Kidney International, Vol. 38 (1990), pp. 323–331

Polymeric IgA and immune complex concentrations in IgA-related renal disease

COLIN L. JONES, HARLEY R. POWELL, PRISCILLA KINCAID-SMITH, and DON M. ROBERTON

Department of Paediatrics, Royal Children's Hospital and Department of Medicine, Royal Melbourne Hospital, University of Melbourne, Australia

Polymeric IgA and immune complex concentrations in IgA-related renal disease. Polymeric IgA (PIgA) and immune complex concentrations in IgA-related renal disease were measured in cross sectional and longitudinal studies to establish the relationship between these parameters and both mucosal infection and renal dysfunction. These studies were performed in 50 patients with IgA nephropathy (IgAN), 17 patients with Henoch Schönlein purpura nephritis (HSPN), 11 control patients with IgA negative, diffuse mesangial proliferative glomerulonephritis (DMPGN) and 50 healthy controls. Total PIgA ($PIgA_T$) and PIgA subclass concentrations were measured using a secretory component binding enzyme immunoassay and isotype specific immune complex concentrations were measured using conglutinin (K) binding immunoassays. In cross sectional studies patients with IgAN were found to have increased concentrations of $PIgA_T$, $PIgA_1$, $K\text{-}IgA_1$ and $K\text{-}IgA_2$ compared to controls. In the longitudinal studies controls and patients had significant increases in $PIgA_T$ and $PIgA_1$ concentrations during infection. However, in patients with IgAN, the increases were greater, persisted for longer, and $PIgA_2$ concentrations were also increased. $K\text{-}IgA_1$ and $K\text{-}IgA_2$ concentrations increased significantly during episodes of infection in IgAN patients in contrast to controls. Patients with HSPN had results similar to those of IgAN patients. No significant correlation was found between PIgA or K-IgA concentrations, and either serum creatinine concentrations or the degree of hematuria. The results indicate that patients with IgA-related renal disease have abnormal regulation of PIgA and immune complexed IgA, and that these abnormalities are exaggerated during mucosal infection.

Mesangial IgA nephropathy (IgAN) and Henoch-Schönlein nephritis (HSPN) are considered to be IgA immune complex deposition diseases [1–5]. A murine model of IgAN in which hematuria was induced demonstrated that immune complexes containing polymeric IgA (PIgA) were deposited in the glomerular mesangium, but that immune complexes containing the same monomeric antibody and the same antigen were not deposited [6]. Early human studies suggested the mesangial IgA deposits contained PIgA by demonstrating that secretory component bound to the deposits [7–9] and that J-chain was present in the mesangial deposits even in the absence of IgM [8, 10, 11]. Recent studies employing the molecular sizing of immunoglobulin eluted from renal tissue confirmed the presence of PIgA in the mesangial deposits in IgAN [12, 13]. Serum PIgA concentrations are also increased in patients with IgAN and HSPN [14–16]. Sancho, Egido and Gonzalez [17] have described increased concentrations of PIgA-containing immune complexes in patients with IgAN. The presence of increased numbers of PIgA producing cells was also demonstrated in the blood of patients with higher urine red blood cell (RBC) counts [18].

The origin of the mesangial PIgA is unknown. The vast majority of PIgA producing cells are found in mucosal associated lymphoid tissue. In normal humans serum PIgA is produced in response to mucosal and parenteral antigen challenge [19–21]. The IgA response is initially dominated by PIgA production but monomeric IgA becomes dominant later [20, 21]. In patients with IgAN mucosal infections often precipitate exacerbation of hematuria and occasionally renal impairment [22].

The aim of this study was to estimate concentrations of serum PIgA and serum IgA immune complexes in patients with IgAN and HSPN under conditions of mucosal infection and freedom from such infection to determine whether these conditions provided a pathogenic link between the clinical events of mucosal inflammation and disease exacerbation. The study was divided into a cross sectional study involving all patients enrolled, and a longitudinal study of those patients and controls followed over the course of a mucosal infection in a prospective manner.

Methods

These studies were performed following approval from the Medical Ethics Committees, Royal Children's Hospital and Royal Melbourne Hospital, and informed consent was obtained from all patients and controls.

Patients and sample collection

Fifty patients with biopsy proven IgAN (33 males, 17 females; aged 8 to 42, median 23 years), 17 patients with HSPN (8 males, 9 females; aged 6 to 35, median 16 years), 11 control patients with IgA-negative diffuse mesangial proliferative glomerulonephritis (DMPGN, 5 males, 6 females; aged 18 to 35, median 26 years), and 50 healthy controls (26 males, 24 females, aged 16 to 45, median 24 years) were enrolled in the study. Cross sectional studies were performed on samples collected from patients and controls on enrollment in the study, provided that the subjects had no infection at the time. Longitudinal studies during the course of a mucosal (upper respiratory tract

Received for publication July 28, 1989
and in revised form January 22, 1990
Accepted for publication March 7, 1990

in all cases apart from one gastrointestinal) infection were performed prospectively. All patients were reviewed every six to ten weeks, and samples of serum and midstream urine (MSU) were collected. When the patients or controls developed symptoms of an infection they were asked to notify the study immediately. They were examined that day, and an infection was confirmed if there was an oral temperature of greater than 38°C, upper respiratory tract mucosal inflammation, or bacteriological (tonsil swab) or virological (post nasal mucus culture) evidence of infection. The first day of infection was defined as the day on which the onset of symptoms could be clearly recalled. The patients had morning fasting serum and MSU samples collected on three occasions during the period of infection: when first seen (days 1 to 3), and then between days four and six, and days seven to ten following the onset of symptoms. The patients were then reviewed 6 to 12 weeks later, and further serum and MSU samples collected. Altogether, 21 patients with IgAN, 11 patients with HSPN, 2 patients with DMPGN and 15 controls were studied during an episode of a mucosal infection.

Measurement of PIgA concentrations

The relative total serum PIgA ($PIgA_T$) and PIgA subclass ($PIgA_1$ and $PIgA_2$) concentrations were estimated using a novel immuno-assay assay [23]. Briefly, purified human secretory component was passively adsorbed to the surface of the immunoplate wells. Duplicate serum samples were adsorbed with Sepharose bound goat antihuman IgM to prevent competition between IgM and PIgA for binding to secretory component, and then the sera were diluted and added to the immunoplate wells. Peroxidase conjugated goat antihuman IgA antisera was added in the third step of the assay, and followed by the addition of orthophenylene diamine substrate. The subclass assays utilized the addition of monoclonal anti-IgA subclass antibodies after the sample incubation step, followed by peroxidase conjugated goat antimouse IgG antisera and then substrate. The assays were quantitated against serial titrations of human IgA_1 and IgA_2 myeloma sera and the results were expressed with respect to the concentrations of these proteins. Control and myeloma reference sera were included on all immunoplates. The interassay coefficients of variation for the $PIgA_T$, $PIgA_1$ and $PIgA_2$ immunoassays were 12, 9 and 12%, respectively.

