Relationships Between Autoantibody Responses to Deletion Mutants of Ki Antigen and Clinical Manifestations of Lupus

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ABSTRACT. *Objective.* To determine the relationships between subtypes of anti-Ki antibodies and clinical manifestations of systemic lupus erythematosus.

Methods. The cDNA encoding full-length bovine Ki antigens or N- or C-terminal fragments were produced by polymerase chain reaction, and the fragments of Ki antigen were expressed as GST fusion proteins. Immunoreactivities of anti-Ki antibodies were tested by Western blotting.

Results. Of 60 sera reactive with full-length Ki antigen (KiF), 21 sera recognized only KiF. KiC5, a fragment containing the last 69 C-terminal amino acids, was recognized by 23 sera. Since no significant difference was observed in prevalence of reactivities between fragments from KiC2 to KiC5, a domain within the last 69 C-terminal amino acids was suggested to be the most common antigenic domain expressed among the GST fusion proteins. All 11 sera reacting with a fragment containing the initial 81 N-terminal amino acids also recognized all other fragments. A domain homologous to SV40 nuclear localization signal was required for N-terminal recognition by 8 sera. Reactivity to the last 69 C-terminal amino acids and the initial 81 N-terminal amino acids showed specificities to systemic lupus erythematosus without and with Sjögren's syndrome, respectively. Sicca was significantly more prevalent in patients whose sera reacted with both N- and C-terminal fragments, while prevalence of anti-SSA/Ro antibody was low.

Conclusion. Ki antigen contains multiple epitopes recognized by autoimmune sera. Autoantibody profiles revealed distinctive immune responses, associated with certain clinical subtypes. (J Rheumatol 2003;30:1208–14)

Key Indexing Terms:

ANTI-KI ANTIBODY	PA28	KI ANTIGEN
SYSTEMIC LUPUS ERYTHEMATOSUS	SJÖGREN'S SYNDROME	EPITOPE

Anti-Ki antibody is an autoantibody detected in 6.7 to 21.4% of patients with systemic lupus erythematosus (SLE). Clinical features associated with anti-Ki antibodies have been studied by several investigators, including persistent arthritis, pericarditis, pulmonary hypertension[1], noninfectious fever[2], and central nervous system (CNS) involvement[3]. Ki antigen was also suggested to be identical to sicca lupus (SL) antigen. Autoantibody to SL antigen was originally reported by Harmon, *et al* as an autoantibody specific for SLE and Sjögren's syndrome (SS) and associated with rash, pleuritis, and sicca complex[4].

The target antigen Ki has been characterized as a 32 kDa nonhistone nuclear protein without associated RNA, having an isoelectric point of 4.3. Results of high pressure liquid chromatography analysis suggested that native Ki antigen exists as a 224 kDa polymer[3]. The cDNA encoding bovine and human Ki antigens have been isolated and the deduced amino acid sequence predicted that Ki antigen was a 29.5 kDa polypeptide with highly hydrophilic and weakly acidic characteristics. The nucleotide and amino acid sequence of Ki is well conserved during evolutions; bovine Ki antigen is 96.5% identical to human Ki antigen; and the antigenicity of recombinant bovine Ki antigen has been well characterized[3,5-7].

The cellular function and association with nuclear components of Ki antigen was poorly understood until several reports established that Ki was identical with a 20S proteasome activator, PA28γ[8-10]. PA28γ (Ki) is one of the proteasome activators that is composed of 2 homologous subunits, PA28α and PA28ß. The proteasome exists in large complexes and PA28 complex is also known to play an important role in peptide presentation by major histocompatibility complex (MHC) class I molecules and in cell cycle regulation[9-16]. PA28γ forms a single homopolymer

complex (hexamer or heptamer) and stimulates multiple peptidase activities of the 20S proteasome, and plays a role as a positive allosteric activator binding to 20S proteasome. Most proteasome complexes are mainly located in cytoplasm. Recent studies have revealed that the proteasome complex locates both in cytoplasm and in nucleus, and the localization of PA28γ is restricted to the nucleus[12,17,18]. The function of PA28γ in the nucleus *in vivo* remains to be determined.

