Kidney International, Vol. 48 (1995), pp. 1998–2013

NEPHROLOGY FORUM

Immunogenetic aspects of primary IgA nephropathy

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Case presentations

Patient 1. A 27-year-old man was referred to the Division of Nephrology at the University of Bari because of recurrent episodes of gross hematuria concomitant with febrile upper respiratory tract infections. Several urinalyses had revealed persistent microscopic hematuria. On admission, he presented with an additional episode of gross hematuria accompanied by sore throat and a flu-like illness. Neither dysuria nor oliguria was present. He did not complain of any pain or discomfort in his lumbar region.

Physical examination was remarkable only for pharyngitis and tonsillitis and an oral temperature of 38.7°C. Blood pressure was 110/80 mm Hg. No abnormalities were found in his chest, abdomen, or extremities. Ophthalmologic and audiologic examinations were normal. The patient was treated with an antibiotic (amoxicillin) for 5 days.

Laboratory investigation revealed: glucose, 93 mg/dl; BUN, 21 mg/dl; serum creatinine, 1.1 mg/dl; creatinine clearance, 91 ml/min; sodium, 140 mEq/liter; potassium, 4.4 mEq/liter; chloride, 105 mEq/liter; bicarbonate, 26 mEq/liter; total protein, 7.4 g/dl; albumin, 3.5 g/dl; cholesterol, 170 mg/dl; triglycerides, 135 mg/dl; uric acid, 5.6 mg/dl; AST, 166 units/liter; and bilirubin, 0.3 mg/dl. The 24-hour urinary protein excretion was 590 mg. Microscopic examination of the urinary sediment revealed many erythrocytes and some leukocytes. A urine culture was negative. An intravenous pyelogram was normal. Immunologic studies revealed normal serum levels of IgG, IgM, C4, C3, and factor B; the IgA was 380 mg/dl (normal value, 50–326 mg/dl).

A percutaneous renal biopsy was performed. Light microscopy revealed mesangial proliferation in less than 50% of glomeruli: no glomerular sclerosis or capsular adhesion was found. No tubulointerstitial changes or vascular abnormalities were present. Immunofluorescent study showed only mesangial deposits of IgA and C3.

Three years after the initial presentation, high blood pressure was detected; enalapril, 10 mg twice daily, was prescribed. Now, 9 years after the diagnosis of hypertension, he is active and healthy, with normal blood pressure under pharmacologic control and normal renal function. Proteinuria ranges between 400 and 700 mg/day. Another episode of gross hematuria occurred during this period of observation, but renal function and blood pressure remained normal during the episode.

His father has persistent microscopic hematuria (Fig. 1). His brother has had frequent episodes of gross hematuria; one year ago, at age 28, a renal biopsy performed at another hospital disclosed IgA nephropathy. Light microscopic examination revealed moderate mesangial proliferation, rare adhesions, and small crescents; focal tubular atrophy and moderate sclerosis of vessel walls were present. Immunofluorescent examination showed mesangial deposits of IgA and C3; capillary deposits were found in some glomeruli. The daughter of the patient's brother also has experienced persistent microscopic hematuria.

Patient 2. A 46-year-old man was referred to us soon after the diagnosis of IgA nephropathy had been established by renal biopsy. Microscopic hematuria and mild proteinuria (600–800 mg/day) had persisted for 5 years before the renal biopsy. In addition, he had been slightly hypertensive (150/100 mm Hg). At the time of the biopsy, he was in apparent good health, and physical examination revealed no discernible abnormality, save that his blood pressure was 145/100 mm Hg and ophthalmologic evaluation revealed grade-1 retinopathy; audiologic examination was normal. Laboratory findings were: glucose, 89 mg/dl; BUN, 20 mg/dl; serum creatinine, 1.1 mg/dl; creatinine clearance, 140 ml/min; sodium, 143 mEq/liter; potassium, 4.3 mEq/liter; chloride, 104 mEq/liter; bicarbonate, 25 mEq/liter; calcium, 8.9 mg/dl; phosphate, 3.0 mg/dl; uric acid, 7.7 mg/dl; total protein, 7.0 g/dl; albumin, 3.5 g/dl; cholesterol, 234 mg/dl; triglycerides, 96 mg/dl. The 24-hour urinary protein excretion was 950 mg. Urinalysis revealed numerous erythrocytes but no white cells or fatty casts. Immunologic studies revealed normal serum levels of IgG, IgM, C3, C4, and factor B and an elevated value of IgA (515 mg/dl). Ultrasonography showed two normal kidneys.

Light microscopic evaluation of the biopsy specimen revealed mesangial cell proliferation with focal and segmental lesions; adhesions and small crescents were present in some glomeruli. Twenty percent of the glomeruli were obsolescent. Mild focal interstitial edema and infiltrates were found. Immunofluorescent microscopy revealed moderate IgA and C3 mesangial deposits; similar deposits also were found in the capillary walls of some glomeruli.

Over the following 10 years, his blood pressure has been well controlled by enalapril, 10 mg twice daily. Proteinuria ranges between 450 and 1000 mg/day.

The patient's sister developed hypertension at age 43 (160/110 mm Hg); microscopic hematuria and mild proteinuria (500–1000 mg/day) were noted. A renal biopsy revealed mesangial cell proliferation in less than 50% of glomeruli, rare areas of sclerosis and adhesions, mild tubular atrophy, and interstitial infiltrate. Immunofluorescent microscopy revealed mild mesangial and parietal deposits of IgA, IgM, and C3. The patient's daughter was found to have intermittent microscopic hematuria (Fig. 1).

No members of either of these two patients' families had deafness or ocular defects.

Discussion

DR. F. PAOLO SCHENA (*Chairman, Institute of Nephrology, and Professor of Nephrology, University of Bari, Polyclinic, Bari, Italy*): These case reports describe two families, each of which had two

The Nephrology Forum is funded in part by grants from Amgen, Incorporated; Merck & Co., Incorporated; Marion Merrell Dow, Incorporated; Dialysis Clinic, Incorporated; and R & D Laboratories.

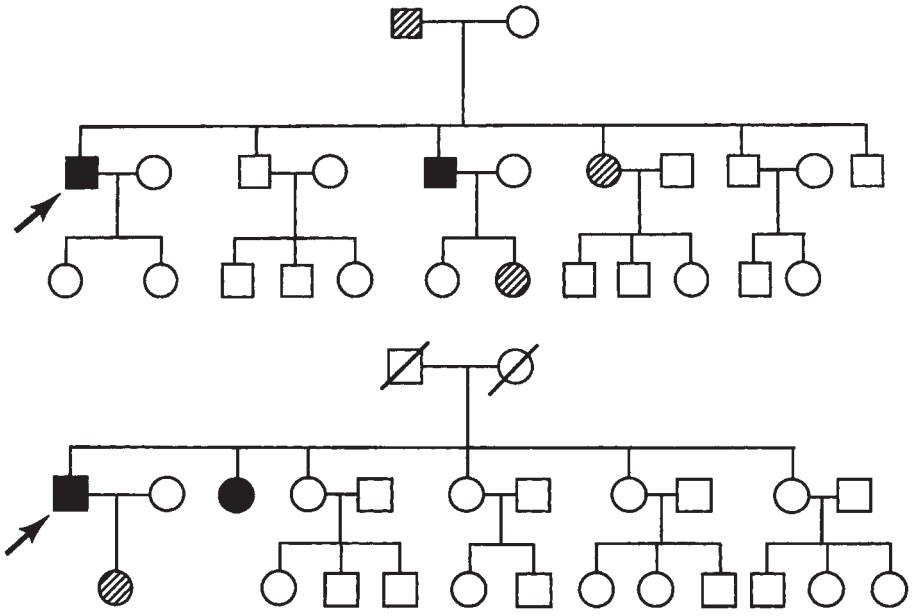

Fig. 1. *Pedigrees of 2 families with pIgAN.* Upper panel: family from Patient 1. Lower panel: family from Patient 2. Black symbols indicate affected individuals; open symbols indicate unaffected individuals; hatched symbols indicate individuals with persistent microhematuria. A line through the symbol indicates deceased members. Arrows indicate probands.

siblings with clinical and renal biopsy-proven primary IgA nephropathy (pIgAN), or Berger's disease, and other members with persistent microhematuria. Primary IgA nephropathy is characterized by persistent hematuria and mesangial deposits of IgA on renal biopsy in the absence of any recognizable systemic disease (for example, lupus erythematosus, Henoch-Schönlein purpura, cryoglobulinemia) or liver disease [1]. Worldwide, pIgAN is the most frequent cause of glomerulonephritis among patients who undergo renal biopsy. For example, in Asia pIgAN accounts for 29.2%; in Australia, 12%; in Europe, 10.7%; and in North America, 5% [2]. The differences in the prevalence of primary IgA nephropathy in different geographic regions of the world might be due to differences in renal biopsy policies and in the use of urinalysis in health screening.

The concept that the documented prevalence of pIgAN is influenced by the local policy for renal biopsy is supported by data from Scottish nephrologists [3]. Propper et al pointed out that the 4% prevalence of pIgAN, previously reported by Sissons et al [4], was based on renal biopsy data from symptomatic patients. In contrast, Propper and colleagues demonstrated pIgAN in 37% of individuals with asymptomatic hematuria who underwent renal biopsy [5]. Thus, it is misleading to compare the prevalence of pIgAN among various regions of the world unless the indications for renal biopsy are taken into consideration.

Differences in utilization of screening urinalysis also strongly influence the reported prevalence of pIgAN. In fact, in Asia, there are large differences among Japan, Singapore, Korea, and Hong Kong because in 1973 the Japanese School Health Law imposed routine urinalysis screening for all school children [6, 7]. In Singapore, army recruits have a regular medical examination, and individuals with recurrent micro- or macrohematuria undergo renal biopsy [8]. In conjunction with the frequent use of renal biopsy in subjects with recurrent microscopic, as well as macroscopic, hematuria, such screening likely contributes to the observed high prevalence of pIgAN in Asia.

In Italy, the Italian Immunopathology Group, which collected data from 45 nephrology centers for the Renal Biopsy National Registry, found major differences in the prevalence of pIgAN; rates ranged between 10% and 40% of all renal biopsies performed in one year. The mean value of 22.6% in 1987 [9] was similar to that observed in 1991 (19.4%) among 2036 renal biopsies examined [10]. In our Division, the percentage of pIgAN in the renal biopsies in the last 4 years ranged between 20% and 28.6%. Interestingly, using the classification scheme proposed by Lee et al [11] and comparing renal biopsy reports of 1990 and 1993, we found an increased frequency of patients with mild renal lesions (G1 and G2), from 33% to 61%, a slight increase of patients with moderate renal damage (G3), from 16% to 24%, and a reduced percentage of patients with severe renal lesions, from 50% to 15%. These data presumably fit with the progressive liberalization of our renal biopsy policy during the last 4 years.

The weighted mean length of followup (2–5 years) obtained from different studies [2] after the performance of renal biopsy showed decreased renal function in 23% of the patients, but the percentage of patients with renal dysfunction rose to as high as 50% after 20 years [11]. Because pIgAN appears mainly in patients between 10 and 30 years of age and is a slowly progressive glomerulonephritis with a high prevalence of chronic renal insufficiency and/or end-stage renal disease, this disease clearly has important social and public health implications. Due to its high prevalence, I believe that a program of routine urinalysis screening in schools and workplaces, followed by an appropriate renal biopsy policy, should be adopted.

The clinical features of pIgAN have been well described by many investigators [2, 12]. Microhematuria, typically the first symptom of the disease, occurs more frequently in Asia (67.4%) than in Europe (39.5%), North America (38.1%), and Australia (30.2%). Macroscopic hematuria is more frequent in North American, Australian, and European patients with pIgAN (56%, 46.5%, and 39.7%, respectively) than in Asian patients (11.5%). Only a small percentage of patients present with mild proteinuria (13%), nephrotic syndrome (2.8% to 6.4%), and/or arterial

Table 1. Immunogenetic associations on the sixth chromosome in patients with pIgAN from several geographic regions

Reference No.	Country	No. of patients	No. of controls	Antigen association	Frequency in Patients (%)	Frequency in Controls (%)	*P* values	Relative risk
HLA-A, B, C								
13	Australia	13	—	Bw35	46	—	—	—
14	France	29	591	Bw35	48	19	0.005	3.9
15	France	43	105	Bw35	39	13	0.02	4.2
16	UK	17	210	Cw1	29	4	0.05	10.5
17	USA	17	100	B12	59	20	0.05	5.7
18	Japan	24	139	Bw44	33	9	0.01	4.8
19	Japan	130	472	Bw35	30	15	0.02	2.3
HLA-D								
20	Japan	40	115	DRw6	46	18	0.02	3.8
21	France	37	—	DRw4	43	13	0.01	—
22	France	45	113	DR4	49	19	0.001	3.9
23	UK	46	385	DR1	49	24	0.008	2.5
18	Japan	24	64	DR4	66	29	0.001	4.7
24	Japan	104	135	DR4	60	36	0.02	2.6
25	France	58	—	DR4	41	19	0.01	—
26	Japan	70	100	DR4	58	34	0.03	2.7
19	Japan	130	472	DR4	60	41	0.01	2.1
HLA complement								
27	USA	48	102	C4	15	4	0.04	4.2
28	USA	141	117	C4	12	5	0.05	2.5
29	W. Germany	67	50	Bf	10	0	0.05	5.8

hypertension (7.6% to 15%). In the remainder of this Forum, I plan to focus on the immunogenetic and familial aspects of pIgA nephropathy.

