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Serum IgA preferentially binds to cationic polypeptides in IgA nephropathy

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SUMMARY

The observation of negatively charged IgA in the mesangium of patients with primary IgA nephropathy (IgA-GN) prompted us to study the charge of serum IgA in IgA-GN, Henoch Schönlein purpura (HSP), alcoholic liver cirrhosis (ALC), membranous nephropathy (MGN) and systemic lupus erythematosus (SLE). Since no abnormal distribution of IgA isoelectric points was detected by isoelectric focusing studies, we developed a sensitive charge-dependent assay using plates coated with either cationized BSA (cBSA) or poly-L-lysine. In 15 IgA-GN sera, the amount of IgA reacting specifically with cBSA (cBSA-IgA) was almost linearly correlated with the poly-L-lysine-binding IgA ($r=0{\cdot}97$, $P=0{\cdot}0006$), suggesting that both assays detect charge-dependent interactions and thus probably measure anionic IgA. Significantly high serum levels of cBSA-IgA were observed in 56% of IgA-GN patients and in 40% of ALC patients. In contrast, normal serum levels of cBSA-IgA were detected in HSP, MGN and SLE. Both, the mono- or polymeric IgA bound to cBSA in a patient's serum studied. Contrasting with the presence of anionic IgA, no increase of cBSA-IgG was observed in IgA-GN. IgA rheumatoid factor (IgA-RF) assay showed high levels in IgA-GN (39%) and in ALC (25%). IgA-RF levels did not correlate with the amount of cBSA-IgA. When 18 patients with IgA-GN were tested after kidney transplantation, increased levels of cBSA-IgA and/or IgA-RF were found to be associated with the recurrence of mesangial IgA deposits in the graft. This suggests that both negatively charged IgA and IgA-RF may play a role in the recurrence of mesangial IgA deposits.

Keywords negatively charged IgA IgA rheumatoid factor IgA nephropathy glomerulonephritis kidney transplantation

INTRODUCTION

The charge of immunoglobulins can play a role in their glomerular deposition, as demonstrated by experimental models (Gallo *et al.*, 1983; Gauthier, Striker & Mannik, 1984). In human glomerulonephritis (GN) only a few data have been reported. In primary IgA nephropathy (IgA-GN), the mesangial IgA has a restricted anionic range contrasting with the broad range of normal human IgA (Monteiro *et al.*, 1985). By contrast, no data concerning the charge of serum IgA in IgA nephropathies have been described. However, earlier works have detected in IgA-GN increased amounts of IgA binding to C1q (Woodroffe *et al.*, 1980), although IgA is not a complement-fixing immunoglobulin, and to bovine conglutinin (Lesavre, Digeon & Bach, 1982) in a complement-independent reaction since it occurred in the presence of EDTA. This suggested the presence of electrostatic interactions with IgA, since both C1q and bovine conglutinin are highly cationic proteins.

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The present study was performed to analyse the *in vitro* electrostatic interactions in IgA nephropathies between serum IgA and proteins in their native or chemically cationized forms, compared to isoelectrofocusing analysis. We demonstrate that increased levels of IgA binding to cationic probes and of IgA rheumatoid factor (IgA-RF) were present in sera from patients with IgA-GN and ALC, although no apparent changes in isoelectric points of serum IgA were observed in these patients. We also showed that abnormal levels of IgA-RF and/or IgA-binding to cationized BSA were present in transplanted patients secondarily to IgA-GN only when mesangial deposits of IgA recurred in the grafted kidney.

MATERIALS AND METHODS

Patients and controls

Primary IgA nephropathy. Two groups of patients were studied:

Group 1. Serum samples from 31 patients (23 males and 8 females; mean age 30 ± 11 years, range 15 to 58 years) with biopsy-proven IgA-GN were studied.

Group 2. Serum samples from 18 patients (15 males and 3 females; mean age 38 ± 9 years, range 30 to 52 years) with end-stage renal failure secondary to IgA-GN who underwent renal

transplantation; immunological studies were performed 10 years after transplantation. As demonstrated by kidney biopsies, recurrence of the original lesions was observed in 9 of the 18 patients.

Other glomerulonephritis. Serum samples from 20 patients under 15 years old (15 males and 5 females) with active HSP nephritis and with mesangial IgA deposits demonstrated by kidney biopsies, were studied. Serum samples from 18 patients with SLE glomerulonephritis (6 males and 12 females; mean age 32 ± 12, range 18 to 48 years) and from 20 patients with idiopathic membranous glomerulonephritis (MGN) (9 males and 11 females; mean age 39 ± 10, range 28 to 55 years) were studied.

Alcoholic liver cirrhosis. Serum samples from 20 patients with ALC (16 males and 4 females; mean age 48 ± 13, range 32 to 68 years) were studied.

