

Circulating immune complexes containing secretory IgA in jejunoileal bypass disease

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SUMMARY Thirteen patients were studied after jejunoileal bypass (JIB) surgery. Seven developed arthritis and six did not. Circulating immune complexes containing IgG and IgA were detected in the sera of patients with and without arthritis. IgA complexes were shown to contain secretory component, a protein predominantly associated with intraluminal IgA, in significantly higher levels in patients with arthritis. Analytical ultracentrifugation showed complexes of approximately 10·8S, consistent with the size of secretory IgA. Arthritis after JIB appears to be associated with circulating immune complexes containing secretory IgA.

Key words: bypass arthritis, IgA immune complexes, immune complex disease.

Intestinal bypass surgery has been used as a treatment for morbid obesity for over 25 years. Originally jejunocolic bypass operations were done, but they were abandoned because of severe metabolic complications.¹ For several years jejunoileal bypass (JIB) surgery was used but it too has been associated with multiple complications including arthritis,^{2–8} hepatitis,^{9,10} renal damage,^{10,11} dermatitis,^{12–18} panniculitis,¹⁹ and haematological abnormalities.²⁰ Although other methods are currently being used for the control of morbid obesity, the JIB complications have provided a unique clinical opportunity to study the pathogenesis of a putative immune complex disease in man.

Evidence supporting the role of immune complexes in the arthritis and dermatitis seen after JIB has been frequently reported.^{4,6,7,15,16,18,21} In addition the presence of bacterial antigen and specific antibacterial antibodies in immune complexes from these patients has been shown.^{7,18,22,23} The present study reports further characterisation of the complexes by immunoglobulin class and complex size. We present data suggesting an association between disease activity, shown by acute arthritis, and

circulating immune complexes (CIC) containing secretory IgA.

Patients and methods

SELECTION OF PATIENTS

Questionnaires were sent to 44 consecutive patients who had undergone JIB surgery by one surgical team between 1972 and 1976. Twelve of these patients were studied. Six had developed signs of arthritis that were observed by at least one of the investigators after the surgery. Six patients had no rheumatological complaints.

One additional patient was referred for evaluation of arthritis which occurred after JIB. This patient subsequently underwent reanastomosis of her bowel and pre- and postoperative serum specimens were studied.

SELECTION OF CONTROLS

Blood specimens were obtained without regard to current therapy from 20 patients with rheumatoid arthritis (RA) seen in the Rheumatology Clinic of the University of Utah Medical Center, from 10 patients with dermatitis herpetiformis (DH) on sulphone therapy, and from randomly chosen Red Cross blood donors. RA patients were selected as controls because these patients may have raised levels of circulating immune complexes containing

IgG.²⁴ DH patients were selected as controls because these patients are known to have raised levels of CIC containing IgA.²⁵

SERUM SAMPLES

Blood was drawn without regard to dietary intake and allowed to clot at 37°C. Serum was separated at 37°C, aliquoted and stored at -70°C until studies were performed.

RAJI CELL IMMUNORADIOMETRIC ASSAY

The Raji cell immunoradiometric assay was performed as previously reported.^{25 26}

ANTISERA

The IgG fraction of goat antihuman IgA α chain specific and goat antihuman IgG γ chain specific were purchased commercially (Cappel Laboratories, Cochranville, PA). The goat antihuman IgA produced a single precipitin line on immunoelectrophoresis with whole human serum and with purified serum IgA (Cappel Laboratories). The goat antihuman IgG produced a single precipitin line on immunoelectrophoresis with whole human serum and with Cohn's fraction II (Miles Labs, Elkhart, IN). Whole rabbit antiserum to human secretory component (SC) was purchased commercially (Cal Biochem-Behring Corporation, La Jolla, CA). The anti-SC antibody did not react on immunoelectrophoresis with normal human serum or with purified serum IgA. A precipitin line was obtained on immunoelectrophoresis when anti-SC was reacted against defatted and decaseinated human colostrum. The localisation was similar to that observed using anti-IgA antiserum indicating that the anti-SC antiserum reacted with an additional component of exocrine IgA.

