The presence in experimental animals of a colon specific Mr 40 000 protein(s) with relevance to ulcerative colitis

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Abstract

In patients with ulcerative colitis a colon tissue bound IgG and serum antibodies against an Mr 40 000 colonic protein(s) has been identified. Using an anti-Mr 40 000 protein monoclonal antibody, 7E₁₂H₁₂, by an immunocytochemical method, the protein was localised in human tissue exclusively to colonic epithelial cells. In this study the presence of the Mr 40 000 protein was assessed in experimental animals by the direct and inhibition enzyme linked immunosorbent assays (ELISAs) using the anti-Mr 40 000 protein monoclonal antibody, 7E₁₂H₁₂ (IgM isotype). In addition, a total of 129 specimens including colon, small intestine, gall bladder, biliary tract, and kidney from nine strains of rats and mice, and from human tissue were studied by the immunocytochemical method using 7E₁₂H₁₂. All colon specimens from both humans and animals reacted with 7E₁₂H₁₂ in the immunocytochemical and ELISA assays. None of the non-colonic organs reacted with 7E₁₂H₁₂. While in human colon 7E₁₂H₁₂ recognised the absorptive epithelial cells, in all the animals it recognised mainly the colonic goblet cells. Extracts of animal colon but not of small intestine inhibited the binding of 7E₁₂H₁₂ to the human colon extract. This study shows the presence of an organ specific Mr 40 000 colonic epithelial protein(s) in humans and experimental animals. A differing cellular localisation of the Mr 40 000 protein(s) in human *v* animal tissue was also shown. Further characterisation of the Mr 40 000 protein(s) may provide important clues regarding the autoimmune mechanisms in ulcerative colitis.

Autoimmunity has been implicated in the pathogenesis of ulcerative colitis.¹⁻¹¹ The precise nature of the involved autoantigen(s) and antibodies is unknown, however. We earlier reported the presence of an IgG antibody bound to colonic tissue from patients with ulcerative colitis and termed it CCA-IgG or colitis colon associated IgG antibody.¹² The CCA-IgG preferentially binds to colonic tissue extracts¹³ containing an Mr 40 000 protein(s) from both normal and diseased colon.¹⁴ Eluted tissue bound IgG from Crohn's disease or normal colon did not recognise this protein.

Circulating antibodies reactive against the Mr 40 000 protein(s) have also been recently shown in patients with ulcerative colitis.¹⁵ Using an anti-Mr 40 000 protein monoclonal antibody (7E₁₂H₁₂, IgM isotype) in an immunocytochemical analysis, the protein has been localised

in human tissue to colonic epithelial cells but not in non-colonic epithelial tissue from 13 other organs.¹⁶ These findings suggest that this protein represents at least one of the autoantigen(s) in ulcerative colitis.

In this study we investigated the presence of the Mr 40 000 protein in various experimental animals. The organ and species specificity of the Mr 40 000 protein was evaluated by the immunocytochemical method, direct enzyme linked immunosorbent assay (ELISA), and inhibition ELISA assays, using an anti-Mr 40 000 protein monoclonal antibody.

Method

IMMUNOCYTOCHEMICAL LOCALISATION OF THE MR 40 000 PROTEIN IN ANIMAL TISSUE

Tissue specimens

A total of 129 formalin fixed tissue specimens were obtained from normal human colon (5) and small intestine (3); Wistar rat colon (11) and small intestine (5); Lewis rat colon (12), small intestine (3), kidney (4), and biliary tract (6); Sprague Dawley rat colon (10) and small intestine (4); BALB/c mouse colon (12), small intestine (7), biliary tract (6), gall bladder (4), and kidney (4); SJL mouse colon (9), small intestine (4), and biliary tract (4); B10.D2 mouse colon (4); DB.A2 mouse colon (4); B6.D2.F11 mouse colon (4); and B6 mouse colon (4). Samples of human colon and ileal tissue were obtained from normal subjects who underwent colonoscopy. The animals were sacrificed by cervical dislocation and specimens of colon, small intestine, kidneys, and biliary tract were fixed in 10% formalin. All animals were males, 4 to 8 weeks old.

