Re: Whither smooth muscle antibodies in the third millennium?

We read with great interest the article by Silvestrini et al. We present a patient (table 1) who presented with malaise, arthralgia, thrombocytopenia, low complement values, abnormal liver function tests, a strong homogeneous antinuclear antibody (ANA) result, and smooth muscle antibodies. We demonstrated that this patient's smooth muscle antibodies were specific for actin using Hep2 cells, a result that is suggestive of type 1 autoimmune hepatitis, and which was later confirmed by liver biopsy. Since then, we have tried to determine the target specificity of smooth muscle antibodies in our laboratory, which serves three hospitals and carries out over 10,000 autoantibody tests each year. Unlike the confirmatory tests described in the literature, Hep2 cells are routinely available. The human epithelial cell monolayer has been regarded as unreliable for the detection of actin specific antibodies, because the staining for actin varies from speckles, as a result of truncated cables, to randomly distributed filaments. However, we have now used Hep2 cells (fig 1) to confirm the actin specificity in 18 patient samples (table 1) following characteristic staining on rat liver, kidney, and stomach. We have had no difficulty in distinguishing actin specific antibodies from other cytoskeletal antibodies. We have recently started using a composite block for routine autoantibody screening, consisting of rodent kidney and stomach, and primate liver, together with human epithelial cells with good actin expression.

We are currently assessing the proportion of smooth muscle antibodies that are actin specific, and its predictive value for autoimmune hepatitis. We also describe three patients with biopsy confirmed type 1 autoimmune hepatitis (AIH) and antibodies directed against double stranded DNA, but no actin specific antibodies (table 2).

Although highly specific for type 1 AIH, actin specific antibodies have been described in primary biliary cirrhosis (PBC), alcoholic liver disease, connective tissue disorders, and healthy people. In our cohort, patients 8, 12, 17, and 18 had no actin specific antibodies.

### Table 1

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<th>Patient</th>
<th>Sex</th>
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<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
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<th>SMA</th>
<th>DNA abs</th>
<th>Alb</th>
<th>AST</th>
<th>Bili</th>
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<td>45</td>
<td>20</td>
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Normal ranges incorporating ranges from three different hospitals: IgG, 5–16; IgA, 0.7–4.0; IgM, 0.4–2.3; Alb, 32–51; ALP, 40–159; ALT, 10–36; AST, 5–40; bilirubin, 0–23. IgG, IgA, IgM, and albumin were measured in units of g/l; AST, ALT, and ALP were measured in units of IU/l; bilirubin was measured in units of µmole/l. *Liver biopsy performed; †ALT measured instead of AST.

ANA, antinuclear antibodies; Alb, albumin; ALP, alkaline phosphatase; ALT, alanine amino transferase; AST, aspartate amino transferase; Bili, bilirubin; SMA, smooth muscle actin.
and 13 have thyroid disease. Patient 13 also had anti-Hu antibody (also known as ANNA-1, anti-neuronal nuclear antibody 1), dementia, and oligoclonal bands in the cerebrospinal fluid, suggestive of a paraneoplastic disorder. Patient 6 has M2 antimitochondrial antibodies, suggestive of a PBC/AIH overlap. Patients 1, 2, 3, and 14 have had liver biopsies, the results of which were compatible with type 1 AIH.

We feel that the use of Hep2 cells is an easily applicable confirmatory test for actin antibodies. However the sensitivity and specificity of actin antibodies for type 1 autoimmune hepatitis should still be regarded with some caution.

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References

The Fas–FasL system and colorectal tumours

We read with interest the article of Bennett et al showing that the coexpression of Fas–FasL in colorectal adenocarcinomas lacks IELs or apoptosis. These authors previously found a progressive decrease in intraepithelial lymphocytes (IELs) during the progression from hyperplastic polyps to adenomas, then incipient adenocarcinomas, and finally advanced adenocarcinomas.1 The potential role that FasL counterattack plays in this decrease in IELs has not yet been established, and may prove difficult to do. However, it is interesting that apoptotic bodies have been observed at the margins of adenomas, and these could represent lymphocytes undergoing apoptosis while attempting to infiltrate the dysplastic lesions.2 Although we can only speculate that FasL counterattack plays a role in immune escape in adenomas, there is substantial evidence that FasL causes apoptosis and depletion of tumour infiltrating lymphocytes (TILs) in colon adenocarcinomas. In a study of 41 colorectal adenocarcinomas, Okada and colleagues recently demonstrated a significant association between FasL expression in the tumours and apoptosis of TILs.3 Furthermore, a high rate of apoptosis of TILs was associated with metastases and significantly poorer prognosis;4 this corroborates other evidence that low numbers of TILs are generally associated with a worse prognosis. We have found that even within individual colorectal adenocarcinomas, regional variation in the expression of FasL correlates with apoptotic depletion of TILs.5 Fasl positive tumour nests had fewer TILs and increased apoptosis of TILs relative to matched FasL negative tumour nests within the same tumours. Indeed, we have found that the presence of a vigorous inflammatory response within colorectal cancers is associated with clearance of micrometastases and improved survival.6 A good anti-tumour immune response appears to be vital

Table 2 The patients with biopsy confirmed type 1 autoimmune hepatitis and antibodies to double stranded DNA but no actin specific antibodies

<table>
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<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>IgG</th>
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<th>IgM</th>
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IgG, IgA, IgM, and albumin were measured in unit of g/l; AST, ALT, and ALP were measured in units of IU/l; bili was measured in units of µmole/l. ANA, antinuclear antibodies; Alb, albumin; ALP, alkaline phosphatase; AST, aspartate amino transferase; Bili, bilirubin, SMA, smooth muscle actin.

