

Soluble interleukin-2 receptors, antineutrophil cytoplasmic antibodies, and other autoantibodies in patients with ulcerative colitis

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Abstract

Ulcerative colitis (UC) is an inflammatory bowel disease of unknown aetiology. In this study, serum samples from 80 patients with UC were studied for the presence of various autoantibodies and soluble interleukin-2 receptor molecules (sIL-2Rs) in an attempt to determine the degree of activation of the immune system in this disease process. Autoantibodies detected included rheumatoid factors (in 5% of patients), antinuclear antibodies (in 51.3%), anti-Ro(SSA) (in 1.3%), anticardiolipin antibodies (IgG and/or IgM classes in 26.3%), anti-double stranded DNA (IgG or IgM classes in 45%), and antineutrophil cytoplasmic antibodies (ANCA, in 30%). The ANCA had a perinuclear pattern (p-ANCA) in 95.8%, without antimyeloperoxidase activity, at least in an enzyme linked immunosorbent assay (ELISA) system. Raised concentrations of sIL-2R were found in 32.5% of patients (26/80, 18 with active and eight with inactive UC). The mean (SD) sIL-2R concentrations were significantly higher in patients with active UC (595 (219) u/ml *v* 406 (162) u/ml, $p=0.0001$) and in patients with ANCA (584 (177) u/ml in ANCA positive *v* 447 (212) u/ml in ANCA negative patients, $p<0.01$). The sIL-2R concentrations were correlated with increased serum concentrations of C3c ($r=0.23$, $p<0.05$) or C4 ($r=0.4$, $p<0.001$) components of the complement system and erythrocyte sedimentation rate (ESR, $r=0.44$, $p=0.0001$). Platelets, ESR, and C3c were not associated with disease activity ($p=0.06$, 0.33 and 0.86) whereas mean (SD) serum concentrations of C4 were higher in active disease (37.4 (11.9) mg/dl *v* 32.3 (10.3) mg/dl, $p<0.05$). The sIL-2Rs had 53% sensitivity and 82.6% specificity for disease activity whereas platelet counts had 53% sensitivity and 58.7% specificity. To conclude, UC is accompanied by an autoimmune response that results in the production of several autoantibodies and cellular immune activation, as shown by the high sIL-2R concentration, is also present. The identification of the target antigen(s) of p-ANCA would possibly act as an indicator of disease activity if this distinct subset of ANCA can be attributed to the pathogenesis of UC. The sIL-2R concentrations seem to be a useful laboratory marker for assessing activity of the disease.

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Ulcerative colitis (UC) is an inflammatory bowel disease of unknown aetiology characterised by recurrent episodes of bleeding with mucus or

pus. Immunological abnormalities have long been considered as one of the multiple factors contributing to the aetiology of the disease.¹

High frequencies of several autoantibodies, including rheumatoid factors, antinuclear antibodies (ANAs), precipitating antibodies to extracted cellular antigens (Ro(SSA), La(SSB), Sm, and U₁nRNP), anti-double stranded DNA (anti-dsDNA), anticardiolipin (anti-CL), and antineutrophil cytoplasmic antibodies (ANCA), have long been established in many autoimmune rheumatic diseases.^{2,3} Among them, anti-dsDNA antibodies are highly specific for the diagnosis of systemic lupus erythematosus,^{4,5} whereas ANCA have been previously reported in serum samples from patients with Wegener's granulomatosis.^{6,7} Moreover, these two autoantibodies are often correlated with disease activity.^{5,8} The ANCA in Wegener's granulomatosis seem to be directed against an elastolytic proteinase and give generalised cytoplasmic granular neutrophil staining by indirect immunofluorescence (c-ANCA).⁹ By contrast, patients with renal diseases caused by idiopathic necrosis or crescentic glomerulonephritis have been found to react mainly with myeloperoxidase and to give a perinuclear staining (p-ANCA).¹⁰

We and others have previously shown that disorders associated with cellular activation, such as autoimmune, neoplastic, and infectious diseases, are characterised by increased serum concentrations of soluble interleukin-2 receptors (sIL-2Rs), which correlate with disease activity.^{11,12} In inflammatory bowel disease well defined biochemical and haematological indicators such as, C reactive protein, α_1 acid glycoprotein, erythrocyte sedimentation rate (ESR), and platelet counts do not satisfactorily quantify activity of disease.¹³

In this study, serum samples from 80 patients with UC were studied for the presence of several autoantibodies (including ANCA) and the concentrations of sIL-2Rs in an attempt to determine the degree of activation of the immune system in the disease process. The possibility that the activity, severity, and extent of the disease correlated, with the presence of autoantibodies or increased sIL-2R concentrations was also investigated.