Control sera. Serum samples from 32 normal subjects (blood donors and laboratory staff personnel) were used as a control group (NHS).

Serum IgA and IgG levels. Measurements of IgA and IgG were performed by laser nephelometry (Hyland Laboratories, Costa Mesa, CA). The levels (mean $\pm$ s.e.m.) of serum IgA or IgG in 32 NHS was $1{\cdot}7 \pm 0{\cdot}8$ and $11{\cdot}4 \pm 2{\cdot}3$ mg/ml, respectively. An IgA and IgG deficient serum (<2 ng/ml) was used as negative serum control.

Buffers

PBS, phosphate-buffered saline; PBS-Tw, PBS containing 0·1% Tween 20; BBS, borate-buffered saline (0·2 M borate buffer, pH 8·2, containing 0·075 M NaCl); BBS-Tw, BBS containing 0·1% Tween 20.

Immunoglobulins and antisera

Normal polyclonal human IgA and IgG, as well as normal goat IgG were purified as previously described (Chevailler *et al.*, 1987).

Affinity purified goat anti-human α-chain and anti-human γ-chain antibodies (Cappel Laboratories, West Chester, PA) were immunoadsorbed by either IgG- or IgA-Sepharose (Pharmacia). Anti-BSA antibody was purchased from Dakopatts (Copenhagen, Denmark). $F(ab')_2$ fragments were prepared as described (Stanworth & Turner, 1973) and purified on AcA-54 Ultrogel (IBF, Villeneuve-la-Garenne, France) and protein-A Sepharose (Pharmacia). The $F(ab')_2$ fragments were homogeneous as assessed by SDS-PAGE analysis.

Labelling procedures

Antibodies, $F(ab')_2$ fragments or proteins were labelled with $Na^{125}I$ (Amersham) using iodogen coated tubes (Pierce Chemical Co., Rockford, IL) (Fraker & Speck, 1978). The specific activity was about 10 μCi/μg. Affinity purified goat anti-human α-chain $F(ab')_2$ fragments were biotinylated in our laboratory (activated biotin, IBF).

Analytical isoelectric focusing (IEF)

Thin-layer polyacrylamide gels (0·5 mm) contained 5% (w/v) acrylamide and 3% (w/v) carrier ampholytes (Ampholine 3·5–9·5, LKB Instruments, Bromma, Sweden) according to the manufacturer's instructions. Ultrathin agarose gels (0·5 mm) contained 1% agarose (agarose EF, LKB), 10% sorbitol and 3% (w/v) carrier ampholytes (Ampholine 3·5–9·5, LKB), as described by Elkon (1984).

For narrow pH gradients, we used polyacrylamide gels containing immobilines, as described (Charlionet, Sesboüé & Davrinche, 1984). An immobilized 4·5–5·5 pH gradient was obtained using distilled methacrylic acid (Merck Laboratories, Darmstadt, W. Germany), itaconic acid (Merck) and dimethylaminopropyl-methacrylamide (Polysciences, Inc. Warrington, PA).

After focusing, the gels were then either immunofixed with anti-IgA antibodies (Dakopatts, Copenhagen, Denmark), extensively washed and stained with 0·1% Coomassie blue, or overlaid with nitrocellulose paper (Schleicher & Schüll, Dassel, W. Germany). After transfer, the sheets were postcoated with a 10% (v/v) FCS, 1% (v/v) gelatine in PBS-Tw and incubated (and washed) successively with a biotinylated goat $F(ab')_2$ anti-human α-chain antibody, and streptavidin-biotinylated horseradish peroxidase complex (Amersham) for 30 min at room temperature. Finally, the detection system included 3,3 diamino-benzidine tetrahydrochloride (Sigma).

Cationization of BSA (cBSA)

BSA (99% purity, from Sigma) was chemically cationized according to the method of Danon *et al.* (1972), using l-ethyl-3(3 dimethyllaminopropyl) carbodiimide hydrochloride (EDC; Sigma) as activator and N,N-dimethyl-1-3-propanediamine (DMPA; Eastman Kodak, Rochester, NY) as a nucleophile to replace carboxyl groups.

Charge-dependent binding assay

Microplates (Dynatech Laboratories, Alexandra, VA) were coated overnight at 4°C with 50 μg/ml solution of native BSA (nBSA), cationized BSA (cBSA) or poly-L-lysine (Sigma) in BBS (100 μl/well). The wells were washed four times with BBS-Tw and then saturated by incubation with 200 μl/well of BBS supplemented with 10 mg/ml (w/v) gelatin (Merck) for 1 h at room temperature. After four more washes in BBS-Tw, 100 μl of diluted serum samples in BBS-Tw were added, and the plates were incubated for 1 h at room temperature. Plates were again washed four times in BBS-Tw, and 100 μl of radiolabelled (^{125}I) anti-human α-, γ-chain, anti-BSA antibodies, $F(ab')_2$ anti-human α-chain fragments or normal goat IgG as control (100,000 ct/min/well) in BBS-Tw were added and incubated for 1 h at room temperature. After four washes in BBS-Tw, the wells were cut and the radioactivity was evaluated by a gamma counter. Two background ct/min were substracted from each cBSA-bound ct/min value: (a) the nBSA-bound ct/min; (b) the cBSA-bound ct/min obtained with an IgA and IgG deficient serum, as a negative control. The total background binding never exceed 8–10% of the counts observed in cBSA-binding Ig assay.

