CLINICAL NEPHROLOGY—EPIDEMIOLOGY—CLINICAL TRIALS

Polymorphism of the cytokine genes and IgA nephropathy

JAANA SYRJÄNEN, MIKKO HURME, TERHO LEHTIMÄKI, JUKKA MUSTONEN, and AMOS PASTERNACK

Medical School, University of Tampere, Department of Medicine and Department of Clinical Chemistry, Tampere University Hospital, Tampere, Finland

Polymorphism of the cytokine genes and IgA nephropathy.

Background. IgA nephropathy (IgAN) is a form of chronic glomerulonephritis of unknown etiology and pathogenesis. Cytokine gene polymorphisms regulate cytokine production and play a role in immune and inflammatory responses; these immunological responses thus are possibly involved in the etiology and pathogenesis of IgAN.

Methods. We studied by polymerase chain reaction (PCR) polymorphisms of important cytokine genes of inflammation *interleukin-1 (IL-1β), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1 receptor antagonist (IL-1Ra)* in 167 patients with IgAN and 400 healthy blood donor controls. IgAN patients had been followed up for 6 to 17 (median 11) years from renal biopsy.

Results. Carriage of the *IL-1β* allele 2 (*IL1β2*) or *IL-1Ra* allele 2 (*IL1RN*2*) was associated with an increased risk of IgAN. These alleles were highly linked and the odds ratio (OR) of IgAN for carriage of both alleles was 1.8 (95% confidence interval 1.2 to 2.6; $P = 0.002$). Carriage of the *TNF-α* allele 2 (*TNF2*) was associated with a decreased risk of IgAN (OR 0.5, range 0.3 to 0.7; $P = 0.001$). The risk of IgAN was found to be highest in those carrying *IL1β2* and *IL1RN*2* but not *TNF2* as compared to those who did not carry both of these *IL-1* cluster genes and were carriers of *TNF2* (OR 5.0 (2.4–10.3); $P < 0.001$). None of the polymorphisms studied was associated with poor prognosis.

Conclusion. Carriage of *IL1β2* and *IL1RN*2* together with non-carriage of *TNF2* is associated with increased susceptibility, but not with a prognosis of IgAN.

IgA glomerulonephritis (IgAN) is an immune-mediated disorder and the most frequent type of glomerulonephritis in humans [1, 2]. Its clinical course is variable, ranging from spontaneous clinical remission to terminal renal failure. About 10 to 20% of IgAN patients progress to end-stage renal disease (ESRD) within 10 years [1, 2]. Several factors, for example hypertension, proteinuria, renal insufficiency, hypertriglyceridemia and hyperuricemia at the time of diagnosis, are associated with progression of IgAN [1–3]. The cause of IgAN is unknown, as are the factors increasing susceptibility to IgAN.

The clinical presentation of IgAN is variable. Gross hematuria episodes coinciding recurrently with microbial infections or other antigenic challenge is a typical manifestation. Another common finding is microscopic hematuria with or without proteinuria [1]. The mode by which various antigenic challenges affect IgAN is unknown. It is suspected that the disorder is somehow connected with mucosal immune response and that the systemic IgA response is abnormal. Several microbial or dietary antigens are possibly of significance in the pathogenesis of IgAN in susceptible persons [4]. Genetic factors are likely to contribute to the development and progression of IgAN [5]. Gharavi and coworkers recently demonstrated by a genome-wide analysis linkage of IgAN to chromosome 6q22-q23 [6].

Interleukin (IL-1) and tumor necrosis factor-α (TNF-α) are pro-inflammatory cytokines, and the IL-1 receptor antagonist (IL-1Ra) is anti-inflammatory, while interleukin-6 (IL-6) has both of these properties. The network of cytokines should function at an optimal level to guarantee the successful eradication of antigens and to avoid excessive damage to the host. The development of glomerular inflammation in IgAN has been associated with various cytokines [7–10]. Cytokines produced during infections or other antigenic challenges may play a role in the pathogenesis of IgAN. One explanation is that host genetic factors in conjunction with infections and/or environmental factors determine the immune and inflammatory responses to the unknown antigen(s). Cytokines also operate in the proliferation and differentiation of lymphoid cells. Patients with IgAN have an increased memory repertoire of IgA1-producing B-lymphocytes in their bone marrow together with high plasma levels of IgA1 [11]. Major effects of the cytokines IL-1 and TNF-α include T- and B-cell help, with IL-1Ra blocking the activity of IL-1, while IL-6 affects B-cell differentiation and maturation.