The nature of the polymeric IgA detected has been previously reported [23]. The proportion of total serum IgA detected was 0.5 to 2.0%, a value similar to others reported using solid phase secretory component binding techniques [24, 25]. Monomeric IgA, heat aggregated monomeric IgA, colostral IgA and salivary IgA did not bind to secretory component or cause interference in the assays.

Conglutinin binding immune complex assays

Conglutinin was isolated using the method of Lachmann and Hobart [26], modified by using pepsin digestion as described by Maire, Barnet and Lambert [27]. Enzyme immunoassays (EIAs) were developed, by modifying the method of Doi et al [28], to measure concentrations of conglutinin binding total IgA ($K\text{-}IgA_T$), $K\text{-}IgA_1$, $K\text{-}IgA_2$, K-IgG and K-IgM. Ninety-six well microtiter plates (Immunoplate 1, Nunc, Kamstrup, Denmark) were coated with conglutinin by incubation of 100 μl of conglutinin (2.5 μg/ml) in 0.06 M carbonate buffer, pH 9.6, for three hours at 37°C and overnight at 4°C. The plates were washed three times between subsequent steps with Veronal buffered saline (0.14 M NaCl, 5 μM sodium diethylbarbiturate, 0.15 mM $CaCl_2$, 1.0 mM $MgCl_2$) containing 0.05% Tween 20 (VBS-Tw). After blocking with 100 μl of VBS-Tw containing 2% human serum albumin for one hour at 37°C, 50 μl of serum samples diluted 1 in 10 in VBS-Tw were incubated, in duplicate, for two hours at 37°C. Either 50 μl of peroxidase conjugated affinity-purified goat antihuman IgA $F(ab)_2$, IgG or IgM antisera (Tago Inc, Burlingame, Vermont, USA), or murine monoclonal antihuman IgA_1 or antihuman IgA_2 antibody (clones 1-155-1 and 14-3-26, respectively, Becton Dickinson Immunocytometry Systems, Mountain View, USA), at the appropriate dilution [23] in VBS-Tw containing 2% bovine serum albumin (VBS-dil) were incubated at 37°C for two hours. Following use of the monoclonal antibodies, 50 μl of peroxidase conjugated goat antimurine IgG in VBS-dil were added for two hours at 37°C. After washing, 50 μl of 0.06 M citrate phosphate buffer containing orthophenylene diamine and 0.003% H_2O_2 were added for 15 minutes. The reaction was ended by addition of 25 μl of 4 N H_2SO_4 and the absorbance was quantitated at 492 nm using a Titertek Multiscan (Flow Laboratories Ltd., Scotland, UK).

The complement and calcium dependence of the assays were confirmed using the method described by Casali et al [29]. Each assay was quantitated in relative units [30] with respect to the duplicate serial titrations of a serum which had been found to have high concentrations of conglutinin binding immunoglobulin (K-Ig). The means of these absorbances were used to construct a curve of absorbance versus dilution of the reference serum and test sample absorbances were read against this curve. A relative unit value of 100 was assigned to a dilution of the reference serum that had a value near the upper end of the sigmoidal reference curve. For the $K\text{-}IgA_T$, $K\text{-}IgA_1$, $K\text{-}IgA_2$, K-IgG and K-IgM assays the intraassay coefficients of variation were 3, 5, 5, 4 and 4%, respectively, and the interassay coefficients of variation were 14, 18, 20, 9 and 9%, respectively.

Microbiological cultures and MSU examinations were performed by the Microbiology Department of the Royal Children's Hospital, Melbourne. Serum creatinine concentrations were estimated by the Biochemistry Department of the Royal Children's Hospital, using a Kodak Ektachem 700 Analyzer.

Statistical analyses

Statistical evaluation was performed on an IBM personal computer, using Minitab (University of Pennsylvania). For comparisons between independent groups, the Chi-square test and the Mann-Whitney U test were performed. For comparison of a parameter's value within a group over a period of time, the Sign test was used. A P value of 0.05 or less was taken as significant. Correlations were sought using the Spearman rank order correlation test.

Results

Cross sectional studies in the absence of infection

No correlation between age and any of the PIgA or K-IgA assay results was found in any of the control or patient groups.

1. Concentrations of PIgA. The concentrations of $PIgA_T$, $PIgA_1$ and $PIgA_2$ when patients and controls were well and free from infection or disease exacerbation are shown in Figure 1,

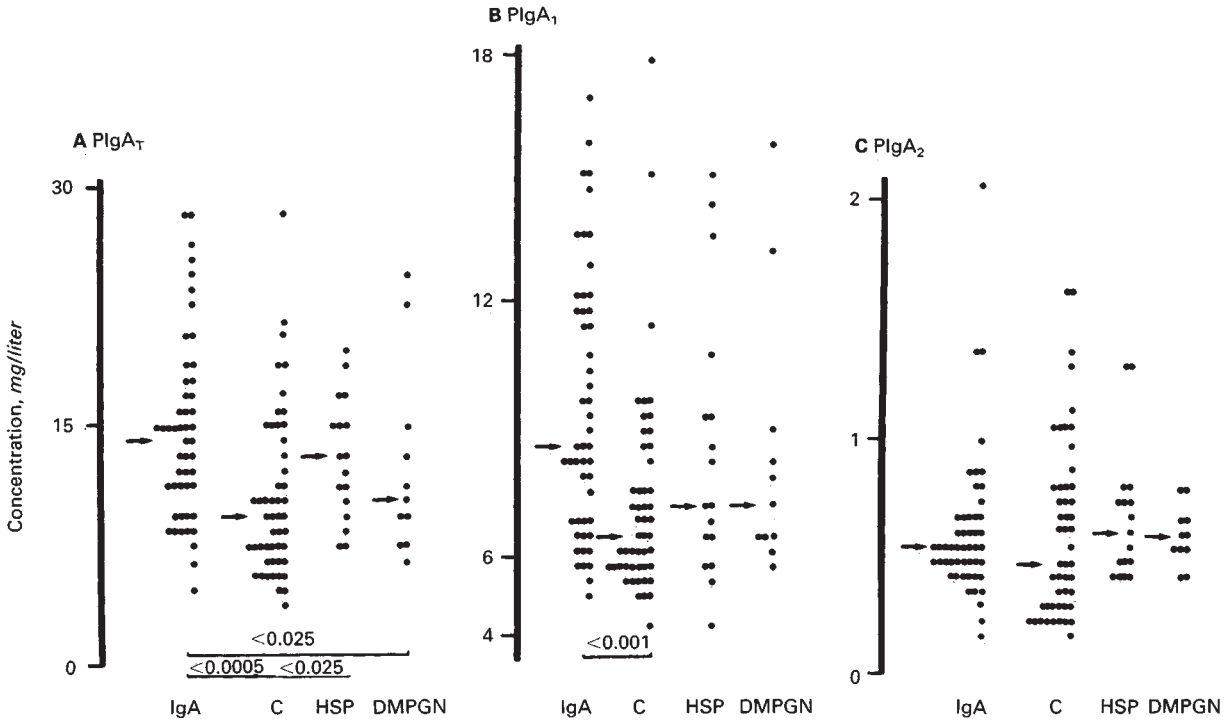

Fig. 1. *Cross sectional study of total PIgA (PIgA$_T$), PIgA$_1$ and PIgA$_2$ concentrations in patients with IgAN (IgA, N = 50), controls (C, N = 50), HSPN (HSP, N = 17), and DMPGN (N = 11) in the absence of infection.* The arrows represent the median values. The results for the groups were compared using the Mann-Whitney U test, and significant differences between the groups are indicated.

