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Glomerulonephritis associated with MRSA infection: A possible role of bacterial superantigen

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Glomerulonephritis associated with MRSA infection: A possible role of bacterial superantigen. We report 10 cases of glomerulonephritis following methicillin-resistant *Staphylococcus aureus* (MRSA) infection. The clinical features of this syndrome were an abrupt or insidious onset of rapidly progressive glomerulonephritis (RPGN) with nephrotic syndrome and occasionally purpura, following MRSA infection. The renal histologic findings showed a variety of types of proliferative glomerulonephritis with varying degrees of crescent formation; immunofluorescence revealed of glomerular deposition of IgA, IgG, and C3. Laboratory findings showed polyclonal increases of serum IgA and IgG, with high levels of circulating immune complexes (ICs). Increased numbers of DR^+CD4^+, and DR^+CD8^+ T cells were observed in the peripheral circulation, with a high frequency of T cell receptor (TCR) $V_\beta{}^+$ cells. MRSA produced enterotoxins C and A and toxic shock syndrome toxin (TSST)-1, all of which are known to act as superantigens. From the above observations, we speculate that post-MRSA glomerulonephritis may be induced by superantigens causing production of high levels of cytokines, and polyclonal activation of IgG and IgA. The formation of ICs containing IgA and IgG in the circulation result in development of glomerulonephritis and vasculitis. Accordingly, microbial superantigens may play an important role in the pathogenesis of this unique syndrome of nephritis and vasculitis.

The histopathologic changes of post-infectious glomerulonephritis are variable and depend on a number of factors related to both the host and the infecting organism. The association between group A hemolytic streptococcal infection and the subsequent development of acute nephritis is well recognized. While post-streptococcal glomerulonephritis is now relatively uncommon in developed countries, it has become apparent that other infections may produce a variety of clinical syndromes and glomerular lesions [1]. Many factors are likely involved in the changing epidemiology of infection-associated glomerular disease, including socioeconomic status and environmental health.

In spite of pharmacologic advances, many antibiotic-resistant bacterial infections are being reported. Infection with MRSA is one of the major opportunistic hospital infections [2], and patients with MRSA infection frequently develop septicemia or toxic shock syndrome (TSS), which are caused by the enterotoxins (SEs) of MRSA [3]. Staphylococcal infections have also been identified as causal agents of glomerulonephritis. Most reports linking staphylococcal infection and glomerulonephritis have emphasized two clinical settings: *Staphylococcus epidermidis* bacteremia with ventriculo-jugular shunts (VJS), and *Staphylococcus aureus* bacteremia with endocarditis [4–6].

SEs have recently been recognized to act as "superantigens" [7–10]. SEs can bind directly to major histocompatibility complex (MHC) class II molecules on antigen presenting cells and the specific V_β chain of the TCR. The SEs stimulate resting T cells to proliferate and cause massive T cell activation and a subsequent release of T cell-derived lymphokines such as interleukin (IL)-2, IL-6, and other cytokines including IL-1, TNF and interferon-γ [11–13]. $TCR^+V_\beta{}^+$ cells are usually present at levels less than 5% in peripheral blood lymphocytes [7–10, 13].

We report 10 cases of MRSA-associated glomerulonephritis with polyclonal increases of IgA and IgG and massive T cell activation. In this study, we analyzed the clinical and immunologic characteristics of these patients and investigated the role of SEs as superantigens in the pathogenesis of this unique form of glomerulonephritis.

Methods

Patients

We studied 10 patients with glomerulonephritis occurring after MRSA infection and 10 patients with MRSA infection without nephritis; all patients were admitted to Tsukuba University Hospital (Table 1). Fifty-one patients with IgA nephropathy and 24 normal individuals were used as controls. The criteria of clinical syndromes were defined by WHO criteria [14]. Case definition of toxic shock syndrome was as defined by Reingold et al [15].

Bacteriologic analysis

Identification of MRSA. Samples from patients were cultured and the sensitivities of bacteria to antibiotics were tested using an antibiotic sensitivity kit (Eiken Kagaku Co., Tokyo, Japan).

Identification of coagulase types and SEs. Culture supernatants were assayed with a staphylococcal enterotoxin detection kit using reversed passive latex agglutination (Denka Seiken Co., Tokyo, Japan) and immunoblotting; coagulase types were determined using a detection kit (Denka Seiken).

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Table 1. Patient profiles and clinical findings in patients with nephritis, and underlying conditions, types of infections, and types of bacteria

No.	Sex	Age	Onset after infection *weeks*	Clinical syndromes of renal disease	Purpura	Outcome of GN
A MRSA infection with nephritis						
1	M	21	16	RPGN + NS	yes	HD
2	M	27	5	NS	yes	improved
3	M	60	11	RPGN	no	improved
4	M	61	4	RPGN + NS	no	improved
5	M	69	6	NS	no	death due to underlying disease
6	M	65	2	RPGN + NS	no	HD to S_{Cr} 2.2 mg/dl (improved)
7	M	84	2	RPGN + NS	no	death due to underlying disease
8	F	66	5	RPGN	no	death due to underlying disease
9	M	60	8	RPGN + NS	no	HD
10	M	65	10	NS	no	improved

No.	Sex	Age	Underlying conditions	Types of infection	Bacteria	Types of coagulase and enterotoxin
B MRSA infection with nephritis						
1	M	21	trauma	abscess of abdominal cavity + sepsis	MRSA	Coagulase type II, enterotoxin C and TSST-1
2	M	27	retroperitoneal tumor	abscess of abdominal cavity + sepsis	MRSA	Coagulase type II, enterotoxin C and TSST-1
3	M	60	phlegmone	phlegmone + sepsis	St. pyogenes/ MRSA	Coagulase type II, enterotoxin A, B, C and TSST-1
4	M	61	postthymectomy-abscess	abscess of pleural cavity + sepsis	MRSA	Coagulase type II, enterotoxin A, C and TSST-1
5	M	69	lung cancer	pneumonia + sepsis	MRSA	Coagulase type II, enterotoxin A, C and TSST-1
6	M	65	arthritis	purulent arthritis + sepsis	MRSA	Coagulase type II, enterotoxin C and TSST-1
7	M	84	subdural hematoma	pneumonia + sepsis	MRSA	ND
8	F	66	gall bladder cancer	abscess of abdominal cavity + sepsis	MRSA	Coagulase type II, enterotoxin A, C and TSST-1
9	M	60	peritonitis, pancreatitis	abscess of abdominal cavity + sepsis	MRSA	Coagulase type II, enterotoxin A, C and TSST-1
10	M	65	fistula-colon	abscess of abdominal cavity + sepsis	MRSA	Coagulase type II, enterotoxin A, C and TSST-1
C MRSA infection without nephritis						
1	F	58	acute pancreatitis	abscess of abdominal cavity + sepsis	MRSA	Coagulase type II, enterotoxin A and TSST-1
2	M	68	IVH-sepsis	pneumonia + sepsis	MRSA	Coagulase type II, enterotoxin C and TSST-1
3	M	74	ureter cancer, ARF due to shock	pneumonia + sepsis	MRSA	Coagulase type II, enterotoxin A and TSST-1
4	F	79	perforation of small intestine	abscess of abdominal cavity + sepsis	MRSA	Coagulase type II, enterotoxin C and TSST-1
5	M	65	meningioma-postop	brain abscess + sepsis	MRSA	Coagulase type II, enterotoxin C and TSST-1
6	F	29	ulcer of leg	abscess of abdominal cavity + sepsis	MRSA	Coagulase type II, enterotoxin C and TSST-1
7	M	51	traffic accident	infection of skin graft + sepsis	MRSA	Coagulase type II, enterotoxin A and C
8	M	50	colon cancer, ileus	abscess of abdominal cavity + sepsis	MRSA	Coagulase type II, enterotoxin A, C and TSST-1
9	M	54	emphysema	pneumonia + sepsis	MRSA	Coagulase type II, enterotoxin A, C and TSST-1
10	M	68	myocardial infarction	sepsis	MRSA	Coagulase type II, enterotoxin C and TSST-1

