

IgA autoreactivity: a feature common to inflammatory bowel and connective tissue diseases

L. KAZEMI-SHIRAZI*†, C. H. GASCHÉ*, S. NATTER†, A. GANGL*, J. SMOLEN‡, S. SPITZAUER§, P. VALENT¶, D. KRAFT† & R. VALENTA†
*Department of Internal Medicine IV, Division of Gastroenterology & Hepatology, †Department of Pathophysiology, ‡Department of Internal Medicine III, Division of Rheumatology, §Clinical Institute of Medical and Chemical Laboratory Diagnostics and ¶Department of Internal Medicine I, Division of Hematology and Hemostaseology, University of Vienna, Austria

(Accepted for publication 21 December 2001)

SUMMARY

The immunopathogenic mechanisms in inflammatory bowel disease (IBD) are not yet fully established. The aim of this study was to determine the profile and magnitude of IgA and IgG autoantibodies in IBD patients. The autoantigen profile defined by IgA and IgG antibodies from 24 IBD (14 Crohn's disease [CD], 10 ulcerative colitis [UC]), three coeliac, 12 connective tissue disease (CTD) patients and 10 healthy individuals was studied in human cellular extracts by Western blotting. The magnitude of the IgA and IgG₁₋₄ subclass responses was measured by ELISA. IBD patients could not be distinguished from healthy individuals on the basis of IgG autoantibodies to Western blotted proteins. IgG subclass analysis indicated no clear bias towards Th1 or Th2 immune responses in IBD or CTD. In accordance with previous work, we found that IgA autoreactivity was strongest in coeliac disease patients. Unexpectedly, IBD as well as CTD patients exhibited strong IgA autoantibody reactivities to components of similar molecular weights (16–80 kD) in intestinal and non-intestinal epithelial cell lines. Our data indicate immunopathogenic similarities between IBD and CTD.

Keywords IgA autoantibodies IgG autoantibodies inflammatory bowel diseases-IBD connective tissue diseases-CTD

INTRODUCTION

The role of autoimmune mechanisms in Crohn's disease (CD) and ulcerative colitis (UC) remains enigmatic. IgG subclass analysis [1] and the cytokine pattern indicate that Th1 immune mechanisms may be prominent in Crohn's disease [2], whereas a Th2-like immune reactivity profile has been reported for ulcerative colitis [3]. While there is evidence for IgG autoantibody secretion and perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) in ulcerative colitis, it has been reported that patients suffering from Crohn's disease exhibit weak autoantibody production [3,4]. Disease specificity and a pathogenic role for IgG autoantibodies in IBD have not been firmly established. Prominent mucosal neutrophil infiltration but normal T-cell reactivity have been reported for ulcerative colitis, whereas Crohn's disease patients contain hyperreactive T cells [3]. A recent study reports the presence of

mucosal lymphoid aggregates consisting of gamma/delta T cells in ulcerative colitis [5].

Although previous studies provided evidence for increased production of total IgA, as well as altered patterns of monomeric IgA and IgA₁, by IBD intestinal mononuclear cells [6], almost no information is available regarding the presence and specificity of IgA autoantibodies in IBD patients [7]. Since IgA antibodies are the major class of secretory immunoglobulins, production of IgA autoantibodies could indicate local autoantigen stimulation and/or play a pathogenic role in inflammatory bowel disease.

The aim of this study was to determine the autoantigen profile in IBD as defined by IgA and IgG autoantibodies. For this purpose, nitrocellulose-blotted human cellular extracts were probed with sera from IBD patients and healthy individuals. Connective tissue disease (CTD) and coeliac disease patients containing elevated IgG and IgA autoantibody levels, respectively, were included as additional controls. The magnitude of the IgA and IgG subclass responses to human antigens was determined by ELISA. The unexpected finding that IBD patients, as well as CTD patients, mount strong IgA autoantibody responses against a variety of human antigens indicates immunological similarities between these diseases.

Correspondence: Dr Rudolf Valenta, Molecular Immunopathology Group, Department of Pathophysiology, University of Vienna Medical School, Vienna General Hospital, Währinger Gürtel 18–20, A-1090 Vienna, Austria.

E-mail: Rudolf.Valenta@akh-wien.ac.at

MATERIALS AND METHODS

IBD Patients

Sera from 24 patients with IBD, 14 with Crohn's disease (nine females, five males: mean age 39 years, age range 28–74) and 10 with ulcerative colitis (four females, six males: mean age 52.5 years, age range 26–78) were studied (Table 1). Patients with either indeterminate colitis or a disease history of less than 3 years were excluded from the study. At the time of venipuncture, none of the patients had received high-dose steroids (>20 mg/day) or other immunosuppressive drugs. Twelve patients (five with Crohn's disease, seven with ulcerative colitis) received mesalazine or salazosulfapyridine (Table 1). The diagnosis of ulcerative colitis and Crohn's disease was based on clinical, radiological, endoscopic examination and histopathology [8,9]. Disease activity was determined by the colitis activity index (CAI: <4 , remission; 4–7, mild to moderate disease; >7 , active disease) [10] and the Crohn's disease activity index (CDAI: index ≤ 150 , remission; >150 , active disease; >350 , severe disease) [11], respectively (Table 1).

