Clin Exp Immunol 1993; 92:367–368

EDITORIAL REVIEW

Type II autoimmune hepatitis: the conundrum of cytochrome P450IID6

D. VERGANI & G. MIELI-VERGANI *Departments of Immunology and Child Health, King's College School of Medicine and Dentistry, London, UK*

(Accepted for publication 5 April 1993)

Liver kidney microsome-1 (LKM-1) antibody defines a subtype of autoimmune hepatitis referred to as type II [1]. This type of autoimmune hepatitis preferentially affects children or young adults, mainly females, and runs a particularly severe clinical course, with frequent evolution to cirrhosis despite apparent response to immunosuppressive treatment. Although its onset is usually insidious, it can present with acute hepatic failure. Histological examination of the liver shows severe mononuclear inflammatory cell infiltration of the portal tracts, which extends into the parenchyma surrounding dying hepatocytes. A major target for the diagnostic LKM-1 antibody is the cytochrome P450IID6 [2–4], which metabolizes debrisoquin and several other drugs [2–5]. Autoimmune hepatitis type II, LKM-1 antibody and its targets have recently been the focus of a lively debate. The observation that short amino acid sequences are shared in common between cytochrome P450IID6 and hepatitis C virus (HCV) has led to the proposal that HCV triggers a cross-reactive autoimmune response which could play an important role in the development of autoimmune hepatitis type II [6]. A link between HCV and LKM-1 reactivity is confirmed in this issue by Seelig *et al.* [7], who demonstrate the presence of HCV RNA in the serum of approximately half of the patients positive for LKM-1. The patients positive for both HCV and LKM-1, however, were mainly males and older than those positive for LKM-1 only, an intriguing observation which has been made previously [8–10]. Although Seelig *et al.* [7] found that virtually all LKM-1-positive sera reacted with recombinant P450IID6, other works indicate that the target of LKM-1 is different in autoimmune hepatitis type II and chronic HCV infection [10,11]. This suggests that, on the basis of the available data, it is unwise to draw conclusions on the role of HCV in triggering autoimmune hepatitis type II.

An additional paper in this issue by Yamamoto *et al.* [12], which concentrates on the classical autoimmune hepatitis type II, addresses another hot question: does LKM-1 play a direct pathogenic role? For LKM-1 to be pathogenically relevant, its target, cytochrome P450IID6, should be easily accessible to the immune system on the outer aspect of the hepatocyte plasma membrane. Making use of a variety of refined techniques, these authors concluded that in all likelihood P450IID6 is not present on the cell surface of normal human hepatocytes. Recently, however, Loeper *et al.* [13], addressing the same question and using a variety of similarly refined techniques, concluded that cytochrome P450IID6 is present and functional on the plasma membrane of human hepatocytes, and that LKM-1 antibodies recognize epitopes expressed on the outer surface. Who is right? Who is wrong? The two studies have a number of themes in common such as human antisera, affinity purified antibodies, and the use of monodispersed live human hepatocytes and subcellular fractions as targets. Since the detection of LKM-1 can present technical and interpretative difficulties, as Seelig *et al.* [7] note, differences between the two studies could be readily explained if the human sera containing the LKM-1 antibody had been obtained from different sources. Both groups, however, used sera in which the LKM-1 positivity has been confirmed by the laboratory of J. C. Homberg in Paris. Both groups observed a positive membrane staining when these sera were used against monodispersed human hepatocytes in immunochemical techniques, although Yamamoto *et al.* [12] described the staining as 'slight'. The presence of anti-liver membrane antibody in the sera of patients with autoimmune hepatitis is hardly surprising, since these patients are usually seropositive for antibodies directed to well defined autoantigenic targets on the outer aspect of the hepatocyte plasma membrane, such as the asialoglycoprotein receptor [14,15]. To overcome this reactivity, Yamamoto *et al.* affinity-purified their LKM-1-positive antiserum against the peptide 254–271, a major epitope of the P450IID6 protein. Using this purified reagent, no membrane staining was observed, prompting the conclusion that P450IID6 was not present on the membrane. During the purification process, however, the titre of the LKM-1 reactivity decreased more than 10 times, readily accounting for the disappearance of the 'slight' fluorescent staining. Moreover, although the peptide used for the immunopurification represents an immunodominant epitope, other epitopes may be exposed on the membrane. Lastly, the peptide used for purification is linear, and may produce a reagent lacking in antibodies directed to conformational epitopes.