Results of the charge-dependent assay are expressed in standard deviation units (SDU) according to the formula: $SDU = (Xt - X_n)/SD_n$, where Xt represents the mean value (ct/min) of each test sample, Xn the mean value of a basic set of samples from at least 32 healthy blood donors assayed simultaneously and SD_n the standard deviation of the mean value obtained for the panel of healthy donors. In cBSA-binding IgA assay (cBSA-IgA), $X_n = 658$ ct/min and $SD_n = \pm 564$ ct/min; in cBSA-binding IgG assay (cBSA-IgG), $X_n = 189$ and

$SD_n = \pm 204$ ct/min. Results were considered positive when the mean value of a test sample run in triplicate exceeded that of the basic set of normal donors by at least 2 SDU.

IgA-RF assay

IgA-RF was measured by an ELISA technique (Sinico *et al.*, 1986). Results of the IgA-RF assay are expressed in SDU as described above for the charge-dependent assay.

Statistical analyses

Differences between groups were determined by the non-parametric Mann–Whitney rank sum test. Associations between the variables studied were determined by the Spearman rank-order correlation test.

RESULTS

Isoelectric points (pI) of IgA in the sera studied

The pI of serum IgA was determined by IEF on either polyacrylamide or agarose gels containing ampholines (pH ranged from 3·5 to 9·5), and on polyacrylamide gels containing immobilines (with a narrow pH gradient ranging from 4·5 to 5·5), as described in Methods. The pI of IgA in 30 NHS ranged from about 4·5 to 7·0 as described previously (Elkon, 1984). No significant differences were obtained in the sera from 30 IgA-GN patients, even when very narrow pH gradients were used (data not shown).

Charge-dependence of the in vitro *cBSA-IgA assay*

Although no significant differences were obtained in IEF studies, we first developed a charge dependent assay using *in vitro* interactions between serum IgA and coated cBSA. The coating of cBSA or nBSA in polystyrene plates was similar, as measured in preliminary experiments (not presented here) in which radiolabelled BSA, cationized or not, was found to bind approximately to the same extent to the plastic sites. Gelatin was used to saturate the free plastic sites, since gelatin is a neutral protein (Sela & Arnon, 1960). The accuracy and reproducibility of the methodology employed for the cBSA-IgA assay were assessed by testing three serum samples in triplicate on five separate days: the coefficient of variation was 5·6, 5·2 and 6·3% respectively.

In order to confirm the charge-dependent interaction of serum IgA and cBSA, we tested the binding of IgA to another cationic synthetic polypeptide, poly-L-lysine. Fifteen sera from patients with IgA-GN, positive in the charge-dependent assay, were simultaneously analysed in both assays. The cBSA-IgA were almost linearly correlated with the poly-L-lysine binding IgA ($r = 0{\cdot}97$, $P = 0{\cdot}0006$), thus confirming that both IgA binding assays selected the negatively charged IgA, which has a stronger affinity for cationic proteins (Fig. 1).

The cBSA-IgA observe here was not dependent on the serum IgA levels, on the possible presence of anti-BSA antibodies (Sancho *et al.*, 1983) or on the presence of IgA-RF. First, the cBSA-IgA titration of pooled sera of 10 IgA-GN patients or 10 NHS show that a 1:20 dilution of IgA-GN serum retains a significant cBSA-IgA activity when compared to 1:5 NHS, even though these serum dilutions contained 250 μg/ml and 375 μg/ml IgA for IgA-GN and NHS, respectively. Also, no significant correlation was observed between IgA levels and cBSA-IgA in the serum of patients studied (see below). This demonstrates

