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Abstract

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KEYWORDS: ADCC assay, anti-LP-1, anti-TAMM-Horsfall glycoprotein, chronic liver disease

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STUDIES ON ANTI-LP-1 AND ANTI-TAMM-HORSFALL GLYCOPROTEIN IN CHRONIC LIVER DISEASE USING ADCC ASSAY AGAINST ANTIGEN-COATED TARGET CELLS

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Abstract. To study autoantibodies against liver cell surface membrane clinically, anti-LP-1 and anti-Tamm-Horsfall glycoprotein (THGP) were determined in the sera of patients with various liver diseases. They were detected by ADCC assay using antigen-coated cells as the target. A high incidence of anti-LP-1 was seen in chronic hepatitis (CH), liver cirrhosis (LC), primary hepatic cancer with cirrhosis (PHC), and primary biliary cirrhosis. The incidence of anti-THGP was also high in CH, LC, and PHC. Both anti-LP-1 and anti-THGP were detected in 2 of 3 patients with lupoid hepatitis. The patients studied here had no obvious evidence of renal tubular acidosis or pyelonephritis. Serum alanine transaminase activity, serum γ-globulin content, and the presence of rheumatoid factors were not associated significantly with the presence of anti-LP-1 or anti-THGP in chronic liver disease. In 7 cases of CH tested serially during their clinical course, anti-LP-1 and/or anti-THGP tended to appear during acute exacerbations. The demonstration of anti-LP-1 and anti-THGP suggested that their appearance was related to the development of chronic liver disease.

Key words : ADCC assay, anti-LP-1, anti-Tamm-Horsfall glycoprotein, chronic liver disease.

Development of chronic liver disease has been considered to be due in part to autoimmunity against liver-cell surface antigens (1). In other words, the antigens against which the autoimmunity in chronic liver disease is directed should be present on the surface of liver cells. As the antigen on the liver cell surface (2), liver-specific membrane lipoprotein (LP-1) (3), a soluble liver membrane antigen (LM-Ag) (4), a cell membrane protein (5), and a Tamm-Horsfall glycoprotein (THGP) (6, 7) have been reported by many investigators. Of these, LP-1 is one of the most investigated antigens clinically.

LP-1 is prepared from normal human liver by column chromatography (3), is considered to be present on liver cell surface (8), and separates into more than 13 subunits on polyacrylamide gel electrophoresis in sodium dodecyl sulfate (9). Chronic active hepatitis has been induced in rabbits by long-term immunization of LP-1 (10). Antibody or cellular immunity against LP-1 has been detected in human chronic liver disease (11). Antibody to LP-1 is most frequently present in the serum of patients with chronic active hepatitis, with or

without HBsAg (11). Also, it is reported that patients' sera with primary biliary cirrhosis (12), or acute hepatitis (13, 14) have the autoantibody to LP-1. Antibody to LP-1 has been detected mainly by radioimmunoassay (RIA) (15, 16) or antibody-dependent cell-mediated cytotoxicity (ADCC) assay (17-19). Since it is thought that in the ADCC mechanism, one or two antibody molecules are enought to initiate the effective reaction (20), the ADCC assay is considered one of the most sensitive tests for detecting a certain antibody. Here, ADCC assay was used to detect antibody to LP-1, and the target was antigen-coated non-liver cell because of exclusion of antigens on liver cells other than LP-1.

Tamm-Horsfall glycoprotein (THGP) extracted from human urine (6) is a homogeneous glycoprotein composed of subunits with molecular weights of approximately 80,000 (21). THGP has been detected on epithelial cells of the renal distal tubule and the ascending limb of the loop of Henle (22), and on liver cell membrane (7). Tsantoulas *et al.* reported that the leukocytes of patients with autoimmune liver disease with renal tubular acidosis showed abnormal responses against THGP in leukocyte migration tests (7). Antibody to THGP has not yet been studied in liver diseases.

In this paper, ADCC assay using antigen-coated non-liver cells as target was persued to detect antibody against LP-1 or THGP for studying the significance of the autoantibody against liver-cell membrane in chronic liver diseases.