IMMUNOPEROXIDASE TECHNIQUE USING THE ANTI-MR 40 000 MONOCLONAL ANTIBODY, 7E₁₂H₁₂ Preliminary experiments were performed on human and animal tissues to evaluate the effect of several fixation procedures on antigen preservation. Because formalin fixation did not affect the antigen(s) and gave the best preservation, formalin fixed, paraffin embedded tissues were used. Five µm sections were deparaffinised, rehydrated, and incubated for 30 minutes in 0.1 mol/l phosphate buffer, pH 7.4, containing 0.3% H₂O₂ in order to block the endogenous peroxidase activity. After washings in phosphate buffer, sections were incubated for two hours with diluted (1/50) normal goat serum (100 µl) to block non-specific binding to the section. After

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incubation excess serum was wiped from around the sections, and the sections were incubated overnight with different dilutions of the monoclonal antibody 7E₁₂H₁₂ (IgM isotype). The dilution for 7E₁₂H₁₂ ranged from 1:20 through 1:300. After washings with 0.1 mol/l phosphate buffer (pH 7.4), biotinylated goat antimouse IgM (Vector Lab, Burlingame CA) was added for 90 minutes, followed, after washings in phosphate buffer, by avidin-biotin-peroxidase complex (ABC, Vector Lab, Burlingame CA) for 90 minutes. The reaction was then developed by incubating the sections in 1 mg/ml 3-3' diaminobenzidine in 0.1 mol/l Tris-HCl, pH 7.6, 0.02% H₂O₂ for 10 minutes in the dark. Slides were then washed, dehydrated, and mounted. All incubations were performed at room temperature except for the overnight incubation, which was at 4°C. Other sections from the same tissue block were deparaffinised, rehydrated, and incubated with an unrelated IgM monoclonal antibody as control. Sections were also stained with haematoxylin and eosin for routine histology.

EXTRACTION OF TISSUE PROTEINS

For human samples, normal segments of colon specimens from two patients who underwent colectomy for colonic carcinoma were used. The colon or small intestine from animals was removed and rinsed with phosphate buffered saline. The cleaned tissue was weighed and the colon transferred to a cold disposable tube, to which, per g of tissue, was added 2.5 ml of ice cold phosphate buffered saline containing 0.5 µg/ml leupeptin, 5 µg/ml pepstatin, 2 mmol/l ethylenediamine tetra-acetate and 2 mmol/l phenyl methyl sulphonyl fluoride. The tissues were homogenised in an ice bath by Polytron for

5 minutes. The homogenate was clarified by centrifugation at 3000 g for 30 minutes at 4°C and further by ultracentrifugation at 100 000 g for one hour at 4°C. Finally, the protein concentration of the supernatant fraction was evaluated by the Bradford protein assay (BIO-RAD).

EXAMINATION OF HUMAN AND ANIMAL TISSUE EXTRACTS FOR THE PRESENCE OF MR 40 000 PROTEIN USING 7E₁₂H₁₂ BY DIRECT ELISA

ELISA plates were coated with 0.2 µg of total tissue extracts per well and diluted in carbonate buffer (pH 9.6), at a final volume of 100 µl per well. As antigen, we used colon and small intestine tissue extracts from humans (2), Wistar rats (3), Lewis rats (3), Sprague Dawley rats (3), BALB/c mice (5), SJL mice (2), DB.A2 mice (2), B10.D2 mice (2), B6.D2.F11 mice (2), and B6 mice (2). As blank, additional wells were coated with 100 µl bovine serum albumin (0.5 µg/well). After overnight incubation at 4°C, non-specific binding was blocked with 100 µl of 0.1 mol/l phosphate buffered saline (pH 7.4) per well with 2% bovine serum albumin, for two hours at 37°C. After three washings in phosphate buffered saline/0.05% Tween 20 (200 µl/well), as first antibody, different dilutions of 7E₁₂H₁₂ (1:100, 1:200, and 1:400) were used (100 µl/well). After two hours' incubation at 37°C and washings in phosphate buffered saline/0.05% Tween 20, as second antibody, 100 µl of alkaline phosphatase conjugated goat antimouse IgM was added to each well (at 1:1000 dilution). After one hour incubation at 37°C and washings with phosphate buffered saline/0.05% Tween 20, 100 µl of substrate p-nitrophenylphosphate, disodium (Sigma 104, Chemical Co, St Louis MO) was added to each well (5 µg/ml) and incubated for one hour at room temperature. The plates were then read at 405 nm in a Vmax ELISA reader (Molecular Devices). Results are expressed as mean optical density of triplicate determinations.

