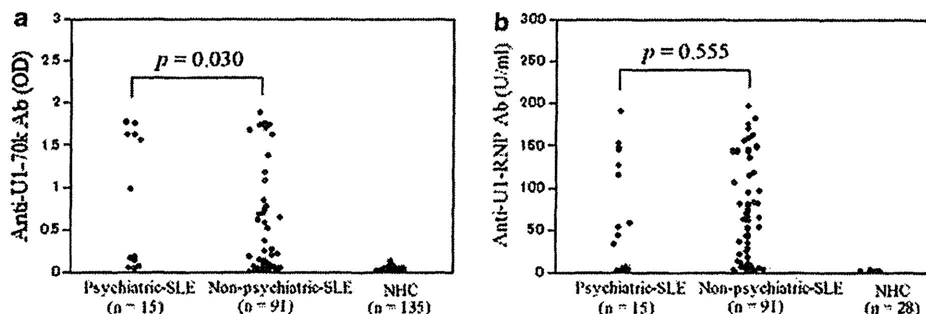

Fig. 4 Comparison of enzyme-linked immunosorbent assay (ELISA) autoantibody titers against 70-kDa polypeptide of the U1-ribonucleoprotein (RNP) complex (U1-70k) (**a**) and U1-RNP (**b**) between systemic lupus erythematosus (SLE) patients with or without psychiatric syndromes ($n = 15$ and 91, respectively) and between 135 normal healthy controls (NHCs) with anti-U1-70k antibody ELISAs or 28 normal healthy controls with anti-U1-RNP antibody ELISAs. Levels of anti-U1-70k antibodies were significantly elevated in SLE patients with psychiatric disorders compared with SLE patients without psychiatric disorders ($p = 0.030$ by the Steel–Dwass multiple comparison test) (**a**). By contrast, no significant difference was observed in the levels of serum anti-U1-RNP antibodies in SLE patients with or without psychiatric syndromes (**b**) ($p = 0.555$)

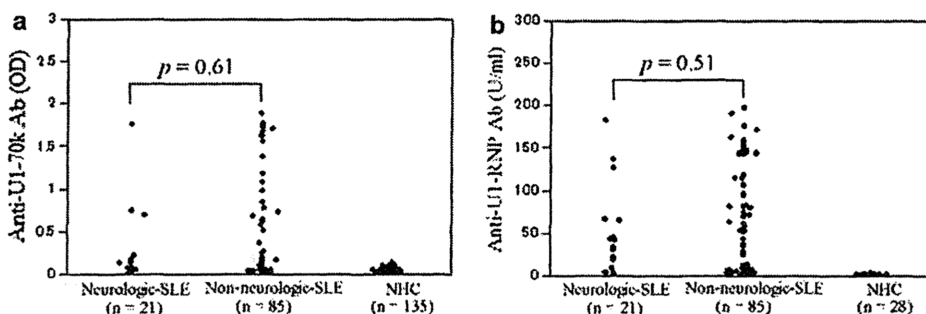

Fig. 5 Comparisons of enzyme-linked immunosorbent assay (ELISA) autoantibody titers against 70-kDa polypeptide of the U1-ribonucleoprotein (RNP) complex (U1-70k) (**a**) and U1-RNP (**b**) between systemic lupus erythematosus (SLE) patients with or without neurologic syndromes ($n = 21$ and 85, respectively) and between 135 normal healthy controls (NHCs) with anti-U1-70k antibody ELISAs and 28 NHCs with anti-U1-RNP antibody ELISAs. No significant difference was observed in the levels of serum anti-U1-70k antibodies (**a**) or anti-U1-RNP antibodies (**b**) in SLE patients with or without neurologic syndromes ($p = 0.61$ and 0.51, respectively by the Steel–Dwass multiple comparison test)

pathogenic in the CNS, but they may cause damage to the blood–brain barrier. If other factors increase the permeability of the blood–brain barrier, serum anti-U1-70k antibodies could be directly pathogenic in the CNS. Because the CSF was not sampled in this study, and because no circulating biomarkers of blood–brain barrier permeability were assessed, the question of whether autoantibodies were present within the intrathecal space was not addressed. Moreover, interferogenic activity, the ability of the serum or the CSF to induce interferon (IFN)-α synthesis in the presence of an IFN-producing cell, in the CSF of SLE patients is significantly correlated with serum anti-U1-RNP antibody levels but not with other known antinuclear antibodies [40]. Therefore, anti-U1-RNP antibodies and their immune complexes in the CSF may have pathogenic roles in NP-SLE [23].

In NP-SLE, levels of serum autoantibodies do not always reflect their behavior in the CSF [5]. Actually, Sato et al. [23] reported that the frequency of anti-U1-RNP antibodies in the CSF and the anti-U1-RNP index is higher in SLE patients with CNS syndromes than in those without, whereas no association was observed between the presence of serum anti-U1-RNP antibodies and CNS syndromes in SLE. However, the reason for these conflicting results may be explained simply by our finding of associations between serum anti-U1-70k antibodies and psychiatric syndromes, but not with CNS syndromes as a whole, in SLE patients. Alternatively, this finding may be related to the heterogeneity of patients with CNS lupus or the methodological differences between Sato et al.'s RNA immunoprecipitation assay and our ELISA.

Anti-U1-70k antibodies were not present in serum samples obtained from some SLE patients with psychiatric syndromes, probably because several different autoimmune and inflammatory mechanisms are likely to play roles in the pathogenesis of NP-SLE [6]. Again, a single pathogenic mechanism is unlikely to be responsible for all of them. Lupus-specific mechanisms underlying NP disease include vasculopathy of the intracranial vessels, local or systemic

production of inflammatory mediators, and generation of specific autoantibodies.

Because some elements in this study design may limit its strength, the novel findings, although promising, require cautious interpretation and further investigation before leading to firm conclusions. First, study validity could be improved by a larger sample size and a nonretrospective study design, although our sample size was comparable with that of the majority of previous studies dealing with anti-U1-RNP antibodies or CNS syndromes in SLE patients. Second, because participants were mostly Japanese, it is not clear whether anti-U1-70k antibodies have different effects on psychiatric/CNS syndromes in patients of different ethnic backgrounds. In fact, ethnic differences have been reported in the frequency of end-organ involvement in the Miami MCTD versus the Missouri Caucasian MCTD study groups [16]. Third, although we speculate that patients with high anti-U1-RNP antibody titers but without anti-U1-70k antibodies measured with ELISA also possessed antibodies against U1-A, U1-C, or U1-RNA, some of the serum samples might have reacted with epitopes altered by the U1-RNA binding to U1-70k [33], which we did not assess. Finally, the biological relationship between the ubiquitous protein (i.e., U1-70k) and CNS specificity was not clear, although we speculated above on several possible pathological mechanisms, citing literature information.

In conclusion, despite its limitations, this study suggests that anti-U1-70k antibodies in serum are associated with psychiatric syndromes but not with CNS syndromes as a whole or with neurologic syndromes in SLE patients. The anti-U1-70k antibodies might be involved in pathological mechanisms of SLE psychiatric syndromes. Determining the precise role of anti-U1-70k antibodies in the pathogenesis of CNS syndromes and their usefulness as biomarkers in SLE patients will require further study, including investigations employing animal models.

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Conflict of interest None.

References

1. Hanly JG, Harrison MJ. Management of neuropsychiatric lupus. Best Pract Res Clin Rheumatol. 2005;19:799–821.
2. ACR Ad Hoc Committee on Neuropsychiatric Lupus Nomenclature. The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. Arthritis Rheum. 1999;42:599–608.
3. Nishimura K, Harigai M, Omori M, Sato E, Hara M. Blood-brain barrier damage as a risk factor for corticosteroid-induced psychiatric disorders in systemic lupus erythematosus. Psychoneuroendocrinology. 2008;33:395–403.
4. Zandman-Goddard G, Chapman J, Shoenfeld Y. Autoantibodies involved in neuropsychiatric SLE and antiphospholipid syndrome. Semin Arthritis Rheum. 2007;36:297–315.
5. Fragoso-Loyo H, Cabiedes J, Orozco-Narvaez A, Davila-Maldonado L, Atisha-Fregoso Y, Diamond B, et al. Serum and cerebrospinal fluid autoantibodies in patients with neuropsychiatric lupus erythematosus. Implications for diagnosis and pathogenesis. PLoS ONE. 2008;3:e3347.
6. Hanly JG, Urowitz MB, Siannis F, Farewell V, Gordon C, Bae SC, et al. Autoantibodies and neuropsychiatric events at the time of systemic lupus erythematosus diagnosis: results from an international inception cohort study. Arthritis Rheum. 2008;58:843–53.
7. Colasanti T, Delunardo F, Margutti P, Vacirca D, Piro E, Siracusano A, et al. Autoantibodies involved in neuropsychiatric manifestations associated with systemic lupus erythematosus. J Neuroimmunol. 2009;212:3–9.
8. Efthimiou P, Blanco M. Pathogenesis of neuropsychiatric systemic lupus erythematosus and potential biomarkers. Mod Rheumatol. 2009;19:457–68.
9. Nojima Y, Minota S, Yamada A, Takaku F, Aotsuka S, Yokohari R. Correlation of antibodies to ribosomal P protein with psychosis in patients with systemic lupus erythematosus. Ann Rheum Dis. 1992;51:1053–5.
10. Gono T, Kawaguchi Y, Kaneko H, Nishimura K, Hanaoka M, Kataoka S, et al. Anti-NR2A antibody as a predictor for neuropsychiatric systemic lupus erythematosus. Rheumatology (Oxford). 2011;50:1578–85.
11. Katsumata Y, Kawaguchi Y, Baba S, Hattori S, Tahara K, Ito K, et al. Identification of three new autoantibodies associated with systemic lupus erythematosus using two proteomic approaches. Mol Cell Proteomics. 2011;10:M110.005330.
12. Katzav A, Solodeev I, Brodsky O, Chapman J, Pick CG, Blank M, et al. Induction of autoimmune depression in mice by anti-ribosomal P antibodies via the limbic system. Arthritis Rheum. 2007;56:938–48.
13. Matus S, Burgos PV, Bravo-Zehnder M, Kraft R, Porras OH, Farias P, et al. Antiribosomal-P autoantibodies from psychiatric lupus target a novel neuronal surface protein causing calcium influx and apoptosis. J Exp Med. 2007;204:3221–34.
14. Gono T, Takarada T, Fukumori R, Kawaguchi Y, Kaneko H, Hanaoka M, et al. NR2-reactive antibody decreases cell viability through augmentation of Ca(2+) influx in systemic lupus erythematosus. Arthritis Rheum. 2011;63:3952–9.
15. Migliorini P, Baldini C, Rocchi V, Bombardieri S. Anti-Sm and anti-RNP antibodies. Autoimmunity. 2005;38:47–54.
16. Maldonado ME, Perez M, Pignac-Kobinger J, Marx ET, Tozman EM, Greidinger EL, et al. Clinical and immunologic manifestations of mixed connective tissue disease in a Miami population compared to a Midwestern US Caucasian population. J Rheumatol. 2008;35:429–37.
17. Greidinger EL, Hoffman RW. Autoantibodies in the pathogenesis of mixed connective tissue disease. Rheum Dis Clin N Am. 2005;31:437–50, vi.
18. Greidinger EL, Zang Y, Jaimes K, Hogenmiller S, Nassiri M, Bejarano P, et al. A murine model of mixed connective tissue disease induced with U1 small nuclear RNP autoantigen. Arthritis Rheum. 2006;54:661–9.