The specificity of the antiserum for bound SC as opposed to free SC was evaluated by G-200 gel filtration separation of various components of human colostrum (obtained less than 48 hours after parturition) and human milk (six months after parturition) by the technique of Van Munster *et al.*²⁸ Bound SC is present in high molecular weight fractions (> 200 000) and free SC in low molecular weight fractions (<100 000). Human colostrum and human breast milk were defatted by centrifugation at 30 000 g for 30 minutes. The casein was precipitated by HCl at pH 4. This was followed by Sephadex G-200 filtration. Elution peaks were collected, concentrated, and pooled according to molecular weight (fraction I >230 000; fraction II 230 000-100 000; fraction III <100 000). Fractions were examined by immunoelectrophoresis with the antihuman IgA and antihuman SC antisera. Both the anti-SC and anti-IgA antisera reacted with

fraction I with similar localisation on immunoelectrophoresis. No reactivity with fractions II or III was noted, indicating that the anti-SC antibody reacts predominantly if not exclusively with bound SC. All the antisera used were radiolabelled with ¹²⁵I by the chloramine T technique.²⁸

IMMUNOADSORPTION

Immunoabsorption experiments were carried out in order to establish whether the SC binding activity detected in serum by the Raji cell technique was associated with the IgA CIC activity or whether it represented independent binding of free SC to Raji cells. A serum sample from a post-JIB patient with high levels of IgA CIC and SC binding activity was chosen for investigation.

An immunoabsorption column for IgA was prepared by coupling 10 mg of antigen-purified α chain specific sheep antihuman IgA (Cappel Laboratories) to 1 g of cyanogen bromide activated Sepharose 4B, with 0.2 M sodium citrate as the coupling buffer. A control column of deactivated beads was prepared in an identical way except that no antihuman IgA was added. A running buffer of 0.07 M phosphate, 0.5 M sodium chloride at pH 7.2 was used. 1.0 ml serum samples were diluted 1:1 in running buffer and then applied to the column. The column was capped and gently rotated at 4°C for three hours. Additional running buffer was applied and the eluate collected until no further protein could be identified at the 280 nm spectrophotometer reading. Identical sample volumes of 3 ml were obtained from both columns. All specimens were immediately dialysed against 0.07 M phosphate, 0.12 M sodium chloride buffer. The dialysed specimens were tested for IgA concentration with the Beckman immunochemistry system. The specimens were also tested without further dilution by the Raji cell technique for anti-IgA and anti-SC activity. Results were expressed in counts per minute (cpm) because dilution of samples made reference to normal undiluted sera inappropriate. Values of an individual sample were expressed as cpm after an RPMI 1640 blank had been subtracted from the original reading.

SUCROSE DENSITY GRADIENT

ULTRACENTRIFUGATION

25 μ l of serum was placed on a 5.2 ml continuous gradient of 10-40% sucrose in distilled water. The gradient was centrifuged for 15 hours at 47 000 g in a Beckman SW50.1 rotor at 4°C and was fractionated from the bottom into 20-drop aliquots. Fractions were dialysed against phosphate-buffered saline (PBS) for 15 hours at 23°C and Raji cell immunoradiometric assay for IgA CIC was carried out on each sample. ¹²⁵I-labelled monomeric IgG (Miles

Labs) was used as the 7S marker and ^{125}I -labelled $\alpha 2$ macroglobulin (Sigma Chemical Company, St Louis, MO) as the 19S marker.

STATISTICAL ANALYSIS

Group comparisons between JIB patients with and without arthritis, and controls were done with the two-tailed Student's *t* test.

Results

CLINICAL DISEASE ACTIVITY

A complete medical history was known for each patient and physical examination was performed in every case, as well as the indicated laboratory evaluations. Results of these studies are summarised in Table 1.

