Liver abnormalities and liver membrane autoantibodies in systemic lupus erythematosus

KOICHI KUSHIMOTO, KOHEI NAGASAWA, AKIRA UEDA, TAKEHITO MAYUMI, YOSHITOMO ISHII, YASUO YAMAUCHI, YOSHIFUMI TADA, HIROSHI TSUKAMOTO, TOMOHIRO KUSABA, AND YOSHIYUKI NIHO

From the First Department of Internal Medicine, Faculty of Medicine, Kyushu University, 3-1-1 Maidashi, Higashi-Ku, Fukuoka 812, Japan

SUMMARY The hepatic involvement of 57 patients with systemic lupus erythematosus (SLE) was studied with special reference to liver membrane autoantibody (LMA). Liver abnormalities were found predominantly in patients with active SLE (27/48 (56%) in active SLE v 3/20 (15%) in inactive SLE). They were, however, rather mild or moderate and tended to disappear as the disease activity of SLE decreased. In this respect the liver abnormalities observed in this study differed from those in patients with lupoid hepatitis. The incidence of LMA in active SLE (8/11 (73%)) was significantly greater than that in inactive SLE (4/12 (33%)). The mean LMA index value in active SLE was 8.3, which was also greater than the 2.9 in inactive SLE. Furthermore, in active SLE the mean LMA titre was significantly higher in patients with liver abnormalities than in those without. These results suggest that LMA may be associated with the activity of SLE and may be one of the factors which cause transient liver abnormalities.

It is well known that systemic lupus erythematosus (SLE) often presents various clinical features owing to multiple organ involvement. For example, lesions in the kidney, lung, or central nervous system have been widely investigated and commonly called lupus nephritis, lupus pneumonitis, and central nervous system lupus respectively.¹ On the other hand, clinical or aetiological aspects of hepatic involvement have not been seriously studied,¹⁻³ though a relatively high incidence of some liver abnormalities has been observed—20–70% in cases of SLE.³⁻⁸

Liver membrane autoantibody (LMA) was first detected in man by Hopf *et al*⁹ in 1976 and has been found predominantly in patients with autoimmune chronic active hepatitis¹⁰⁻¹² or lupoid hepatitis.¹³ To our knowledge, however, little is known about the role of LMA in the liver abnormalities occurring in patients suffering from SLE.

In this study we investigated the incidence and clinical features of liver abnormalities and the significance of LMA in association with SLE.

Patients and methods

PATIENTS

Fifty seven patients with SLE, one male and 56 female, seen at Kyushu University Hospital between 1978 and 1987, were enrolled in this study. They fulfilled the 1982 revised criteria for the classification of SLE as proposed by the American Rheumatism Association.¹⁴ The patients' mean age was 32.5 years (range 15–64). The patients were divided into four groups based on their state—that is, with or without SLE activity and with or without liver disease. From these 57 patients, 68 samples (three different serum samples from one patient and two from nine patients) were examined.

THE DISEASE ACTIVITY OF SLE

SLE activity was assessed according to a statistically validated lupus activity criteria count.⁸ The presence of any two or more of the following nine features or groups of features constitutes active SLE: (a) arthritis; (b) laboratory abnormalities, namely positive LE cell preparation or white cell count $<4 \times 10^9/l$ or decreased CH₅₀ or C3, or presence of DNA antibodies; (c) rash or mucous membrane ulcers or alopecia; (d) pleuritis or pericarditis; (e) seizures,

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Correspondence to Dr Koichi Kushimoto, First Department of Internal Medicine, Faculty of Medicine, Kyushu University, 3-1-1 Maidashi, Higashi-Ku, Fukuoka 812, Japan.

psychosis, organic brain syndrome, or headache due to SLE; (f) vasculitis; (g) haematuria.

With these criteria the SLE activity was classified as active or inactive.