Clinical syndromes of renal disease are defined by the WHO criteria [10]. Abbreviations are: RPGN, rapidly progressive glomerulonephritis; NS, nephrotic syndrome; Onset, onset of glomerulonephritis (occurrence of proteinuria, hematuria, and edema); MRSA, methicillin-resistant *Staphylococcus aureus*; St. pyogenes, *Streptococcus pyogenes*; TSST-1, toxic shock syndrome toxin-1; ND, not done.

Histologic examination

Renal biopsy was performed in five cases and autopsy was performed in one case. For light microscopy, tissues were fixed in buffered formalin, processed in the usual fashion, and stained with hematoxylin-eosin, periodic acid-Schiff and periodic acid-silver methenamine reagent. A portion of each specimen was frozen with n-hexane in solid carbon-dioxide-acetone for immunofluorescence microscopy (IF). The 4-μm tissue sections were stained in the standard manner, using FITC-labeled Ab against human IgG, IgA, IgM, C3, and rabbit anti-SEs (Cappel, Organon Teknika Corp. West Chester, PA, USA): A, B, C, D, and TSST-1 Abs (Denka Seiken Co.) or mouse monoclonal Abs (Chemunex,

Maisons-Alfort, France), rabbit-anti-protein A Ab (Rockland, Inc., Gilbertsville, PA, USA), mouse monoclonal anti-*Staphylococcus aureus* (Chemicon International, Inc., Temecula, CA, USA) and FITC-anti-rabbit or FITC-anti-mouse IgG or IgM (Cappel), as secondary Abs.

Lymphocyte subset analysis

Peripheral lymphocytes from patients with MRSA infection with nephritis, MRSA infection without nephritis, healthy volunteers and patients with IgA nephropathy were obtained. Mononuclear cells were isolated by density centrifugation. Lymphocyte subsets were analyzed on an FACScan (Becton Dickinson Immunocytometory Systems, San Jose, CA, USA), using the following biotin, phycoerythrin (PE) or FITC-labeled monoclonal Abs: anti-CD4 (Leu-3a) (mouse IgG1), anti-CD3 (Leu-4) (mouse IgG1), anti-CD19 (Leu-12) (mouse IgG1), and anti-HLA-DR (mouse IgG2a) Abs (Beckton Dickinson). The variable regions of human TCR were analyzed by three color analysis, using the Ab TCR screening panel 1F, which contained Abs against six V_β subsets [V_β5.2 + 5.3Ag (V_β5a), V_β5.3Ag (V_β5b), V_β5.1 (V_β5c), V_β6.7 (V_β6a), V_β8 subfamily (V_β8a), V_β12.1 (V_β12a)], one Vα2 subfamily (αV2a) (T Cell Diagnostics Inc., Cambridge, MA, USA) and anti-CD4, CD8 Abs.

Immune complex (IC) detection by ELISA

Serum samples were added to ELISA plates, and coated with mouse monoclonal anti-C3d Ab, and peroxidase-conjugated monoclonal Ab anti-human IgG (mouse IgG1) and monoclonal Ab anti-human IgA (mouse IgG1) were used as secondary Abs (immune complex detection test, QUIDEL, Advanced Research Technologies, San Diego, CA, USA). Amounts of IgG ICs were expressed as μg equivalent of human IgG, and those of IgA ICs were expressed as OD units.

Detection of autoantibodies

Anti-glomerular basement membrane (GBM) Ab and antinuclear Ab were analyzed by indirect immunofluorescence using normal human kidney sections and HEp-2 cells (HEPANA test; Medical & Biological Laboratories, Nagoya, Japan). Anti-neutrophil cytoplasmic antibodies (ANCA) were detected by indirect immunofluorescence using human neutrophils. Cryoglobulins were assayed by the cryoglobulin precipitation method. Rheumatoid factor (RF) was detected by the latex agglutination test.

Statistical analysis

Statistical analysis for T cell-subsets was performed by analysis of variance (ANOVA) and by the Mann-Whitney test. Differences in enterotoxins between patients with nephritis and without nephritis were analyzed by Fisher's exact probability test.

Results

Clinical features of patients with MRSA infection with or without nephritis and outcome of the nephritis

The clinical features of the 10 patients with MRSA with glomerulonephritis are shown in Table 1A. Within 10 weeks after the onset of MRSA infections, massive proteinuria with hematuria appeared in 8 of the 10 cases. In 7 cases the clinical presentation was RPGN, and 8 patients had the nephrotic syndrome. Purpura was observed in 2 cases. Two patients ultimately required therapy with continuous hemodialysis (HD). In 5 cases, the nephritis remitted with cure of the infection. None of these 10 patients had TSS or evidence of endocarditis, or hypotension during the course of nephritis.

In case 3 the cause of infection was a scratch wound. Bacteria found in the early stage of infection were *St. pyogenes* and MRSA. This patient developed ARF due to aminoglycosides and received HD. He recovered four weeks after the initiation of HD. At that point, there was no proteinuria or hypotension, and no evidence of TSS or endocarditis. Lymphocyte subsets were measured before the onset of glomerulonephritis. Four weeks after the last HD therapy his urinary protein gradually increased, accompanied by a polyclonal rise in IgG and IgA and development of RPGN.

