PostScript

CORRESPONDENCE

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Re: Whither smooth muscle antibodies in the third millennium?

We read with great interest the article by Silvestrini *et al*.[1] In November 1999, patient 1 (table 1) presented with malaise, arthralgia, thrombocytopaenia, 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,[1] 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.[2] 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.[3 4]. In our cohort, patients 8, 12,

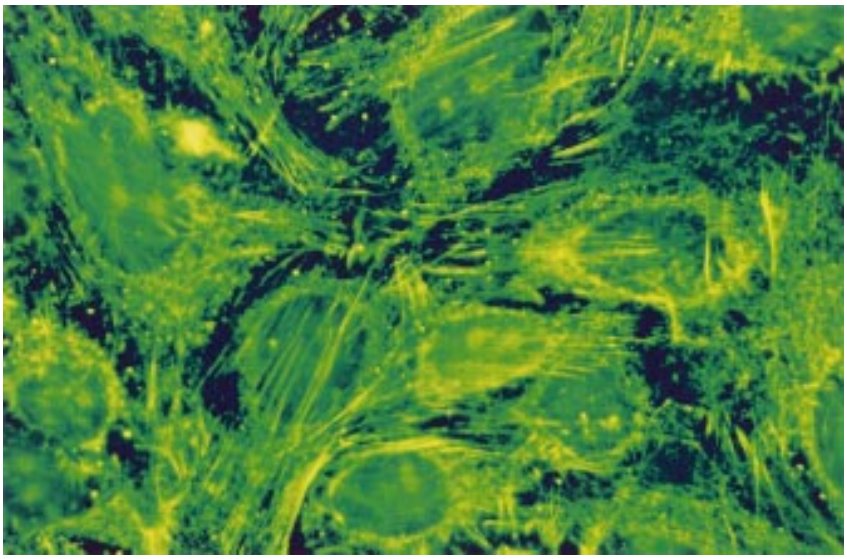

Figure 1 Characteristic staining of microfilaments in human epithelial cells by an anti-actin antibody.

Table 1 Eighteen patients with actin specific antibodies

Patient	Sex	Age	IgG	IgA	IgM	ANA result	SMA	DNA abs	Alb	AST	Bili	ALP
1*	F	15	61.4	2.2	1.76	>1/640 Homogeneous	>1:640	<30	26	752	116	332
2*	M	53	50.7	16.5	0.53	Negative	1/80	<30	13	54	48	99
3*	F	18	43	<0.07	1.57	1/40 Homogeneous	1/320	171	35	1092	209	141
4*	F	57	41.3	7.6	3.16	1/80 Homogeneous	1/160	42.5	27	1462	121	193
5*	F	62	34.6	1.6	2.4	1/80 Speckled	1/160	32	40	32	22	296
6	F	60	28.2	4.26	4	Negative	1/320	<30	41	74†	8	470
7*	F	56	18.4	1.66	1.11	1/40 Nucleolar	1/640	<30	39	121	7	52
8	F	78	17.4	7.25	1.21	1/160 Homogeneous	1/160	<30	38	59	6	115
9	M	84	16.4	7.56	2.2	>1/640 Homogeneous	1/80	<30	38	15†	32	338
10	F	69	15.7	0.94	0.92	1/160 Speckled	1/160	<30	45	24	12	93
11	M	75	14.6	3.45	1.34	Negative	1/80		41	26	21	110
12	M	72	10.8	3.29	1.2	Negative	1/160	<30	34	41†	23	127
13	F	57	9.5	1.65	0.56	1/320 Speckled	1/40	<30	42	15	9	38
14	M	51	9.4	1.67	1.3	Negative	1/80		45	84	20	67
15	F	76				Negative	1/640	<30	38	26	5	63
16	F	55				Negative	1/40		44	15†	12	115
17*	F	27				Negative	1/160	<30	42	460†	32	317
18	F	38				1/320 Homogeneous	1/80	35	45	20	10	75

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; bili 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.

Table 2 The 3 patients with biopsy confirmed type 1 autoimmune hepatitis and antibodies to double stranded DNA but no actin specific antibodies

Patient	Sex	Age	IgG	IgA	IgM	ANA result	SMA	DNA abs	Alb	AST	Bili	ALP
1	F	66	28.9	3.41	1.57	>1/640 Homogeneous	Neg	>300	37	1104	143	147
2	F	20	39.1	2.65	1.8	>1/640 Homogeneous	Neg	427	35	1076	90	327
3	F	22	21.6	2.4	1.17	>1/640 Speckled	Neg	76	45	343	8	206

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.

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 1–5, 7, and 17 (table 1) 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.

S J Katona, P C Cooper, M E Cramp, E R Kaminski
Department of Immunology, Level 7, Derriford Hospital, Plymouth PL6 8DH, UK; katonas@doctors.org.uk

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The Fas–FasL system and colorectal tumours

We read with interest the article of Bennett *et al*,[1] showing that the coexpression of Fas–Fas ligand (FasL) did not elicit increased apoptosis in colonic tumour cells. They suggested that colonocytes acquire resistance to Fas mediated apoptosis early in the transformation process. The Cork Group has greatly contributed to unravelling the riddle of the mechanism(s) behind the (CD95/APO1) receptor (Fas)–FasL system, which enables apoptosis to occur in neoplastic tissues.