CTD patients

The group with connective tissue diseases comprised six patients suffering from systemic lupus erythematosus (SLE), three with systemic sclerosis (SSC) and three with mixed connective tissue disease (MCTD). ANAs were detected by an indirect immunofluorescence test on Hep-g-2 cells (Kallestad, Minnesota, USA) and in all these patients, the anti-nuclear antibody (ANA) titres

were $\geq 1:1280$. Classification of SLE, SSC and MCTD was performed according to established criteria.

Coeliac disease patients

Sera from three patients with untreated coeliac disease were examined for control purposes. All had high levels of anti-endomysial antibodies (EMA) and anti-gliadin antibodies (AGA). IgA-EMA were determined by indirect immunofluorescence using fixed cryostat sections of monkey oesophagus (INOVA, San Diego, CA, USA) as a substrate. AGA levels were measured by a commercial gluten EIA (Pharmacia -Upjohn Diagnostics, Uppsala, Sweden) [12].

Healthy individuals

For control purposes, sera from 10 healthy individuals were included in all sets of experiments.

Laboratory variables for IBD patients

C-reactive protein (CRP) (normal range: ≤ 1) and oroso mucoid concentrations (normal range: 40–120 mg/dl) were determined by routine laboratory methods. Total serum IgG (normal range: 700–1600 mg/dl) and total serum IgA (normal range: 70–400 mg/dl) were determined by nephelometry using a Behring nephelometric analyser (Behring, Marburg, Germany).

IgA and IgG immunoblotting

The human epithelial cell line A431 (non-intestinal), established from an epidermoid vulvar carcinoma, and the human colon

Table 1. Demographic, clinical and laboratory data of patients with inflammatory bowel disease

	Patients	Age (y)	Sex	Disease location	Extraintestinal manifestations	CDAI/CAI	Medication	Total IgA (mg/dl)	Total IgG (mg/dl)	CRP (mg/dl)	O.m. (mg/dl)
CD	1	34	F	Terminal ileum	Stomatitis	306		174	738	7.6	188
	2	37	F	Ileocolon	Perianal fistula, joints	226	5-ASA	486	885	8.6	217
	3	29	M	Ileocolon	Joints	229		174	885	8	260
	4	31	F	Ileocolon	Joints	298		192	598	3.8	128
	5	32	M	Upper GI		99		201	1150	1.6	188
	6	32	F	Ileocolon	Perianal fistula	35		192	835	1.2	131
	7	37	F	Terminal ileum		212	5-ASA	110	1378	2.2	189
	8	66	F	Terminal ileum		202		258	554	1.2	128
	9	37	M	Terminal ileum	Joints	114	5-ASA	268	1150	2.8	176
	10	74	M	Upper GI		110		288	1498	0.9	105
	11	28	F	Ileocolon		57		201	1095	0.5	88
	12	32	M	Ileocolon	Perianal fistula	23		313	1060	<0.5	110
	13	29	F	Terminal ileum	Joints	164	5-ASA	172	1040	<0.5	94
	14	52	F	Ileocolon	Perianal fistula, Joints	111	5-ASA, S	313	1200	<0.5	79
UC	1	78	M		Joints	7	5-ASA, S	299	738	1.7	119
	2	54	M		Joints	4	5-ASA	448	738	0.5	70
	3	26	F		PSC	3		219	2438	0.5	115
	4	70	M			2	5-ASA	309	1262	0.5	200
	5	49	M		PSC, joints	2		387	2013	0.6	102
	6	40	M			3	5-ASA	342	1378	0.9	72
	7	70	F			0	5-ASA	174	1378	0.7	94
	8	59	M			0	5-ASA	141	1150	0.5	101
	9	48	F			0		210	989	0.5	81
	10	31	F			3	5-ASA	268	1540	<0.5	73

CD = Crohn's disease; UC = ulcerative colitis; Age: y = years; Sex: F = female, M = male; Extraintestinal manifestations: PSC = primary sclerosing cholangitis; CDAI = Crohn's disease activity index (Remission ≤ 150), CAI = colitis activity index (Remission <3); Medication = at time of investigation: 5-ASA = 5-amino salicylic acid, S = steroid (<20 mg/day). IgA: normal range: 70–400 mg/dl; IgG: normal range: 700–1600 mg/dl; CRP: normal range <1 ; O.m. Oroso mucoid: normal range: 40–120 mg/dl.