IMMUNE COMPLEX DETERMINATIONS

Circulating immune complexes containing IgG. Three of the 12 patients had raised levels of CIC

containing IgG (Table 2) and the JIB patients had a significant increase of IgG CIC compared with normals ($p < 0.05$). As would be expected several RA patients had raised levels of IgG CIC, and DH patients had normal levels. There was no difference between IgG CIC levels in JIB patients with and without arthritis ($p > 0.1$).

Circulating immune complexes containing IgA. Nine of the 12 JIB patients had raised levels of CIC containing IgA (Table 2), and the JIB patients had a significant increase of IgA CIC compared with normals ($p < 0.005$). Fig. 1 shows a grouped comparison of IgA CIC levels between JIB patients with and without arthritis, patients with RA, and patients with DH. Again there is no difference in CIC levels between patients with and without arthritis ($p > 0.1$). The RA patients have normal levels of IgA CIC, and the DH patients have raised levels as previously reported.^{25 35}

Circulating immune complexes containing secretory component. Eight of the 12 patients had

Table 1 *Clinical characteristics of patients postjejunoileal bypass with and without arthritis*

Patient	Sex	Age	Wt. loss (pounds)	Arthritis	ESR* (mm/h)	Other postoperative complications
1	M	46	85	+	32	Cholecystitis, incisional hernia
2	M	41	NK	+	41	None
3	F	50	165	+	35	None
4	F	35	105	+	10	Diarrhoea > 15 stools/day, haemorrhoids
5	F	23	117	+	21	Cholecystitis
6	F	27	130	+	7	Cholecystitis
7	F	27	77	-	ND	None
8	F	40	90	-	ND	Headaches, undetermined aetiology
9	F	40	50	-	ND	None
10	M	37	0	-	ND	Lipaemia
11	F	51	105	-	ND	None
12	F	28	103	-	ND	None

NK=not known. ND=not done. *ESR=erythrocyte sedimentation rate.

Table 2 *Characterisation of immune complexes found in the sera of postjejunoileal bypass patients with and without arthritis*

Patient	Arthritis	IgG CIC*	IgA CIC*	SC CIC*
1	+	4.1	7.1	3.2
2	+	2.4	4.4	5.4
3	+	0.6	4.4	7.1
4	+	1.0	2.1	5.8
5	+	0.7	2.3	0.5
6	+	0.3	0.9	5.1
7	-	1.5	4.3	3.4
8	-	1.8	3.4	2.7
9	-	0.8	2.0	1.4
10	-	0.7	1.0	1.9
11	-	3.3	1.9	3.0
12	-	1.2	7.2	1.8

*CIC test units.

raised levels of CIC containing the SC of IgA (Table 2), and the JIB patients had a significant increase of SC containing CIC compared with normals ($p < 0.001$). Fig. 2 shows a grouped comparison of SC containing CIC between JIB patients with and without arthritis. Patients with arthritis at the time of evaluation had higher levels ($p < 0.02$) of SC containing CIC than those without arthritis. Moreover patients with DH who had raised levels of IgA CIC had normal levels of SC containing CIC.

Pre- and post-small bowel reanastomosis. One patient was followed before and after reanastomosis and had diffuse symmetrical arthritis involving her peripheral joints, as well as a pustular dermatitis, before her small bowel anatomy returned to normal. Fig. 3 shows the decline in the patient's CIC levels, which correlated with improvement of her rheumatological and dermatological complaints.

Immunoabsorption. IgA levels of the eluate (3 ml) from the anti-IgA column were 29 mg/dl (0.29 g/l) compared with 39 mg/dl (0.39 g/l) in the control column of deactivated beads. IgA CIC measurements of the eluate of the anti-IgA column and the

