Short Report

Production of Monoclonal Antibodies against Recombinant HBcAg

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UENO, Y., KOBAYASHI, K., SUZUKI, H., YAMAMOTO, T. and TOYOTA, T. Production of Monoclonal Antibodies against Recombinant HBcAg. Tohoku J. Exp. Med., 1990, 161 (3), 253-255 — Three monoclonal antibodies against recombinant HBcAg were obtained from hybridomas fused between mouse myeloma line NS1 and splenocytes of immunized Balb/C mice. They specifically bound to recombinant HBcAg. Subtypes of these monoclonal antibodies were IgM and IgA. — recombinant HBcAg; monoclonal antibodies; anti-HBc

Monoclonal antibodies have several advantages to polyclonal antibodies, such as their purity and specific binding to an epitope. Up to now, there are a few reports of making monoclonal antibodies against recombinant hepatitis B core antigen (HBcAg) (Hložánek et al. 1987). We established hybridoma clones which produced antibodies specific for HBcAg.

Female Balb/C mice, 8 weeks of age, were injected intraperitoneally at first with $10\mu$g of recombinant HBcAg in complete Freund's adjuvant, then with the same amount of antigen in incomplete Freund's adjuvant 2 weeks later, and finally with the same amount of HBcAg alone 4 weeks later. Four days prior to hybridization, mice were injected intravenously with the same dose of the antigen alone. Myeloma NS-1 cells were successfully fused with splenocytes using polyethylene glycol method (Kohler and Milstein 1975). Cells producing anti-HBc were detected by enzyme linked immunosorbent assay (ELISA). Cloning of the cells producing anti-HBc were repeated twice with limiting dilution method. Briefly, 0.2 cells per well were incubated in 96 well culture plates, and 3 clones, 4C8, 8E3 and 8F9, were obtained: single colony forming possibilities of these clones at the second cloning were 16%, 32% and 18%, respectively. Isootypes of immunoglobulins produced by these clones are IgA; K chain, IgM; K chain and IgM; K chain, respectively. All supernatants were negative for anti-HBe (data not shown). Every supernatant from each clone contained immunoglobulins bound with recombinant HBcAg in a dose dependent manner (Fig. 1). In addition, the binding of these antibodies to HBcAg was effectively blocked by addition of rabbit polyclonal anti-HBc (DAKO, Santa Barbara, CA, USA) (Fig. 2). Based on these data, we concluded that the monoclonal antibodies prepared here recognized the antigenic sites of HBcAg.

HBcAg produces anti-HBc of IgG, IgM and IgA subclasses in patients infected with human hepatitis B virus. Among these subclasses, IgA type anti-HBc may be responsible for liver cell injury (Nomura et al. 1985). But the significance of anti-HBc in terms of hepatic necrosis is still unknown because of limited amount of anti-HBc available from...

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Abbreviations: anti-HBc, anti-hepatitis B core antibody; anti-HBe, anti-hepatitis B envelope antibody.
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Patients' sera. And production of anti-Hbc may be regulated by anti-(anti-Hbc) idotype, which will be accurately detected with HbcAg-specific monoclonal antibody. The availability of the monoclonal antibodies described here should make feasible further approaches to these questions.

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References