ASSESSMENT OF LIVER ABNORMALITIES

As the liver abnormalities in most patients were rather short term and transient, evaluations of liver abnormalities were made by four serum enzymes: serum aspartate transaminase (AST), serum alanine transaminase (ALT), serum alkaline phosphatase, and lactic dehydrogenase. When two or more enzymes, including at least one of the transaminases, were increased liver abnormalities were considered to be present. Other causes of increased enzyme activities, especially heart or muscle disease, were excluded. Previous experience was used to define serum enzyme increases as follows: serum AST >45 IU/l (normal range 0–40), serum ALT >40 IU/l (0–35), serum alkaline phosphatase >120 IU/l (30–110), and lactic dehydrogenase >280 IU/l (120–250).

ASSAY OF LIVER MEMBRANE AUTOANTIBODY

Liver membrane autoantibody titres were assayed in 38 serum samples obtained from 33 patients with SLE. In addition to the normal controls, four patients with hepatitis B surface antigen (HBsAg) positive chronic active hepatitis, which had been histologically diagnosed, were also examined.

LMA titres were measured using rat liver cell coating plates, according to the radioimmunoassay techniques of Kronborg *et al.*¹⁵ Fresh rat liver cell coating plates (2×10^5 cells/well) were fixed with 3% paraformaldehyde solution. Serum samples (50 μ l) were added to each well with 500 μ l of TRIS buffered saline (50 mM TRIS; 2% bovine serum albumin; 20 mM EDTA-2Na; 0.05% Tween; 10% sheep serum; 0.05% NaN_3 ; pH 7.5), and the plates were incubated for 16 hours at 25°C on the shaker. The plates were washed three times with 0.05% Tween-saline, then 500 μ l of ^{125}I labelled protein A in phosphate buffered saline (PBS) (10^5cpm) was added to each well, followed by one hour's incubation at 25°C on the shaker. The plates were then washed three times with 0.05% Tween-saline, 500 μ l of 0.5 M acetic acid was added to each well, and free ^{125}I labelled protein A was removed. Finally, each well was counted for radioactivity. The LMA titre was expressed as an index value (IV), which was defined by the formula: (sample counts – normal control counts) $\times 10$ /normal control counts, and the LMA was judged as + (IV ≥ 2.0), $\pm$ ($2.0 > \text{IV} \geq 1.0$), or – (IV < 1.0). Fourteen serum samples from healthy persons were used as controls.

LIVER MEMBRANE AUTOANTIBODY DETECTION BY IMMUNOFLUORESCENCE

Fresh rat liver cells were cultured on cover glasses in wells (2×10^5 cells/well) for two days at 37°C. The cover glasses were washed with PBS, fixed with acetone for 10 minutes, and then dried. Serum samples, diluted 1:10 with PBS, were then placed on the cover glasses and incubated in a 100% moisturised plastic box for one hour at 37°C. The cover glasses were washed with PBS, dried, and then filled with 1:5 diluted fluorescein isothiocyanate conjugated goat antihuman immunoglobulin (MBL, Nagoya, Japan). They were again incubated in a 100% moisturised plastic box for one hour at 37°C. Thereafter, they were washed with PBS and mounted with 50% glycerin-PBS for observation by a fluorescence microscope. For immunofluorescence in living hepatocytes, suspended cells (1×10^6) were incubated with 100 μ l serum diluted 1:10 at 4°C. After 30 minutes of incubation the cells were washed three times with PBS, then incubated with 50 μ l of 1:50 dilution of fluorescein isothiocyanate conjugated goat antihuman immunoglobulin at 4°C for 30 minutes, and washed three times with PBS. The final cell pellets were suspended in 20 μ l of 50% glycerin-PBS, mounted on slide glasses, and examined by fluorescence microscopy.

STATISTICAL ANALYSIS

A χ^2 test was used to assess the differences in the incidence of liver dysfunction and that of LMA. Student's *t* test was used to assess the differences in mean LMA titre among the groups. Results are expressed as means (SD).