MATERIALS AND METHODS

Sera from patients. Sera for detecting the antibody to LP-1 or THGP were separated from the blood of patients: acute hepatitis (AH), 6 cases including 2 cases of type A AH; chronic hepatitis (CH), 28 cases including 22 cases of chronic aggressive hepatitis (CAH), 3 cases of chronic persistent hepatitis (CPH), and 3 cases of lupoid hepatitis (LH) (23); liver cirrhosis (LC), 6 cases; primary hepatic cancer with liver cirrhosis (PHC), 6 cases; primary biliary cirrhosis (PBC), 5 cases; miscellaneous liver diseases, 5 cases including 3 cases of fatty liver, a case of Gilbert's disease, and a case of hepatic hemangioma; other autoimmune diseases, 11 cases including 7 cases of Sjögren's syndrome, 2 cases of systemic lupus erythematosus, a case of rheumatoid arthritis, and a case of polymyositis. CAH, CPH, LC, and fatty liver were diagnosed histologically (24). For determination of the normal range of % cytotoxicity, sera were taken from 11 healthy subjects. None of the above had any obvious evidence of renal tubular acidosis or pyelonephritis. These sera were heat-inactivated at 56℃ for 30 min and stored at −20℃. In the ADCC assay, these were diluted 400-fold with RPMI-1640.

Hepatitis B surface antigen (HBsAg) was determined by passive hemagglutination. Serum alanine transaminase (S-GPT) activity, serum γ-globulin content, and rheumatoid factor (RF) were measured in the Central Clinical Laboratory, Okayama University Hospital.

Preparations of antigens. LP-1 was prepared from a normal human liver (25) by the method of McFarlane *et al.* (9). THGP was purified from human urine (24) by the method of Fletcher *et al.* (6).

ADCC assay for detecting antibody to LP-1 or THGP. As previously reported (25), ADCC assays were conducted in microtestplates (Fig. 1). In brief, target cells were prepared by

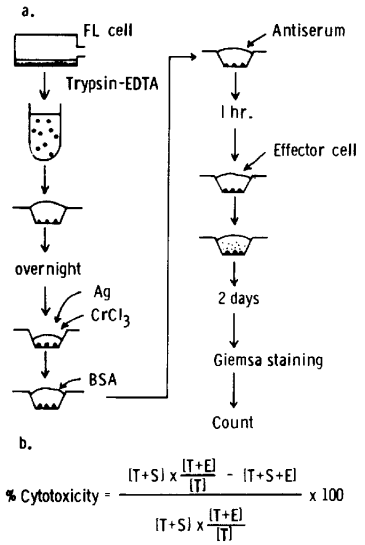


Fig. 1. a) Schema for coating FL cells with antigens and ADCC assay for detecting antibodies against the corresponding antigens (25). b) Formula for calculating % cytotoxicity of the serum.
[ ] indicates numbers of attached cells in a well. T: target cell, S: serum, E: effector cell.

coating FL cells (a cell line derived from human amnion) with antigens by chromic chloride method (26). Target cells were incubated with diluted patient's serum at 37 ℃ for 1 h followed by cultivation for 2 days with non-phagocytic mononuclear cells taken from a single healthy subject as effector cells. In this assay, the ratio of target cells to effector cells was 1:50. Thereafter, the number of attached target cells remaining at the bottom of each well of the plates was counted and the % cytotoxicity was calculated. Fig. 1 shows an outline of the assay and the formula for calculating the % cytotoxicity.

RESULTS

The mean + 2 × standard deviation (M + 2SD) of the % cytotoxicity against LP-1 for healthy subjects was 24.7 (n = 11, M ± SD = 4.5 ± 10.1) and against THGP was 25.4 (n = 9, M ± SD = 4.8 ± 10.3). Therefore, sera with values over these were interpreted as positive for antibody against the corresponding antigen.

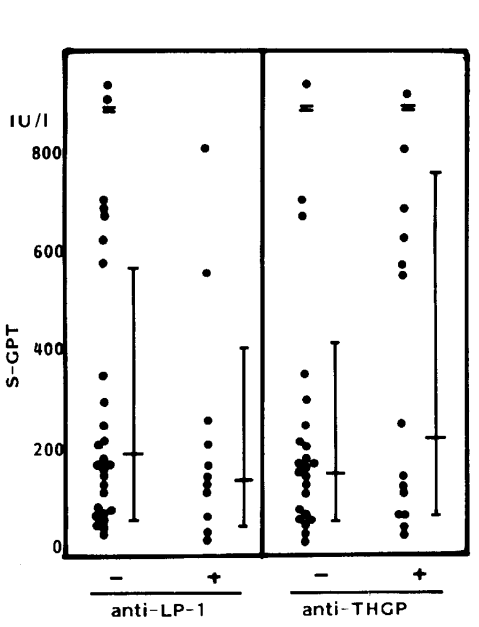
The positivity of anti-LP-1 was high in the sera of patients with CH, LC, PHC, and PBC, especially with LH (all of 3 patients) (Table 1). In 2 cases of AH who had anti-LP-1 within 2 months of the onset, the anti-LP-1 disappeared more than 2 months after onset. None of the patients with CPH had anti-LP-1. In other autoimmune disease, 3 cases of Sjögren's syndrome with normal liver function and one case of systemic lupus erythematosus (SLE) were positive for anti-LP-1. In this SLE case, anti-LP-1 was positive when the S-GPT activity was 114 IU/l, and negative when the S-GPT activity was 41 IU/l.