Autoantigenic domains recognized by anti-Ki antibodies have been studied rarely. However, the diversity of reported prevalences of anti-Ki antibodies according to different antibody detection systems suggests the presence of multiple autoantigenic domains on Ki antigen, which might be altered through antigen preparation[19,20]. From this point of view, we studied autoantibody responses to Ki antigen, focusing on a domain located between amino acids 82 and 91. As an antigen, we used a synthetic peptide whose amino acid sequence had been reported to be homologous to SV 40 large T-antigen nuclear localization signal (SV40 NLS). The results indicated that the domain represents an epitope on Ki antigen that is detectable in both native and denatured conditions, and also suggested discoid rash and sicca complex are associated with this subtype of anti-Ki antibodies[7,21].

In this study, to more fully characterize the subtypes of anti-Ki antibodies, we analyzed immunoreactivities of anti-Ki sera using truncated fusion proteins from the N- and C-termini and investigated relationships with certain clinical features of SLE.

MATERIALS AND METHODS

Patients and sera. Reactivity of sera to Ki antigen was confirmed by Western blotting using recombinant Ki antigen; 60 positive sera were selected, 24 from SLE patients with Sjögren's syndrome (SS), 24 from SLE patients without SS, and 12 from patients with primary SS. All patients with SLE met the American Rheumatism Association diagnostic criteria[22]. All patients with SS satisfied the San Diego criteria for definite SS[23]. Clinical and laboratory features in SLE patients were defined according to the 1982 revised criteria[22] or according to accepted clinical practice (e.g., sicca refers to xerostomia and xerophthalmia, cardiac involvement refers to abnormal results on electrocardiogram and/or echocardiography, CNS involvement refers to psychosis, convulsion, consciousness disturbance or stroke).

Preparation of full-length and N-, and C-terminal truncated Ki antigens. Bovine cDNA encoding the nucleotide sequence of Ki antigen, pb-Ki-1, was cloned from a cDNA library obtained from bovine retina as described[5]. Nine different sizes of cDNA encoding full-length, N-, or C-terminal truncated bovine Ki antigen were produced from pb-Ki-1 by polymerase chain reactions (PCR) using primers designed to contain *Eco* RI restriction sites at both 5'- and 3'- terminal ends (Figure 1A). PCR products were separated on 1% agarose gels and purified using Suprec (Takara, Shiga, Japan). Each purified PCR product was digested by *Eco* RI and inserted into an expression vector, pGEX (Amersham Pharmacia Biotec, Buckinghamshire, England), and this plasmid DNA was introduced into a competent *Escherichia coli* strain DH-5α (Gibco BRL, Grand Island, NY, USA). Expression of fusion protein was induced in the presence of 1 mM isopropyl-ß-D-thiogalactopyranoside (IPTG) for 4 h at 37°C.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

and Western blotting. SDS-PAGE was performed to confirm production of GST fusion proteins as described[24]. The slab gel consisted of 12.5% acrylamide and 0.1% SDS in Tris-HCl (pH 8.8) with 15 mm of stacking gel containing 5% acrylamide and 0.1% SDS in Tris-HCl (pH 6.8). Each sample was dissolved in sample buffer (3% SDS, 5% 2-mercaptoethanol, 55 mM Tris HCl, pH 6.8, 10% glycerol, bromphenol blue), boiled for 5 min, and electrophoresed. Resulting bands were stained with Coomassie blue R250.

For Western blots, recombinant Ki antigens were subjected to electrophoresis on a 12.5% SDS polyacrylamide gel and transferred to nitrocellulose at 55 V for 3 h in 12.5 mM Tris-HCl (pH 8.3), 96 mM glycine, and 20% methanol[25]. The nitrocellulose was stained with 1% Ponceau S (Sigma) in 5% acetic acid to confirm transfer efficiency, and then saturated in phosphate buffered saline (PBS)/Tween containing 5% nonfat dry milk (PBS-Tween/milk) at room temperature for 4 h to block excessive background staining. The nitrocellulose was cut into strips and incubated with antibodies diluted 1:100 in PBS-Tween/milk at room temperature for 90 min, followed by washing in PBS-Tween for 10 min with 2 changes.