Results

INCIDENCE OF LIVER ABNORMALITIES

Sixty eight serum samples from 57 patients with SLE were evaluated for liver functions. Liver abnormalities were seen in 29 of 48 (60%) samples from patients with active SLE and in six of 20 (30%) inactive SLE samples. Two patients in the former group and one in the latter were found to have fatty liver and two patients in the latter were diagnosed as having drug induced hepatitis. When these samples were excluded the incidence of liver abnormalities probably due to SLE was 27 of 48 (56%) in the group with active SLE and three of 20 (15%) in the inactive SLE group. These results suggested that liver abnormalities were found predominantly in patients with active SLE ($p < 0.005$). In most of our patients liver abnormalities developed at the onset or during the exacerbation of SLE. Furthermore, none of the patients took non-steroidal anti-

Fig. 1 Changes of (a) serum aspartate transaminase (AST) and (b) serum alanine transaminase (ALT) values as the systemic lupus erythematosus (SLE) changes from active to inactive during a period of two to 12 months.

inflammatory drugs, including aspirin. We gave immunosuppressive drugs to some patients, two of whom developed obvious drug induced liver dysfunctions as mentioned above. We used no other drugs that were supposed to induce liver abnormalities. Hence these liver abnormalities were considered to be associated with the SLE itself and not with drugs.

LIVER ENZYME VALUE IN ACTIVE SLE WITH LIVER ABNORMALITIES

Of the 27 patients with liver abnormalities and active SLE, increases of serum AST, serum ALT, lactic dehydrogenase, and serum alkaline phosphatase were generally mild or moderate (mean (SD): serum AST 278 (314) IU/l, serum ALT 201 (189) IU/l, lactic dehydrogenase 428 (159) IU/l, serum alkaline phosphatase 165 (173) IU/l). Only five

Fig. 2 Liver membrane autoantibody (LMA) index values in patients with or without disease activity of systemic lupus erythematosus (SLE) and liver abnormalities.

serum samples presented with serum AST>500 IU/l, and only one serum sample showed serum AST>1000 IU/l. The number of cases with serum ALT>500 IU/l was only two. In 24 of the 27 cases (89%) increases of transaminases were transient and decreased in parallel with the inactivation of SLE by treatment. Figure 1 shows the changes in serum AST and serum ALT values as the SLE changes from active to inactive. No patient had jaundice or hyperbilirubinaemia.

LIVER MEMBRANE AUTOANTIBODY TITRES

Thirty eight serum samples from 33 patients with SLE were assayed for LMA (Table 1 and Fig. 2). We found LMA in 21/25 (84%) samples from active SLE and in only 5/13 (38%) from patients with inactive SLE ($p<0.005$). In active SLE samples LMA was found in 13/14 (93%) of those with liver abnormalities and in 8/11 (73%) of those without abnormalities, but the difference was not statistically significant. On the other hand, the incidence of LMA in inactive SLE without liver abnormalities was 4/12 (33%), significantly lower than that in active SLE samples ($p<0.05$). In addition, as shown

Table 1 Incidence of liver membrane autoantibody in patients with or without disease activity of systemic lupus erythematosus and liver abnormalities

State of SLE†	No (%) of LMA† positive samples		Total No (%)
	With liver abnormalities	Without liver abnormalities	
Active (n=25)	13/14 (93)	8/11 (73)*	21/25 (84)**
Inactive (n=13)	1/1 (100)	4/12 (33)	5/13 (38)

* $p<0.05$ compared with inactive SLE without liver abnormalities; ** $p<0.005$ compared with inactive SLE.
†SLE=systemic lupus erythematosus; LMA=liver membrane autoantibody.

