Letters

Colonic angiodysplasia

I was very interested by the suggestion by Roskell et al that colonic angiodysplasia may be related in some way to abnormalities in vascular basement membranes. The idea is appealing, and the illustration of staining for type IV collagen does indeed appear to show a difference between the strong positivity in submucosal vessels (fig 1A) and absence in the lamina propria vessels (1B). However, in colonic mucosa there is a basement membrane between the columnar epithelium and the stroma. This contains type IV collagen. If we are to trust the apparent absence of staining of the vascular basement membranes in the lamina propria, can the authors explain why the adjacent epithelial basement membranes also appear to be completely negative in their illustrations?

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Authors’ response

Dr Furness is correct in pointing out that the epithelial basement membrane in the colon would be expected to stain for type IV collagen, and indeed in all of our cases and controls some staining was seen. However, at the antibody concentration used in our study the epithelial basement membrane staining was very weak and focal compared to the strong staining of the submucosal blood vessels. Indeed epithelial basement membrane staining has been in use since 1953 to assess the weak staining of the mucosal vessels seen in angiodysplasia.

Our observation of apparent deficiency of type IV collagen in the mucosal vessels represents as a relative deficiency compared to the strong submucosal vascular staining, and is not compared to the epithelial basement membrane. If we had used a concentration of antibody high enough to result in strong epithelial basement membrane staining sufficient for photography, the differences in vascular staining would not have been visible.

Derek Roskell, Simon Biddolph, Bryan Warren
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Immunofluorescent patterns associated with ANCA

We would like to comment on a number of issues raised by Savige et al in their recent paper in the Journal. First, they state that dual positivity for anti-MPO (myeloperoxidase) and anti-PR3 “usually indicates non-specific binding”—that is, a false positive. Our experience is different. We find dual positivity is a relatively rare phenomenon and tends to represent true positivity. This may in part result from our practice to include a control (unfixed neutrophils) to check for non-specific ELISA binding. Reviewing our last 500 referred samples reveals only one dual positive. This sample was from a 50 year old female with long standing biopsy proven Wegener’s granulomatosis. Immunofluorescence was C-ANCA pattern.

Next, we agree that antibodies such as antimitochondrial and anti-smooth muscle can stain neutrophils, but with the exception of antinuclear antibody (ANA), these rarely mimic true ANCA and seldom cause recognition problems in routine analysis. With regard to the problem of ANCA interference, we still find the use of formalin fixed neutrophils helpful in the differentiation of ANA and P-ANCA, though the method has its critics. In our ulterior, MPO antibodies are rarely formalin negative. A recent review of our data shows that, in a three month period, 0/56 sera showing nuclear stain on ethanol fixed neutrophils and negative for antimitochondrial and antismooth muscle antibodies (that is, ANA or atypical P-ANCA) were anti-MPO positive. Thus in the context of necrotising vasculitides it offers a quick and useful method for selecting sera for anti-MPO ELISA.

Lastly, Savige et al mention a range of atypical immunofluorescence staining patterns, such as the so called “flat CANCA.” We are strongly of the view that, while these are of research interest, they can be communicated clearly to ward doctors not as ANCA positive but instead as atypical reactivity of doubtful clinical significance.

R J Lock
D J Unsworth
Immunology, Southend Hospital, Broadstairs B160 5NB, UK


Authors’ response

Thank you for the opportunity to reply to Drs Lock and Unsworth. Their letter has highlighted some of the difficulties with ANCA testing that have been addressed by a group of clinicians and scientists in the International consensus document on ANCA testing and reporting.

The Consensus document states that the minimum requirements for ANCA testing in new patients are the indirect immunofluorescence (IIF) examination of all sera on normal neutrophils. Sera that contain ANCA, any other cytoplasmic fluorescence, or an ANA that results in a homogeneous or peripheral nuclear pattern should then be tested promptly in ELISA for both proteinase 3 (PR3) and MPO-ANCA.

The Consensus document recommends that reports use the terms “C-ANCA” for cytoplasmic granular fluorescence with interlobular accentuation, “C-ANCA (atypical)” for other types of cytoplasmic fluorescence, “P-ANCA” for any type of perinuclear or granulocyte specific nuclear fluorescence, and “atypical” for other less common patterns, such as mixed cytoplasmic and perinuclear fluorescence. Antigen specificities are described as PR3- and MPO-ANCA. Reports should indicate that positive neutroph IIF alone is not diagnostic for Wegener’s granulomatosis or microscopic polyangiitis, and that decisions about treatment should not be based solely on the ANCA results.

