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Autoimmunity in ulcerative colitis: tropomyosin is not the major antigenic determinant of the Das monoclonal antibody, 7E12H12

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SUMMARY

Ulcerative colitis (UC) has a proposed autoimmune pathogenesis. A 40-kD antigen (P40) has been isolated from UC colon, bound to immunoglobulin. Tropomyosin has been reported as the target antigen of a MoAb (7E12H12) raised against P40. We set out to investigate whether tropomyosin is the major antigenic determinant for 7E12H12. Formalin-fixed, paraffin-processed and cryostat sections of fresh frozen colon from patients with UC, Crohn's disease and normals, were immunostained with 7E12H12 and commercial anti-tropomyosin antibodies. In addition, the immunoreactivity of 7E12H12 with cytoskeletal components was examined on human endothelial cells (HUVEC) using anti-tropomyosin as a positive control. Con-focal microscopy was used to determine the subcellular localization of signal. An extract of total colonic protein from UC colon was prepared. Using a combination of Western and immunoblotting (dot-blots), the immunoreactivities of both tropomyosin (porcine and chicken) and colon protein extract with either 7E12H12 or commercial anti-tropomyosin were examined. Immunocytochemically, 7E12H12 localized to the apical and basolateral regions of plasma membrane, and to the supranuclear cytoplasm in colonic epithelium. Using anti-tropomyosin antibody it was not possible to identify the cytoskeleton in colonic epithelium. Cytoskeletal components were identifiable in HUVEC cultures with anti-tropomyosin antibody but not with 7E12H12. P40 antigen was identified in the colon protein extract by immunoblotting with 7E12H12. There was clear immunoreactivity between anti-tropomyosin antibody and both chicken and porcine tropomyosin, and the colon protein extract. 7E12H12 did not bind to either chicken or porcine tropomyosin in appropriately controlled systems. We conclude that the pattern of immunostaining with 7E12H12 is not cytoskeletal, and there is no reactivity in immunoblots, between tropomyosin and 7E12H12. Tropomyosin is not the major target antigen of this antibody in ulcerative colitis.

Keywords ulcerative colitis autoimmunity tropomyosin pathogenesis

INTRODUCTION

An autoimmune pathogenesis has been proposed for ulcerative colitis (UC). A variety of circulating antibodies have been identified in UC, including anti-colon antibodies, x-anti-neutrophil cytoplasmic antibodies (ANCA) and anti-smooth muscle antibodies [1,2]. Several mucosal antigens have been proposed as the trigger for the formation of *in situ* immune complexes within the colonic mucosa [3–5]. The Das P40 protein has been reported to be a promising potential auto-antigen; it is a 40-kD protein, characterized recently as tropomyosin [6]. The observation that the 40-kD antigen co-localized with both IgG1 and components of the complement cascade using triple immunostaining [7], plus its presence in extra-colonic sites of disease [8], make it of considerable interest in terms of pathogenesis.

Following the original identification of a colon tissue-bound immunoglobulin (CCA-IgG), a 40-kD target antigen was identified using Western blotting and radiolabelled CCA-IgG [9]. Studies revealed that although the 40-kD protein was also present in normal colon and Crohn's disease, it was only bound to immunoglobulin in UC colon. Attempts to characterize the antigen involved partial purification and enrichment of the 40-kD protein by anion-exchange chromatography. The 40-kD colonic protein was then used as an antigen to generate an IgM murine MoAb, 7E12H12, which gave strong immunocytochemical staining of colonic mucosa. Competitive binding experiments confirmed that the MoAb 7E12H12 and CCA-IgG bound to the same epitope. It is notable that in both immunohistochemical and immuno-electronmicroscopic studies of

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7E12H12 binding, signal was observed predominantly at the plasma membrane in the apical and basolateral regions of the luminal and crypt cell epithelium, a distribution that is not readily consistent with a cytoskeletal element [6].

Two tryptic fragments of P40, separated by high pressure liquid chromatography (HPLC) were found, by internal amino acid sequencing, to have 93–100% homology in 14–16 amino acids with tropomyosin from different sources (human fibroblast, cytoskeleton, muscle, rat muscle and Japanese quail). In addition, P40 reacted with anti-tropomyosin in immunotransblot analysis, confirming that P40 contained tropomyosin as one of its constituent peptides. Recent data suggest that UC patients have significantly elevated titres of circulating IgG antibodies to tropomyosin [6]. However, these results do not confirm that tropomyosin is the antigen for either CCA-IgG or 7E12H12.

The purpose of this study was to use a combination of morphological and protein immunoblotting techniques, to confirm or refute the hypothesis that tropomyosin is the major antigen for the MoAb 7E12H12.

MATERIALS AND METHODS

Immunocytochemistry

The method used was adapted from that originally used by Das *et al.* [10].