together with P values for comparisons between groups where a significant difference was found.

2. Concentrations of conglutinin binding immune complexes. The concentrations of K-IgA$_T$, K-IgA$_1$ and K-IgA$_2$ in the same samples on which PIgA measurements were performed are shown in Figure 2. All test samples had detectable concentrations of IgA and IgA$_1$ binding to conglutinin. However, 46% of controls, 55% of patients with DMPGN, 32% of patients with IgAN and 43% of patients with HSPN had no detectable IgA$_2$ binding to conglutinin.

There were no significant differences between the groups with regard to K-IgG and K-IgM concentrations.

The number of patients having a result greater than the 95th centile of the control group was significant in both the IgAN and HSPN groups for K-IgA$_T$ ($\chi^2 = 18.3$, $P < 0.0001$, and $\chi^2 = 20.0$, $P < 0.0001$, respectively) and K-IgA$_1$ ($\chi^2 = 12.8$, $P < 0.0005$, and $\chi^2 = 4.5$, $P < 0.05$, respectively), and in the IgAN group for K-IgA$_2$ ($\chi^2 = 4.6$, $P < 0.05$).

3. Correlation between K-IgA concentrations and PIgA concentrations. The correlations between PIgA$_T$ and K-IgA$_T$ were significant for patients with IgAN and patients with HSPN, although the r values were low. The rank order correlations for PIgA$_T$ and K-IgA$_T$ concentrations were: for IgAN patients $N = 50$, $r_s = 0.34$, $P = 0.01$; for HSPN patients $N = 17$, $r_s = 0.42$, $P < 0.05$; for controls $N = 50$, $r_s = 0.18$, $P > 0.05$; and for DMPGN patients $N = 11$, $r_s = 0.3$, $P > 0.05$. No significant correlation was found between PIgA subclass concentrations and K-IgA subclass concentrations in the other groups.

Studies during the course of upper respiratory tract mucosal infection

The two control patients with DMPGN followed during a mucosal infection had results close to the modal results for the healthy control group and, as the results of the cross sectional data revealed the two groups were similar with regard to these parameters, the results have been included with those of the control group. The infective agents, where identified microbiologically, are listed in Table 1.

The number of infections due to any one specific microbiological cause was small, but no trend was noted for the PIgA or K-IgA immune response to differ with regard to bacterial or viral etiological agents.

1. Concentrations of PIgA within each group during infections. The concentrations of PIgA before infection, at the three sequential periods during infection and after infection are shown in Figure 3. The sequential changes in the PIgA concentrations have been evaluated using the Sign test in Table 2.

Within the control group concentrations of PIgA$_T$ and PIgA$_1$ increased from the preinfection time to the period four to six days following infection and then decreased to the period 6 to 12 weeks following infection.

Within the IgAN group concentrations of PIgA$_T$ and PIgA$_1$ increased from before infection to all periods during infection reaching a maximum found seven to ten days after the onset of infection. Concentrations of PIgA$_2$ increased from before infection to all times during infection, and the median value was highest one to three days after the onset of infection. The

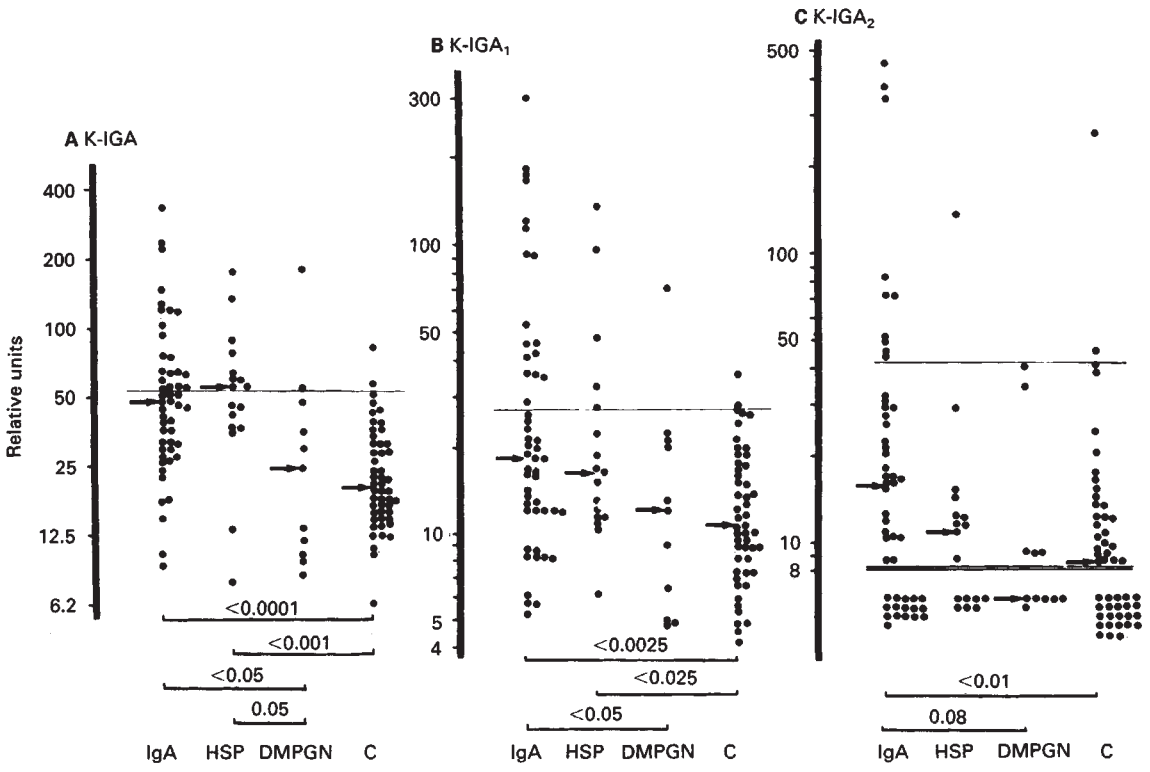

Fig. 2. *Cross sectional study of K-IgA, K-IgA$_1$ and K-IgA$_2$ concentrations in the patient groups in the absence of infection.* The 95th centiles for the results for the control group (C) are indicated by the single horizontal lines. The double horizontal line (for K-IgA$_2$ results) indicates the lower limit of sensitivity of the assay. The results for the groups have been compared using the Mann-Whitney U test (results shown in the figure), and Chi-square test for the number of patient results above the 95th centile of the control group (results are in the text).