Cytokine genes are polymorphic and their polymorphism may influence the levels of corresponding cytokines [12, 13]. The *IL-1* gene cluster on chromosome 2 (2q12-q21) [13] contains three related genes within a 430 kilobase region, which encode the proinflammatory cytokines IL-1α and IL-1β as well as their endogenous receptor antagonist IL-1Ra. Carriage of the more infrequent allele 2 of the *IL-1*β gene at position −511 (*IL1β2*) is associated with increased IL-1 production [12], and carriage of *IL-1Ra* allele 2 (*IL1RN*2*) with increased production of IL-1Ra [14]. The enhancing effect of *IL1RN*2* on plasma IL-1Ra levels requires the presence of *IL1β2*, which implies that the *IL-1β* gene participates in the regulation of IL-1Ra production [14]. TNF genes locate on chromosome 6 (6p21.3) [13]. Carriage of the more infrequent allele 2 of the *TNF* gene at position −308 (*TNF2*) has been linked to increased TNF transcription in vitro [15, 16], but contradictory results are also reported [17, 18]. *IL-6* gene locates on chromosome 7 (7p21-p14) [13]. *IL-6* gene G/C polymorphism at position −174 also is located in the promoter region of the gene and carriage of the C allele has been associated with decreased IL-6 levels [19].

Genetic polymorphisms of *IL-1* (*α* and *β*), *TNF* (*α* and *β*) and *IL-1Ra* are implicated in several inflammatory diseases [12, 20], such as alopecia areata, rheumatoid arthritis and systemic lupus erythematosus. The carriage rate of *IL1RN*2* has been reported to be higher in diabetic patients with nephropathy than in those without nephropathy [21]. *IL-1Ra* gene polymorphism in IgAN has been reported in two recent studies [22, 23]. Shu and colleagues found an excessive carriage of *IL1RN*2* in patients as compared to healthy controls [23], whereas no such association was found in an earlier study [22]. However, in both studies the carriage rate of *IL1RN*2* was significantly higher in IgAN patients with gross hematuria than in controls. Carriage of *TNF2* also associated with gross hematuria in IgAN patients [23]. The impact of *IL-1* and *IL-6* gene polymorphism on IgAN has not been reported.

It seems possible that both pro- and anti-inflammatory cytokines play a role in the development and progression of IgAN. As polymorphisms in the cytokine genes may determine the production of different cytokines, we investigated the contribution of *IL-1*, *TNF-α*, *IL-1Ra* and *IL-6* gene polymorphism to susceptibility and to prognosis of IgAN in a well-defined, homogeneous patient population. Our patients were Finns in whom IgAN had been diagnosed between 1980 and 1990 and who were still living in our hospital area. They had been followed up for a fairly long period ranging from 6 to 17 (median 11) years from renal biopsy.

METHODS

Patients and controls

All subjects were Caucasians from the area of Pirkanmaa, Finland. Data on the patients have recently been described [3]. Briefly, the original patient population consisted of all 223 IgAN patients diagnosed between January 1980 and December 1990 in Tampere University Hospital, which serves the area of Pirkanmaa with 440,000 inhabitants. The indications for renal biopsy were: microscopic hematuria and proteinuria (≥0.15 g/24 h) in 179 patients, microscopic hematuria alone in 28, proteinuria without hematuria in 13 and acute renal failure in three. Six patients had nephrotic syndrome and 78 (35%) patients had a history of gross hematuria. During the follow-up period, 30 of the original patients died and 15 moved away from Pirkanmaa. The 178 patients still living in the area were invited to attend for assessment between October 1996 and January 1997; 168 (94%) agreed to participate. DNA could be extracted from whole blood samples from all but one of these 168 patients. Thus, the study population comprised 167 patients, 102 (61%) of whom were men and 65 (39%) women. Their mean age (range) at the time of renal biopsy was 38 (8 to 78) years. The median follow-up time (range) from biopsy was 11 (6 to 17) years and from the first signs of IgAN (episode of macroscopic hematuria, discovery of microscopic hematuria, or proteinuria or renal insufficiency) 14 (6 to 57) years. Since diagnosis the patients have been under the control of our hospital or a local health care center. Data on medication, occurrence of gross hematuria, blood pressure, 24-hour urinary protein excretion and serum creatinine were studied at the time of renal biopsy and at the assessment visit.

There were no cases of systemic lupus erythematosus or liver cirrhosis. At the time of renal biopsy eight patients had purpura, in two cases associated with arthritis and abdominal pain (Henoch-Schönlein purpura), one with only arthritis and two with only abdominal pain.

Four hundred healthy blood donors from same area of Finland were used as controls. Blood samples were obtained from the Finnish Red Cross Transfusion Center, Tampere, Finland. The donors were adults, their mean age (range) was 41 (18 to 60) years; 50% were males and 50% females.