19. Greidinger EL, Zang Y, Fernandez I, Berho M, Nassiri M, Martinez L, et al. Tissue targeting of anti-RNP autoimmunity: effects of T cells and myeloid dendritic cells in a murine model. Arthritis Rheum. 2009;60:534–42.
20. Keith MP, Moratz C, Tsokos GC. Anti-RNP immunity: implications for tissue injury and the pathogenesis of connective tissue disease. Autoimmun Rev. 2007;6:232–6.
21. Greidinger EL, Hoffman RW. The appearance of U1 RNP antibody specificities in sequential autoimmune human antisera follows a characteristic order that implicates the U1–70 kd and B'/B proteins as predominant U1 RNP immunogens. Arthritis Rheum. 2001;44:368–75.
22. Okada J, Hamana T, Kondo H. Anti-U1RNP antibody and aseptic meningitis in connective tissue diseases. Scand J Rheumatol. 2003;32:247–52.
23. Sato T, Fujii T, Yokoyama T, Fujita Y, Imura Y, Yukawa N, et al. Anti-U1 RNP antibodies in cerebrospinal fluid are associated with central neuropsychiatric manifestations in systemic lupus erythematosus and mixed connective tissue disease. Arthritis Rheum. 2010;62:3730–40.
24. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1982;25:1271–7.
25. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997;40:1725.
26. Katsumata Y, Harigai M, Kawaguchi Y, Fukasawa C, Soejima M, Takagi K, et al. Diagnostic reliability of cerebral spinal fluid tests for acute confusional state (delirium) in patients with systemic lupus erythematosus: interleukin 6 (IL-6), IL-8, interferon-alpha, IgG index, and Q-albumin. J Rheumatol. 2007;34:2010–7.
27. Katsumata Y, Harigai M, Kawaguchi Y, Fukasawa C, Soejima M, Kanno T, et al. Diagnostic reliability of magnetic resonance imaging for central nervous system syndromes in systemic lupus erythematosus: a prospective cohort study. BMC Musculoskelet Disord. 2010;11:13.
28. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 1988;31:315–24.
29. Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Arthritis Rheum. 1980;23:581–90.
30. Vitali C, Bombardieri S, Moutsopoulos HM, Coll J, Gerli R, Hatron PY, et al. Assessment of the European classification criteria for Sjogren's syndrome in a series of clinically defined cases: results of a prospective multicentre study. The European Study Group on Diagnostic Criteria for Sjogren's Syndrome. Ann Rheum Dis. 1996;55:116–21.
31. Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol. 2005;58:840–6.
32. Katsumata Y, Ridgway WM, Oriss T, Gu X, Chin D, Wu Y, et al. Species-specific immune responses generated by histidyl-tRNA synthetase immunization are associated with muscle and lung inflammation. J Autoimmun. 2007;29:174–86.
33. Murakami A, Kojima K, Ohya K, Imamura K, Takasaki Y. A new conformational epitope generated by the binding of recombinant 70-kd protein and U1 RNA to anti-U1 RNP autoantibodies in sera from patients with mixed connective tissue disease. Arthritis Rheum. 2002;46:3273–82.
34. Petri M, Kim MY, Kalunian KC, Grossman J, Hahn BH, Sammaritano LR, et al. Combined oral contraceptives in women with systemic lupus erythematosus. N Engl J Med. 2005;353:2550–8.
35. Luyckx A, Westhovens R, Oris E, Papisch W, Bossuyt X. Clinical relevance of measurement of antibodies to individual snU1-RNP proteins. Clin Chem. 2005;51:1888–90.
36. Koren E, Reichlin MW, Koscec M, Fugate RD, Reichlin M. Autoantibodies to the ribosomal P proteins react with a plasma membrane-related target on human cells. J Clin Invest. 1992;89:1236–41.
37. Okawa-Takatsuji M, Aotsuka S, Uwatoko S, Sumiya M, Yokohari R. Enhanced synthesis of cytokines by peripheral blood monocytes cultured in the presence of autoantibodies against U1-ribonucleoprotein and/or negatively charged molecules: implication in the pathogenesis of pulmonary hypertension in mixed connective tissue disease (MCTD). Clin Exp Immunol. 1994;98:427–33.
38. Okawa-Takatsuji M, Aotsuka S, Fujinami M, Uwatoko S, Kinoshita M, Sumiya M. Up-regulation of intercellular adhesion molecule-1 (ICAM-1), endothelial leucocyte adhesion molecule-1 (ELAM-1) and class II MHC molecules on pulmonary artery endothelial cells by antibodies against U1-ribonucleoprotein. Clin Exp Immunol. 1999;116:174–80.
39. Okawa-Takatsuji M, Aotsuka S, Uwatoko S, Takaono M, Iwasaki K, Kinoshita M, et al. Endothelial cell-binding activity of anti-U1-ribonucleoprotein antibodies in patients with connective tissue diseases. Clin Exp Immunol. 2001;126:345–54.
40. Santer DM, Yoshio T, Minota S, Moller T, Elkon KB. Potent induction of IFN-alpha and chemokines by autoantibodies in the cerebrospinal fluid of patients with neuropsychiatric lupus. J Immunol. 2009;182:1192–201.

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Original article

Urinary free light chain is a potential biomarker for ISN/RPS class III/IV lupus nephritis

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Abstract

Objectives. To evaluate the use of urinary free light chains (FLCs) as a biomarker for proliferative LN and the potential association between the intensity of plasma cell infiltration of the kidney and urinary FLC levels in LN.

Methods. Forty-three SLE patients were consecutively enrolled in the study. These patients were divided into an International Society of Nephrology and Renal Pathology Society (ISN/RPS) class III/IV LN subset ($n = 18$) and an ISN/RPS class I/II/V (class non-III/IV) LN subset ($n = 25$). The expression of κ-LCs, λ-LCs, CD19 and CD138 in kidney specimens was also evaluated with immunohistochemical staining. To measure FLC levels before and after treatment, an additional six patients with class III/IV LN were consecutively enrolled.

Results. Urinary FLCs were significantly higher in the class III/IV LN subset than in the class non-III/IV LN subset. Urinary λ-FLC levels were significantly correlated with the urinary protein-creatinine ratio in the class III/IV LN subset ($r_s = 0.67$, $P < 0.01$). Moreover, the LC-secreting $CD19^-/CD138^+$ cell counts in the kidney specimens were higher in the class III/IV LN subset than in the class non-III/IV LN subset. Total urinary FLC levels were correlated with the numbers of $CD138^+$ cells in the kidney ($r = 0.71$, $P = 0.03$). Following treatment, urinary λ-FLCs could not be detected in any of the patients.

Conclusion. The intensity of plasma cell infiltration of the kidney is associated with urinary FLC levels. Urinary FLCs are potentially useful biomarkers in ISN/RPS class III/IV LN or proliferative LN.

Key words: lupus nephritis, free light chain, plasma cell, disease activity.

Introduction

SLE is an autoimmune disease with multiple organ manifestations, including skin lesions, arthritis, serositis, nephritis and neuropsychiatric and haematological disorders. LN is a common complication of SLE; the frequency of LN is approximately 31-65% among SLE patients in the USA and Europe and 45-86% in Japan [1]. The long-term prognosis for LN has improved [2]. However, WHO class IV LN is one of the most common contributors to end-stage renal failure (ESRF). The frequency of ESRF is 40.9% in patients with WHO class IV LN compared with 2.6% in patients with non-class IV LN [3]. In general, combination therapy with corticosteroids and immunosuppressive agents (IAs), such as cyclophosphamide and mycophenolate mofetil, is recommended for class III/IV LN, as defined by the International Society of Nephrology and the Renal Pathology Society (ISN/RPS). The early diagnosis and appropriate management of ISN/RPS class III/IV LN is critical for improving the renal and overall survival of SLE patients.

Conventional clinical parameters of SLE, such as the levels of serum complement, anti-dsDNA antibodies, creatinine and proteinuria, are assessed to evaluate disease activity and predict complications of ISN/RPS class III/IV LN in SLE patients [4]. However, these markers are not always sufficiently sensitive or specific to detect ongoing disease activity and early relapses of LN. Numerous novel biomarkers, such as serum and urinary cytokines,

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chemokines, adhesion molecules and growth factors, have been evaluated for monitoring treatment response and detecting early renal flares in LN [5]. In particular, urinary biomarkers are more promising than serum biomarkers, possibly because the former result directly from kidney inflammation or injury [5].