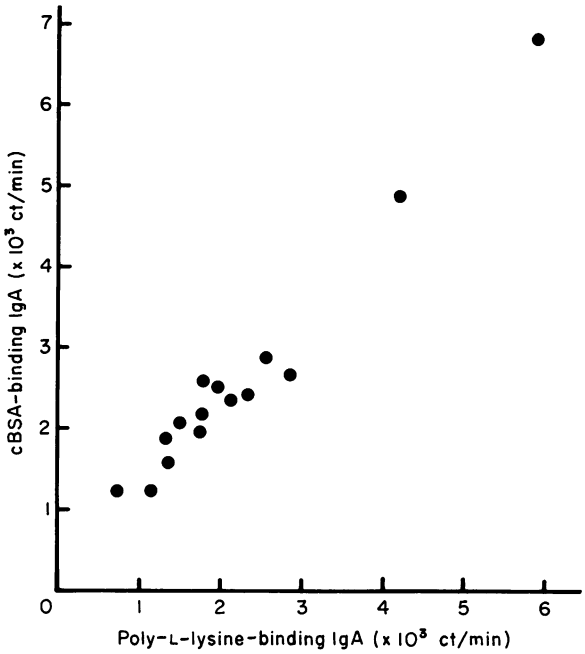

Fig. 1. Specificity of the charge-dependent assay. Correlation between cBSA-binding IgA and poly-L-lysine-binding IgA assays in 15 sera of IgA-GN patients using a Spearman rank-order correlation test ($r = 0{\cdot}97$, $P = 0{\cdot}0006$). 1:40 serum dilutions were added to cBSA and poly-L-lysine-coated wells and were incubated in BBS-Tw for 1 h at room temperature. After washing, ^{125}I-radiolabelled anti-human α-chain antibody was added to the wells. After incubation for 1 h at room temperature, the wells were washed, cut and radioactivity was evaluated by a gammacounter. Results were expressed in ct/min. Each point represents the mean of triplicate determinations.

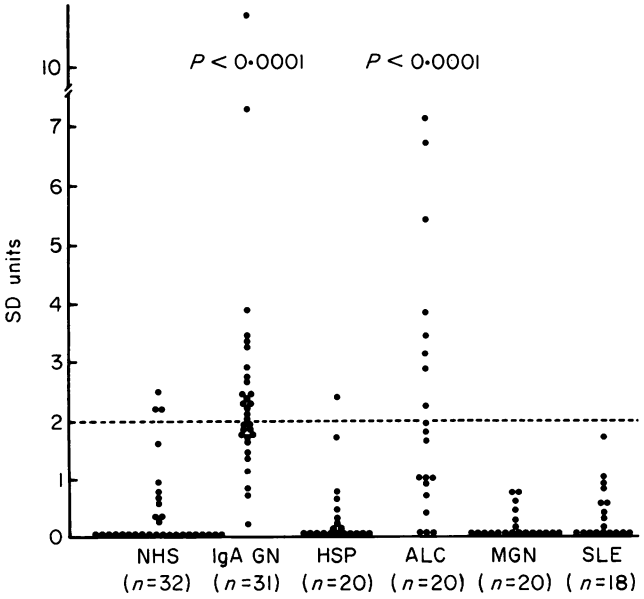

Fig. 2. Negatively charged IgA levels measured in sera from controls and patients. The charge-dependent assay was performed using 1:40 diluted sera, as described in Methods. Results were expressed in standard deviation (SD) units.

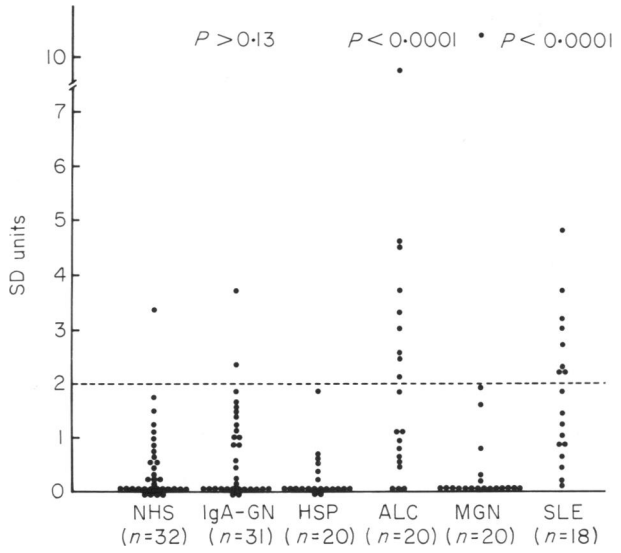

Fig. 3. Negatively charged IgG levels measured in sera from controls and patients. The charge-dependent assay was performed using 1:40 diluted sera, as described in Methods, revealed by ^{125}I-radiolabelled goat anti-human γ-chain antibody. Results were expressed in standard deviation (s.d.) units.