cancer cell line CCL 227 (intestinal) were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). Protein extracts were prepared from the human epithelial cell line A431 and the colon cancer cell line CCL as previously described [13], separated by preparative 12.5% SDS-PAGE [14] and blotted onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) [15]. Nitrocellulose membranes were incubated twice for 5 min and once for 30 min in buffer A (50 mM sodium phosphate buffer, pH 7.5, containing 0.5% w/v bovine serum albumin, 0.5% v/v Tween 20 and 0.05% w/v NaN₃). After blocking in buffer A, the membranes were incubated overnight with diluted patients' sera at 4°C. Patients' sera were diluted 1:1000 in buffer A for the detection of both IgA and IgG autoantibodies. The membrane strips were then washed three times in buffer A. For detection of membrane-bound IgG antibodies, the strips were incubated at 4°C overnight with rabbit anti-human IgG antibodies (Dako, Glostrup, Denmark) diluted 1:1000 in buffer A. Bound rabbit antibodies were visualized by incubation with an ¹²⁵I-labelled donkey anti-rabbit antiserum (Amersham, UK) as described [16].

IgA autoantibodies were detected with a monoclonal mouse anti-human IgA antibody (CLB, Amsterdam, Netherlands). The monoclonal mouse anti-human IgA antibody was ¹²⁵I-labelled using the chloramine T method [17]. Nitrocellulose strips were incubated overnight with 100 000 cpm/ml of the ¹²⁵I-labelled anti-IgA antibody at room temperature. Bound ¹²⁵I-labelled antibodies were visualized by autoradiography as previously described [12].

ELISA measurements

Protein extracts were prepared by homogenizing 5×10^6 CCL cells in 20 ml phosphate-buffered saline (PBS), containing 1 mM PMSF and 0.5 mM benzamide, using an Ultraturrax (Ika, Stauffen, Germany). The homogenate was centrifuged (7000 $\times$ g, 4°C, 10 min) and the supernatant-derived protein extract was collected and placed on ice. ELISA plates (Nunc, Maxisorb, Roskilde, Denmark) were immediately coated with the freshly-prepared protein extracts (100 μ l/well) and left overnight at 4°C. The plates were washed twice with PBS containing 0.05% v/v Tween 20, 0.5% w/v BSA. They were then blocked with PBS containing 1% w/v BSA for 2 h at 37°C, washed again and incubated overnight at 4°C with sera diluted 1:100 in PBS, 0.05% v/v Tween 20, 0.5% w/v BSA. The plates were then washed five times and exposed to 1:1000 diluted monoclonal mouse anti-human IgG₁₋₄ antibodies (Pharmingen, San Diego, CA, USA), or a monoclonal mouse anti-human IgA antibody (CLB, Amsterdam, Netherlands), for 1 h at 37°C and for 1 h at 4°C. After washing five times, the bound mouse antibodies were detected with a 1:2000 diluted horseradish peroxidase (HRP)-labelled rabbit anti-mouse immunoglobulin antiserum (Amersham) for 1 h at 37°C and 1 h at 4°C. After extensive washing, 100 μ l/well ABTS solution (2,2'-azino-bis-(3-ethylbenthiazoline-6-sulphonic acid)) were added. The optical density (O.D.) corresponding to the amount of bound antibodies was determined using an ELISA reader (Dynatech, Denckendorf, Germany). All measurements were performed in duplicate.

RESULTS

Coeliac disease, CD, UC and CTD patients exhibit IgA reactivity to nitrocellulose-blotted human cellular extracts

The presence of IgA autoantibodies in sera from IBD and control individuals was studied by immunoblotting using human intestinal

(CCL) and non-intestinal epithelial (A431) cell extracts (Fig. 1a,b). IgA-reactive bands of comparable molecular weights were detected in both extracts by sera from patients with chronic inflammatory diseases (CD, UC, coeliac disease and CTD) (Fig. 1a,b). Sera from patients with CD and UC displayed IgA reactivity to components between 16 kD and 80 kD (Fig. 1a,b). The 16 kD band and bands between 25 and 35 kD represented the most frequently detected IgA-reactive components. These were also detected by sera from patients with coeliac and connective tissue diseases. Components of 65 kD and 38 kD were recognized strongly and exclusively by one CD (CD patient 10) and one UC (UC patient 1) patient, respectively (Fig. 1a,b). The most intensive IgA reactivity to the nitrocellulose-blotted human proteins was observed with sera from coeliac disease, UC and, unexpectedly, with sera from CTD patients. Sera from patients with CTD also reacted strongly and frequently with a 42 kD (patients 1–4) and a 62 kD moiety (patients 10 and 11). Weak or no IgA reactivity to blotted human cellular extracts was observed in healthy individuals (Fig. 1a,b).