Table 1. The results of microbiological investigations of the causes of the mucosal infections in the longitudinal study

Infective organism	Number of isolations in each patient group		
	Control (N = 17)	IgAN (N = 21)	HSPN (N = 11)
Parainfluenza viruses	1	1	1
Enterovirus		1	
Rhinovirus	1	2	3
Influenza A virus	1		
Influenza B virus	1		
Campylobacter jejuni			1
Group A, beta-hemolytic streptococcus	3	1	

qualitative nature of the changes in PIgA$_T$ and PIgA$_1$ concentrations within the HSPN group were similar to those found in patients with IgAN.

2. Comparison of PIgA concentrations between the patient and control groups during infection. Concentrations of PIgA$_T$ and PIgA$_1$ were higher in the patients with IgAN than in the control group at all times before, during and after infection (Mann Whitney U test, $P < 0.05$ for all time periods). The maximum difference was found seven to ten days following onset of infection for PIgA$_T$ concentrations, and at four to six days following onset of infection for PIgA$_1$ concentrations. No significant difference in PIgA$_2$ concentrations was found between patients with IgAN and controls, despite the significant rise in PIgA$_2$ concentrations within the IgAN group.

The IgAN and HSPN groups had similar time related changes in concentrations of PIgA. No significant differences were found at any time between these two groups. Likewise, no significant difference was found between the PIgA$_T$ concentrations for the patients with HSPN and the control group at any time, despite the increased PIgA$_T$ concentrations found in the cross sectional studies in the HSPN group (where there were greater control and HSPN patient numbers). However, a significant difference in PIgA$_1$ concentrations was found in the periods four to six days, seven to ten days and 6 to 12 weeks following onset of infection in the HSPN group compared to the control group ($P < 0.05$ for each case).

3. Concentrations of K-Ig within each group during infections. The data for each assay are displayed in Figure 4. The significance of the K-IgA concentration changes has been tabulated (Table 3).

Conglutinin binding immunoglobulin concentrations remained fairly constant within the control group, although a significant decrease in K-IgA$_T$ concentrations was found between 7 and 10 days following onset of infection and 6 to 12 weeks after infection.

Within the IgAN group K-IgA$_T$, K-IgA$_1$ and K-IgA$_2$ concentrations increased to highest median concentrations one to three and four to six days following the onset of symptoms. K-IgG concentrations increased from prior to infection to the time four to six days following the onset of infection ($P < 0.05$), and

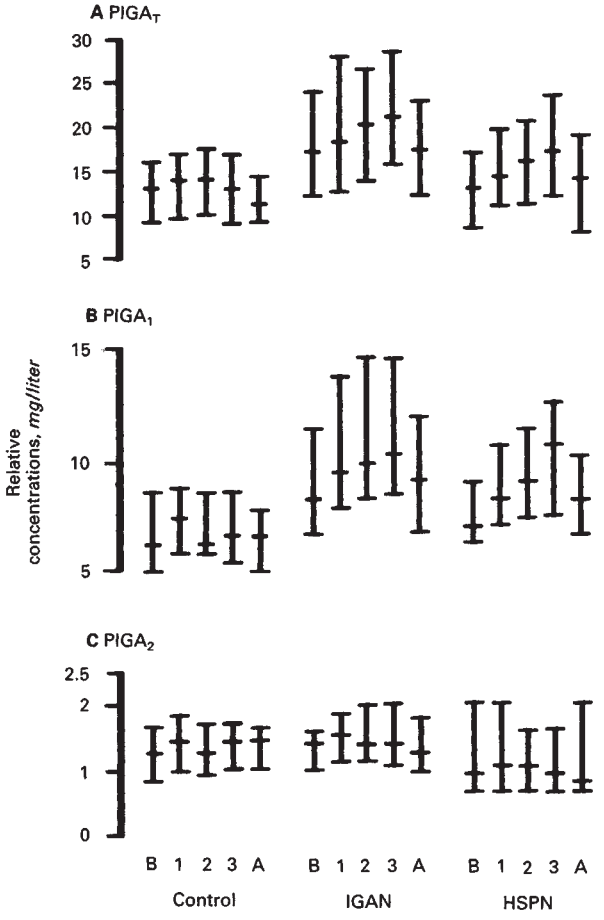

Fig. 3. *Longitudinal study of PIgA$_T$ (PIgA$_T$), PIgA$_1$ and PIgA$_2$ concentrations during a mucosal infection.* The times of sampling are indicated by B (6–12 weeks before infection); 1, 2, 3 (1–3 days, 4–6 days, and 7–10 days following the onset of infection, respectively), and A (6–12 weeks after the onset of infection). The 25th, median, and 50th percentile values are indicated by the lower, middle and upper horizontal bars, respectively, at each time interval. The groups are controls (N = 17), patients with IgAN (N = 21) and patients with HSPN (N = 11).

decreased from four to six days following onset of infection to the periods seven to ten days following onset of infection and six weeks following infection ($P < 0.05$ for each case).

The K-IgA concentrations within the HSPN group were maximal seven to ten days following the onset of infection. K-IgM concentrations decreased between four and six days following onset of infection and six weeks post-infection ($P < 0.05$).

4. Comparison of K-IgA changes between patient and control groups during infection. A significantly lower concentration of K-IgA was found in the control group compared to that found in the IgAN and HSPN groups at all times (Mann Whitney U test, $P < 0.01$ for K-IgA$_T$, and $P < 0.05$ for K-IgA$_1$ and K-IgA$_2$ at each time).

The changes in K-IgA$_T$, K-IgA$_1$ and K-IgA$_2$ concentrations were significantly greater for the periods one to three and four to six days following the onset of infection in patients with IgAN compared to those of the control group ($P < 0.05$ in each case). The changes in K-IgA$_T$ concentrations were also significantly greater in patients with IgAN than in patients with HSPN ($P = < 0.01$) for the time one to three days following the onset of infection, but not later. This reflects the slower rise in K-IgA$_T$ concentrations seen in the HSPN group (Fig. 4).

The changes in K-IgA$_1$ concentrations were significantly greater in HSPN patients than controls for the times one to three, four to six and seven to ten days following the onset of infection ($P < 0.05$ in each case), although similar results were not found for K-IgA$_T$ or K-IgA$_2$ concentrations.

