Correspondence

Immunohistochemical detection of Ki67 antigen in formalin fixed, paraffin wax embedded sections

We read with interest the recent paper by Torp *et al*[1] reporting the detection of the Ki67 antigen in formalin fixed, paraffin wax embedded glioblastoma tissue sections using a monoclonal antibody directed against Ki67, purchased from Dako, Glostrup, Denmark. The authors did not recommend the use of this antibody on paraffin wax sections. We have also tested the antibody on sections from routine blocks from human gingiva, tonsils and lymph nodes using different time schedules for microwave pretreatment and application of the primary antibody. Two crucial procedures for detecting Ki67 antigen successfully were identified. Firstly, slides should be pretreated in a microwave oven in citrate buffer (pH 6.0) for over 20 minutes (4 × 5 minutes).[2] Secondly, Ki67 antibody (diluted 1 in 100–200) should be incubated overnight at 4°C to produce strong staining.

Torp *et al* pretreated sections by microwaving for only 10 minutes (2 × 5 minutes) and incubated the Ki67 antibody for only one hour. Under these conditions, we could not achieve satisfactory staining on sections of the three tissues described above. Bankfalvi *et al*[3] have reported that almost identical results were found with the Ki67 and MIB1 antibodies. In their method, microwave heating was carried out for 35 minutes (7 × 5 minutes) and the Ki67 monoclonal antibody was applied overnight.

However, it should be noted that microwave treatment has certain drawbacks compared with autoclave treatment. Human gingival tissue sections were sometimes disrupted and detached from slides during microwave heating, presumably because of the violent boiling procedure, whereas tonsil and lymph node sections were resistant to this procedure even after one hour in our laboratory. Furthermore, another disadvantage of the Ki67 antibody was background staining on epithelium and various cell types except polymorphs, although these were easily distinguishable from genuinely reactive structures. Thus, Ki67 monoclonal antibody is as useful as MIB1 even in formalin fixed, paraffin wax sections, if the tissue sections to be examined can withstand more than 20 minutes in a microwave oven.

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1 Torp SH, Johannesen E, Lindboe CF. Comparison of different Ki67 antibodies in human glioblastomas. *J Clin Pathol: Mol Pathol* 1995; **48:**M191–3.
2 Munakata S, Hendricks JB. Effect of fixation and microwave oven heating time on retrieval of the Ki-67 antigen from paraffin-embedded tissue. *J Histochem Cytochem* 1993;**41:**1241–6.
3 Bankfalvi A, Navabi H, Bier B, Bocker W, Jasani B, Schmid W. Wet autoclave pretreatment for antigen retrieval in diagnostic immunohistochemistry. *J Pathol* 1994;**174:**223–8.

Drs Torp, Johannesen and Lindboe comment:

We recently reported our experience with the prototypic Ki67 monoclonal antibody and a new Ki67 antiserum on frozen and paraffin wax section of human glioblastomas.[1] It was not the intention of this study to optimise the staining procedures but to use the standard procedures in our laboratory—that is, microwave oven heating 2 × 5 minutes (600 W), citrate buffer (pH 6.0) and incubation of the antibody for one hour at room temperature. Under these conditions, the prototypic Ki67 antibody produced variable immunostaining whilst the Ki67 antiserum yielded nice immunostaining on paraffin wax sections. Thus, we concluded that the latter can be recommended for use on paraffin wax sections.

We realise that time of fixation, microwave irradiation and antibody incubation as well as the pH of the buffer may all influence staining quality.[2–4] Comparison of data is more difficult when different immunostaining techniques are used. Therefore, we are of the opinion that on paraffin wax sections, only those antibodies that give satisfactory immunostaining under standard conditions should be recommended.

1 Torp SH, Johannesen E, Lindboe CF. Comparison of different Ki67 antibodies in human glioblastomas. *J Clin Pathol: Mol Pathol* 1995; **48:**M191–3.
2 Munakata S, Hendricks JB. Effect of fixation time and microwave oven heating time on retrieval of the Ki-67 antigen from paraffin-embedded tissue. *J Histochem Cytochem* 1993;**41:**1241–6.
3 Cuevas EC, Bateman AC, Wilkins BS, Johnson PA, Williams JH, Lee AHS, *et al.* Microwave antigen retrieval in immunocytochemistry: a study of 80 antibodies. *J Clin Pathol* 1994; **47:**448–52.
4 Imam SA, Young L, Chaiwun B, Taylor CR. Comparison of two antigen retrieval solutions in unmasking epitopes in formalin-fixed tissue for immunostaining (abstract). *Anticancer Res* 1995;**15:**1812.

Book reviews

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Adhesion Molecules and the Lung. Ward PA, Fantone JC (eds). Volume 89 in the series **Lung biology in Health and Disease.** Lenfant C (executive editor). (Pp 416; $165.00.) Marcel Dekker. 1996. ISBN 0 8217 9517 2.