CROSS REACTIVITY BETWEEN ANIMAL AND HUMAN COLON EXTRACTS: INHIBITION ELISA

Inhibition ELISA was performed by following the method described by Klatser *et al*¹⁷ with some modifications. Wells of the ELISA plates were coated with 0.2 µg Lewis rat colon extract diluted in carbonate buffer (pH 9.6), at a final volume of 100 µl per well. As blank, additional wells were coated with 100 µl of bovine serum albumin (0.5 µg/well). Plates were then incubated overnight at 4°C. A blocking agent, phosphate buffered saline with 2% bovine serum albumin (100 µl/well), was then added to each well and plates were incubated for two hours at 37°C. The presence of a specific inhibition of the binding of the Lewis rat colon extract with 7E₁₂H₁₂ by colon and small intestine extracts from humans (2), Wistar rats (3), Lewis rats (3), Sprague Dawley rats (3), BALB/c mice (5), and SJL mice (2), was tested by evaluating their ability to compete with the Lewis rat colon extract for binding with 7E₁₂H₁₂. For this purpose equal volumes of 7E₁₂H₁₂ diluted at 1:50 in 50 µl (final dilution 1:100) were incubated for

TABLE I Immunoperoxidase (129 tissue specimens tested)

		7E ₁₂ H ₁₂ monoclonal antibody dilutions						
		1:20	1:40	1:75	1:80	1:100	1:200	1:300
Wistar rat								
Colon	(11)	+	+	+	+	+	+	-
Small intestine	(5)	-	-	-	-	-	-	-
Lewis rat								
Colon	(12)	+	+	+	+	+	+	+
Small intestine	(3)	-	-	-	-	-	-	-
Biliary tract	(6)	-	-	-	-	-	-	-
Kidney	(4)	-	-	-	-	-	-	-
Sprague Dawley rat								
Colon	(10)	+	+	+	+	+	+	+
Small intestine	(4)	-	-	-	-	-	-	-
BALB/c mice								
Colon	(12)	+	+	+	+	+	+	+
Small intestine	(7)	-	-	-	-	-	-	-
Biliary tract	(6)	-	-	-	-	-	-	-
Gall bladder	(4)	-	-	-	-	-	-	-
Kidney	(4)	-	-	-	-	-	-	-
SJL mice								
Colon	(9)	+	+	+	+	+	+	-
Small intestine	(4)	-	-	-	-	-	-	-
Biliary tract	(4)	-	-	-	-	-	-	-
B10.D2 mice								
Colon	(4)						+	-
DB.A2 mice								
Colon	(4)						+	-
B6.D2.F11 mice								
Colon	(4)						+	-
B6 mice								
Colon	(4)						+	-
Human								
Colon	(5)		+	+		+	+	-
Small intestine	(3)		-	-	-	-	-	-

Colon specimens n=75; small intestine specimens n=26; biliary tract specimens n=16; gall bladder specimens n=4; kidney specimens n=8; + = positive staining; - = negative staining.

three hours at 37°C with 50 μ l of the tissue extracts at different serial concentrations. Triplicate wells were incubated with 11 serial dilutions of the tissue extracts – for example, 0.003 μ g to 4 μ g per well. After washing three times with phosphate buffered saline/0.05% Tween 20, the wells were incubated with alkaline phosphatase conjugated goat antimouse IgM followed by p-nitrophenylphosphate, as described in the direct ELISA. In separate experiments inhibition ELISA assays were performed after coating the plates with human colon extracts and competing with animal tissue extracts as described above.