Patients and methods

PATIENTS

Serum samples from 80 consecutive unselected patients with UC were studied. The diagnosis of UC was based on clinical, radiological, endoscopic and histological evaluation.¹⁴ The activity,

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TABLE I Association of anatomical extent and specific severity of disease in 80 patients with ulcerative colitis

Severity of disease	Anatomical extent		
	Proctitis (%) (n=10)	Left colitis (%) (n=47)	Pancolitis (%) (n=23)
Mild	100	29.8	0
Moderate	0	27.7	21.7
Severe	0	42.5	78.3

extent, and severity of UC (Table I) were defined according to Truelove's criteria.¹⁵ The activity of UC in particular was further defined according to the definition of Binder *et al.*,¹⁴ modified by us as follows. Active disease was recorded when bowel movements were more than two a day with daily presence of blood, or pus and with or without systemic symptoms (fever, tachycardia, or weight loss). Inactive disease was recorded when the patients had normal bowel function without blood, mucous, or pus, and without systemic symptoms.¹⁶ Forty six patients (23 men and 23 women, age range 18 to 64 years, mean age 50, duration of disease 58.8 (71.7) months) had inactive disease, and 34 patients (13 men and 21 women, age range 14 to 75 years, mean age 40, duration of disease 54.4 (62.9) months) had active disease. Nine out of 46 patients with inactive disease and six out of 34 patients with active disease were taking steroids or other immunosuppressive agents (azathioprine) before and on the day of the collection of serum samples. Pairs of serum samples taken from 10 patients with active disease and during a previous clinical remission were also available.

HAEMATOLOGICAL STUDIES

Blood was collected for concurrent measurement of ESR, platelet counts, haemoglobin concentration, packed cell volume, and leucocyte counts by standard procedures.

SEROLOGICAL STUDIES

Coded serum samples were stored at -30°C until tested. Activity of rheumatoid factors was detected by the latex fixation test (positive rheumatoid factor titre $\geq 1:40$) and ANAs by the indirect immunofluorescence technique with Hep-2 epithelial cells as substrate (positive ANA titre

$\geq 1:80$). The presence of precipitating antibodies to extracted cellular antigens Ro(SSA), La(SSB), Sm, and U₁nRNP, was detected by counterimmunoelectrophoresis against calf thymus extract. The concentrations of IgG and IgM, anti-CL, and anti-dsDNA antibodies were measured by quantitative isotype specific solid phase enzyme linked immunosorbent assays (ELISAs), as described previously with modifications for the anti-dsDNA ELISA.³⁸ Briefly, native DNA (Sigma) was dissolved in phosphate buffered saline (PBS) to a final dilution of 50 $\mu\text{g}/\text{ml}$. Polystyrene microtitre plates were pretreated with 50 $\mu\text{g}/\text{ml}$ poly-L-lysine in PBS for two hours at 37°C . Poly-L-lysine was then removed and the wells were washed twice with PBS. The DNA preparation (50 μl) was added to each well and the plates were incubated for one hour at 37°C and subsequently overnight at 4°C . The DNA was then removed and the wells were washed three times with PBS and then treated with 100 μl of S₁ nuclease solution of 50 IU/ml; (incubation for one hour at 37°C). After removal of S₁ nuclease and washing, the remaining free binding sites were blocked by addition of 100 μl of 10% bovine serum in PBS (BS-PBS) to each well (and incubation for one hour at room temperature). After washing, 50 μl of 1:50 serum solution in 10% BS-PBS were added to duplicates and incubated for one hour at room temperature. After a further washing, 50 μl alkaline phosphatase conjugated goat antihuman IgG or antihuman IgM (diluted to 1:1000 in 10% BS-PBS) was added to each well. Incubation for 90 minutes at room temperature was followed by five washings with PBS. Subsequently, 50 μl of substrate solution (*p*-nitrophenyl phosphate dissolved in 1 mg/ml substrate buffer) were added to each well and the plates were incubated for 30 minutes at 37°C . The reaction was stopped by adding 50 μl 3N NaOH to each well. The optical density of each well was read at 405 nm in a microplate reader (Dynatech, England). In each assay the between day variation of optical density values was eliminated by referring optical density values of every tested sample to binding units as described previously.¹⁷ The results were expressed as a binding index calculated by dividing the binding units of every sample by the mean binding units of the control group plus 3 SDs (for anti-dsDNA) or 4 SDs (for anti-CL) multiplied by 100. According to this formula, a binding index of 100 was defined as the cut off point. The properties of the isotype specific ELISAs have been examined and standardised in extensive preliminary experiments.^{3,8} Also, the adoption of these stringent cut off points for positivity precluded the possibility of false positive results.^{8,18}