Qualitative analysis of IgG-defined autoantigens

The results obtained for IgG autoantibodies were different from those obtained for IgA. Sera from IBD patients, but also from the control groups (coeliac disease, CTD and healthy subjects), exhibited variable IgG reactivity to multiple bands ranging from 16 kD to 100 kD in blotted intestinal and non-intestinal epithelial cell extracts. Neither the pattern nor the recognition intensity of IgG autoantigens appeared to differ between the group of IBD, coeliac disease patients and healthy individuals (Fig. 2). Sera from patients with CTD showed the most intense IgG autoreactivity, and reacted with antigens of similar molecular weights in intestinal and non-intestinal epithelial cell extracts (Fig. 2). For example, all MCTD patients recognized a 68–70 kD band in A431 and CCL extracts, possibly corresponding to the U1 70 kD nRNP [18]. Furthermore, a 46 kD A431 and CCL-derived antigen was detected by most SLE and SSC patients.

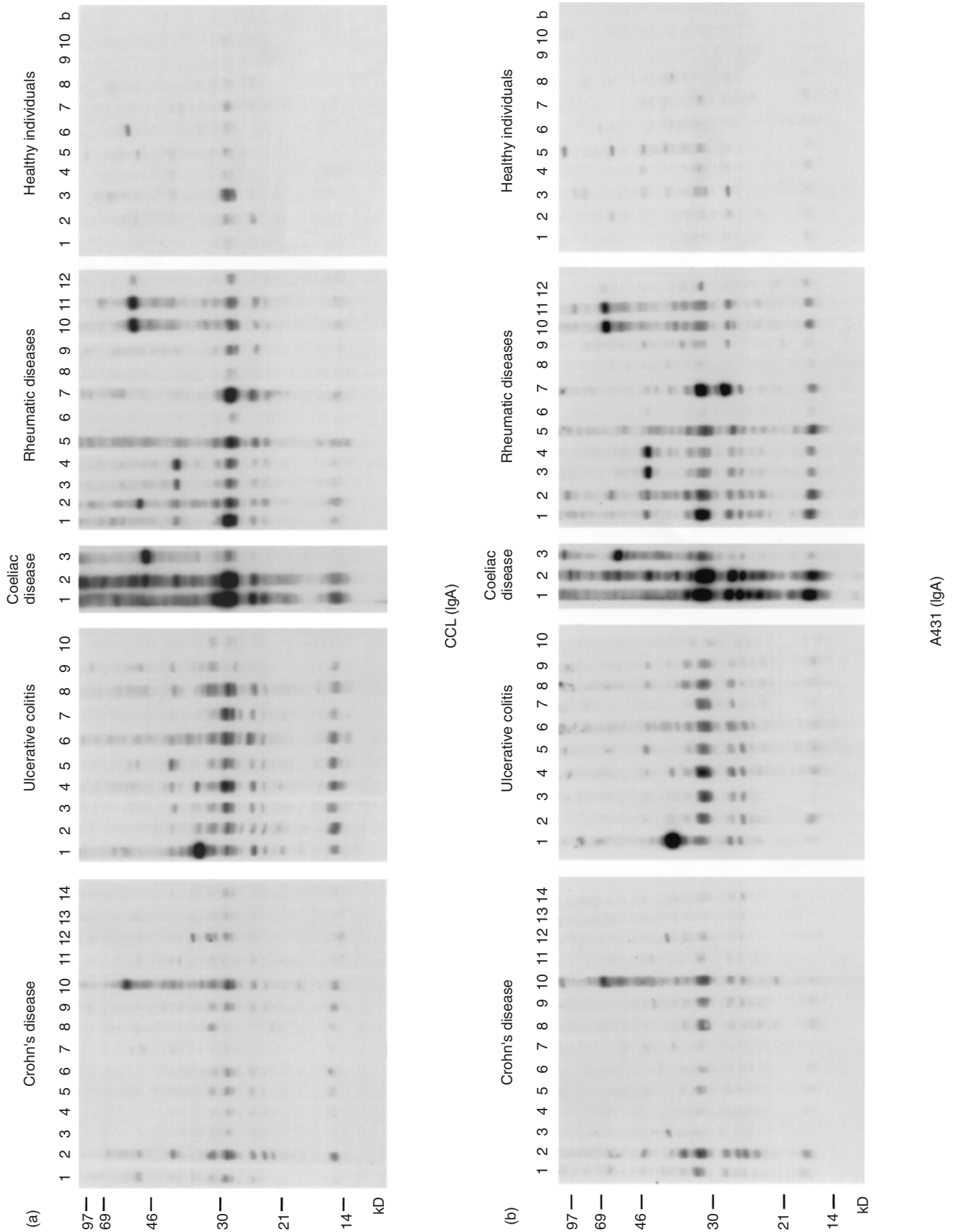
Quantitative analysis of IgA and IgG autoreactivity

IgG₁₋₄ subclass and IgA reactivity to human intestinal protein extracts was analysed by ELISA using sera from patients with IBD and control individuals. The results for each serum and mean antibody reactivities for each group are shown in Table 2.