Immunogenetic associations

Studies on the role of genetics in pIgAN began in the mid-1970s, when investigators described a strong association between this disease and the HLA BW35 antigen [13–15]. These reports were followed by contributions from other countries (Table 1), with indications of a strong association between some HLA antigens and pIgAN [16–29]. The discrepancy among these studies might be due to differences in the selection of patients and differences in the genetic makeup of different countries. In fact, the HLA antigen association varied from country to country and, in some countries, from area to area. In addition, it is likely that more than one gene determines susceptibility to the disease; conversely, the effects of these genes could be modified by environmental factors in the population. Of particular interest is the report of a strong association between HLA-DR4 and pIgAN, in which the relative risk ranged between 2.1 and 4.7 [18, 19, 21, 22, 24–26]. Because the DR locus appears to influence various aspects of humoral and cellular immune response—particularly the efficiency of interaction among T-cells, B-cells, and macrophages—the sequence of immunologic processes in pIgAN likely is genetically controlled by the HLA-DR genes. Several findings, such as high serum levels of IgA [mainly in polymeric (p) form], high levels of IgA-bearing cells and an increased in-vitro spontaneous production of IgA by peripheral blood mononuclear cells (PBMC), argue in favor of an abnormal IgA immune response in pIgAN. Several investigators who studied the influence of DR4 antigen on the deterioration of renal function in patients with pIgA nephropathy reported conflicting data [18, 19, 30]. Other investigators have suggested that C4 genes could contribute to susceptibility to the disease [27, 28].

Traditionally, HLA typing is accomplished by serologic or cellular techniques that require the presence of detectable levels of HLA proteins on the lymphocyte surface. The recent application of HLA polymorphism analysis at the genic level has overcome the limitation of traditional typing methods [31]. In fact, some alleles do not contain a unique sequence and must be identified with a combination of probes that detect sequences present in two or more alleles. Three class-II products, DP, DQ, and DR, are crucial for presentation of processed antigen to specific T-cells. Each class-II antigen is a heterodimer consisting of α and β chains, which are encoded by A and B genes, respectively. In the last 4 years, several investigators have studied the gene polymorphism of these class-II products in patients with pIgA nephropathy (Table 2). Moore and colleagues demonstrated that polymorphic variations of the DP region genes were not important in conferring susceptibility to pIgAN or in influencing disease expression [32], but a close association of DQB1 with the disease was found in Caucasoid British patients [33, 34]. Specifically, Li et al reported an increased frequency of the DQw7 (formally called DQ7) in Caucasoid patients with pIgAN (75%) compared with controls (28%) [34]. The same investigators recently described a high frequency of homozygous DQβ3b(DQ7) in Chinese patients with pIgAN [35]. Abe and coworkers observed an increased frequency of DQB (DQw4/8/9) and D-DR4 (DR4) in Japanese patients with pIgAN and Henoch-Schönlein purpura [36]. Rambausek et al performed a similar study in patients with pIgAN but found no increased frequency of DQB-1 (DQw7) [37]. In light of these findings, the putative gene(s) could reside within or adjacent to the DQ and DR subregions. Therefore, a linkage disequilibrium between the DR and DQ loci, in which DQ alleles can be inherited more frequently together with certain DR alleles, could contribute to susceptibility to the disease.

If the disease-causing gene is located close to an HLA molecule-encoding gene, a family study would be the most convenient

Table 2. Genotypic studies on HLA-D loci in patients with IgAN

Reference No.	Yr	Pts	Race	Ethnic group	Methods	HLA		*P* value	rr[a]
						DP	DQ		
33	1990	73	Caucasoid	British	RFLPs		DQβ	0.0001	5.5
34	1991	36	Caucasoid	British	RFLPs + ASO		DQw7	0.001	6.2
32	1992	213	Caucasoid	British Italian Finnish	RFLPs	ns			
37	1992	67	Caucasoid	German	RFLPs		DQα1A	0.005	2.6
35	1994	79	Oriental	Chinese	RFLPs + SSP		DQβ3b	0.01	3.2
							DQA_2 U (CRF)	0.0003	5.4
							$DQ\alpha_2$ (CRF)	0.03	0.2
							$DQ\alpha_2$ (NRF)	0.03	4.1

[a] rr = relative risk; CRF = chronic renal failure; NRF = normal renal function; RFLP = restriction fragment length polymorphism; ASO = allele-specific oligonucleotide typing; SSP = sequence specific primers typing.

and likely the only way to identify the association. Three different approaches can be used to identify an HLA gene marker cosegregating with pIgAN. The first is the lod score analysis, which can individualize the mode of inheritance and penetrance. The second is the "affected-only" analysis, which is used to determine whether nonaffected individuals might obscure potential linkage. This approach is independent of penetrance, and all individuals who are not coded as affected may be recorded as unknown. A linkage software package (LINKAGE) [38] is used for these approaches. The third approach is the "affected-pedigree-member" method, which does not depend on the mode of inheritance of the disease and is used because the results of the first two approaches are subject to inaccuracies in the assumed genetic model. This third method tests for excess sharing of alleles at the marker locus among related affected individuals but does not necessarily trace the segregation of alleles with a disease in families. This method allows one to evaluate allelic identity-by-state among affected individuals. The specific genetics program packages (APMMULT-version 2.0 and SIMMULT) are used for this method [39, 40]. However, for these studies, it is necessary to collect many "multiplex" families, that is, families in which two or more members have biopsy-proven pIgAN.

Family studies

After the first report by de Werra et al in 1973 on the possible presence of relatives with presumed pIgAN (unfortunately these cases were not documented by renal biopsy) in a family of a patient with IgAN [41], other larger familial studies were performed by Egido and colleagues [42] and later by Levy and Lesavre [43]. Affected members of these families were either parent and a child, or two or more siblings. In addition, other relatives frequently had other types of glomerulonephritis, mainly Henoch-Schönlein purpura. Thus, some family members can have mild urinary abnormalities, such as persistent microhematuria and/or proteinuria, whereas others manifest recurrent macrohematuria or chronic renal failure. Of course, renal biopsy is an entirely appropriate diagnostic approach in the latter group, but in the former, histologic evaluation of renal involvement typically is not performed. Two factors militate against genetic studies in family members of patients with IgAN. First, investigators are disinclined to perform renal biopsy on family members presenting with only minimal urinary findings. Although the biopsy is necessary to define the immunohistologic pattern of pIgAN, its invasive nature makes it unjustifiable. Second, the resistance to performing renal biopsy on sporadic patients with occasional episodes of micro- or macrohematuria clearly reduces the possibility of genetic studies.

The true incidence of familial pIgAN is unknown because few renal units systematically examine the urine of family members and perform renal biopsy in those individuals with persistent microhematuria. The more distant relations of patients with pIgAN were studied in the USA and Italy [44, 45]. In the first study, 60% of patients from eastern Kentucky were related to at least one other patient. Epidemiologic investigations did not reveal a common environmental factor; occupation, type of residence, and food differed. In the Italian study, a familial clustering of several cases of chronic glomerulonephritis in patients belonging to three related families was reported. The higher gene frequency (of HLA-Dw 8.1, related to DRB8, DQβ3a, and DQα1b) in the affected and unaffected pedigree members than in Italian controls, suggested that these patients had inherited a susceptibility to develop mesangial glomerulonephritis. Other unknown factors likely act in concert with the basic genetic predisposition.

We had the opportunity to study 269 asymptomatic, first-degree relatives from 48 families of patients with documented pIgAN from three generations [46]. Microhematuria and proteinuria were sought by N-Multistix (Ames, Miles, Elkhart, Indiana) on urine specimens collected at least three times in three months. When the microhematuria persisted, urine sediments were examined under the microscope and an Addis count was performed. Patients with persistent microhematuria underwent renal echotomography. Interestingly, some young family members who had shown a negative urinalysis at the beginning of our study developed microhematuria later during the course of our investigation (2 years). Urinalysis revealed persistent microhematuria in 42 of 269 relatives (15.6%), persistent proteinuria in 8 members (3%), and both abnormalities in 11 relatives (4%) (Fig. 2). Twenty-two families had 2 or more members with urinary abnormalities, and 6 families had one member with persistent microhematuria or proteinuria. Finally, biopsy-proven pIgAN was documented in 4 relatives who had urinary abnormalities. We compared the prevalence of urinary abnormalities among the relatives of affected individuals with that in a large number of young people from the general population (8255 students who ranged in age between 6 and 18 years) and found a significant difference: only 4.3% of

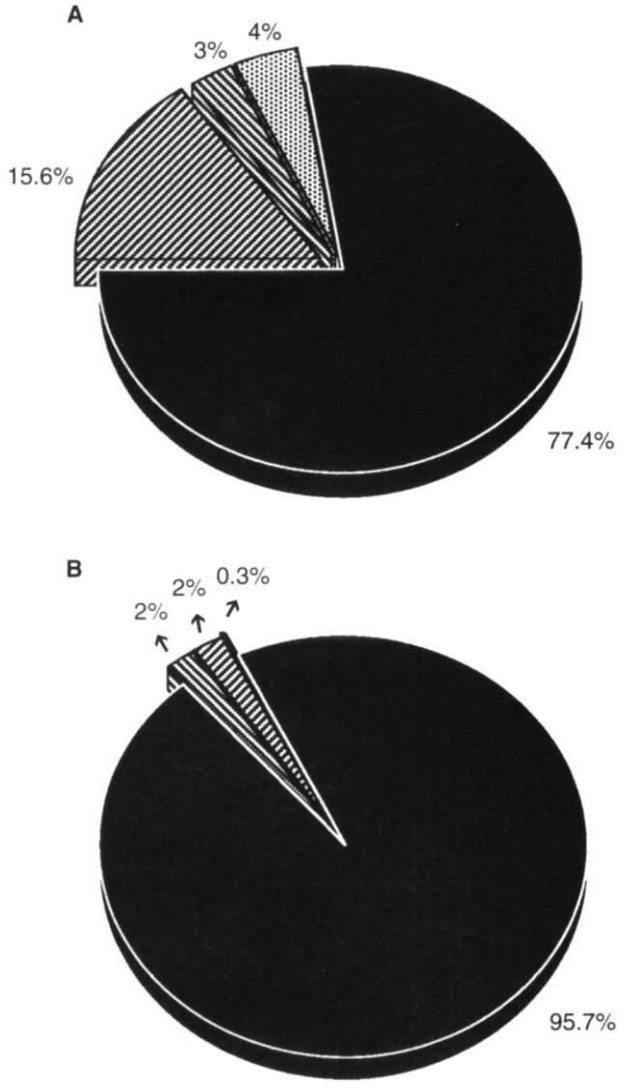

Fig. 2. *Incidence of urinary abnormalities in 269 relatives from 48 families of patients with pIgAN (A) and in 8255 students (B).* Symbols are: (▨) microhematuria; (▧) proteinuria; (□) microhematuria + proteinuria; (■) normal urine.

students had persistent urinary abnormalities (2%, microhematuria; 2%, proteinuria; and 0.3%, microhematuria and proteinuria) (Fig. 2) (Aquilino A et al, unpublished data). In conclusion, our study demonstrated that urinary abnormalities occurred in 22.6% of relatives of patients with pIgAN. Thus, nearly one-quarter of family members might be affected by pIgAN or other mesangial glomerulonephritides.

Phenotypic serum abnormalities in relatives. Several investigators have shown various abnormalities in the immune regulation of the IgA system in patients with pIgAN and their relatives [47–52]. We studied phenotypic serum abnormalities in 54 of 120 first-degree relatives of 11 patients with IgAN who belonged to 9 unrelated pedigrees [53]. Of 54 relatives, 36 (66%) had at least one serum IgA abnormality (high levels of IgA, pIgA, IgA rheumatoid factor, or circulating IgA1/IgM or IgA1/IgG immune complexes), and 15 of 54 relatives (27%) showed more than one abnormality. Moreover, serum IgA abnormalities were present in 9 of 13 family members who had persistent microhematuria (69%).