C reactive protein and C3c and C4 components of the complement system were measured by single radial immunodiffusion (Behringwerke, Germany). The normal range of values for reactive protein (0–0.8 mg%), C3c (53–125 mg/dl), and C4 (20–49 mg/dl) were those used in our laboratory. The ANCA were detected by the indirect immunofluorescence technique with isolated granulocytes (approximately 10^5 neutrophils from a single normal subject per slide) fixed in 100% ethanol at 4°C for five minutes, air dried, and stored at -20°C , as substrate. The

TABLE II Frequency of various autoantibodies

Autoantibodies	Normal controls		Patients	
	n	Positive (%)	n	Positive (%)
RF	170	0	80	5*
ANA	170	0	80	51.3†
Anti-Ro (SSA)	170	0	80	1.3‡
Anti-La (SSB)	170	0	80	0
Anti-Sm	170	0	80	0
Anti-U ₁ nRNP	170	0	80	0
Anti-dsDNA (IgG and/or IgM)	119	0	80	45†
Anti-CL (IgG and/or IgM)	261	2.3	80	26.3¶
ANCA	65	0	80	30§
Anti-MPO	65	0	80	0
Autoantibodies (of at least one specificity)	261	2.3	80	76.3¶

Fisher's exact test, * $p < 0.02$; † $p < 0.0005$; ‡NS; § $p < 0.001$; ¶ χ^2 test $p < 0.0005$. RF=rheumatoid factors; ANA=antinuclear antibodies; Anti-dsDNA=antibody to double stranded DNA; Anti-CL=anticardiolipin; ANCA=antineutrophil cytoplasmic antibodies; Anti-MPO=antibodies binding to human myeloperoxidase.

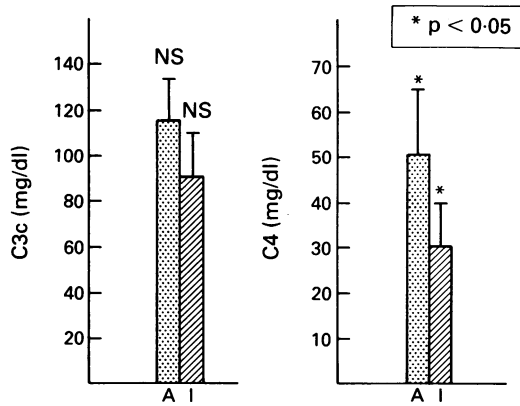


Figure 1: Mean (SD) C3c and C4 concentrations in paired serum samples from 10 patients with active (A) and inactive (I) ulcerative colitis. * $p < 0.05$.

coded serum samples were tested at a dilution of 1:20 and stained with fluorescein labelled F(ab')₂ γ chain specific antibody as described elsewhere (positive ANCA titre $\geq 1:20$).^{6,9} A direct binding ELISA was used to detect antibodies binding to human myeloperoxidase (anti-MPO, Calbiochem, Behringer, La Jolla, CA).¹⁰ According to the manufacturer's instructions a sample with optical density values three times that of the control was defined as positive for anti-MPO antibodies. The concentrations of sIL-2Rs in serum samples were measured by a sandwich ELISA method with monoclonal antibodies against distinct epitopes of IL-2R (anti-Tac and 7G7/B6, provided by Drs T A Waldmann and D L Nelson, NIH, Bethesda, Maryland) as previously described.¹⁷ A reference reagent consisting of culture supernatant of seven days phytohaemagglutinin stimulated normal peripheral blood lymphocytes was used. The 1:10 dilution of the culture supernatant was arbitrarily assigned a value of 500 sIL-2R units/ml and accordingly all the optical density values of samples tested were converted to sIL-2R units/ml. Every sample with values more than the mean value of healthy controls plus 3 SDs was defined as positive for increased sIL-2R concentration (cut off point).

STATISTICAL ANALYSIS

Data were analysed by unpaired *t* test, χ^2 (two by two with Yate's correction or two by three), Fisher's exact test, simple regression analysis, and analysis of variance (ANOVA) where applicable.