Results

IMMUNOCYTOCHEMICAL LOCALISATION OF THE MR 40 000 PROTEIN BY 7E₁₂H₁₂ MONOCLONAL ANTIBODY

All 75 colon specimens from mice, rats, and humans reacted with 7E₁₂H₁₂, but none of the 26 small intestine specimens from human or any of the experimental animals reacted with 7E₁₂H₁₂ (Table I). Kidney (8 specimens), biliary tract (16 specimens), and gall bladder (4 specimens) from the rats and mice did not react with 7E₁₂H₁₂. A difference in the degree of immunoreactivity against the Mr 40 000 protein by 7E₁₂H₁₂ was evident among the different species (Table I). Colon specimens from Lewis rats, Sprague Dawley rats, and BALB/c mice reacted with 7E₁₂H₁₂ when used at a higher dilution (1:300), but other colon specimens including those from humans were negative at this dilution. Figure 1B shows that in animal colon 7E₁₂H₁₂ reacted mainly with the goblet cells. In human colon, however, immunoreactivity was found in the absorptive epithelial cells, mainly along the apical and basolateral areas of the plasma membrane (Figure 1C). Neither the epithelial cells nor the goblet cells in the small intestine from humans and animals reacted with 7E₁₂H₁₂.

PRESENCE OF MR 40 000 PROTEIN IN THE HUMAN AND ANIMAL TISSUE EXTRACTS AS DETECTED BY THE DIRECT ELISA

To further examine the presence and quantify the Mr 40 000 protein in human and animal colon extracts, a direct ELISA assay using 7E₁₂H₁₂ has been developed. The specific binding of 7E₁₂H₁₂ against colon and small intestine extracts from all the species is shown in Figure 2. The direct ELISA assay confirmed the findings of immunoperoxidase staining, indicating the presence of the specific immunoreactivity of 7E₁₂H₁₂ against all the colon specimens tested from both human and animal samples. On the contrary, none of the small intestine extracts reacted with 7E₁₂H₁₂. The data also showed a wide variation in the degree of binding of 7E₁₂H₁₂ to the Mr 40 000 colonic protein(s) present in the colon extracts among the different species and strains. Higher immunoreactivity of 7E₁₂H₁₂ was observed against colon extracts from BALB/c mice and Lewis rats (Fig 2).

CROSS REACTIVITY BETWEEN ANIMAL AND HUMAN COLON EXTRACTS: INHIBITION ELISA

The cross reactivity of the antigen(s) present in the colon from humans, Wistar rats, Lewis rats, Sprague Dawley rats, BALB/c mice, and SJL mice was further investigated by using different colon extracts to compete with the Lewis rat colon extract and human colon extract for binding against 7E₁₂H₁₂ in an inhibition ELISA assay. Figure 3 and Table II show the cross reactivity among various species and the relative differences in the expression of the immunoreactive epitope(s). As Table II shows all the colon specimens tested were capable of inhibition, although at different concentrations. A 50% inhibition of the binding of 7E₁₂H₁₂ to the Lewis

Figure 2: Immunoreactivity of 7E₁₂H₁₂ (1:100) against human and animal tissue extracts as evaluated by direct ELISA. The histograms show optical density values (mean (SEM)) from three different experiments. Blank values (range 0.070–0.090) have been deducted. The means (SEM) for different groups are: human 0.425 (0.031), Wistar rat 0.590 (0.092), Sprague Dawley rat 0.408 (0.070), Lewis rat 0.684 (0.092), SJL mice 0.380 (0.090), BALB/c mice 0.785 (0.154), DB. A2 mice 0.355 (0.042), B10. D2 mice 0.252 (0.042), B6. D2. F11 0.311 (0.031), B6 mice 0.450 (0.074). All the colon extracts reacted with 7E₁₂H₁₂, and the higher immunoreactivity was present in colon extracts from BALB/c mice and Lewis rats. None of the small intestine extracts reacted with 7E₁₂H₁₂ (not shown here).