Table 3. In-vitro IgM and IgA production by peripheral blood mononuclear cells of pIgAN patients and their relatives [54][a]

	Controls (n = 18)	Patients (n = 22)	Relatives	
			With microhem. (n = 12)	Without microhem. (n = 32)
IgM				
Spontaneous prod.	472 ± 106	1012 ± 163[b]	727 ± 175	670 ± 106
Stimulated prod.	1084 ± 183	2557 ± 294[b]	2625 ± 655[b]	2572 ± 437[b]
-Fold increase	2.3 ± 0.7	2.6 ± 0.5	3.2 ± 0.6	3.7 ± 0.6
IgA tot.				
Spontaneous prod.	1262 ± 334	4115 ± 538[b]	2232 ± 482	1850 ± 247
Stimulated prod.	2322 ± 510	9124 ± 835[b]	8366 ± 1836[b]	7774 ± 788[b]
-Fold increase	1.3 ± 0.3	1.8 ± 0.4	3.4 ± 1.0[b]	4.5 ± 0.6[b]
IgA1				
Spontaneous prod.	991 ± 259	3594 ± 479[b]	1743 ± 490	1664 ± 239
Stimulated prod.	2070 ± 508	7294 ± 751[b]	7347 ± 1600[b]	6578 ± 756[b]
-Fold increase	1.7 ± 0.6	1.7 ± 0.4	4.4 ± 1.2[b]	4.6 ± 0.8[b]
IgA2				
Spontaneous prod.	142 ± 45	274 ± 50	175 ± 38	338 ± 122
Stimulated prod.	257 ± 71	465 ± 78	427 ± 104	418 ± 56
-Fold increase	1.2 ± 0.3	0.8 ± 0.1	1.7 ± 0.5	1.0 ± 0.2

[a] Immunoglobulin concentrations are expressed in ng/ml. Values are mean ± SEM. The mean-fold increase for each group is the mean of the -fold increases of the individual samples.
[b] Significant difference ($P < 0.05$, ANOVA) compared to control group.

When age distribution was taken into account, 53% of relatives younger than 18 years had serum IgA abnormalities; the frequency rose to 69% in family members between 19 and 40 years, and to 85% in those between 40 and 60 years. In older relatives (>60 years), 62% had serum IgA abnormalities. The serum levels of pIgA were higher in patients with pIgA nephropathy and in their relatives who either had microhematuria or no urinary abnormalities than in controls. High values of IgM also were found in patients with IgAN and their relatives regardless of the presence of urinary abnormalities. Serum levels of IgG were not significantly increased. These findings confirm the high frequency of serum IgA abnormalities in relatives of patients with pIgAN. However, it is interesting to note that high serum levels of IgM also were present in relatives.

We also investigated the in-vitro immunoglobulin synthesis by peripheral blood mononuclear cells (PBMC) in a relatively small number of relatives (39 subjects); blood samples from children were not available in our studies [53, 54]. High pokeweed mitogen (PWM)-induced cell production of IgA, IgA1, and IgM, pIgA, and IgA rheumatoid factor was found in 64% of relatives from 8 families. The production increased both in relatives with urinary abnormalities and in those with normal urinalyses (Table 3). No difference in basal or stimulated production of IgG and their subclasses was found [54]. Our findings [54], in agreement with those reported by others [55, 56], demonstrate that the increased production of IgA is restricted to the subclass A1. Investigators now agree that IgA1 represents the predominant subclass of immunoglobulins in mesangial deposits of pIgAN and in circulating immune complexes [57, 58]. Increased IgA1-producing plasma

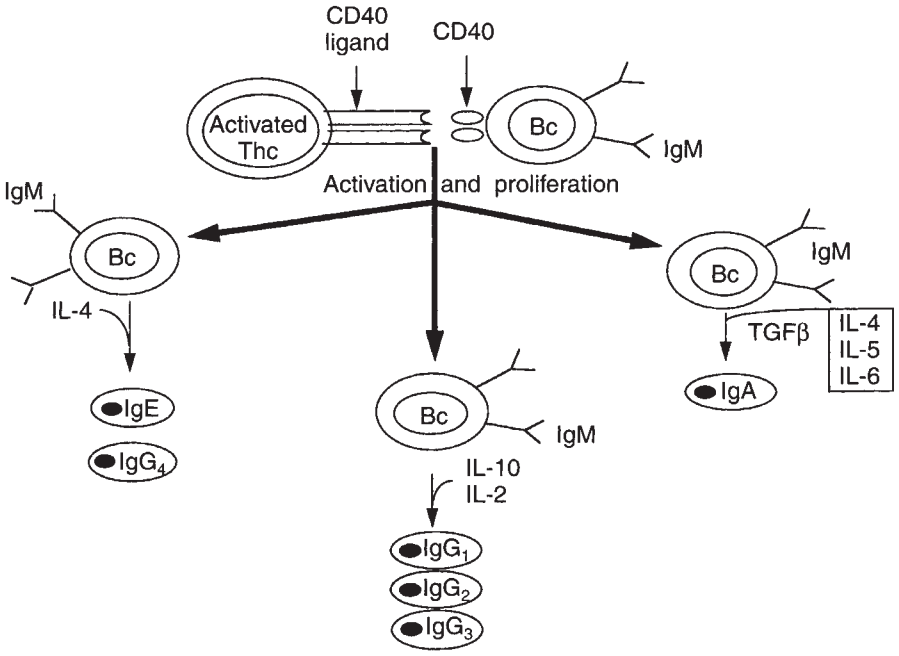

Fig. 3. *Specific cytokines implicated in modulating the Ig class switch process.* Abbreviations: Thc (T-helper cell); Bc (B-cell).

cells have been described in tonsils and bone marrow of patients with pIgAN [59, 60]. A dysregulation of the IgA1, and not the IgA2, system is thus well established as a contributor to some pathogenetic aspects of pIgAN. We also confirmed reports by Hale et al [61] and others [62, 63] concerning high serum levels of IgM and abnormal production of this immunoglobulin by stimulated PBMC in relatives [53].

On the basis of these findings, one can hypothesize that a common genetic substrate affects B-lymphocyte function in patients and their relatives. It is noteworthy that PBMC isolated from relatives showed an increased mitogen-induced synthesis of IgA and IgA1 when compared with controls and even with patients. Asymptomatic and unaffected relatives lack the spontaneous hyperproduction of IgA1 and IgM that specifically characterizes the patients, however. Thus, the reduced suppressor activity claimed to explain basal IgA1 and IgM hyperproduction in patients with pIgAN may be superimposed on a general hyperactivity of specific IgA1 and IgM production in relatives, as revealed by in-vitro stimulation with mitogens.

Cytokine production by PBMC of relatives. Our findings, as well as those of other authors regarding abnormal production of IgA in patients with pIgAN and their relatives, support the view that the imbalanced regulation of immunoglobulin synthesis is not limited to an altered switch from IgM to IgA1 production, but involves a more complicated imbalance in the regulation of IgA synthesis [53, 54, 62–65]. An altered production of cytokines might play an important role within this framework, given recent evidence suggesting that IL-2, IL-4, IL-5, IL-6, IL-10, and TGFβ regulate immunoglobulin isotype production [66–69] (Fig. 3). In addition, other investigators also have reported a role for IL-12 and IL-13 [70, 71].

We studied the spontaneous and mitogen-induced production of some of these cytokines by PBMC from 34 patients with pIgAN and 44 of their first-degree relatives, 10 of whom had persistent microhematuria [72]. Peripheral blood mononuclear cells from patients and relatives with microhematuria showed increased spontaneous production of IL-2, whereas IL-4, IL-6, and IFN-γ production were normal. Phytohemagglutinin stimulation increased production of all the cytokines tested [72]. Interestingly, relatives of pIgAN patients with microhematuria had the same profile of cytokine production as did patients with pIgAN. Conversely, relatives with normal urinalyses did not display any significant difference in cytokine synthesis from normals (Table 4). We [73] and others [74–77] observed an overproduction of cytokines (IL-2 and others) in patients with pIgAN, whereas such hyperproduction of T-cell cytokines was not found in patients with other forms of glomerulonephritis. In addition, we demonstrated that this aberrant cytokine synthesis also was present in patients' relatives with microhematuria [72].

Some of these cytokines play a key role in immunoglobulin class switching and influence the synthesis of IgA and IgE by PBMC from patients with pIgAN [63, 78]. Thus, an abnormally high production of these cytokines could favor an imbalance in the distribution of isotypes in antibody responses in patients and relatives. Such an imbalance, expressed as a loss of mucosal tolerance, might underlie defects in the population of antigen-specific IgA+ cells in the mucosal lamina propria, promoting antigen penetration of the mucosal barrier [79–81].

IgM/IgA switch and cytokine regulation

Immunoglobulin class switch recombination allows B-cells to sequentially express antibodies that have identical specificities but that differ in class and thus in effector function. Isotype switching requires collaboration between antibody-synthesizing B-cells and helper CD4+T cells. B-cells first express antibodies of the IgM and IgD isotypes on the plasma membrane. During the primary immune response, IgM is the predominant isotype expressed, the secondary immune response being characterized by a switch to one of the other immunoglobulin isotypes (IgG/IgA). The end result of isotype switching is the production of antibodies that use

Table 4. In-vitro cytokine production by peripheral blood mononuclear cells of pIgAN patients and their relatives [72][a]

	Controls (n = 34)	IgAN patients[b] (n = 34)	Relatives		
			With microhematuria (n = 10)	Without microhematuria (n = 34)	Other GN (n = 17)
IL-2					
Spontaneous	62 ± 5	121 ± 14[c]	115 ± 22[c]	86 ± 11	94 ± 23
Stimulated	626 ± 55	1274 ± 168[c]	1153 ± 210[c]	730 ± 100	1017 ± 191
IL-4					
Spontaneous	64 ± 4	76 ± 5	70 ± 8	62 ± 4	73 ± 13
Stimulated	211 ± 26	574 ± 98[c]	519 ± 148[c]	310 ± 55	313 ± 69
IL-6					
Spontaneous	1082 ± 181	1727 ± 191	1977 ± 405	1347 ± 282	1996 ± 382
Stimulated	22957 ± 1501	27142 ± 1814	26786 ± 2481	23750 ± 905	25640 ± 2589
IFN-γ					
Spontaneous	51 ± 4	53 ± 5	56 ± 9	53 ± 4	57 ± 8
Stimulated	243 ± 20	331 ± 39[c]	367 ± 69[c]	253 ± 19	271 ± 45

[a] Cytokine concentrations are expressed in pg/ml. Values are mean ± SEM.
[b] IgAN, IgA nephropathy; GN, glomerulonephritis.
[c] Significant difference ($P < 0.05$, ANOVA) compared with control group.

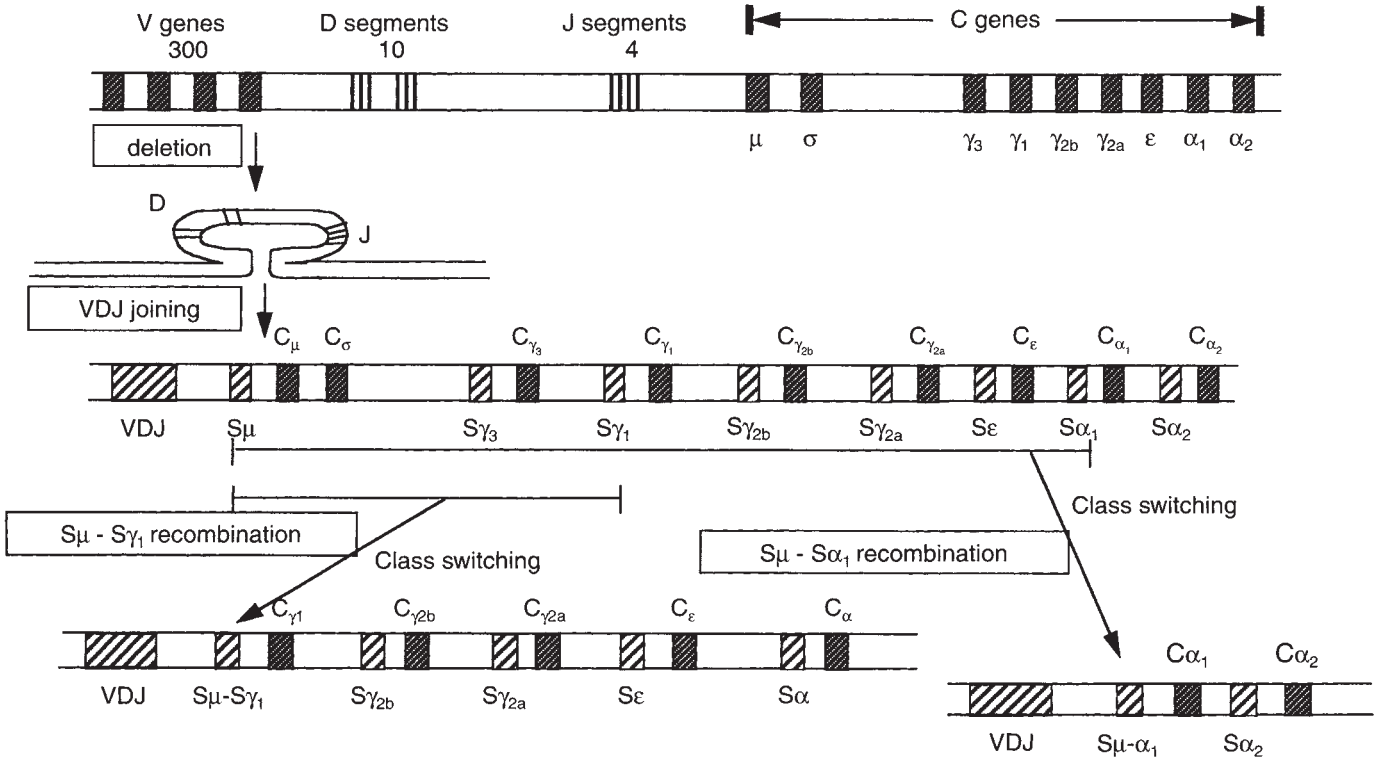

Fig. 4. *Heavy chain gene assembly throughout the deletion mechanism and two successive switches (Sμ-Sγ1 recombination and Sμ-Sα1 recombination).* Abbreviations: V, variable; J, joining; D, diversity; S, switching.

the same V (variable) region and therefore have identical antigen-binding properties, but which use a new C (constant) region. Due to isotype switching, the four IgG subclasses predominate in serum, while IgA is prominent in mucosal secretion. The V, D (diversity), and J (joining) gene segments are juxtaposed in frame and together encode the variable domain of the immunoglobulin heavy chain. The constant domains of the immunoglobulin isotypes are encoded by C genes located 3′ to Cμ on the same chromosome 14. The formation of VDJ gene and isotype switching each involve DNA recombination. VDJ recombination is targeted by highly conserved sequences; switch recombination can occur at multiple sites throughout noncoding S (switching) regions (Fig. 4). DNA rearrangement during class switching of heavy genes occurs via an intramolecular looping out-deletion mechanism that generates a circular DNA excision product.