After washing, strips were incubated with alkaline phosphatase labeled anti-human IgG (KPL, Gaithersburg, MD, USA) diluted 1:5000 with 3% PBS-Tween/milk at room temperature for 90 min. After washing with PBS-Tween for 10 min with 2 changes, the bound conjugate was detected by incubation with substrate solution BCIP/NBT Phosphatase Substrate (KPL). Strips were washed with distilled water after an appropriate incubation period.

Other methods. Statistical significance was assessed by chi-squared analysis with Yates' correction (2 tailed test).

RESULTS

Expression of Ki fusion proteins. N- and C-terminal truncated cDNA were constructed by PCR using cDNA encoding bovine Ki antigen as a template (Figure 1A). KiN1 and KiN2 as well as KiC2 and KiC3 were designed to compare the antigenicity of fragments with and without a domain homologous to SV40 NLS (AA 82 to 91; Figure 1A).

Expression of N- and C-terminal truncated Ki antigens was confirmed by SDS-PAGE (Figure 1B). Fusion protein d (KiF), containing the full-length amino acid sequence of Ki antigen, showed a band at 54 kDa in addition to the bacterial proteins (Figure 1B, lane d). In contrast, the expression vector pGEX without an insert produced a 26 kDa protein (Figure 1B, lane P). SDS-PAGE analysis of the other fusion proteins (KiN1 to 3 and KiC1 to 5) yielded polypeptides of 35, 36, 42, 49, 45, 44, 38, and 33 kDa, respectively.

Reactivities against N- and C-terminal fragments of the Ki antigen by Western blot. To compare immunoreactivities of anti-Ki antibodies, Western blotting was performed using N- and C-terminal fragments of Ki antigen. As shown in Figure 2, various reaction patterns were observed. Of 60 sera, 11 reacted with all 8 fragments prepared for this study. In contrast, 21 sera did not recognize any antigen except KiF, which consisted of the amino acids of full-length Ki antigen (Figure 2).