5. Correlation between PIgA, KIgA, MSU RBC counts, and creatinine. In the cross sectional studies no significant correlations were found between either PIgA or K-IgA concentrations, and either MSU RBC counts or serum creatinine concentrations. During mucosal infection every patient with IgAN, HSPN and DMPGN had an increase in MSU RBC counts and all had increases in serum K-IgA and serum PIgA concentrations. However, the correlation between MSU RBC counts and either PIgA or KIgA was significant in only a few patients. Of the 21 patients with IgAN patients followed during infection, two had a significant correlation between PIgA$_T$ and MSU RBC counts, and five had a correlation between KIgA$_T$ and MSU RBC counts. For the 11 HSPN patients the correlations were both significant in three patients. MSU RBC counts were highest at one to three days after the onset of symptoms in all except four patients with IgAN and four patients with HSPN patients.

Discussion

The principal findings of this study were that patients with IgA-related renal disease had abnormally increased serum concentrations of polymeric IgA$_1$ and conglutinin binding IgA$_1$ and IgA$_2$. These concentrations, as well as polymeric IgA$_2$ concentrations, were abnormally increased during mucosal infection. The magnitudes of the IgA$_1$ concentration changes were greater than those of the IgA$_2$ concentration changes. These findings provide information on the possible link between the clinical association of disease exacerbation associated with infection and the immunohistological finding of mesangial polymeric IgA deposition in these diseases [1, 7–13, 22], as discussed below. Of course, the findings do not necessarily imply that a mucosal associated site is the source of the polymeric IgA or that monomeric IgA is not important in the pathogenesis of these diseases.

The cross sectional study findings of increased concentrations of PIgA in patients with IgA-related renal diseases confirm the findings of earlier studies [14–16], and the findings of increased concentrations of K-IgA confirm the findings of increased IgA immune complex concentrations where conglutinin was used [2, 3] or whether other methodologies were employed [1, 4, 5]. The correlation between PIgA$_T$ and K-IgA$_T$ suggest that IgA immune complexes in IgAN and HSPN patients contain significant amounts of PIgA, as found by Sancho et al [17]. These correlations were not found in controls, and this indicates a qualitative difference in the nature of K-IgA in patients with IgAN and HSPN compared to controls and patients with DMPGN.

In the longitudinal studies performed of febrile mucosal infections the median concentrations of PIgA in the control group were highest one to three days following the onset of symptoms of infection. In the HSPN and IgAN patient groups

Table 2. Results of the comparison of PIgA concentrations from earlier time periods before or during infection, to later periods during or after infections using the Sign test

Earlier time of comparison	PIgA assay	Later time of comparison: Controls (N = 27) Timing[a] 1–3	4–6	7–10	After	IgAN group (N = 21) Timing[a] 1–3	4–6	7–10	After	HSPN group (N = 11) Timing[a] 1–3	4–6	7–10	After
Before infection	$PIgA_T$	NS	0.05	NS	NS	0.01	0.01	0.01	NS	0.05	0.05	0.05	NS
	$PIgA_1$	0.01	0.05	NS	NS	0.01	0.01	0.01	NS	0.01	0.01	0.01	NS
	$PIgA_2$	NS	NS	NS	NS	0.05	0.05	0.05	NS	NS	NS	NS	NS
1–3 Days from onset of infection	$PIgA_T$		NS	NS	NS		NS	0.05	NS		NS	0.05	NS
	$PIgA_1$		NS	NS	0.05		0.05	0.01	NS		NS	NS	0.05
	$PIgA_2$		NS	NS	NS		NS	NS	0.05		NS	NS	NS
4–6 Days from onset of infection	$PIgA_T$			NS	0.05			0.01	0.01			NS	0.05
	$PIgA_1$			NS	0.05			NS	0.01			NS	0.05
	$PIgA_2$			NS	NS			NS	0.05			NS	NS
7–10 Days from onset of infection	$PIgA_T$				NS				0.01				0.05
	$PIgA_1$				NS				0.01				0.05
	$PIgA_2$				NS				0.05				NS

Results are reported at the 0.05 and 0.01 level of confidence. NS, the result was not significant.
[a] Timing refers to the time at which tests were performed: 1–3, 4–6, or 7–10 days following the onset of infection or 6–12 weeks after infection.

PIgA and PIgA subclass concentrations had started to increase one to three days following the onset of symptoms. The assays used in these studies measure nonspecific polyclonal PIgA [23], and there is no comparable data in the literature. Studies of specific polymeric polyclonal antibodies have shown that following influenza infection [19], parenteral tetanus toxoid administration [20] or Campylobacter jejuni infection [21], specific PIgA production was maximal one to two weeks after symptoms began or after immunization. The short time interval to maximal PIgA concentrations noted in the control group in this study may have occurred for several reasons. A latent period of asymptomatic infection before illness notification would result in the reported day of onset of symptoms (taken as day 1) being later than the day of antigen exposure, whereas, in the above studies, the time of antigen administration was known. Also, there may be a polyclonal activation of non-specific PIgA production induced by nonspecific mediators of inflammation. Thus, the early changes in PIgA concentrations may not be due to the production of specific PIgA directed toward the infective agents. In the control group there are no later changes (that is, at the times four to six and seven to ten days following onset of infection) in PIgA concentrations suggestive of an increase in specific PIgA antibody production. This is expected as PIgA comprises 10 to 20% of total serum IgA [14–16], and the response to any specific antigen is likely to be small in comparison with the total amount of IgA present.

In contrast to controls, the $PIgA_2$ concentrations in patients with IgAN and HSPN started to increase by one to three days following the onset of symptoms and PIgA concentrations were maximal at that time, although concentrations of $PIgA_T$ and $PIgA_1$ were maximal for at least seven to ten days, and possibly longer, after the onset of infection. This time interval would be consistent with the production of specific $PIgA_T$ and $PIgA_1$. Defective clearance of PIgA could be responsible for the increase in PIgA concentrations as the half-life of PIgA is three days [31]. However, the clinical setting of an acute infection, where increased antibody production is expected, makes this less likely than increased PIgA production.