Three gene families localized on chromosomes 2, 14, and 22, respectively, control the organization and expression of the heavy and kappa and lambda light chains of the immunoglobulins.

Because the polymorphism of the immunoglobulin heavy chain switch region genes is associated with differences in the variable heavy chain region, alterations in the Sμ and Sα gene regions probably influence the immunoglobulin heavy chain switch region. The first report on the switch region sequences in patients with pIgA nephropathy showed differences for the Sμ and Sα1 genotype and phenotypes that control the antigen recognition repertoire [82]. A significant increase in the Sα1 genotype number was observed in English and German patients with pIgAN. These results suggested that genes within the immunoglobulin heavy chain loci may play an important role in the pathogenesis of pIgAN. However, Moore et al, in a similar study performed in pIgAN patients from the UK, Finland, and Italy, did not confirm the significant differences in the genotypic frequencies of Sμ and Sα alleles [83].

Three cytokines (IL-4, IL-5, and TGFβ) play pivotal roles in regulating the IgA switch process. Specifically, in humans, TGFβ appears to be an IgA-specific switch factor; moreover, IL-6 also might be implicated in the switch mechanism. These cytokines induce the production of specific immunoglobulin isotypes by altering the chromatin state and inducing the transcription of the constant heavy (CH) genes to which they direct switching. IL-4 stimulates accumulation of germline ε transcripts, IFN-γ induces accumulation of germline γ2a transcript, and TGFβ induces accumulation of germline α transcripts [67]. The accumulation of germline transcripts appears to be a very early event in isotype switching. The cytokines may target specific switch regions for DNA recombination by regulating their accessibility to a switch recombinase. However, a second signal is necessary for isotype switching. CD40 is required for completing isotype switching to IgE, while IL-5 provides for isotype switching of IgG1, IgE, and IgA.

Recent genetic studies on diseases caused by primary hyperimmunoglobulinemia have shown that the IL-4 gene is a major candidate for IgE responsiveness and atopy [84], the CD40 ligand gene defect is responsible for X-linked hyper-IgM syndrome [85], the deficient local IgA response is found in mice with targeted disruption of the gene encoding IL-6 [86], and impaired immune and acute phase responses occur in the IL-6-deficient mouse [87]. The restoration of the IL-6 gene improved the IgA antibody response. Moreover, the evidence of an impaired IgA switching has been reported in patients with IgA deficiency [88]. These data suggest that investigating the gene expression of TGFβ, IL-4, IL-5, and IL-6 in patients with pIgAN might help to reveal a defect of isotype switching.

Approximately 10% of the DNA in the genome consists of repeats of two nucleotides that are usually approximately 250 base pairs long. These repeats were originally considered non-coding sequences, and they were called satellite DNA. The length of each satellite DNA varies from one person to another; this polymorphism can serve as chromosomal markers [89]. Some short runs of tandemly repeated DNA, designated "hypervariable minisatellites," "variable number of tandem repeats," or "variable tandem repeats," arise from the head-to-tail concatenation of short-sequence motifs 10 to 100 bp long. These arrays, through a mutational process that increases or decreases the number of repeat motifs in a given allele, are frequently unstable and produce highly polymorphic loci that often possess dozens of alleles. Other short runs of tandemly repeated DNA are the "microsatellites" that are sequence motifs 1 to 6 bp long. Consequently, mini- and microsatellites are ideal genetic markers for the investigation of a wide range of genetic variations. Recently, these short runs of tandemly repeated DNA have been observed in cytokine genes such as IL-4 and IL-6 [90, 91]. Polymorphisms for the TGFβ gene, which codifies for the specific cytokine of the IgA switch process, have not yet been reported. By using the DNA satellites, however, it is possible to analyze the linkage of some of their cytokine polymorphic markers to pIgAN in affected families.

Is pIgAN a familial disease?

The occurrence of pIgAN aggregates in some families in which about 25% of first-degree relatives have biopsy-proven IgAN or persistent microscopic hematuria and/or proteinuria and in which over 66% of relatives have immunologic abnormalities of the IgA system [53] (which might depend on altered cytokine production) suggests that pIgAN may be an inherited disease. A genetic background responsible for the observed abnormalities could interact with environmental factors during life and promote development of the disease. An additional argument in favor of pIgAN being an inherited disease is the existence of the large pedigrees in which many pIgAN patients in several generations have been reported in various parts of the world [44, 45, 92]. Arguments against this hypothesis are: (1) genotyping studies have not shown that HLA antigens uniformly cosegregate with pIgAN, and (2) families with several members affected by pIgAN have not evidenced the same Mendelian inheritance pattern.

Many obstacles hinder the evaluation and design of genetic studies of pIgAN. First, the occurrence of multiple pathogenetic mechanisms—such as primary B- or T-cell hyperactivity, primary lymphokine hyperproduction, and others—indicate a probable polygenic disease. Second, environmental factors, such as diet and exogenous antigenic challenge, might play a major role in the pathogenesis. Therefore pIgAN might be caused by the interaction of environmental factors with several genes at different loci, each of them with an additive effect. Finally, despite an increasing awareness of the familial and regional clustering of patients with pIgAN, no true serologic marker is available. The diagnosis therefore requires renal biopsy, an invasive procedure especially hard to justify in apparently healthy subjects whose only abnormality is persistent microhematuria and/or asymptomatic, mild proteinuria.

The two general approaches to uncovering the molecular basis for a polygenic disease are: (1) seeking a "candidate gene" and (2) performing a "blind hunt" with batteries of probes for single-point restriction fragment length polymorphisms or hypervariable regions [93]. Candidate genes for pIgAN are as various as those encoding immunoglobulins, complement proteins, T-cell receptors, cytokines, and some antigens of the major histocompatibility complex. The data in the IgAN literature indicate that this disease, characterized by polymeric IgA1 renal deposits with λ chain, has four fundamental aspects. First, the serum contains both high levels of IgA, mainly λ-IgA1 with anionic charge and in polymeric form, and increased numbers of IgA-bearing B-lymphocytes and activated Tα helper cells (CD4+ and IL-2r+). Second, some "immunologic-rich" tissues such as tonsils and bone marrow produce abnormal amounts of IgA1. Third, cultured peripheral blood lymphocytes produce increased amounts of λ-IgA1 and cytokines (IL-2, IL-4, IL-5, IL-6, TGFβ, and IFN-γ). Finally, recent studies of CD4+ cells from patients with IgAN have shown

augmented mRNA expression of those cytokines (IL-4 and TGFβ) that are devoted to regulating the IgA switch process [94].

On the basis of these findings, I think it would be worthwhile to study the genetic variations of the factors that regulate the immunoglobulin class switch process in IgAN patients and their relatives. These studies should be done in large, multiple-generation families using the "sib-pair" analysis, which consists of comparing affected with non-affected siblings. Studies of twins should be especially useful in defining the genetic component of pIgAN.

Conclusions

The study of classical genetics focuses on dominant and recessive diseases, whereas the study of genetics at the molecular level entails a much more complicated set of genotype/phenotype associations. Awareness is growing that the clinical risk for a given disease stems from the interaction between susceptible genotypes and deleterious environmental factors. I think pIgAN is one of those diseases. Although the genetic structure underlying IgA production remains constant throughout life, specific genes are probably developmentally regulated so that their influence on IgA production is expressed only at a certain age. We observed that some relatives of patients with pIgAN initially have negative urinalyses but develop microhematuria years later. Moreover, abnormalities in serum IgA appear to increase with age in relatives of affected patients. Thus, the expression and activity of the IgA system in the families might be modulated by the combined interaction of a number of genetically influenced intermediate phenotypes, each acting with environmental factors (for example, infections, dietary antigens). Some of these environmental factors could be transmitted within family units; other factors could be randomly dispersed throughout the population. The principal approach in revealing the genetic factors should consist in (1) identifying the intermediate phenotypes, which have a strong genetic component, and (2) applying molecular techniques to identify the genotypes. The distribution of the identified genotypes within high-risk pedigrees will subsequently allow us to determine how they interact and whether they can be used as pre-clinical markers of susceptibility to pIgAN. Much more work is needed to identify a substantial number of genotypes and to understand how each contributes to inducing pIgAN.

Questions and answers

DR. NICOLAOS E. MADIAS (*Chief, Division of Nephrology, New England Medical Center, Boston, Massachusetts*): Is there any evidence that progression to renal dysfunction is different in familial versus nonfamilial cases of IgAN? Do any hereditary features appear to predict progression?

DR. SCHENA: Two groups of nephrologists, Egido et al in Spain [42, 48] and Julian et al in the US [50], have investigated whether the clinical manifestations of pIgAN in patients with familial disease differ from those of patients with sporadic pIgAN. Both groups concluded that clinical findings cannot distinguish the two groups; the mean age at onset of the disease, age at the time of renal biopsy, degree of microhematuria, and daily proteinuria were similar. In contrast, immunologic abnormalities of the IgA system were more evident in familial IgAN. Thus, there is a genetic predisposition to these IgA abnormalities, but that does not predict the outcome of the disease.

DR. LANDINO ALLEGRI (*Institute of Medical Clinic and Nephrology, University of Parma, Parma, Italy*): You mentioned in your presentation that many studies had found IgA nephropathy in first-degree relatives of affected patients. What is the prevalence of IgA nephropathy in your own experience?

DR. SCHENA: Unfortunately, I do not have a precise answer to your question. In a previous study, we performed urinalyses in 54 first-degree relatives of 9 unrelated pedigrees of pIgAN patients [53]. The relatives were distributed over three generations, and persistent microhematuria was found in 24% of subjects. In the last 3 years, we expanded our familial study, analyzing urine of 275 relatives of 50 unrelated pedigrees. We found persistent microhematuria in 25% of the relatives. Interestingly, microhematuria was most prevalent in the second and third decade. Given the invasive nature of renal biopsy, IgAN was documented by renal biopsy only in 5 relatives.

DR. JEROME P. KASSIRER (*Editor-in-Chief, New England Journal of Medicine, Boston, Massachusetts*): It seems to me that your own conclusions about the genetic and environmental influences of IgA nephropathy contradict your earlier conclusions about the explanation for the difference in prevalence of IgA nephropathy in various parts of the world, biopsy practices, for example. Unless the genotype frequency is the same in India, Europe, and the USA, which seems quite unlikely, the two conclusions appear to contradict each other.

DR. SCHENA: The contradiction is only apparent. Indeed, different biopsy policies can amplify differences due to genetic and/or environmental factors. If we consider some Asian countries that have adopted a similar renal biopsy policy, we still find a different prevalence of the disease: 40% in Japan and 52% in Singapore. Additionally, the same environmental factor can influence the IgA system in a different way, as shown by a recent multicenter study. Coppo et al demonstrated that sera of pIgAN patients from Italy, Australia, and Japan had different patterns of IgA immune complexes, IgA antibodies against dietary antigens, and also a different lectin-binding activity of IgA [95].

DR. GREGORY G. VOSNIDES (*Chief, Division of Nephrology, Laiko General Hospital, Athens, Greece*): Nephritis accompanying Henoch-Schönlein purpura shares similarities with IgA nephropathy. In addition, mesangial IgA deposits are found in patients with alcoholic liver disease and in patients with immunologic diseases, for example, ulcerative colitis and ankylosing spondylitis. Is there any immunologic or immunogenetic link between these conditions and idiopathic IgAN?

DR. SCHENA: The occurrence of IgAN and Henoch-Schönlein syndrome in members of the same family was reported first by Meadow and Scott [96] and subsequently by others. In a cooperative study, M. Levy and the French Society of Nephrology collected clinical data from 15 families in which 2 or more members were affected by pIgAN or anaphylactoid purpura [97]. Cases of IgAN evolving into Henoch-Schönlein syndrome have been reported by others. These and other diseases share a common finding, IgA deposition in the glomerular mesangium. Patients with IgAN can synthesize IgA molecules with an altered composition consisting of a deficiency of galactose residues in the hinge region of the IgA [98]; this alteration results in reduced recognition of this protein by the sialoglycoprotein receptor. A marked deficiency in galactose residues of IgA1 from patients with pIgAN might be responsible for inefficient plasma clearance and persistent mesangial deposition. An analogous alteration

might be present in the diseases that you have previously mentioned.