Results

Studies in our laboratory of serum samples from healthy blood donors (12 to 53 years of age) have shown no evidence of rheumatoid factors, ANAs, anti-Ro(SSA), anti-La(SSB), anti-Sm, anti-U₁nRNP (170 serum samples), anti-dsDNA (119 samples), or ANCA (65 samples), and only a few displayed low concentrations of anti-CL antibodies (6/261 (2.3%), three with IgG and three with IgM anti-CL antibodies) (Table II). Furthermore, none of the blood donors studied (48 samples) had increased sIL-2R concentra-

tions or C3c or C4 components of the complement system.

Increased concentrations of C reactive protein were found in only three patients with active UC (1.3–7.05 mg%, 8.8%). No statistical difference was found for components of the complement system between patients (C3c (mean (SD) 98.7 (21.3) mg/dl) and C4 (34.4 (11.2) mg/dl) and controls (C3c 100 (35.3) mg/dl and C4 32.5 (13) mg/dl). Patients with active UC, however, had significantly higher concentrations of C4 than did patients with inactive disease, whereas C3c was not associated with activity of disease (Table III). Moreover, the mean concentrations of C3c and C4 in paired serum samples from 10 patients during active disease and after a clinical remission confirmed this association (Fig 1). Hypocomplementaemia was found in seven patients (8.8%), all but one with inactive UC. This was not significant (data not shown). No statistical differences were found between the ESR, leucocytes, platelets, packed cell volume, and haemoglobin and the activity (Table III), severity (ANOVA, $p=0.22$, $p=0.07$, $p=0.14$, $p=0.10$, and $p=0.11$ respectively), or extent of the disease (ANOVA, $p=0.53$, $p=0.44$, $p=0.20$, $p=0.06$, and $p=0.18$ respectively). The mean concentrations of C3c and C4 components of the complement system were not affected by the immunosuppressive treatment of the patients (unpaired *t* test, $p=0.97$ for C3c and $p=0.59$ for C4).

AUTOANTIBODIES

Rheumatoid factors were found in 5% (mean titre 1:140, range 1:40–1:320) of patients (two with active and two with inactive disease). Precipitating antibodies to extracted cellular antigens were found in only one patient (1.3%, anti-Ro(SSA)). Antinuclear antibodies (ANAs) were found in 41 patients (51.3%, mean titre 1:186, range 1:80 to 1:320). No predominance of specific ANA type was found between the patients studied. Antinuclear antibodies were not associated with the activity (Table III), anatomical extent, severity, or duration of the disease, or the current treatment with immunosuppressive agents ($\chi^2=1.56$,

TABLE III. Correlation of serological variables with activity of ulcerative colitis

	Ulcerative colitis	
	Active Mean (SD)	Inactive Mean (SD)
Hb (g%)	12.3 (2.2)	12.8 (2)
ESR (mm/h)	23 (20)	19 (21)
Platelets ($\times 10^3/\text{mm}^3$)	350 (131)	305 (77)†
Leucocytes ($\times 10^3/\text{mm}^3$)	7.3 (1.9)	7.05 (2.8)
Increased CRP (%)	8.8	0
C3c (mg/dl)	97 (20.5)	100 (19.5)
C4 (mg/dl)	37.4 (11.9)	32.3 (10.3)*
Positive ANA (%)	55.9	47.8
Positive ANCA (%)	38.2	23.9
(IgM anti-dsDNA)‡	95 (82)	120 (126)
(IgG anti-dsDNA	83 (52)	94 (74)
(IgM anti-CL	83 (29)	80 (52)
(IgG anti-CL	76 (32)	81 (31)
sIL-2R (U/ml)	598 (219)	406 (162)§

* $p < 0.05$; † $p < 0.10$; ‡anti-dsDNA and anti-CL antibodies are given as binding index; § $p = 0.0001$. Hb=haemoglobin; ESR=erythrocyte sedimentation rate; CRP=C reactive protein; sIL-2R=soluble interleukin 2 receptor molecules; other abbreviations as for Table II.