In case 6 the cause of infection was trauma. The patient had septicemia and also had arthritis due to MRSA infection. He received aminoglycosides and developed ARF. After recovery from ARF, proteinuria developed with progression of RPGN with nephrotic syndrome. There was no evidence of TSS, or endocarditis, as judged by echocardiography.

Among 10 cases of MRSA infection without nephritis, 7 cases showed varying levels of hematuria at the start of observation, and 3 cases still showed hematuria at the end of observation. None of these patients showed deterioration in renal function.

Bacteriologic analysis

Results of the bacteriologic analysis are shown in Table 1 B and C. MRSAs were detected in all cases, and *St. pyogenes* was also detected in one case. The coagulase was consistently type II in all cases, and SE-C, A, and TSST-1 were common toxins in these patients. There were no significant differences of SE types between patients with MRSA infection with nephritis and those without nephritis ($\chi^2 = 0.867$, $P = 0.833$).

Laboratory data

The serum levels of IgA in patients with nephritis were significantly higher than those in patients without nephritis ($P < 0.0004$; Tables 2 and 3).

In the control group, the levels of serum IgA were increased in four cases and primary selective IgA deficiency was observed in one case (case 5).

The serum complement levels were within normal limits, or elevated in patients with nephritis compared to normal individuals.

Autoantibodies, including anti-DNA Ab, anti-nuclear Abs, RF, ANCA, and Ab anti-GBM were not detected. Cryoglobulins were also not detected.

Histopathological study

Various types of proliferative glomerulonephritis with varying degrees of crescent formation were observed in six cases (Table 4, Figure 1). Mononuclear cell infiltration into the tubular epithelium and the interstitium (tubulointerstitial nephritis) was observed. On IF examination IgA, IgG, and C3 deposits in both the mesangium and peripheral capillary walls were observed. There was no significant staining for SEs (SE-A, B, C, D, E, TSST-1),

Table 2. Laboratory data at the onset, the nadir of renal function, and the end of observation

No.	Urine protein at onset *g/day*	Hematuria at onset *grade*	S_{Cr} at onset *mg/dl*	Max urine protein *g/day*	Max S_{Cr} *mg/dl*	Urine protein	Hematuria	S_{Cr}
						at the end of follow-up		
A MRSA infection with nephritis								
1	1.82	3+	0.60	9.00	HD	HD	HD	HD
2	12.00	2+	0.50	20.00	1.5	0.2	(−)	0.6
3	0.30	3+	1.20	1.20	2	(−)	(−)	1.9
4	0.40	2+	0.80	5.40	2.5	4.3	3+	1.5
5	2.20	1+	1.00	5.10	1.8	7.6	1+	1.7
6	1.00	3+	9.90	5.50	HD-2.1	0.6	3+	2.2
7	3.00	1+	1.10	10.00	3.5	2	3+	3.5
8	1+	3+	1.5	2.5	7.3	1.4	3+	7.3
9	1+	3+	1	16.4	HD	HD	HD	HD
10	1.4	2+	1.3	6.4	1.3	0.1	2+	1.3
B MRSA infection without nephritis								
1	(−/+−)	2+	0.5	(−)	0.6	(−)	(−)	0.5
2	(+/−)	3+	2.5	(+/−)	3.7	0.26	2+	2.2[a]
3	(−)	3+	1.3	(+/−)	HD	HD	HD	HD[b]
4	(+/−)	1+	0.4	(−)	0.5	(−)	(+/−)	0.5
5	(+/−)	3+	0.9	(−)	0.9	(−)	(−)	0.5
6	(−)	(−)	0.6	(−)	0.9	(−)	(−)	0.6
7	(+/−)	1+	0.6	(+/−)	0.8	(−)	3+	0.6
8	(−)	(−)	0.8	(−)	1	(−)	(−)	1.1
9	(−)	3+	0.6	(+/−)	1	(−)	(−)	0.8
10	(−)	(+/−)	1.1	(−)	1.1	(−)	1+	1.1

Abbreviations are: S_{Cr}, serum creatinine; HD, hemodialysis.
[a] Due to aminoglycoside
[b] Due to DIC/MOF

Staphylococcus aureus (SA) antigen or protein A from SA in the glomeruli. Cutaneous biopsy in two patients revealed leukocytoclastic vasculitis with IgA deposition.

Lymphocyte subsets

We analyzed the lymphocyte subsets in 10 cases with MRSA infection with nephritis, and compared those to 10 cases with MRSA infection without nephritis, 24 normal individuals, and 49 patients with IgA nephropathy. The ratios of both DR^+CD4^+ and DR^+CD8^+ cells were increased in patients with MRSA infection with glomerulonephritis, when compared with those of normal individuals and patients with IgA nephropathy (Fig. 2 A, B).

We analyzed the variable regions of TCR in 10 patients with MRSA infection with nephritis, 10 patients with MRSA infection without nephritis, 6 patients with IgA nephropathy, and 10 normal individuals. In patients with MRSA infection with nephritis, the ratios of TCR-V_βs [$V_\beta5.1^+$, $V_\beta(5.2 + 5.3)^+$, $V_\beta6.7,^+$ $V_\beta8^+$ family, $V_\beta12.1^+$], were significantly increased (Fig. 2C). In contrast, in patients with MRSA infection without nephritis, TCR-V_βs ratios were significantly lower than those in patients with MRSA infection with nephritis.

In one patient (case 3), we followed changes in the V_β regions from before onset through the course of the disease. The ratios of TCR-$V_\beta5.1^+$ at onset were significantly increased compared to those before onset; these ratios gradually decreased during vancomycin therapy (Fig. 3C). The ratios of DR^+CD4^+ and DR^+CD8^+ cells at the onset were significantly increased compared to those before the onset (Fig. 3 A, B). The subsets of cells that were increased were mainly $TCR^+CD4^-CD8^-$ [double negative (DN) cells] in this case. The ratio of these TCR-V_βDN cells was five times higher than that before the onset of nephritis (Fig. 4). In all cases combined, TCR $V^+{}_\beta$s cells and TCR^+DN cells were significantly increased compared to those in the other groups (Fig. 5).

