Fc receptor function and circulating immune complexes in gluten sensitive enteropathy – possible significance of serum IgA

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Abstract

The capacity to clear IgG containing immune complexes from the circulation was studied in patients with coeliac disease (n=13), dermatitis herpetiformis (n=8), and coeliac disease with concomitant serum IgA deficiency (n=4). A small group of patients with active ulcerative colitis (n=4) was included as a bowel disease control group. Clearance was estimated by measuring the disappearance rate of a bolus dose of intravenously injected IgG coated autologous erythrocytes. The mean $T_{1/2}$ of clearance was prolonged in both coeliac disease (86 (24) minutes) and dermatitis herpetiformis (111 (35) minutes), compared with healthy subjects (20 (5) minutes) and coeliac patients with concomitant serum IgA deficiency ($T_{1/2}$ =17 (6) minutes). Patients with ulcerative colitis had a prolonged clearance, with a $T_{1/2}$ of 195 (63) minutes. Values of circulating immune complexes were measured by four assays; C1q binding and C3, IgG, and IgA containing immune complexes. C1q binding immune complexes were detected only in IgA deficient gluten sensitive enteropathy. Patients with coeliac disease and dermatitis herpetiformis had higher values of C3, IgG, and IgA containing immune complexes than control subjects and serum IgA deficient patients with coeliac disease. The clearance rate was inversely correlated to the amount of immune complexes for the subgroups of gluten sensitive enteropathy.

The term gluten sensitive enteropathy has been introduced for the small bowel damage seen in coeliac disease and dermatitis herpetiformis. In gluten sensitive enteropathy, dietary wheat gluten and similar grain proteins cause a typical small bowel damage, histologically characterised by crypt hyperplasia/villous atrophy and infiltration of leukocytes into the mucosa. The mechanisms behind the bowel damage are presumed to be of autoimmune nature.¹ A link with histocompatibility genes has been reported – for example up to 90% of patients are reported to possess HLA DR3, DQw2 antigen.²⁻⁷ Autoimmune conditions are frequently associated with gluten sensitive enteropathy, with the highest reported prevalence in dermatitis herpetiformis, where up to 25% of patients have one or more autoimmune manifestations.⁸

The immunological response in gluten sensitive enteropathy involves formation of anti-gladin antibodies.¹ In untreated coeliac disease, the serum IgA concentration is increased, possibly as a consequence of the vigorous mucosal

immune response, and increased values of circulating immune complexes in serum, containing both IgA⁹⁻¹² and IgG,^{13,14} have been reported.

The spectrum of clinical disease in gluten sensitive enteropathy is wide. About 20% of the patients with this bowel inflammation disease also develop dermatitis herpetiformis.¹⁵ Patients with dermatitis herpetiformis may have less pronounced bowel damage, and their main symptom is usually a vesicular and strongly pruritic skin rash.¹⁵ Skin biopsy specimen shows subepidermal deposition of IgA and complement factor C3, but the antigenic composition of the deposited complexes has not been identified.¹⁶ A link with gluten has been clearly shown for the skin condition, as most patients recover, albeit slowly, from the skin rash on a gluten free diet.¹⁷

Immune complexes containing IgM or IgG (at high density) have the capacity to activate complement, which ultimately leads to deposition of C3 fragments (C3b and iC3b) on the immune complex surface, thereby making them accessible for binding to the C3b receptors (CR1, CD 35) on erythrocytes.¹⁸ The erythrocyte bound immune complexes are transported to the macrophages in the liver and in the spleen where they bind to the iC3b (CR3, CR4) and Fc receptors and are subsequently eliminated by phagocytosis.^{19,20} It has been reported that clearance of IgG coated erythrocytes is dependent mainly on splenic Fc receptor function, while soluble immune complexes and IgM – that is, complement-coated erythrocytes – are cleared mainly by the liver macrophages in primates and humans.^{19,21-24} A relation between the complement and Fc receptor mediated pathways can be expected in that impaired clearance via one pathway leads to accumulation of immune complexes and subsequently to saturation of receptors and decreased clearance via the other pathway.