Definitions of progression of IgAN, proteinuria and hypertension

The initial measurement of serum creatinine was made at the time of renal biopsy, values ≤125 μmol/L in men or ≤105 μmol/L in women being considered normal. Progression of IgAN was defined as an elevation of serum creatinine above the normal level at the assessment visit and over 20% from baseline. Creatinine values also

had been measured at about one-year intervals during the follow-up period and were used in studying the time to impaired renal function (defined as above) for the purposes of renal survival analysis. Measurement of urinary protein excretion was made quantitatively by 24-hour collection; proteinuria was defined as 24-hour urinary protein excretion ≥ 1 g. History of gross hematuria was registered at the time of renal biopsy and history of gross hematuria was also asked at the time of the assessment visit. The criterion for hypertension was the use of antihypertensive medication or systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg measured by sphygmomanometer in sitting position after rest during hospitalization for the renal biopsy or at assessment.

Analysis of gene polymorphisms

Genomic DNA was isolated from the blood samples using the salting out method [24]. Polymerase chain reaction (PCR)-based genotyping of *IL-1β* (base exchange polymorphism at position −511), *TNF-α* (base exchange polymorphism at position −308), *IL-1Ra* (variable number of tandem repeats in intron 2) and *IL-6* (base exchange G/C at position −174) was carried out as previously described [19, 25–27]. The *IL-1β* gene has two alleles at position −511: the more common allele 1 (*IL1β1*) and the more infrequent *IL1β2*. The most common *IL-1Ra* allele 1 (*IL1RN*1*) contains four repeats, *IL1RN*2* two, *IL-1Ra* allele 3 (*IL1RN*3*) five, *IL-1Ra* allele 4 (*IL1RN*4*) three, and *IL-1Ra* allele 5 (*IL1RN*5*) six. The *TNF-α* gene has two alleles at position −308: the more common allele 1 (*TNF1*) and the more infrequent *TNF2*.

Statistical methods

The SPSS package was used for statistical analysis. Allele frequency was calculated as the number of occurrences of the test allele in the population divided by the total number of alleles. The carriage rate was calculated as the number of individuals carrying at least one copy of the test allele divided by the total number of individuals. Differences in proportions in different patient groups were compared by chi-square test. Odds ratios (OR) (equivalent to approximate relative risk) were calculated for carriage of different alleles or their combinations in IgAN patients compared with controls. OR for carriage of these alleles also were calculated in IgAN patients with and without gross hematuria compared with controls. Confidence limits (CI) of 95% for OR are also shown. Renal survival analysis in patients carrying the specific cytokine gene allele was carried out by the Kaplan-Meier technique. The difference between survival curves was tested by log-rank test. Two-sided *P* values are reported, 0.05 being taken as the level of statistical significance. Tests for Hardy-Weinberg equi-

Table 1. Allele frequencies of cytokine genes in IgAN patients and controls

| | Allele frequency % | | |
| | IgAN | Control | |
Gene	($N = 167$)	($N = 400$)	*P* value[a]
IL1β1	51.8	59.3	0.02
IL1β2	48.2	40.8	
*IL1RN*1*	59.9	70.1	0.002[b]
*IL1RN*2*	38.0	28.9	
*IL1RN*3*	2.1	1.0	
TNF1	91.0	83.7	0.002
TNF2	9.0	16.2	
IL-6 (G)	45.3	44.6	NS
IL-6 (C)	54.3	55.4	

Abbreviations are: IgAN, IgA nephropathy; IL, interleukin; TNF, tumor necrosis factor.
[a] χ^2 test
[b] Patients and controls carrying the very infrequent allele ILRN*3 are omitted in the analysis

librium were calculated using the Arlequin program version 2.000 [28].

Approval of Ethics Committee

This study was approved by the Ethics Committee of Tampere University Hospital. All patients gave their informed consent to participate.

RESULTS

Progression of IgAN, occurrence of proteinuria, hypertension and gross hematuria

One hundred and thirty-six patients (81%) had normal and 31 (19%) elevated serum creatinine at the time of renal biopsy. Eighteen of the 136 patients (13%) who initially had normal serum creatinine showed progression during the follow-up. In 8 out of the 31 (26%) with elevated serum creatinine at biopsy there was a further rise. In another 10 of these 31 (32%), serum creatinine normalized, but in 13 (42%) the renal functional impairment was unchanged. Thus, 26 of the study patients (16%) evinced progression of renal disease according to the definition. ESRD (that is, serum creatinine ≥ 700 μmol/L or requiring dialysis) developed in 10 (6%) patients 2 to 16 years (median 8 years) after the renal biopsy. Proteinuria ≥ 1 g/24 h at biopsy and at assessment was found in 48 (29%) and 35 (21%) patients, respectively. Sixty-four patients (38%) had a history of gross hematuria. Hypertension was found in 80 patients (48%) at the time of renal biopsy and in 114 (68%) at the assessment visit.