It was recently reported that large numbers of anti-dsDNA antibody-secreting plasma cells were present in the kidneys of NZB/W mice and that differentiated (long-lived) plasma cell infiltration of the kidney medulla was associated with more severe LN in SLE patients [6]. A normal immunoglobulin molecule is composed of two light chains (LCs) and two heavy chains. During normal immunoglobulin synthesis by B cells/plasma cells, most LCs bind to heavy chains. Unbound LCs are released from B cells/plasma cells as free light chains (FLCs) [7]. Serum FLC levels are strongly correlated with global disease activity in SLE and may have applications as biomarkers [8]. Furthermore, urinary FLC levels may be useful as quantitative markers of *in vivo* polyclonal B cell activity [9]. Moreover, more severe renal inflammation and a higher risk of disease relapse are associated with increases in urinary FLCs in SLE patients [10, 11]. The findings of the above studies indicate that FLC synthesis by B cells/plasma cells is activated both systematically and locally (in the kidneys) in LN.

However, relationships between serum and urinary FLC levels, associations between serum or urinary FLC levels and pathohistological findings such as ISN/RPS class III/IV LN and class non-III/IV LN and correlations between FLC levels and the infiltration of B cells/plasma cells in the kidney have not yet been evaluated by previous studies in LN. In the present study, we measured both serum and urinary FLC levels and investigated the differences between ISN/RPS class III/IV LN and ISN/RPS class non-III/IV LN at the FLC level.

Materials and methods

Patients

A total of 43 patients with SLE were enrolled in the study. These patients were admitted to our hospital between 2004 and 2006 and consecutively underwent renal biopsies after written informed consent was obtained. All the patients were diagnosed with SLE based on the criteria of the ACR [12] and were admitted to our institution because of active SLE-associated symptoms. Of these 43 patients, 41 were Japanese and 2 were non-Japanese Asians.

To evaluate the serum and urinary FLC levels before and after treatment, another six Japanese patients with ISN/RPS class III/IV LN were consecutively enrolled from 2009 to 2010. This study was approved by the ethics committee of our institution (Institute of Rheumatology, Tokyo Women's Medical University), in accordance with the Declaration of Helsinki.

Data collection

Information that included clinical manifestations and laboratory data was obtained from the patients' medical records. Urinary measurements, including proteinuria and haematuria (by dipstick), urine sediment, protein-creatinine ratio, serum albumin, creatinine, complement components (C3 and C4), IgG and anti-dsDNA antibodies, were evaluated upon admission and prior to the renal biopsy. C3 and C4 were measured using the standard method. Anti-dsDNA antibodies were detected with a radioimmunoassay, with <6 IU/ml considered normal. Antibodies against SS-A, U1-snRNP and Sm were measured with double immunodiffusion. Antibodies against cardiolipin and β2-glycoprotein I were measured with ELISA. The estimated glomerular filtration rate (eGFR) was calculated according to a previously described method using parameters that included serum creatinine levels, age and sex [13]. The SLE disease activity of each patient at admission was assessed using the SLEDAI-2K [14]. The SLEDAI-2K renal scores included urinary casts [haeme-granular or red blood cell (RBC) casts], haematuria [>5 RBC/high-power field (HPF)], proteinuria (urinary protein-creatinine ratio >0.5) and pyuria [>5 white blood cells (WBC)/HPF].

Measurement of serum and urinary FLCs

Sera and spot urine samples were obtained shortly before the renal biopsies were performed and were stored at -80°C. Both serum and urinary FLCs were measured with a nephrometric assay (the Freelite). The normal values for serum κ-FLCs and λ-FLCs were 3.3-19.4 and 5.7-26.3 mg/l, respectively [15]. The normal values for urinary κ-FLCs and λ-FLCs had not been previously determined, although FLCs are present in the urine of healthy individuals only at extremely low concentrations [7]. The FLC values from the spot urine samples correlated well with those from the 24-h urine samples ($r = 0.71$, $P < 0.01$, data not shown). Therefore the FLC values from the spot urine samples were not corrected for urinary creatinine, as with the urinary protein-creatinine ratio.

Evaluation of renal pathohistology

The renal pathohistological findings were categorized according to the 2003 ISN/RPS classification system [16, 17]. In addition, we evaluated the infiltration of immunoglobulin LC-secreting B cells/plasma cells into the kidney cortex and medulla in ISN/RPS class III/IV LN and compared the measurements with those from the ISN/RPS class V samples. We selected biopsy samples that included sufficient portions of the medulla (with a cortex/medulla proportion <1). Ultimately five ISN/RPS class III/IV samples and four ISN/RPS class V samples fulfilled the criteria described above.

Immunohistochemical staining was performed for κ-LCs (DAKO, Tokyo, Japan, A0191), λ-LCs (DAKO, A0193), CD19 (DAKO, M7296) and CD138 (DAKO, M7228) using the standard method. To evaluate the intensity of the cellular infiltration, positively stained cells were counted in all the fields of the samples. The total cell count of each sample was divided by the entire area of the sample (cell count per mm^2).

Statistical analyses

Statistical analyses were performed using a χ^2 test to compare frequencies, a *t*-test to compare mean values and the Mann-Whitney *U* test to compare median values. Correlation coefficients were calculated as a Pearson's correlation coefficient or Spearman's rank correlation if applicable. The urinary protein-creatinine ratio, the anti-dsDNA antibody levels and the serum and urinary FLC levels before and after treatment were compared using the Wilcoxon signed-rank test. The data were analysed with JMP software (SAS Institute, Cary, NC, USA). *P*-values < 0.05 indicated statistical significance.

Results

Comparison of clinical manifestations between ISN/RPS class III/IV and class non-III/IV LN subsets

The 43 enrolled patients were divided into the following two subsets: an ISN/RPS class III/IV LN subset and an ISN/RPS class I, II or V (class non-III/IV) LN subset. The combined classes III and V and classes IV and V were referred to as class III and class IV, respectively. The frequencies of LN classified as ISN/RPS classes I, II, III, IV and V were 9 (21%), 7 (16%), 8 (19%), 10 (23%) and 9 (21%), respectively. As shown in Table 1, the median age, sex, frequency of prednisolone (PSL) or IA administration and PSL dosage did not differ between the two subsets. The urinary protein-creatinine ratio was higher ($P = 0.01$) and the serum albumin level was lower ($P = 0.03$) in the ISN/RPS class III/IV LN subset than in the ISN/RPS class non-III/IV LN subset. The complement level was lower, and the anti-dsDNA antibody titre and the SLEDAI-2K total score were higher in the ISN/RPS class III/IV LN subset.

Comparison of serum and urinary FLC levels between ISN/RPS class III/IV and class non-III/IV LN subsets

As shown in Table 2, the serum κ-FLC levels were significantly higher in the ISN/RPS class III/IV LN subset than in the ISN/RPS class non-III/IV LN subset, although the median values and interquartile ranges were within the normal limits in each subset. There were no significant differences in serum λ-FLCs between the two subsets.

In contrast, both urinary κ-FLCs and λ-FLCs were significantly higher in the ISN/RPS class III/IV LN subset than in the ISN/RPS class non-III/IV LN subset ($P = 0.02$ for both). In the ISN/RPS class non-III/IV LN subset, no urinary λ-FLCs were detected in 13 (52%) of 25 patients.

Relationship between serum and urinary FLCs

No significant correlations were found between the serum and urinary κ-FLCs in both the ISN/RPS class non-III/IV LN subset and the ISN/RPS class III/IV LN subset (Fig. 1A). In addition, there were no significant correlations between serum and urinary λ-FLCs in either subset (Fig. 1B).

Associations between SLEDAI-2K scores and serum/urinary FLCs

We analysed the correlations between the SLEDAI-2K total or renal score and serum or urinary FLCs in all the

Table 1 Comparison of the clinical manifestations of the ISN/RPS class III/IV LN subset and the class non-III/IV LN subset

	Class III/IV ($n = 18$)	Class non-III/IV ($n = 25$)	*P*-value
Age at renal biopsy, years	32 (23–48)	31 (25–42)	0.66
Female, *n* (%)	18 (100)	24 (96)	1.00
Patients who received PSL, *n* (%)	5 (28)	11 (44)	0.35
Dosage of PSL, mg/day	0 (0–15)	5 (0–17.5)	0.43
Patients who received IA, *n* (%)	1 (6)	2 (8)	1.00
Urinary protein-creatinine ratio	0.85 (0.10–1.54)	0.07 (0.03–0.24)	0.01
Serum albumin, g/dl	3.3 (2.9–3.5)	3.8 (3.3–4.1)	0.03
Serum creatinine, mg/dl	0.54 (0.45–0.59)	0.51 (0.46–0.58)	0.68
eGFR, ml/min/1.73 m^2	115 (89–124)	114 (91–135)	0.79
C3, mg/dl	44.0 (32.5–70.3)	65.0 (44.8–86.5)	0.06
C4, mg/dl	4.0 (2.3–7.5)	9.0 (3.8–15)	0.03
IgG, mg/dl	1956 (1635–2706)	1908 (1467–2564)	0.64
Anti-dsDNA Ab, IU/ml	298.5 (150.3–623.8)	20.5 (7.5–29.3)	<0.01
Anti-SS-A Ab positivity, *n* (%)	11 (61)	10 (40)	0.22
Anti-U1 snRNP Ab positivity, *n* (%)	6 (33)	15 (60)	0.12
Anti-Sm Ab positivity, *n* (%)	1 (6)	10 (40)	0.01
Anti-CLβ2GP I Ab positivity, *n* (%)	2 (11)	2 (8)	1.00
SLEDAI-2K total score	15 (10–21)	9 (4–12)	0.01