Immunocytochemistry on both paraffin-processed and cryostat sections of fresh frozen colon was performed in order to compare the binding patterns of 7E12H12 (gift from Dr K. M. Das, New Brunswick, NJ) and commercial anti-tropomyosin (Sigma Chemical Co., Poole, UK). Tissues were obtained from UC patients ($n = 10$), patients with Crohn's disease ($n = 10$), or non-inflammatory disease controls ($n = 10$), diagnosed using standard clinical, radiological and histological criteria. Human umbilical vein endothelial cells (HUVEC) were grown to confluence by established methods and were used as a source of a discontinuous cell line of human origin that could be shown to contain tropomyosin by immunocytochemistry.

Immunoperoxidase technique

Sections (5 μm) of formalin-fixed tissues from patients were used for immunostaining with 7E12H12. These were deparaffinized in xylene and taken to water through alcohol.

Immunoreactivity was examined using a three-stage technique. Briefly, sections were incubated in 7E12H12 at a dilution of 1:50. Second stage incubation in biotinylated goat anti-mouse (GAM^{b}) (Southern Biotechnology Assoc., Birmingham, AL) was followed by avidin-biotin complex, and colour development reaction achieved with 3,3 diaminobenzidine tetrahydrochloride (Sigma). Non-specific binding was blocked using 5% normal goat serum (NGS) and endogenous peroxidase activity quenched with 3% H_2O_2 in methanol. All reagents were titrated for optimum immunostaining, although the dilution of 7E12H12 was the same as that used by Das. Sections were counter-stained and mounted in DPX (BDH Chemicals Ltd., Poole, UK).

Indirect immunofluorescence and con-focal microscopy

The sub-cellular localization of 7E12H12 binding was visualized by indirect immunoflourescent labelling and con-focal microscopy. Briefly, unfixed 5-μm cryostat sections from patients were incubated with 7E12H12 at a 1:50 dilution. Second stage incubation was performed with a 1:10 dilution of a flourescein-linked goat anti-mouse (GAM-^{FITC}) conjugate (Southern Biotechnology), and sections were then mounted in glycerol:PBS (1:1) solution containing an anti-fading agent (Citiflour, Canterbury, UK).

This protocol was also used to study the binding pattern of both 7E12H12 and anti-tropomyosin to HUVEC. The primary antibody was omitted in negative controls. Anti-tropomyosin (Sigma, cat. no. T2780, clone TM311, raised against chicken gizzard tropomyosin) or 7E12H12, were applied to multi-chamber slides (Lab-tech, Nunc Inc., Naperville, IL), supporting confluent monolayer cultures (passages 3–5) of HUVEC. Sections were examined using both UV and con-focal microscopy.

Protein extraction from intestinal resection specimens

For preparation of the intestinal homogenate, 5 g of colonic tissue, which had been stored deep frozen, from one patient with UC, was divided from serosal fat and thawed in an ice bath. All subsequent manipulations were carried out at 4°C. The sample was finely chopped, washed in PBS buffer containing 2 mM EDTA, 2 mM PMSF, and 2 mM Na azide, washed five times and filtered (Whatmans no.1 filter paper; Maidstone, UK); 10 volumes of PBS were added.

Homogenization was performed at a speed setting of × 5 using an ultra turrax homogenizer (Janke and Kuntal GmBh, Staufen, Germany) for four cycles each of 10 s. The solubilized extract was centrifuged at 2000 *g* for 20 min at 4°C and the supernatant ultracentrifuged at 20 000 g for 1 h at 4°C. The resulting supernatant was removed, and the protein concentration estimated using a BioRad protein assay. The protein extract, 1 mg/ml, was stored frozen in 1-ml aliquots at −80°C.

Immunoblotting

Western blotting was consistently unsuccessful in our hands, presumably due to epitope lability of the antigen for 7E12H12 in the preparative stages. Therefore, we elected to demonstrate antigen–antibody reactivity using an immunoblot (dot-blot) method. Both porcine tropomyosin (cat. no. T2400; Sigma) and chicken gizzard-derived tropomyosin (cat. no. T3026; Sigma) were used as the antigenic substrates for dot-blot experiments. In previous experiments using the same porcine tropomyosin (Sigma T2400) as the antigenic substrate for an ELISA, clear immunoreactivity between the sera of patients with UC and this antigen was identified [6]. The lyophilized powders were diluted in 1 M KCl to achieve a 1 mg/ml solution of the antigen.

The commercial anti-tropomyosin antibody chosen was a murine IgG MoAb, raised against chicken gizzard tropomyosin (Sigma clone TM311). This MoAb is known to react with chicken and porcine tropomyosin isotypes (Sigma). Aliquots (20 μl) containing 20 μg protein of chicken gizzard tropomyosin (T3026 Sigma), porcine tropomyosin (T2400 Sigma), and colon protein extract were applied to strips of nitrocellulose (Sartorius AG, Gottingen, Germany) using a micropipette.

Nitrocellulose membranes were air dried, baked for 2 h at 37°C, transferred to Sterilin containers (Sterilin, Stone, UK) and incubated for 2 h with 2 ml 5% dried milk powder, Marvel (Nestlé, Stafford, UK) in TBS (20 mM Tris, 500 mM NaCl, pH 7·5) to block non-specific binding sites. They were

then washed for 5 min with 2 ml Tween–TBS (0·05% v/v Tween 20; BDH Chemicals Ltd) in TBS.