Fig. 3 Relation between liver membrane autoantibody (LMA) index and CH_{50} values.

in Fig. 2a, the mean LMA index value in active SLE samples was significantly higher than that in inactive SLE ($p < 0.05$). It was also found that among patients with active SLE the LMA index value was significantly higher in patients with liver abnormalities than in those without ($p < 0.05$) (Fig. 2b). The four patients with HBsAg positive chronic

Fig. 4 Changes of liver membrane autoantibody (LMA) index values as the systemic lupus erythematosus (SLE) changes from active to inactive during a period of two to 12 months.

active hepatitis all showed a negative test for LMA (data not shown).

Figure 3 shows that the LMA titre is inversely correlated with the CH_{50} value, which is one of the indices of disease activity. This result may support the above mentioned relation between LMA and disease activity of SLE. Moreover, in five patients whose SLE was initially active LMA titre fell as the disease activity of SLE decreased during a period of two to 12 months (Fig. 4). These results indicate that LMA may be associated with the disease activity of SLE and, if not entirely, then partly, with the liver abnormalities in SLE.

RELATION BETWEEN LIVER MEMBRANE AUTOANTIBODY AND ANTINUCLEAR ANTIBODIES

As antinuclear antibodies are found in the serum samples of most patients with SLE there was a

Fig. 5 Fixed rat hepatocytes stained by indirect immunofluorescence with liver membrane autoantibody (LMA) positive or negative serum. (a) Bright staining of the surface and cytoplasm in addition to the nuclei when the LMA positive systemic lupus erythematosus (SLE) serum was used. (b) Staining of the nuclei only, with dark surface and cytoplasm, when the LMA negative SLE serum was used.

Fig. 6 Relation between liver membrane autoantibody (LMA) index values and antinuclear antibody (ANA) titres.

possibility that the LMA detected by radioimmunoassay might merely represent the presence of antinuclear antibodies. To rule out this possibility we observed the LMA by indirect immunofluorescence. The surface and cytoplasm of fixed rat hepatocytes were brightly stained, in addition to the staining of the nuclei, when LMA positive SLE serum was used (Fig. 5a). By contrast, only the nuclei were found to be stained with the dark surface and cytoplasm when LMA negative SLE serum was used (Fig. 5b). These results were confirmed using living rat hepatocytes; LMA positive SLE serum linearly stained only the cell surface, whereas LMA negative serum did not (photos not shown). In addition, the relation between LMA and antinuclear antibody titres was investigated. No specific relation between those two antibody titres was found (Fig. 6). These results indicate that the LMA assayed in this study is not a reflection of antinuclear antibodies.

Discussion

In this study we investigated the hepatic involvement in SLE and the association of LMA with the disease activity of SLE and liver abnormalities.

Rothfield reported raised liver enzyme activities in about 30% of patients with SLE at the time of diagnosis when active disease was evident and found that they returned to normal with corticosteroid treatment.³ Morito *et al* also observed a coincidental transaminase increase in 11/23 patients during an exacerbation of SLE.⁷ In our study liver abnormalities not due to drugs, but probably due to SLE, were found in 27/48 (56%) patients with active SLE and in only 3/20 (15%) of those with inactive SLE, which is compatible with previous reports. The increase of serum transaminase concentration was mild or

moderate, and no patient had jaundice or a increase of total bilirubin. Dubois and Tuffanelli found jaundice in 3.8% of 520 patients with SLE. Kofman *et al* observed hyperbilirubinaemia in 27% and jaundice in 12% of 25 patients with SLE.¹⁰

In patients with SLE histologically non-specific and mild liver changes are common, but severe damage is rare.^{8, 16-19} Benner *et al*, analysing 37 cases of SLE with hepatic involvement in 18 publications, concluded that the liver damage was not severe and would never be the cause of death.¹¹ On the other hand, in several papers severe liver damage in SLE has been reported,^{5, 20} but its difference from cases of autoimmune hepatitis is still controversial.²¹⁻²⁵ There seem to be no clear criteria for distinguishing hepatic involvement in SLE from autoimmune hepatitis. Although we did not examine our patients histologically, we found no clinical evidence of chronic active hepatitis. Thus there seems to be a type of liver abnormality in patients with SLE which is clinically distinct from lupoid or autoimmune hepatitis because of its transiency, mild or moderate transaminase increase or absence of an increase in total bilirubin. Liver abnormalities of this type in SLE may be identical to 'hepatic lupus', first named by Mackay *et al*,¹⁸ which means a mild liver dysfunction found in SLE which is clinically and histologically different from lupoid hepatitis.