The time course of K-IgA concentrations in patients with HSPN have been followed during the course of mucosal infection by Kaufmann et al [32], and Coppo et al [33] followed

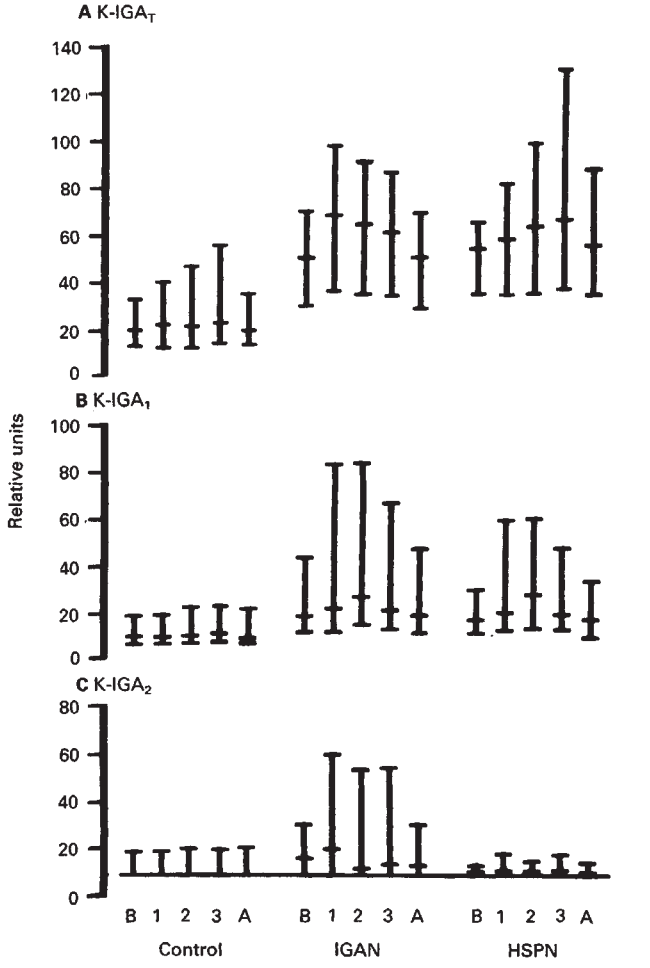

Fig. 4. *Longitudinal study of total K-IgA$_T$ (K-IgA), K-IgA$_1$, and K-IgA$_2$ concentrations during a mucosal infection.* The time intervals and groups are as described for Figure 3. The horizontal line on the K-IgA$_2$ panel represents the sensitivity of that assay.

K-IgA concentrations in patients with IgAN and HSPN during periods of disease activity and inactivity. Both groups found K-IgA concentrations to be increased at times of infection or

Table 3. Results of the comparison of K-IgA concentrations from earlier time periods before or during infection to later periods during or after infections using the Sign test

Earlier time of comparison	K-IgA assay	Later time of comparison: Controls (N = 17) Timing[a] 1–3	4–6	7–10	After	IgAN group (N = 21) Timing[a] 1–3	4–6	7–10	After	HSPN group (N = 11) Timing[a] 1–3	4–6	7–10	After
Before infection	K-IgA$_T$	NS	NS	NS	NS	0.01	0.01	0.01	NS	NS	NS	0.05	NS
	K-IgA$_1$	NS	NS	NS	NS	0.01	0.01	0.01	NS	NS	0.05	0.05	NS
	K-IgA$_2$	NS	NS	NS	NS	0.01	0.05	NS	NS	0.05	NS	NS	NS
1–3 Days from onset of infection	K-IgA$_T$		NS	NS	NS		NS	NS	0.01		NS	NS	0.05
	K-IgA$_1$		NS	NS	NS		NS	NS	0.01		NS	NS	0.05
	K-IgA$_2$		NS	NS	NS		NS	NS	0.05		NS	NS	0.05
4–6 Days from onset of infection	K-IgA$_T$			NS	NS			NS	0.01			NS	0.05
	K-IgA$_1$			NS	NS			0.01	0.01			NS	0.01
	K-IgA$_2$			NS	NS			NS	NS			NS	NS
7–10 Days from onset of infection	K-IgA$_T$				0.05				0.01				0.01
	K-IgA$_1$				NS				0.01				NS
	K-IgA$_2$				NS				NS				NS

Results have been reported at the 0.05 and 0.01 level of confidence. NS, results were not significant.
[a] Timing refers to the time at which tests were performed: 1–3, 4–6, or 7–10 days following the onset of infection or 6–12 weeks after infection.

increased disease activity as found in this study. The cause of the slower increase in K-IgA$_T$ concentration in the HSPN group is unclear.

In the control group K-IgA$_T$ concentrations increased during infection in some patients, as reflected by the increase in the 75th percentile value (Fig. 4A) and the significant decrease in the control concentrations between seven and ten days and 6 to 12 weeks following infection. Thus, it is possible that the differences found in patients with IgA-related renal disease are a quantitative exaggeration of normal changes rather than a qualitatively different immune response.

The lack of a close correlation between PIgA and quantitated hematuria and between K-IgA and hematuria does not imply that glomerular damage is not associated with these two parameters. Indeed, over the course of mucosal infection all patients had increased hematuria and increased concentrations of PIgA and K-IgA. MSU RBC counts reflect the concentration of urine as well as the amount of hematuria. K-IgA represents only a portion of immune complexed IgA [32], and it is unlikely that a direct correlation exists between the formation of immune complexes, glomerular deposition of such complexes, inflammatory mediated defects in glomerular capillaries and red blood cell loss proportional to the degree of glomerular damage. However, the lack of a close correlation between these immune parameters and hematuria is evidence that PIgA and K-IgA are not the direct effectors of glomerular injury causing increased hematuria in the setting of mucosal infection.

This study adds to a large literature circumstantially supporting muscosal immune involvement in these diseases. The vast majority of episodes of macroscopic hematuria in these diseases occur in association with upper respiratory or gastrointestinal infections, and the specific etiological agents include all those listed in Table 1 [1, 22, 34–37]. IgAN has been associated with celiac disease [38] and dermatitis herpetiformis [39], and a gluten free diet has been reported to result in decreased IgA immune complex concentrations and disease activity [40]. Increases in the ratio of IgA to IgG producing cells in tonsillar tissue have been found [41, 42] and increased salivary and breast milk IgA concentrations have been reported [43–45]. Antigens usually associated with mucosal sites (food protein and viral antigen) have been found in the glomerular mesangium by some investigators [46–48], but the problem of non-specific immunological staining has cast doubt on these reports [49]. An animal model of IgAN induced by oral immunization has been developed [50].

The increased concentrations of K-IgA and PIgA indicate altered control of the IgA immune response under conditions of antigen challenge associated with mucosal inflammation, as well as at times of apparent freedom from infection. The longitudinal studies demonstrating that mucosal infection was associated with increased hematuria, increased PIgA and increased K-IgA concentrations are evidence that the mucosal immune system may be involved in the causation of these immune abnormalities. The mucosal immune system could be the source of the PIgA and K-IgA$_1$; it could be the source of a factor stimulating the systemic production of PIgA and K-IgA, or perhaps a source of a factor inhibiting PIgA and K-IgA clearance.