Cytokine genotypes

The observed distribution of homozygotes and heterozygotes conformed to Hardy-Weinberg expectations. Table 1 shows the allele frequencies of *IL-1*, *TNF-α*, *IL-1Ra* and *IL-6* genes in patients and healthy controls. *TNF*

Table 2. Carriage rate of cytokine alleles in IgAN patients and controls and odds ratio (OR) of IgAN for carriage of the corresponding allele

| | Carriage rate % | | | |
	IgAN ($N = 167$)	Control ($N = 400$)	OR (95% CI)	P value[a]
IL1β2	74.9	63.5	1.7 (1.1–2.6)	0.009
*IL1RN*2*	59.9	48.5	1.6 (1.1–2.3)	0.01
IL1β2 and *IL1RN*2*	53.3	39.0	1.8 (1.2–2.6)	0.002
IL-6 (G)	69.5	70.5	1.0 (0.6–1.4)	NS
TNF2	17.5	31.3	0.5 (0.3–0.7)	0.001

[a] χ^2 test

Table 3. Risk of IgAN (OR) for subjects carrying different combinations of *IL1β2* and *IL1RN*2* alleles using subjects with neither the *IL1β2* nor *IL1RN*2* allele as reference

*IL1β2/IL1RN*2*	Patients[a] ($N = 167$)	Controls[a] ($N = 400$)	OR (95% CI)	P value[b]
IL1β2+/*IL1RN*2*+	89	156	2.0 (1.2–3.2)	0.005
IL1β2+/*IL1RN*2*−	36	98	1.2 (0.7–2.2)	0.38
IL1β2−/*IL1RN*2*+	11	38	1.0 (0.5–2.2)	0.98
IL1β2−/*IL1RN*2*−	31	108	Reference	

[a] Number of patients or controls with corresponding combination of alleles
[b] χ^2 test

Table 4. Risk of IgAN (OR) for subjects carrying different combinations of *IL1β2* and *TNF2* alleles using subjects without the *IL1β2* allele and with the *TNF2* allele as reference

IL1β2/TNF2	Patients[a] ($N = 166$)	Controls[a] ($N = 400$)	OR (95% CI)	P value[b]
IL1β2+/*TNF2*−	101	177	4.6 (1.9–11.0)	0.001
IL1β2+/*TNF2*+	23	77	2.4 (0.9–6.3)	0.08
IL1β2−/*TNF2*−	36	98	2.9 (1.2–7.5)	0.02
IL1β2−/*TNF2*+	6	48	Reference	

[a] Number of patients or controls with corresponding combination of alleles
[b] χ^2 test

Table 5. Risk of IgAN (OR) for subjects carrying different combinations of the *IL1RN*2* and *TNF2* alleles using subjects without the *IL1RN*2* allele and with the *TNF2* allele as reference

*IL1RN*2/TNF2*	Patients[a] ($N = 166$)	Controls[a] ($N = 400$)	OR (95% CI)	P value[b]
*IL1RN*2*+/*TNF2*−	80	130	4.2 (2.0–8.8)	<0.001
*IL1RN*2*+/*TNF2*+	20	64	2.1 (0.9–5.0)	0.09
*IL1RN*2*−/*TNF2*−	57	145	2.7 (1.2–5.7)	0.01
*IL1RN*2*−/*TNF2*+	9	61	Reference	

[a] Number of patients or controls with corresponding combination of alleles
[b] χ^2 test

Table 6. Risk of IgAN (OR) for subjects carrying different combinations of *IL-1* cluster2[a] alleles and *TNF2* alleles using subjects without the *IL-1* cluster2 alleles and with the *TNF2* allele as reference

IL-1 cluster2[a]/*TNF2*	Patients[b] ($N = 166$)	Controls[b] ($N = 400$)	OR (95% CI)	P value[c]
IL-1 cluster2+/*TNF2*−	70	107	5.0 (2.4–10.3)	<0.001
IL-1 cluster2+/*TNF2*+	19	49	2.9 (1.3–6.7)	0.01
IL-1 cluster2−/*TNF2*−	67	168	3.0 (1.5–6.2)	0.002
IL-1 cluster2−/*TNF2*+	10	76	Reference	

[a] *IL-1* cluster2+ means those subjects who carried both *IL1β2* and *IL1RN*2* alleles, and *IL-1* cluster2− were those who did not carry these alleles simultaneously
[b] Number of patients or controls with corresponding combination of alleles
[c] χ^2 test

genotyping could not be carried out in one patient sample. The rates of carriage of the more infrequent alleles are given in Table 2. Excessive carriage of *IL1β2* and *IL1RN*2* as well as non-carriage of *TNF2* was found in the patient group as compared with the normal controls (Table 2). Odds ratios with 95% confidence intervals (CI) of IgAN for carriage of these alleles are also given in Table 2. The odds ratio of IgAN for non-carriage of *TNF2* was 2.1 (1.4 to 3.4).