TABLE IV C3c and C4 components of complement system and the haematological variables associated with anti-dsDNA anti-CL, ANCA, and autoantibodies in total (data are given as mean (SD))

	Anti-dsDNA (IgG and/or IgM)		Anti-CL (IgG and/or IgM)		ANCA		Autoantibodies	
	+ =36	- =44	+ =21	- =59	+ =24	- =56	+ =61	- =19
Hb (g%)	13.2 (1.7)	12.9 (2.3)	12.7 (2.1)	13.2 (2)	13.1 (2.1)	13.4 (2)	12.9 (2)	13.7 (2.2)
PCV (%)	41.7 (4.7)	41.1 (6.5)	40.9 (6.2)	41.5 (5.7)	39.8 (6.5)	42.1 (5.3)	40.9 (5.8)	42.8 (5.6)
ESR (mm/h)	21.8 (18.9)	19.7 (22.6)	16.6 (17.8)	22.1 (21.9)	23.4 (20.6)	19.5 (21.1)	20.1 (18.2)	22.4 (28.5)
Platelets ($\times 10^3/\text{mm}^3$)	316.5 (104)	329 (106)	307 (91)	329 (109)	367.6 (127)*	305 (88)*	327 (111)	314 (84)
Leucocytes ($\times 10^3/\text{mm}^3$)	6.9 (2.6)	7.4 (2.4)	7.1 (2.3)	7.1 (2.5)	7.0 (3.3)	7.2 (2.1)	7.2 (2.5)	7.1 (2.3)
C3c (mg/dl)	98.6 (21.9)	98.8 (21)	100.5 (23.3)	98.1 (20.7)	102 (20.7)	97.5 (21.5)	97.5 (22.2)	102.8 (18)
C4 (mg/dl)	34.2 (11.6)	34.6 (11.1)	35.6 (11.8)	34.1 (11.1)	35.8 (12.9)	33.9 (10.6)	34.2 (10.9)	35.3 (12.5)

* $p=0.02$; †=positive; - =negative; PCV=packed cell volume; other abbreviations as for Tables II and III.

$p=0.21$). Also, ANAs were not associated with C3c and C4 concentrations (unpaired t test, $p=0.85$ and $p=0.93$ respectively), platelet numbers ($p=0.50$), leucocyte numbers ($p=0.38$), haemoglobin concentration ($p=0.99$), packed cell volume ($p=0.93$), or other autoantibody specificity (data not shown). Antinuclear antibodies were associated with higher ESR (25.5 (20.1) mm/h in ANA positive patients *v* 15.9 (21) mm/h in ANA negative patients, $p<0.05$).

Anti-CL and anti-dsDNA antibodies (IgG or IgM classes) were detected in 26.3% and 45% of patients respectively, but were not associated with disease activity (Table III), anatomical extent ($\chi^2=1.23$, $p=0.54$ and $\chi^2=1.38$, $p=0.50$), severity ($\chi^2=0.25$, $p=0.88$ and $\chi^2=4.8$, $p=0.09$), the duration of the disease (unpaired t test, $p=0.97$ and $p=0.10$), or the current immunosuppressive treatment of the patients ($\chi^2=0.08$, $p=0.77$ and $\chi^2=1.68$, $p=0.20$). These autoantibodies mainly represent an IgM response (66.7% of anti-CL and 77.8% of anti-dsDNA positive patients). The binding index in patients positive for anti-CL antibodies was 133 (27) (IgG, $n=8$) and 147 (25) (IgM, $n=14$) whereas for anti-dsDNA antibodies the binding index was 162 (17) (IgG, $n=9$) and 172 (55) (IgM, $n=28$). In general, however, the binding index for these autoantibodies in UC patients did not differ statistically from the binding index of healthy controls. Moreover, no association was found between anti-CL and anti-dsDNA antibodies and C3c and C4 components of the complement system and the haematological variables studied (Table IV). None of the anti-CL positive patients had any manifestations of the so called antiphospholipid syndrome (thrombosis, thrombocytopenia, and recurrent fetal loss).^{18,19} ANCAs were found in 30% of patients (mean titre 1:48, range 1:20–1:160). The detectable ANCAs had a perinuclear pattern in 95.8% of patients and one patient had a mixed pattern (perinuclear and diffuse cytoplasmic). With the ELISA technique, anti-MPO antibodies were not found in any of the patients. The ANCAs could not be associated with the activity (Table III), severity ($\chi^2=2.77$, $p=0.25$), or duration of UC (unpaired t test, $p=0.14$), or the current immunosuppressive treatment of the patients ($\chi^2=1.56$, $p=0.21$). By contrast, ANCAs tended to be predominant in patients with left colitis (62.5%) or pancolitis (37.5%) but this was not significant ($\chi^2=5.28$, $p<0.10$). ANCAs were detected in younger patients (age 39.3 (SD 17.5) yr *v* 47.6 (SD 16.6) yr, $p<0.05$) and were associated with increased platelet

counts, but not with C3c, C4, or haemoglobin concentrations, ESR, or leucocyte numbers (Table IV).