Authors’ reply

We appreciate the comments of Rubio and Jacobsson regarding our recent paper in the Journal of Clinical Pathology, which demonstrated early upregulation of FasL (ligand for Fas) in colon carcinogenesis. It is interesting that these authors previously found a progressive decrease in intraepithelial lymphocytes (IELs) during the progression from hyperplastic polyps to adenomas, then incipient adenocarcinomas, and finally advanced adenocarcinomas. The potential role that FasL counterattack plays in this decrease in IELs has not yet been established, and may prove difficult to do. However, it is interesting that apoptotic bodies have been observed at the margins of adenomas, and these could represent lymphocytes undergoing apoptosis while attempting to infiltrate the dysplastic lesions. Although we can only speculate that FasL counterattack plays a role in immune escape in adenomas, there is substantial evidence that FasL causes apoptosis and depletion of tumour infiltrating lymphocytes (TILs) in colon adenocarcinomas. In a study of 41 colorectal adenocarcinomas, Okada and colleagues recently demonstrated a significant association between FasL expression in the tumours and apoptosis of TILs. Furthermore, a high rate of apoptosis of TILs was associated with metastases and significantly poorer prognosis; this corroborates other evidence that low numbers of TILs are generally associated with a worse prognosis. We have found that even within individual colorectal adenocarcinomas, regional variation in the expression of FasL correlates with apoptotic depletion of TILs. Fasl positive tumour nests had fewer TILs and increased apoptosis of TILs relative to matched Fasl negative tumour nests within the same tumours. Indeed, we have found that the presence of a vigorous inflammatory response within colorectal cancers is associated with clearance of micrometastases and improved survival. A good anti-tumour immune response appears to be vital

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for the containment of primary tumour growth, and also to prevent metastasis. As an important mediator of immune downregulation, expressed early in colonic tumorigenesis, we agree with Rubio and Jacobsson that FasL could represent a target for future immunotherapeutic approaches.

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References


Rationalising the use of polymerase chain reaction based tests for diagnosis of common viral infections of the central nervous system

Polymerase chain reaction (PCR) based tests have proved to be useful for establishing the aetiology of many infections of the central nervous system (CNS). As a result there has been a rise in the demand for these relatively expensive tests. A recent study has shown that the detection of herpes simplex virus (HSV) by PCR is highly unlikely if the leucocyte count and protein concentration of the cerebrospinal spinal fluid (CSF) are within the normal range. They suggest that not performing PCR tests for HSV on CSF specimens with a normal leucocyte count and protein concentration will result in considerable savings without decreasing sensitivity for the detection of common viral infections of the CNS.

We reviewed the leucocyte count, protein concentration, and glucose concentration of all CSF specimens that were tested for viral pathogens using PCR during a 12-month period (March 2000 to February 2001). The PCR tests were performed at the Public Health Laboratory, Addenbrookes Hospital, Cambridge, UK. All specimens were initially screened using two in house testing panels, which included HSV, varicella zoster virus, enteroviruses, and ECHO 22 virus by a method described by Read et al. Further tests for individual viruses were performed if the screening tests were positive.

Forty five CSF specimens were tested by PCR. Thirty of 45 specimens had a raised leucocyte count (> 4 /cm$^3$) or protein concentration (> 600 mg/litre). Six of these 30 CSF specimens with a raised leucocyte count or protein concentration were positive for encephalitis (five) or HSV (one). Only one of 15 “normal” CSF specimens (normal leucocyte count and protein concentration) was PCR positive for HSV. However, this patient had advanced AIDS with severe neutropenia.

Although the number of patients studied is relatively small, our results not only confirm the observations of Tang et al but show that the PCR tests for other common viruses are also very unlikely to be positive in CSF specimens with a normal leucocyte count and protein concentration, except in immunocompromised patients.

We are aware that many clinicians frequently use a negative PCR to stop empirical aciclovir treatment for patients with suspected HSV encephalitis. We are unable to find the scientific evidence for this strategy. The question is whether negative PCR exclude HSV encephalitis? There is evidence that even PCR may be negative in early HSV$^+$ patients. We entirely agree that empirical treatment should be initiated for patients with suspected HSVE, but subsequent clinical progress, CSF findings, electroencephalogram results, and computed tomography scans, rather than a negative HSV PCR, should determine the need to continue treatment. Further details of events to be included should be sent to Maggie Butler, Technical Editor JCP, The Cedars, 36 Queen Street, Castle Hedingham, Essex CO9 3HA, UK; email: maggie.butter2@topworld.com

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