In reply to specific points raised by Drs Lock and Unsworth:

The ability to distinguish between ANCA and other autoantibodies that stain neutrophils depends very much on the quality of the neutrophil preparation and the skill of the observer. Because of this variability, the consensus document has indicated that all sera that produce positive IIF should be tested for PR3- and MPO-ELISA. The absence of binding in these assays will indicate that the diagnosis of Wegener’s granulomatosis and microscopic polyangiitis is unlikely. With respect to IIF patterns, the consensus document recommends that a “flat ANCA” is called a “C-ANCA atypical.” However, it is not always possible to distinguish this from a “C-ANCA” and for this reason ELISA for PR3- and MPO-ANCA should be performed to diagnose Wegener’s granulomatosis and microscopic polyangiitis.

The consensus document does not suggest formalin fixation to differentiate ANA and P-ANCA. This is because the success of this technique varies in different laboratories, ANA and P-ANCA may occur together, the procedure involves another “run” of IIF, and an ELISA is still necessary if the IIF remains positive, at least to determine the antibody level.

Finally, most laboratories, in Australia at least, use commercial kits for PR3- and MPO-ANCA and do not immerse the binding to uncoated wells for individual sera. Under these conditions “dual positivity” usually represents non-specific binding and is often “borderline” in amount. If background binding is subtracted, then “non-specific positivity” is more likely to represent a “true” ANCA.

Judy Savige (on behalf of the other authors)
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Long before the influence of Calman on training, my only guidance for preparation for MRCPath IV was "read Topley from cover to cover." There were two volumes then. Now there are six. New in the 9th edition are the international authorship, coverage of mycology and parasitology, and the CD version. This is now a massive text to review. I would not expect to find factual fault with any of the contributions from experts, nor did I, even in the areas on which I am qualified to opine. I shall concentrate on the new electronic format.

The CD offers the advantage at least of saving shelf space, but the book becomes "virtual" and there is no feeling of where one is in the text—nor indeed how much of it there is. I find it uncomfortable to read more than a small amount of text from the screen. It was easier to print individual sections that concerned me. I find it uncomfortable to read more than a small amount of text from the screen. I wanted to study (you can do this for up to 50 paragraphs).

The software allows text searches with varying degrees of complexity, rapid jumps, and personalisation of electronic annotation and colour highlighting of text. One can cut and paste text (for example references) into personal documents. This does facilitate plagiarism but that is more of a problem in students' assessed work.
than in professional shuddery. However, all the otherwise hidden tags that allow the jumps and popups are carried too.

The quality of the graphics varies from poor to acceptable. Electron micrographs were generally clear and fungal cultures looked good (fuzziness helps). The blood films of malaria did not adequately show dots and clefts. Some of the "extra illustrations exclusive to the electronic version" turned out to be very poor quality. This was a disappointment. Large tables were particularly tricky to navigate if they did not fit into one screen, and vertical labels were very hard to read. I got round that by highlighting columns in colour blocks—very useful in following a long vertical list of biochemical reactions in the identification of bacteria.

Where the paper reviewer can delight in finding typos, the electronic reviewer has the additional opportunity to look for bugs (even more fun in a book on infection). There are lots. Searching for *Mycobacterium* *bovis* takes you to all the occurrences of that species but also to *Mycoplasma* *bovis*. Some jumps don't get anywhere and some labels make no sense (tunnel vision?). Therefore it is very helpful to have illustrations next to text on histopathology. An atlas so closely matches the paper version its full potential can never be realised.

These are small gripes. This remains a very well written book which I would certainly recommend to all those clinicians, pathologists, and researchers with an interest in muscle diseases. Although not primarily intended as a pathologist's handbook, the book is well written and throughout the book the index appears to be helpful. In addition, there are up to date references. The majority of the 100 photographs are of very good quality. My main criticisms are somewhat trivial. Figure 1.1 is placed in chapter 3, which is rather irritating. In addition, I feel that some of the introductions to chapters are a little repetitive and would have benefited from some editorial pruning. In summary, this is a well written book which I would certainly recommend to all those clinicians, pathologists, and researchers with an interest in muscle diseases. Although not primarily intended as a pathologist's handbook, the book is well written and throughout the book the index appears to be helpful. In addition, there are up to date references. The majority of the 100 photographs are of very good quality.