Alternatively, disordered kinetics of the IgA immune response may be present in these diseases. Following experimentally induced influenza A infection [19] or acute Campylobacter jejuni enteritis [21], the IgA response is initially dominated by polymeric IgA production, but later monomeric IgA production becomes dominant. Similar changes in the predominant molecular form of IgA have been described following parenteral treatment with rabbit anti-thymocyte globulin [51] or parenteral tetanus toxoid immunization [20]. If these kinetics represent a generalized phenomenon in the IgA immune response, it could be speculated that an abnormality in the switch of PIgA$_1$ production to monomeric IgA$_1$ production results in prolonged PIgA$_1$ production and thus gives rise to the abnormal magnitude and duration of the PIgA response reported here.

IgAN and HSPN are diseases that are different clinically, but both share a tendency for increased hematuria, often macroscopic in degree, to accompanying mucosal infection. In this study, changes in the concentrations and in the time course of changes in concentrations of PIgA and IgA immune complexes were found in association with mucosal infection. The site of origin of these IgA abnormalities is unknown, but the involve-

ment of mucosal factors in the aberrant IgA immunoregulation appears likely.

Acknowledgments

Dr. C. Jones was supported by a National Health and Medical Research Council (Australia) scholarship. Serum creatinine concentrations were performed by the Department of Biochemistry, Royal Children's Hospital, Melbourne.

Reprint requests to Dr. Colin Jones, Department of Nephrology, Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8.

References

1. Woodroffe AJ, Gormly AA, McKenzie PE, Wootton AM, Thompson AJ, Seymour AE, Clarkson AR: Immunologic studies in IgA nephropathy. *Kidney Int* 18:366–374, 1980
2. Coppo R, Basolo B, Martina G, Roccinoc DE, Marchi M, Giacchino F, Mazzucco G, Messina M, Piccoli G: Circulating immune complexes containing IgA, IgG and IgM in patients with primary IgA nephropathy and with Henoch-Schönlein nephritis. Correlation with clinical and histological signs of activity. *Clin Nephrol* 18:230–237, 1982
3. Lesavre PK, Digeon M, Bach JF: Analysis of circulating IgA and detection of immune complexes in primary IgA nephropathy. *Clin Exp Immunol* 48:61–69, 1982
4. Hall RR, Lawley TJ, Heck JA, Katz SI: IgA-containing circulating immune complexes in dermatitis herpetiformis, Henoch-Schönlein purpura, systemic lupus erythematosus and other lesions. *Clin Exp Immunol* 40:431–437, 1980
5. Levinsky RJ, Barratt TM: IgA immune complexes in Henoch-Schönlein purpura. *Lancet* i:1100–1103, 1979
6. Rifai A, Small PA, Teague PO, Ayoub EA: Experimental IgA nephropathy. *J Exp Med* 150:1161–1173, 1979
7. Egido J, Sancho J, Mampaso F, Lopez-Trascasa M, Sanchez-Crespo M, Blasco R, Hernando L: A possible common pathogenesis of the mesangial IgA glomerulonephritis in patients with Berger's disease and Schönlein-Henoch Syndrome. *Proc Eur Dial Transplant Assoc* 17:660–666, 1980
8. Bene MC, Faure G, Duheille J: IgA nephropathy: Characterization of the polymeric nature of mesangial deposits by in vitro binding of free secretory component. *Clin Exp Immunol* 47:527–532, 1982
9. Rajaraman S, Goldblum RM, Cavallo T: IgA associated glomerulonephritides: A study with monoclonal antibodies. *Clin Immunol Immunopathol* 39:514–522, 1986
10. Donini U, Casanova S, Zini N, Zucchali P: The presence of J chain in mesangial immune deposits of IgA nephropathy. *Proc Eur Dial Transplant Assoc* 19:655–662, 1982
11. Lomax-Smith JD, Zabrowarny LA, Howarth GJ, Seymour AE, Woodroffe AJ: The immunochemical characterization of mesangial IgA deposits. *Am J Path* 113:359–364, 1983
12. Tomino Y, Sakai H, Miura M, Endoh M, Nomoto Y: Detection of polymeric IgA in glomeruli from patients with IgA nephropathy. *Clin Exp Immunol* 49:419–425, 1982
13. Monteiro RC, Halbwachs-Mecarelli L, Roque-Barreira MC, Noel LH, Berger J, Lesavre P: Charge and size of mesangial IgA in IgA nephropathy. *Kidney Int* 28:666–671, 1985
14. Lopez-Trascasa M, Egido J, Sancho J, Hernando L: IgA glomerulonephritis (Berger's disease): Evidence of high serum levels of polymeric IgA. *Clin Exp Immunol* 42:247–254, 1980
15. Valentijn RM, Radl J, Haaijman JJ, Vermeer BJ, Weening JJ, Kaufmann RH, Daha MR, Van Es LA: Circulating and mesangial secretory component-binding IgA in primary IgA nephropathy. *Kidney Int* 26:760–766, 1984
16. Newkirk MM, Klein MH, Katz A, Fisher MM, Underdown BJ: Estimation of polymeric IgA in human serum: An assay based on binding of radiolabelled human secretory component with applications in the study of IgA nephropathy, IgA monoclonal gammopathy and liver disease. *J Immunol* 130:1176–1181, 1983
17. Sancho J, Egido J, Gonzalez E: A simple method for determining polymeric IgA immune complexes. *J Immunol Meth* 60:305–317, 1983
18. Lozano L, Garcia-Hoyo R, Egido J: IgA nephropathy: Association of a history and macroscopic haematuria episodes with increased production of polymeric IgA. *Nephron* 45:98–103, 1987
19. Brown TA, Murphy BR, Radl J, Haaijman JJ, Mestecky J: Subclass distribution and molecular form and immunoglobulin A haemaglutinin antibodies in sera and nasal secretions after experimental secondary infection with influenza A virus in humans. *J Clin Microbiol* 22:259–264, 1985
20. Mascart-Lemone F, Duchateau J, Conley ME, Delacroix DL: A polymeric IgA response in serum can be produced by parenteral immunization. *Immunology* 61:409–414, 1987
21. Mascart-Lemone F, Duchateau JR, Oosterom J, Butzler J-P, Delacroix DL: Kinetics of anti-campylobacter jejuni monomeric and polymeric immunoglobulin A1 and A2 responses in serum during acute enteritis. *J Clin Microbiol* 25:1253–1257, 1987
22. Kincaid-Smith P, Nicholls K: Mesangial IgA nephropathy. *Am J Kid Dis* 111:90–102, 1983
23. Jones C, Mermelstein N, Kincaid-Smith P, Powell H, Roberton D: Quantitation of human serum polymeric IgA, IgA_1 and IgA_2 immunoglobulin by enzyme immunoassay. *Clin Exp Immunol* 72:344–349, 1988
24. Radl J, Swart ACW, Mestecky J: The nature of the polymeric serum IgA in man. *Proc Soc Exp Biol Med* 150:482–484, 1975
25. van der Wall Bake AWL, Daha MR, Haaijman JJ, Radl J, van der Ark, van Es LA: Elevated production of polymeric and monomeric IgA by the bone marrow in IgA nephropathy. *Kidney Int* 35:1400–1404, 1989
26. Lachmann PJ, Hobart MJ: Complement technology, in *Handbook of Experimental Immunology*, edited by Weir DM, Oxford, Blackwell Scientific, 1978, pp. 5A.1–5A.23
27. Maire MA, Barnet M, Lambert PH: Purification of bovine conglutinin using pepsin digestion. *Molec Immunol* 18:85–89, 1980
28. Doi T, Kanatsu K, Sekita K, Yoskida H, Nagai H, Hamashima Y: Circulating immune complexes of IgG, IgA and IgM classes in various glomerular diseases. *Nephron* 32:335–341, 1982
29. Casali P, Bossus A, Carpentier NA, Lambert PH: Solid-phase enzyme immunoassay or radio-immunoassay for the detection of immune complexes based on their recognition by conglutinin: Conglutinin-binding test. *Clin Exp Immunol* 29:342–354, 1977
30. Bishop RF, Cipriani E, Lund JS, Barnes GL, Hosking CS: Estimation of rotavirus immunoglobulin G antibodies in human serum samples by enzyme linked immuno-sorbent assay: Expression of results as units derived from a standard curve. *J Clin Microbiol* 19:447–452, 1984
31. Delacroix DL, Elkom KB, Geubel AP, Hodgson HF, Dive C, Vaerman JP: Changes in size, subclass, and metabolic properties of serum immunoglobulin A in liver diseases and in other diseases with high serum immunoglobulin A. *J Clin Invest* 71:358–367, 1983
32. Kaufmann RH, Herrman WA, Meyer CJLM, Daha MR, van Es LA: Circulating IgA-immune complexes in Henoch-Schönlein purpura. A longitudinal study of their IgA disease activity and vascular deposition of IgA. *Am J Med* 69:859–866, 1980
33. Coppo R, Basolo B, Piccoli G, Mazzucco G, Bulzomi MR, Roccatello D, De Marchi M, Carbonara AO, Di Belgiojoso GB: IgA_1 and IgA_2 immune complexes in primary IgA nephropathy and Henoch Schönlein nephritis. *Clin Exp Immunol* 67:583–590, 1984
34. Clarkson AR, Seymour AE, Thompson AJ, Haynes WDG, Chan Y-L, Jackson B: IgA nephropathy: A syndrome of uniform morphology, diverse clinical features and uncertain prognosis. *Clin Nephrol* 8:459–471, 1977
35. Imbasciatti E, Colosanti G, de Belgioioso GB, Banfi G, Durante A, Ragni A, Ponticelli C, Minetti L, D'Amico G: Long term follow up of IgA mesangial deposits glomerulonephritis. *Proc Eur Dial Transplant Assoc* 14:472–477, 1977
36. Sissons JGP, Woodrow DF, Curtis JR, Evans DJ, Gower P, Sloper JC, Peters DK: Isolated glomerulonephritis with mesangial IgA deposits. *Brit Med J* 3:611–613, 1975
37. Allen DM, Diamond LK, Howell DA: Anaphylactoid purpura