rat colon extract was achieved with 0.022 µg of autologous colon extract, though 0.054 to 1 µg of colon extracts from the other species were needed to induce the same degree of inhibition (Table II). Inhibition of the binding of 7E₁₂H₁₂ to Lewis rat colon extract by different concentrations of colon and small intestine extracts from human, Lewis rats, and BALB/c mice is shown in Figure 3. These data suggest a difference in the affinity of the Mr 40 000 colonic protein to 7E₁₂H₁₂ among the different species. The highest specific immunoreactivity against 7E₁₂H₁₂ was present in the Lewis rats and BALB/c mice colon extracts (Fig 3 and Table II). The small intestine extracts from humans and animals did not interfere with the binding of 7E₁₂H₁₂ with Lewis rat colon extract. The binding of 7E₁₂H₁₂ to human colon extract was also inhibited by all the animal colon extracts but

Figure 3: Inhibition ELISA: the inhibition of 7E₁₂H₁₂ binding to Lewis rat colon extract by serial dilutions of colon or small intestine extract from humans, Lewis rats, and BALB/c mice. All the colon extracts, and none of the small intestine extracts, inhibited the binding of 7E₁₂H₁₂ to the antigen coating the plate. The inhibition was stronger using the Lewis rats and BALB/c mice colon extracts.

not small intestine extracts (Table II). The lowest amount of protein required to induce a 50% inhibition of the binding of 7E₁₂H₁₂ to the human colon extract was observed with colon extract from Lewis rats followed by homologous colon extract.

Discussion

In this study the presence of an Mr 40 000 associated protein(s) has been shown in the colon from humans and various animals using the anti-Mr 40 000 monoclonal antibody 7E₁₂H₁₂. The organ specificity of this protein in human tissue was shown earlier,¹⁶ and in this study we have identified the protein in various experimental animals and confirmed the organ specificity. Using serial dilutions a different degree of expression of the 7E₁₂H₁₂ reactive epitope on Mr 40 000 colonic protein(s) was observed among the different species. The highest specific immunoreactivity against 7E₁₂H₁₂ was present in colon from Lewis rats and BALB/c mice. Results from immunoperoxidase reactivity in the immunocytochemical analysis and direct and inhibition ELISA assays using 7E₁₂H₁₂ indicated that the Mr 40 000 protein is present only in colon from all the species and not in small intestine, biliary tract, and kidney.

In this study heterogeneity in the localisation of the Mr 40 000 colonic protein(s)/epitopes in human *v* animals was noted. In humans 7E₁₂H₁₂ reacts with biliary epithelium in addition to colon,¹⁸ but in the rats and mice none of the 20 specimens of biliary tract and gall bladder reacted with 7E₁₂H₁₂. Furthermore, results from immunoperoxidase reactivity indicated that while in humans 7E₁₂H₁₂ mainly recognised the columnar colonic epithelium (confirming our previous data¹⁶), in all the animals 7E₁₂H₁₂ reacted with the colonic goblet cells. This finding may be explained on the basis of a common origin of all cell types in the colonic epithelium from undifferentiated cells at the crypt base in experimental animals.¹⁹ The unitarian theory of a pluripotential stem cell postulates that columnar, goblet, endocrine, and Paneth cells all develop from the same precursor.¹⁹ It can be postulated that the 7E₁₂H₁₂ reactive Mr 40 000 epitope is expressed at different stages of cell differentiation in colon from humans *v* animals. A second monoclonal antibody, 7E₆A₅,²⁰ which recognises the intestinal goblet cells in humans reacted with neither colon nor small intestine from animals in the present study (data not shown). Such a species specificity was reported for goblet cell mucin, where human was cross reactive with dog, monkey, and rabbit mucin, but not with mucin of rat, pig, toad, and oyster.²¹