Reactivities against N- and C-terminal fragments of Ki antigen are summarized in Table 1. KiN1, containing the initial 81 N-terminal amino acids, was recognized by 11 sera. On the other hand, 19 sera reacted with KiN2, an N-
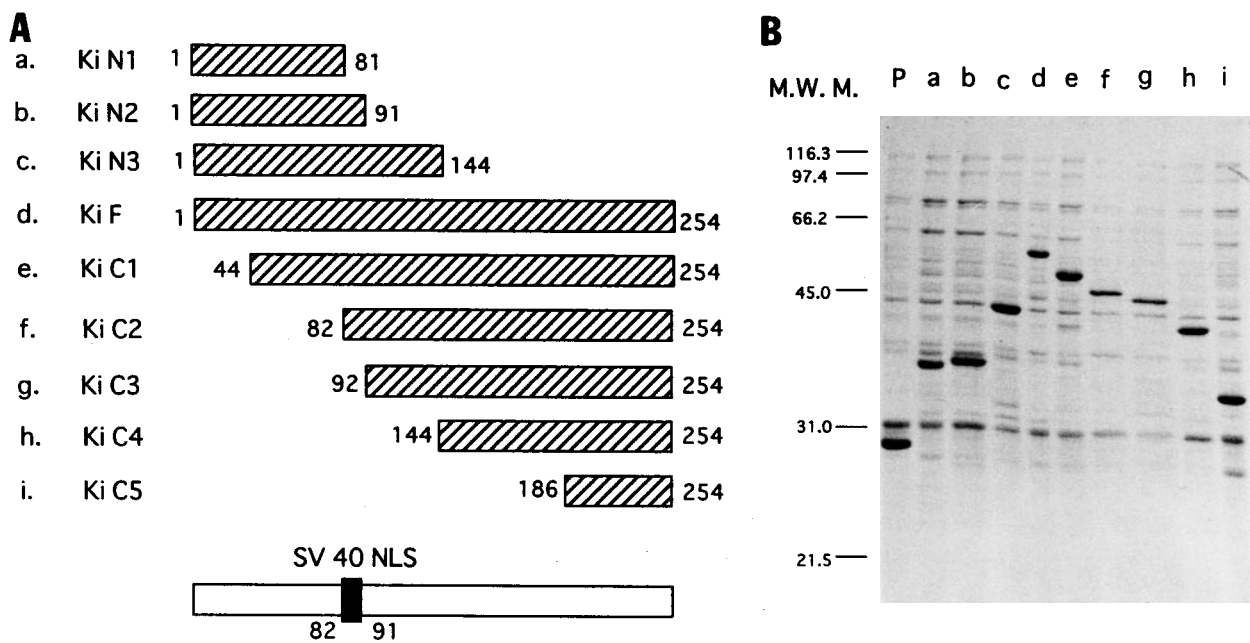
Figure 1. N- or C-terminal deletions and expression of Ki fusion proteins. A. Ki antigens produced as GST fusion proteins. Fragments KiN1 to 3 begin at amino acid residue 1 and extend to the residue number to the right of each fragment. KiN1 includes the initial 81 N-terminal amino acids, and KiN2 contains a domain homologous to SV 40 NLS, in addition to KiN1. KiF is full-length Ki antigen. KiC1 to 5 lack the N-terminus of Ki antigen. KiC1 contains residues 44 to the C-terminal residue 254; KiC2 consists of a domain homologous to SV 40 NLS, while the domain is truncated in KiC3; KiC4 consists of residues 144 to the C-terminal residue 254; KiC5 represents the 69 C-terminal amino acids. Numbers denote the amino acids at N- and C-terminal fragments. A domain homologous to SV 40 NLS, amino acid residues within 82 to 91, is shown by the shaded area. KiN1 and KiN2 as well as KiC2 and KiC3 were digested to compare autoantigenicity of the domain. B. SDS-PAGE analysis of Ki fusion proteins under reducing conditions. N- and C-terminal truncated Ki antigens were produced as GST fusion proteins in the presence of IPTG. Lane P shows GST fusion protein, while lanes a to i were produced from cDNA constructed by PCR as shown in panel A. Molecular weight markers are at left.

terminal fragment 10 amino acids longer than KiN1 that consisted of a domain homologous to SV40 NLS. This result suggested that these additional 8 sera required the presence of a domain within AA 82 to 91 for N-terminal recognition. All 11 sera that recognized KiN1 also reacted with all other fragments, indicating that these sera recognized epitopes at both N- and C-terminal ends. Since no significant difference was observed between prevalence of

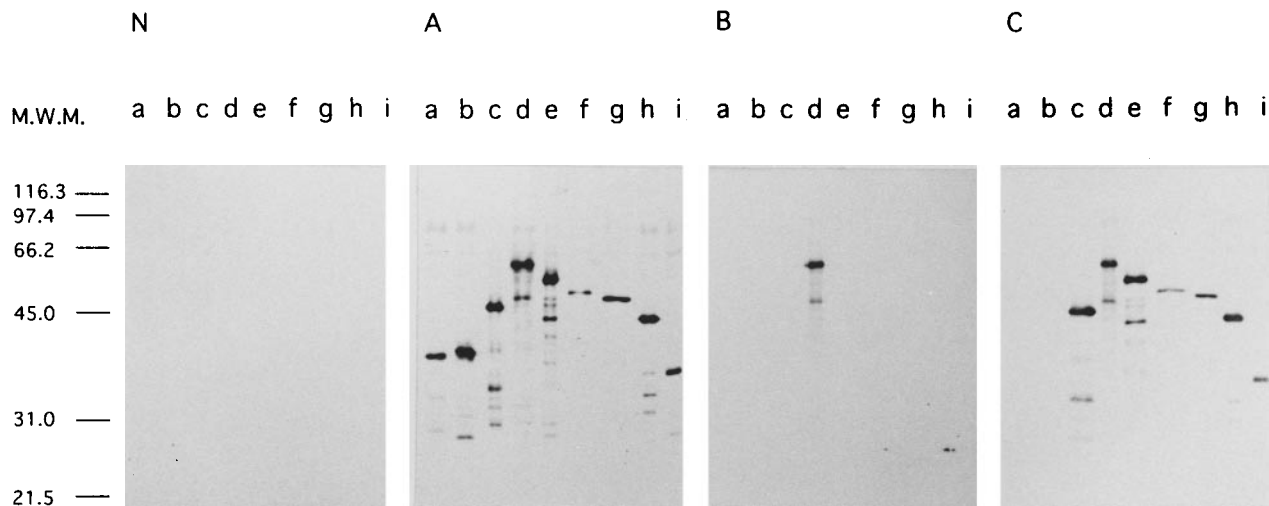*Figure 2*. Immunoreactivity of anti-Ki sera against Ki fusion proteins shown by Western blots. N: normal human serum; A: serum reactive with all fragments; B: serum reactive only with full-length Ki (KiF); C: serum reactive with all C-terminal fragments (KiC1 to 5) and the 144 N-terminal amino acids (KiN3). Lanes a to i correspond to cDNA in panel A of Figure 1. Molecular weight markers are at left.