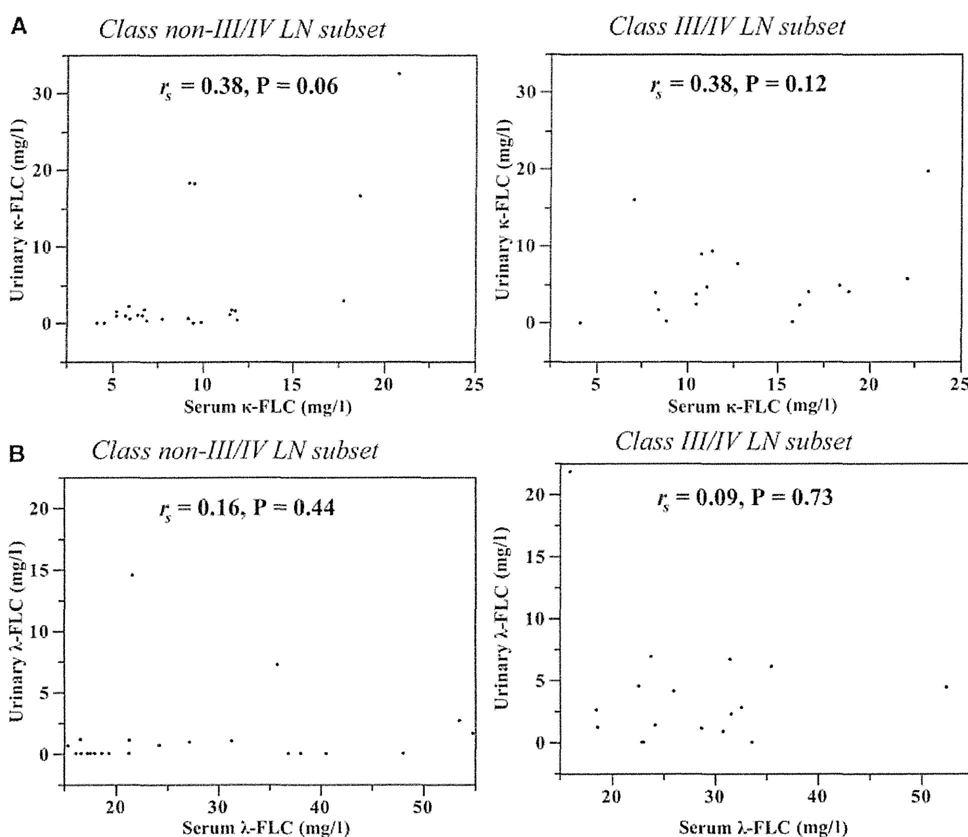
Except for the *n* (%) values, all values listed above represent median (IQR). *P*-values were established using a χ^2 test or Mann-Whitney *U* test. CLβ2GP I: cardiolipin and β2-glycoprotein I; SLEDAI-2K: systemic lupus erythematosus disease activity index 2000; SLEDAI-2K renal score: SLEDAI score with renal involvement, including urinary casts, haematuria, proteinuria and pyuria.

TABLE 2 Comparison of serum and urinary FLC levels in the ISN/RPS class III/IV subset and the ISN/RPS class non-III/IV subset

	Class III/IV ($n=18$)	Class non-III/IV ($n=25$)	*P*-value
Serum κ-FLC (mg/l)	11.3 (8.8-17.1)	9.2 (5.9-11.7)	0.04
Serum λ-FLC (mg/l)	27.4 (22.8-32.9)	21.3 (17.0-36.4)	0.13
Urinary κ-FLC (mg/l)	4.05 (2.16-8.01)	1.0 (0.47-1.99)	0.02
Urinary λ-FLC (mg/l)	2.69 (1.04-6.25)	0 (0-1.11)	0.02

Values listed above represent median (IQR). *P*-values were established using the Mann-Whitney *U* test.

FIG. 1 Associations between serum and urinary FLCs in the ISN/RPS class non-III/IV LN subset and in the ISN/RPS class III/IV LN subset.



An association between serum κ-FLC and urinary κ-FLC (**A**) and an association between serum λ-FLC and urinary λ-FLC (**B**) in each subset.

43 enrolled patients. The serum FLC levels were not significantly correlated with the SLEDAI-2K total score (κ-FLC: $r_s=0.13$, $P=0.40$ and λ-FLC: $r_s=0.20$, $P=0.20$). There was a correlation between the SLEDAI-2K total score and the urinary λ-FLC levels ($r_s=0.40$, $P=0.02$), although no significant correlation existed between the SLEDAI-2K total score and the urinary κ-FLC levels (κ-FLC: $r_s=0.23$, $P=0.15$) and between the SLEDAI-2K renal score and urinary κ- or λ-FLC levels. Moreover, there was no significant difference in serum or urinary FLC levels between the active disease (SLEDAI-2K total score >4) subset and the inactive disease subset in either the ISN/RPS class non-III/IV LN subset and the class III/IV LN subset.

Association between urinary FLCs and conventional biomarkers

Significant correlation was found between urinary λ-FLC and anti-dsDNA Ab ($r_s=0.42$, $P=0.01$), although there was no significant correlation between urinary λ-FLC

and C3 ($r_s = -0.14$, $P = 0.44$) in the ISN/RPS class III/IV subset. There were no statistically significant differences in the urinary FLC levels between the presence of active urinary sediments (RBC > 5/HPF, WBC > 5/HPF or haeme-granular or RBC casts) subset and the absent subset.

Correlation between urinary FLCs and urinary protein-creatinine ratio

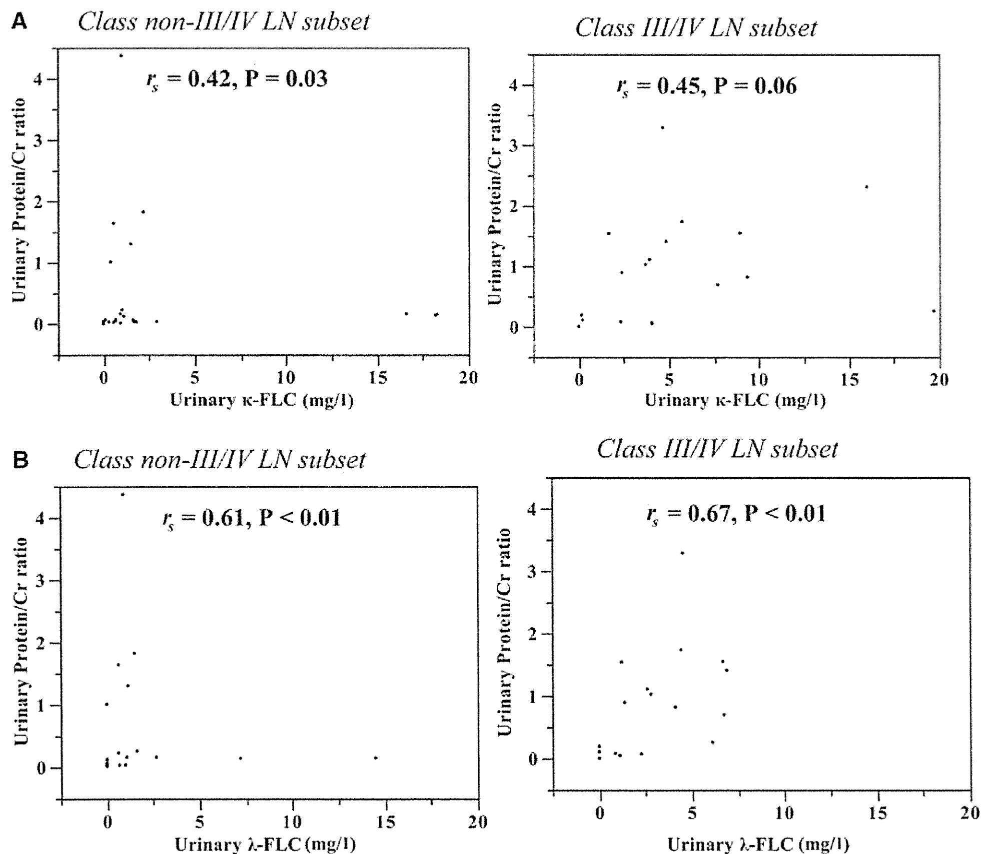
The serum κ-FLCs and λ-FLCs were not correlated with the urinary protein-creatinine ratio in either the ISN/RPS class non-III/IV LN subset or the ISN/RPS class III/IV LN subset. As shown in Fig. 2A, there was also no significant association between the urinary κ-FLCs and the urinary protein-creatinine ratio in the ISN/RPS class III/IV LN subset ($r_s = 0.45$, $P = 0.06$), although the urinary κ-FLCs correlated with the urinary protein-creatinine ratio in the ISN/RPS class non-III/IV LN subset ($r_s = 0.42$, $P = 0.03$). The urinary λ-FLCs were significantly positively correlated with the urinary protein-creatinine ratio in both the ISN/RPS class non-III/IV LN subset ($r_s = 0.61$, $P < 0.01$) and the ISN/RPS class III/IV LN subset ($r_s = 0.67$, $P < 0.01$) (Fig. 2B). On the other hand, the C3 and anti-dsDNA antibody levels were not associated with the urinary protein-creatinine ratio.

To investigate an association between the levels of urinary FLC and proteinuria in detail, we compared the ISN/RPS class III/IV subset with the class V subset. The median value of the urinary protein-creatinine ratio was 0.8 and 0.9 in the ISN/RPS class III/IV subset and the class V subset, respectively. There was no statistically significant difference in the urinary protein-creatinine ratio between the two subsets. In contrast, the ratio of the urinary κ- or λ-FLC levels to the urinary proteinuria concentration was higher in the class III/IV subset than in the class V subset ($P = 0.05$ and 0.01 in urinary κ-FLC and urinary λ-FLC, respectively).

Immunohistochemical staining of kidney specimens with immunoglobulin light chains, CD19 and CD138 in ISN/RPS classes III/IV and V LN

To compare the ISN/RPS class III/IV LN patients ($n = 5$) with the ISN/RPS class V LN patients ($n = 4$) in terms of the infiltration of B cells/plasma cells and the status of the LCs synthesized by those cells in the kidney, immunohistochemical staining was performed for κ-LCs, λ-LCs,

Fig. 2 Correlations between urinary protein-creatinine ratios and urinary FLCs in the ISN/RPS class non-III/IV LN subset and the ISN/RPS class III/IV LN subset.



Correlations between (**A**) the urinary protein-creatinine ratio and urinary κ-FLC and (**B**) the urinary protein-creatinine ratio and urinary λ-FLC in each subset.

CD19 and CD138 in both patient subsets. As shown in a representative ISN/RPS class III/IV LN case (Fig. 3A), κ-LCs and λ-LCs were synthesized mainly by CD138$^+$ cells. The κ-LC$^+$ cell counts were significantly higher ($P = 0.03$) in the ISN/RPS class III/IV LN subset than in the ISN/RPS class V LN subset (Fig. 3B). In addition, the λ-LC$^+$ cell counts and the CD 138$^+$ cell counts were higher in the class III/IV LN subset than in the class V LN subset, although this difference was not significant (Fig. 3C and E). The CD19$^+$ cell counts did not differ between the two subsets (Fig. 3D). Among all of the infiltrating CD138$^+$ cells, there were no significant differences between the κ-LC$^+$ cell count and the λ-LC$^+$ cell count. Moreover, the CD138$^+$ cells expressed virtually no CD19 in the kidney specimens from the ISN/RPS class III/IV LN subset. Almost all of the CD138$^+$ cells were located around the glomeruli or the tubulointerstitium of the margin between the renal cortex and the medulla.

