LETTERS TO THE EDITOR

Serum and tissue autoantibodies to colonic epithelium in ulcerative colitis

SIR,—I read with interest and disappointment the above article by Snook *et al* which appeared recently.[1] The authors confirmed previous reports from us and others regarding the presence of cytotoxic serum antibodies in a proportion of patients with inflammatory bowel disease, especially those with active disease, except that their percentage of positivity was much lower (20%) than all the other published reports (which were about 50% or more) and most frequent in ulcerative colitis.[2,3] One reason for this could be the use of different target cells – for example in this study the authors used HT-29 colon cancer cells compared with other studies (including ours) where RPMI-4788 colon cancer cells or normal colon epithelial cells were used as targets. Indeed, this is important because one of the target molecules on the colon cells recognised by ulcerative colitis serum antibody in cytotoxicity assay is associated with the Mr 40K protein[4] which acts as an autoantigen in ulcerative colitis.[5,6] Monoclonal and polyclonal anti-Mr 40K antibodies block the cytotoxicity of ulcerative colitis sera on RPMI-4788 and DLD-1 colon cancer cells,[4,7] showing the role of Mr 40K protein in this recognition. While normal colon epithelial cells, RPMI-4788, and DLD-1 colon cancer cells express Mr 40K protein, several colon cancer cell lines, including HT-29, which was used by Snook *et al*, did not express detectable amounts of the Mr 40K protein. Therefore, the use of normal colon cells or selection of appropriate colon cancer target cells is very important for the cytotoxicity study. On the basis of the cytotoxicity data using a single cell line – that is HT-29 – it is indeed misleading for Snook *et al* to conclude that 'lack of association with disease, extent and activity . . . lack of cytotoxic activity all strongly suggest this antibody is merely an epiphenomenon.'

Using 'ulcerative colitis (UC)-colon extracted IgG (CCA-IgG)', these authors reported completely negative results in their 'functional studies' such as 'cytoxicity assay' using HT-29 target cells and 'immunohistochemical staining' of colon tissue. These results are in contrast to a number of our independent reports[5-8] and the results of many other investigators.[9-12]

There are several major problems with the study of Snook *et al*. Firstly, the authors did not provide any evidence of intact immunoglobulin or immunoreactive Fab'_2 fragments being present in their ulcerative colitis colon extracted materials ('CCA-IgGs'). The presence of IgG reactivity was reported only with ELISA. Fragmented IgG and Fc fragments will react in the ELISA as performed by them, but will be functionally inactive in the assays they have used. Indeed, six of the 'CCA-IgG' preparations prepared by these authors were examined by us for Ig analysis and immunoreactivity to tissue antigen(s). None of the samples had detectable intact IgG when we analysed them several times by SDS-polyacrylamide gel electrophoresis. This was communicated to the authors well before the publication of the article.

Secondly, as mentioned above, HT-29 cells do not seem to be the right target cells for the cytotoxicity study as they do not express any detectable amount of Mr 40K protein.

Therefore, the 'negative functional studies' can easily be explained by the fact that the 'CCA-IgG' extracted by the authors are most likely fragmented by proteases, which are plentiful in colon particularly in ulcerative colitis. Such fragmentations are common if the extraction procedure is performed at room temperature.

A striking increase of local IgG production in ulcerative colitis,[9,10] with reactivity to colonic epithelial antigen(s)[11] have been reported by many investigators, besides our several independent studies.[5-8] The immunoreactive colon antigen, Mr 40K protein has been analysed by us using two sensitive techniques, by immunotransblot analysis[5,7] and by immunocytochemistry using monoclonal antibody.[8] Recently, Trond Halstensen *et al*[12] beautifully demonstrated by two colour immunofluorescence technique the presence of colon epithelial bound IgG (IgG_1 subtype) and also epithelial deposition of activated complement products (C_3b and terminal complement complex) in active ulcerative colitis. All these studies indicate the presence of autoantibodies against epithelial cellular protein(s) in ulcerative colitis which might play an important role in the pathogenesis.

While we share the 'disappointment' of the authors for their 'uniform failure in their efforts of extraction of functionally active tissue autoantibodies' in ulcerative colitis, their conclusion of 'no evidence of extractable, epithelial-bound autoantibody' is unfounded.

