Monoclonal Antibodies

Monoclonal Antibodies Against Hepatitis B Core Antigen

Antigen Used for Immunization

Hepatitis B core antigen (HBcAg) was obtained from Fitzgerald (cat. no. 30-AH39; Concord, MA) and used for immunizations and ELISA.

Method of Immunization

Six- to 8-week-old female BALB/c mice were immunized intraperitonally. Each mouse was immunized with 2 μg injection of the HBcAg in 100 μL phosphate-buffered saline (PBS) mixed with 100 μL complete Freund's adjuvant (Sigma, Steinheim, Germany). Immunizations were repeated at days 15, 30, and 45 with incomplete adjuvant (Sigma). Four days before the fusion, a booster injection of 1 μg of HBcAg in 0.1 mL PBS without adjuvant was given.

Parental Cell Line Used for Fusion

The lymphocytes from spleen and lymph nodes of the BALB/c mouse immunized with HbcAg, as described above, and mouse myeloma cells F0 (ATTC CRL 1646) were used in fusion.

Selection and Cloning Procedure

The standard fusion protocols were followed. Polyethylene glycol 4000 (Merck, Darmstadt, Germany) was used as fusion agent. Hybridomas were selected in HAT-containing medium (100 mM hypoxanthine, 0.4 mM aminopterin, and 16 mM thymidine). The indirect enzyme-linked immunosorbent assay (ELISA) was used for screening the hybridoma supernatant and plasma of the mice.

Heavy and Light Chains of Immunoglobulin

The immunoglobulin class of the antibody was determined using a mouse subisotyping kit (Behring Diagnostics, La Jolla, CA), according to the manufacturer's protocol. The immunoglobulin class of antibody was identified as IgM isotype (μ heavy chain).

Specificity

The specificity and cross-reactivity of monoclonal antibody was determined by ELISA and Western blot analysis. It was shown that monoclonal antibody (MAM12C9) had high affinity for the HbcAg.

Specific Antigen Identified

Table 1 shows comparison of the reactivity (OD405) of the monoclonal antibody (MAM12C9) with the hepatitis antigens and other proteins.

Availability

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Tissue culture supernatant</th>
<th>Ascitic fluid</th>
<th>Hybridoma cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAb</td>
<td>Yes ✓</td>
<td>Yes ✓</td>
<td>Yes ✓</td>
</tr>
</tbody>
</table>

Table 1. Comparison of Reactivity (OD405) of the 12C9 Hybrid Cell With Hepatitis Antigens and Other Proteins

<table>
<thead>
<tr>
<th>Hybrid cell</th>
<th>HBcAg</th>
<th>HBsAg</th>
<th>HBeAg</th>
<th>Human serum</th>
<th>BSA</th>
<th>Transferin</th>
</tr>
</thead>
<tbody>
<tr>
<td>12C9</td>
<td>1.892</td>
<td>0.149</td>
<td>0.123</td>
<td>0.186</td>
<td>0.155</td>
<td>0.168</td>
</tr>
</tbody>
</table>

HBcAg, hepatitis core antigen; HBsAg, hepatitis surface antigen; HBeAg, hepatitis e antigen; BSA, bovine serum albumin.
Acknowledgment

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References