DR. DANIEL CORDONNIER (*Centre Hospitalier Universitaire, Grenoble, France*): Did you obtain blood pressure measurements in family members of patients with sporadic and familial IgAN? I'm interested in knowing whether the familial predisposition to essential hypertension is linked in any way to the familial predisposition to IgAN.

DR. SCHENA: The frequency of hypertension in pIgAN patients without renal insufficiency is higher than that found in the healthy population matched for gender and age and living in the same geographic area. At the onset of the disease, hypertension is present in 5% to 10% of patients. At the time of renal biopsy, this percentage is higher (20% to 30%) and it increases with long-term followup, during which about 50% of patients become hypertensive [99]. Differences in the prevalence of hypertension between patients with sporadic IgAN and those with familial IgAN have not been reported. Additionally, no data have been published on the prevalence of hypertension in family members of patients with IgAN. In our family study, we did not measure blood pressure. However, nephrologists from Heidelberg reported a possible increased genetic risk of hypertension in patients with chronic glomerulonephritis; mesangial IgA deposits were found in 0.6% of post-mortem kidney specimens collected from 337 hypertensive patients [100, 101].

DR. KOSTAS C. SIAMOPOULOS (*Associate Professor of Medicine/Nephrology, University Hospital of Ioannina, Ioannina, Greece*): Regarding the progression of IgAN to renal failure, what is your opinion about the role of interstitial lesions, particularly in patients with minor glomerular lesions? Have you seen patients with interstitial lesions more severe than glomerular lesions?

DR. SCHENA: That is an interesting question because the role of the interstitial lesions in the progression of the renal damage in pIgAN has been emphasized recently. Interstitial infiltrates are more frequent in patients with moderate and severe glomerular lesions (G3–G5) than in those with minimal or minor changes (G1–G2); Lee's classification of pIgAN takes note of this fact [11]. Moreover, the presence of extensive interstitial infiltrates is a poor prognostic marker. The interstitial infiltrates comprise monocyte/macrophages and T-lymphocytes, which produce cytokines and growth factors in large amounts. The decrease of renal function in patients during the course of 8 to 15 years of followup correlates with the presence of tubulo-interstitial damage but not with glomerular lesions. However, in my experience I have never found severe tubulo-interstitial lesions in patients with minimal or minor glomerular changes.

DR. MADIAS: Does a correlation exist between the production of IgA or cytokines by peripheral blood mononuclear cells and activity of the disease?

DR. SCHENA: We have found an increased number of cells actively secreting IgG and IgA in patients with pIgAN during the active phase of the disease, as defined by fever, upper respiratory tract infection, and macrohematuria [102]. In addition, we detected increased serum levels of IgA1-IgG immune complexes and an augmented production of IL-2 by peripheral blood mononuclear cells during the relapse of the disease [58, 73]. Increased T-cell activation and cytokine release also have been observed by other investigators [76, 77].

DR. L. A. VAN ES (*Department of Nephrology, Leiden University, Leiden, The Netherlands*): You have concentrated on cytokines influencing IgA production by peripheral blood mononuclear cells. Would you elaborate on the role of cytokines within the kidney itself, especially with regard to progression of disease?

DR. SCHENA: Several studies support the role of cytokines and growth factors produced by resident, that is, glomerular, endothelial, tubular, and interstitial, renal cells as well as by cellular infiltrates. Recent experimental studies have shown that cytokines are responsible for the in-vitro production of some complement factors, for example, C3 and C4, by mesangial and tubular cells [103, 104]. Moreover, Montinaro and colleagues demonstrated that the combination of IL-1 and IL-6 intensified mesangial hypercellularity in a model of experimental IgAN [105]. The synergy of these cytokines with the nephritogenicity of the antigens could lead to the rapid progression of renal damage. Growth factors also might be implicated in determining mesangial cell proliferation in IgAN. We demonstrated an increased glomerular expression of PDGFβ receptor in patients with moderate to severe renal lesions [106].

DR. JESÚS EGIDO (*Associate Chief of Nephrology, Fundación Jiménez Díaz, Madrid, Spain*): Many relatives of patients with IgA nephropathy have abnormalities in the regulation of IgA and also in the synthesis of IL-2 and IL-4. Have you found in a multivariate analysis any differences between that group of relatives and the patients themselves? Have you had the opportunity to restudy patients and healthy relatives?

DR. SCHENA: As I said earlier, the relatives of pIgAN patients revealed a normal spontaneous production of cytokines, which increased considerably after phytohemagglutinin stimulation of PBMC. These findings demonstrate that the relatives of pIgAN patients are hyper-responsive to stimuli and might produce increased amounts of cytokines during viral or bacterial infections. When we restudied immunoglobulin production in patients with pIgAN, we confirmed our earlier results. By contrast, we did not repeat the cytokine study in relatives, because it is not easy to obtain collaboration from apparently healthy subjects.

DR. PHILIPPE LESAVRE (*Department of Nephrology, Hôpital Necker, Paris, France*): I have two comments and one question. First, the rare familial cases of IgAN have been studied by Micheline Lévy and the collaborative study group of the Société de Néphrologie [97]. Familial clustering was more frequent than previously thought, given that 40 families, each with at least 2 members with biopsy-confirmed IgA nephropathy, were collected. The main characteristics of the patients were: male predominance, young age at the apparent onset of the disease, and severity. Familial cases therefore do not appear different from isolated cases. The interval between the onset of the disease in relatives can reach 15 to 30 years, thus favoring a genetic predisposition rather than environmental factors. In addition, this study pointed to the links between IgA nephropathy and Henoch-Schönlein purpura. Both diseases were found in 23 families [107].

Second, together with Micheline Lévy, we set up an INSERM network to study candidate genes in the sporadic form of IgA nephropathy. HLA-D polymorphism was studied by PCR/oligonucleotide at DQA1, DQB1, and DRB1 loci by Virginia LePage (INSERM U93, Hôpital Saint-Louis, Paris). No association was found. Similarly, no association was found with HLA-A, -B, -DR, or -DQ determined serologically. The HLA-D negative results were disappointing to us and have not yet been published (Lepage V, personnal communication). Then Marie Paule LeFranc (URA CNTS 1191, Montpellier, France) studied RFLP α, β, γ, and δ

chains of T-cell receptor (TcR), the constant region of IgG, and κ and γ light chain; again, no association was detected. These negative results were reported only in abstract form during the 11th International Histocompatibility Workshop and Conference (Yokohama, 1991). The only positive results obtained were observed by studying the RFLP of the alpha switch region using a switch alpha probe; the patients were characterized by the presence of new allele fragments not found in the control population. This finding might be important, because the switch α region gene precedes the constant domain gene coding for IgA and facilitates isotype switching. The positive observation was published in *Human Genetics* [108]. Don't you think that the immunogenetic studies are biased by publishing positive results and not negative results? Do you think that we really have evidence of HLA-D association in IgAN?

DR. SCHENA: As I mentioned before, many investigators performed phenotypic and genetic studies on the HLA-D region and obtained different data. In my opinion, these studies are lacking in two aspects. First, the number of controls, which normally should be three times the number of IgAN cases, frequently was low. Second, if the candidate gene is in the HLA-D region or in the switch α region, it is necessary that familial studies be performed instead of population studies. We are carrying out a similar study in the families of our patients, and preliminary data are not very encouraging. Nevertheless, I agree with you that negative results in this field should be published.

DR. FERNANDO VALDERRÁBANO (*Chairman, Department of Nephrology, Hospital General Universitario Gregorio Marañon, Madrid, Spain*): The Spanish Registry of Glomerulonephritis has very similar data to the Italian Registry. In our last report published in 1994, studying more than 1000 biopsies per year, we found a prevalence of IgA nephropathy of 22% in adults and 25% in children among all glomerulonephritides, including the secondary forms [109]. During the last 20 years, the incidence of IgA nephropathy has increased simultaneously with the decrease of membranoproliferative glomerulonephritis without apparent variations in the criteria for renal biopsies. The last report noted a decrease in macroscopic hematuria as a presenting symptom of IgAN [109]. What is your opinion regarding macroscopic hematuria as a prognostic factor in IgAN?

DR. SCHENA: There are contrasting reports on the prognostic role of the macrohematuria. Some studies have shown that isolated or recurrent episodes of macrohematuria are more common in patients with a benign course of the disease. In contrast, others report gross hematuria as a poor prognostic sign, since crescents and impaired renal function are often present. Nicholls and coworkers demonstrated a strong correlation between hematuria, defined as red blood cells more than 10^5/ml, and the presence of crescents [110]. Additionally, in some reports, 3% of patients with macrohematuric episodes presented with acute renal insufficiency and red blood cell cast formation in tubules. The long-term prognosis is poor in these patients when more than 40% of glomeruli are sclerosed.

DR. ALEX M. DAVISON (*Consultant Renal Physician, St. James's University Hospital, Leeds, UK*): In view of your findings regarding the frequency of abnormalities of urinalysis and the immunogenetic links between patients and their relatives, would you be willing to transplant a kidney from an identical twin or another relative to a patient with end-stage renal failure due to IgA nephropathy?

DR. SCHENA: Your question is very interesting because pIgAN is one of the glomerulonephritides that recurs in renal transplant patients. Let me mention the first report from Berger et al, in which the recurrence of IgAN was more frequent in patients who received the kidney from living-related donors (80%) than in those who received a cadaveric kidney (25%) [111]. These data favor a familial predisposition to the disease in family members who develop pIgAN in various periods of their life. The recurrence of IgAN has been reported by other nephrologists, who found a higher recurrence rate of IgAN in recipients of living-related transplants (83%) when compared with recipients of cadaveric kidneys (14%) [112]. Therefore, some caution appears in order in using related donors, especially HLA-identical siblings, when we transplant kidneys into patients with renal failure caused by IgAN. On the other hand, keep in mind that only a minority of patients with recurrent pIgAN progress to renal failure after receiving a transplant.

DR. VAN ES: Let me tell you what we are doing. We are performing a study in all transplant recipients who originally had biopsy-proven IgA nephropathy. The risk of recurrence of IgA deposits in the glomeruli is higher in patients who receive a kidney from a related donor than in patients with an unrelated donor. However, very few recipients have hematuria, and the GFR remains stable in the large majority of patients. In view of the excellent results that can be obtained in HLA-identical combinations, I would proceed with the transplantation, when the donor is genuinely motivated.

DR. COHEN: Dr. van Es, in those instances in which IgAN recurs in the HLA-identical transplant, do we know whether the donor has an increased incidence of urinary abnormalities after donation?

DR. VAN ES: We recalled all our individuals who donated kidneys to IgA nephropathy patients since 1966 and found a 10% prevalence of hypertension, not significantly different from the control population. We did not find hematuria in these donors.

DR. MICHEL LESKI (*Division of Nephrology, Hôpital Cantonal Universitaire, Geneva, Switzerland*): Are you prescribing ACE inhibitors for all patients with IgA nephropathy, even when they are normotensive and do not have proteinuria?

DR. SCHENA: We normally prescribe ACE inhibitors in pIgAN patients whose daily proteinuria exceeds 1 g. In our hands, ACE inhibitors are not efficient when proteinuria is more than 3 g/day. We do not administer these drugs in pIgAN patients without proteinuria, although some recent studies have shown that ramipril, an ACE inhibitor of the latest generation, can reduce the increase in DNA synthesis induced by fetal calf serum and PDGF BB in cultured human mesangial cells (unpublished data). The potential benefits of ACE inhibitors, administered in patients with mild or moderate renal changes, might be inferred by the inhibition of angiotensin II formation. Indeed, ACE inhibitors reduce the interstitial deposition of type-IV collagen in a model of chronic ureteral obstruction in the rat [113].

DR. TILMAN DRÜEKE (*Professor, Department of Nephrology, Hôpital Necker, Paris*): I have a comment and a question. You said that there was no relation between cytokine or growth factor levels in the circulation and the severity of glomerular disease in IgAN. I think that the recent paper by Lai et al demonstrates a correlation between TGFβ production by peripheral CD4+ T-cells and the degree of glomerulosclerosis in IgA nephropathy [94].

You showed an increased IgA production by PBMC in relatives of patients with IgA nephropathy who had no evidence of renal abnormalities. The increase was present in more than 50% of relatives. Would this observation favor an environmental factor rather than a genetic factor?

DR. SCHENA: We demonstrated in vitro a moderate, spontaneous production of IgA by PBMC of apparently healthy relatives, which increased significantly after PWM stimulation, regardless of the occurrence of urinary abnormalities [53]. We did not check for a Mendelian distribution of this abnormality; however, using linkage analysis, a systematic study is being performed. Actually, it is hard to envision how an environmental factor could be responsible for this familial abnormality of the IgA system, unless we accept an underlying predisposing genetic factor.