The incidence of anti-THGP was high in the sera of patients with CH, LC, and PHC. The presence of both anti-LP-1 and anti-THGP was shown in 2 of 3 patients with LH, and in 2 of 5 with PHC.

TABLE 1. INCIDENCE OF ANTI-LP-1 AND ANTI-TAMM-HORSFALL GLYCOPROTEIN (THGP) IN VARIOUS DISEASES

Diagnosis	Anti-LP-1	Anti-THGP	Anti-LP-1 and anti-THGP
Acute hepatitis			
from onset: < 2 months	2/4	1/3	0/3
> 2 months	1/4	0/3	0/3
Chronic hepatitis:	7/28 (1/13)	11/27 (5/12)	4/27 (1/12)
chr. aggressive hepatitis	4/22 (1/12)	9/22 (5/12)	2/22 (1/12)
chr. persistent hepatitis	0/3 (0/1)	0/2	0/2
lupoid hepatitis	3/3	2/3	2/3
Liver cirrhosis	3/6 (2/4)	2/5 (2/3)	1/5 (1/3)
Primary hepatic cancer with cirrhosis	3/6 (1/3)	2/5 (1/2)	2/5 (1/2)
Primary biliary cirrhosis	2/5	1/5	0/5
Miscellaneous liver diseases	0/5	0/5	0/5
Other autoimmune disease	4/11	2/11	0/11

( ): HBsAg-positive cases



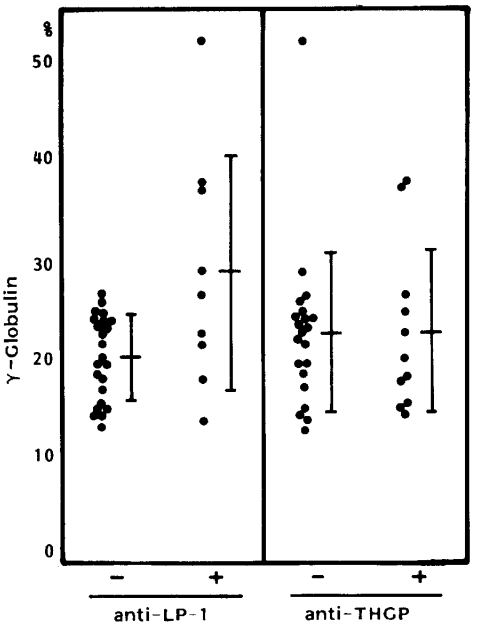


Fig. 2. *(left)* Relationship between serum alanine transaminase (S-GPT) activity and the presence of anti-LP-1 or anti-THGP. Mean ± standard deviation of S-GPT activity was calculated from the logarithm of each value.

Fig. 3. *(right)* Relationship between serum γ-globulin levels and the presence of anti-LP-1 or anti-THGP.

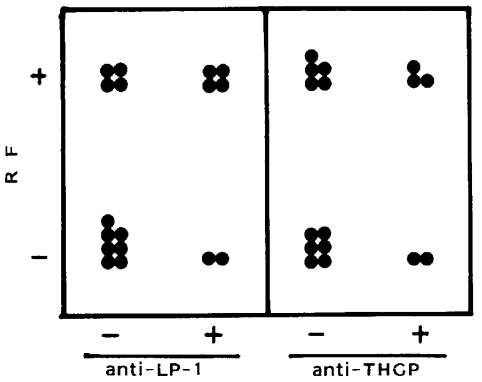


Fig. 4. Relationship between the presence of rheumatoid factor (RF) and anti-LP-1 or anti-THGP.