Table 3. Immunologic data at the nadir of renal function

N.R.	IgG (800–2000)	IgA (80–400)	IgM (30–300)	C3 (40–120)	C4 (13–59)	
No.	*mg/dl*					Autoantibodies
A MRSA infection with nephritis						
1	2950	1143	160	87	28	(−)
2	1286	534	110	73	48	(−)
3	2677	698	116	83	45	(−)
4	1432	569	83	76	29	(−)
5	2422	630	104	110	48	(−)
6	2040	574	104	51	29	(−)
7	1370	512	130	89	53	(−)
8	4292	656	534	64	22	(−)
9	1644	650	95	93	43.7	(−)
10	2460	601	196	124	35	(−)
B MRSA infection without nephritis						
1	2565	441	126	99	41	(−)
2	1663	421	91	85	46	(−)
3	ND	ND	ND	ND	ND	ND
4	2619	418	176	89	29	(−)
5	3101	8	241	115	57	(−)
6	974	191	258	151	44	(−)
7	1896	269	149	137	44	(−)
8	1526	471	339	113	59	(−)
9	683	165	161	100	52	(−)
10	ND	ND	ND	ND	ND	ND

Abbreviations are: NR, normal range; ND, not done.
Autoantibodies are: anti-glomerular basement membrane Ab, anti-neutrophil cytoplasma Abs, anti-DNA Ab, anti-nuclear Abs, cryoglobulins, rheumatoid factor.

Table 4. Renal histopathologic findings

No.	LM findings: Glomerular lesion	TIN	IF findings: IgG	IgA	IgM	C3
1	Diffuse mesangioproliferative GN with crescents	3+	P(++)M(+−)	P(+)M(++)	P(+−)M(−)	P(+)M(++)
2	Mesangiocapillary GN with crescents	1+	P(++)M(+)	P(++)M(+)	P(−)M(−)	P(++)M(+)
3	Segmental necrotizing GN	1+	P(++)M(+)	P(++)M(++)	P(−)M(−)	P(++)M(++)
4	ND	ND	ND	ND	ND	ND
5	ND	ND	ND	ND	ND	ND
6	Diffuse endo-mesangioproliferative GN	2+	P(+)M(++)	P(+)M(+−)	P(+−)M(−)	P(++)M(+)
7	ND	ND	ND	ND	ND	ND
8	Mild mesangial proliferative GN		P(+)M(+)	P(+)M(+)	P(+−)M(−)	P(+)M(+)
9	ND	ND	ND	ND	ND	ND
10	Mild mesangial proliferative GN		P(+)M(+)	P(+)M(+)	P(+−)M(−)	P(+)M(+)

Abbreviations are: LM, light microscopy; IF, immunofluorescence microscopy; TIN, tubulointerstitial nephritis; P, peripheral capillary wall; M, mesangium; ND, not done.

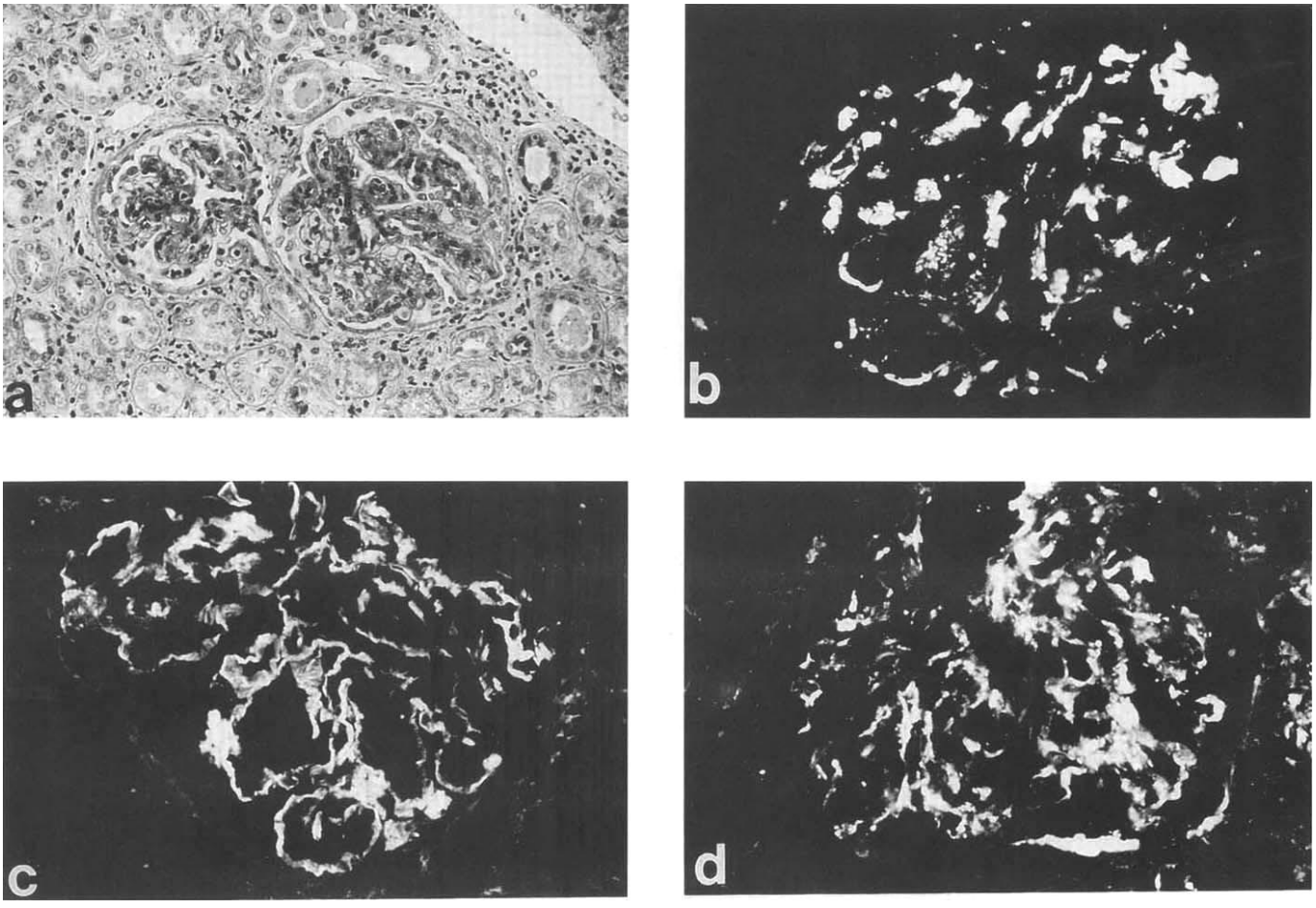

Fig. 1. *Light microscopic finding of case 1(A).* Diffuse mesangioproliferative glomerulonephritis with crescent formation was observed. Mononuclear cell infiltration into tubular epithelium and the interstitium was also observed. (Periodic acid-Schiff stain × 66). Depositions of IgA (**B**), IgG (**C**) and C3 (**D**) in both mesangium and peripheral capillary walls were observed.

IC detection

Amounts of IgA-ICs in patients with MRSA infection and nephritis were significantly increased compared to those with MRSA infection without nephritis and normal individuals (Fig. 6).