There was a strong association between carriage of *IL1*β2 and *IL1RN*2* in both patients and controls. In patients 89 out of 125 (71%) of carriers of *IL1*β2 were carriers of *IL1RN*2* versus 11 out of 42 (26%) of non-carriers of *IL1*β2 (χ^2 test, $P < 0.001$). The corresponding figures in controls were 156 out of 254 (61%) versus 38 out of 146 (26%) (χ^2 test, $P < 0.001$). The combined action of *IL1*β2 and *IL1RN*2* or the action of either of them on the risk of IgAN were therefore compared to their absence. The results with 95% CI are given in Table 3, which shows the risk of IgAN to be associated with carriage of both of these alleles.

Carriage of *TNF2* was associated with a decreased risk of IgAN (Table 2) and carriage of both *IL1*β2 and *IL1RN*2* with increased risk of this disease (Table 3). The *IL-1* gene cluster and *TNF* genes are located in different chromosomes and are not known to be in linkage. There was no association between non-carriage of *TNF2* and carriage of *IL1*β2 and *IL1RN*2* in patients or controls. Table 4 shows the effect of different combi-

nations of carriage and non-carriage of *IL1*β2 and *TNF2* on the risk of IgAN as compared to non-carriage of *IL1*β2 and carriage of *TNF2*. The effect of different combinations of carriage and non-carriage of *IL1RN*2* and *TNF2* on the risk of IgAN as compared to non-carriage of *IL1RN*2* and carriage of *TNF2* is given in Table 5. Table 6 shows the effect of different combinations of carriage and non-carriage of both *IL1*β2 and *IL1RN*2* as well as *TNF2* on the risk of IgAN as compared to those who did not carry both of these *IL-1* cluster genes and carried *TNF2*.

Prognosis of IgAN and cytokine polymorphisms

There were no significant differences with respect to presence of hypertension or proteinuria at time of biopsy or assessment visit according to different cytokine geno-

Table 7. Carriage rates of various cytokine alleles according to history of gross hematuria

	Clinical picture			
Cytokine allele	Carriage rate in pts without gross hematuria % (N = 103)	Carriage rate in pts with gross hematuria % (N = 64)	OR (95% CI)[a]	P value[a]
IL1β2	81.6	64.1	2.5 (1.2–5.1)	0.01
IL1RN*2	63.1	54.7	1.4 (0.8–2.7)	0.3
TNF2	20.4	12.7	1.7 (0.7–4.3)	0.2
IL-6 (−174) G	71.8	65.6	0.7 (0.4–1.5)	0.4

[a] χ^2 test; patients without gross hematuria are compared to those with gross hematuria

types or carriage of different cytokine alleles. Progression or renal survival (studied by Kaplan-Meier technique) did not differ significantly according to different cytokine genotypes or carriage of different cytokine alleles (data not shown). No deleterious effects were found on prognosis of carriage of *IL1β2*, *IL1RN*2*, or both of these alleles, neither of carriage of *TNF2* or either of the *IL-6* alleles. Progression was observed in 15% of carriers versus 16% of non-carriers of *IL1β2*, in 13% of carriers versus 19% of non-carriers of *IL1RN*2*, in 12% of carriers versus 19% of non-carriers of both of these alleles, and in 10% of carriers versus 17% of non-carriers of *TNF2*.

Gross hematuria and cytokine polymorphisms

Carriage of *IL1β2* differed according to the history of gross hematuria (Table 7). Carriage of *IL1β2* in the patients who did not present gross hematuria differed significantly from controls (81.6% in patients vs. 63.5% in controls; P = 0.001), while there was no significant difference in those with gross hematuria. Likewise, carriage of *IL1RN*2* differed significantly from controls only in the subgroup of patients without gross hematuria (63.1% in patients vs. 48.5% in controls; P = 0.008). Carriage of *TNF2* was significantly less frequent in patients both without (20.4%) and with (12.7%) gross hematuria as compared to controls (31.3%; P = 0.03 and P = 0.002, respectively).

DISCUSSION

This study illustrates the association between carriage of *IL1β2*, *IL1RN*2* and non-carriage of *TNF2* and susceptibility to IgAN. Carriage of both *IL1β2* and *IL1RN*2* together with non-carriage of *TNF2* was associated with highly increased risk of IgAN. None of the cytokine allele polymorphisms studied affected the prognosis of IgAN.

Referral patterns and indications for renal biopsy vary in different studies, which probably influences the proportion of patients with progressive disease. Also, racial differences and management of patients affect the outcome of IgAN. Our patient population was homogeneous and representative of IgAN, and the criteria for biopsy were applied consistently throughout the study period. Our policy has been to perform a renal biopsy also in cases of minor urinary abnormalities. This is reflected in a fairly favorable prognosis of IgAN; the pattern is, however, quite similar to that in another European study [29]. Furthermore, the strength of the present study was a large study population with a long follow-up and a low number of patients lost to follow-up. Thirty of the original 223 patients had died during follow-up (5 of them from uremia and 25 of other causes [3]) and their cytokine gene polymorphism was not analyzed. This may affect the results with respect to progression, which was more common among those who died. However, 26 patients could be analyzed who showed progression during the follow-up.

