Monoclonal antibodies against an immunodominant and neutralizing epitope on hepatitis A virus antigen

Brief Report

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Summary. Two monoclonal antibodies (813 and 10.09) were raised against hepatitis A virus (HAV). They recognize an immunodominant epitope and a neutralizing site on HAV.

Hepatitis A virus (HAV) is the etiological agent of human hepatitis A [9]. For the development of a HAV vaccine, a panel of monoclonal antibodies (Mabs) is required for identifying the neutralization immunogenic sites of HAV. Several Mabs to HAV have been previously produced in different laboratories [2, 7, 10, 13, 15]. In this paper we report the development and characterization of two of these Mabs to HAV obtained from our laboratory.

Cell culture adapted HAV strain CF53 was propagated in the human hepatoma cell line PLC/PRF/5 with release of virus into the cell culture medium [3, 5]. HAV was concentrated by 10% polyethyleneglycol 6,000 precipitation as previously described [3]. The concentrated suspension was extracted by trichloro-trifluoroethane (Merk). The stock virus was quantified by the RIA endpoint titration [14] and infectious titer determination [1]: antigen titer was $1.4 \times 10^4$ and infectious HAV titer was $10^{6.7}$ TCID₅₀/ml. Six-week old female BALB/C mice were immunized with two subcutaneous inoculations of 150 μl of stock virus suspended in complete Freund’s adjuvant. Immune response was monitored using a competitive radio-immuno assay (RIA) previously described [5]. The animal with the highest specific antibody level was selected and boosted intraperitoneally 3 days before cell fusion with 200 μl of stock virus. Cell fusion
was performed according to Köhler and Milstein [12]. Mabs were purified from ascites fluids either by dialysis against distilled water for mouse IgG3 or by DEAE trisacryl chromatography for the other Ig isotypes [8]. Mabs were isotyped by double immuno diffusion with isotype-specific antisera (NORDIC) and were iodinated using chloramine-T method [11]. Supernatant fluids from hybridoma cultures, ascitic fluids, mouse sera or purified Mabs were tested for anti-HAV reactivity by competitive RIA [5]. Selected Mabs were characterized by indirect immunofluorescence assay [16] and virus neutralization test [13] on PLC/PRF/5 cell culture. Mab reactivity on various HAV strains was studied by RIA according to Purcell’s method [17].

Two Mabs have been selected by competitive RIA: (i) 813; (ii) 10.09. Isotyping of Mabs 813 and 10.09 showed that they were mouse G3K and G1K immunoglobulins, respectively. In the hepatoma cell line PLC/PRF/5 infected with HAV strain CF53, Mabs 813 and 10.09 specifically stained HAVAg by immunofluorescence (Fig. 1). Ascitic fluids and purified Mabs were able to block up to 70% of the radiolabeled anti-HAV human IgG binding, in the competitive RIA used for Mab screening. Cross competition assay [6] between 813 and 10.09 was performed using purified and radiolabeled Mabs. When 813 was used as probe, unlabeled 10.09 gave 67% inhibition of binding. When 10.09 was used as probe, 813 gave 90% inhibition of binding. Each Mab was able to neutralize HAV infectivity in PLC/PRF/5 cell culture. The infectious HAV titer decrease was 2.0 and 2.5 log_{10} after pre-incubation of the stock virus suspension with Mab 813 and 10.09 respectively. Moreover radiolabeled 813 or 10.09 as radiolabeled anti-HAV human IgG were able to detect various HAV strains

Fig. 1. Indirect immunofluorescence of PLC/PRF/5 cells infected (A) or not infected (B) with HAV strain CF53. Mabs provide a specific granular cytoplasmic staining in HAV-infected cells. Magnification, ×500