Table 1. Reactivities against N- and C-terminal fragments of Ki antigen. Reactivity refers to number of reactive sera among 60 specimens.

Fragments	Fusion Proteins	Reactivity
KiN1	AA 1–81	11
KiN2	AA 1–91	19
KiN3	AA 1–144	20
KiF	AA 1–254	60
KiC1	AA 44–254	34
KiC2	AA 82–254	22
KiC3	AA 92–254	23
KiC4	AA 144–254	21
KiC5	AA 186–254	23

AA: amino acids.

reactivity to KiN2 (AA 1 to 91) and KiN3 (AA 1 to 144), the domain between AA 92 and 144 apparently was not required for antibody binding.

KiC1, the longest C-terminal fragment, contained 210 amino acids and was recognized by 34 sera. Numbers of sera reacting with the other C-terminal fragments (KiC2 to 5) ranged between 21 and 23, suggesting that these sera were mainly targeting a domain within 69 amino acids of the C-terminus.

Relationship between diagnosis and subtype of anti-Ki antibodies. Since we found that anti-Ki sera showed varying reactivities to Ki deletions, relationships between diagnostic groups and immunoreactivity pattern were studied further. Patients were classified into 3 disease groups according to the reported disease specificities (SLE without SS, primary SS, and SLE with SS). Table 2 presents immunoreactivity patterns and clinical patterns observed in this study; 21 sera reacted with only full-length Ki, 23 with KiC5, 11 with KiN1 in addition to KiC5, and 8 with KiN2 but not KiN1. All sera that reacted with KiN1, the shortest N-terminal fragment, also reacted with all fragments prepared for this study.

Of 24 sera from SLE patients without SS, 6 reacted only with KiF. Reactivity to KiC5, a C-terminal fragment with 69 amino acids, was observed most frequently in this disease group. Although not statistically significant, prevalence of KiC5 tended to be higher in these patients than in primary SS. Of 11 sera that reacted with C-terminal fragments, 2 also recognized the initial 81 N-terminal amino acids. Seven sera reacted with KiN2 but not KiN1, which indicated that these

sera required presence of a domain between AA 82 and 91 for N-terminal recognition. Most occurrences of this immunoreactivity pattern were in the SLE group.

Half of the 12 sera from patients with primary SS reacted only with KiF. Reactivity to the last 69 C-terminal amino acids was observed in only one serum specimen among those from patients with primary SS (8.3%).

Considering 24 sera from SLE patients with SS, reactivity to the last 69 C-terminal amino acids was detected more frequently than in the group of the SLE patients without SS (11 sera, 45.8%). Interestingly, most sera in this group also recognized KiN1, amounting to a prevalence significantly higher than in SLE without SS ($p < 0.01$). Further, of 11 sera that reacted with KiN1, 8 were from SLE patients with SS. These results suggested that the reactivity to the 69 C-terminal amino acids was a feature in common with SLE, while reactivity to the 81 N-terminal amino acids was specific for SLE with SS.