Loeper *et al.* [13] affinity-purified their LKM-1-positive antiserum with a microsome-derived, SDS–PAGE-separated, cytochrome P450IID6. Using this purified reagent they obtained positive membrane staining on 40–60% of hepatocytes—although one would like to have seen more convincing immunofluorescence pictorial evidence. To emphasize the positive staining of the hepatocyte membrane, the authors used as negative controls peripheral blood lymphocytes from healthy subjects, i.e. cells whose membrane is devoid of cytochrome P450IID6. Lymphocytes were first incubated with LKM-1-positive sera and then, as a second step, with labelled anti-IgG antisera deprived of their Fc fragment, to avoid their non-

Correspondence: Dr D. Vergani, Departments of Immunology and Child Health, King's College School of Medicine and Dentistry, Bessemer Road, London SE5 9PJ, UK.

specific engagement with Fc receptors present on lymphocytes. Although the negative controls appear negative, two caveats should be directed at these results: first, Fc receptors are present also on hepatocytes [16] and $F(ab)_2$ fragments should have been used as the second step reagent in all experiments; and second, sera from patients with autoimmune hepatitis contain anti-lymphocytic antibodies [17], making it surprising that no staining was seen on lymphocytes. The uncertainty as to whether P450IID6 is present on the outer aspect of the hepatocyte plasma membrane is augmented by further experiments in which no staining of monodispersed liver cells was seen when an anti-P450IID6 MoAb was used. It is possible, as the authors suggest, that the epitope recognized by this MoAb is not exposed on the hepatocyte membrane. Immunocytochemical techniques, therefore, do not provide a definite answer. Are the answers to be found in cellular fractionation studies? Loeper *et al.* [13] provide convincing evidence that P450IID6 is present and functional in a human hepatocyte plasma membrane fraction, the purification of which was documented by enrichment in membrane markers and by the minimal contamination with microsomal markers. Yamamoto *et al.* [12] in electromicroscopical studies, in which an anti-LKM-1-positive serum is incubated with rat hepatocyte microsomal fractions, showed that epitopes recognized by the serum were localized only on the cytoplasmic side of the endoplasmic reticulum membrane. They argue that, since the position of P450IID6 depends on the position of the protein in the endoplasmic reticulum, P450IID6 cannot be on the outer aspect of the plasma membrane. Additional studies by both groups, including transfection of Chinese hamster ovary cells with P450IID6 DNA, surface labelling of hepatocytes with sulphosuccinimidyl 2-(biotinamido) ethyl 1,3-dithiopropionate, liver perfusion with LKM-1-positive sera, fall short of answering the key question of cytochrome P450IID6 being a readily accessible target on the liver cell plasma membrane for LKM-1 antibodies. Do other studies hold the answer to this question?

In 1984 Lenzi *et al.* [18] reported in this Journal that some patients with autoimmune hepatitis have in their serum LKM-1 and anti-liver membrane antibodies. Wondering whether antibodies to liver cell microsomes and plasma membrane are directed against the same target, they performed cross-absorbtion experiments. Sera containing both LKM-1 and anti-liver cell membrane antibody were absorbed with: (i) microsomes and tested against monodispersed mouse hepatocytes; and (ii) monodispersed live hepatocytes and tested on cryostat sections for residual LKM-1 positivity. In their neat experiments, Lenzi *et al.* [18] showed that microsomes removed membrane fluorescence, and membranes (i.e. live hepatocytes) removed the LKM-1 pattern. The argument against the conclusions of this seminal work is that since a proportion of the hepatocytes used were dead, the LKM-1 pattern may have been removed by exposed microsomal material, and not by plasma membranes. Moreover, the membrane pattern described was that of liver membrane antibody (LMA), subsequently reported to recognize epitopes on the inner side of the plasma membrane [19].

With the evidence produced, are we in the position to answer the initial question regarding the cellular location of the target of LKM-1 antibody? On the membrane or not on the membrane? On the membrane must be the answer. On the outer aspect of it? This remains the problem.

REFERENCES

1 Homberg JC, Abuaf N, Bernard O *et al.* Chronic active hepatitis associated with anti-liver/kidney microsome antibody type 1: a second type of "autoimmune" hepatitis. Hepatology 1987; **7**:1333–9.