Primary antibodies, diluted in antibody buffer (1% Marvel in TBS), were then added to the incubation containers. These included anti-tropomyosin IgG isotype (Sigma), at 1 : 100 dilution, and 7E12H12 IgM isotype at 1:50; for the negative controls the primary antibody was omitted. Nitrocellulose strips were left overnight at 4°C in blocking solution. They were then washed three times with 3 ml Tween–TBS.

Detection of bound antibody was achieved using an avidin–biotin system. The nitrocellulose strips were incubated for 1 h at room temperature in the presence of biotinylated anti-mouse link antibodies: for 7E12H12, the link was a μ-chain-specific biotinylated goat anti-mouse (GAM^b); for anti-tropomyosin the link conjugate used was a γ-chain-specific biotinylated rabbit anti-mouse (RAM^b) (Dako, High Wycombe, UK). Both of these antibodies were used at a dilution of 1:100 in antibody buffer. Following incubation the nitrocellulose strips were washed three times in 3 ml Tween–TBS. Streptavidin conjugated to alkaline phosphatase (Dako) was added at a dilution of 1:50 for 30 min and the nitrocellulose strips then washed twice for 5 min with 50 ml Tween–TBS. The blots were then washed, in TBS alone, to remove Tween, which may interfere with the colour reaction.

Colour development

Nitro blue tetrazolium (NBT; 30 mg) was dissolved in 1 ml of a 70% solution of NN-dimethyl-formamide (DMF). Bromo-chloro-indolyl-phosphate (BCIP; 15 mg) was dissolved in 1 ml DMF. Immediately before use the NBT and BCIP solutions were mixed and added to 100 ml bicarbonate buffer containing 0·1 M $NaHCO_3$, 1 mM $MgCl_2$ pH 9·8. This developer solution (5 ml) was added to the sterilin pots, which were left on a roller mixer for 5 min. The colour reaction was terminated by washing repeatedly in 10% acetic acid in double distilled H_2O.

SDS–PAGE

SDS–PAGE gradient gels (4–20%; BioRad, Hemel Hempstead, UK) were used for the electrophoresis of antigens. Briefly, 20-μg aliquots of tropomyosin (chicken and porcine), colon protein extract and rainbow markers (Amersham International, Little Chalfont, UK), were mixed with a reducing buffer containing 3·75% β-mercaptoethanol and 0·03% bromophenol blue in 1-ml Eppendorf tubes, and placed in boiling water for 2 min. Samples (20 μl) were loaded into the stacking wells of the gel and electrophoresed at a constant current of 35 mA. Identification of the electrophoresed proteins was achieved by overnight incubation in coomassie blue and gels were destained in 10% acetic acid.

Western blotting

Following SDS–PAGE electrophoresis, gels were incubated in transfer buffer (39 mM glycine, 48 mM Tris, 0·0375% SDS, 20% methanol); transfer to nitrocellulose membranes was carried out at a constant current of 108 mA for 1 h using a semi-dry transfer cell (Transblot SD; BioRad) The nitrocellulose strips were washed in TBS and the immunoidentification performed using an identical method to that for the dot-blots.

The experiment was repeated using non-reducing conditions in SDS–PAGE gels. To achieve a non-reducing environment, samples were added to sample buffer that did not contain mercaptoethanol, and were added at room temperature to the stacking gel.

RESULTS

Immunocytochemistry

Immunoperoxidase staining and light microscopy showed an identical pattern of binding by 7E12H12 to colonic epithelium to that described previously by Das *et al.* [10]. Antibody binding localized to the luminal and crypt epithelial plasma membrane. Similar patterns were observed using indirect immunofluorescence (IIF) and both light and con-focal microscopy (Fig. 1). Supranuclear intracytoplasmic localization of the P40 antigen was apparent using the con-focal image acquisition system (Fig. 2). Application of the commercial anti-tropomyosin antibody to colonic tissue sections produced no recognizable immunostaining at the light microscopic level.

There was no demonstrable immunostaining when the reactivity of 7E12H12 with HUVEC was examined. This was despite a range of antibody concentrations, including the dilution which had successfully stained frozen and paraffin sections of colonic mucosa. When these cells were stained with commercial anti-tropomyosin there was clear immunofluorescence of cytoskeletal elements (Fig. 3). In addition, there was no evidence of any positive staining in the absence of either 7E12H12 or anti-tropomyosin. These reactions were all carried out in the presence of appropriate controls.

Immunoblotting

Initial experiments confirmed that it was possible to demonstrate clear immunoreactivity between both chicken and porcine tropomyosin and commercial anti-tropomyosin antibody (Fig. 4). Using this same system we demonstrated that the anti-tropomyosin antibody could identify human tropomyosin contained in the colon protein extract (Fig. 4). In each case, no signal was seen in the negative controls.