Defective handling of immune complexes via one pathway or both leads to accumulation of immune complexes which have the potential capacity to modulate immune response mediated by B and T cells, for example via Fc receptors, the C3d receptor, CR2, the C3a receptor, CR1, and possibly via the IL 2 receptor,²⁵⁻³⁰ which are all factors involved in autoimmunity.

Defective Fc receptor function in vivo has been reported in patients with dermatitis herpetiformis.³¹ However, no comparative study of Fc receptor function and composition of circulating immune complexes has been made in patients with different subgroups of gluten sensitive enteropathy. In this study the clearance rate of IgG coated erythrocytes and values of IgA,

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TABLE I Clinical data on patients with gluten sensitive enteropathy investigated by Fc receptor mediated clearance of autologous sensitised erythrocytes

Patient no	Age	Sex	Year of diagnosis	Diet	AGA	Small bowel histology	Extent of ulcerative colitis
Coeliac disease							
1	63	F	1971	GFD	9	N	
2	72	F	1984	GFD	77	PVA	
3	50	F	1987	GFD	20	N	
4	47	F	1988	N/GFD	200/100	PVA	
5	42	M	1986	GFD	320	PVA	
6	68	F	1988	N	60	PVA	
7	48	F	1983	GFD	59	N	
8	51	M	1985	GFD	16	N	
9	48	M	1985	GFD	56	N	
10	60	M	1977	GFD	11	N	
11	59	M	1985	GFD	110	PVA	
12	27	F	1988	N	64	PVA	
13	35	F	1985	GFD	5	PVA	
Dermatitis herpetiformis							
14	29	M	1988	GFD	49	PVA	
15	31	M	1989	GFD	60	PVA	
16	57	M	1976	GFD	29	N	
17	49	F	1987	GFD	6	N	
18	58	F	1975	GFD	39	N	
19	31	F	1988	GFD	30	N	
20	57	F	1979	GFD	57	N	
21	43	M	1980	N	180	PVA	
Coeliac with serum IgA deficiency							
22	54	F	1979	GFD	0	PVA	
23	52	M	1980	GFD	0	N	
24	44	M	1976	GFD	0	N	
25	42	F	1977	GFD	0	PVA	
Ulcerative colitis							
26	31	M	1983				Total
27	24	M	1986				Left side
28	25	M	1984				Total
29	44	M	1981				Total

GFD=gluten free diet, N=normal diet. AGA=gladin antibody titre (IgA; reference range <48). PVA=partial villous atrophy, N=normal villous architecture.

IgG, and C3 containing and C1q binding immune complexes in patients with coeliac disease, with or without dermatitis herpetiformis, have been measured. In an attempt to explain the role of serum IgA in coeliac disease, the same parameters have been studied in a group of patients with serum IgA deficiency. Patients with ulcerative colitis and a group of healthy test subjects are included as control subjects.

Methods

TEST SUBJECTS

Thirteen patients with coeliac disease (eight women and five men) aged 27 to 72 years (mean, 52 years), eight patients with dermatitis herpe-

TABLE II Composition of immune complexes in patients with gluten sensitive enteropathy, ulcerative colitis, and healthy controls. All estimates of significance are made compared with healthy controls. Data are given as mean (SD)

	C3IC (ng/ml)	IgGIC (µg/ml)	IgAIC (ng/ml)	C1qIC (µg/ml)	Haemolytic activity	
					CPW	APW
Dermatitis herpetiformis (n=8)	162 (28)‡	3.47 (1.2)‡	988 (207)†	12 (2)	105 (4)	127 (7)
Coeliac disease (n=13)	108 (14)*	2.50 (0.44)‡	771 (11)‡	18 (2)	104 (2)	119 (4)
Coeliac disease IgA deficient (n=4)	78 (18)	1.70 (0.13)†	59 (31)†	34 (13)†	99 (6)	110 (8)
Ulcerative colitis (n=4)	194 (33)†	1.90 (0.90)	828 (267)*	ND	ND	ND
Normal subjects (n=14)	62 (16)	0.82 (0.12)	356 (56)	14 (2)	100 (1)	100 (4)

ND=not determined. *p<0.005; †p<0.01; ‡p<0.0001.

