Characterization of the Aspartate Aminotransferase–Immunoglobulin M Complex in a Japanese Woman, Koichi Asanuma, Atsuhito Yagihashi, and Naoki Watanabe* (Dept. of Lab. Diagnosis, Sapporo Medical Univ. School of Med., South 1, West 16, chuo-ku, Sapporo, 060, Japan; *author for correspondence: fax 81-11-622-7502)

Alterations in serum aspartate aminotransferase (AST) concentration usually correlate with the clinical stages of organ-specific disease. However, persistently increased AST concentration is sometimes due to the formation of a macrocomplex between immunoglobulin (Ig) and AST. In most healthy cases, AST-Ig complexes were detected after detailed studies to determine the cause of a persistent high concentration of serum AST. If high AST concentrations are found in the absence of organ-specific disease, we should suspect the presence of an AST-Ig complex. In eight previously reported healthy individuals with a high AST concentration [1–6], the Ig that was bound to AST was always IgG. Here we characterize the immunological and biochemical properties of the AST-IgM complex in an individual without serious disease.

The patient, a 61-year-old woman, was referred to our hospital for evaluation of persistently high serum AST concentration of 9 years duration. Her AST concentration was measured six times between February and July 1992 in our hospital. Each time, AST was high but all the other liver function tests were normal. Laboratory tests at the time of admission showed (reference range): AST, 294 (5–40) U/L; IgM, 2.83 (0.7–1.70) g/L; C3, 0.53 (0.63–0.99) g/L; antimacrosomal antibody, ×400 (<×100). Results of hepatitis B surface and core antibody assays were positive. Computed tomography and ultrasonography of the abdomen revealed no evidence of liver disease such as hepatocellular carcinoma, liver cirrhosis, or fatty liver. A liver biopsy sample was normal.

Total AST activities were measured by an Olympus AU-560 automated analyzer (Olympus, Tokyo, Japan) with a commercially available kit (Wako Pure Chemical Co., Osaka, Japan). AST isoenzymes were separated by electrophoresis on a Titan-III lipocellulose acetate membrane (Helena Labs., Beaumont, TX) in a pH 8.7 buffer containing 25 mmol/L sodium pyrophosphate and 11 mmol/L barbital, according to the method described previously [7]. To visualize AST activity, the membrane was stained with an AST isoenzyme reagent staining kit (Wako Pure Chemical Co.) consisting of 60 mmol/L Tris-HCl, pH 7.4 (1.4 mL), 0.1 mol/L sodium l-asparaginase (0.8 mL), 0.1 mol/L α-ketoglutaric acid (0.2 mL), 1 g/L pyridoxal-5 phosphate (0.05 mL), and Fast Violet B salt, 3 mg; a principle of this procedure is the diazo reaction. The patient’s serum produced a single band on the membrane between supernatant AST (s-AST) on the anodal side and mitochondrial AST (m-AST) on the cathodal side, suggesting that something was bound to AST. To determine the heavy-chain and light-chain classes and the type of a suspected Ig complex, we performed electroimmunosyneresis (without deproteinization) [8] and immunoturbidimetry [9, 10] by using antiseras against human IgG, IgA, and IgM, and light-chain κ and λ (Dakopatts, Glostrup, Denmark). With electroimmunosyneresis, the bands of the patient’s AST activities that precipitated with anti-IgM and anti-λ antibody were shifted to the cathodal side (Fig. 1). Immunoturbidimetry showed that the absorbances in tubes to which anti-IgM or anti-λ antibody had been added were higher than those in tubes containing control serum. Absorbance at 570 nm was measured by means of a Toshiba TBA-180 analyzer (Toshiba Medical, Tokyo, Japan). The Ig bound to AST was judged to be IgM of the λ type. To dissociate the AST-IgM complex, the patient’s serum was treated with 0.1 mol/L 2-mercaptoethanol and incubated for 60 min at 37 °C. After this treatment, the AST activity remained unchanged, and the electrophoretic pattern of the s-AST isoenzyme normalized. These results suggest that the binding of IgM to s-AST did not affect s-AST activity. Further testing with in vitro recombination was used to study the binding affinity of Ig in the enzyme-Ig complex for human AST isoenzymes. To dissociate the Ig from the complex, the patient’s serum was dialyzed for 20 h against 50 mmol/L glycine-HCl buffer (pH 3.4). The dissociated IgM was purified by Sephadex S-300 (Pharmacia Fine Chemicals, Uppsala, Sweden) gel filtration with the same buffer used as eluant [11]. To purify s-AST and m-AST, liver tissue obtained at autopsy was homogenized, then layered on 25 mmol/L sucrose–20 g/L human albumin, and centrifuged at 1000 rpm for 5 min. The supernatant was further centrifuged at 13 000 rpm for 3 min. This supernatant and sediment were used as the starting materials for the purification. s-AST and m-AST were purified by DEAE-Sephadex A-50 ion-exchange chromatography. Upon completion of the purification, the bands of the supernatant containing s-AST and m-AST were cut out from the gel and homogenized. For each band, 0.3 mL of 0.1 mol/L 2-mercaptoethanol was added, and the mixture was incubated for 60 min at 37 °C. After this treatment, the AST activity of the s-AST band remained unchanged, whereas the AST activity of the m-AST band was lost.