Using the same system with 7E12H12, we confirmed that the colon protein extract contained an antigen to which this antibody bound (Fig. 4). It was concluded therefore that the colon protein extract contained both tropomyosin and the P40 antigen (Fig. 4). Subsequent application of 7E12H12 to either porcine or chicken tropomyosin, using the colon protein extract as a positive control, showed no immunoreactivity (Fig. 4). This was not altered either by increasing the antigen load applied to nitrocellulose from 20 μg to 60 μg, or by decreasing the concentration of the antibody 7E12H12.

SDS–PAGE electrophoresis and Western blotting

Bands were identified for chicken tropomyosin (36 and 38 kD), and for porcine tropomyosin (41 kD) following SDS–PAGE (Fig. 5). The molecular weights were calculated from semi-log plot of the Rf (electrophoretic mobility) of molecular weight standards *versus* the molecular weight values. The results matched exactly the data sheet estimates of the molecular weights of the two preparations of tropomyosin.

Attempts by our group to use 7E12H12 to identify the P40 antigen in the colon protein extract by Western blotting, under both reducing and non-reducing conditions, were unsuccessful. This suggested that the epitopes in the P40 antigen, that confer antigenicity to 7E12H12, were lost following reduction in

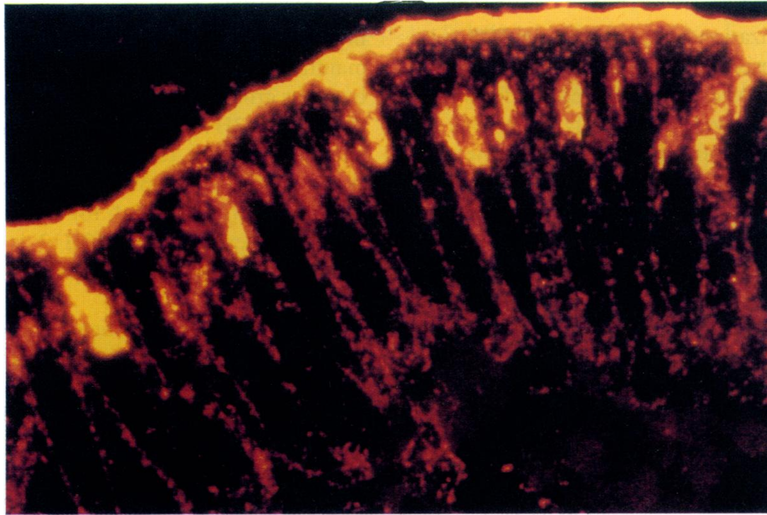

Fig. 1. Con-focal image of immunostaining of normal colonic epithelium with 7E12H12, showing luminal plasma membrane localization. (Mag. × 40.)

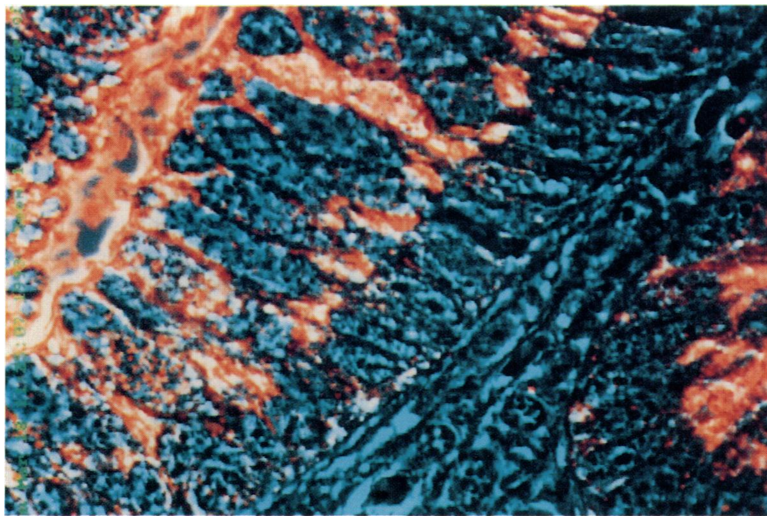

Fig. 2. Con-focal image of immunostaining of normal colonic epithelium with 7E12H12 showing intracellular, supranuclear localization of antigen. (Mag × 100.)