IgA autoreactivity

IgA levels were higher in all patient groups compared with healthy subjects. The highest IgA autoantibody levels were found in the coeliac disease patient group (1.374–2.5 O.D. units: mean, 2.124), followed by patients with CTD (0.576–2.086 O.D. units: mean, 1.143), CD (0.464–1.722 O.D. units: mean, 0.926) and UC

Fig. 1. IgA reactivity to nitrocellulose-blotted human (a) intestinal (CCL) and (b) non-intestinal epithelial (A431) cellular extracts. Sera from patients with Crohn's disease ($n = 14$), ulcerative colitis ($n = 10$), coeliac disease ($n = 3$), rheumatic (connective tissue) diseases ($n = 12$) (lanes 1–6: systemic lupus erythematosus; lanes 7–9: systemic sclerosis; lanes 10–12: mixed connective tissue disease) and healthy individuals ($n = 10$) were exposed to nitrocellulose-blotted proteins. Lane b represents the buffer control. Molecular weights (kD) are displayed on the left side. Numbering of patients corresponds to patient numbers in Tables 1 and 2, respectively.



(0.098–1.785 O.D. units: mean, 0.846). Sera from healthy subjects contained low IgA autoantibody levels (0.314–0.649 O.D. units: mean, 0.535) (Table 2). The results obtained in the ELISA experiments were thus in agreement with those obtained by immunoblotting.

IgG₁₋₄ autoreactivity

IgG₁ autoantibody levels were highest in the CTD group (0.104–0.718 O.D. units: mean, 0.261), and CD patients mounted higher IgG₂ autoantibody levels (0.091–1 O.D. units: mean, 0.204) than healthy persons (Table 2). In contrast to published data [1], no clear bias towards IgG_{2/3}-subclass autoreactivity, which is indicative of a prominent Th1-immunoreactivity, was found for IBD patients. No obvious differences regarding IgG₄ autoantibody levels were observed between any of the patient groups and healthy controls (Table 2).

Lack of association between antibody reactivity profiles, levels and disease activity

The vast majority of IBD patients had normal total serum IgA and IgG levels. There was no association between total serum IgA and IgG titres, and parameters of acute disease (i.e. disease activity indices, CAI/CDAI, Table 1).

The results of immunoblotting and ELISA experiments demonstrate that patients with IBD, CTD and coeliac disease contain elevated IgA autoantibody levels. Furthermore, they exhibit IgA autoantibodies to components of similar molecular weights in extracts of different cell types. IBD patients could not be distinguished from healthy subjects on the basis of total IgG autoantibodies. The pattern, intensity and levels of IgG and IgA autoreactivity of sera from IBD patients were not associated with acute disease activity or laboratory parameters indicative of acute inflammation (CRP, oroso-mucoid) (Tables 1,2).

DISCUSSION

Our study reveals an unexpected similarity regarding the presence of IgA autoantibodies in IBD and CTD patients. IgA autoantibodies from IBD, coeliac and CTD patients recognized several antigens of comparable molecular weight in nitrocellulose-blotted human intestinal and non-intestinal epithelial cell extracts. Although not proven, this finding suggests that the IgA-defined autoantigens may be proteins with conserved biological functions occurring in different cell types, as reported for many other prominent autoantigens [19]. While the observed IgA autoantibody production may result from the stimulation of 'natural' autoantibody responses [20], this possibility appears less likely in view of the different IgA autoantibody reactivity profiles in the various groups studied. In the case of increased 'natural' autoantibody formation, one would expect similar patterns of antigens to be detected with different intensities. IgA autoreactivity might result from cross-reactivity to food, microflora antigens or infectious agents in patients with mucosal barrier impairment. Alternatively, mucosal tissue damage caused by exogenous agents may lead to exposure of self antigens that trigger local and systemic IgA autoantibody production. Whether these IgA autoantibodies lack inflammatory activity or trigger inflammatory responses upon binding to appropriate receptors cannot be resolved at this stage [21].

In contrast, IgG autoantibodies from IBD and CTD patients were directed to antigens of different molecular weights, and it

Table 2. IgG subclass and IgA reactivity to human CCL extracts defined by ELISA for sera from patients with Crohn's disease (CD), ulcerative colitis (UC), connective tissue diseases (CTD), coeliac disease (C) and healthy individuals (H)