Fig. 1. Electroimmunosyneresis (without deproteinization) pattern of the patient’s serum identified as IgM-λ type.

AST activities on precipitin lines were observed in lanes 4 and 6. Lane 1, patient’s serum; lane 2, patient’s serum, anti-IgG serum; lane 3, patient’s serum, anti-IgA serum; lane 4, patient’s serum, anti-IgM serum; lane 5, patient’s serum, anti-κ light-chain serum; lane 6, patient’s serum, anti-λ light-chain serum; lane 7, control serum from a patient with acute myocardial infarction.
exchange chromatography (Pharmacia Fine Chemicals) by using a 0–0.2 mol/L NaCl gradient in a stepwise elution with 15 mmol/L phosphate buffer, pH 7.2, at a flow rate of 10 mL/h. The dissociated Ig was incubated with the purified AST isoenzymes (s-AST 386 U/L, m-AST 986 U/L) in a ratio of 4:1 by volume at 25 °C for 60 min, and the recombined enzyme-Ig complex was detected by immunoturbidimetry [11]. Immunoturbidimetry showed that the absorbances in tubes to which anti-IgM and the patient’s IgM and s-AST had been added were higher than in tubes containing m-AST (Table 1). The results showed that the purified IgM was able to recombine with s-AST. These results show that the patient’s increased serum AST was related to the presence of an s-AST-IgM-λ-type complex.

We found 53 published cases of high serum AST concentrations that were due to the formation of an Ig-AST complex [12]. In 28 cases, the macro form of AST was found to be an IgA-AST complex; in 18 cases, an IgG-AST complex [1, 3, 5, 6, 9, 12–18]; in 1 case, an IgM-AST complex [19]; and in 6 cases, AST was found to be complexed with IgG and IgA [12, 20]. The reported cases with Ig-AST complexes can be divided into three groups, according to the clinical characteristics of the patients: (a) 40 patients with various liver diseases, malignancies, and diseases involving immune disorders; (b) 8 apparently healthy individuals [1–6]; and (c) 5 patients whose disease was not described. However, three of the healthy individuals with IgG-AST complex had received medication (one, an oral contraceptive [1]; one, a griseofulvin [5]; and one, a weight-reducing drug [5]), and one healthy individual with IgG-AST complex had hepatitis B vaccination before the increased serum AST activity was detected. Our patient showed a high antimicrosomal antibody concentration, suggesting an autoimmune disorder. These reports suggest that not only the patients with serious liver disease or immune disorder, but also most of the healthy individuals had experienced possibly hepatotoxic or host immune response-modifying episodes before the increased serum AST activity was detected [15], and these episodes or diseases may be involved in the production of Ig-AST complexes.

Our patient was discharged with no treatment. After 3 years, the patient is still clinically well, and her AST serum concentrations are still high. We are following her enzyme activity to determine the duration of the high AST activity and whether the IgM bound to AST will switch to another class of Ig. When high concentrations of serum AST are observed in the absence of organ-specific disease, the presence of an Ig-enzyme complex should be investigated to avoid unnecessary or invasive examinations.

### Table 1. Recombination of dissociated IgM and AST by immunoprecipitation reaction in free liquid media.

<table>
<thead>
<tr>
<th>Absorbance at 570 nm</th>
<th>Anti-IgG</th>
<th>Anti-IgA</th>
<th>Anti-IgM</th>
<th>Anti-k</th>
<th>Anti-λ</th>
</tr>
</thead>
<tbody>
<tr>
<td>s-AST 0.1338</td>
<td>0.1333</td>
<td>0.2068</td>
<td>0.1325</td>
<td>0.1467</td>
<td></td>
</tr>
<tr>
<td>m-AST 0.1333</td>
<td>0.1306</td>
<td>0.1299</td>
<td>0.1307</td>
<td>0.1313</td>
<td></td>
</tr>
</tbody>
</table>

Novel Reductant for Determination of Total Plasma Homocysteine, Brian M. Gilfix,1,2* David W. Blank,2 and David S. Rosenblatt1 (1 Div. of Med. Genetics, 2 Div. of Clin. Biochem., Royal Victoria Hosp., 687 Pine Ave. W., Montreal, QC H3A 1A1, Canada; * author for correspondence: fax 514-843-1499, e-mail mcgb@musica.mcgill.ca)

Homocysteine (Hcy), increasingly being recognized as a risk factor for vascular disease [1], is found primarily in plasma in the form of homocysteine and mixed disulfides, both protein-bound and unbound; total Hcy (tHcy) is the...