Autoantibodies of at least one specificity were detected in 61 (76.3%) patients. No association could be found between the presence of autoantibodies and age, sex (data not shown), anatomical extent ($\chi^2=0.99$, $p=0.61$), activity ($\chi^2=1.87$, $p=0.17$), severity ($\chi^2=0.89$, $p=0.64$), or duration of the disease (unpaired t test, $p=0.23$), the current immunosuppressive treatment of the patients ($\chi^2=1.7$, $p=0.19$), or with C3c and C4 concentrations, and the haematological variables studied (Table IV).

SOLUBLE INTERLEUKIN-2 RECEPTORS

The mean (SD) sIL-2R concentrations (U/ml) in the serum samples from 34 patients with active UC (598 (219)) were significantly higher ($p=0.0001$, Table III, Fig 2) than in the 46 patients with inactive disease (406 (162)), and both subgroups had significantly higher concentrations (active, $p<0.0005$ and inactive, $p<0.001$) than healthy controls (309 (83)). Raised concentrations of sIL-2Rs (higher than the cut off point, 550 U/ml) were found in 32.5% (26/80, 18 with active and eight with inactive UC) of patients, in association with disease activity ($\chi^2=9.7$, $p<0.01$). Also, paired serum samples from 10 patients during active disease and after a clinical remission were also evaluated. We found significantly higher sIL-2R concentrations (Fig 3) in the active stage of UC in the same

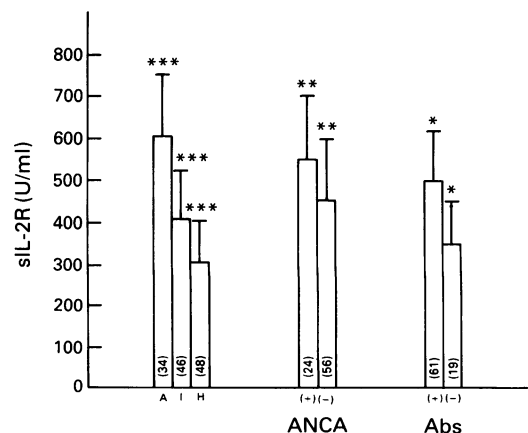


Figure 2: Mean (SD) (sIL-2R) concentrations in patients (No in parentheses) with active (A) or inactive (I) disease and healthy blood donors (H), as well as with antineutrophil cytoplasmic antibodies (ANCA) or with at least one specific autoantibody (Abs). * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

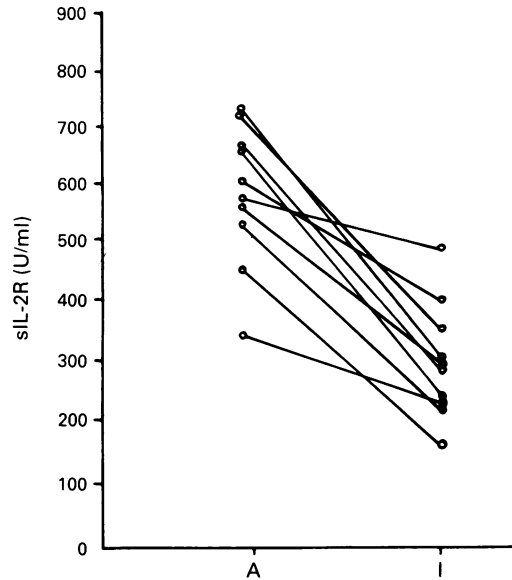


Figure 3: Pairs of interleukin-2 receptor (sIL-2R) concentrations in the 10 patients with active (A) and inactive (I) disease.

patient population (574 (137) U/ml than after a clinical remission (297 (100) U/ml $p < 0.001$). The mean sIL-2R concentrations were associated with the presence of ANCA (Fig 2) as well as with the presence of at least one specific autoantibody (Fig 2). By contrast, sIL-2R was not associated with the extent (ANOVA, $p = 0.38$), severity (ANOVA, $p = 0.28$), the duration of the disease (regression analysis, $p = 0.97$), or age and sex (data not shown). A positive correlation was found between the concentrations of sIL-2R and C3c and C4 concentrations (Figs 4 and 5). Concentrations of sIL-2Rs were positively correlated with ESR (Fig 6) but ESR was not associated with activity of disease ($p = 0.33$; Table III). No correlation was found between sIL-2R concentrations and the other haematological variables studied (data not shown) or the current immunosuppressive treatment of the patients (unpaired t test, $p = 0.37$).