Correlation between urinary FLC levels and the number of CD138$^+$ cells infiltrating the kidney

As shown in Fig. 3F, the total (κ + λ) urinary FLC levels were significantly correlated ($r = 0.80$, $P < 0.01$) with the numbers of CD138$^+$ cells infiltrating the kidney of nine patients described above (five patients with ISN/RPS class III/IV LN and four with ISN/RPS class V LN), although there were no such associations with the total serum FLC levels.

Comparison of clinical parameters before and after immunosuppressive treatment

As shown in Fig. 4A and B, the urinary protein-creatinine ratio and the anti-dsDNA antibody titre were lower after treatment than before treatment ($P = 0.03$ and 0.06, respectively) in six patients with ISN/RPS class III/IV LN. Both serum κ-FLCs and serum λ-FLCs decreased, although their values were almost within normal limits prior to treatment (Fig. 4C and D). Both urine κ-FLCs and urine λ-FLCs also decreased after treatment compared with before treatment (Fig. 4E and F). Notably, no urine λ-FLCs could be detected in any patient after treatment.

Discussion

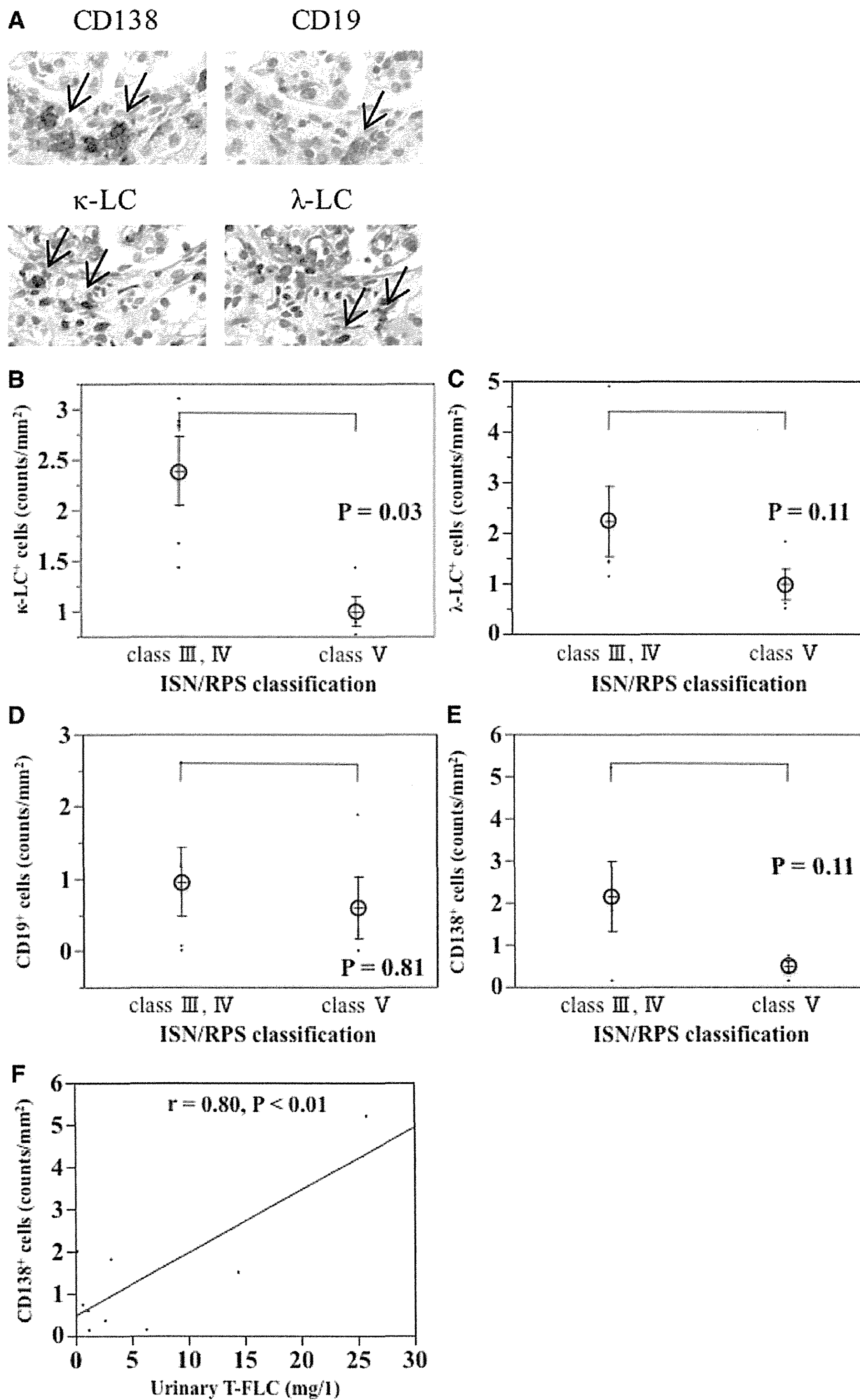
The present study demonstrated that urinary FLC levels were elevated and were associated with the intensity of plasma cell infiltration of the kidney in ISN/RPS class III/IV LN patients. During normal immunoglobulin synthesis, B cells/plasma cells release FLCs, which pass through the glomerular filtration barrier rapidly (with a serum half-life of 2–6 h) [7]. The urinary FLC levels are low in healthy individuals [18, 19]. Serum FLC levels are elevated in many autoimmune/inflammatory diseases, and FLC levels are strongly correlated with other markers of B cell/plasma cell activation [8, 20, 21]. Urinary FLC levels might represent quantitative markers of real-time, *in vivo* polyclonal B cell/plasma cell activity; SLE relapse has been associated with increases in urinary FLCs [9, 10]. According to the previous report described by Tsai *et al.* [11], urinary FLCs were not detected in patients with inactive LN (24-h urinary excretion; range 3–20 g/day). In the present study, the ratio of the urinary FLC levels to the proteinuria levels was higher in the ISN/RPS class III/IV LN subset than the ISN/RPS class V LN subset, although there was no significant difference in the levels of proteinuria between the two subsets. These results indicate that urinary FLC levels led to not only the synthesis of FLCs or the urinary protein excretion concentrations, but also the disease activity of LN. Urinary FLC levels may better reflect the disease activity of LN in real time than conventional markers.

A recent study revealed that plasma cell infiltration into the renal medulla is correlated with the amount of inflammation and the disease severity of patients with LN. A study of NZB/W mice revealed that anti-dsDNA antibodies were produced by long-lived plasma cells located in the kidney [6]. CD138$^+$ cells that express low levels of CD19 are compatible with a long-lived plasma cell phenotype [22]. Long-lived plasma cells are capable of continuously secreting antibodies. These cells live primarily in the bone marrow and secondarily in niches in the spleen and chronically inflamed tissues, such as the kidneys in SLE or the synovia in RA [23]. The present study demonstrated that LCs were synthesized mainly by CD19$^-$/CD138$^+$ cells and that the total urinary FLC levels were significantly correlated with the numbers of kidney-infiltrating CD138$^+$ cells in ISN/RPS class III/IV LN. In contrast, the ISN/RPS class non-III/IV (e.g., class V) LN patients exhibited low or undetectable urinary FLC levels, and the CD138$^+$ cells infiltrating their kidneys were scarcely detectable. These findings revealed that the infiltration of long-lived plasma cells into the kidney may play a role in local inflammation and that the urinary FLC levels reflect the intensity of the plasma cell infiltration of the kidney in proliferative types of LN, such as ISN/RPS class III/IV LN.

In the present study, urinary λ-FLCs were not detected after immunosuppressive therapy in any of the ISN/RPS class III/IV LN patients. In contrast, urinary κ-FLC levels were significantly decreased (but detectable) after treatment, although there was no significant difference between κ-LC$^+$/CD138$^+$ cell counts and λ-LC$^+$/CD138$^+$ cell counts in the ISN/RPS class III/IV LN kidney specimens. κ-LCs and λ-LCs are encoded by different chromosomes. λ-FLCs have a dimeric structure, whereas κ-FLCs have a monomeric structure [24]. In amyloid light chain amyloidosis, λ-LCs are involved in amyloid deposition more often than κ-LCs [25]. Although the precise molecular functions of each type of FLC remain unknown, the molecular differences between κ-LCs and λ-LCs might cause the discrepancies in the serum and urinary levels between κ-FLC and λ-FLC.