Using a rat neu transgenic (rNeu-TG) mouse model, Céfai *et al* demonstrated that the FasL mediated escape from immune rejection of breast tumours correlated with the apoptosis of infiltrating T cells.[2]

During studies of similar colorectal lesions as those reported by Bennett *et al*, we investigated the occurrence of infiltrating lymphocytes.[3] In that study, intraepithelial lymphocytes (IELs) were recorded in 70% of 102 hyperplastic polyps and tubular adenomas, in > 80% of 75 villous and serrated adenomas, in 14 of 28 incipient adenocarcinomas, and in only nine of 50 advanced carcinomas. IELs were CD3 positive but major histocompatibility complex class II (MHCII) and TIA1 negative in the normal colorectal mucosa and in hyperplastic polyps. In contrast, MHCII and TIA1 were upregulated in IELs and Fas was downregulated in dysplastic cells from tubular, serrated, and villous adenomas, and in incipient and advanced carcinomas.[3] Proliferation and transmission electron microscopy studies provided no indication that the adenomatous cells were undergoing apoptosis. On the other hand, the accumulation of apoptotic granules between the base of the dysplastic cells and the basement membrane strongly suggested that the apoptotic bodies belonged to wandering cells trying to enter the dysplastic epithelium from the subjacent stroma.[4] Whereas the neoplastic associated lymphocytes are often intraepithelial in colorectal adenomas, they are often peritumoral in advanced carcinomas.[5] Patients with advanced rectal carcinomas who have peritumoral lymphocytes have a good five year tumour free interval, but those lacking peritumoral lymphocytes do not.[5]

Against that background, some questions seem pertinent, namely: What is the role played by the Fas–FasL system in advanced colorectal carcinomas lacking IELs? In contrast, what is the role played by peritumoral lymphocytes on the Fas–FasL system? These questions remain unanswered for patients without treatment because they will eventually succumb to the carcinoma, independent of the presence or the absence of intratumoral or peritumoral lymphocytes. However, in treated patients, thc peritumoral lymphocytes seem to master the "metastatic" tumour cells because after tumour removal by surgery those patients have a better tumour free five year interval than operated patients carrying tumours without peritumoral lymphocytes.[5] It has been suggested[4] that the "struggle" between IELs and adenoma cells with downregulated Fas molecules prevents rapidly proliferating adenomas from becoming clinically apparent cancer; a process that usually takes between 10 and 20 years. These notions are substantiated by the finding that a colonotropic carcinogen inducing slow growing colonic adenomas in rats often evoked IELs and pronounced apoptosis, whereas carcinogens able to induce fast growing adenomas seldom elicited IELs or apoptosis.[6]

The importance of Fas–FasL in surgically removed advanced colorectal carcinomas, either having or lacking peritumoral lymphocytes, remains elusive. However, that knowledge could be seminal for the design of immunotherapeutic strategies against advanced colorectal malignancies.

C A Rubio, B Jacobsson
Gastrointestinal and Liver Research Laboratory, Department of Pathology, Karolinska Hospital, 171 76 Stockholm, Sweden; Carlos.Rubio@onkpat.ki.se

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Authors' reply

We appreciate the comments of Rubio and Jacobsson regarding our recent paper in the *Journal of Clinical Pathology*,[1] which demonstrated early upregulation of Fas ligand (FasL) in colonic tumorigenesis. It is interesting that these authors previously found a progressive decrease in intraepithelial lymphocytes (IELs) during the progression from hyperplastic polyps through to adenomas, then incipient adenocarcinomas, and finally advanced adenocarcinomas.[2] 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.[3] 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 colonic 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.[4] 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 colonic 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 antitumour immune response appears to be vital

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.

M W Bennett, J O'Connell, A Houston, J Kelly, J K Collins, F Shanahan
Department of Medicine, National University of Ireland, Cork, Ireland; j.oconnell@ucc.ie

G C O'Sullivan
Department of Surgery, National University of Ireland

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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.[1]

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*.[2] 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 ($> 4/cm^2$) or protein concentration (> 600 mg/litre). Six of these 30 CSF specimens with a raised leucocyte count or protein concentration were positive for enteroviruses (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.[1]

We are aware that many clinicians frequently use a negative PCR to stop empirical aciclovir treatment for patients with suspected HSV encephalitis (HSVE). We are unable to find the scientific evidence for this strategy. The question is does negative PCR exclude HSV encephalitis? There is evidence that even PCR may be negative in early HSVE.[3] We entirely agree that empirical treatment should be initiated for patients with suspected HSVE, but subsequent clinical progress, CSF findings, electroencephogram results, and computed tomography scans, rather than a negative HSV PCR, should determine the need to continue treatment. Furthermore, in many hospitals such as ours, facilities for performing HSV PCR are not available in house. Dependence on negative PCR results to stop aciclovir treatment will entail referral of specimens to a reference laboratory and the continuation of treatment until the results are available.

We conclude that PCR based tests are best reserved for those patients in whom the CSF findings are compatible with viral meningitis or encephalitis; that is, raised white cell count ($> 4/cm^2$) and raised protein (> 600 mg/litre). Such a strategy will promote cost effective use of laboratory resources without compromising clinical care.

S Chakrabarti, D Garvie, K RayChaudhuri, G Gopal Rao
Department of Microbiology, University Hospital Lewisham, London SE13 6LH, UK; gopal.rao@uhl.nhs.uk

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