The definitions of impaired renal function and progression of IgAN were based on serum creatinine levels, which were used routinely in the follow-up of all patients [3]. It is well established that substantial reductions in renal function may occur before serum creatinine becomes abnormal; thus it is possible that in some patients defined as having normal renal function it was actually slightly decreased. We also included an elevation of creatinine of over 20% in the definition of progression of IgAN to avoid misclassification of patients with stable disease.

There are no earlier reports of carriage of *IL1β2* in IgAN, but carriage of *IL1RN*2* has been studied. In accord with our results Shu and colleagues found an association between carriage of *IL1RN*2* and IgAN [23]. The carriage rates of *IL1RN*2* in both patients and controls, however, differed from our current study: 4% in the controls and 20% in the patients in their study [23] versus 49% in controls and 60% in patients our study. This discrepancy probably reflects racial differences and shows that the carriage of *IL1RN*2* per se cannot have an independent role in the etiology and pathogenesis of IgAN. In an earlier study Liu and coworkers found an association between excessive carriage of *IL1RN*2* and Henoch-Schönlein nephritis and IgAN with recurrent gross hematuria, but not with other clinical patterns [22]. This is quite the opposite of our results, where an associa-

tion between carriage of *IL1RN*2* and IgAN was found only in patients who did not have gross hematuria. Liu and coworkers [22] found that the carriage rate of *IL1RN*2* again was different from our study results and those of Shu and colleagues [23]. They reported carriage rate of 25% in controls, 27% in all IgAN patients and 35% in those with recurrent gross hematuria [22]. An association between carriage of *IL1RN*2* and diabetic nephropathy also has been reported [21], and in the Caucasian population in question the carriage rate in controls was quite similar (42%) to our observation. An association between *IL1RN*2* and ESRD due to diabetes or other diseases has been found by genetic linkage analysis in African Americans as well [30].

Our study demonstrates that simultaneous carriage of both *IL1β2* and *IL1RN*2* increases the risk of IgAN. The mechanism underlying this association remains a matter of speculation. Pro-inflammatory cytokines, especially IL-1, are of central importance in T- and B-cell help and the initiation, for example, of humoral immune responses such as IgA production. Enhanced IgA responses have been observed after parental or oral vaccination of IgAN patients [31, 32]. In IgAN, IgA is defectively glycosylated [33–35] and the deposition in the mesangium is followed by mesangial proliferation and chronic glomerular inflammation. IL-1 is expressed in the glomeruli of these patients [8, 10], and it may be involved in mesangial cell proliferation [36] and extracellular matrix production [37]. IL-1Ra also plays a role as a natural blocking agent in the inflammation mediated by IL-1. In IgAN the association between alleles of the *IL-1* cluster genes, *IL1β2* and *IL1RN*2*, has not previously been reported. The risk of IgAN was associated with simultaneous carriage of *IL1β2* and *IL1RN*2*, but not with carriage of either *IL1β2* or *IL1RN*2* alone. This may be explained by *IL1RN*2*, or an allele closely associated to it, being probably the strongest up-regulator of in vitro IL-1β levels [38], and thus, the effect of carrying both these alleles is probably pro-inflammatory. Although there are several hypothetical links between IL-1, IL1Ra and IgAN, it is possible that both *IL1β2* and *IL1RN*2* are markers of a linked locus that is the real disease-associated locus. The observed association between the alleles of the *IL-1* cluster genes or of *TNF* genes and IgAN cannot be explained by the earlier findings of Gharavi and coworkers on the linkage of IgAN to chromosome 6q22-q23 [6], as the locations of these cytokine genes are different.

We found a significantly higher number of noncarriers of *TNF2* among patients with IgAN than in controls. In the study of Shu and coworkers the carriage rate of *TNF2* was also less frequent in patients than in controls, though the difference was not statistically significant (11 vs. 21%) [23]. They found an excessive carriage of *TNF2* in IgAN patients with gross hematuria [23], whereas in our

patients with gross hematuria the carriage of *TNF2* was rare. These opposite results are difficult to interpret. The occurrence of gross hematuria was different in these studies (38% in our study vs. 12% in the study by Shu and coworkers), which may affect the results. Although increased *TNF* transcriptional activity has been associated with the *TNF2* (−308) allele, it has not been shown to have any relationship to circulating levels of TNF-α, but local concentration of TNF-α at the site of inflammation may be influenced [39]. In patients with systemic lupus erythematosus *TNF2* is associated with a reduced risk of lupus nephritis [39]. TNF-α has a dual function, being pro-inflammatory in the initial infection and anti-inflammatory or immunoregulatory in the later phases of the response [39]. This may be important in interpreting the results of the present study in the case of patients with chronic inflammation in their kidneys triggered by an unknown antigen(s).