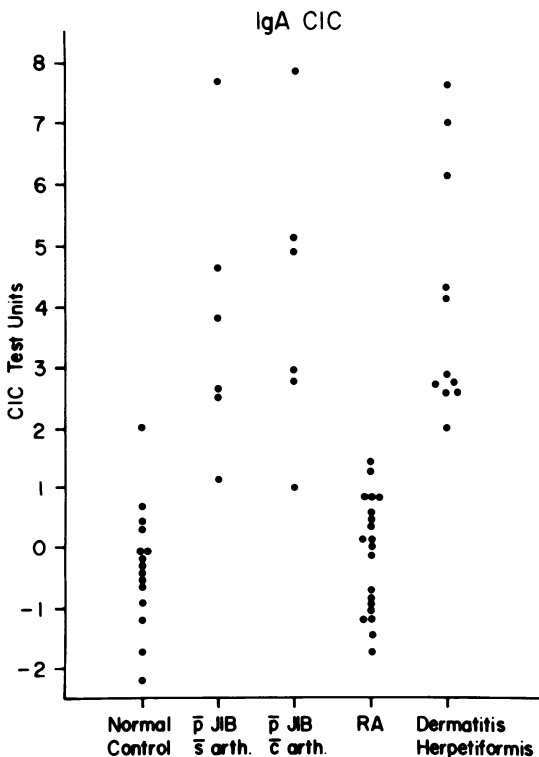


Fig. 1 Quantitative evaluation post-JIB of IgA containing CIC in patients with RA, with DH, and for normals.

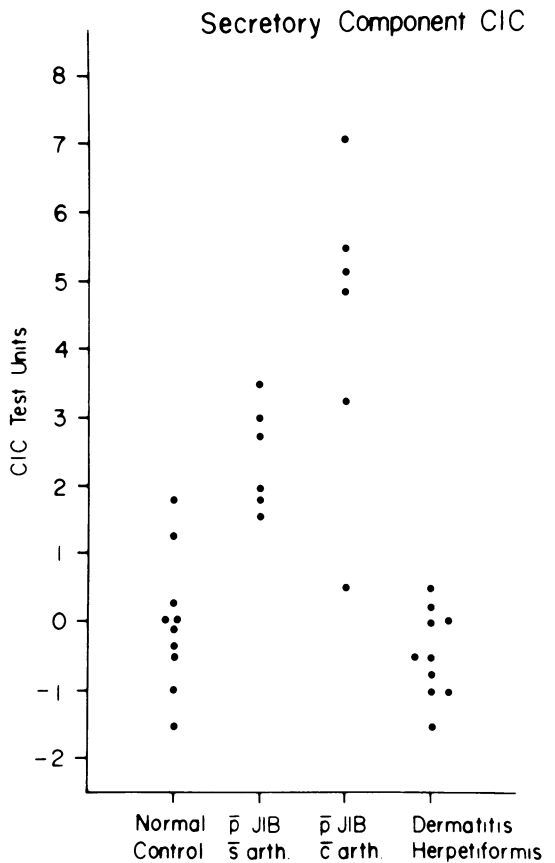


Fig. 2 Quantitative evaluation post-JIB of SC containing CIC in patients with DH, and for normals.

deactivated beads were 13 000 and 18 000 cpm respectively. SC containing CIC values of the eluates fell in a similar manner, with eluates from the anti-IgA column and the deactivated beads giving values of 42 000 and 87 000 cpm respectively.

Therefore the anti-IgA immunoabsorption column was effective in removing a portion of serum IgA and IgA CIC from the test serum. Removal of IgA CIC activity from serum with monospecific anti-IgA antibody also decreased the SC activity measured by the Raji cell assay, indicating that the SC activity being detected is probably bound to IgA rather than present in the free form.

COMPLEX SIZE

Analytical sucrose density ultracentrifugation was done with radiolabelled IgG as the 7S marker and radiolabelled $\alpha 2$ macroglobulin as the 19S marker.