KIRON M DAS
Division of Gastroenterology and Hepatology, Department of Medicine, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, New Brunswick, NJ 08903-0019, USA

1 Snook JA, Lowes JR, Wu KC, Priddle JD, Jewell DP. Serum and tissue autoantibodies to colonic epithelium in ulcerative colitis. *Gut* 1991; **32**: 163–6.
2 Nagai T, Das KM. Demonstration of an assay for specific cytolytic antibody in sera from patients with ulcerative colitis. *Gastroenterology* 1981; **80**: 1507–12.
3 Auer IO, Grosch L, Hardorfer C, Roder A. Ulcerative colitis specific cytotoxic IgG-autoantibodies against colonic epithelial cancer cells. *Gut* 1988; **29**: 1639–47.
4 Biancone L, Ebert EC, Das KM. The Mr K40,000 colonic protein is associated with antibody dependent cell-mediated cytolysis (ADCC) by ulcerative colitis (UC) sera. *Gastroenterology* 1990; **98**(5): A159.
5 Takahasi F, Das KM. Isolation and characterization of a colonic autoantigen specifically recognized by colon tissue-bound IgG from idiopathic ulcerative colitis. *J Clin Invest* 1985; **76**: 311–8.
6 Takahasi F, Shah H, Wise L, Das KM. Circulating antibodies against human colonic extract enriched with a 40 kDa protein in patients with ulcerative colitis. *Gut* 1990; **31**(9): 1016–20.
7 Das KM, Sakamaki S, Vecchi M. Ulcerative colitis: specific antibodies against a colonic epithelial Mr 40,000 protein. *Immunological Investigations* 1989; **18**: 459–72.
8 Das KM, Vecchi M, Sakamaki S, Diamond B. The production and characterization of monoclonal antibodies to a human colonic antigen associated with ulcerative colitis: Cellular localization of the antigen using the monoclonal antibody. *J Immunol* 1987; **139**: 77–84.
9 Kett K, Rogmem TO, Brandtzaeg P. Mucosal subclass distribution of IgG producing cells is different in ulcerative colitis and Crohn's disease of the colon. *Gastroenterology* 1987; **93**: 919–24.
10 Scott MG, Nagm MH, Macke K, Nash GS, Bertovich MJ, MacDermott RP. Spontaneous secretion of IgG subclasses by intestinal mononuclear cells: differences between ulcerative colitis, Crohn's Disease, and controls. *Clin Exp Immunol* 1986; **66**: 209–15.
11 Hibi T, Aiso M, Ishikawa M, Watanabe M, Yoshida T, Kobayashi K, Asakura H, Tsuru S, Tsuchiya M. Circulating antibodies to the surface antigens on colonic epithelial cells in ulcerative colitis. *Clin Exp Immunol* 1983; **54**: 163–8.
12 Halstensen TS, Mollnes TE, Garred P, Fausa O, Brandtzaeg P. Epithelial deposition of immunoglobulin G_1 and activated complement (C_3b and terminal complement complex) in ulcerative colitis. *Gastroenterology* 1990; **98**: 1264–71.

Reply

SIR,—We are grateful to Dr Das for his comments, and especially for the information that HT-29 cells do not express the Mr 40K protein. We were also unable to show this protein by immunohistochemical staining using cytospin preparations, but did not use more sensitive methods.

We were, however, somewhat surprised by his comments about our negative findings for CCA-IgG being due to fragmentation of IgG. Dr Das rightly informed us that he was unable to detect CCA-IgG in our samples nor could he detect intact IgG. We therefore rechecked not only the frozen aliquots of the samples but also reconstituted freeze dried material which had been sent to Dr Das. The IgG content in the original aliquots and the freeze dried samples differed by less than 10%, and intact IgG was present. Furthermore, we sent the samples to an independent laboratory within the John Radcliffe Hospital which confirmed our findings. These results were communicated to Dr Das who was also sent a copy of the manuscript before it was submitted to *Gut*.

We conclude that CCA-IgG could not be detected in our patients, although we accept that the use of other targets may have shown cytotoxicity.

J A SNOOK
D P JEWELL
Gastroenterology Unit, The Radcliffe Infirmary, Oxford OX2 6HE

Correspondence to: Dr Snook.

Oesophageal complications in epidermolysis bullosa

SIR,—Drs Walton and Bennett have written a good overview of oesophagocutaneous diseases (*Gut* 1991; **32**: 694–7). Such diseases are rare and information on their appropriate management is therefore limited. We have an interest in epidermolysis bullosa (EB) and have recently reviewed 258 cases covering all major forms of EB to determine the prevalence of oesophageal lesions, among other features.[1] Some comments made by Walton and Bennett need to be qualified.

Firstly, oesophageal lesions needing dilatation can occur in dominant dystrophic EB. Some 20% of our 57 patients with dominant dystrophic EB had dysphagia, and oesophageal dilatation was needed in about half of these.[2] Dysphagia was most common in recessive dystrophic EB (72% of 36 patients), but oesophageal dilatation was needed in a similar proportion. Secondly, cervical strictures are not 'easily dilated'. We try to perform dilatation under general anaesthetic at the same time that the patient is having another operative procedure (such as division of acquired syndactyly), and this requires expert anaesthetic care to avoid trauma to the orofacial tissues, apart from negotiating with a guide wire a stricture that may start 18 cm from the teeth. We are