The present study showed no association between progression of IgAN and any of the cytokine gene polymorphisms studied. In contrast to our results, Shu et al found an association between carriage of *IL1RN*2* and progression [23]. Their definition of progression differed from ours. The most striking difference between these studies was the proportion of patients who had reached ESRD: 31% in their study as compared to 6% in ours. While the indications for renal biopsy differed less markedly, only 52% of all patients diagnosed were studied with regard to cytokine polymorphism in their study compared to 75% here. Differences in patient material may partly explain these discrepant results. Shu and coworkers also found no association between *TNF* (−308) polymorphism and progression [23]. It also is possible that the cytokine polymorphism found to be associated with susceptibility has no effect on disease severity and progression, and that nonimmunologic mechanisms such as hypertension, proteinuria and metabolic disturbances play a more important role.

In conclusion, the present study shows that carriage of *IL1β2* together with *IL1RN*2* increases the risk of IgAN twofold as compared to non-carriage of either of these alleles. Carriage of *TNF2* was found to be protective against IgAN. Carriage of *IL1β2* and *IL1RN*2* together with non-carriage of *TNF2* increased the risk of IgAN fivefold, suggesting that different cytokines probably function in concert in the development of IgAN. No association was found between progression and cytokine gene polymorphisms.

ACKNOWLEDGMENTS

Reprint requests to Jaana Syrjänen, M.D., Medical School, FIN-33014 University of Tampere, Tampere, Finland.
E-mail: jaana.syrjanen@uta.fi