Patients	IgG ₁	IgG ₂	IgG ₃	IgG ₄	IgA	
CD	1	0.148	0.14	0.197	0.147	1.722
	2	0.126	0.137	0.141	0.13	1.571
	3	0.124	0.132	0.156	0.094	0.825
	4	0.126	0.133	0.177	0.105	0.854
	5	0.119	0.156	0.199	0.119	0.854
	6	0.144	0.165	0.266	0.117	1.298
	7	0.14	0.132	0.12	0.102	0.492
	8	0.111	0.092	0.099	0.086	0.702
	9	0.12	0.091	0.096	0.088	0.866
	10	0.124	1.0	0.096	0.159	1.132
	11	0.11	0.103	0.108	0.107	0.69
	12	0.122	0.114	0.168	0.109	0.659
	13	0.139	0.376	0.126	0.122	0.464
	14	0.116	0.091	0.082	0.101	0.828
UC	Mean	0.126	0.204	0.145	0.113	0.926
	1	0.123	0.082	0.065	0.088	1.785
	2	0.101	0.085	0.075	0.087	0.098
	3	0.127	0.089	0.080	0.084	0.724
	4	0.13	0.115	0.169	0.117	0.972
	5	0.14	0.111	0.097	0.114	1.198
	6	0.098	0.077	0.064	0.068	0.963
	7	0.102	0.079	0.059	0.071	0.62
	8	0.091	0.081	0.068	0.081	0.699
	9	0.1	0.078	0.065	0.073	0.811
	10	0.118	0.109	0.13	0.097	0.592
	Mean	0.113	0.09	0.087	0.088	0.846
	1	0.135	0.083	0.121	0.096	>2.5
	2	0.105	0.088	0.085	0.084	>2.5
C	3	0.107	0.094	0.09	0.105	1.374
	Mean	0.116	0.088	0.099	0.095	2.124
	1	0.167	0.125	0.093	0.111	1.345
	2	0.104	0.079	0.061	0.071	1.539
	3	0.158	0.085	0.066	0.121	0.883
	4	0.255	0.1	0.06	0.079	0.689
	5	0.718	0.148	0.069	0.091	1.636
	6	0.135	0.103	0.137	0.093	0.576
	7	0.323	0.178	0.101	0.112	2.086
	8	0.109	0.079	0.068	0.083	0.719
	9	0.135	0.128	0.069	0.078	0.92
	10	0.358	0.104	0.077	0.29	1.504
	11	0.303	0.153	0.073	0.339	1.016
	12	0.368	0.127	0.142	0.234	0.803
H	Mean	0.261	0.117	0.085	0.142	1.143
	1	0.138	0.112	0.089	0.084	0.314
	2	0.116	0.102	0.106	0.083	0.487
	3	0.155	0.125	0.174	0.097	0.612
	4	0.153	0.121	0.116	0.147	0.649
	5	0.156	0.118	0.133	0.101	0.608
	6	0.136	0.144	0.186	0.124	0.48
	7	0.167	0.176	0.179	0.123	0.619
	8	0.157	0.146	0.154	0.131	0.481
	9	0.18	0.271	0.127	0.237	0.583
	10	0.133	0.179	0.106	0.103	0.521
	Mean	0.149	0.149	0.137	0.123	0.535