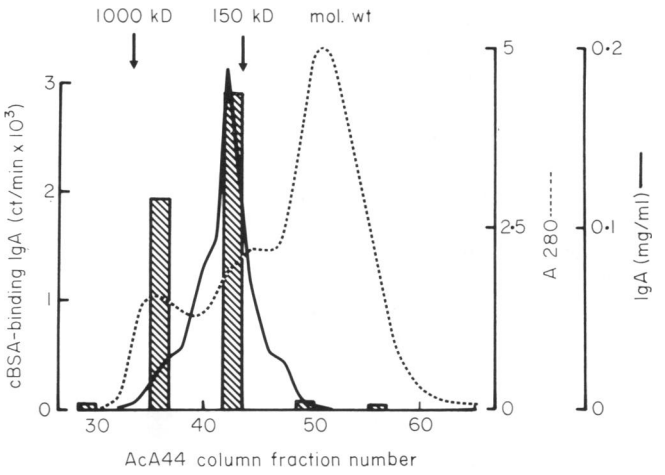

Fig. 4. Molecular weight determination of negatively charged IgA from an IgA-GN patient's serum. Three millilitres of serum were analysed on an Ultrogel AcA-44 chromatography column (IBF, 2 × 88 cm), with 10–130 kD fractionation range (exclusion limit > 200 kD). Two ml fractions were collected at a flow rate of 8 ml/h. The column was previously standardized with IgG and IgM, as molecular weight markers (150 and 1,000 kD, respectively). IgA was detected in the fractions by ELISA. The IgA pools were adjusted to the same IgA concentration and tested in the cBSA-binding IgA assay.

that the level of serum IgA did not account for cBSA-IgA. Furthermore, the increase in ionic strength between 0·035 and 0·6 M NaCl led to 80% inhibition of cBSA-IgA in a dose-dependent fashion in patient sera, while antigen–antibody interaction of cBSA with a radiolabelled rabbit anti-BSA antibody was almost not affected (< 15% inhibition) by this salt concentration (not shown here). The inhibition of cBSA-IgA was not due to removal of cBSA from plastic sites by high ionic strength, since a similar cBSA-IgA was obtained in coated plates whether pre-treated or not by 0·6 M NaCl. Finally, two additional studies were performed in 15 sera from IgA GN patients with positive cBSA-IgA and in 15 NHS to examine the possibility that IgA (or IgG) acted as rheumatoid factor antibodies and recognized goat IgG antibodies: (a) the radiolabelled antibody was replaced by radiolabelled normal goat IgG in the final incubation step of the charge-dependent assay, as described above, and no significant binding was obtained in all sera studies; (b) $F(ab')_2$ anti-human α-chain fragments, free of Fc fragments, led to identical results, ruling out a role of rheumatoid factor activity for these IgA bound to cBSA.

Serum levels of negatively charged IgA or IgG in controls and patients

Significantly higher levels of cBSA-IgA were demonstrated in 56% (17/31) of primary IgA-GN patients and in 40% (8/20) of ALC patients, compared to normal serum controls ($P < 0·0001$ and $P < 0·0001$, respectively) and patient sera with HSP nephritis and other primary GN (Fig. 2). The serum levels of cBSA-IgA detected in patients with IgA GN did not correlate with serum IgA levels (mean ± s.d. = 4·3 ± 1·8 mg/ml, ranging from 2·63 to 11·2) ($r = 0·07$, $P > 0·6$). In contrast, the serum levels of cBSA-IgA detected in ALC patients were significantly correlated with their serum IgA levels (mean ± s.d. = 6·5 ± 3·3 mg/ml, ranging from 2·7 to 15) ($r = 0·57$, $P < 0·01$). Normal levels of cBSA-IgA were obtained in HSP patients independently of their serum IgA levels (mean 2·7 ± 1·2 mg/ml, ranging from 1·01 to 5·5) ($r = 0·41$, $P < 0·06$). On the other hand, only a few sera were plotted in the upper SDU limit for coated nBSA binding assay (1/32: NHS; 2/31: IgA-GN; 1/20: HSP; 3/20: ALC; 0/20: MGN and 0/18: SLE), thus excluding the possibility of IgA anti-BSA antibodies.

Serum levels of cBSA-IgG were significantly increased in 45% (9/20) of ALC patients and 44% (8/18) of SLE patients ($P < 0·0001$ and $P < 0·0001$, respectively), contrasting with the normal range observed for IgA-GN, HSP and MGN patients (Fig. 3). The serum levels of cBSA-IgG detected in ALC and SLE patients correlated with their serum IgG levels ($r = 0·56$, $P = 0·01$ and $r = 0·45$, $P < 0·05$, respectively).

In one patient with high levels of serum cBSA-IgA, we determined the molecular weight of the cBSA-IgA after gel chromatography on AcA 44 Ultrogel. Both monomeric and polymeric IgA were found to bind to cBSA (Fig. 4). By contrast, no significant cBSA-IgA was obtained when NHS was fractionated on AcA 44 columns.