Increased concentrations of sIL-2R (550 U/ml) were found in 18 patients with active disease (sensitivity 53%) whereas decreased concentrations of sIL-2R were found in 38 patients with inactive disease (specificity 82.6%). By contrast, platelet counts were 53% sensitive and

58.7% specific. The sensitivity for disease activity was increased when the presence of a positive value in the fixed sIL-2R ELISA and raised platelet counts were found together (25/34, 73.5%), but then the specificity was decreased (30/46, 65.2%). Also, if three patients were excluded the specificity of sIL-2R for disease activity increased (38/43, 88.4%). Those excluded were in clinical remission for intestinal symptoms but had symptoms of the disease outside the intestine (for example chronic active hepatitis or pericholangitis) possibly as a result of the increased sIL-2R concentrations.²⁰

Discussion

In our study, we found a high incidence of non-organ specific autoantibodies (76.3%), but in low titres, among UC patients. The increased frequency of anti-CL antibodies, anti-dsDNA antibodies, and ANAs among UC patients, may indicate that various foreign antigens drive their production. The high prevalence of anti-CL and anti-dsDNA antibodies, however, mainly represents an IgM response that for both autoantibodies carries low clinical significance.^{5, 18} Furthermore, the titres of anti-CL and anti-dsDNA antibodies found in UC patients were significantly lower than those usually found in patients with other autoimmune diseases such as systemic lupus erythematosus, primary Sjogren's syndrome, or primary antiphospholipid syndrome.^{3, 8, 18, 19} Obviously, anti-dsDNA antibodies (mainly IgG) are highly specific for systemic lupus erythematosus but these autoantibodies are also detectable in low concentrations, in other autoimmune diseases (for example, classic autoimmune chronic active hepatitis).²¹ A possible explanation is that these autoantibodies are different from those found in systemic lupus erythematosus. This has also been suggested for patients with autoimmune chronic active hepatitis²² as well as by the fact that antibodies against extractable nuclear antigens are not detectable in patients with UC.

Antineutrophil cytoplasmic antibodies similar to those detected in UC have been previously reported in patients with primary sclerosing cholangitis either with or without UC.²³⁻²⁵ Whether the p-ANCA associated with primary sclerosing cholangitis and UC are reactive to the

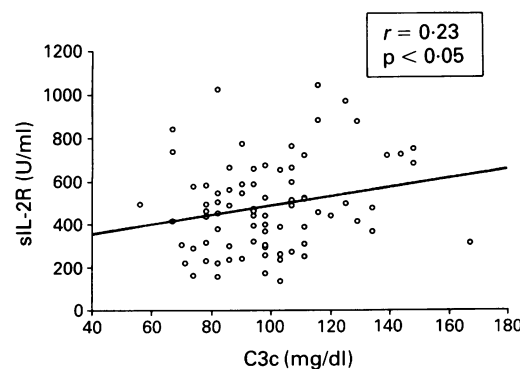


Fig 4

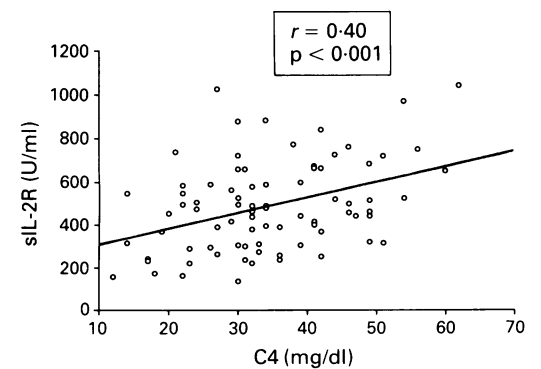


Fig 5

Figures 4 and 5: Correlation of interleukin-2 receptor (sIL-2R) concentrations with C3c and C4 complement components in the 80 patients with ulcerative colitis.

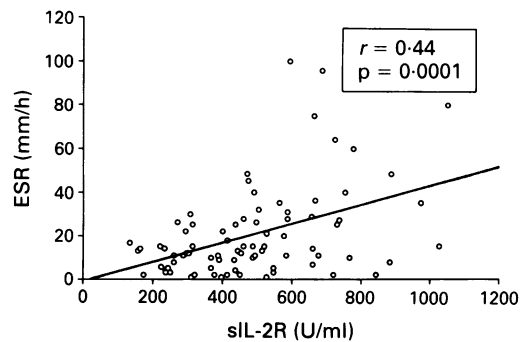


Figure 6: Correlation of interleukin-2 receptor (sIL-2R) concentrations with erythrocyte sedimentation rate (ESR) in the 80 patients with ulcerative colitis.