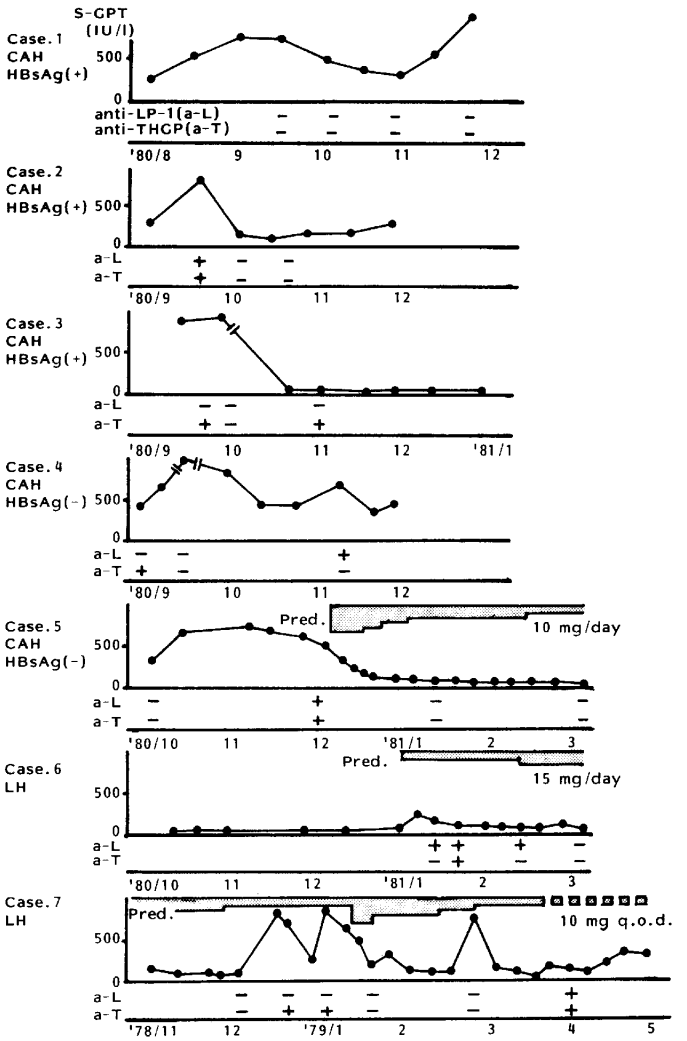


Fig. 5. Clinical course of patients with chronic hepatitis, anti-LP-1, and anti-THGP. CAH: chronic aggressive hepatitis, LH: lupoid hepatitis, HBsAg: hepatitis B surface antigen.

There was no correlation between HBsAg and these antibodies, although the positivity of anti-LP-1 in patients with HBsAg-negative CAH tended to occur more than in HBsAg-positive CAH (Table 1).

In chronic liver disease including CH, LC, and PBC, no statistically significant differences between S-GPT activity or serum γ-globulin content and anti-LP-1 or anti-THGP were seen (Figs. 2, 3). Also, RF were not correlated to anti-LP-1 or anti-THGP (Fig. 4).

The clinical course of 7 cases (3 cases of HBsAg-positive CAH, 2 cases of HBsAg-negative CAH, and 2 cases of LH) and the changes of anti-LP-1 and anti-THGP are shown in Fig. 5. In case 1, anti-LP-1 and anti-THGP were negative during the period of observation. In case 2-7, anti-LP-1 and/or anti-THGP tended to be detected during acute exacerbations of the liver function test ("Schub") or within one month after "Schub". However, at the time of maximum disease activity as indicated by more than 1,000 units of S-GPT activity, these antibodies were not detected. In case treated with corticosteroid (cases 5, 6), anti-LP-1 and anti-THGP disappeared after the beginning of treatment. In case 7, in which the corticosteroid was administered before the author's observation, decrease in the dose of corticosteroid caused "Schub" three times accompanied, or followed, by the occurrence of anti-LP-1 and/or anti-THGP.

DISCUSSION

Antibody-dependent cell-mediated cytotoxicity (ADCC) assay was used here to detect anti-LP-1 or anti-THGP. For detecting anti-LP-1 in patients' sera, radioimmunoassays (RIA) (13, 15, 16), or ADCC assays (17-19) were reported. Since only one or two antibody molecules are required for cytolysis by the ADCC mechanism (20), the ADCC assay for detecting antibody against an antigen may be one of the most sensitive methods. The antibody class reacting in ADCC mechanism was thought to be IgG and IgM (27). Therefore, antibodies detected in the ADCC assay are considered not to belong to any particular immunoglobulin class.