Figure 1: (A) Clearance of IgG coated erythrocytes from the circulation of a normal subject with a $T_{1/2}$ of approximately 16 minutes. (B) Clearance of IgG sensitised autologous erythrocytes from the circulation of 8 patients with DH (□), 13 patients with coeliac disease (○), 4 patients with coeliac disease with concomitant serum IgA deficiency (■), 4 patients with ulcerative colitis (●) and 14 healthy control subjects (△).

tiformis (four women and four men) aged 29 to 58 years (mean, 44 years), and four patients with coeliac disease and concomitant deficiency of serum IgA, defined as serum IgA less than 0.1 g/l (two women and two men) aged 42 to 54 years (mean, 48 years) were studied. These patients were compared with a previously described reference group consisting of 14 healthy subjects (seven women and seven men) aged 26 to 64 years (mean, 39 years).²⁴ A group of four patients with ulcerative colitis was also studied (four men) aged 24 to 44 years (mean, 32). The patients' clinical data are given in Table I. In the subgroup of coeliac patients with serum IgA deficiency we determined HLA class II antigens; all patients were found to possess DR 3, DQw2 antigen.

PROTEIN AND ANTISERUM PREPARATIONS

Anti-human IgG antiserum was raised in rabbits and purified as previously described.²⁴⁻³² Staphylococcal protein A (Pharmacia AB, Sweden) was labelled with ¹²⁵I by a lactoperoxidase method using Enzymobeads (Biorad Laboratories, Richmond, CA, USA) in accordance with the manufacturer's instructions.

CLEARANCE OF IgG COATED ERYTHROCYTES

Blood was drawn into vacutainer tubes containing EDTA. Ten millilitres of the blood were washed in Ringer acetate infusion liquid (Pharmacia, AB, Sweden) and coated with human IgG containing 62 µg of anti-D antibodies (Rhesonative, Kabi Vitrum, Sweden) for 45 minutes at 37°C as previously described.²⁴

After the incubation, the erythrocytes were washed three times, resuspended to a volume of 10 ml, and reinjected into the test subject. Vacutainer tubes containing EDTA were used to draw 5 ml blood samples from the same vein before injection and every 10 minutes for 70 minutes after injection.

The clearance rate of the IgG sensitised erythrocytes was measured by a previously described immunoassay.²⁴ Briefly, four aliquots of washed blood cells from each sample were incubated in two steps with anti-human IgG antiserum followed by ¹²⁵I labelled protein A. The cell bound radioactivity was monitored in a gamma counter and the half life time ($T_{1/2}$) of the reinjected IgG coated erythrocytes – that is, the time after which half the amount of the sensitised cells had disappeared from the circulation of the test subject – was calculated, if necessary by extrapolation (Fig 1A).

FACS ANALYSIS OF IgG MOLECULES AND C3 FRAGMENTS ON ERYTHROCYTES

After coating, the ability of the cell bound IgG to activate complement was tested by incubation of the cells with autologous serum for 30 minutes at 37°C, after which the washing procedure described above was repeated. The occurrence of IgG molecules and C3 fragments on the erythrocytes was determined by means of flow cytometry.^{33,34} After incubation, aliquots of 1×10^6 cells were washed with PBS and incubated with 5 µg of mouse monoclonal antibodies against C3b, iC3b, C3d, and IgG at 37°C for 30 minutes, followed by FITC conjugated anti-mouse mAb at room temperature for 15 minutes. After subsequent washing, fluorescent cells were analysed in a FACStar (Becton Dickinson) analyser/sorter, equipped with a 5W argon ion laser run at 0.5W and emitting at 488 nm. Green fluorescence was collected through a 530/30 nm filter. Data were processed in a Hewlett-Packard 217 computer (Ford Collins, CO) using Consort 30 (Becton Dickinson) software.