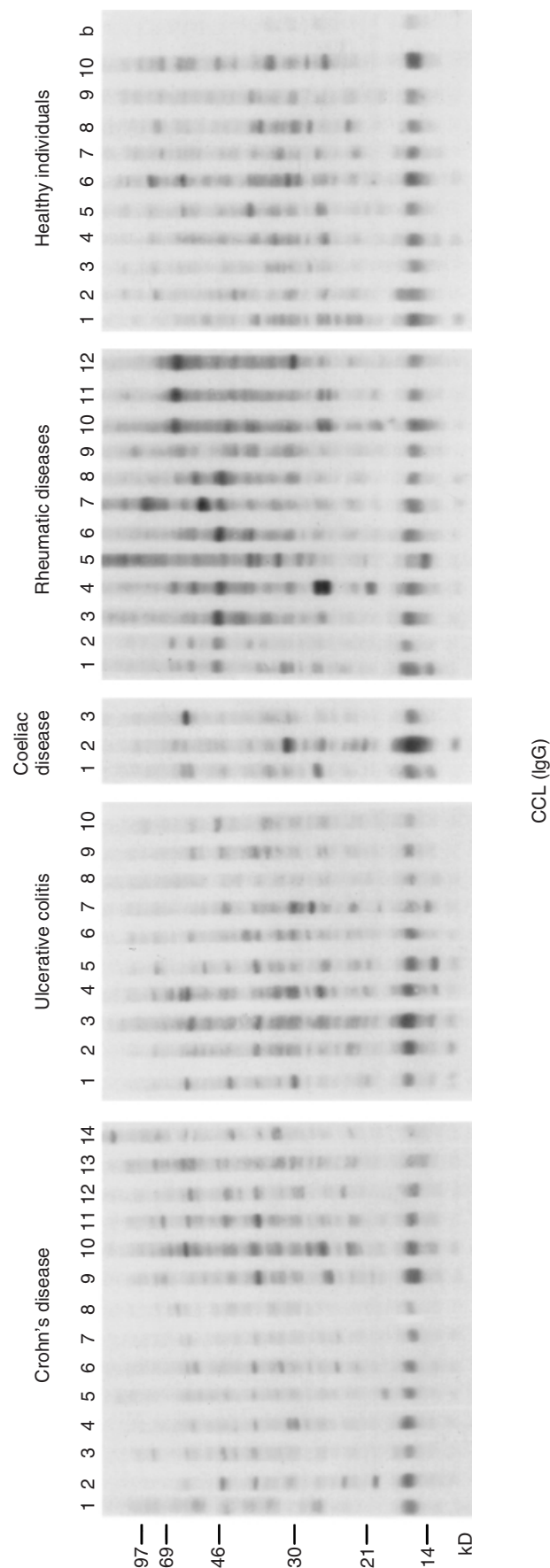


Fig. 2. IgG reactivity to nitrocellulose-blotted human intestinal extracts. Sera from patients with Crohn's disease ($n = 14$), ulcerative colitis ($n = 10$), coeliac disease ($n = 3$), rheumatic (connective tissue) diseases ($n = 12$) (lanes 1–6: systemic lupus erythematosus; lanes 7–9: systemic sclerosis; lanes 10–12: mixed connective tissue disease) and healthy individuals ($n = 10$) were exposed to nitrocellulose-blotted proteins. Lane b represents the buffer control. Molecular weights (kD) are displayed on the left side. Numbering of patients corresponds to patient numbers in Tables 1 and 2, respectively.

was not possible to discriminate IBD patients from healthy subjects on the basis of IgG autoreactivity.

The IgA-defined autoantigens described in our study are different from the autoantigens previously identified in IBD because of their different molecular weight and the fact that they were not recognized by IgG autoantibodies. In contrast to the most frequently reported autoantigen, a colonic protein of 40 kD which is exclusively recognized by IgG eluted from the colon affected by ulcerative colitis [22,23], the IgA-defined autoantigens described by us seem not to be specific for IBD. The lack of IgG reactivity against the 40 kD autoantigen in our experiments could be due to the use of only two cell lines, thus limiting the spectrum of detectable autoantigens. Sera from IBD, CTD and coeliac disease patients contained IgA autoantibodies mainly against the 16, 25 and 35 kD moieties. Two out of three mixed connective tissue disease (MCTD) patients displayed IgA reactivity to a 68 kD component, which, although not yet proven, might be related to the U1 nRNP autoantigen [18]. Further studies are necessary to reveal the molecular nature of the other antigens, and whether IBD, CTD and coeliac disease patients react with the same molecules. The occurrence of IgA autoantibodies in coeliac disease is well established. However, it is unlikely that the IgA autoreactivity in our patients is directed against tissue transglutaminase, a prominent autoantigen described for coeliac disease, because nitrocellulose-blotted tissue transglutaminase reportedly lacks antibody binding capacity [24].

In accordance with the results obtained by Western blotting, we detected by quantitative ELISA high levels of IgA autoantibodies in sera from coeliac disease, IBD and CTD patients (coeliac disease > CTD > IBD). Nevertheless, IgA autoreactivity in IBD was not associated with markers of acute inflammation. The unexpected finding that both IBD and CTD patients contained elevated levels of IgA autoantibodies might be an indicator of common pathological mechanisms in these diseases. In this context, it is noteworthy that extra-intestinal manifestations affecting the joints and systemic features of autoimmunity (e.g. vasculitis) are frequently observed in IBD [25–28]. In view of the possibility that immune cells from the gut may home to the synovium, and probably also to other organs, there may be a link between intestinal immunity and chronic arthropathies [29].

In summary, our study is, to the best of our knowledge, the first description of the co-occurrence of IgA autoantibodies in IBD and CTD. Further studies are needed to clarify the molecular nature of these autoantigens and to determine whether IgA autoantibodies may be responsible for tissue destruction. The IgA autoantibodies described in our study may be useful as diagnostic parameters for monitoring chronic tissue destruction in these disease entities.

ACKNOWLEDGEMENTS

This work was supported by grant F0506 of the Austrian Science Fund and by the ICP Program of the Austrian Federal Ministry for Education, Science and Culture, as well as a grant from the Hans und Blanca Moser Stiftung.