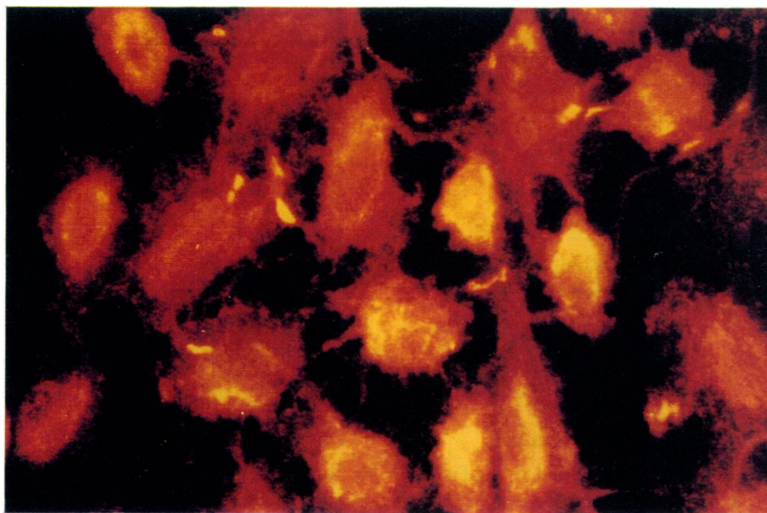

Fig. 3. Con-focal image of immunostaining of the cytoskeleton of human endothelial cells (HUVEC) using anti-tropomyosin antibody. (Mag. × 40.)

mercaptoethanol and linearization of the protein in SDS gels. Similarly, using electrophoresis under non-reducing conditions, it was not possible to demonstrate the P40 epitope following Western transfer (data not shown). Conversely, there was no epitope loss following Western transfer of the porcine and chicken tropomyosin (Fig. 6). The immunoidentification clearly identified a 42/46-kD doublet for chicken tropomyosin and a 41/48-kD doublet for porcine tropomyosin. The molecular weights of the constituent bands of tropomyosin in the Western blot were several kD higher than those identified on SDS gel electrophoresis. This Western blot also identified the presence of human tropomyosin in the colon protein extract. Immunoidentification showed a band at 48 kD indicating the presence of human tropomyosin.

DISCUSSION

The above data do not support the contention that tropomyosin is the major 40-kD antigen to which the MoAb 7E12H12 binds.

Morphological evidence against P40 being tropomyosin

Initially, the original pattern of localization of the P40 antigen as detected by immunocytochemical staining of the colon with 7E12H12 was confirmed. Tropomyosin, an ubiquitous cytoskeletal protein, is present in intestinal tissues such as small intestine and stomach, that have not been reported to bind 7E12H12 [10]. Although regional differences in isoforms of tropomyosin might explain this observation, there is no evidence for this. Perhaps more significantly, the subcellular localization of 7E12H12 binding is not consistent with tropomyosin, which was readily demonstrable in primate cells maintained *in vitro*. The pattern of immunostaining reported by other groups for tropomyosin is substantially different from that of 7E12H12. With tropomyosin, staining of the rootlet filaments and the terminal web is seen, and restricted to the apical and basal cytoplasm [11]. The localization for 7E12H12 is not therefore typical for this cytoskeletal element. The pattern observed for 7E12H12 is consistent with the local synthesis and export of an epithelial cell-associated molecule.

Immunoblotting evidence against P40 being tropomyosin

We produced an extract of colonic protein using similar methods to Das *et al.* [10]. The cross-species reactivity demonstrated in our studies, using this antibody raised against chicken gizzard tropomyosin, suggests marked interspecies conservation of the antigenic epitopes of tropomyosin. It was considered reasonable at this stage to extrapolate to human tropomyosin, observations concerning immunoreactivity of 7E12H12, using purified antigen from other species.

The lack of binding between 7E12H12 and tropomyosin implies that the latter is not the major antigenic determinant of this antibody. It could be argued that human tropomyosin has a unique antigenic determinant, to which 7E12H12 binds, that is not shared by other animal species. So far it has not been possible to obtain pure human tropomyosin to clarify this point. However, the morphological studies are evidence against this.

It might be argued that the absence of binding to purified tropomyosin in dot-blots is due either to inadequate amounts of antigen, or to excessive dilution of the antibody. The first argument seems unlikely, since the colon protein extract