Liver-specific membrane lipoprotein (LP-1) is a component of normal liver cell membrane (3, 8) and is considered a target antigen of autoimmunity in chronic active liver diseases (11). Anti-LP-1 has been detected in chronic active liver disease or chronic active hepatitis (13, 15-19), acute phase of acute hepatitis (13-15), and primary biliary cirrhosis (PBC) (12) consistent with the results in this paper (Table 1). Cochrane *et al.* reported that the cytotoxicity of lymphocytes of patients with chronic active hepatitis against isolated hepatocytes was due to the ADCC mechanism (28). Although the target of the ADCC assay in this paper was not the liver cell but LP-1-coated non-liver cell, patient's sera with anti-LP-1 induced cytolysis of the target cells, as hypothesized for the

pathogenesis of active chronic hepatitis (1), and destruction of hepatic parenchyma in PBC (12). Cell-mediated immune responses to LP-1 have also been thought to contribute to active chronic hepatitis and PBC (29). Whether cellular or humoral immune responses involving ADCC against LP-1 play a role in the pathogenesis of chronic liver disease needs investigation.

Anti-THGP has been detected in the sera of patients with urinary tract infection and renal parenchymal lesions (30), but not with liver diseases. Cellular immunity against THGP as detected by the leukocyte migration test was found by Tsantoulas *et al.* (7) in patients with PBC and chronic active hepatitis with renal tubular acidosis. In this paper, anti-THGP was detected in CH, LC, PHC without any clinical evidence of renal tubular acidosis or pyelonephritis. It was interesting that in lupoid hepatitis anti-THGP was detected together with anti-LP-1. This suggests that autoantibodies to not only LP-1 but other antigens, for example THGP or other antigens on the liver cell surface membrane, are involved in the pathogenesis and development of lupoid hepatitis.

No relationship between S-GPT or γ-globulin, and anti-LP-1 or anti-THGP was observed in chronic liver diseases (Figs. 2, 3). Since neither anti-LP-1 nor anti-THGP was detected in CPH, the activity of the disease might be related to the presence of these autoantibodies. As there was no correlation between RF and anti-LP-1 or anti-THGP (Fig. 4), the antibodies detected were not thought to be anti-IgG against IgG contained in the antigen preparation (31).

Changes in autoantibodies dependent on the stage of the disease have not yet been studied in chronic hepatitis. Jensen *et al.* showed that the titer of antibody against LP-1 was related to the histological and biochemical activity of chronic active hepatitis (13). In this paper, the author observed that the positivity of anti-LP-1 and/or anti-THGP tended to appear at "Schub" (Fig. 5), although there was no significant correlation between positive antibodies and biochemical indices in the population of chronic liver diseases studied here. Whether these antibodies are pathogenic to chronic liver disease, concerned in the development of such disease, or appeared only as the result of liver cell destruction needs investigation. To clarify this problem, serial determination of autoantibodies to the liver cell membrane from earlier stages of disease in certain cases might be helpful.

In other autoimmune diseases, 4 patients had anti-LP-1 antibodies and 2 patients had anti-THGP (Table 1). Apart from one case of systemic lupus erythematosus with anti-LP-1, in which anti-LP-1 disappeared after the S-GPT returned to normal, these cases were not accompanied by any evidence of liver disease. This suggests that the combination of autoantibodies against liver cell membrane and other factors, such as persistent viral infections, might induce the liver cell damage.

In chronic liver disease, the presence of antibodies reacting with various tissue components such as anti-nuclear factors, smooth muscle antibodies, or

anti-mitochondrial antibodies was reported (32). These tissue autoantibodies were not organ specific and were thought to be the result of stimulation by antigens released from damaged liver cells, hence not pathogenic to chronic liver disease (1).

Lupoid hepatitis was described by Mackay *et al.* as a disease process of active chronic hepatitis with positive LE cell tests and manifestations similar to systemic lupus erythematosus (24). The characteristics of active chronic hepatitis described by Mackay *et al.* (33) included the response of the disease activity to immunosuppressive drugs. Case 6 and 7 in Fig. 5 were lupoid hepatitis treated with corticosteroid that showed improvement throughout the period of investigation. Since not only S-GPT but also anti-LP-1 and anti-THGP improved in these cases responding to corticosteroid therapy, these autoantibodies may be related to the activity of lupoid hepatitis.