REFERENCES

1. D'AMICO G: The commonest glomerulonephritis in the world: IgA nephropathy. *Q J Med* 245:709–727, 1987
2. PACKHAM D: Prognosis in IgA nephropathy. *Nephrol* 3:231–235, 1997
3. SYRJÄNEN J, MUSTONEN J, PASTERNACK A: Hypertriglyceridaemia and hyperuricaemia are risk factors for progression of IgA nephropathy. *Nephrol Dial Transplant* 15:34–42, 2000
4. RANTALA I, MUSTONEN J, HURME M, *et al*: Pathogenetic aspects of IgA nephropathy. *Nephron* 88:193–198, 2001
5. HSU SI, RAMIREZ SB, WINN MP, *et al*: Evidence for genetic factors in the development and progression of IgA nephropathy. *Kidney Int* 57:1818–1835, 2000
6. GHARAVI AG, YAN Y, SCOLARI F, *et al*: IgA nephropathy, the most common cause of glomerulonephritis, is linked to 6q22–23. *Nat Genet* 26:354–357, 2000
7. MONTINARO V, HEVEY K, AVENTAGGIATO L, *et al*: Extrarenal cytokines modulate the glomerular response to IgA immune complexes. *Kidney Int* 42:341–353, 1992
8. YOSHIOKA K, TAKEMURA T, MURAKAMI K, *et al*: In situ expression of cytokines in IgA nephritis. *Kidney Int* 44:825–833, 1993
9. CHEN A, CHEN WP, SHEU LF, LIN CY: Pathogenesis of IgA nephropathy: In vitro activation of human mesangial cells by IgA immune complex leads to cytokine secretion. *J Pathol* 173:119–126, 1994
10. YANO N, ENDOH M, NOMOTO Y, *et al*: Phenotypic characterization of cytokine expression in patients with IgA nephropathy. *J Clin Immunol* 17:396–403, 1997
11. VAN DEN WALL BAKE AW, DAHA MR, HAAIJMAN JJ, *et al*: Elevated production of polymeric and monomeric IgA1 by the bone marrow in IgA nephropathy. *Kidney Int* 35:1400–1404, 1989
12. HURME M, LAHDENPOHJA N, SANTTILA S: Gene polymorphisms of interleukins 1 and 10 in infectious and autoimmune diseases. *Ann Med* 30:469–473, 1998
13. BIDWELL J, KEEN L, GALLAGHER G, *et al*: Cytokine gene polymorphism in human disease: On-line databases. *Genes Immun* 1:3–19, 1999
14. HURME M, SANTTILA S: IL-1 receptor antagonist (IL-1Ra) plasma levels are co-ordinately regulated by both IL-1Ra and IL-1beta genes. *Eur J Immunol* 28:2598–2602, 1998
15. KROEGER KM, CARVILLE KS, ABRAHAM LJ: The -308 tumor necrosis factor-alpha promoter polymorphism effects transcription. *Mol Immunol* 34:391–399, 1997
16. WILSON AG, SYMONS JA, MCDOWELL TL, *et al*: Effects of polymorphism in the tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci USA* 94:3195–3199, 1997
17. BRINKMAN BM, ZUIJDEEST D, KAIJZEL EL, *et al*: Relevance of the tumor necrosis factor alpha (TNF alpha) −308 promoter polymorphism in TNF alpha gene regulation. *J Inflamm* 46:32–41, 1995
18. STUBER F, UDALOVA IA, BOOK M, *et al*: −308 tumor necrosis factor (TNF) polymorphism is not associated with survival in severe sepsis and is unrelated to lipopolysaccharide inducibility of the human TNF promoter. *J Inflamm* 46:42–50, 1995
19. FISHMAN D, FAULDS G, JEFFERY R, *et al*: The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest* 102:1369–1376, 1998
20. WILSON AG, DI GIOVINE FS, DUFF GW: Genetics of tumour necrosis factor-alpha in autoimmune, infectious, and neoplastic diseases. *J Inflamm* 45:1–12, 1995
21. BLAKEMORE AI, COX A, GONZALEZ AM, *et al*: Interleukin-1 receptor antagonist allele (IL1RN*2) associated with nephropathy in diabetes mellitus. *Hum Genet* 97:369–374, 1996
22. LIU ZH, CHENG ZH, YU YS, *et al*: Interleukin-1 receptor antagonist allele: is it a genetic link between Henoch-Schönlein nephritis and IgA nephropathy? *Kidney Int* 51:1938–1942, 1997
23. SHU KH, LEE SH, CHENG CH, *et al*: Impact of interleukin-1 receptor antagonist and tumor necrosis factor-alpha gene polymorphism on IgA nephropathy. *Kidney Int* 58:783–789, 2000
24. MILLER SA, DYKES DD, POLESKY HF: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 16:1215, 1988
25. DI GIOVINE FS, TAKHSH E, BLAKEMORE AI, DUFF GW: Single base polymorphism at -511 in the human interleukin-1 beta gene (IL1 beta). *Hum Mol Genet* 1:450, 1992
26. WILSON AG, DI GIOVINE FS, BLAKEMORE AI, DUFF GW: Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product. *Hum Mol Genet* 1:353, 1992
27. TARLOW JK, BLAKEMORE AI, LENNARD A, *et al*: Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. *Hum Genet* 91:403–404, 1993
28. SCHNEIDER S, ROESSLI D, EXCOFFIER L: *Arlequin ver. 2.000: A Software for Population Genetics Data Analysis.* Geneva, Genetics and Biometry Laboratory, University of Geneva, 2000
29. ALAMARTINE E, SABATIER JC, GUERIN C, *et al*: Prognostic factors in mesangial IgA glomerulonephritis: An extensive study with univariate and multivariate analyses. *Am J Kidney Dis* 18:12–19, 1991
30. FREEDMAN BI, YU H, SPRAY BJ, *et al*: Genetic linkage analysis of growth factor loci and end-stage renal disease in African Americans. *Kidney Int* 51:819–825, 1997
31. LEINIKKI PO, MUSTONEN J, PASTERNACK A: Immune response to oral polio vaccine in patients with IgA glomerulonephritis. *Clin Exp Immunol* 68:33–38, 1987
32. PASTERNACK A, MUSTONEN J, LEINIKKI P: Humoral immune response in patients with IgA and IgM glomerulonephritis. *Clin Exp Immunol* 63:228–233, 1986
33. ALLEN AC, HARPER SJ, FEEHALLY J: Galactosylation of N- and O-linked carbohydrate moieties of IgA1 and IgG in IgA nephropathy. *Clin Exp Immunol* 100:470–474, 1995
34. HIKI Y, HORII A, IWASE H, *et al*: O-linked oligosaccharide on IgA1 hinge region in IgA nephropathy. Fundamental study for precise structure and possible role. *Contrib Nephrol* 111:73–84, 1995
35. TOMANA M, MATOUSOVIC K, JULIAN BA, *et al*: Galactose-deficient IgA1 in sera of IgA nephropathy patients is present in complexes with IgG. *Kidney Int* 52:509–516, 1997
36. WARDLE EN: Cytokine growth factors and glomerulonephritis. *Nephron* 57:257–261, 1991
37. BORDER WA, NOBLE NA: From serum sickness to cytokines: Advances in understanding the molecular pathogenesis of kidney disease. *Lab Invest* 68:125–128, 1993
38. SANTTILA S, SAVINAINEN K, HURME M: Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production in vitro. *Scand J Immunol* 47:195–198, 1998
39. HAJEER AH, HUTCHINSON IV: TNF-alpha gene polymorphism: Clinical and biological implications. *Microsc Res Tech* 50:216–228, 2000