Serum levels of IgA-RF

Serum levels of IgA-RF were significantly elevated in 39% (12/31) of IgA-GN patients and 25% (5/20) of ALC patients when compared to normal serum controls ($P < 0·0001$ and $P < 0·001$, respectively) or sera from HSP GN and other chronic primary GN (Fig. 5). There was no significant correlation between serum IgA-RF levels and levels of cBSA-IgA for IgA-GN patients ($r = 0·082$, $P > 0·6$) and for ALC patients ($r = 0·36$, $P > 0·1$). By contrast, a weak but statistically significant correlation was observed between serum IgA-RF levels and levels of serum IgA in IgA-GN and ALC ($r = 0·44$, $P < 0·01$ and $r = 0·47$, $P < 0·05$, respectively). The levels of IgA-RF did not correlate with the presence and intensity of the IgG deposits in the mesangium from patients with IgA-GN, analysed by immunofluorescence in a double blind study ($r = 0·24$, $P > 0·17$).

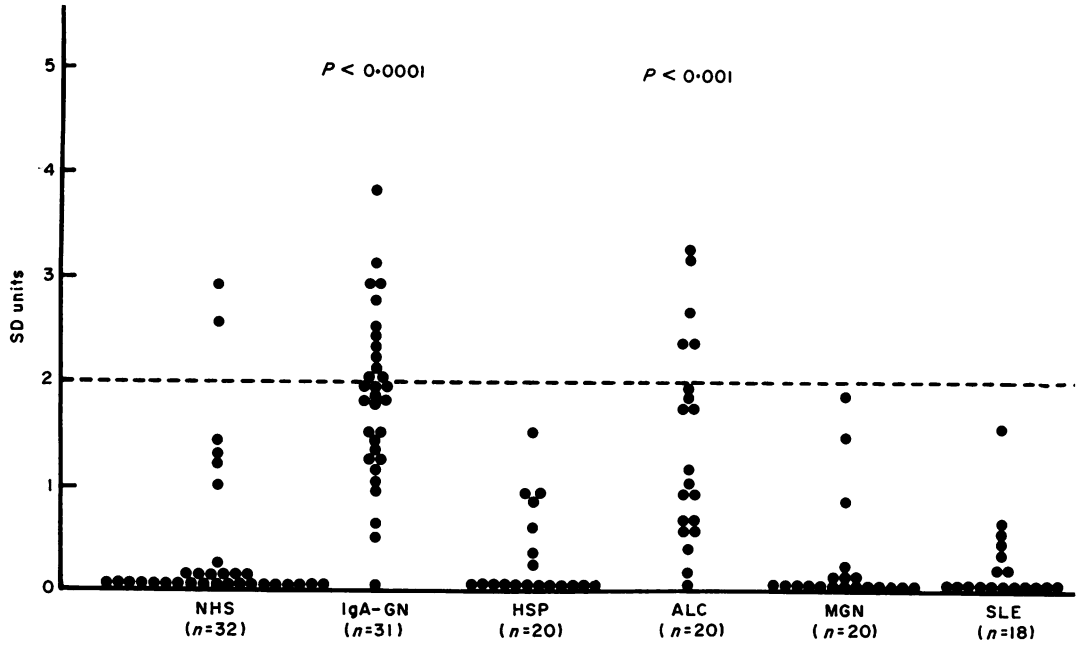

Fig. 5. IgA-RF levels in sera from controls and patients. Diluted sera (1:40) were assayed for IgA-RF assay. The results were expressed in standard deviation (SD) units, as described in Methods. Each point represents the mean of triplicate determinations.