Autoantibodies to liver membranes are commonly classified into two types. One is the antibody to liver specific membrane lipoprotein^{26, 27} and the other is LMA.⁹ Some studies have shown that these antibodies are each directed against different antigens of liver membranes.^{10, 28} Although antibodies to liver specific membrane lipoprotein have been detected in both acute and chronic hepatitis independently of HBsAg,²⁶⁻²⁹ LMA has been found predominantly in patients with autoimmune chronic active hepatitis.^{11, 12, 30} So far, 40-70% of patients with autoimmune chronic active hepatitis have been reported to be positive for LMA.^{11, 12, 30} Frazer *et al* reported that LMA titres correlated with the activity of autoimmune chronic active hepatitis.¹¹ The pathogenetic significance of these autoantibodies is still unsettled, but some investigators consider that antibody dependent, cell mediated cytotoxic reaction is a major effector mechanism.^{31, 32}

It seems, however, that not very much attention has been paid to LMA in association with SLE or hepatic involvement of SLE. In the limited number of publications available the incidence of LMA in patients with SLE has been reported to be low. Frazer *et al* found no difference in LMA titres of 14 patients with SLE and normal individuals,¹¹ and Wiedmann *et al* rarely observed LMA in patients

with SLE.¹² They scarcely discussed the role of LMA in SLE. In contrast, we showed a high incidence of LMA in patients with active SLE, and also showed that LMA titres fell in parallel with the decrease of SLE activity in five patients. It was also found that LMA titres correlated inversely with CH₅₀. These results suggest that LMA may be associated with the disease activity of SLE. Moreover, the titres of LMA were significantly higher in patients with SLE with liver abnormalities than in those without them, indicating that LMA may be involved in the liver damage observed in SLE. LMA was negative in all four patients with HBsAg positive chronic active hepatitis. This is compatible with other reports, which showed a tendency for LMA to be less commonly detected in patients with HBsAg positive chronic active hepatitis than in those with autoimmune chronic active hepatitis.^{12 13 30} For example, Toda *et al* reported that LMA was detected more often in patients with HBsAg negative liver cirrhosis than in those with the HBsAg positive form (six of 13 v two of 11).³⁰

When the serum samples of LMA positive patients with SLE were used fluorescence staining was observed more brightly on the surface and cytoplasm of fixed rat hepatocytes and the surface of living rat hepatocytes than in the nucleus. This staining pattern confirmed the results obtained by radioimmunoassay, which indicated that LMA is against the antigen of the membrane rather than against the nucleus of hepatocytes. These facts exclude the possibility that LMA obtained by radioimmunoassay may reflect antinuclear antibodies, which most patients with SLE possess.

In general, various autoantibodies, such as anti-nuclear antibody, rheumatoid factor, or platelet associated IgG, appear in association with hypergammaglobulinaemia in active SLE. LMA may also be one of them. As there was statistical significance between the LMA titres of patients with and without liver abnormalities, however, we suggest that LMA is an autoantibody which appears during the active phase of SLE and which participates, if not entirely, then partly, in the autoimmune pathogenesis of the hepatic involvement in SLE. The results obtained here also indicate that the liver abnormalities due to SLE may share the same cause as lupoid or autoimmune hepatitis, though their clinical features are clearly distinct. Further studies are needed to clarify the clinical significance and the basal mechanism of LMA.

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