This is the 89th volume in a continuing series on lung biology in health and disease and addresses the exponentially increasing information on adhesion molecules. As with all texts, we probably have our own views on how they should be presented and developed. Ideally, the book should start with a basic chapter on the whole process of cell recruitment, outlining the rolling of cells, loose adhesion, firm adhesion, and then cell migration. These processes involve different adhesion molecules. With this background, it might have been more appropriate to have started with selecting, proceeded to a chapter on the beta 2 integrins and then the other cell adhesion molecules.

There is some repetition throughout the chapters, as would be expected in such a complicated field, but this certainly does not detract from the strength of the book, which is very comprehensive, well referenced and generally easy to follow. The introduction is good and it is strong from the basic science point of view. It includes a chapter on signal transduction, the lesser known role of adhesion molecules in lung morphogenesis, the adhesion processes between the epithelial cells, and the underlying matrix. There are also chapters on bacterial adherence, a process which is a prerequisite for invasive infection, and the role of adhesion molecules in malignant metastases. From the clinical point of view, there are sections on experimental lung injury, wound healing, transplantation, and pneumonia.

One great omission from this book is the genetics of adhesion deficiency. The whole field of adhesion molecules was established following the observation of defective cell migration, shown to be related to deficiency of the adhesion molecules. There have been great advances in the understanding of the basic genetic defect and it would have been appropriate to have had a chapter on this. In addition, there is little on the immune response, which clearly requires very specific cell adhesion and what evidence is included in the text is found in sections such as those on lung transplantation. Again, it might have been better to have pulled out several chapters on cell specific adhesion processes such as those that involve the lymphocyte or the neutrophil. However, once again, this probably reflects personal preference rather than true criticisms of the book.

In summary, this is an important addition to the lung biology in health and disease series. It contains a lot of information that overlaps other fields of medicine and will appeal to both basic scientists and clinical scientists alike.

R A STOCKLEY

Gel Electrophoresis: Proteins. Dunn MJ. Series: **Introduction to Biotechniques.** (Pp 176; £17.95.) Bios Scientific Publishers in association with the Biochemical Society. 1996. ISBN 1 8 72748 21 X.

This soft back edition sets out to offer a practical introduction to undergraduates, postgraduate scientists and technicians in the field of electrophoretic protein separation methods. Overall, I found this book to be clearly written, well laid out, amply illustrated, and well referenced. I found the concept of summarising transcription, translation and protein structure in two and a half pages of text quite commendable, even if erring a little on the basic side. The book concentrates primarily on polyacrylamide gel electrophoresis (PAGE) and gives little space to any other forms of protein electrophoresis.

On the whole, I thought that the book dealt well with the theoretical and practical considerations of PAGE methods, but would have benefitted from clearer presentation of methodological details. If the reader requires an introduction to the theory behind PAGE methods, then it can be said that the book adequately fulfils its remit. However, the reader hoping to extract practical methodology from the text would be better advised to purchase—for example, the IRL practical approach series publication *Gel Electrophoresis of Proteins*, the latter being just one example of the many already popular publications with which Dunn's book will be competing. Verdict: not a bad book, but given the number of publications on the market, it is difficult to see what area *Gel Electrophoresis: Proteins* covers that are not already adequately covered by others.

S AFFORD

Molecular Basis of Oncology. Freireich EJ, Stass SA (eds). (Pp 000; £39.50.) Blackwell Science. 1995. ISBN 0-865-42254.

This book will be of interest to cancer pathologists and oncologists, but probably of most use to haematologists and those with a special interest in leukaemias. It must be said that there are elements of the volume which are particularly good but these are compromised by failings in other components, probably the result of uncertainties about the prospective readership. The weakest parts are the general review chapters which open the book. These are of undergraduate level and could not be considered a satisfactory preparation for the detailed material which follows. A glossary is included, a feature which often attracts potential readers and which if done comprehensively can be an invaluable aid to understanding the jargon of today's scientific literature. Unfortunately, the listing here omits many of the terms which are essential to translating reports of molecular analysis into scientific understanding. However, the book does have some excellent contributions on DNA mismatch repair defects (which review the contribution of such abnormalities to diseases other than cancer), tumour angiogenesis and a series of chapters on the molecular pathogenesis of individual types of leukaemia. There is an odd balance to the coverage of solid malignancies; with detailed review of the genetics of prostate cancer and brain tumours (to be applauded as these tumour types have received too little attention) but no chapter on breast cancer. Is the book a worthy purchase for your library? In the crowded market in which it competes it is outperformed both as a general introduction and specialist review by rival texts, some of which have the additional attraction of being cheaper.

N R LEMIONE

Cellular and molecular pathogenesis- Sirica AE (ed). (Pp 575; £76.00.) Lippincott-Raven. 1995. ISBN 07817 03018.