in children (Schönlein-Henoch Syndrome). *Am J Dis Child* 99:833–854, 1960
38. KATZ A, DYCK RF, BEAR RA: Coliac disease associated with immune complex glomerulonephritis. *Clin Nephrol* 11:39–44, 1979
39. PAPE JF, MELLBYE OJ, OYSTESE B, BRODWALL ER: Glomerulonephritis in dermatitis herpetiformis. A case study. *Acta Med Scand* 203:445–448, 1978
40. COPPO R, BASOLO B, ROLLINO C, ROCCATELLO D, MARTINA G, AMORE A, PICCOLI G: Dietary gluten and primary IgA nephropathy. *N Engl J Med* 315:1167–1168, 1986
41. BENE ML, FAURE G, HURAULT DE LIGNY B, KESSLER M, DUHEILLE J: Immunoglobulin A nephropathy: Quantitative immunohistomorphology of the tonsillar plasma cells evidences an inversion of the immunoglobulin A versus immunoglobulin G secreting cell balance. *J Clin Invest* 71:1342–1347, 1983
42. EGIDO J, BLASCO R, LAZANO L, SANCHO J, GARCIA-HOYO R: Immunological abnormalities in the tonsils of patients with IgA nephropathy: Inversion of the ratio of IgA:IgG bearing lymphocytes and increased polymeric IgA synthesis. *Clin Exp Immunol* 57:101–106, 1984
43. TOMINO Y, ENDOH M, KANESUIGE H, NOMOTO Y, SAKAI H: Increase of IgA in pharyngeal washings from patients with IgA nephropathy. *Am J Med Sci* 286:15–21, 1983
44. YAMABE H, OZAWA K, FUKUSHI K, OHSAWA H, CHIBA N, ONODERA K: Elevated salivary IgA in patients with IgA nephropathy. (letter) *Nephron* 45:176, 1987
45. BENE MC, FAURE GC, DUHEILLE J: Mucosal immunity and IgA nephropathy: Review of experimental models, in *Proceedings on Mucosal Immunity IgA and Polymorphonuclear Neutrophils*, 1985, pp. 170–172, Paris, Foundation Franco-Allemande Suresnes
46. NAGY J, UJ M, SZUES G, TRINN CS, BURGER T: Herpes virus antigens and antibodies in kidney biopsies and sera of IgA glomerulonephritis patients. *Clin Nephrol* 21:259–262, 1984
47. GREGORY MC, HAMMOND ME, BREWER ED: Renal deposition of cytomegalovirus antigen in immunoglobulin-A nephropathy. *Lancet* 1:11–13, 1987
48. RUSSELL MW, MESTECKY J, JULIAN BA, GALLA JH: IgA-associated renal diseases: Antibodies to environmental antigens in sera and deposition of immunoglobulins and antigens in glomeruli. *J Clin Immunol* 6:74–86, 1986
49. WALDO FB, BRITT WJ, TAMARA M, JULIAN BA, MESTECKY J: Non-specific mesangial staining with antibodies against cytomegalovirus in immunoglobulin-A nephropathy. *Lancet* 1(8630):129–131, 1989
50. EMANCIPATOR SN, GALLO GR, LAMM ME: Experimental IgA nephropathy induced by oral immunization. *J Exp Med* 157:572–582, 1983
51. HIEMSTRA PS, BALDWIN WM, VAN DER VOORT EAM, PAUL LC, VAN ES LA, DAHA MR: Polymeric IgA antibody response to rabbit anti-thymoctye globulin in renal transplant recipients. *Transplantation* 45:701–705, 1988