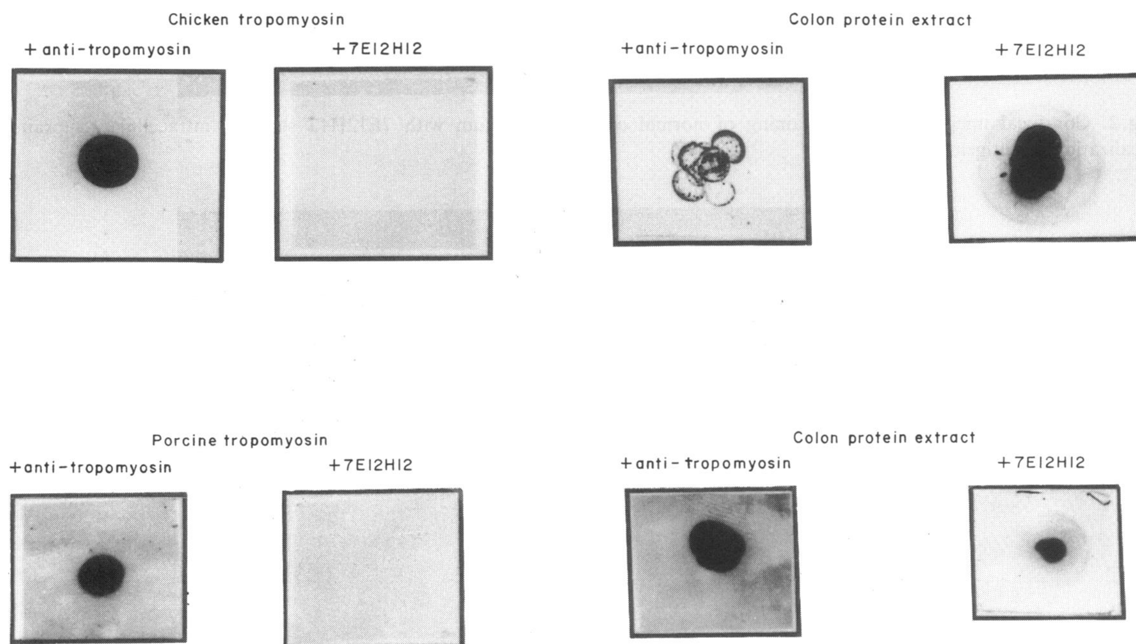

Fig. 4. Dot-blots showing positive immunoidentification of chicken and porcine tropomyosin using commercial anti-tropomyosin. P40 contained in the colon protein extract is identifed by 7E12H12. Tropomyosin contained in the colon protein extract is identified by anti-tropomyosin.There is no immunoreactivity between either chicken or porcine tropomyosin and 7E12H12.

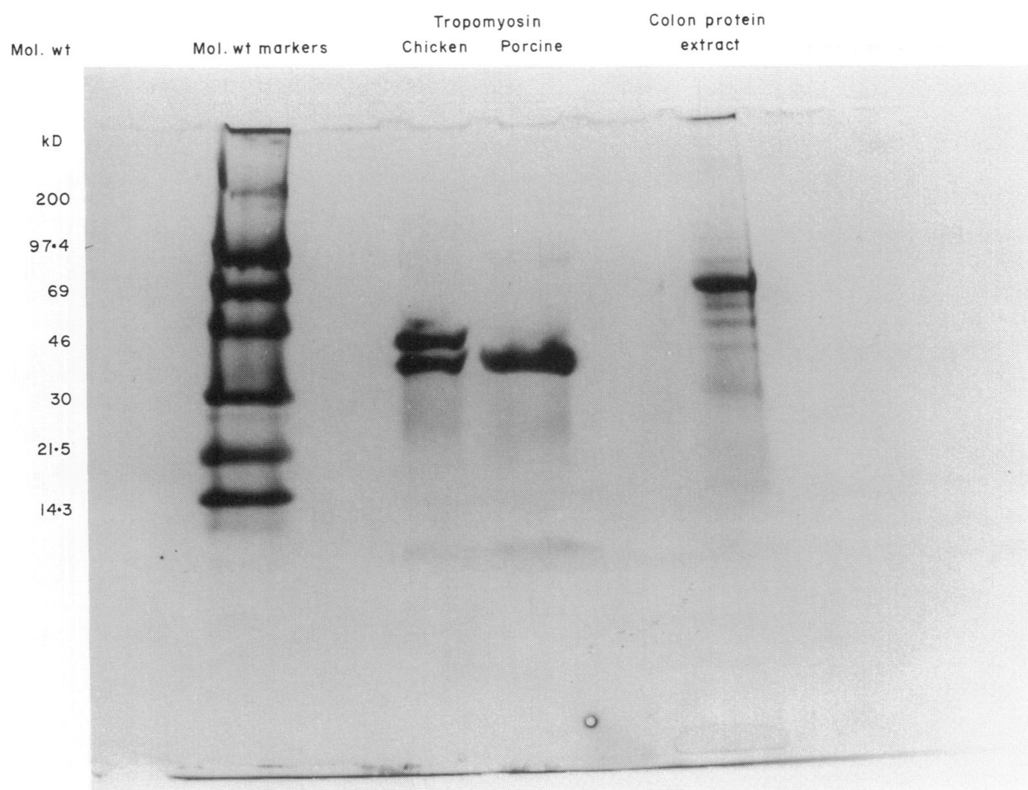

Fig. 5. SDS–PAGE of chicken, porcine tropomyosin and colon protein extract, showing molecular weights of tropomyosin isotypes.

contained a total of 1 mg/ml protein: both human tropomyosin and P40 are likely to represent only a small proportion of the total proteins present. Conversely, when increased amounts of tropomyosin were used in an attempt to detect immunoreactivity with 7E12H12, none was seen, whereas both P40 and tropomyosin were readily detected in the colon protein extract using the same system. In addition, increasing the concentration of 7E12H12 from 1:50 to 1:10 did not produce a positive signal with tropomyosin.