Discussion

MRSA strains were initially detected in 1961 in the United Kingdom [16] shortly after methicillin came into clinical use. They have subsequently been isolated in many other parts of the world, including Japan. As a nosocomial pathogen, MRSA infection is a serious problem from both clinical and epidemiologic standpoints.

Staphylococcal infections were identified as causal agents of glomerulonephritis by Powell in 1961 [4]. Reports of staphylococcal infection with glomerulonephritis have described two main types: *Staphylococcus epidermidis* bacteremia in children with VJS [5, 17–19] and *Staphylococcus aureus* bacteremia in adults with

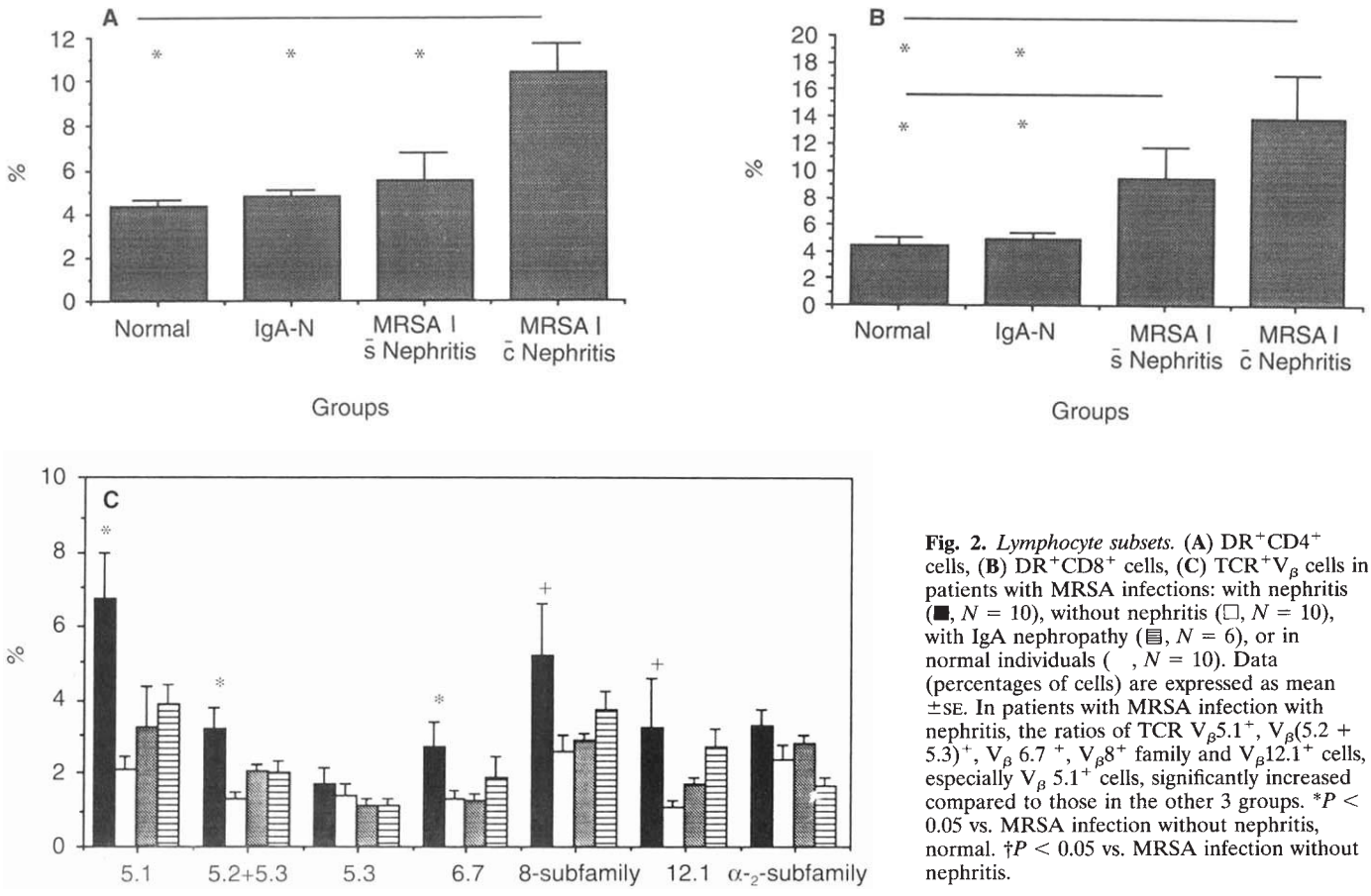

Fig. 2. *Lymphocyte subsets.* (**A**) DR^+CD4^+ cells, (**B**) DR^+CD8^+ cells, (**C**) TCR^+V_β cells in patients with MRSA infections: with nephritis (■, $N = 10$), without nephritis (□, $N = 10$), with IgA nephropathy (▤, $N = 6$), or in normal individuals (, $N = 10$). Data (percentages of cells) are expressed as mean ±SE. In patients with MRSA infection with nephritis, the ratios of TCR $V_\beta 5.1^+$, $V_\beta(5.2 + 5.3)^+$, V_β 6.7^+, $V_\beta 8^+$ family and $V_\beta 12.1^+$ cells, especially V_β 5.1^+ cells, significantly increased compared to those in the other 3 groups. $^*P < 0.05$ vs. MRSA infection without nephritis, normal. $\dagger P < 0.05$ vs. MRSA infection without nephritis.

endocarditis [6, 20]. The clinical course is variable, but usually mild in VJS nephritis. The complement levels are low in the majority of patients [5, 17–19]. The histologic findings in VJS nephritis show either mesangial hypercellularity or diffuse proliferative glomerulonephritis often with a lobulated or mesangiocapillary type. Immunofluorescence microscopy in VJS nephritis usually shows IgG, C3, and IgM in capillary loops or mesangium. In constant, patients with glomerulonephritis and acute *Staphylococcal aureus* endocarditis generally show signs of sepsis and acute renal failure. The serum C3 and C4 levels are usually decreased [6, 20]. Pathologic findings are acute proliferative glomerulonephritis, and in some cases, a membranoproliferative type. Immunofluorescence usually reveals granular deposition of C3 but no IgA [5, 18].

Spector et al have reported three patients with visceral *Staphylococcus aureus* infection without endocarditis who developed acute glomerulonephritis [21]. Renal biopsy in two patients showed mesangial proliferative glomerulonephritis and mesangial deposits containing IgA, IgG and C3. These findings were different from those found in the well-understood syndromes of glomerulonephritis associated with endocarditis or infected VJS.

