Contribution of secretory IgA, polymeric IgA and IgA/secretory component-containing circulating immune complexes to the serum hyper-IgA in diabetes mellitus

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Summary. The relative contribution of secretory IgA, monomeric and polymeric IgA and IgA/secretory component-containing immune complexes was investigated in sera of diabetic patients. Secretory IgA and immune complexes containing IgA and secretory component seem to participate in the hyper-IgA of patients with Type 2 (non-insulin-dependent) diabetes only, suggesting an altered hepatic clearance via secretory component receptors on hepatocytes. In Type 1

(insulin-dependent) diabetes, the high serum IgA levels might be explained by an increase in IgA production in response to antigenic stimuli. Evidence is also accumulated that immune complexes containing IgA of mucosal origin may be involved in microangiopathy production in Type 2 diabetes.

Key words: Type 1 diabetes, Type 2 diabetes, secretory IgA, IgA/immune complexes.

Increased concentrations of serum IgA are observed in various diseases [1]. Patients with liver disease frequently exhibit hyper-IgA with a preferential increase in the proportion of the polymeric form [2]. There is also experimental [3] and clinical [4] evidence that polymeric IgA are involved in tissue injury in some diseases.

Patients with diabetes mellitus frequently show hyper-IgA, the aetiology of which is at present unknown. Recently we have demonstrated an increase in the prevalence of circulating immune complexes containing IgA in diabetes, closely associated with the presence of microangiopathy [5].

The aim of this study was to investigate the relative contribution of secretory IgA, monomeric and polymeric IgA, and IgA-immune complexes in healthy subjects and patients with diabetes mellitus, to evaluate whether a positive correlation between these parameters occurs in diabetes and whether polymeric IgA, secretory IgA, and IgA-immune complexes could be regarded as indices of tissue injury.

Subjects and methods

Subjects

Sera of 30 patients with Type 1 diabetes (mean age 23 ± 16 years, range 7–68 years; mean duration of diabetes 6 years) and 22 patients with Type 2 diabetes (mean age 57 ± 14 years, range 42–72 years; mean duration of diabetes 10 years) were studied. As the control group, sera of 60 healthy subjects of equivalent age were selected.

Total IgA and secretory IgA concentrations in serum

Total IgA was measured by immunonephelometry, an assay that is virtually unaffected by the molecular size of IgA [6]. Secretory IgA was measured by a competitive solid-phase enzyme immunoassay. This assay measures all forms of secretory component in serum,

namely secretory component bound to IgA and IgM, free secretory component being virtually absent in sera [7].

Detection of circulating immune complexes containing IgA

IgA-circulating immune complexes were measured by a solid-phase anti-C3 enzyme-linked immunosorbent assay [5]. This method was partially modified for detecting secretory component-containing circulating immune complexes in a selected group of subjects. Sera were diluted 1:10 in 0.05 mol/l phosphate buffered saline (PBS, pH 7.2) containing 2% bovine serum albumin and 0.05% Tween 20 and incubated for 2 h in the anti-C3 coated solid-phase. Anti-human secretory component produced in rabbit was added at a dilution of 1:1000 in the same buffer, and after 1 h incubation anti-secretory component bound to the solid-phase was determined by the addition of anti-rabbit IgG conjugated with alkaline phosphatase. Positivity was defined by optimal density values (O.D., 405 A) exceeding the mean values plus three standard deviations of a control population.

Size distribution of IgA in serum

Sera of two patients with hyper-IgA and of a normal subject were diluted 1:2 in PBS and 300 μ l portions were placed at the top of 4.7 ml tubes containing 10–40% sucrose gradient in PBS.

Sera were centrifuged at 45000 rev/min for 15 h with a SW50.1 Beckman rotor in a Beckman L5-65 ultracentrifuge (Beckman, Fullerton, California, USA). Purified IgG and IgM were centrifuged in parallel gradients as markers. Fifteen fractions were collected from the bottom of the tubes and analysed for levels of IgA using commercially available low-concentration radial immunodiffusion plates (Behringwerke, AG).

Results

Total serum IgA

Unselected patients with Type 2 diabetes had significantly higher serum IgA levels (324 ± 135 mg/dl) than control subjects (210 ± 72 mg/dl) and patients with

Table 1. Serum levels of total IgA and secretory IgA

Subjects	Total serum IgA (mg/dl)		Serum secretory IgA (μ g/ml)	
	Mean $\pm$ SD	No. subjects > 350 mg/dl	Mean $\pm$ SD	No subjects > 23.75 μ g/ml
Type 1 diabetic patients ($n=30$)	234 $\pm$ 119	8 (27%)	13.0 $\pm$ 5.6	1 (3%) ^a
Type 2 diabetic patients ($n=22$)	324 $\pm$ 135 ^b	8 (36%)	19.8 $\pm$ 11.3	7 (32%)
Control subjects ($n=60$)	210 $\pm$ 72	—	14.5 $\pm$ 5.1	1 (2%)

^a Patient with a recent hepatitis B infection; ^b $p < 0.02$ statistically significant (versus Type 1 diabetic and control subjects)

Type 1 diabetes (234 $\pm$ 119 mg/dl; Table 1). Eight out of 30 patients with Type 1 diabetes and eight out of 22 patients with Type 2 diabetes had IgA concentrations in the upper part of the normal range (> 350 mg/dl).