MEASUREMENTS OF IMMUNE COMPLEXES

C3, IgG, and IgA containing immune complexes,³⁵ C1q binding immune complexes containing IgG,³⁶ and functionally active complement components activated via the alternative or classic pathway³⁷ were measured using previously described methods.

STATISTICAL ANALYSIS

Statistical analysis of the data was performed using a non-parametric method (the Mann-Whitney U test). Simple regression analysis was used for correlations.

The study was approved by the Ethical

Figure 2: Correlation between half life of injected IgG sensitised erythrocytes and (A) IgA containing; (B) IgG containing; and (C) C3 containing immune complexes. The individual groups are from left to right coeliac disease with concomitant IgA-deficiency (■), healthy control subjects (△), coeliac disease (○), dermatitis herpetiformis (□), and ulcerative colitis (●). Values are mean (SD).

Committee of the Medical Faculty, Uppsala University. Patients and controls gave their informed consent to participation in the study.

Results

CLEARANCE OF IgG COATED ERYTHROCYTES

In patients with coeliac disease (excluding patients with serum IgA deficiency), the $T_{1/2}$ for clearance of sensitised erythrocytes varied between 30 to more than 200 minutes. The mean (SD) $T_{1/2}$ (86 (24) minutes, $n=13$) was significantly prolonged compared with healthy individuals (mean 20 (5) minutes, $n=14$, $p<0.001$). The $T_{1/2}$ for patients with dermatitis herpetiformis ranged from 45 to more than 200 minutes with a mean of 111 (35) minutes, $n=8$

($p < 0.0001$, compared with healthy controls and $p = 0.29$ compared with coeliac disease). After 60 minutes, a mean (SD) of 71 (6)% of the sensitised erythrocytes had been eliminated in the patients with gluten sensitive enteropathy and 43 (10)% in the patients with dermatitis herpetiformis ($p < 0.05$). The $T_{1/2}$ in the four patients with coeliac disease and concomitant serum IgA deficiency fell within the range of the healthy individuals ($p = 0.23$); 12–25 minutes, mean 17 (6) minutes. In ulcerative colitis the mean (SD) $T_{1/2}$ was 195 (63) minutes ($p < 0.005$). The individual $T_{1/2}$ for all patients, by groups, is shown in Figure 1B.

No significant correlation between clearance rate of sensitised erythrocytes and duration of disease ($r = 0.45$), histopathological status of small bowel biopsy specimen ($r = 0.014$), and adherence to a gluten free diet ($r = 0.18$) was found in gluten sensitive enteropathy.

DETECTION OF IgG MOLECULES AND C3 FRAGMENTS ON ERYTHROCYTES

FACS analysis of the IgG sensitised erythrocytes showed that more than 90% of the cells were coated, while no IgG was detected before incubation with the anti-D antiserum. Incubation of the labelled cells in autologous serum at 37°C for 30 minutes did not give complement activation resulting in deposition of C3 on the cell surface. This is indicated by the negligible amount of monoclonal anti-C3 antibodies bound after incubation (not shown).

IMMUNE COMPLEX VALUES

As shown in Table II, increased values of C3, IgG, and IgA containing immune complexes were found in all patient groups, with the exception of the coeliac patients with serum IgA deficiency. This group showed a modest increase in IgG containing immune complexes ($p < 0.01$), normal values of C3 containing immune complexes, and no IgA containing immune complexes ($p < 0.01$) compared with normal subjects. Increased C1q binding immune complexes were seen only in the group of patients with gluten sensitive enteropathy and concomitant serum IgA deficiency ($p < 0.01$). There were inverse relations between the values of IgA, IgG, and C3 containing immune complexes and the average clearance rate in each subgroup with gluten sensitive enteropathy (Fig 2).

The haemolytic activity was slightly increased in all subgroups of gluten sensitive enteropathy (Table II).