Further study of the significance of autoimmunity against liver cell surface membrane should investigate serially cellular and humoral markers indicating autoimmunity in several cases of chronic liver disease. Also, better purification and characterization of target antigens on the liver cell surface membrane is required.

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REFERENCES

1. Eddleston, A.L.W.F. and Williams, R.: Inadequate antibody response to HBAg or suppressor T-cell defect in development of active chronic hepatitis. *Lancet* **ii**, 1543-1545, 1974.
2. Nagashima, H., Arima, T., and Shimomura, H.: Immunology in liver disease. *Rinsho Meneki* **12, (Suppl. 1)** S147-158, 1980 (in Japanese).
3. Meyer zum Büschenfelde, K.H. and Miescher, P.A.: Liver specific antigens: purification and characterization. *Clin. Exp. Immunol.* **10**, 89-102, 1972.
4. Meyer zum Büschenfelde, K.H., Manns, M., Hütteroth, T.H., Hopf, U., and Arnold, W.: LM-Ag and LSP—two different target antigens involved in the immunopathogenesis of chronic active hepatitis? *Clin. Exp. Immunol.* **37**, 205-212, 1979.
5. Neville, D.M.: Isolation of an organ specific protein antigen from cell-surface membrane of rat liver. *Biochim. Biophys. Acta* **154**, 540-552, 1968.
6. Fletcher, A.P., Neuberger, A., and Ratcliffe, W.A.: Tamm-Horsfall urinary glycoprotein: the chemical composition. *Biochem. J.* **120**, 417-424, 1970.
7. Tsantoulas, D.C., McFarlane, I.G., Portmann, B., Eddleston, A.L.W.F., and Williams, R.: Cell mediated immunity to human Tamm-Horsfall glycoprotein in autoimmune liver disease with renal tubular acidosis. *Br. Med. J.* **4**, 491-494, 1974.
8. Hopf, U., Meyer zum Büschenfelde, K.H., and Freudenberg, J.: Liver-specific antigens of

different species. II. localization of a membrane antigen at cell surface of isolated hepatocytes. *Clin. Exp. Immunol.* **16**, 117-124, 1974.

9. McFarlane, I.G., Wojcicka, B.M., Zucker, G.M., Eddleston, A.L.W.F., and Williams, R.: Purification and characterization of human liver-specific membrane lipoprotein (LSP). *Clin. Exp. Immunol.* **27**, 381-390, 1977.
10. Meyer zum Büschenfelde, K.H., Kössling, F.K., and Miescher, P.A.: Experimental chronic active hepatitis in rabbits following immunization with human liver protein. *Clin. Exp. Immunol.* **10**, 99-108, 1972.
11. Eddleston, A.L.W.F.: Immune responses to the liver-specific membrane lipoprotein. In *Immune Reactions in Liver Disease*, ed. A.L.W.F. Eddleston, J.C.P. Weber, and R. Williams, Pitman Medical Publishing, Kent, pp.2-11, 1979.
12. Tsantoulas, D., Perperas, A., Portmann, B., Eddleston, A.L.W.F., and Williams, R.: Antibodies to a human liver membrane lipoprotein (LSP) in primary biliary cirrhosis. *Gut* **21**, 557-560, 1980.
13. Jensen, D.M., McFarlane, I.G., Portmann, B.S., Eddleston, A.L.W.F., and Williams, R.: Detection of antibodies directed against a liver-specific membrane lipoprotein in patients with acute and chronic active hepatitis. *N. Engl. J. Med.* **299**, 1-7, 1978.
14. Manns, M., Arnold, W., Meyer zum Büschenfelde, K.H., Nagai, S., and Hoffmann, H.: Studies on anti-LSP auto antibodies in acute and chronic non-B hepatitis—evidence for lack of anti LSP in non-A, non-B (NANB) viral hepatitis. *Klin. Wochenschr.* **59**, 685-689, 1981.
15. Kakumu, S., Arawaka, Y., Goji, H., Kashio, T., and Yata, K.: Occurrence and significance of antibody to liver-specific membrane lipoprotein by double-antibody immunoprecipitation method in sera of patients with acute and chronic liver disease. *Gastroenterology* **76**, 665-672, 1979.
16. Manns, M., Meyer zum Büschenfelde, K.H., Hutteroth, T.H., and Hess, G.: Detection and characterization of liver membrane autoantibodies in chronic active hepatitis by a solid-phase radioimmunoassay. *Clin. Exp. Immunol.* **42**, 263-272, 1980.
17. Vogten, A.J.M., Hadzic, N., Shorter, R.G., Summerskill, W.H.J., and Taylor, W.F.: Cell-mediated cytotoxicity in chronic active liver disease: a new test system. *Gastroenterology* **74**, 883-889, 1978.
18. Behrens, U.J., Vernace, S., and Paronetto, F.: Studies on "liver-specific" antigens. II. detection of serum antibodies to liver and kidney cell membrane antigens in patients with chronic liver disease. *Gastroenterology* **77**, 1053-1061, 1979.
19. Adachi, M., Sano, M., Imura, H., and Ito, K.: Studies on the humoral anti-hepatocyte membrane antibody in active chronic hepatitis (continued): investigation by a new method using ADCC system. *Nippon Shokakibyo Gakkai Zasshi* **78**, 883-889, 1981. (in Japanese)
20. Möller, G. and Svehag, S.E.: Specificity of lymphocyte mediated cytotoxicity induced by in vitro antibody-coated target cells. *Cell. Immunol.* **4**, 1-19, 1972.
21. Fletcher, A.P., Neuberger, A., and Ratcliffe, W.A.: Tamm-Horsfall urinary glycoprotein: the subunit structure. *Biochem. J.* **120**, 425-432, 1970.
22. McKenzie, J.K. and McQueen, E.G.: Immunofluorescent localization of Tamm-Horsfall mucoprotein in human kidney. *J. Clin. Path.* **22**, 334-339, 1969.
23. Mackay, I.R., Taft, L.I., and Cowling, D.C.: Lupoid hepatitis. *Lancet* **ii**, 1323-1326, 1956.
24. De Groote, J., Desmet, V.J., Gedigk, P., Korb, G., Popper, H., Poulsen, H., Scheuer, P.J., Schmid, M., Thaler, H., Uehlinger, E., and Wepler, W.: A classification of chronic hepatitis. *Lancet* **ii**, 626-628, 1968.