This text is an extremely comprehensive compilation of the basic mechanisms of disease. There are 35 authors contributing 20 chapters, and it can be justifiably claimed that most of the mechanisms that we currently understand to be involved in the development of disease processes have been covered and brought together in one volume. In order to achieve this end, certain aspects may have been sacrificed, so that the work is in danger of falling between the two stools of comprehensive work for academic researchers and textbook for the student. For instance, it is a pity that the fashionable subject of apoptosis is mentioned only in passing, and that the discussion of signal transduction mechanisms is largely limited to a treatise on protein kinase C. The book is targeted at advanced graduate students and post-doctoral researchers working in fields related to molecular pathology. Unfortunately, the reader is, in most cases, unable to go back to the original scientific publications; instead each chapter ends with a list of "suggested readings". This may be a ploy to save space and thus to allow the book to be published in one binding, but it is in my view a mistake to discourage the student and obstruct the research scientist from referring to the original work.

As is so often the case with edited compilations, the style and quality of the individual chapters varies considerably. In some the stated aim of the book, to introduce the student "to fundamental concepts and principles...using important paradigms and experimental models..." is followed, and there are excellent reviews on immunodeficiency disorders, cytokine networks and the central role of inflammation in pathology. In others, though, we are introduced to a string of "facts" in true student textbook style. This can be dangerous in a book that is so up to date; today's fact can be tomorrow's perjury, something that the student may not necessarily realise. This potential problem is exacerbated, again, by the reader's inability to refer easily back to the original work. Only in one chapter did I find the cautionary note that "this chapter represents only our current understanding, guaranteed to change as knowledge...continues to evolve". How I wish that we could engrave this statement in our students' minds rather than clog them with information.

On the whole, this textbook is a worthy addition to the field. Covering subjects from interleukins to acid rain, at £76.00 it represents reasonable value for money. I hope that its success will permit the future publication of a truly comprehensive, referenced and multi-volume edition.

D J BUTTLE

Transacting Functions of Human Retroviruses. Volume 193 in the series **Current Topics in Microbiology and Immunology.** Chen ISY, Koprowski H, Srinivasan A, Vogt PK (eds). (Pp 239; DM 177.00.) Springer Verlag. 1995. ISBN 3-540-57901-X.

Some 12 years after the identification of the causative agent of AIDS, HIV is now by far the most well understood of viruses. It is easy to forget that reverse transcriptase activity was discovered many years previously, in 1970. This discovery overturned the central dogma of biology at the time, that information flows from DNA to RNA to protein, and opened the field of retrovirology as a new area of scientific inquiry. HIV research has subsequently contributed greatly to this field. Nevertheless, we have not witnessed the great advances in antiretroviral therapy or HIV vaccinology that such knowledge may have been expected to fuel. Some of the problems encountered in this respect include the large degree of genome variation generated through viral replication, and the fact that by infecting CD4 cells, HIV directly damages the cells which comprise the mainstay of viral clearance. In addition, however, the more complex retroviruses encode a whole array of "regulatory proteins", which may upregulate, downregulate and coordinate gene expression, contribute to latency and interact with host proteins. There is increasing recognition of the importance of such proteins in the maintenance of retroviral infections. This of course makes such unique proteins a point of viral vulnerability, and has led to a concerted effort to develop antiviral strategies based on these targets. It is in this context that *Transactivating Functions of Human Retroviruses* should be approached.

This volume is one of the successful series of *Current Topics in Microbiology and Immunology*, covering the biology, structure and function of regulatory proteins of HIV, HTLV-1 and also a chapter on foamy viruses. There is a great emphasis on tat, rev, nef, vif, vpx, and vpu proteins of HIV. A considerable amount of information has accrued regarding the mechanism by which HIV tat regulates gene expression through binding to HIV RNA (TAR), and how rev affects viral specific mRNA stability. These respective chapters are highly condensed, and a more liberal use of diagrams may have made them more accessible to the non-specialist reader. I was also disappointed that the potential use of these proteins in antiviral gene therapy approaches is not given greater prominence. Any summary of the nef protein is difficult, given the array of contradictory data published over the past five years. The authors of the nef chapter have done well to provide an in depth, up to date, and well organised review. Adequate space is given to the nef deletion data from the SIV macaque model, which is of considerable interest with regard to the potential for live attenuated HIV vaccines (with nef deletions). Unfortunately, the publication of this volume preceded the availability of relevant information on nef variation in long term HIV infected survivors. The volume also benefits from a chapter on the use of transgenic mice in understanding the pathogenic role of regulatory proteins.

In summary, this volume is of variable quality. It includes a large amount of up to date information which is not always presented well, and more could be made of therapeutic implications. It will be most useful to those with an existing research or teaching involvement with retrovirology.

D PILLAY

Pathways for Cytolysis. Griffiths GM, Tschopp J (eds). (Pp 224; DM177.00.) Springer Verlag. 1995. ISBN 3-540-58725-X.

Pathways for Cytolysis is a recently published compilation of review articles by investigators from 11 of the leading laboratories carrying out research on mechanisms of cytotoxicity by T cells or natural killer (NK) cells. These reviews provide an extensive, in depth summary of current knowledge both about perforin mediated cytotoxicity and apoptotic death. During the past year or two, there has been a dramatic increase in our understanding of the mechanisms by which cytolytic effector lymphocytes mediate killing of susceptible targets. The development of perforin knock-out mice by several investigators has been the key advance that led both to the