Targets of Antibodies to Soluble Liver Antigen in Patients with Autoimmune Hepatitis

To the Editor:

Antibodies to a cytosolic soluble liver antigen (SLA), originally detected by an inhibition ELISA using cytosolic liver fractions and proposed as marker of a third type of autoimmune hepatitis (AIH) negative for other autoantibodies, have been also reported in anti-nuclear and/or -smooth muscle antibody type 1 AIH, liver kidney microsomal type 2 AIH, and autoimmune sclerosing cholangitis (1–3). Anti-SLA is specific for these autoimmune liver diseases, in which it is associated with a more severe disease course, whereas it is virtually absent in non-hepatic autoimmune disorders (1–3). The target of anti-SLA has recently been identified by several groups as a UGA serine tRNA-associated protein complex [tRNP(Ser)Sec], through the screening of cDNA libraries (4–6).

On the basis that not all of the anti-SLA-positive sera identified by inhibition ELISA react with tRNP(Ser)Sec and referring to studies indicating that SLA is a mixture of distinct antigens, Ballot et al. (7) questioned the identity of tRNP(Ser)Sec as the molecular target of anti-SLA antibodies. These investigators set out to reassess the matter, using anti-SLA-positive sera against rat liver cytosolic fraction in one- and two-dimensional immunoblotting analyses. Through peptide mass fingerprint analysis after matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, the authors identified four isoforms of α-enolase, a cytosolic enzyme of 50 kDa, as the major target of anti-SLA-positive sera. Volkman et al. (8) questioned their proposal on both technical and clinical grounds. In their reply to Volkman et al. (8), Johanet et al. (9) restated that the question of what is the major target of SLA remains open. Because anti-SLA is a specific serologic marker for autoimmune liver diseases and because of the wide availability of assays based on the use of recombinant SLA/liver pancreas (LP) polypeptide, the knowledge of whether SLA/LP is the correct antigen is of considerable clinical relevance. Not only does anti-SLA positivity identify patients with autoimmune liver disease, it also predicts a more severe course of the disease (2, 3).

We would like to make a comment and give some experimental data:

- At variance with anti-tRNP(Ser)Sec antibodies, which have a >95% specificity for autoimmune liver diseases, anti-α-enolase can be found not only in AIH and primary sclerosing cholangitis, but also in some biliary cirrhosis, viral hepatitides, inflammatory bowel diseases, systemic lupus erythematosus, mixed cryoglobulinemia, systemic sclerosis, vasculitis, rheumatoid arthritis, Behcet disease, and Hashimoto encephalopathy; thus it lacks the disease specificity of anti-SLA (1–6, 10–12).

- The 50-kDa band recognized by anti-SLA-positive sera could theoretically be α-enolase, tRNP(Ser)Sec, or both. Ballot et al. (7) concluded their work by stating that the 50-kDa band is attributable to α-enolase; they did not, however, perform critical inhibition studies with the purified antigen to corroborate their statement. They went on to suggest that tRNP(Ser)Sec is not detected because it is probably present in trace amounts in the supernatant of liver homogenate and thus is barely identifiable in immunoblot experiments.

We tested serum samples from patients with AIH, positive for SLA by a modified inhibition ELISA (13) and radioligand assay (3), by immunoblotting on strips electrophoretically separated primate liver homogenate containing the cytosolic fraction (3). We obtained patterns highly reminiscent of those described by Ballot et al. (7) (Fig. 1) with a band of ~50 kDa being consistently immunofixed by anti-SLA-positive sera. When we repeated the experiment after preincubating SLA-positive sera with the solid-phase recombinant UGA suppressor tRNP(Ser)Sec (Euroimmun), the 50-kDa band disappeared (Fig.
1). Results of preabsorption experiments similar to ours have also been described previously (4–6).

These data support the notion that tRNP\textsuperscript{Ser/Sec} is the main 50-kDa target of anti-SLA antibodies. The discrepancy between our data and those of Ballot may be attributable to our use of primate, rather than rodent, liver homogenate.

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References


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**Drs. Ballot, Bruneel, and Johanet respond:**

*To the Editor:*

In their letter, Bogdanos et al. interestingly discussed our results obtained with rat liver and a proteomic approach concerning ζ-enolase as a possible target of anti-soluble liver antigen (SLA) antibodies (1). They rightly stated that the presence of anti-ζ-enolase antibodies has been reported in various other autoimmune diseases, such as primary biliary cirrhosis and systemic lupus erythematosus, as well as in viral hepatitis. They also described the disappearance of the 50-kDa band stained by anti-SLA-positive sera after preincubation with a primate UGA serine tRNA-associated protein [tRNP\textsuperscript{Ser/Sec}], underlining the importance of this recombinant protein as a target for anti-SLA antibodies.

Concerning their point about the presence of anti-ζ-enolase antibodies, using the “gold standard”, inhibition ELISA (2), we failed to find anti-SLA antibodies in 52 patients with primary biliary cirrhosis, 105 patients with hepatitis C virus infection, 24 patients with systemic lupus erythematosus, and 15 patients with inflammatory bowel diseases, or in 102 blood donors (3). From these data, we speculate that anti-SLA antibodies in autoimmune hepatitis (AIH) probably exhibit a greater affinity toward specific isoforms of ζ-enolase than anti-enolase antibodies found in other diseases (4).

Concerning the disappearance of the 50-kDa band after incubation with tRNP\textsuperscript{Ser/Sec}, the present experiment clearly demonstrated that anti-SLA antibodies reacted with recombinant tRNP\textsuperscript{Ser/Sec} protein. We do not contest the use of such a recombinant protein as an additional diagnostic tool for AIH. It has been demonstrated that sera from patients with AIH contain autoantibodies against tRNP\textsuperscript{Ser/Sec} complex, which recognized protein(s) of 48 and/or 52 kDa (5). The 48-kDa protein, however, was identified only from cDNA (5, 6), and it is well established that there is a poor correlation between cDNA and protein expression in the liver (7, 8). We believe that it would be interesting to use the sensitivity of nanoelectrospray mass spectrometry to identify tRNP\textsuperscript{Ser/Sec} protein in the liver cytosolic fraction.

In conclusion, at this time, we believe that SLA is a generic term encompassing several cytosolic antigenic targets for anti-SLA antibodies.

References