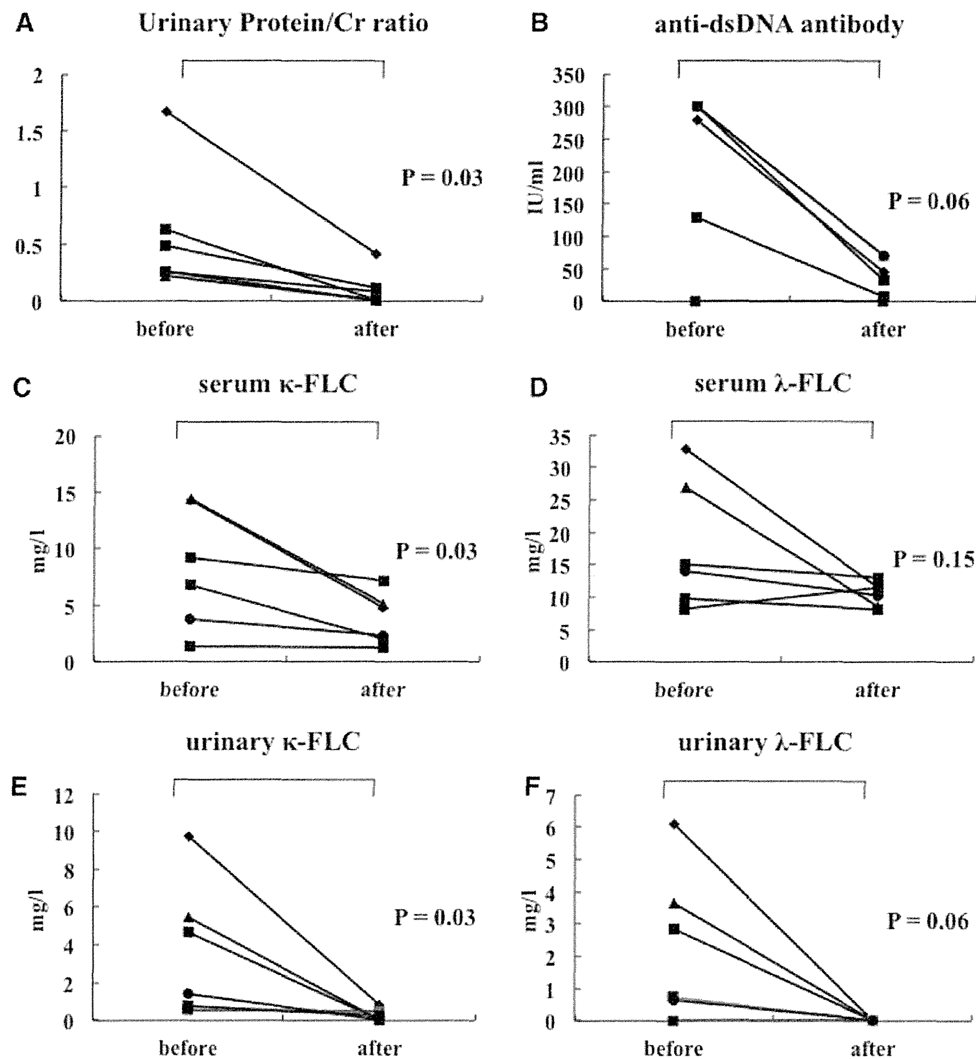
A previous study reported that serum FLC levels were correlated with global disease activity in SLE [8]. However, the present study found no correlation between the SLEDAI-2K scores and the serum FLC levels. A bias in the selection of enrolled patients may have contributed to the differences between the results of the previous study and ours. In this study, urinary FLC levels were correlated with the urinary protein-creatinine ratio, although

FIG. 3 Immunohistochemical staining for immunoglobulin LCs, CD19 and CD138 in ISN/RPS class III/IV and class V LN kidney specimens.



In a representative ISN/RPS class III/IV LN case, κ-LC and λ-LC were synthesized mainly by CD138+ cells (**A**). Immunohistochemical staining for κ-LC (**B**), λ-LC (**C**), CD19 (**D**) and CD138 (**E**) in the class III/IV LN subset ($n = 5$) and in the class V LN subset ($n = 4$). There was a positive correlation between the total urinary FLC levels and the numbers of CD138+ cells infiltrating the kidney (**F**).

FIG. 4 Clinical parameters before and after treatment.



Urinary protein-creatinine ratios (**A**), anti-dsDNA antibody titres (**B**), serum κ-FLC levels (**C**), serum λ-FLC levels (**D**), urinary κ-FLC levels (**E**) and urinary λ-FLC levels (**F**).

there were no significant correlations between the urinary protein-creatinine ratio and serum biomarkers such as FLC, anti-dsDNA Ab and C3. Urinary FLC could reflect the activity of LN. However, urinary FLC is not a specific marker for LN. Urinary FLC may be increasing in the other glomerulonephritis involved with plasma cells infiltration. Moreover, serum and urinary FLC levels can be influenced by several factors, including renal dysfunction and hypergammopathies such as infections and multiple myeloma [7, 10].

There are some limitations to our study. First, the serum and urine samples were stored for long term. This might affect the results of the serum and urinary FLC levels. Second, the sample size was small. We did not longitudinally evaluate the urinary FLC level after treatment. So we are unsure whether urinary FLC is a useful biomarker as a predictor for renal relapse.

In conclusion, urinary FLC is a non-invasive biomarker for ISN/RPS class III/IV LN or proliferative LN. The intensity of the plasma cell infiltration of the kidney is also associated with urinary FLC levels in LN.

Rheumatology key messages

- Urinary FLCs are useful as a non-invasive biomarker for the response to treatment of ISN/RPS class III/IV LN.
- The intensity of the plasma cell infiltration of the kidney is associated with urinary FLC levels in LN.

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References

1 Yokoyama H, Okuyama H, Yamaya H. Clinicopathological insights into lupus glomerulonephritis in Japanese and Asians. Clin Exp Nephrol 2011;15:321-30.

2 Cameron J. Lupus nephritis. J Am Soc Nephrol 1999;10: 413-24.

3 Yokoyama H, Wada T, Hara A *et al*. The outcome and a new ISN/RPS 2003 classification of lupus nephritis in Japanese. Kidney Int 2004;66:2382-8.

4 Wakasugi D, Gono T, Kawaguchi Y *et al*. Frequency of class III and IV nephritis in systemic lupus erythematosus without clinical renal involvement: an analysis of predictive measures. J Rheumatol 2012;39:79-85.

5 Mok CC. Biomarkers for lupus nephritis: a critical appraisal. J Biomed Biotechnol 2010;2010:638413.

6 Espeli M, Bökers S, Giannico G *et al*. Local renal autoantibody production in lupus nephritis. J Am Soc Nephrol 2011;22:296-305.

7 Hutchison CA, Basnayake K, Cockwell P. Serum free light chain assessment in monoclonal gammopathy and kidney disease. Nat Rev Nephrol 2009;5:621-8.

8 Aggarwal R, Sequeira W, Kokebie R *et al*. Serum free light chains as biomarkers for systemic lupus erythematosus disease activity. Arthritis Care Res 2011;63:891-8.

9 Hopper JE, Sequeira W, Martellotto J *et al*. Clinical relapse in systemic lupus erythematosus: correlation with antecedent elevation of urinary free light-chain immunoglobulin. J Clin Immunol 1989;9:338-50.

10 Hopper JE, Golbus J, Meyer C *et al*. Urine free light chains in SLE: clonal markers of B-cell activity and potential link to in vivo secreted Ig. J Clin Immunol 2000; 20:123-37.

11 Tsai CY, Wu TH, Sun KH *et al*. Increased excretion of soluble interleukin 2 receptors and free light chain immunoglobulins in the urine of patients with active lupus nephritis. Ann Rheum Dis 1992;51:168-72.

12 Hochberg M. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:1725.

13 Matsuo S, Imai E, Horio M *et al*. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 2009;53:982-92.

14 Gladman DD, Ibañez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. J Rheumatol 2002;29:288-91.

15 Katzmann JA, Clark RJ, Abraham RS *et al*. Serum reference intervals and diagnostic ranges for free kappa and free lambda immunoglobulin light chains: relative sensitivity for detection of monoclonal light chains. Clin Chem 2002;48:1437-44.

16 Weening J, D'Agati V, Schwartz M *et al*. The classification of glomerulonephritis in systemic lupus erythematosus revisited. J Am Soc Nephrol 2004;15:241-50.

17 Weening J, D'Agati V, Schwartz M *et al*. The classification of glomerulonephritis in systemic lupus erythematosus revisited. Kidney Int 2004;65:521-30.

18 Kaplan B, Livneh A, Sela BA. Immunoglobulin free light chain dimers in human diseases. ScientificWorldJournal 2011;11:726-35.

19 Mogensen CE, Sølling. Studies on renal tubular protein reabsorption: partial and near complete inhibition by certain amino acids. Scand J Clin Lab Invest 1977;37: 477-86.

20 van der Heijden M, Kraneveld A, Redegeld F. Free immunoglobulin light chains as target in the treatment of chronic inflammatory diseases. Eur J Pharmacol 2006; 533:319-26.

21 Gottenberg JE, Aucouturier F, Goetz J *et al*. Serum immunoglobulin free light chain assessment in rheumatoid arthritis and primary Sjogren's syndrome. Ann Rheum Dis 2007;66:23-7.

22 Cepok S, Rosche B, Grummel V *et al*. Short-lived plasma blasts are the main B cell effector subset during the course of multiple sclerosis. Brain 2005;128(Pt 7): 1667-76.

23 Neves M, Alves JD. Factors implicated in the generation and persistence of long-lived plasma cell-mediated autoimmunity. Autoimmun Rev 2011;10:375-82.

24 Nakano T, Matsui M, Inoue I *et al*. Free immunoglobulin light chain: its biology and implications in diseases. Clin Chim Acta 2011;412:843-9.

25 Bellotti V, Mangione P, Merlini G. Review: immunoglobulin light chain amyloidosis—the archetype of structural and pathogenic variability. J Struct Biol 2000;130: 280-9.

東北地方におけるB型肝炎再活性化前向き研究について

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要　旨

既感染B型肝炎関節リウマチ患者157人のうち18ヵ月間でHBV DNAが再活性化した13人では，生物学的製剤，エタネルセプト，メトトレキサート，高用量ステロイド，タクロリムスの使用が多く，再活性化のハザード比はCox回帰ハザード分析で生物学的製剤10.9（p＝0.008），エタネルセプト6.9（p＝0.001），多重ロジスティック回帰解析でタクロリムス11.1（p＝0.0015）であった．

はじめに

我々は，東北地方のリウマチ性疾患患者における，免疫抑制療法後のB型肝炎ウイルス（HBV）再活性化の実態を調査するため，HBIRTH（Hepatitis B virus Infection in Rheumatic diseases in ToHoku area）研究会を立ち上げ，厚生労働省の研究班が提唱したB型肝炎対策ガイドラインが有用であるかどうかを検討する目的で，多施設による前向き研究を行っている．研究のエントリー期間が2013年3月末までであり，すべてのデー

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キーワード：関節リウマチ，B型肝炎，生物学的製剤，免疫抑制薬，コルチコステロイド

タの凍結が終了次第，解析を行う予定である．このため，本稿ではHBIRTHのパイロット研究となった青森県つがる地域における関節リウマチ（RA）患者のHBV DNA再活性化について紹介したい．