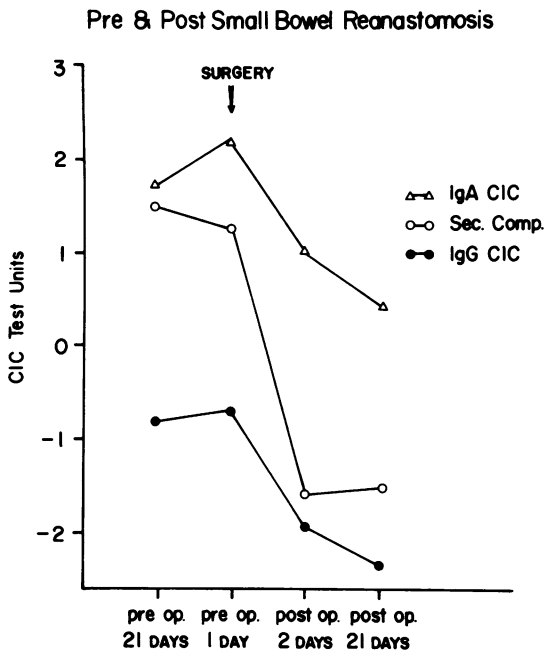


Fig. 3 CIC evaluations of serial sera after return of the small bowel anatomy to normal.

Both IgA and SC activity were found at approximately the 10-8S range. This size is most consistent with dimeric IgA.

Discussion

Arthritis has been recognised as a complication of JIB surgery for the treatment of morbid obesity in at least 25% of patients.⁵⁻⁸ It is usually insidious in onset, with fleeting exacerbations and remissions that leave no permanent stigmata and improve with the passage of time. Occasionally the disease is so severe, as with one patient in this series, that the bowel must be reanastomosed even at the cost of marked weight reaccumulation. The arthritis usually involves the peripheral joints, sparing the axial skeleton, and may be associated with skin, blood, or kidney abnormalities,²⁻²¹ as well as with the frequently recognised metabolic and gastrointestinal diseases.²⁹⁻³² Except for isolated reports^{17 21} there appears to be no relationship between the histocompatibility loci and the development of the arthropathy.

In 1976 Wands *et al.* reported raised levels of cryoprecipitable material in JIB patients' sera.⁴ IgG, IgA, and IgM, C3, C4, and C5, antibodies against bacterial antigens including *Escherichia coli* and

Bacteroides fragilis,^{4 6 7 15-18 20-23} and detectable group D streptococcal antigen⁷ have all been reported in these cryoprecipitates.

The present investigation further characterises the immune complexes that appear to be associated with articular and skin diseases that follow JIB surgery. While immunoglobulins of various classes have been detected after JIB surgery, the best correlation with active arthritis appears to be with CIC containing the SC of IgA. This point is further suggested by the observation that SC containing IgA immune complexes decreases following a return of the small bowel anatomy to normal.

The secretory immune system has been described elsewhere.^{36 37} External secretions, including those in the bowel, are generally characterised by a predominance of IgA. Human serum IgA is found primarily as a monomer, with only a small part in polymeric configurations. In contrast secretory IgA is composed of an IgA dimer, the pairs of heavy chains being linked by a joining or J chain. SC combines with dimeric or secretory IgA during the secretion process through hepatic and mucosal cell surfaces. Secretory IgA has an 11S size, consistent with that found in our study, and assumes importance when it is recognised that this is the form normally found in secretions but only in small amounts in the serum. SC is felt to be obligatory for the secretion of IgA³⁵⁻³⁹ and is not found in association with 7S IgA. An association of SC with IgM has been reported in a patient with IgA deficiency.⁴⁰ Secretory component binding within IgA CIC suggests that these complexes were formed in the gut lumen and later passed into the circulation, presumably through a defective mucosal barrier. In the case of JIB it is possible that chronic bacterial overgrowth in the small bowel produces an altered mucosal barrier, giving IgA immune complexes containing SC and formed in the bowel lumen access to the systemic circulation. This bacterial overgrowth would then provide the bacterial antigen necessary for continued stimulation of the mucosal immune system.²³

While the pathogenesis of JIB disease is still not fully understood, our data and the work of others strongly suggest that CIC containing secretory IgA and enteric bacterial antigens have a role in the development of the arthritis and dermatitis that is frequently seen following jejunoileal bypass surgery. The alterations that result from this surgery provide the potential milieu for these pathological processes to develop.

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