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Production and Characterization of Monoclonal Anti-Ovalbumin Antibodies

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Abstract

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In this study, the results of anti- ovalbumin (OVA) monoclonal antibody production and characterization are reported. Splenocytes from OVA-hyperimmune Balb/C mouse were fused with F0 myeloma cells and resulting hybridoma cells were selected in hypoxanthine-aminopterine-thymidine containing medium. A total of 37 HAT resistant hybridomas were obtained. Upon testing the hybridoma supernatants in OVA and mouse immunoglobulin G specific enzyme-linked immunosorbent assays, we observed that two of the hybridomas secreted OVA specific antibodies. The hybridomas produced IgG class antibodies. The hybridomas were grown and the supernatants from the cultures were also tested in a Western Blot assay. The results of ELISA were confirmed with Western Blot assays. The results indicate that the mAbs produced in this study reacted with the continuous (non- conformational) epitopes present on OVA.

Keywords

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Ovalbumin, monoclonal antibody, hybridoma.

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PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES - Free download as PDF File (.pdf), Text File (.txt) or read online for free. Production and characterization of monoclonal antibodies specific for pseudorabies virus. Production and characterization of monoclonal antibodies specific for pseudorabies virus. checked by means of binding reaction between separated hybridoma. supernatants and ascite liquids. This study aimed at producing monoclonal antibodies (MAbs) against AFM1 for future development of immunoassay-based detection test kit. 2. Methods. 2.1. Immunization of mice. Isotype of monoclonal antibodies were determined by Sigma-Aldrich isotyping kit. Plates were coated with isotyping specific antibodies: IgG1, IgG2a, IgG2b, IgG3, IgM and IgA and incubated at 37ºC for 1 hr. After washing, culture supernatant was added, plates were incubated at 37ºC for 1 hr. After washing, HRP-goat anti-mouse IgG (Fab specific) were added and incubated at 37ºC for 1 hr. The assay was then performed as previously described. Sensitivity and cross reactivity. Large-scale production has revolutionized the market for monoclonal antibodies by boosting its production and becoming a more practical method of production. Production techniques have only had a sizable breakthrough due to molecular techniques. The proposed chapter covers the fundamental aspects of monoclonal antibody production methods, with emphasis on methodologies using immobilized cells, wave bioreactor systems, SUBs, and finally the roller bottles technique. Such techniques have been described in the most recent literature, both for murine monoclonal antibody production and for production of antibodies from modified microorganisms. 2. mAbs production techniques. 2.1. Hybridoma and phage display. The monoclonal antibody has to be subjected to

biochemical and biophysical characterization for the desired specificity. It is also important to elucidate the MAb for the immunoglobulin class or sub-class, the epitope for which it is specific and the number of binding sites it possesses. The stability of the cell lines and the MAbs are important. The objective is to develop bioreactors for the large scale production of monoclonal antibodies. It may be noted that the antigen binding regions of antibody (Fv or Fab fragments) are very crucial, while the Fc portion is dispensable. A schematic representation of the procedure adopted for the production of functional antibody fragments is shown in Fig. Figure 3. Monoclonal antibodies (mAbs) are produced by introducing an antigen to a mouse and then fusing polyclonal B cells from the mouse's spleen to myeloma cells. The resulting hybridoma cells are cultured and continue to produce antibodies to the antigen. Hybridomas producing the desired mAb are then grown in large numbers on a selective medium that is periodically harvested to obtain the desired mAbs. Since the most common methods for producing monoclonal antibodies use mouse cells, it is necessary to create humanized monoclonal antibodies for human clinical use. Mouse antibodies cannot be injected repeatedly into humans, because the immune system will recognize them as being foreign and will respond to them with neutralizing antibodies.