The study shows the presence of a unique Mr 40 000 colonic protein(s) in humans and different animal species and confirms its organ specificity. The results also suggest a heterogeneity of this protein in humans *v* animals. These are shown both by immunocytochemical analysis and by an ELISA. In addition, animal colon extracts, and not small intestinal extracts, inhibited the binding of 7E₁₂H₁₂ with the human colon extracts, suggesting the cross reactivity between the different species. As ulcerative colitis is almost

TABLE II Inhibition of binding of Lewis rat and human colon extracts with 7E₁₂H₁₂

Species	Tissue	% Inhibition	Protein concentration (µg)
Lewis rat colon:			
Lewis rat	Colon	50%	0.022
	Small intestine	None	—
Wistar rat	Colon	50%	0.055
	Small intestine	None	—
Sprague Dawley rat			
	Colon	50%	0.070
	Small intestine	None	—
BALB/c mice			
	Colon	50%	0.054
	Small intestine	None	—
SJL mice			
	Colon	50%	1.2
	Small intestine	None	—
Human			
	Colon	50%	0.280
	Small intestine	None	—
Human colon:			
Lewis rat	Colon	50%	0.030
	Small intestine	None	—
Wistar rat	Colon	50%	0.219
	Small intestine	None	—
Sprague Dawley rat			
	Colon	50%	0.125
	Small intestine	None	—
BALB/c mice			
	Colon	50%	0.109
	Small intestine	None	—
SJL mice			
	Colon	50%	0.438
	Small intestine	None	—
Human			
	Colon	50%	0.046
	Small intestine	None	—

exclusively localised to colonic mucosa, the finding of the organ specificity of the Mr 40 000 colonic protein(s) may be important in the pathogenesis of the disease. Furthermore, the presence of colon tissue bound¹⁴ and circulating IgG antibodies¹⁵ reactive to the Mr 40 000 protein(s) in patients with ulcerative colitis suggest that the Mr 40 000 protein acts as at least one autoantigen(s) in ulcerative colitis. Circulating antibodies from ulcerative colitis react against rat colonic epithelial cells in fluorescence activated cell sorter analysis⁸ and also mediate antibody dependent cell mediated cytotoxicity against rat colonic epithelial cells.²² The Mr 40 000 protein has been localised on the plasma membrane of human colon epithelial cells by immunoperoxidase and immunoelectromicroscopy¹⁶ and recently by fluorescence activated cell sorter on the surface of a colon cancer cell line DLD-1.²³ Sera from ulcerative colitis patients also showed antibody dependent cell mediated cytotoxicity against DLD-1 cells.²⁴ It is not known, however, whether this is related to the recognition of the Mr 40 000 colonic protein(s) in the rat colonic epithelial cells and the colon cancer cell line. Future studies focusing on biochemical characterisation of the 40 000 protein(s), and the immunoreactive epitope(s), from human and animal colon may provide important clues about the autoimmune mechanisms in ulcerative colitis. The results of this study may also help in the development of an animal model of colitis using the Mr 40 000 protein(s).

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Addendum

Recently, after fixation of animal colon with 4% paraformaldehyde in phosphate buffered saline (pH 7.2) for three hours at 4°C we observed that 7E₁₂H₁₂ reacted with columnar epithelial cells in

addition to goblet cells. Small intestine, biliary tract, and skin from the animals were still negative.