same antigen(s) and whether they are of pathogenic importance is not yet known, but the presence of a similar pattern of ANCAs in primary sclerosing cholangitis and UC possibly indicates shared immunopathogenic mechanisms. Antibodies against the myeloid lysosomal enzymes myeloperoxidase (anti-MPO), elastase, or cathepsin G have been reported to show perinuclear immunofluorescence staining on alcohol fixed neutrophils. Anti-MPO antibodies have been primarily detected in patients with crescentic glomerulonephritis¹⁰ but have also been found in patients with different forms of vasculitis²⁶ and more recently in patients with systemic lupus erythematosus.²⁷ Previous studies in our laboratory have shown that about the same proportion of p-ANCAs as is found in UC patients was found in serum samples from patients with systemic lupus erythematosus (11/40, 27.5%) but 63.6% of the patients with systemic lupus erythematosus had anti-MPO activity by the same ELISA technique (Goussia AC *et al* unpublished observations). In our study, none of the patients had detectable anti-MPO antibodies at least with the ELISA technique used. Previous studies have not detected antibodies to MPO, elastase, cathepsin G, or neutrophil primary granules in patients with UC.^{23 24 28 29} The presence of p-ANCA in only 6% of patients with a variety of other forms of colitis (primarily in patients with collagenous colitis)²³ as well as the finding that p-ANCAs also occurred in patients with UC after colectomy²⁴ suggest that p-ANCAs associated with UC and primary sclerosing cholangitis are possibly specific autoantibodies for the two diseases and not simply a reflection of the chronic colonic inflammation.

We found ANCAs in 30% of patients with UC (95.8% with a perinuclear pattern) but these did not correlate with the activity, extent, severity, or duration of the disease. This percentage is similar to those of Schlenker *et al*³⁰ and Jorgensen *et al*³¹ but lower than that of others.^{23 28} Duerr *et al* have reported the presence of p-ANCAs (measured by immunofluorescence) in 60% of patients with UC.^{23 24} Moreover, with a fixed neutrophil ELISA for IgG neutrophil antibodies, these authors showed a higher frequency of ANCAs (85%) than with immunofluorescence. They also found that 63% of patients with liver diseases – other than primary sclerosing cholangitis – showed positive results by this ELISA but none showed the perinuclear

immunofluorescence pattern. The identification of the antigen(s) to which the p-ANCAs in UC patients are reactive may contribute to the understanding of the possible immunopathogenic mechanisms in UC and may allow the development of more sensitive and specific assays to act as an indicator of this disease.

Our results also showed that patients with active UC had significantly increased mean serum sIL-2R concentrations compared with those in patients with inactive disease, whereas both of the groups had higher mean sIL-2R concentrations than control subjects. Furthermore, the presence of at least one specific autoantibody or ANCA alone, as well as the C3c and C4 components of the complement system were correlated with increased serum concentrations of sIL-2R, suggesting an association between such aberrant immunological phenomena. It is well documented that sIL-2R release *in vitro* as well as *in vivo* represents an event associated with lymphocyte activation.³² Crabtree *et al*, however, showed a similar rise in sIL-2R concentration in patients with Crohn's disease, correlated with disease activity, but even during inactive disease, sIL-2R concentrations were increased in patients with UC as well as Crohn's disease compared with controls.³³ This report and ours suggest that similar immune system activation, as well as a persistence of early activation antigens on peripheral or intestinal mononuclear cells are present in the quiescent forms of the two types of inflammatory bowel disease. By contrast with the findings, of Crabtree *et al*,³³ sIL-2R concentrations were positively correlated with ESR but not with platelet counts. Furthermore, the positive correlation between sIL-2R concentrations and C3c or C4 components of the complement system, suggests that the acute phase response is possibly present as the UC relapsed. On the other hand, the serum sIL-2R concentrations have also been found to correlate with various indices of activity of other autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus and progression to extraglandular involvement and pseudolymphoma or lymphoma in patients with primary Sjogren's syndrome.^{11 34}

In conclusion, we suggest that UC is accompanied by an autoimmune response, as shown by the presence of low titres of various non-organ specific autoantibodies and cellular immune activation (indicated by high sIL-2R concentrations). The detection of the distinct subset p-ANCAs is of interest as it has been found mainly in UC and in conditions such as primary sclerosing cholangitis that may accompany UC. The identification of the target antigen(s) of p-ANCA would possibly clarify the specificity of this distinct subset of ANCAs for UC. Until this identification is established, the high specificity of sIL-2R for UC activity seems to be the best laboratory indicator for assessing activity of UC.

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