B型肝炎の再活性化とは

世界人口のおよそ30億人はHBVに感染歴があり，3億5千万人のキャリア患者が存在する[1]．近年，化学療法・免疫療法・移植療法の進歩に伴い，多様な抗がん剤や免疫抑制薬を使用する機会の増加に伴うB型肝炎の再活性化が報告されている[2]．B型肝炎の再活性化は一般的に一過性で，臨床的に問題となることは少ないが，しばしば深刻で，急性の肝不全を引き起こすことがある[3]．

B型肝炎の再活性化には，大きく2つのシナリオが存在する．まずキャリア例からの発症であり，次に既感染からの発症である．B型肝炎の診断は通常HBs抗原の同定によって行われ，HBs抗原の陰性化は通常B型肝炎治癒と考えられていた[4]．実は，これらのB型肝炎治癒（既感染HBV）患者では，数十年間，肝臓と末梢血単核細胞内で低レベルながらHBV複製が継続していることが明らかとなった．そのような患者では，例えばHBV特異的CTL活性が抑制されるなど，HBV複製に対する免疫応答が抑制されている[4]．近年，HBs陰性でHBc抗体ないしHBs抗体陽性例の既感染HBV患者に，リツキシマブなど強力な免疫抑制薬により，HBV再活性化による重症肝炎が発症することが報告され，*de novo* B型肝炎と呼ばれている[5]．既感染B型肝炎合併 RA においても，コルチコステロイド（CS）やメトトレキサート（MTX），および TNF 阻害薬を主とした生物学的製剤を含む免疫抑制療法により，劇症または致死的な肝炎を引き起こすことが報告されている[6]．以上から，我々は既感染B型肝炎RAにおけるHBV再活性化率を明らかにするために，以下の研究を行った[7]．

青森県つがる地域におけるB型肝炎再活性化前向き研究

1．方　法

2008年1月から2009年8月までに我々の外来を受診した，1987年米国リウマチ学会の分類基準を満たす RA 516人に対して，HBs抗原，HBs抗体，HBc抗体を測定した．HBVマーカーは，市販のCLIA法（HBs抗原：アーキテクト HBsAg QT，HBs抗体：アーキテクト Anti-HBs および HBc抗体：アーキテクト Anti-HBc；アボット ジャパン社，東京）を用いて検出した．患者がHBs抗原陽性，もしくはHBs抗原が陰性でHBs抗体 and/or HBc抗体陽性である場合に，HBV DNA量を測定した．最低検出感度は2.1 log copies/m*l* であった．HBV DNAの測定結果が陰性であった場合には測定を3ヵ月ごとに繰り返し実施し，HBV DNAが陽性に転じれば測定を毎月繰り返し実施した．HBV DNA量は，コバス TaqMan HBV「オート」v2.0（ロシュ・ダイアグノスティックス社，東京）を用いて定量した．コバス AmpliPrep を用いて血清から核酸抽出を行い，リアルタイム PCR 装置であるコバス TaqMan を用いて増幅および測定を実施した．生物学的製剤も含めた薬物治療は，HBV DNA 量を問わず中止しなかった．すべての参加施設の倫理委員会において本試験の実施が承認され，すべての患者から文書による同意を取得した．統計解析は，Fisher直接確率検定，Student t 検定および Mann-Whitney U 検定を用いて，ベースラインの患者背景因子をサブグループ間で比較した．両側 p 値が≦0.05 の場合に有意な差と見なした．Cox回帰ハザード分析法で，生物学的製剤，MTX，CSおよび疾患修飾性抗リウマチ薬（DMARD）が HBV

表1 HBV DNA 再活性化（+）群と（−）群における患者背景因子および治療開始前の検査値の比較

	HBV DNA 再活性化（+）(n=13)	HBV DNA 再活性化（−）(n=144)	p 値
年齢（歳）	66.6±10.7（67.6）	64.9±11.8（66.2）	0.670
女性（n）	8（61.5%）	114（77.9%）	0.505
RA 罹患期間（年）	8.0±7.7（4.7）	7.6±9.0（4.0）	0.241
CRP（mg/d*l*）	0.92±2.46（0.09）	1.04±2.11（0.20）	0.218
ESR（mm/h）	26.0±30.0（13.0）	26.1±26.8（15.0）	0.476
IgM RF（IU/m*l*）	46.2±34.0（49.3）	88.0±151.2（24.5）	0.791
AST（U/*l*）	25.5±6.5（27.0）	27.9±16.4（23.0）	0.688
ALT（U/*l*）	19.9±6.8（20.5）	26.0±19.2（19.0）	0.959
IgG（mg/d*l*）	1,454±573（1,382）	1,432±450（1,358）	0.604
好中球数（/μ*l*）	3,326±1,567（2,722）	4,462±2,302（3,868）	0.063
リンパ球数（/μ*l*）	1,503±425（1,431）	1,732±813（1,562）	0.323

数値は平均±標準偏差（中央値）として示す．
RA：関節リウマチ，CRP：C反応性タンパク質，ESR：赤血球沈降速度，Ig：免疫グロブリン，
RF：リウマトイド因子，AST：アスパラギン酸アミノトランスフェラーゼ，
ALT：アラニンアミノトランスフェラーゼ

DNA 複製の再活性化に寄与する因子を評価した．さらに，ステップワイズ変数減少法による多重ロジスティック回帰モデル分析を実施した．分析はすべて，JMP バージョン 8.0 ソフトウェア（JMP ジャパン事業部，東京）を用いて実施した．

2．結　果

516 例の患者の背景因子を表1に示す．HBs 抗原陽性は7例であったのに対し，HBs 抗原が陰性で HBs 抗体 and/or HBc 抗体が陽性のケースは 157 例であった（30.4%）．既感染B型肝炎 RA 患者において，治療開始前の HBV DNA は全例で陰性であった．全例で 18ヵ月間のモニタリングを実施したところ，157 例中 13 例（8.3%）で HBV DNA が再活性化（平均 3.44 log copies/m*l*）したが，全例で肝機能は正常であった（表1）．HBV DNA の再活性化を来した 13 例の詳細を表2に示す．13 例中 10 症例では生物学的製剤を用いた治療中に HBV DNA が検出されたのに対し［エタネルセプト（ETN，n=8），アバタセプト（n=2），アダリムマブ（n=1），インフリキシマブ（n=1），トシリズマブ（n=1），リツキシマブ（n=1）］，3症例では生物学的製剤による治療なしに HBV DNA が検出されている．なお，症例5では2回の HBV DNA 再活性化が認められた．試験中に RA 治療に用いた DMARD および免疫抑制薬のタイプと，各薬物療法を受けた患者の例数を表3に示す．13 例中2例の患者では，抗ウイルス療法を行わずに HBV DNA が陰性となったが，13 例中 10 例ではエンテカビル療法によって HBV DNA が陰性化した（平均 3.3ヵ月）．残り1例は，HBV DNA が陽転化後，原因不明の突然死を来した．

HBV DNA 再活性化に関与する因子について解析を実施した（表3）．既感染B型肝炎 RA 患者に対し使用された薬剤について，HBV DNA 再活性化（+）群と（−）群の間には，生物学的製剤（76.9% 対 36.1%，p=0.006），ETN（61.5% 対 22.2%，p=0.005），MTX（76.9% 対 46.5%，p=0.044），高用量 CS（15.4% 対 1.4%，p=0.035）およびタク

表2 HBV DNA 再活性化を認めた13症例の治療履歴と検査データ

症例	年齢（歳）	RA 罹患期間（月数）	生物学的製剤	MTX（mg 週）	DMARD	プレドニゾロン（mg/日）	HBV DNA（log copies/ml）	エンテカビル	HBV DNA の最終的な状態	HBV DNA 発現からその消失までの期間（月数）	ALT（U/l）
1	77	35	トシリズマブ*1	6	なし	なし	2.0	なし	DNA 陰性	1	27
2	65	53	エタネルセプト	なし	なし	2	5.0	あり	DNA 陰性	5	20
3	46	120	エタネルセプト	8	タクロリムス 1 mg/日	なし	3.7	あり	DNA 陰性	1	30
4	49	60	エタネルセプト	なし	ブシラミン 200mg/日	なし	7.4	あり	DNA 陰性	18	20
5*2	60	36	エタネルセプト	なし	レフルノミド 10mg/日	3	2.0	なし	DNA 陰性	1	25
	61	48	アダリムマブ	2	タクロリムス 1 mg/日	3	2.1	あり	DNA 陰性	1	25
6	75	18	エタネルセプト	8	なし	6	2.4	あり	DNA 陰性	2	26
7	74	19	なし	7.5	なし	5	3.0	あり	DNA 陰性	2	14
8	84	162	リツキシマブ	7.5	タクロリムス 1 mg/日	30	2.2	なし			22
9	74	73	なし	8	ブシラミン 200mg/日	4	2.1	あり	DNA 陰性	1	11
10	69	180	アバタセプト	8		2.5	7.8	あり	DNA 陽性		21
11	60	224	アバタセプト*3	7.5			4.1	あり	DNA 陰性	1	13
12	66	317	エタネルセプト		ブシラミン 200mg/日		2.2	あり	DNA 陰性	1	6
13	72	2		7.5		25	2.1	あり	DNA 陰性	1	18

*1 3種類の生物学的製剤（インフリキシマブ，エタネルセプトおよびトシリズマブ）をスイッチして使用，*2 HBV DNA 再活性化が2回認められた，
*3 2種類の生物学的製剤（エタネルセプトおよびアバタセプト）をスイッチして使用．
RA：関節リウマチ，MTX：メトトレキサート，DMARD：疾患修飾性抗リウマチ薬，ALT：アラニンアミノトランスフェラーゼ