- 1 Broberger O, Perlmann P. Autoantibodies in human ulcerative colitis. *J Exp Med* 1958; **110**: 657-74.
- 2 Broberger O, Perlmann P. In vitro studies of ulcerative colitis. I. Reactions of patients' serum with human fetal colon cells in tissue culture. *J Exp Med* 1963; **117**: 705-15.
- 3 Perlmann P, Broberger O. In vitro studies of ulcerative colitis. II. Cytotoxic action of white blood cell from patients on human fetal colon cells. *J Exp Med* 1963; **117**: 717-33.
- 4 Lagercrantz R, Hammarstrom S, Perlmann P. Immunological studies in ulcerative colitis. III. Incidence of antibodies to colon-antigen in ulcerative colitis and other gastrointestinal disease. *Clin Exp Immunol* 1966; **1**: 263-76.
- 5 Carlsson HE, Sundblad G, Hammarstrom S, Perlmann P, Gustafsson BE. Immunological studies in ulcerative colitis: extract, fractionation and immunological characterization of germ-free rat colon antigen. *Arch Biochem Biophys* 1978; **187**: 366-75.
- 6 Zeromsky J, Perlmann P, Lagercrantz R, Hammarstrom S. Immunological studies in ulcerative colitis. VII. Anticolon antibodies of different immunoglobulin classes. *Clin Exp Immunol* 1970; **7**: 469-75.
- 7 Perlmann P, Hammarstrom S, Lagercrantz R, Cambell D. Autoantibodies to colon in rats and human ulcerative colitis: cross reactivity with E. coli O:14 antigen. *Proceedings of the Society for Experimental Biology and Medicine* 1967; **125**: 975-80.
- 8 Hibi TB, Aiso M, Ishikawa M, et al. Circulating antibodies to the surface antigens on colon epithelial cells in ulcerative colitis. *Clin Exp Immunol* 1983; **54**: 163-8.
- 9 Aronson RA, Cooke SL, Roche JK. Sensitization to epithelial antigens in chronic mucosal inflammatory disease. I. Purification, characterization and immunoreactivity of murine epithelial cell-associated components (ECAC). *J Immunol* 1983; **131**: 2796-844.
- 10 Brandtzaeg P, Baklien K, Fausa O, Hoel PS. Immunohistochemical characterization of local immunoglobulin formation in ulcerative colitis. *Gastroenterology* 1974; **66**: 1123-36.
- 11 Scott BB, Goodell A, Stephenson P, Jenkin D. Rectal mucosal plasma cells in inflammatory bowel disease. *Gut* 1983; **24**: 519-24.
- 12 Das KM, Dubin R, Nagai T. Isolation and characterization of colonic tissue-bound antibodies from patients with idiopathic ulcerative colitis. *Proc Natl Acad Sci USA* 1978; **75**: 4528-32.
- 13 Nagai T, Das KM. Detection of colonic antigen(s) in tissue from ulcerative colitis using purified colitis colon tissue-bound IgG (CCA-IgG). *Gastroenterology* 1981; **81**: 463-70.
- 14 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.
- 15 Takahashi 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.
- 16 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.
- 17 Klatser PR, Le Wit MY, Kolk AH. An ELISA-inhibition test using monoclonal antibody for the serology of leprosy. *Clin Exp Immunol* 1985; **62**: 468-73.
- 18 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.
- 19 Cheng H, Leblond CP. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. V. Unitarian theory of the origin of the four epithelial cell types. *Am J Anat* 1974; **141**: 537-62.
- 20 Vecchi M, Sakamaki S, Diamond B, Novikoff AB, Novikoff PM, Das KM. Development of a monoclonal antibody specifically reactive to gastrointestinal goblet cells. *Proc Natl Acad Sci USA* 1987; **84**: 3425-9.
- 21 Qureshi R, Forstner GG, Forstner JF. Radioimmunoassay of human intestinal goblet cell mucin. Investigation of mucus from different organ and species. *J Clin Invest* 1979; **64**: 1149-51.
- 22 Hibi T, Ohara M, Toda K, et al. Establishment of human anticolon antibody producing cell lines transformed by Epstein-Barr virus. In: MacDermott RP, ed. *Inflammatory bowel disease, current status and future approach*. New York: Elsevier, 1988: 77-81.
- 23 Das KM, Squillante L, Roberston F. An increased expression of the Mr 40,000 colonic epithelial protein in DLD-1 colon cancer cells in response to gamma interferon (γ-IFN) and tumor necrosis factor (TNF). *Gastroenterology* 1989; **96**: A111.
- 24 Biancone L, Ebert EC, Das KM. The Mr 40,000 colonic protein is associated with antibody-dependent cell-mediated cytotoxicity (ADCC) by ulcerative colitis (UC) sera. *Gastroenterology* 1990; **98**: A159.