Discussion

We have shown that patients with dermatitis herpetiformis have a decreased clearance rate of IgG sensitised erythrocytes and increased values of C3, IgG, and IgA containing circulating immune complexes compared with patients with gluten sensitive enteropathy without dermatitis herpetiformis, and that values in both groups were significantly raised in respect of these parameters in comparison with healthy controls. The differences in circulating immune

complex concentrations might explain the deposition of immune complexes in the skin of dermatitis herpetiformis patients and possibly the higher prevalence of autoimmune manifestations reported in dermatitis herpetiformis compared with gluten sensitive enteropathy without dermatitis herpetiformis.

The impairment of clearance of circulating immune complexes in gluten sensitive enteropathy could be the result of a primary defect in Fc receptor function with secondary accumulation of circulating immune complex. The HLA haplotype associated with gluten sensitive enteropathy as well as with a number of other autoimmune conditions, HLA B8DR3, has been reported to be associated with defective Fc receptor function.³¹ An alternative hypothesis is saturation of or hindrance to binding to the Fc receptors by circulating immune complexes. In this context the circulating immune complex composition may be a key factor in delaying immune clearance, as the heavy mucosal IgA response found in these bowel diseases can lead to the production of immune complexes rich in IgA.

In an attempt to explain the role of serum IgA, immune clearance and circulating immune complexes were measured in a group of IgA deficient patients with gluten sensitive enteropathy. It has been shown that 5–10% of these patients are IgA deficient, a condition which is known to lead to increased antigen leakage from the gut.³⁸ In serum IgA deficient subjects, the absence of secretory IgA to protect the mucosal surfaces might further increase the leakage of antigens into the circulation, potentially leading to increased concentrations of circulating immune complexes, as the key function of secretory IgA is immune exclusion. Furthermore, a compensatory increase in secretory IgM is known to exist in the bowel,³⁹ and the histological bowel damage in these subjects is usually more severe than in patients sufficient in IgA.

In this study, normal clearance, low amounts of C3 and IgG containing circulating immune complexes, high amounts of C1q binding circulating immune complexes, and no IgA containing circulating immune complexes were found in a group of patients with IgA deficiency. These patients all have HLA B8DR3, which contradicts the hypothesis of a genetically determined Fc receptor defect as the main cause of slow clearance and circulating immune complex accumulation in coeliac disease.

We found very low concentrations of immune complex C1q in the groups of patients with IgA sufficient gluten sensitive enteropathy, which could be explained by defective complement activation by the classic pathway. The increased levels of haemolytic activity by both pathways in all subgroups of gluten sensitive enteropathy are in agreement with a low level of complement consumption, probably due to the characteristics of the circulating immune complexes. Polymeric IgA can activate complement by the alternative pathway, but the activation by IgA containing immune complexes is probably lower than that of immune complexes containing IgG or IgM, or both, which can activate both pathways.^{40 41}

In conclusion, the evidence presented here

suggests that the difference in clearance rates in patients with IgA sufficient and deficient gluten sensitive enteropathy may be caused by high concentrations of IgA containing circulating immune complexes, rather than by a genetically determined receptor dysfunction, since IgA sufficient patients with gluten sensitive enteropathy had a lower clearance rate than their IgA deficient counterparts, despite similar HLA type. The decreased clearance rate of IgG coated erythrocytes could thus be explained by an interaction either between immune complex bound IgA and the Fc receptor or between polymeric IgA and immune complex bound IgG. No evidence of an interaction between IgA and the Fc receptor has hitherto been reported, but the latter hypothesis is supported by reports suggesting that IgA might interfere with the Fc domain on IgG. These include observations by Wilton,⁴² who reported suppression by IgA of IgG mediated phagocytosis and by Schena *et al*,⁴³ who found that polymeric IgA led to decreased solubility of immune complexes in human serum. However, the full significance of a receptor dysfunction cannot be completely evaluated from these experimental data, suggesting the necessity for further studies of phagocytosis in vitro using macrophages or monocytes or investigations where immune complexes are eliminated in vivo – for example by plasmapheresis.

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