DR. KASSIRER: I do not believe that routine renal biopsies of patients with persistent isolated hematuria are justified. Even though the risk of biopsy is extremely small, there is little evidence that the results will benefit the patient. Collecting data on prevalence of various glomerulopathies is not, in my opinion, a sufficient justification for such a study.

DR. SCHENA: Your comment reflects the renal biopsy policy of many American nephrology units; for this reason, pIgAN apparently is not as frequent in the USA as in Europe and Asia [2]. In the last 10 years, some American nephrologists have focused on this disease. The more frequent use of renal biopsy by some investigators in patients with persistent isolated microhematuria or recurrent macrohematuria has shown a high incidence of IgAN in the general US population (36%) and in southwestern American Indians (35%) [114, 115]. Moreover, I would like to point out the importance of renal biopsy as a prognostic indicator.

DR. MADIAS: Do you recommend a liberal biopsy policy in the context of research projects or as a matter of practice?

DR. SCHENA: I recommend performing renal biopsy in patients with recurrent episodes of macrohematuria and in those with persistent microhematuria and mild proteinuria (more than 1 g/day) after the exclusion of urologic causes. More than 5000 red blood cells/min in the urine are a reliable indicator of disease. Persistent microhematuria with red blood cell counts of more than 10^5/ml likely indicates the presence of crescents affecting glomeruli [110]. In our experience, this policy has revealed a large number of patients with mild renal lesions, and a few with moderate renal lesions, which progress to end-stage renal disease in 50% of patients. Thus, I support the use of renal biopsy for clinical purposes, as I indicated earlier. Moreover, the application of molecular biology techniques to renal biopsies with moderate changes should help collect further information on the role of cytokines and growth factors.

DR. JORDAN J. COHEN (*President, Association of American Medical Colleges, Washington, DC*): The finding that IgA nephropathy recurs in transplanted kidneys more frequently in HLA-identical donor-recipient pairs than otherwise would appear to support the view that genetic factors are important. Is this interpretation correct?

DR. SCHENA: Yes, your interpretation is correct. However, I should emphasize that environmental factors such as infectious agents and dietary habits can contribute to familial pIgAN even though an epidemologic study performed by Julian et al in a 200-year-old pedigree encompassing seven generations did not reveal a common environmental factor [44].

DR. YVES PIRSON (*Associate Professor, University of Louvain Medical School, Brussels, Belgium*): I have two questions. In regard to the outcome of renal transplantation in patients with IgA nephropathy, patients with IgA nephropathy have better graft tolerance than do those with other types of chronic glomerulonephritis [116], possibly because of a protective effect of IgA [117]. Would you comment on this point? Second, with respect to the risk of recurrence of the disease after transplantation, are there any risk factors for recurrence that are identifiable before transplantation?

DR. SCHENA: In 1992 Lim and Terasaki showed that the graft survival rate of patients receiving a first transplanted kidney from a cadaveric donor was 76% at 5 years post transplant in 374 IgAN patients, compared with 50% to 60% among patients with other renal diseases who received transplants [116]. In a subsequent study by the same group, Koka and coworkers demonstrated that one-year graft survival of IgAN patients with IgA anti-HLA class-I antibodies was far superior (91%) to that of those without the antibody (58%) [118]. In contrast, IgG antibodies were deleterious; at one year, graft survival was 74% in patients with IgG antibodies compared with 87% in those without antibodies [118]. This group concluded that IgA antibodies contribute to renal graft survival by blocking IgG or IgM antibodies against the transplanted organ, especially during the early post-transplant phase. In fact, Lim et al, in an in-vitro study, showed that high pretransplant serum levels of IgA antibodies to HLA class-I antibodies are able to block IgG anti-HLA antibodies [117].

Actually, other hypotheses have been advanced, such as the non-complement immune fixation of the IgA. Still, this aspect is controversial; some investigators demonstrated activation of the complement system by IgA in an in-vivo rat model [119, 120]. However, I do not have an answer for your second question, because risk factors have not yet been identified.

DR. MADIAS: I believe that IgA anti-mesangial cell autoantibodies have been described in some patients with IgAN [121]. Have you observed this in your cases, whether familial or non-familial?

DR. SCHENA: We studied the occurrence of another autoantibody, the IgA rheumatoid factor, in patients and their relatives [53]. We found high serum levels of IgA rheumatoid factor in pIgAN patients and in 25% of their relatives. The presence of the IgA rheumatoid factor might indicate the reduced function of Tα suppressor cells and the increased activity of Tα helper cells, which have been demonstrated in patients with pIgAN.

DR. ALLEGRI: In what percentage of patients do IgA renal deposits disappear during the course of the disease, thus simulating another type of nephropathy if biopsy is performed late?

DR. SCHENA: Serial renal biopsies performed in patients with pIgAN have shown the persistence of mesangial IgA deposits, even in those in whom urinalysis became normal. Usually patients with biopsy-proven IgAN do not undergo a second renal biopsy after entering clinical remission. In a few cases, however, immunofluorescent study has not shown the persistence of IgA deposits. Julian and collegues reviewed the literature on this topic [122]. They quoted six papers in which the disappearance of mesangial IgA deposits was described in 10 rebiopsied patients. The disappearance of IgA deposits might be due to many factors, such as the rate of synthesis of IgA and of the clearance of IgA immune complexes.

DR. LESAVRE: In the study of patients with recurrent IgA nephropathy who had received transplants, I believe that immunologic abnormalities help predict recurrence. In the 1980s, we

demonstrated that serum conglutinin reactive IgA, negatively charged IgA, and IgA rheumatoid factor were associated with the recurrence of mesangial IgA deposits in transplanted kidneys. The situation is clear-cut because serum from transplanted patients with or without recurrence were studied blindly [123, 124]. When we combined the three immunologic abnormalities, it appeared that predictability of recurrence was almost total. However, this probably had little clinical interest because relapse has no clinical importance in that disease.

DR. ARTURO BORSATTI (*Professor of Nephrology, University of Padova, Padova, Italy*): Do you believe it is possible to identify an acute phase of IgA nephropathy?

DR. SCHENA: Many nephrologists define the acute phase of pIgAN as the occurrence of fever, infections of the upper respiratory tract, and macrohematuria. In this phase, relapsed patients show an increased number of IgG and IgA secretory cells [99] and high serum levels of circulating IgA1-IgG immune complexes [58]. We observed in vitro an increased spontaneous production of IL-2 by PBMC during the acute phase of the disease, which reversed completely during followup; the same behavior was observed with PHA-stimulated PBMC [73]. These findings demonstrate that the "hyperactive" IgA system of patients with pIgAN is further stimulated during the active phase of the disease, when there is also the participation of the IgG system, which is presumably responsible for the activation of the complement system.

DR. SÁNDOR SONKODI (*Head, Nephrology Section, Albert Szent-Gyorgyi Medical University, Szeged, Hungary*): I would like to ask a question in connection with the link between IgA nephropathy and tonsillitis. You referred to data indicating an increase in the number of IgA1-producing plasma cells in tonsils of patients with IgAN compared with controls. Do you think that tonsillitis is an important factor in the development of the disease? In your experience, is tonsillectomy beneficial?

DR. SCHENA: Actually Bené et al [125] and Egido and colleagues [126] showed an inverted IgG/IgA ratio in the tonsils of patients with pIgAN and hypothesized that this abnormality was due to an increased amount of IgA-producing plasma cells in palatine tonsils or to an abnormal switch mechanism favoring the IgA. In a clinical and immunologic followup study of biologic parameters performed in 34 tonsillectomized patients with IgA nephropathy, Bené and coworkers demonstrated a decrease in serum IgA levels and proteinuria 6 months after tonsillectomy; however, no variation of serum creatinine values was observed [127]. Others recently have claimed that tonsillectomy is a valid therapeutic measure in patients with frequent tonsillitis because it reduces the rate of deterioration of renal function [128]. However, we cannot exclude that other infections, such as pharyngitis and bronchitis in tonsillectomized patients, influence the renal disease.

DR. VOSNIDES: You mentioned the presence of IgA-rheumatoid factor in the serum of patients with IgA nephropathy. How common is this finding?

DR. SCHENA: IgA rheumatoid factor has been found in 41% of patients with pIgAN [129]. Autoantibodies against laminin, ssDNA, endothelial cells, and lymphocytes have been observed in a few cases, and rarely anti-neutrophil cytoplasmic antibodies and anti-GBM IgA class antibodies have been found.

Acknowledgments

The author wishes to thank Drs. Biagio Favoino and Ms. Pina Cerullo for their valuable contributions to our genetic studies, and Dr. Ambrogio Aquilino for the data on the urinary abnormalities in a normal population. This work was supported in part by the CNR (Consiglio Nazionale delle Ricerche) grant (92.749.04), by the MURST (Ministero dell'Università e della Ricerca Scientifica e Tecnologica) grant (93.5382), and by a grant from the Regione Puglia "Prevention of the renal diseases and Culture of the organ donation," 1990–1993.

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References

1. BERGER J, HINGLAIS N: Les dépots intercapillaires d'IgA-IgG. *J Urol Nephrol* 74:694–695, 1968
2. SCHENA FP: A retrospective analysis of the natural history of primary IgA nephropathy worldwide. *Am J Med* 89:209–215, 1990
3. PROPPER DJ, POWER DA, SIMPSON JG, EDWARD N, CATTO GRD: Incidence of IgA nephropathy in U.K. (letter). *Lancet* 1:90, 1988
4. SISSONS JGP, WOODROW DF, CURTIS JR, EVANS DJ, GOWER PE, SLOPER JC, PETERS DK: Isolated glomerulonephritis with mesangial IgA deposits. *Br Med J* 3:611–614, 1975
5. PROPPER DJ, POWER DA, SIMPSON JG, EDWARD N, CATTO GRD: The incidence, mode of presentation, and prognosis of IgA nephropathy in Northeast Scotland. *Semin Nephrol* 7:363–366, 1987
6. KITAGAWA T: Screening for asymptomatic hematuria and proteinuria in school children. Relationship between clinical laboratory findings and glomerular pathology or prognosis. *Acta Paediatr Jpn* 27:366–373, 1985
7. KITAGAWA T: A multicenter clinical study on the epidemiology of chronic glomerulonephritis in children in Japan, in *Recent Advances in Pediatric Nephrology*, edited by MURAKAMI K, et al, Japan, Elsevier, 1987, p 13
8. WOO KT, EDMONDSON RPS, WU AYT, CHING GSC, PWEE HS, LIM CH: The natural history of IgA nephritis in Singapore. *Clin Nephrol* 25:15–21, 1986
9. FERRARIO F, BARBIANO DI BELGIOIOSO G, CAGNOLI L, LUPO A, SCHENA FP, BANFI C: *Le glomerulonefriti primitive e secondarie*. Milano, Wichtig Editore, 1991, pp 1–5
10. GRUPPO DI IMMUNOPATOLOGIA RENALE DELLA SOCIETÀ ITALIANA DI NEFROLOGIA: Rapporto sul Registro Nazionale delle biopsie renali. *G Ital Nefrol* 10:235–241, 1993
11. LEE SMK, RAO VM, FRANKLIN WA, SCHIFFER MS, ARONSON AJ, SPARGO BH, KATZ AI: IgA nephropathy: morphologic predictors of progressive renal disease. *Hum Pathol* 13:314–322, 1982
12. D'AMICO G: The commonest glomerulonephritis in the world: IgA nephropathy. *Q J Med* 245:709–727, 1987
13. MACDONALD IM, DUMBLE LJ, KINCAID-SMITH P: HLA and glomerulonephritis, in *HLA and Disease* (vol 58), INSERM Paris, 1976, p 203
14. NOEL LH, DESCHAMPS B, JUNGERS P, BACH JF, BUSSON M, SUET C, HORS J, DAUSSET J: HLA antigen in three types of glomerulonephritis. *Clin Immunol Immunopathol* 10:19–23, 1978
15. BERTHOUX FC, GAGNE A, SABATIER JC, DUCRET F, LE PETIT JC, MARCELLIN M, MERCIER B, BRIZARD CP: HLA-Bw35 and mesangial IgA glomerulonephritis (letter). *N Engl J Med* 298: 1034, 1978
16. BRETTLE R, PETERS DK, BATCHELOR JR: Mesangial IgA glomerulonephritis and HLA antigens (letter). *N Engl J Med* 299:200, 1978
17. RICHMAN AV, MAHONEY JJ, FULLER TJ: Higher prevalence of HLA-B12 in patients with IgA nephropathy (brief report). *Ann Intern Med* 90:201, 1979
18. KASHIWABARA H, SHISHIDO H, YOKOYAMA T, MIYAJIMA T: HLA in IgA nephropathy. *Tissue Antigens* 16:411–412, 1980
19. HIKI Y, KOBAYASHI Y, OOKUBO M, KASHIWAGI N: The role of HLA-DR4 in the long-term prognosis of IgA nephropathy. *Nephron* 54:264–265, 1990
20. KOMORI K, NOSE Y, INOUYE H, TSUJI K, NOMOTO Y, SAKAI H: Study on HLA system in IgA nephropathy. *Tissue Antigens* 14:32–36, 1979