REFERENCES

- Gouni-Berthold I, Baumeister B, Berthold HK, Schmidt C. Immunoglobulins and IgG subclasses in patients with inflammatory bowel disease. *Hepatogastroenterology* 1999; **46**:1720–3.
- Kakazu T, Hara J, Matsumoto T *et al*. Type I T-helper cell predominance in granulomas of Crohn's disease. *Am J Gastroenterol* 1999; **94**:2149–55.
- Fiocchi C. Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterology* 1998; **115**:182–205.
- Targan SR, Landers CJ, Cobb L, MacDermott RP, Vidrich A. Perinuclear anti-neutrophil cytoplasmic antibodies are spontaneously produced by mucosal B-cells of ulcerative colitis patients. *J Immunol* 1995; **155**:3262–7.
- Yeung MM, Melgar S, Baranov V *et al*. Characterization of mucosal lymphoid aggregates in ulcerative colitis: immune cell phenotype and TcR-gamma/delta expression. *Gut* 2000; **47**:215–27.
- MacDermott RP, Nash GS, Bertovich MJ, Seiden MV, Bragdon MJ, Beale MG. Alterations of IgM, IgG, and IgA synthesis and secretion by peripheral blood and intestinal mononuclear cells from patients with ulcerative colitis and Crohn's disease. *Gastroenterology* 1981; **81**:844–52.
- Mayet WJ, Press AG, Hermann E *et al*. Antibodies to cytoskeletal proteins in patients with Crohn's disease. *Eur J Clin Invest* 1990; **20**:516–24.
- Lennard-Jones JE. Classification of inflammatory bowel disease. *Scand J Gastroenterol* 1989; **24**:2–6.
- Le Berre N, Heresbach D, Kerbaol M *et al*. Histological discrimination of idiopathic inflammatory bowel disease from other types of colitis. *J Clin Pathol* 1995; **48**:749–53.
- Rachmilewitz D. Coated mesalazine (5-aminosalicylic acid) versus sulphasalazine in treatment of active ulcerative colitis: a randomized trial. *BMJ* 1989; **298**:82–6.
- Best WR, Beckett JM, Singleton JW, Kern F Jr. Development of a Crohn's disease activity index – National Crohn's Disease Study. *Gastroenterology* 1976; **70**:439–44.
- Natter S, Granditsch G, Reichel GL *et al*. IgA-cross-reactivity between a nuclear autoantigen and wheat proteins suggests molecular mimicry as a possible pathomechanism in coeliac disease. *Eur J Immunol* 2001; **31**:918–28.
- Valenta R, Maurer D, Steiner R *et al*. Immunoglobulin E response to human proteins in atopic patients. *J Invest Dermatol* 1996; **107**:203–8.
- Fling SP, Gregerson DS. Peptide and protein molecular weight determination by electrophoresis using a high-molarity Tris buffer system without urea. *Anal Biochem* 1986; **155**:83–8.
- Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979; **76**:4350–4.
- Skyllouriotis P, Skyllouriotis-Lazarou M, Natter S *et al*. IgG subclass reactivity to human cardiac myosin in cardiomyopathy patients is indicative of a Th1-like autoimmune disease. *Clin Exp Immunol* 1999; **115**:236–47.
- Harlow E, Lane D (eds). *Antibodies. A Laboratory manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1988.
- Welin-Henriksson E, Wahren-Herlenius M, Lundberg I, Mellquist E, Pettersson I. Key residues revealed in a major conformational epitope of the U1–70K protein. *Proc Natl Acad Sci USA* 1999; **96**:14487–92.
- Tan EM. Autoantibodies in pathology and cell biology. *Cell* 1991; **68**:841–2.
- Coutinho A, Kazatchkine MD, Avrameas S. Natural autoantibodies. *Curr Opin Immunol* 1995; **7**:812–8.
- Van Egmond M, Damen CA, van Sriel AB, Vidarsson G, van Garderen E, van de Winkel JGJ. IgA and the IgA Fc receptor. *Trends Immunol* 2001; **22**:205–11.
- Nagai T, Das KM. Detection of colonic antigen(s) in tissues from ulcerative colitis using purified colitis colon tissue-bound IgG (CCA-IgG). *Gastroenterology* 1981; **81**:463–70.
- Bhagat S, Das KM. A shared and unique epitope in the human colon, eye and joint detected by a monoclonal antibody. *Gastroenterology* 1994; **107**:103–8.

- 24 Dieterich W, Ehnis T, Bauer M *et al.* Identification of tissue transglutaminase as the autoantigen of coeliac disease. *Nat Med* 1997; **3**:797–801.
- 25 Wright V, Watkinson G. The arthritis of ulcerative colitis. *BMJ* 1965; **2**:670–5.
- 26 Gravalese EM, Kantrowitz FG. Arthritic manifestations of inflammatory bowel disease. *Am J Gastroenterol* 1988; **83**:703–9.
- 27 Leirisalo-Repo M. Enteropathic arthritis, Whipple's disease, juvenile spondyloarthropathy, uveitis and SAPHO syndrome. *Curr Opin Rheumatol* 1995; **7**:284–9.
- 28 Orchard TR, Wordsworth BP, Jewell DP. Peripheral arthropathies in inflammatory bowel disease: their articular distribution and natural history. *Gut* 1998; **42**:387–91.
- 29 Brandtzaeg P. Review article: Homing of mucosal immune cells – a possible connection between intestinal and articular inflammation. *Aliment Pharmacol Ther* 1997; **11**:24–39.