When antigenicities were compared between KiN1 and KiN2, 8 sera reacted with KiN2 but not KiN1, indicating that these sera required the presence of a domain lying between AA 82 and 91 for N-terminal recognition. These 8 included 7 specimens from SLE patients without SS.

Clinical significance of antibodies to N- and C-terminal domains of the Ki antigen. We further studied the relationship between individual clinical significance and immunoreactivity patterns of anti-Ki antibodies (Table 3). In patients whose sera reacted only with KiF, relatively high rates of hypergammaglobulinemia and anti-SSB/La antibody positivity were noted. Prevalence of anti-Sm antibody was significantly higher in patients whose sera recognized KiC5. Sicca was observed frequently in patients who had antibodies to both N- and C-terminal fragments, a prevalence significantly higher than for other reactivity patterns. In contrast, the prevalence of anti-SSA/Ro antibody in these patients was significantly low.

DISCUSSION
We compared immunoreactivities of anti-Ki sera against N- or C-terminal fragments of Ki antigen. The Western blot results indicated that the autoantigenic domains were present within both N-terminal 81 and C-terminal 69 amino acids. In addition, 8 sera required a domain between AA 82

Table 2. Relationship between diagnosis and subtypes of anti-Ki antibodies.

	Reactive Only with KiF	Reactive with KiC5	Reactive with KiNi and All Other Fragments	Reactive with KiN2 But Not KiN1
SLE without SS, n = 24 (%)	6 (25.0)	11 (45.8)	2 (8.3)	7 (29.2)**
Primary SS, n = 12 (%)	6 (50.0)	1 (8.3)	1 (8.3)	0 (0.0)
SLE with SS, n = 24 (%)	9 (37.5)	11 (45.8)	8 (33.3)*	1 (4.2)
Total, n = 60 (%)	21 (35.0)	23 (38.3)	11 (18.3)	8 (13.3)

* $p < 0.05$ versus SLE without SS; ** $p < 0.05$ versus SLE with SS.

Table 3. Correlation between autoantibody profiles and clinical and laboratory features.

Clinical and Laboratory Features	Reactive Only with KiF (%)	Reactive with KiC5 (%)	Reactive with KiN1 and All Other Fragments (%)	Reactive with KiN2 But Not KiN1 (%)	Total (%)
Photosensitivity	7/12 (58.3)	8/18 (44.4)	5/10 (50.0)	1/5 (20.0)	17/39 (43.6)
Malar rash	8/14 (57.1)	9/20 (45.0)	5/9 (55.6)	1/5 (20.0)	22/40 (55.0)
Discoid rash	1/14 (7.1)	2/18 (11.1)	1/9 (11.1)	1/5 (20.0)	6/43 (13.9)
Alopecia	5/14 (35.7)	4/20 (20.0)	2/9 (22.2)	1/5 (20.0)	12/45 (26.7)
Oral ulcer	5/14 (35.7)	3/20 (15.0)	2/9 (22.2)	0/5 (0.0)	10/45 (22.2)
Raynaud's phenomenon	3/14 (21.4)	5/19 (26.3)	4/9 (44.4)	1/5 (20.0)	13/44 (29.5)
Sicca	9/15 (60.0)	11/17 (64.7)	9/10 (90.0)*	1/5 (20.0)	28/47 (59.6)
Arthralgia	10/15 (66.7)	15/19 (78.9)	7/9 (77.8)	1/5 (20.0)	32/45 (71.1)
Myositis	1/12 (8.3)	0/17 (0)	0/9 (0)	0/5 (0)	4/39 (10.3)
Lymphadenopathy	0/14 (0)	1/20 (5.0)	0/9 (0)	0/5 (0)	1/44 (2.3)
Cardiac involvement	2/13 (15.4)	0/19 (0)	0/9 (0)	0/5 (0)	3/42 (7.1)
Serositis	1/14 (7.1)	0/18 (0)	0/8 (0)	0/5 (0)	1/42 (2.4)
Interstitial pneumonia	1/14 (7.1)	0/19 (0)	0/9 (0)	0/5 (0)	2/43 (4.7)
Renal involvement	10/13 (76.9)	12/19 (63.2)	5/9 (55.6)	2/5 (40.0)	27/43 (62.8)
CNS involvement	3/13 (23.1)	1/21 (4.8)	0/10 (0)	0/5 (0)	5/44 (11.4)
Leukopenia	6/14 (42.9)	10/21 (47.6)	4/10 (40.0)	3/5 (60.0)	21/47 (44.7)
Thrombocytopenia	1/13 (7.7)	2/21 (9.5)	1/10 (10.0)	0/5 (0)	3/45 (6.7)
Hypergammaglobulinemia	12/15 (80.0)*	10/21 (47.6)	5/9 (55.6)	1/5 (20.0)	25/47 (53.2)
Hypocomplementemia	7/14 (50.0)	8/20 (40.0)	2/10 (20.0)	2/5 (40.0)	20/44 (45.5)
RF	2/13 (15.4)	6/19 (31.6)	4/9 (44.4)	0/5 (0.0)	10/42 (23.8)
Anti-DNA antibody	11/14 (78.6)	11/18 (61.1)	5/10 (50.0)	3/5 (60.0)	27/42 (64.3)
Anti-U1RNP antibody	4/14 (28.6)	7/18 (38.9)	3/9 (33.3)	1/5 (20.0)	14/41 (34.1)
Anti-Sm antibody	1/13 (7.7)	6/19 (31.5)*	2/9 (22.2)	1/5 (20.0)	7/41 (17.1)
Anti-SSA/Ro antibody	9/14 (64.3)	10/18 (55.6)	3/11 (27.3)*	2/5 (40.0)	23/43 (53.5)
Anti-SSB/La antibody	5/15 (33.3)*	1/18 (5.6)	0/10 (0)	1/5 (20.0)	6/42 (14.3)