2 Alvarez F, Bernard O, Homberg JC, Kreibich G. Anti-liver-kidney microsome antibody recognizes a 50,000 molecular weight protein of the endoplasmic reticulum. J Exp Med 1985; **161**:1231–6.

3 Zanger UM, Hauri HP, Loeper J, Homberg JC, Meyer UA. Antibodies against human cytochrome P450 dbl in autoimmune hepatitis type II. Proc Natl Acad Sci US 1988; **27**:8256–60.

4 Manns MP, Johnson EF, Griffin KJ, Tan EM, Sullivan KF. Major antigen of liver kidney microsomal autoantibodies in idiopathic autoimmune hepatitis is cytochrome P450 dbl. J Clin Invest 1989; **83**:1066–72.

5 Gueguen M, Meunier Rotival M, Bernard O, Alvarez F. Anti-liver kidney microsome antibody recognizes a cytochrome P450 from the IID subfamily. J Exp med 1988; **168**:801–6.

6 Manns MP, Griffin KJ, Sullivan KF, Johnson EF. LKM-1 autoantibodies recognize a short linear sequence in P450IID6, a cytochrome P-450 monooxygenase. J Clin Invest 1991; **88**:1370–8.

7 Seelig R, Renz M, Bunger G *et al.* Anti-LKM-1 antibodies determined by use of recombinant P4502D6 in ELISA and Western blot and their association with anti-HCV and HCV-RNA. Clin Exp Immunol 1993; **92**:373–380.

8 Michel G, Ritter A, Gerken G, Meyer zum Büschenfelde KH, Decher G, Manns M. Anti-GOR and hepatitis C virus in autoimmune liver diseases. Lancet 1992; **339**:267–9.

9 Lunel F, Abuaf N, Frangeul L *et al.* Liver/kidney microsome antibody type 1 and hepatitis C virus infection. Hepatology 1992; **16**:630–6.

10 Ma Y, Peakman M, Lenzi M *et al.* Case against subclassification of type II autoimmune chronic active hepatitis. Lancet 1993; **341**:60.

11 Yamamoto AM, Criestiel D, Homberg JC, Alvarez F. Characterization of anti-liver-kidney microsome antibody (anti-LKM) from hepatitis C virus positive and negative sera. Gastroenterology 1993; in press.

12 Yamamoto AM, Mura C, De Lemos-Chiarandini C *et al.* Cytochrome P450IID6 recognized by LKM1 antibody is not exposed on the surface of hepatocytes. Clin Exp Immunol 1993; **92**:381–390.

13 Loeper J, Descatoire V, Maurice M *et al.* Cytochromes P-450 in human hepatocyte plasma membrane: recognition by several autoantibodies. Gastroenterology 1993; **104**:203–16.

14 McFarlane IG, McFarlane BM, Major GN, Tolley R, Williams R. Identification of the hepatic asialoglycoprotein receptor (hepatic lectin) as a component of liver specific membrane lipoprotein. Clin Exp Immunol 1984; **55**:347–54.

15 Poralla T, Treichel U, Lörh H, Fleischer B. The asialoglycoprotein receptor as target structure in autoimmune liver diseases. Semin Liver Dis 1991; **11**:217–22.

16 Hopf U, Meyer zum Büschenfelde K-H, Dierich MP. Demonstration of binding sites for IgG Fc and the third complement component (C3) on isolated hepatocytes. J Immunol 1976; **117**:639–45.

17 Donaldson PT, Hussain MJ, Mieli-Vergani G, Mowat AP, Vergani D. Anti-lymphocytic antibodies in autoimmune chronic hepatitis starting in childhood. Clin Exp Immunol 1989; **75**:41–46.

18 Lenzi M, Bianchi FB, Cassani F, Pisi E. Liver cell surface expression of the antigen reacting with liver-kidney microsomal antibody (LKM). Clin Exp Immunol 1984; **55**:36–40.

19 Gerken G, Manns M, Ramadori G, Poralla T, Dienes HP, Meyer zum Büschenfelde K-H. Liver membrane autoantibodies in chronic active hepatitis. Studies on mechanically and enzymatically isolated rabbit hepatocytes. J Hepatol 1987; **5**:65–74.