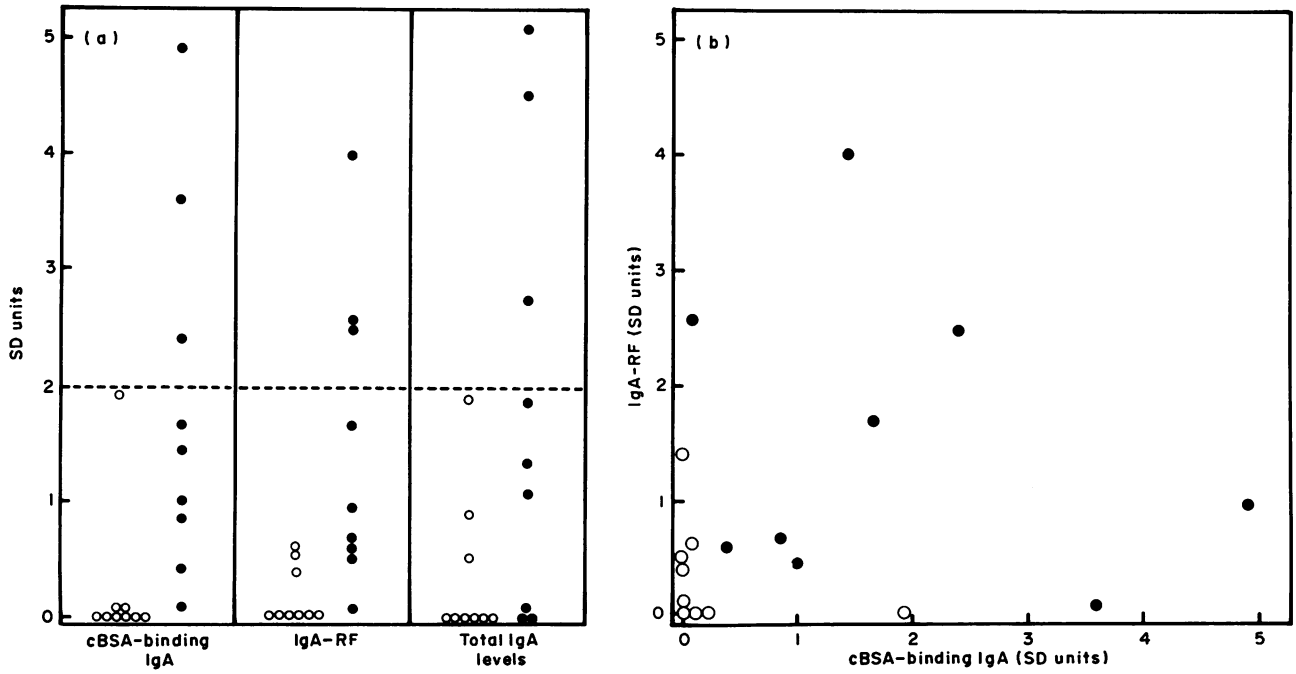

Fig. 6. Correlation between the levels of negatively charged IgA (cBSA-binding IgA), IgA-RF or total serum IgA, and the recurrence of mesangial IgA deposits after transplantation, in IgA-GN patients. Eighteen sera from transplanted patients with (O) or without (O) recurrence of mesangial IgA deposits were studied. cBSA-binding IgA and IgA-RF assays were performed as described in Methods. (A) differences between groups of patients with or without recurrence were determined by the Mann–Whitney rank sum test ($P < 0{\cdot}002$, $P < 0{\cdot}003$ and $P < 0{\cdot}02$ for cBSA-binding IgA, IgA-RF and total serum IgA, respectively). (B) Spearman rank-order correlation test between cBSA-binding IgA and IgA-RF ($r = 0{\cdot}29$, $P > 0{\cdot}22$).

Serum studies in end-stage IgA-GN transplanted patients

The cBSA-IgA and IgA-RF assays were performed with the sera of nine end-stage IgA-GN transplanted patients without recurrence and compared with those of nine end-stage IgA-GN transplanted patients with recurrent IgA-GN, without knowledge of the transplanted kidney biopsy of each patient. Significantly higher levels of cBSA-IgA, IgA-RF or serum IgA were only present in transplanted patients with recurrent IgA-GN (3/9) when compared to patients without recurrence ($P<0{\cdot}002$, $P<0{\cdot}003$ and $P<0{\cdot}02$, respectively) (Fig. 6A). No significant correlation was obtained between cBSA-IgA or IgA-RF levels and serum IgA levels (mean $\pm$ s.d. $= 3{\cdot}4 \pm 2{\cdot}5$ mg/ml, ranging from 0·02 to 8·57) in the nine transplanted patients with recurrent IgA-GN ($r=0{\cdot}25$, $P>0{\cdot}48$ and $r=0{\cdot}5$, $P>0{\cdot}15$, respectively). Although both cBSA-IgA and IgA-RF are associated with recurrence, there was no significant correlation between cBSA-IgA and IgA-RF in the 18 sera studied, as shown on Fig. 7B ($r=0{\cdot}29$, $P>0{\cdot}22$). Nevertheless, six out of nine patients with recurrent IgA-GN were positive (> 2 s.d. units) in at least one of the two tests (Fig. 6B).

DISCUSSION

We previously demonstrated in IgA-GN (Monteiro *et al.*, 1985), that eluted mesangial IgA was mainly dimeric and had a restricted anionic charge (pI: 4·5–5·6) compared to normal serum IgA (pI: 4·5–6·8). These data prompted us to analyse the charge of serum IgA in mesangial IgA nephropathies. We first investigated the isoelectric points (pI) of serum IgA in IgA-GN and in normal control sera using ampholine- or immobiline-IEF. No significant differences in the pI range of serum IgA was observed between patients and controls. However, this technique would not detect uneven distributions of charged groups on IgA molecules, which would not result in a modification of the total net charge, but could be an important determinant of electrostatic interactions with other charged particles. In order to explore this hypothesis, we developed an indirect method to analyse the electrostatic interactions between serum IgA and cationic polypeptides, such as cBSA and poly-L-lysine. The charge-dependence of this assay was strongly suggested by: (a) an almost linear correlation between cBSA- and poly-L-lysine-binding serum IgA; (b) the inhibition of cBSA-IgA by 0·6 M NaCl, an ionic strength which did not affect BSA-anti-BSA interactions. The presence of IgA anti-BSA antibodies (Sancho *et al.*, 1983) was also excluded in this study, since no detectable nBSA-IgA binding (> 2 s.d. Units) was observed in the sera studied; (c) the predominance of cBSA-IgA in anionic serum IgA fractions after ion-exchange chromatography (not presented here). The possibility that cBSA-IgA was dependent on IgA-RF activity was excluded since: (i) the use of $F(ab')_2$ anti-α-chain fragments led to identical results and (ii) no binding was obtained using radiolabelled normal goat IgG in control experiments.