* $p < 0.05$ versus all the patients in the other groups. CNS: central nervous system; RF: rheumatoid factor.

and 91 for N-terminal recognition. These results support our previous findings that amino acid residues 82 to 91 of Ki antigen constituted a sequence homologous to SV40 NLS and represented an epitope recognized by 20 to 37% of anti-Ki sera[7,21]. KiC5 containing C-terminal 69 amino acids was recognized by 23 sera, and truncation of a domain between AA 82 and 185 did not affect reactivity to Ki antigen. In addition to these results, since all sera reacting with KiN1 recognized all fragments prepared for this study, a domain within the last 69 C-terminal amino acids is likely to be the most common antigenic domain expressed among the GST fusion proteins.

Previous reports have described varying frequencies of anti-Ki antibodies. In particular, the reported prevalence of anti-Ki antibodies in lupus sera has differed according to the immunoassay systems: 6.7% by immunoprecipitation, 11.8% by double immunodiffusion (DID), 18.9% by ELISA using recombinant Ki, and 21.4% by ELISA using purified Ki[1-3,6]. We recently compared immunoreactivity of anti-Ki sera detected by DID in other immunoassay systems and found that anti-Ki reactivities were different in individual antibody detection systems[7]. These results suggested that the epitopes on Ki antigen were affected by antigen preparation or detection methods. Although these discrepancies could be explained by different sensitivities of the individual immunoassay systems, the findings more likely

suggest that recognition of epitopes depends upon conformational structure[26,27].

Such discrepancies are also observed in Sm, SSA/Ro, SSB/La, proliferating cell nuclear antigen (PCNA), Ku, and many other antigen-antibody assay systems[28-34]. As discussed by several investigators, the autoantigenic domains typically recognized by autoantibodies are located in the functional and conformational domains of the target antigens, and expression of autoantibodies is antigen-driven[35,36]. Recombinant antigens may fail to acquire the specific conformational structure and posttranslational modification of the native antigen, as well as lacking protein-protein interaction. Recent studies on cellular function and structural analysis of Ki antigen as a proteasome activator revealed that Ki antigen exists as a homopolymer that binds to a 20S proteasome to form a complex, and was specifically identified as present in association with affinity purified PCNA[37-39]. These observations also suggest that the autoepitopes of Ki antigen commonly recognized by autoimmune sera depend on its conformational structure in the same manner as other autoantigens. These findings may explain why we could not identify epitopes recognized by the majority of sera in Western blotting, which lends itself to detection of linear epitopes. In addition to these findings, human autoantibodies recognizing epitopes specific for human antigen might fail to react with recombinant antigen

from different species, even if most of the antigenic domains and amino acid sequence are highly conserved.

