Antibody response to IgA-binding streptococcal M proteins in children with IgA nephropathy

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Abstract

Background. IgA nephropathy (IgAN), the most common glomerulonephritis worldwide, is characterized by mesangial deposits containing predominantly IgA. IgAN commonly occurs or exacerbates after upper respiratory tract infections such as streptococcal pharyngitis. Certain group A streptococci express M proteins with IgA-binding regions (IgA-BRs). We have previously shown that these IgA-BRs co-localize with mesangial IgA in IgAN.

Methods. Blood samples from patients with IgAN (n=21) and age-matched controls (n=83) were assayed by ELISA to detect an IgG antibody response to the IgA-BRs of the M4, M22 and M60 proteins. Antibodies were assayed for each IgA-BR separately and the results were combined.

Results. Antibody levels to the IgA-BRs were significantly higher in IgAN patients than controls (P=0.016), particularly in patients with recent streptococcal infection (P=0.008).

Conclusions. The results suggest that children with IgAN had a previous infection with a streptococcal strain expressing an IgA-binding M protein.

Keywords: children; group A streptococci; IgA nephropathy; M protein

Introduction

IgA nephropathy (IgAN) is a common form of glomerulonephritis characterized by recurrent episodes of macroscopic haematuria, variably progressing to renal failure in which renal biopsies demonstrate predominantly galactose-deficient IgA1 deposits in the mesangium [1–3]. IgAN is often preceded by infections [4–10], primarily of the upper respiratory tract. Many cases are preceded by streptococcal pharyngitis [10] and clinical observations indicate that tonsilllectomy may improve the outcome of IgAN [11].

Group A streptococci (GAS) express surface-localized M proteins, which have an N-terminal hypervariable region that determines the M type of the strain. Certain M proteins bind human IgA-Fc via a semi-variable region [IgA-binding region (IgA-BR)] [12–15]. In a previous study, we showed that most renal biopsies from paediatric IgAN patients contained mesangial deposits of IgA-BRs, which co-localized with IgA, suggesting a pathogenic role for IgA-BRs in IgAN [6]. The aim of the current study was to analyse the antibody response to IgA-BRs in children with IgAN.

Materials and methods

Subjects and blood samples

Patients with IgAN (n=21) and age-matched controls (n=83) were included in the study. Blood (serum or citrated plasma) samples were taken within a median of 3 months (range 0–14 months) after the onset of clinical disease (as defined by the presence of symptoms) in all patients. Detailed information regarding the patients and controls and blood samples taken are available as Supplementary data. The study was approved by the ethics committee of the Medical Faculty, Lund University and blood samples were obtained with informed written consent of all patients or their parents when patients were younger than 15 years.

Evidence for streptococcal infection in patients

Throat cultures and serological assays for streptococcal infection were available for 18 of 21 patients. Evidence for recent streptococcal infection was found in 10 of 18 patients at the initial presentation of IgAN. For the methodology, see the Supplementary data.

Synthetic peptides and rabbit antisera

IgA-BRs of the M4, M22 and M60 proteins were available as synthetic peptides designated Sap4, Sap22 and Sap60, respectively [6]. The N-terminal hypervariable regions of M4 (M4-N) and of the non-IgA-binding M5 protein (M5-N) were also available as synthetic peptides [16]. Rabbit antisera to the peptides were raised as described [6,16].

Detection of IgG antibodies to the IgA-BRs of M4, M22 and M60

Serum IgG antibody levels to the Sap4, Sap22 and Sap60 peptides and also to the non-IgA-binding M4-N and M5-N peptides were measured by ELISA. Immunoblotting was used to test the specificity of the secondary antibody. For detailed descriptions, see the Supplementary data.

Statistics

Statistical evaluation was performed using SPSS version 17.0 (Chicago, IL). Differences in antibody levels were evaluated by the Mann–Whitney
Correlation of antibodies was evaluated using Spearman’s rho test. P-values ≤ 0.05 were considered significant.

Results

Serum antibody response to the IgA-BRs of M4, M22 and M60

For all patients and age-matched controls, serum antibody levels to the IgA-BRs of the M4, M22 and M60 proteins were assayed for each protein separately and the results were combined (Figure 1). Antibody levels for each individual patient or control resulted in three separate observations, representing antibodies to the IgA-BRs of M4, M22 and M60 that were combined for comparison. Thus, results for IgAN patients (n=21) represent a combination of 63 observations, and the results for controls (n=83) represent 249 observations. The non-IgA-binding M5-N peptide, from the M5 protein, served as a control. Antibody levels to the IgA-BRs of the M4, M22 and M60 proteins were significantly higher in patients than in controls (P=0.016). This difference became even more significant when 10 sera of patients with evidence for recent GAS infection were compared with the controls (P=0.008). Patients with evidence for recent GAS infection had higher levels of antibodies to IgA-BR than patients without (P=0.03).

Patient antibody levels to the IgA-BR of M4 correlated significantly with those to M4-N, derived from the most N-terminal region of M4 (r=0.683, P=0.001), suggesting an antibody response to a larger region of M4.

Discussion

In a previous study, we presented evidence that patients with IgAN have mesangial deposits of IgA in complex with a streptococcal M protein fragment that binds IgA-Fc, indicating that infection with an IgA-Fc-binding GAS strain contributes to the pathogenesis of IgAN [6]. The present study provides further support for this notion because patients with IgAN had significantly higher levels of IgG antibodies to streptococcal IgA-BRs. Although IgA-BRs vary in sequence among strains [15], sequence homology may cause cross-reactivity between different IgA-BRs, as described for M4, M22 and M60 [6]. Such cross-reactivity
may have also permitted demonstration of increased antibody levels in patients infected by a GAS serotype not included in our assay. Interestingly, analysis of the M4 system suggested that IgAN patients also have antibodies to the most N-terminal region of M4, suggesting an immunological response to a larger part of or the entire M protein in these patients. Together, these data provide evidence for a general prevalence of infections caused by IgA-binding GAS strains in children with IgAN.

Supplementary data

Supplementary data is available online at http://ndt.oxfordjournals.org.

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Conflict of interest statement. None declared.

References


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