We have not been able to repeat the identification in a Western blot system of P40 using 7E12H12 as described by Das *et al.* [10], although we have consistently identified immunoreactivity between the colon protein extract and 7E12H12 using dot-blots. Similarly, the original authors have experienced difficulty with repeating the Western blot technique (Das *et al.*, 1993, personal communication). Loss of antigenicity in this system persists with non-reducing SDS–PAGE conditions, suggesting that the antigen was labile even under these circumstances. In contrast, the Western blotting of pure tropomyosin isotypes did not affect antigenicity, and it is clearly demonstrable in both the colon protein extract and the pure animal preparations. It is notable that the molecular weights obtained with the Western blot were slightly higher than those identified by coomassie staining of SDS–PAGE gels. This could imply that the conformational epitopes conferring antigenicity on tropomyosin do not represent the majority of the constituent peptide present at lower molecular weights, and the sensitivity of the detection system used may identify larger molecular weight proteins than are easily identified by coomassie staining of the SDS gels.

As we did not detect P40 using Western blotting with 7E12H12, we have been unable to compare the molecular weight of its target antigen with that of tropomyosin. However, the identification of a band for human tropomyosin in the colon protein extract of >40 kD suggests that a large part of the antigenic determinant of human tropomyosin is larger than the target antigen P40.

Circulating antibodies to this partially purified antigen, P40, which have been reported to parallel disease activity [12], have been demonstrated by ELISA. Das *et al.* have also examined sera from patients with UC for circulating antibodies to tropomyosin. They reported the presence of circulating antibodies to tropomyosin in an ELISA assay, using porcine tropomyosin (Sigma T2400) as the antigen [6]. However, others have failed to confirm this finding [13,14].

Reasons why Das may have incorrectly identified the P40 target of 7E12H12 as tropomyosin

Tropomyosin forms part of a large group of closely related actin-binding proteins that are responsible for maintaining cytoskeletal structure and cellular contractility. Tropomyosin exists in all mammalian cell types in various isoforms with molecular weights of 30–45 kD. Das *et al.* demonstrated immunoreactivity between partially purified P40 and anti-tropomyosin, evidence that at least one of the constituent proteins was tropomyosin. However, using a partial purifica-

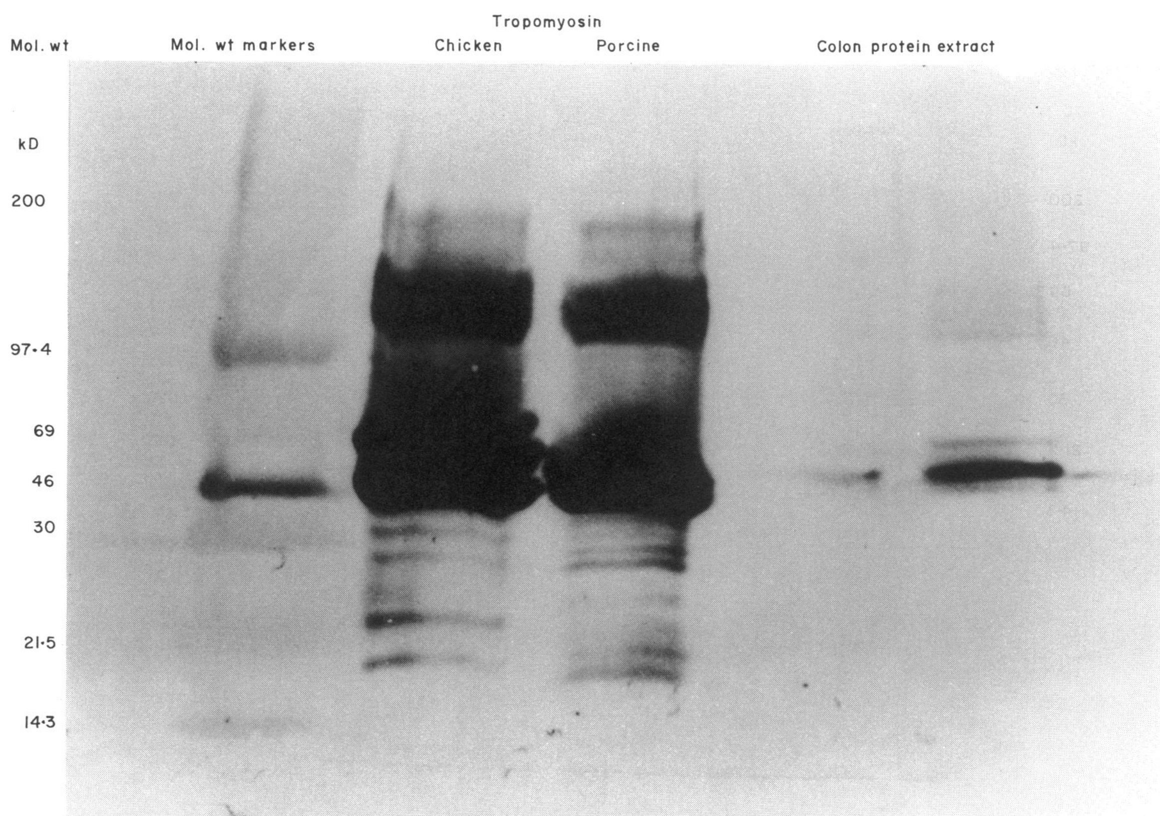

Fig. 6. Western blot, showing identification of human tropomyosin within colon protein extract using anti-tropomyosin, and immunoreactivity between purified tropomyosin and anti-tropomyosin following transfer to nitrocellulose.

tion method, he may have co-purified several different colon-derived proteins, including human tropomyosin.