We report 10 patients with a unique syndrome characterized by an abrupt or insidious onset of RPGN with nephrotic syndrome following MRSA infection. The renal histology showed proliferative glomerulonephritis with varying degrees of crescent formation, and the glomerular deposition of IgA, IgG, and C3. Laboratory findings showed a polyclonal increase in serum IgA and IgG, with high levels of circulating ICs (Fig. 6). These findings are similar to those reported by Spector et al [21].

Recently, SEs have attracted attention as bacterial superantigens, because of their unique role in T cell activation [7–11, 13]. SEs can bind directly to MHC class II molecules on the antigen-presenting cell and specific V_β chain of TCR. Binding to class II molecules, on macrophages could activate these cells to release cytokines. The production of macrophage-derived mediators such as IL-1 and TNF have been demonstrated after toxin stimulation of human cells [11–13]. These cytokines are known to be pathogenic at high levels, causing inflammation and tissue damage [22, 23]. Recently, there have been reports of a role of SEs in the pathogenesis of human diseases, including TSS [3, 23], rheumatoid arthritis [24], Kawasaki disease [25], AIDS [26], Sjögren syndrome [27], and multiple sclerosis [28].

Paliard et al reported that in patients with rheumatoid arthritis the frequency of $V_\beta 14^+$ T cells was significantly higher in the synovial fluid of affected joints than in the peripheral blood [24]. Sumida et al have reported that the repertoires of $V_\beta 2^+$ and $V_\beta 13^+$ genes were predominantly expressed on the T cells of lip specimens of patients with Sjögren syndrome, suggesting a possible role in triggering the autoimmunity of this disease [27]. Abe et al have reported that the acute phase of Kawasaki disease is associated with the expansion of T cells expressing the $V_\beta 2^+$ and $V_\beta 8.1^+$ gene segments [25]. Thus the increase of $TCR^+V_\beta{}^+$ cells is thought to be a marker of superantigen-related diseases.

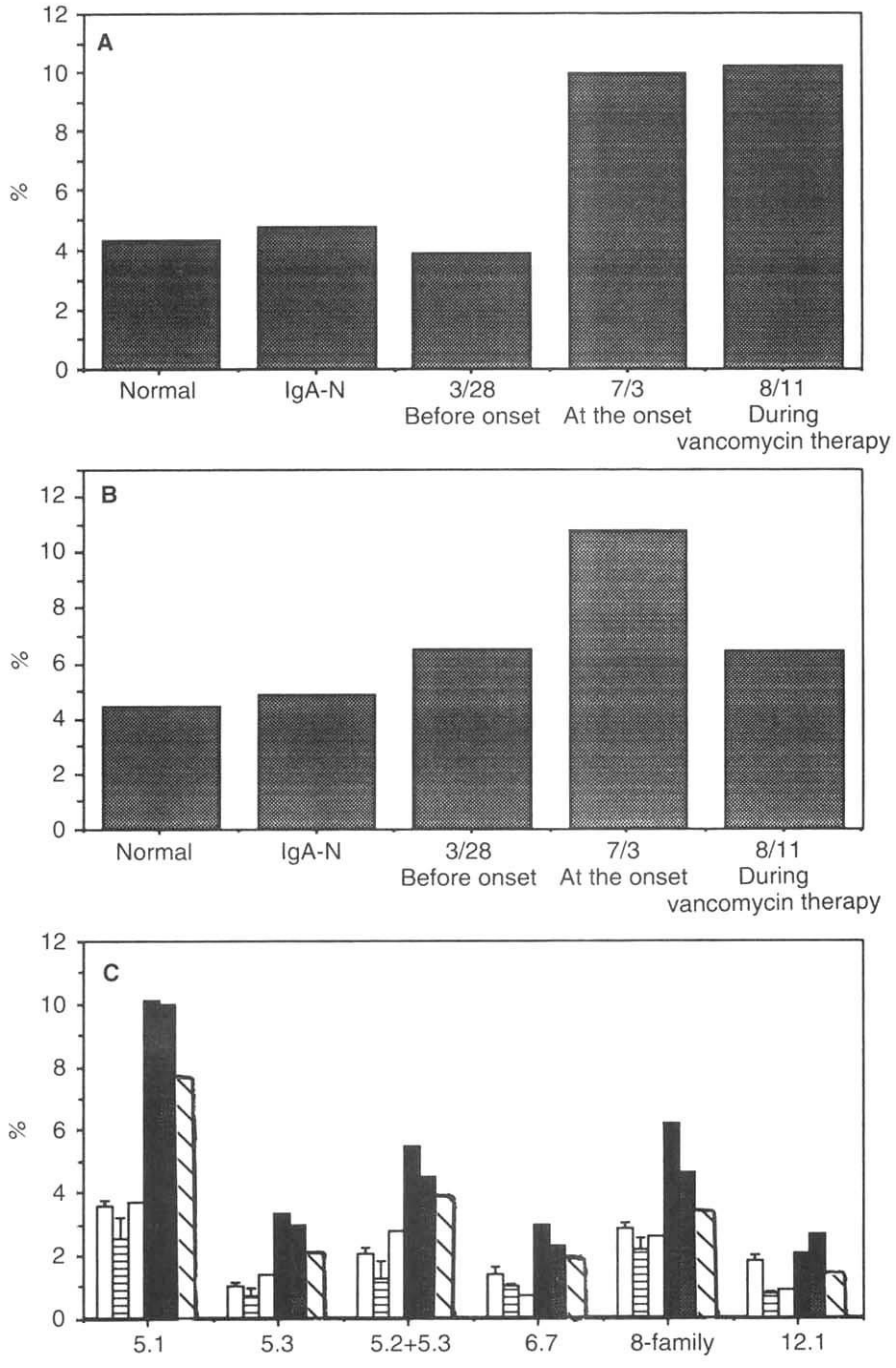

Fig. 3. *Changes in lymphocyte subsets in case 3.* (**A**) DR^+CD4^+ cells, (**B**) DR^+CD8^+ cells, (**C**) TCR-V_β regions. The ratios of DR^+CD4^+ and DR^+CD8^+ cells and $TCR^+V_\beta5.1$, V_β $(5.2 + 5.3)^+$, $V_\beta6.7^+$, $V_\beta8^+$ family and $V_\beta12.1^+$ cells increased at the onset of disease and gradually decreased during vancomycin (VM) therapy. Symbols in **C** are: (□) normal; (▤) MRSA infection with nephritis; (□) before onset 3/28; (■) at onset 7/3; (▨) during VM therapy 8/11; (▧) during VM therapy 10/28.