表3　試験期間中に関節リウマチ（RA）関連併用薬を使用した患者の数
－HBV DNA 再活性化（+）群と（－）群の比較－

	患者数*		p 値	HR（95%CI）
	HBV DNA 再活性化（+）(n=13)	HBV DNA 再活性化（－）(n=144)		
生物学的製剤	10（76.9%）	52（36.1%）	0.006	2.1（1.5～3.1）
アダリムマブ	1（7.7%）	8（5.6%）	0.550	1.4（0.2～10.2）
エタネルセプト	8（61.5%）	32（22.2%）	0.005	2.8（1.6～4.7）
インフリキシマブ	7（53.8%）	17（11.8%）	1.000	0.7（0.1～4.5）
トシリズマブ	1（7.7%）	7（4.9%）	0.507	1.6（0.2～11.9）
アバタセプト	2（15.4%）	3（20.8%）	0.055	7.4（1.4～40.3）
リツキシマブ	1（7.7%）	0	0.080	
メトトレキサート	10（76.9%）	67（46.5%）	0.044	1.7（1.2～2.3）
平均用量	7.1±1.9mg/週	6.8±1.9mg/週	0.707	
コルチコステロイド	6（46.2%）	57（39.6%）	0.770	1.2（0.6～2.2）
平均用量	12.7±15.6mg/日	5.7±5.0mg/日	0.533	
高用量コルチコステロイド（≧0.5mg/kg/日）	2（15.4%）	2（1.4%）	0.035	11.1（1.7～72.3）
スルファサラジン	1（7.7%）	36（25.0%）	0.303	0.3（0.0～2.1）
ブシラミン	3（23.1%）	29（20.1%）	0.729	1.1（0.4～3.3）
タクロリムス水和物	4（30.8%）	8（5.6%）	0.010	5.5（1.9～15.9）
注射金製剤	1（7.7%）	5（3.5%）	0.410	2.2（0.3～17.6）
レフルノミド	1（7.7%）	2（1.4%）	0.230	5.5（0.5～57.1）
D-ペニシラミン	0	2（1.4%）	1.000	
アクタリット	0	1（0.7%）	1.000	
オーラノフィン	0	7（4.9%）	1.000	
シクロスポリン	0	1（0.7%）	1.000	
ミノサイクリン塩酸	0	2（1.4%）	1.000	
シクロホスファミド	0	1（0.7%）	1.000	

* 数値は薬剤を服用している患者の数を示す．患者は複数の薬剤を使用しており，また複数の生物学的製剤をスイッチして使用している症例もある．
HR：ハザード比，95%CI：95% 信頼区間

ロリムス水和物（30.8% 対 5.6%，p＝0.010）の使用に有意差が認められた．Cox 回帰ハザード分析でも，生物学的製剤と ETN の使用が HBV DNA 再活性化の予測因子である可能性が明らかとなった．生物学的製剤および ETN を使用することのハザード比（HR）は，それぞれ 10.9（p＝0.008）および 6.9（p＝0.001）であった．受診時の年齢，RA の罹患期間，男性であること，MTX および CS の使用，MTX および CS の用量，ALT/AST 値，IgG，好中球数およびリンパ球数は，HBV DNA 再活性化に関与は認めなかった．ステップワイズ解析によって抽出された 4 つの変数を，次に HBV DNA 再活性化の予測因子として多重ロジスティック回帰モデルに当てはめ，それぞれの重要性を決定した．こ

表4　関節リウマチ（RA）患者において HBV 複製を予測するロジスティック回帰モデル

	オッズ比（95%CI）	p値
タクロリムス水和物	11.1（2.0～50.6）	0.0015
スルファサラジン	0.3（0.0～1.7）	0.2604
アバタセプト	1.5（0.1～17.4）	0.7726
免疫グロブリンG	1.9（0.0～160.3）	0.7572

95%CI：95% 信頼区間

のモデルの解析結果を表4に示す．予測能が認められたのはタクロリムス水和物の使用のみであった．

3．考　察

CD4$^+$ヘルパーT細胞は，HBV 特異的 CTL の誘導と維持を促進することによって，HBV 感染症コントロールに関与している．MTX とタクロリムスは，この CTL の機能を阻害し，HBV DNA 複製の再増殖を誘導している可能性がある[8)9)]．CS は免疫系を全般的に抑制する間接的な効果に加え，直接的に HBV 増殖を刺激する[3)]．

TNF は，さまざまな刺激や自己免疫状態において重要な炎症誘発性，免疫調節性を来すサイトカインである[10)]．TNF 抑制薬は最近，RA，強直性脊椎炎，乾癬性関節症，クローン病などの自己免疫疾患で用いられるようになっている．TNF 阻害薬は，これらの患者に治療法のパラダイムシフトと劇的な変化をもたらしたが，TNF 阻害薬の使用増加に伴い，HBV 再活性化，それに伴う *de novo* B型肝炎の症例が幾つか報告されている．

TNF 阻害薬の HBV 再活性化には，幾つかの作用機序があると考えられている．B型肝炎患者の血清と肝細胞では HBV 特異的 CTL が分泌され，TNF が増加し HBV に感染した肝細胞にアポトーシスを引き起こしている[11)]．また，TNF は細胞内の HBV の転写を減ずることによって HBV DNA 複製を抑制する[12)]．さらに，TNF ノックアウトマウスでは HBV 特異的 CTL の増殖機能が欠如していることが明らかになっている[13)]．これらの TNF の機序を，TNF 阻害薬は抑制している可能性がある．

RA 患者における TNF 阻害薬によるB型肝炎の再活性化の最初の報告は 2003 年である[14)]．この症例では HBV キャリア例で TNF 阻害薬が開始されているため，彼らは HBV 感染の血清検査を推奨している．HBV キャリアの RA に TNF 阻害薬で治療を行った症例のレビューでは，HBV の再活性化は 17%（35人中6人）と報告し[15)]，HBs 抗原陽性患者に TNF 阻害薬を処方するときは，核酸アナログ製剤の予防投与と肝炎の慎重な臨床的・血清的モニタリングを推奨している．

Vassilopoulos ら[16)]は 14人の HBV キャリア RA で，ラミブジン投与により，TNF 阻害薬治療によるB型肝炎の再活性化が1人を除いて起こらなかったことを示した．再活性化の1例はラミブジンの耐性出現が原因と考えられたので，核酸アナログ製剤投与は慢性B型肝炎患者に対する安全な対策であると彼らは結論している．

Matsumoto ら[17)]は 71歳女性の RA 患者で，インフリキシマブ，MTX とプレドニゾロン治療後 22ヵ月目に *de novo* B型肝炎を発症した症例を報告している．肝炎発症時にエンテカビルが投与されたが，肝不全で死亡した．

このような症例報告から，TNF 阻害薬による *de novo* B型肝炎の幾つかの特徴が明らかになった．第1に，RA 治療開始と *de novo* B型肝炎までの期間は少なくとも1年あること，第2に，患者は TNF 阻害薬だけでなく，それら自体が免疫抑制効果を持つ DMARD や CS も投与されていたこと，第3に，*de novo* B型肝炎には死亡の危険性があること，第4に，数種類の TNF 阻害薬に *de novo* B型肝炎を引き起こす可能性があること，であ

る[18]．

以上より，既感染B型肝炎の RA 患者は HBV DNA レベルにかかわらず，生物学的製剤と MTX，高用量 CS と，タクロリムス水和物を含む免疫抑制薬を投与されている場合，慎重なモニタリングが必要である．しかしながらモニタリングの方法（頻度や期間など）はおそらく症例ごとに異なり，より厳密な前向き調査である HBIRTH の結果がその参考になると思われる．

文　　献

1) Ganem D, et al: Hepatitis B virus infection－natural history and clinical consequences. N Engl J Med 350: 1118–1129, 2004.
2) Hoofnagle J H: Reactivation of hepatitis B. Hepatology 49: S156–165, 2009.
3) Calabrese L H, et al: Hepatitis B virus (HBV) reactivation with immunosuppressive therapy in rheumatic diseases: assessment and preventive strategies. Ann Rheum Dis 65: 983–989, 2006.
4) Rehermann B, et al: The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T–lymphocyte response. Nat Med 2: 1104–1108, 1996.
5) Umemura T, et al: Mortality secondary to fulminant hepatic failure in patients with prior resolution of hepatitis B virus infection in Japan. Clin Infect Dis 47: e52–56, 2008.
6) Madonia S, et al: Occult hepatitis B and infliximab–induced HBV reactivation. Inflamm Bowel Dis 13: 508–509, 2007.
7) Urata Y, et al: Prevalence of Reactivation of Hepatitis B Virus DNA Replication in Rheumatoid Arthritis Patients. *In*: DNA Replication－Current Advances (Seligmann H, eds), p587–594. InTech, Croatia, 2011.
8) Kuwano K, et al: The inhibitory effect of FK506 on cytotoxic T–lymphocyte killing. Immunol Lett 43: 153–157, 1994.
9) Strauss G, et al: Induction of apoptosis and modulation of activation and effector function in T cells by immunosuppressive drugs. Clin Exp Immunol 128: 255–266, 2002.
10) Bradley J R: TNF–mediated inflammatory disease. J Pathol 214: 149–160, 2008.
11) Fang J W, et al: Activation of the tumor necrosis factor–alpha system in the liver in chronic hepatitis B virus infection. Am J Gastroenterol 91: 748–753, 1996.
12) Wong G H, et al: Tumour necrosis factors alpha and beta inhibit virus replication and synergize with interferons. Nature 323: 819–822, 1986.
13) Kasahara S, et al: Lack of tumor necrosis factor alpha induces impaired proliferation of hepatitis B virus–specific cytotoxic T lymphocytes. J Virol 77: 2469–2476, 2003.
14) Ostuni P, et al: Hepatitis B reactivation in a chronic hepatitis B surface antigen carrier with rheumatoid arthritis treated with infliximab and low dose methotrexate. Ann Rheum Dis 62: 686–687, 2003.
15) Carroll M B, et al: Use of tumor necrosis factor alpha inhibitors in hepatitis B surface antigen–positive patients: a literature review and potential mechanisms of action. Clin Rheumatol 29: 1021–1029, 2010.
16) Vassilopoulos D, et al: Long–term safety of anti–TNF treatment in patients with rheumatic diseases and chronic or resolved hepatitis B virus infection. Ann Rheum Dis 69: 1352–1355, 2010.
17) Matsumoto T, et al: Adalimumab–induced lethal hepatitis B virus reactivation in an HBsAg–negative patient with clinically resolved hepatitis B virus infection. Liver Int 30: 1241–1242, 2010.
18) Tanaka E, et al: Risk of hepatitis B reactivation in patients treated with tumor necrosis factor–α inhibitors. Hepatol Res 42: 333–339, 2012.

Prevalence of Reactivation of Hepatitis B Virus DNA Replication in Rheumatoid Arthritis Patients in the TOHOKU Region

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