21. HORS J: Strong association between HLA DRw4 and mesangial IgA nephropathy (Berger's disease) (*abstract 8.5.26*). 4th Int Cong Immunol, Paris, 1980
22. FAUCHET R, LE POGAMP P, GENETET B, CHEVET D, GUÉGUEN M, SIMON P, RAMÉE MP, CARTIER F: HLA-DR4 antigen and IgA nephropathy. *Tissue Antigens* 16:405–410, 1980
23. FEEHALLY J, DYER PA, DAVIDSON JA, HARRIS R, MALLICK NP: Immunogenetics of IgA nephropathy: experience in a U.K. centre. *Dis Markers* 2:493–500, 1984
24. KASAHARA M, HAMADA K, OKUYAMA T, ISHIKAWA N, OGASAWARA K, IKEDA H, TAKENOUCHI T, WAKISAKA A, AIZAWA M, KATAOKA Y, MIYAMOTO R, KOHARA M, NAITO S, KASHIWAGI N, HIKI Y: Role of HLA in IgA nephropathy. *Clin Immunol Immunopathol* 25:189–195, 1982
25. SIMON P, RAMÉE MP, ANG KS, GENETET B, FAUCHET R: Minimum prevalence and evolution of IgA nephropathy in a French region with 250,000 inhabitants: seven year follow-up (abstract). *Kidney Int* 24:137, 1983
26. NAITO S, KOHARA M, ARAKAWA K: Association of class II antigens of HLA with primary glomerulonephropathies. *Nephron* 45:111–114, 1987
27. MCLEAN RM, WYATT RJ, JULIAN BA: Complement phenotypes in glomerulonephritis: Increased frequency of homozygous null C4 phenotypes in IgA nephropathy and Henoch-Schönlein purpura. *Kidney Int* 26:855–860, 1984
28. WYATT RJ, JULIAN BA, WALDO FB, MCLEAN RH: Complement phenotypes (C3 and C4) in IgA nephropathy, in *Recent Advances in Mucosal Immunology*, edited by MESTECKY J, MCGHEE JR, BIENENSTOCK J, ORGA PL, New York, Plenum, 1987, pp 1569–1575
29. RAMBAUSEK M, VAN DER WALL BAKE AWL, SCHUMACHER-ACH R, SPITZENBERG R, ROTHER U, VAN ES LA, RITZ E: Genetic polymorphism of C3 and βf in IgA nephropathy. *Nephrol Dial Transplant* 2:208–211, 1987
30. KASHIWABARA H, SHISHIDO H, TOMURA S, TUCHIDA H, MIYAJIMA T: Strong association between IgA nephropathy and HLA-DR4 antigen. *Kidney Int* 22: 377–382, 1982
31. SAVAGE D, BAXTER-LOWE LA, GORSK J, MIDDLETON D: Molecular methods, in *Histocompatibility Testing. A Practical Approach*, edited by DYER P, MIDDLETON D, Oxford, Oxford University Press, 1992, pp 107–142
32. MOORE RH, HITMAN GA, MEDCRAFT J, SINICO RA, MUSTONEN J, LUCAS EY, D'AMICO G: HLA-DP region gene polymorphism in primary IgA nephropathy: no association. *Nephrol Dial Transplant* 7:200–204, 1992
33. MOORE RH, HITMAN GA, LUCAS EY, RICHARDS NT, VENNING MC, PAPIHA S, GOODSHIP THS, FIDLER A, AWAD J, FESTENSTEIN H, CUNNINGHAM J, MARSH FP: HLA DQ region gene polymorphism associated with primary IgA nephropathy. *Kidney Int* 37:991–995, 1990
34. LI PK-T, BURNS AP, SO AKL, PUSEY CD, FEEHALLY J, REES AJ: The DQw7 allele at the HLA-DQB locus is associated with susceptibility to IgA nephropathy in Caucasians. *Kidney Int* 39:961–963, 1991
35. LI PK-T, POON ASY, LAI KN: Molecular genetics of MHC class II alleles in Chinese patients with IgA nephropathy. *Kidney Int* 46:185–190, 1994
36. ABE J, KOKSAHA T, TANAKA M, KOBAYASHI N: Genetic study on HLA Class II and class III region in the disease associated with IgA nephropathy. *Nephron* 65:17–22, 1993
37. RAMBAUSEK M, RITZ E, MYTILINEOS J, OPELZ G: HLA-DP region gene polymorphism in primary IgA nephropathy: no association (letter). *Nephrol Dial Transplant* 7:1256, 1992
38. LATHROP JM, LALONEL JM, JULIER C, OTT J: Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 81:3443–3446, 1984
39. WEEKS DE, LANGE K: The affected-pedigree-member of linkage analysis. *Am J Hum Genet* 42:315–326, 1988
40. WEEKS DE, LANGE K: A multilocus extension of the affected-pedigree-member method of linkage analysis. *Am J Hum Genet* 50:859–868, 1992
41. DE WERRA P, MOREL-MAROGER L, LEREOUX-ROBERT C, RICHET G: Glomérulites a dépots d'IgA diffus dans le mésangium. Etude de 96 cas chez adulte. *Schweiz Med Wochenschr* 103:797–803, 1973
42. EGIDO J, JULIAN BA, WYATT RJ: Genetic factors in primary IgA nephropathy. *Nephrol Dial Transplant* 3:134–142, 1987
43. LEVY M, LESAVRE P: Genetic factors in IgA nephropathy (Berger's disease). *Adv Nephrol* 21:23–51, 1992
44. JULIAN BA, QUIGGINS PA, THOMPSON JS, WOODFORD SY, GLEASON K, WYATT RJ: Familial IgA nephropathy—Evidence of an inherited mechanism of disease. *N Engl J Med* 321:202–208, 1985
45. SCOLARI F, AMOROSO A, SAVOLDI S, PRATI E, SCAINI P, MANGANONI A, BORELLI I, MAZZOLA G, CANALE L, SACCHI G, MIGLIETTI N, CRISTINELLI L, CURTONI SE, BINDA PL, BONOMELLI D, MAIORCA R: Familial occurrence of primary glomerulonephritis: evidence for a role of genetic factors. *Nephrol Dial Transplant* 7:587–596, 1992
46. SCHENA FP, SCIVITTARO V, RANIERI E: IgA nephropathy: Pros and cons for a familial disease, in IgA Nephropathy. The 25th year. *Contrib Nephrol* 104:36–45, 1993
47. EGIDO J, BLASCO R, SANCHO J, HERNANDO L: Immunological abnormalities in healthy relatives of patients with IgA nephropathy. *Am J Nephrol* 5:14–20, 1985
48. EGIDO J, GARCIA-HOYO R, LOZANO L, GONZALES-CABRERO J, DE NICOLAS R, HERNANDO L: Immunological studies in familial and sporadic IgA nephropathy. *Semin Nephrol* 7:311–314, 1987
49. SAKAI H, NOMOTO Y, ARIMORI S, KOMORI K, INOUYE H, TSUJI K: Increase of IgA-bearing peripheral blood lymphocytes in families of patients with IgA nephropathy. *Am J Clin Pathol* 72:452–456, 1979
50. JULIAN BA, WOODFORD SY, BACHLER RW, MCMORROW RG, WYATT RJ: Familial clustering and immunogenetic aspects of IgA nephropathy. *Am J Kidney Dis* 12:366–370, 1988
51. WALDO FB, BEISCHEL L, WEST CD: IgA synthesis by lymphocytes from patients with IgA nephropathy and their relatives. *Kidney Int* 29:1229–1233, 1986
52. JACKSON S, GALLA JH, KIRK KA, THORN BT, JULIAN BA: Epstein-Barr virus transformation of B lymphocytes from IgA nephropathy patients and first-degree relatives results in increased immunoglobulin synthesis not restricted to IgA. *Am J Kidney Dis* 17:55–61, 1991
53. SCHENA FP, SCIVITTARO V, RANIERI E, SINICO R, BENUZZI S, DI CILLO M, AVENTAGGIATO L: Abnormalities of the IgA immune system in members of unrelated pedigrees from patients with IgA nephropathy. *Clin Exp Immunol* 92:139–144, 1993
54. SCIVITTARO V, RANIERI E, DI CILLO M, AVENTAGGIATO L, EMANCIPATOR SN, SCHENA FP: In vitro immunoglobulin production in relatives of patients with IgA nephropathy. *Clin Nephrol* 42:1–8, 1994
55. ROTHSCHILD E, CHATENOUD L: T cell subset modulation of immunoglobulin production in IgA nephropathy and membranous glomerulonephritis. *Kidney Int* 25:557–564, 1984
56. ROSTOKER G, PECH MA, PRATO SD: Serum IgG subclass and IgM imbalance in adult IgA mesangial glomerulonephritis and idiopathic Henoch-Schönlein purpura. *Clin Exp Immunol* 75:30–35, 1989
57. VALENTIJN RM, RADL J, HAAIJMAN JJ, VERMEER BJ, WEENING JJ, KAUFFMANN RH, DAHA MR, VAN ES LA: Circulating and mesangial secretory component binding IgA-1 in primary IgA nephropathy. *Kidney Int* 26:760–766, 1984
58. SCHENA FP, PASTORE A, LUDOVICO N, SINICO RA, BENUZZI S, MONTINARO V: Increased serum levels of IgA1-IgG immune complexes and anti-F(ab')2 antibodies in patients with primary IgA nephropathy. *Clin Exp Immunol* 77:15–20, 1989
59. LAI KN, LAI FM, CHIU SM: Studies of lymphocytes subpopulations and immunoglobulin production in IgA nephropathy. *Clin Nephrol* 28:281–287, 1987
60. VAN DEN WALL BAKE AWL, DAHA MR, RADL J, HAAIJMAN JJ, VAN DER ARK A, VALENTIJN RM, VAN ES LA: The bone marrow as production site of the IgA deposited in the kidneys of IgA nephropathy. *Clin Exp Immunol* 72:321–325, 1988
61. HALE GM, MCINTOSH SL, HIKI Y, CLARKSON AR, WOODROFFE AJ: Evidence for IgA-specific B cell hyperactivity in patients with IgA nephropathy. *Kidney Int* 29:718–724, 1986
62. VAN DEN WALL BAKE AWL, DAHA MR, VAN DER ARK A, HIEMSTRA PS, VAN ES LA: Serum levels and in vitro production of IgA subclasses in patients with primary IgA nephropathy. *Clin Exp Immunol* 74:115–120, 1988
63. VAN DEN WALL BAKE AWL, CROWLEY-NORWICK PA, KULHAVY R, HERMANS J, JACKSON S, JULIAN BA, MESTECKY J: Cytokine-induced immunoglobulin production in primary IgA nephropathy. *Am J Kidney Dis* 20:611–617, 1992