It can be concluded from the results of this study that the epitope for 7E12H12 is not shared by mammalian isotypes of tropomyosin. In his original report, Das used anion-exchange chromatography to partially purify P40 for use as an immunogen in the production of a panel of MoAbs. Das *et al.* have now identified tropomyosin as the antigen for 7E12H12, showing that the amino acid constituents of the peptides within P40 had > 90% homology with tropomyosin. The method of identification used in this latest report [6] involved protein sequencing and HPLC analysis, following a similar method of partial purification. These methods may have been appropriate, but the results of our study show that there has been co-purification of at least one contaminating protein of similar molecular weight.

The HPLC plot showed that although the two predominant peptide peaks selected for subsequent amino acid sequencing had significant homology with tropomyosin, there were also a number of other peptides that were not sequenced which formed a significant part of P40 [6]. In addition, the amino acid content differed in comparison with known isoforms of tropomyosin, in that it contained large amounts of both glycine and phenylalanine. These data further suggest that several proteins may have been co-purified.

It is notable that the tissue specificity of P40, which included its localization to the organs involved in the systemic manifestations of UC, has recently been challenged by the original workers. They have reported that P40, detected by 7E12H12, has been found to localize to the abnormal epithelium found in Barrett's oesophagus [15].

The nature of the antigen for 7E12H12 remains to be established.

ACKNOWLEDGMENTS

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REFERENCES

1 Colombel JF, Reumaux D, Duthileul P *et al.* Anti-neutrophil cytoplasmic antibodies in inflammatory bowel disease. Gastroenterol Clin Biol 1992; **16**:656–60.
2 Oudkerek Pool M, Ellerbrook PM, Ridwan BU *et al.* Serum anti-neutrophil antibodies in inflammatory bowel disease. Gut 1993; **34**:46–50.
3 Chadwick VS. Immunology of inflammatory bowel disease. Curr Opin Gastroenterol 1992; **8**:634–40.
4 Cees G, Kallenberg M, Leontine Muller A, Tervaert J. Anti-neutrophil cytoplasmic antibodies: a still growing class of antibodies in inflammatory disorders. Am J Med 1992; **93**:675–82.
5 Hibi T, Ohara M, Toda K *et al.* *In vitro* anticolon antibody production by mucosal or peripheral blood lymphocytes from patients with ulcerative colitis. Gut 1990; **31**:1371–6.

6 Das KM, Dasgupta A, Mandal A, Geng X. Autoimmunity to cytoskeletal protein tropomyosin. A clue to the pathogenetic mechanism for ulcerative colitis. J Immunol 1993; **150**:2487–93.

7 Halstensen TS, Das KM. Brandtzaeg P. Epithelial deposits of immunoglobulin G1 and activated complement colocalise with the M(r) 40 kD putative autoantigen in ulcerative colitis. Gut 1993; **34**:650–7.

8 Das KM, Vecchi M, Sakamaki S. A shared and unique epitope(s) on human colon, skin, and biliary epithelium detected by a monoclonal antibody. Gastroenterology 1990; **98**:464–9.

9 Takahashi F, Das KM. Isolation and characterization of a colonic autoantigen specifically recognized by colon tissue-bound immunoglobulin G from idiopathic ulcerative colitis. J Clin Invest 1985; **76**:311–8.

10 Das KM, Sakamaki S, Vecchi M, Diamond B. The production and characterization of monoclonal antibodies to a human colonic antigen associated with ulcerative colitis: cellular localization of the antigen by using the monoclonal antibody. J Immunol 1987; **139**:77–84.

11 Drenckhan D, Grosche-Stewart U. Localisation of myosin, actin, and tropomyosin in rat intestinal epithelium: immunohistochemical studies at the light and electron microscope levels. J Cell Biol 1980; **86**:475–82.

12 Takahasi F, Shah HS, Wise LS, Das KM. Circulating antibodies against human colonic extract enriched with a 40 kDa protein in patients with ulcerative colitis. Gut 1990; **31**:1016–20.

13 Lee JCW, Cevallos AM, Naeem A, Lennard-Jones JE, Farthing MJG. Anti-colonocyte and anti-tropomyosin antibodies in patients with ulcerative colitis. Gut 1994; AF195.

14 Khoo Y, Bjarnson I, Forgacs I, Williams R, Macpherson A. Are anti-epithelial tropomyosin antibodies important in the pathogenesis of ulcerative colitis? Gastroenterology 1994; **106**:A710.

15 Gujral N, Ruemmler-Fisch C, Amenta PS, Das KM. Barrett's epithelium and gastric intestinal metaplasia: an immunocyochemical difference using the novel monoclonal antibody 7E12H12. Gastroenterology 1994; **106**:A84.