Serum secretory IgA

Mean levels of secretory IgA were similar in diabetic and normal sera (Table 1). However, seven out of 22 patients with Type 2 diabetes had values above the normal range (> 23.75 μ g/ml), whereas this occurred only in one case with Type 1 diabetes, namely in a patient who suffered from hepatitis B infection.

IgA/secretory component-circulating immune complexes

IgA circulating immune complexes were detected in 10 out of 30 patients with Type 1 diabetes and in eight out of 22 patients with Type 2 diabetes. Serum IgA were significantly higher in the sera with circulating immune complexes (440 $\pm$ 115 mg/dl) than in those without (253 $\pm$ 90 mg/dl; $p < 0.01$). In patients with Type 2 diabetes, serum secretory IgA were higher in circulating immune complex-positive (25.5 $\pm$ 13.1 μ g/ml) than in circulating immune complex-negative sera (16.5 $\pm$ 8.9 μ g/ml); the difference, however, was not significant. Secretory component-circulating immune complexes were investigated in a selected group of patients with ($n=10$) or without ($n=9$) IgA-circulating immune complexes. Secretory component circulating immune complexes were detected in 80% of IgA-circulating immune complex-positive sera only, of which two had normal values of serum total IgA and secretory IgA.

Size distribution of serum IgA

Sucrose density gradient separation showed that 80–87% of diabetic IgA were polymeric, whereas < 20% of polymeric IgA was found in normal serum.

Relationship between total serum IgA and secretory IgA

A positive correlation was found between serum total IgA and secretory IgA in patients with Type 2 diabetes ($p < 0.05$) (Fig. 1b). The same did not occur in Type 1 diabetes (Fig. 1a).

Clinical study

All patients with Type 2 diabetes and IgA-circulating immune complexes (50% of which associated with in-

creased levels of total IgA and secretory IgA) had clinical evidence of microangiopathy. Only three out of ten patients with Type 1 diabetes with IgA-circulating immune complexes had evidence of microangiopathy and none showed increased levels of secretory IgA with the above mentioned exception.

Discussion

The present study shows that many diabetic patients have increased levels of total serum IgA. These elevations are accompanied by an increase in the proportion of the polymeric to the monomeric form, probably reflecting failure of normal hepatic clearance and/or increased synthesis of polymeric IgA. Elevations of total serum IgA were accompanied by a preferential increase of secretory IgA only in Type 2 diabetes. The finding of a positive correlation between levels of total IgA and secretory IgA only in Type 2 diabetes also suggests at least two different routes for IgA increases in diabetes.

Elevations of secretory IgA have been reported in subjects with altered liver function [2, 8]. The liver, in fact, seems to be involved in the selective removal of IgA via receptors for the secretory fragment [9]. A defective secretory component-mediated mechanism of hepatobiliary transport of IgA might be present in such patients as the result of liver dysfunction. In agreement with this hypothesis, secretory IgA were found to be increased in Type 1 diabetes only in a patient that had suffered from a hepatitis B surface antigen-positive acute hepatitis. Indeed, in a recent retrospective study we found an elevated prevalence of liver damage only in patients with Type 2 diabetes (unpublished observations).

In Type 1 diabetes the increase in serum IgA concentration might be most likely explained by an increase in IgA production in response to an increased availability of factors, such as bacteria, viruses, dietary antigens, a phagocytic cell dysfunction [10], probably resulting in a persistence of antigens in the circulation, forming a continuous stimulus to antibody production. According to this point of view, studies on IgA synthesis in vitro by diabetic peripheral blood lymphocytes are in progress in our laboratories to demonstrate whether an enhanced synthesis rather than, or in addition to, a decreased catabolism contributes to the high IgA levels in diabetes. Circulating immune complexes containing IgA were frequently detected in patients with hyper-

Fig. 1. A and B Relationship between serum levels of total IgA and secretory IgA in **A** Type 1 diabetic patients ($r = 0.010$, NS) and **B** Type 2 diabetic patients ($r = 0.45$; $p < 0.05$). $\blacktriangle$ = sera positive for IgA-containing circulating immune complexes

IgA. IgA-circulating immune complexes were closely associated with microangiopathy only in Type 2 diabetes (duration of diabetes was similar in IgA-circulating immune complex-positive patients with both Type 1 and Type 2 diabetes), where an increase of secretory IgA also occurred, suggesting that immune complexes containing IgA of mucosal origin may play a role in the vascular complications of diabetes. Indeed, in this study we present evidence of a frequent association of IgA-circulating immune complexes with circulating immune complexes containing the secretory fragment. Increasing evidence is accumulating that IgA-circulating immune complexes are involved in the vascular damage of some immunological and related disease [11] and although the origin of circulating and deposited IgA has been a controversial subject, IgA2 (IgA subclass of prevalent mucosal origin) and secretory component have been reported in vascular and mesangial renal deposits in IgA nephropathy [12] and in alcoholic cirrhosis [13].

Recently our non-conclusive studies demonstrated the presence of IgA deposits in dermal vessel walls of a small group of unselected diabetic patients (unpublished observations). However, to date we have no information on the presence of the secretory fragment in such deposits.

In conclusion, we have documented a close association between hyper-IgA, secretory IgA, IgA/secretory component-circulating immune complexes and microangiopathy. Further analysis, as well as prospective studies, are necessary to clarify the role of mucosal IgA in late diabetic complications.

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