Our results showed that about one-third of anti-Ki sera failed to recognize the longest N- and C-terminal fragments, while these fragments included 100 overlapping amino acids. In Western blots performed under denatured conditions, antibodies are thought to recognize only linear epitopes. However, several investigators have observed similar results and suggested that some autoantibodies can bind to a conformational structure, despite the denatured condition in Western blotting[20,40-43]. It has been suggested that the presence of conformational or discontinuous epitopes, which were labile in the reducing condition or refolded by partial renaturation after the denatured proteins, are transferred to the nitrocellulose membrane[44,45]. Thus, it is possible that antibodies that could react with only full-length Ki recognize the conformational structure that required the presence of both N- and C-terminal domains for antigen recognition. Although these reports and our data may support this hypothesis, further investigation will be required to determine the presence of a conformational structure on the GST fusion protein under denatured conditions, and whether the weak reactivities against smaller fragments of recombinant antigen with low titer anti-Ki sera were not observed in this assay system.

To study the relationship between clinical features and immunoresponses of anti-Ki antibodies, reactive patterns were classified into 4 representative groups. As depicted in Table 2, the immunoresponse to Ki antigen differed in relation to different diagnoses. In the study of reactivity to C-terminal fragments, 22 of 23 sera (95.7%) that reacted with KiC5 were obtained from patients with SLE, while one-half of sera from patients with SS recognized only full-length Ki. These results suggest that reactivity to the last 69 C-terminal amino acids might be specific for SLE. When reactivities to N-terminal fragments were compared between sera reacting with KiC5, most sera from patients with SLE with SS and also all sera from patients who had SLE without SS reacted with KiN2. In contrast, reactivity to KiN1 showed dissociation, occurring in 8 of 11 sera (72.7%) from SLE patients with SS but in only 2 sera (18.2%) from SLE patients without SS. This suggests that the autoantibody to the initial 81 N-terminal amino acids has a strong association with features of SS in lupus patients. Detection of this reactivity therefore would be helpful for diagnosis of sicca complex.

Profiles of anti-Ki antibodies and specific clinical or laboratory features showed some other significant associations. Anti-Sm antibody, known to be a diagnostic marker for SLE, was frequently detected among patients whose sera reacted with KiC5. This corresponds with our finding that the reactivity to KiC5 was specific for SLE. When we focused on association with other autoantibodies, anti-SSA/Ro antibody was frequently detected[2]. The highest prevalence of anti-SSA/Ro antibody was observed in sera reacting for Ki only with the full-length antigen. The largest population of this group was patients with SS, and anti-SSB/La antibody was significantly more common in this group than in others. In contrast, the highest prevalence of sicca complex was observed in patients whose sera recognized both N- and C-terminal domains of Ki antigen. This patient group showed specificity to SLE with SS, although the prevalence of anti-SSA/Ro antibody was significantly lower than in other groups. Thus, sicca complex in this group was strongly associated with autoantibodies targeting the initial 81 N-terminal amino acids, but not with the anti-SSA/Ro antibody. These findings strongly suggest that development of SS in these 2 patient groups might be induced by different mechanisms.

In this study, we identified multiple autoantigenic domains on Ki antigen. Immunoreactivity to the C-terminal domain of Ki antigen was specific for SLE, while the initial 81 N-terminal amino acids were more important in the autoantibody response in SLE patients with sicca complex. This suggests the possibility of different mechanisms in development of sicca complex between patients with these subtypes of anti-Ki antibodies. However, Ki antigen is a component of a multi-protein complex, and further studies of its structure are required to understand how antigen presentation of this subnuclear particle influences autoantibody expression. This understanding should enhance knowledge of how the profile of autoantibody responses is related to specific clinical features.

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