64. SAKAI H, MIYAZAKI M, ENDOH M, NOMOTO Y: Increase of IgA-specific switch T cells in patients with IgA nephropathy. *Clin Exp Immunol* 78:378–382, 1989
65. YASUMOTO Y, SUGA T, MIURA M, ENDOH M, NOMOTO H, TOMINO Y, NOMOTO Y, SAKAI H: Subpopulation of T-alpha cells in patients with IgA nephropathy: correlation between T-alpha 4 cells and in vitro IgA production. *Clin Immunol Immunopathol* 51:232–239, 1989
66. KIM PH, KAGNOFF NF: Transforming growth factor β1 increases IgA isotype switching at the clonal level. *J Immunol* 145:3773–3778, 1990
67. PURKERSON J, ISAKSON P: A two-signal model for regulation of immunoglobulin isotype switching. *FASEB J* 6:3245–3253, 1992
68. SNAPPER CM, MOND JJ: Towards a comprehensive view of immunoglobulin class switching. *Immunol Today* 14:15–17, 1993
69. ZHANG K, MILLS FC, SAXON A: Switch circles from IL-4 directed ϵ class switching from human B lymphocytes. *J Immunol* 152:3427–3435, 1994
70. MORRIS SC, MADDEN KB, ADAMOVICZ JJ, GANSE WC, HUBBARD BR, GATELY MK, FINKELMAN FD: Effects of IL-12 on in vivo cytokine gene expression and Ig isotype selection. *J Immunol* 152: 1047–1056, 1994
71. PUNNONEN J, DE VRIES JE: IL-13 induces proliferation, Ig isotype switching, and Ig synthesis by immature human fetal B cells. *J Immunol* 15:1094–1102, 1994
72. SCIVITTARO V, GESUALDO L, RANIERI E, MARFELLA C, SCHEWN SA, EMANCIPATOR SN, SCHENA FP: Profiles of immunoregulatory cytokine production in vitro in patients with IgA nephropathy and their kindred. *Clin Exp Immunol* 96:311–316, 1994
73. SCHENA FP, MASTROLITTI G, JIRILLO E, MUNNO I, PELLEGRINO N, FRACASSO AR, AVENTAGGIATO L: Increased production of interleukin 2 and IL-2 receptor in primary IgA nephropathy. *Kidney Int* 35:875–879, 1989
74. LAI KN, LEUNG JCK, LI PKT, LUI SF: Cytokine production by peripheral blood mononuclear cells in IgA nephropathy. *Clin Exp Immunol* 85:240–245, 1991
75. OHTA K, TAKANO N, SENO A: Detection and clinical usefulness of urinary interleukin-6 in the diseases of the kidney and the urinary tract. *Clin Nephrol* 38:185–189, 1992
76. LAI KN, LEUNG JCJ, MAC MONNE LAI F, TAM JS: T-lymphocyte activation in IgA nephropathy: serum soluble interleukin-2 receptor level, interleukin-2 production, and interleukin-2 receptor expression by cultured lymphocytes. *J Clin Immunol* 9:485–492, 1989
77. PARERA M, RIVERA F, EGIDO J, CAMPOS A: The role of interleukin-2 and serum soluble IL-2 receptor cells in idiopathic IgA nephropathy. *Clin Immunol Immunopathol* 63:196–199, 1992
78. YANO N, ENDOH M, MIYAZAKI M, YAMAUCHI F, NOMOTO Y, SAKAI H: Altered production of IgE and IgA induced by IL-4 in peripheral blood mononuclear cells from patients with IgA nephropathy. *Clin Exp Immunol* 88:295–300, 1992
79. EMANCIPATOR SN, OVARY Z, LAMM ME: The role of mesangial complement in the hematuria of experimental IgA nephropathy. *Lab Invest* 57:269–276, 1987
80. GESUALDO L, LAMM ME, EMANCIPATOR SN: Defective oral tolerance promotes nephritogenesis in experimental IgA nephropathy induced by oral immunization. *J Immunol* 145:3684–3691, 1990
81. EMANCIPATOR SN: Nephrology Forum: Immunoregulatory factors in the pathogenesis of IgA nephropathy. *Kidney Int* 38:1216–1229, 1990
82. DEMAIN AG, RAMBAUSEK M, KNIGHT JF, WILLIAMS DG, WELSH KI, RITZ E: Relation of mesangial IgA glomerulonephritis to polymorphism of immunoglobulin heavy chain switch region. *J Clin Invest* 81:611–614, 1988
83. MOORE RH, HITMAN GA, SINICO RA, MUSTONEN J, MEDCRAFT J, LUCAS EY, RICHARDS NT, VERNING MC, CUNNINGHAM J, MARSH FP, D'AMICO G: Immunoglobulin heavy chain switch region gene polymorphisms in glomerulonephritis. *Kidney Int* 38:332–336, 1990
84. MARSH DG, NEELY E, BREAZEALE DR, GHOSH B, FREIDHOFF LR, EHRLICH-KAUTZKY E, SCHON C, KRISHNASWAMY G, BEATY TH: Linkage analysis of IL-4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 264:1152–1156, 1994
85. ALLEN RC, ARMITAGE RJ, CONLEY ME, ROSENBLATT H, JENKINS NA, COPELAND NG, BEDELL MA, EDELHOFF S, DISTECHE CM, SIMONEAUX DK, FAUSLOW WC, BELMONT J, SPRIGGS MK: CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. *Science* 259:990–993, 1993
86. RAMSAY AJ, HUSBAND AJ, RAMSHAW IA, BOA S, MATTHACI KI, KOEHLER G, KOPF M: The role of interleukin-6 in mucosal IgA antibody responses in vivo. *Science* 264:561–563, 1994
87. KOPF M, BAUMANN H, FREER G, FREUDENBERG M, LAMERS M, KISHIMOTO T, ZINKERNAGEL R, BLUETHMAN H, KOHLER G: Impaired immune and acute phase responses in interleukin-6 deficient mice. *Nature* 368:339–342, 1994
88. ISLAM KB, BASKIN B, NILSSON L, HAMMARSTROM L, SIDERAS P, SMITH CIE: Molecular analysis of IgA deficiency. Evidence for impaired switching to IgA. *J Immunol* 152:1442–1452, 1994
89. LEWIN B: Organization of simple sequence DNA, in *Genes IV*, edited by LEWIN B, Oxford, Oxford University Press, 1990, pp 530–539
90. MOUT R, WILLEMZE R, LANDEGENT JE: Repeat polymorphism in the interleukin-4 gene (IL-4) (abstract). *Nucleic Acids Res* 19:3763, 1991
91. BOWCOCK AM, RAY A, ERLICH H, SEHGAL PB: Rapid detection and sequencing of alleles in the 3′ flanking region of the interleukin-6 gene. *Nucleic Acids Res* 17:6855–6864, 1989
92. O'CONNELL PJ, IBELS LS, THOMAS MA, HARRIS M, ECKSTEIN RP: Familial IgA nephropathy. A study in an Australian Aboriginal family. *Aust N Z J Med* 17:27–33, 1987
93. WEATHERALL DJ: Molecular genetics and common diseases, in *New Genetics and Clinical Practice*, Oxford, Oxford University Press, 1991, pp 193–200
94. LAI KN, HO RTH, LEUNG CK, LAI FMM, LI PKT: Increased mRNA encoding for transforming factor-β in CD4+ cells from patients with IgA nephropathy. *Kidney Int* 46:862–868, 1994
95. COPPO R, AMORE A, ROCCATELLO D, GRANOGLIO B, MOLINO A, PICCOLI G, CLARKSON A, WOODROFFE AJ, SAKAI H, TOMINO Y: IgA antibodies to dietary antigens and lectin-binding IgA in sera from Italian, Australian and Japanese IgA nephropathy patients. *Am J Kidney Dis* 17:480–487, 1991
96. MEADOW SR, SCOTT DG: Berger disease: Henoch-Schönlein syndrome without the rash. *J Pediatr* 106:27–32, 1985
97. LEVY M: Cas familiaux de maladie de Berger ou de maladie de Berger et de purpura rheumtoide. Etude coopérative de al Société Francaise de Néphrologie. *Néphrologie* 10:175–182, 1989
98. MESTECKY J, TOMANA M, CROWLEY-NOWICK PA, MOLDOVEANU Z, JULIAN BA, JACKSON S: Defective galactosylation and clearance of IgA1 molecules as a possible etiopathogenic factor in IgA nephropathy. *Contrib Nephrol* 104:172–182, 1993
99. SCHENA FP: IgA nephropathies, in *Oxford Textbook of Clinical Nephrology* (vol 1), edited by CAMERON S, DAVISON AM, GRÜNFELD J-P, KERR D, RITZ E, Oxford, Oxford University Press, 1992, pp 339–369
100. SCHMID M, MEYER S, WEGNER R, RITZ E: Increased genetic risk of immune complex-associated glomerulonephritis? *J Hypertens* 8:573–577, 1990
101. HAUER C, VALDHERR R, RITZ E: Prevalence of immune complex-associated glomerulonephritis in hypertensive subjects. *J Hum Hypertens* 8:181–183, 1994
102. SCHENA FP, MASTROLITTI G, FRACASSO AR, PASTORE A, LADISA N: Increased immunoglobulin secreting cells in the blood of patients with active idiopathic IgA nephropathy. *Clin Nephrol* 26:163–168, 1986
103. BROOIMANS RA, STEGMANN APA, VAN DORP WT, VAN DER ARK AAJ, VAN DER WOUDE FJ, VAN ES LA, DAHA MA: Interleukin-2 mediates stimulation of complement C3 biosynthesis in human proximal tubular epithelial cells. *J Clin Invest* 88:379–384, 1991
104. MONTINARO V, SERRA L, PERISSUTTI S, RANIERI E, TEDESCO F, SCHENA FP: Biosynthesis of C3 by human mesangial cells. Modulation by proinflammatory cytokines. *Kidney Int* 47:829–836, 1995
105. MONTINARO V, HEVEY K, AVENTAGGIATO L, FADDEN K, ESPARZA A, CHEN A, FINBLOOM DS, RIFAI A: Extrarenal cytokines modulate the glomerular response to IgA immune complexes. *Kidney Int* 42:341–353, 1992
106. GESUALDO L, DI PAOLO S, MILANI S, PINZANI M, GRAPPONE C,

RANIERI E, PANNARALE G, SCHENA FP: Expression of platelet-derived growth factor in normal and diseased human kidney. An immunohistochemistry and in situ hybridization study. *J Clin Invest* 94:50–58, 1994
107. LÉVY M: Multiplex families in IgA nephropathy. *Contrib Nephrol* 104:46–53, 1993
108. KEYEUX G, NUSBAUM P, ALEXANDER D, LESAVRE P, LEFRANC MP: New RFLPs of the immunoglobulin switch alpha region in mesangial IgA glomerulonephritis. *Hum Genet* 86:624, 1991
109. REGISTRO ESPAÑOL DE GLOMERULONEFRITIS (REGN): Evolución de la frecuencia de las glomerulonefritis primarias y secundarias in España en los años 1991 y 1992. *Nephrologia* 14:288–296, 1994
110. NICHOLLS KM, FAIRLEY KF, DOWLING JP, KINCAID-SMITH P: The clinical course of mesangial IgA associated nephropathy in adults. *Q J Med* 53:22–50, 1984
111. BERGER J, YANEVA H, NABARRA B, BARNABEL C: Recurrence of mesangial deposition of IgA after renal transplantation. *Kidney Int* 7:232–241, 1975
112. BACHMAN V, BRAVA C, AMEND W, FEDUSKA N, MELZER J, SALVATIERRA O, VINCENTI F: The clinical course of IgA nephropathy and Henoch-Schönlein purpura following renal transplantation. *Transplantation* 42:511–515, 1986
113. KANETO H, MORRISAY J, MCCRAKEN R, REYES A, KLAHR S: Enalapril reduces collagen type IV synthesis and expression of the interstitium in the obstructed rat kidney. *Kidney Int* 45:1637–1647, 1994
114. PONTIER PJ, PATEL TG: Racial differences in the prevalence and presentation of glomerular disease in adults. *Clin Nephrol* 42:79–84, 1994
115. MELEG-SMITH S, TUNG KSK: Incidence of IgA-related nephritides in American Indians in New Mexico. *Hum Pathol* 16:181–184, 1985
116. LIM EC, TERASAKI PI: High survival rate of kidney transplants in IgA nephropathy patients. *Clin Transplant* 6:100–105, 1992
117. LIM EC, CHIA D, GJERTSON DW, KOKA P, TERASAKI PI: In vitro studies to explain high renal allograft survival in IgA nephropathy patients. *Transplantation* 55:996–999, 1993
118. KOKA P, CHIA D, TERASAKI PI, CHAN H, CHIA J, OZAWA M, LIM E: The role of IgA anti-HLA class I antibodies in kidney transplant survival. *Transplantation* 56:207–211, 1993
119. STAD RK, BOGERS WMJM, THOOMES-VAN DER SLUYS ME, VAN ES LA, DAHA MR: In vivo activation of complement by IgA in rat model. *Clin Exp Immunol* 87:138–143, 1992
120. VAN ES LA: Nephrology Forum: Pathogenesis of IgA nephropathy. *Kidney Int* 41:1720–1729, 1992
121. BALLARDIE FW, BRENCHLEY PEC, WILLIAMS S, O'DONOGHUE DJ: Autoimmunity in IgA nephropathy. *Lancet* 2:588–592, 1988
122. JULIAN BA, CANNON VR, WALDO FB, EGIDO J: Macroscopic hematuria and proteinuria preceding renal IgA deposition in patients with IgA nephropathy. *Am J Kidney Dis* 17:472–479, 1991
123. LESAVRE P, DIGEON M, BACH JF: Analysis of circulating IgA and detection of immune complexes in IgA nephropathy. *Clin Exp Immunol* 48:61–69, 1982
124. MONTEIRO RC, CHEVAILLER A, NOËL LH, LASAVRE P: Serum IgA preferentially binds to cationic polypeptides in IgA nephropathy. *Clin Exp Immunol* 73:300–306, 1988
125. BENÉ MC, FAURE G, HURAULT DE LIGNY B, KESSLER M, DUHEILLE J: Quantitative immunohistomorphometry 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
126. EGIDO J, BLASCO R, LOZANO L, SANCHO J, GARCIA-HOYO R: Immunological abnormalities in the tonsils of patients with IgA nephropathy: inversion in the ratio of IgA: IgG bearing lymphocytes and increased polymeric IgA synthesis. *Clin Exp Immunol* 57:101–106, 1984
127. BENÉ MC, HURAULT DE LIGNY B, KESSLER M, FOLIGNET B, FAURE GC: Tonsils in IgA nephropathy. *Contrib Nephrol* 104:153–161, 1993
128. IINO Y, AMBE K, KATO Y, NAKAI A, TORIYAMA M, SAIMA K, YOSHIMOTO K: Chronic tonsillitis and IgA nephropathy. Clinical study of patients with and without tonsillectomy. *Acta Otolaryngol* (Stockh) (suppl) 508:29–35, 1993
129. SINICO RA, FORNASIERI A, ORENI N, BENUZZI S, D'AMICO G: Polymeric IgA rheumatoid factor in idiopathic IgA mesangial nephropathy (Berger's disease). *J Immunol* 137:536–541, 1986

This Forum was presented at the Third European Nephrology Forum conference at the Tufts University European Center in Talloires, France, in September 1994.