Introducing the blood-brain barrier into textbooks. The book comprises 50 chapters by a leading international group of basic and clinical scientists (including several distinguished pathologists) and is divided into five sections concerning the methodology appropriate for the study of the blood-brain barrier, its transport biology, with more specific sections on signal transduction and immunology, and finally pathophysiology in disease states. The last of these chapters proved to be the most instantly appealing, with a wide ranging series of excellent contributions, from microvascular abnormalities in dementia to multiple sclerosis, from blood-brain disease with particularly interesting contributions on infectious diseases including HIV, cerebral malaria, and bacterial meningitis. The first section on methodology, containing a discussion of the clinical applications of barrier function methodology using MRI and PET technology, was particularly interesting and informative. The book has been well edited and produced, with numerous illustrations in monochrome. The index is excellent and the chapters are well

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Images are the key ingredient for a histopathological diagnosis—and a picture tells more than a thousand words. Further biology is so diverse that the number of morphological appearances is almost infinite. The CD-ROM display lists in almost the same format as the book. The first step has been taken to transform this reference text into electronic format, but as long as the CD version so closely matches the paper version its full potential can never be realised.


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**Letters, CD-Rom, Book reviews, Notices, Correction**

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The anonymous preface to this book claims that "adequate knowledge of the blood-brain barrier forms an essential component in the complete understanding of a large proportion of medical disciplines," and laments the general ignorance of its structure, function, and investigative techniques appropriate for this important structure. The book itself attempts to redress this problem. To what extent does it succeed? The answer is almost completely, as both the content and scope of the book belie a mere "introduction."

The book comprises 50 chapters by a leading international group of basic and clinical scientists (including several distinguished pathologists) and is divided into five sections concerning the methodology appropriate for the study of the blood-brain barrier, its transport biology, with more specific sections on signal transduction and immunology, and finally pathophysiology in disease states. The last of these chapters proved to be the most instantly appealing, with a wide ranging series of excellent contributions, from microvascular abnormalities in dementia to multiple sclerosis, from blood-brain disease with particularly interesting contributions on infectious diseases including HIV, cerebral malaria, and bacterial meningitis. The first section on methodology, containing a discussion of the clinical applications of barrier function methodology using MRI and PET technology, was particularly interesting and informative. The book has been well edited and produced, with numerous illustrations in monochrome. The index is excellent and the chapters are well

The second edition of this classic book, largely written by two international experts in the field and with contributions by a further eight authors, many of whom are well known experts themselves, offers a comprehensive and authoritative account of the morphology and pathophysiology of occupational lung disease. Important contributions on the clinical evaluation and on (American) legal aspects result in a broad perspective and make this book of interest also for others active in the management of patients suffering from these diseases.

The book provides a wealth of data, which are generally easily accessible through numerous tables and lists. Illustrations are also numerous and include a plethora of excellent micrographs of tissue sections, many chest radiographs, macroscopic specimen photographs, and scanning EM pictures and x ray spectra of a very large variety of inhaled dusts. The text is detailed, at times to such a degree that even the interested reader needs to make a conscious effort to finish reading the book in its entirety. Perhaps the layout of the book, which is a bit grey, and has a make up of text and tables which I personally consider rather uninviting, is partly to blame here. However, this should not interfere with our judgement of the essentials, to which these criticisms do not apply.

Pathologists with a special interest in lung disease should seriously consider buying this book. Basically, there is no competition: it is unique. One should, however, bear in mind that the large pulmonary pathology textbook (Dail and Hammar, Spencer/Hasleton, Thurlbeck) also offer much detailed information on the pathology of occupational lung disease.

Pathologists who do not have a special interest in this area may find that these more general texts are adequate for their requirements. Those, however, who wish to obtain a really detailed account of the pathology of this group of diseases and who are interested in the many additional bonuses of this text (chemical, clinical, mineralogical, forensic, legal) will not want to do without their personal copy of this extraordinary and outstanding book.

W J M OOI

Correction

In the Leader “Classification of acute leukaemias: the need to incorporate cytogenetic and molecular genetic information” by B J Bain (vol 51, June, pages 420–3), the fusion gene in M3-like AML associated with the (t(11;17)(q23;q21)) is given incorrectly in the text. The correct fusion gene, PLZF-RARα, is given in table 2.
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Immunofluorescent patterns associated with ANCA.

R J Lock and D J Unsworth

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