The serum levels of negatively charged IgA were increased in IgA-GN patients (56%) and in ALC patients (40%), but were in the normal range for acute HSP patients and other glomerulonephritides studied. Interestingly, Knight *et al.* (1987) recently demonstrated in acute HSP patient's plasma, an oligoclonal polymeric IgA with a pI 5·92, more cationic than broad range of plasma IgA. In our cBSA-IgA assay, this serum IgA binding to cBSA seems to be not dependent on its mono or polymeric nature, since after gel filtration of one IgA-GN patient serum, both molecular weight forms of IgA bind to cBSA. Interestingly, the presence of negatively charged IgA was not correlated with the levels of serum IgA in IgA-GN. By contrast, in ALC the cBSA-IgA correlates with the levels of serum IgA. These data suggested that the excess of negatively charged IgA in IgA-GN is not due to the high serum IgA concentration, but to a qualitative IgA alteration. In addition, the increased cBSA-binding immunoglobulins were restricted to IgA, since the serum levels of negatively charged IgG were normal in IgA-GN. By contrast, the level of negatively charged IgG was increased in ALC patients (45%) and in SLE patients (44%). This is probably secondary to the high IgG levels in these diseases, since a significant correlation was observed between cBSA-IgG and serum IgG level.

In the present study, the abnormal serum levels of cBSA-IgA detected in IgA-GN patients probably reflects the excess of negatively charged IgA. This could result from a particular carbohydrate composition or a high content of acidic amino acids in the variable region. The net charge of serum IgA in IgA-GN was not modified as shown by undetectable pI changes in IEF studies, suggesting an abnormal distribution of negatively charged groups on the IgA molecules. Alternatively, negatively charged IgA may be either due to covalent binding of an anionic molecule such as α1 microglobulin (Vincent *et al.*, 1985) or to the binding of an anionic antigen. Although mesangium trapping is mainly size-dependent (Germuth, Senterfit & Dressman, 1972; Rifai *et al.*, 1979), some experimental models using charge-modified antigens or antibodies suggested that negative molecules are preferentially deposited in this region (Isaacs & Miller, 1982 and 1983; Gallo *et al.*, 1983; Gauthier, Striker & Mannik, 1984). By analogy with these experimental results, our data allow us to postulate a pathogenic role for the circulating anionic IgA (or IgA containing immune complexes) in the mesangial IgA deposition observed in IgA-GN.

We detected IgA-RF in about 40% of IgA-GN and in 25% of ALC patients. The increased levels of IgA-RF detected in IgA-GN patients agree with previous reports (Czerkinsky *et al.*, 1986; Sinico *et al.*, 1986). By contrast, the normal levels of IgA-RF detected in active HSP-GN sera disagree with a previous study (Saulsbury, 1986). In IgA-GN and ALC patients, the serum level of IgA-RF and serum level of IgA were significantly correlated suggesting that both increases may be secondary to one single mechanism, namely IgA polyclonal activation. Indeed, a spontaneous IgA synthesis by blood mononuclear cells has been observed in IgA-GN (Hale *et al.*, 1986) and in ALC patients (van De Wiel *et al.*, 1987). A potential role for IgA-RF in IgA-GN was not established in this study, since no correlation between serum IgA-RF levels and mesangial IgG deposits was observed.

On the basis of similarities between IgA-GN and HSP, it has been suggested that these two diseases represent variants of one nosological entity (Weiss *et al.*, 1978). IgA-CIC have also been demonstrated in HSP (Kauffmann *et al.*, 1980), these complexes are larger than 21S (Kauffmann, van Es & Daha, 1981) whereas those from IgA-GN patients are between 11 and 21S (Lopez-Trascasa *et al.*, 1980). We have also obtained different results for these diseases, with high serum levels of negatively charged IgA and IgA-RF in IgA-GN, contrasting with the normal levels in HSP, suggesting different pathogenic mechanisms resulting in mesangial IgA deposition.

Finally, the crucial point of this study is the statistically significant detection of either negatively charged serum IgA or IgA-RF only in transplanted patients with recurrent IgA-GN, without any correlation between these two variables. Interestingly, we have previously demonstrated in IgA-GN (Lesavre, Digeon & Bach, 1982), that high levels of IgA-binding to bovin conglutinin (a cationic protein) were correlated with IgA deposit recurrence. These results suggest that, although independent, both negatively charged IgA and IgA-RF may play a role in the recurrence of mesangial IgA deposits.

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