We used FACS scanning to compare the T cell subsets and the frequency of the V_β region of TCR in patients with MRSA infection with nephritis to those in patients without nephritis or IgA nephropathy, and control subjects. Increases in DR^+CD4^+ and DR^+CD8^+ T cells, and TCR^+V_β cells were found in patients with MRSA infection with nephritis. In one case (case 3), we followed the changes in the V_β regions before and after the onset of glomerulonephritis. Ratios of $TCR^+V_\beta{}^+$ cells, especially $V_\beta{}^+5.1^+$ cells, were significantly increased after the onset and gradually decreased during vancomycin treatment. Interestingly, subsets of the increased $TCR^+V_\beta{}^+$ cells were mainly DN cells. In all cases combined, subsets of the TCR^+DN and TCR^+CD4^+ cells were increased (Fig. 5). These TCR^+DN cells have been reported to be markedly expanded in patients with autoimmune diseases [29–32].

Though we measured only 5 of the 21 reported V_βs [33, 34], our results showed V_β $(5.2 + 5.3)^+$, 5.1^+, and 6.7^+ cells were increased significantly in patients with MRSA infection with nephritis, compared to those in normal individuals. The V_β specificities of the SEs for human and murine V_βs have been

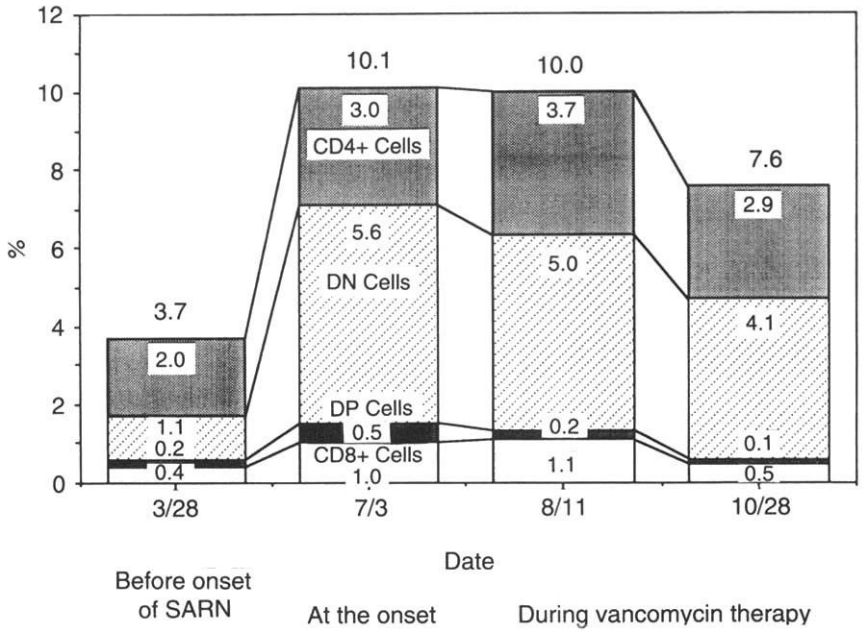

Fig. 4. *Subsets of TCR $V_\beta 5.1^+$ cells before onset and during the disease.* The subsets of increased cells were mainly $CD4^-CD8^-$ (DN) cells. The ratio of these TCR $V_\beta 5.1^+$ DN cells at the onset of nephritis increased fivefold compared to that before onset. Symbols are: (▨)$CD4^+CD8^-$; (□)$CD4^-CD8^-$; (▩)$CD4^+CD8^+$; (▧)$CD4^-CD8^+$.

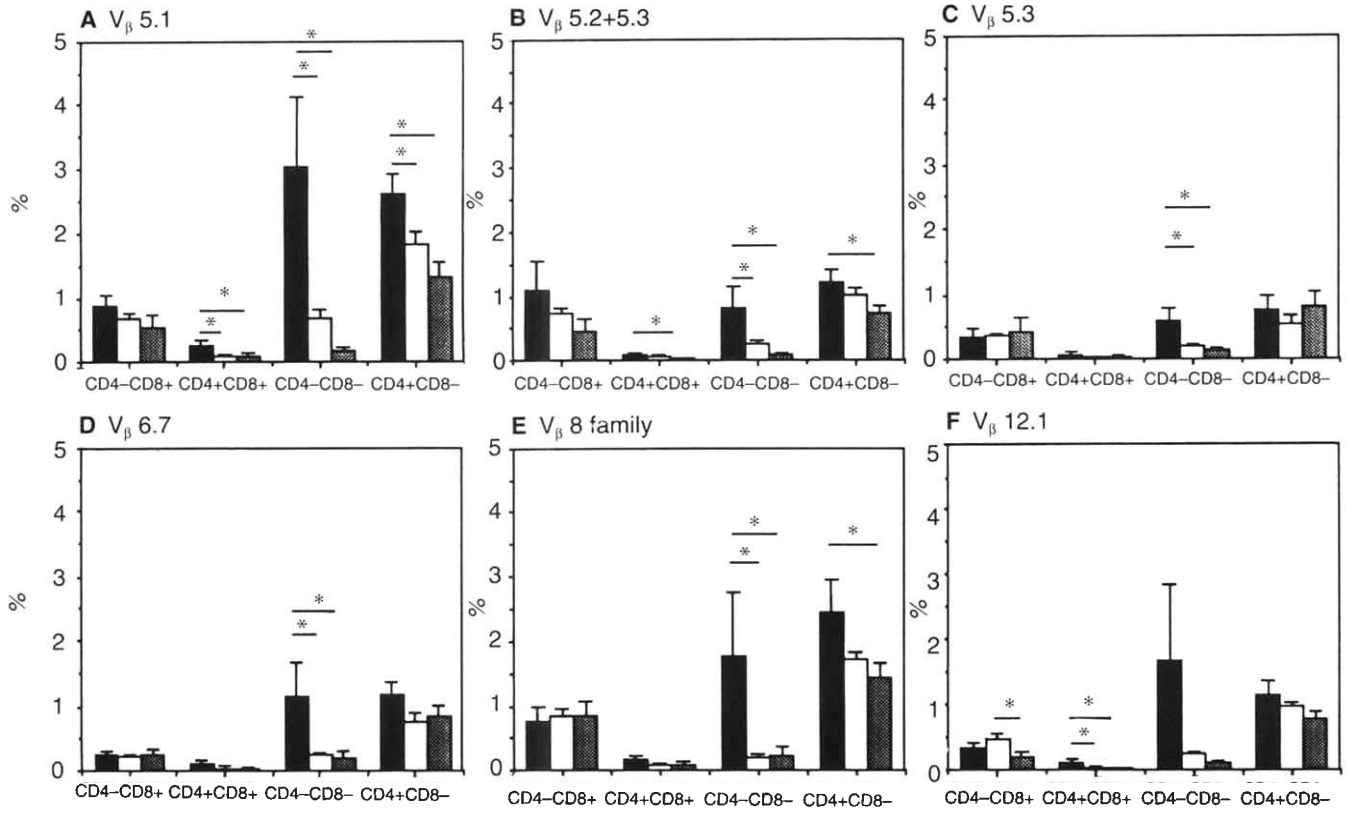

Fig. 5. *Subsets of TCR V_β cells in MRSA infection with nephritis.* Increased subsets in patients with MRSA infection with nephritis were TCR DN cells and TCR $CD4^+$ cells. $^*P < 0.05$. Data (percentages of cells) are expressed as mean ± SE. Symbols are: (■) MRSA infection with nephritis, $N = 10$; (□) Normal, $N = 10$; (▨) MRSA infection without nephritis.

