still hold up well in 2000. Similarly favourable comments could probably be made about the other chapters of the book, considering the distinguished authors, but I am less familiar with these other topics than with emphysema.

Choices are inevitable in selecting topics for a book on such a broad field as the molecular biology of the lung from the perspective of disease. Clearly, this book includes many of the important areas, but one wonders why interstitial lung disease with its intriguing features of fibrosis and inflammation was not included. Also, I would have enjoyed an introduction by the editor so that I could read his overall perspective on the field of lung cell and molecular biology, and his rationale for the topics he chose to include in the book.

ROBERT M SENIOR

Notice

Therapeutic Applications of Leucocyte Filtration

Hammersmith Hospital, Imperial College, London, UK

7 July 2000

This prestigious meeting has been organised by Professor K Taylor and Dr T Gourlay who have invited a guest panel of international speakers. Additional areas include leucocyte filtration of salvaged blood; total leucocyte control for cardiovascular surgery; and new therapeutic applications for use in sepsis patients in ICU and for patients undergoing angioplasty, etc. Speakers will include Gr W O'Neill, Professor KJ Oldhafer, Dr M Cross, Dr G Matheis, Professor S Homer-Vanniasinkam, Dr J Parker Gott, BS Allen, Dr A Fabbri, Dr L van der Waterings, Dr KA Brown, and Dr D Teacher.

Abstracts are invited for poster presentations and prizes will be awarded to the best three.

For further details, abstract forms, and registration forms please contact: Jean Bryant, Wolfson Centre, Imperial College of Science, Technology and Medicine, Hammersmith Hospital, Du Cane Road, London W12 ONN, UK; tel +44 (0) 208 383 3117; fax: +44 (0) 208 383 2428; email: wcc@rpms.ac.uk