25. Shimomura, H.: Studies on ADCC assay using antigen-coated cells as target for detection of anti-LP-1 and anti-Tamm-Horsfall glycoprotein. *Gastroenterol. Jpn.* **17**, 109-116, 1982.
26. Goding, J.W.: The chromic chloride method of coupling antigens to erythrocytes: definition of some important parameters. *J. Immunol. Methods* **10**, 61-66, 1976.
27. Cochrane, A.M.G., Moussouros, A., Thomson, A.D., Eddleston, A.L.W.F., and Williams, R.: Antibody-dependent cell-mediated (K cell) cytotoxicity against isolated hepatocytes in chronic active hepatitis. *Lancet* **i**, 441-444, 1976.
28. Wåhlin, B., Perlmann, H., and Perlmann, P.: Analysis by a plaque assay of IgG- or IgM-dependent cytolytic lymphocytes in human blood. *J. Exp. Med.* **144**, 1375-1380, 1976.
29. Miller, J., Smith, M.G.M., Mitchell, C.G., Reed, W.D., Eddleston, A.L.W.F., and Williams, R.: Cell-mediated immunity to a human liver-specific antigen in patients with active chronic hepatitis and primary biliary cirrhosis. *Lancet* **i**, 296-297, 1972.
30. Hanson, L.A, Fasth, A., and Jodal, U.: Autoantibodies to Tamm-Horsfall protein, a tool for diagnosing the level of urinary tract infection. *Lancet* **i**, 226-228, 1976.
31. Arima, T., Matsuura, H., Shimomura, H., Suwaki, K., Yasuhara, T., Narumoto, J., Koide, N., Sakaguchi, K., and Nagashima, H.: The characterization of liver-specific membrane lipoprotein (LP-1). In *Shokaki to Meneki* No.7, ed. M. Tsuchiya, Ishiyaku Shuppan, Tokyo, pp.273-276, 1981 (in Japanese).
32. Golding, P.L., Smith, M., and Williams, R.: Multisystem involvement in chronic liver disease: studies on the incidence and pathogenesis. *Am. J. Med.* **55**, 772-782, 1973.
33. Mackay, I.R., Weiden, S., and Hasker, J.: Autoimmune hepatitis. *Ann. N. Y. Acad. Sci.* **124**, 767-780, 1965.