summarized by Herman et al [13]. The SEs found in these patients were mainly SE-C, A, and TSST-1. It is well known that SEs activate specific TCR V_βs positive cells, and that they also activate several TCR V_βs. Finding specific activation of multiple TCR V_βs consequently suggests an involvement of superantigen in the pathogenesis of this form of glomerulonephritis.

The limitations of this study are measurement of TCR V_β^+ cells by FACS scan, because the V_β specificity of some toxins in

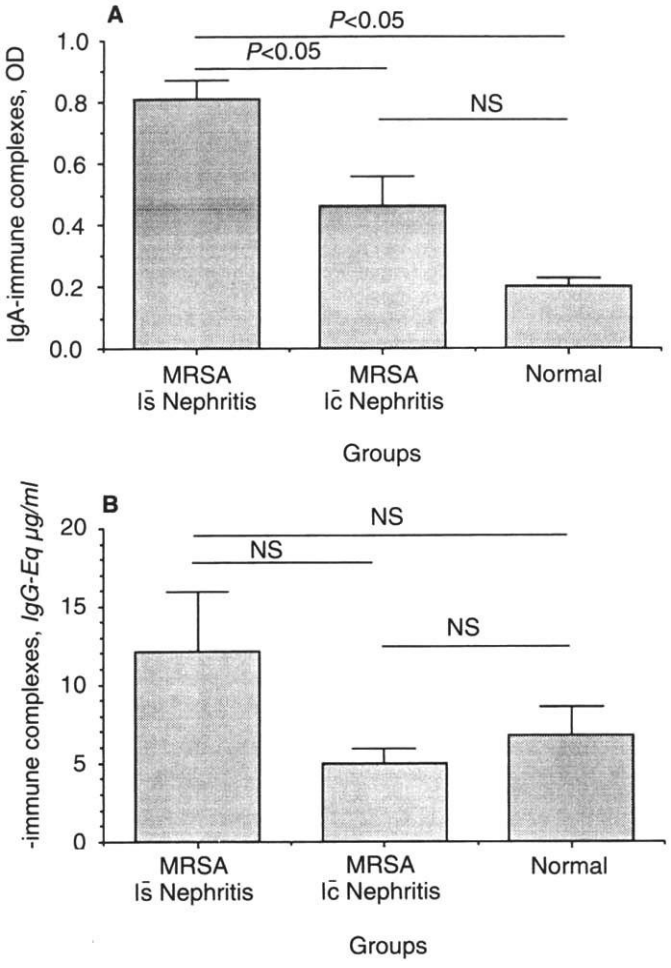

Fig. 6. *Amounts of IgA-ICs* (**A**) *and IgG-ICs* (**B**) *in patients with MRSA infection with nephritis, without nephritis, and normal individuals.* In patients with MRSA infection with nephritis, levels of IgA-ICs were significantly higher than those in patients with MRSA infection without nephritis and normal individuals ($P < 0.05$).

human has been evaluated with a limited number of available antibodies to human V_βs. Therefore there may be nephritis patients without significantly increased TCR V_β^+ cells. However, it seems likely that measurement of TCR V_β^+ cells by FACS scan is better than that of mRNA levels of V_β genes by PCR, because TCR V_β^+ expression can be more directly and accurately estimated by FACS scan.

Recently, Friedman et al have suggested a potential role for microbial superantigens in the pathogenesis of systemic autoimmune disease [35]. The microbial superantigens might promote an abnormal form of cognate T helper-B cell interaction analogous to that which may occur during graft versus host disease, and cause B cell activation and systemic autoimmunity. The resting human B cells bind microbial superantigens and present them to superantigen-reactive autologous T helper cells, resulting in T cell activation and polyclonal IgM and IgG production. In our case, although polyclonal activation of IgA and IgG occurred, we did not detect autoantibodies in the patients. The reasons why superantigens induce the polyclonal activations of IgA and IgG, and not IgM is not clear. Superantigens may selectively activate the T cells that release class-switching lymphokines [36–38].

The histopathologic findings by immunofluorescence microscopy in the patients with MRSA infection with nephritis resemble those seen in IgA-related nephropathies, such as IgA nephropathy and Henoch-Schönlein purpura nephritis. We compared the T cell subsets and frequency of the V_β region of TCR in patients with MRSA infection with nephritis to those in patients with IgA nephropathy, and there were significant differences between the two patient groups.

In these patients with MRSA infections with nephritis, one of the characteristic features of MRSA infection is susceptibility to prolonged septicemia. This is an important precondition for the development of glomerulonephritis, because SEs may be in the circulation and may activate T cells for a prolonged period. Interestingly, in many patients with MRSA infection without nephritis, the ratios of TCR V_β^+ cells were significantly lower than those in patients with MRSA infection with nephritis. These phenomena suggest that clonal suppression or deletion by SEs may occur in patients with MRSA infection without nephritis. Some evidence suggests that superantigens occasionally depress the immune system. The T cell clones stimulated by superantigens often disappear or become inactive after being stimulated [32]. The factors that determine which patients will or will not develop glomerulonephritis remain unknown; there may be differences in the immunogenetic background of patients which contribute to this susceptibility [39, 40].

Though superantigens induce B cell activation both polyclonal and Ag-specific Ab responses [34], we could not detect SEs in the glomeruli or autoantibodies, such as a-GBM Abs, anti-nuclear Abs, cryoglobulins or ANCA. Further studies are necessary to investigate the nature of ICs, including the other autoantibodies deposited in the glomerulonephritis.

From the above observations, we speculate that the nephritis occurring during an MRSA infection may be induced by superantigens. These may be causing the production of high levels of cytokines, and the polyclonal activation of IgG and IgA, resulting in the formation of ICs containing IgA and IgG in the circulation. These changes may result in glomerulonephritis and vasculitis. However, further work will be required to clarify the role and mechanism of these superantigens in the pathogenesis of glomerulonephritis.

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