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Production and characterization of monoclonal antibodies (MAb) against flounder serum immunoglobulin (Ig)

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Abstract

Specific polyclonal and/or monoclonal antibodies (MAbs) to immunoglobulins (Igs) and their subunits have proved to be valuable tools in immunological research and in immunological assays. In this study, we developed and characterized MAbs against flounder serum Igs. To obtain the pure flounder serum Igs, mouse IgG (mIgG) was immunized to flounder. Flounder Igs were purified by using mIgG-agarose affinity column chromatography. The structure of purified flounder Ig was observed, on denatured SDS-PAGE, to be composed of two heavy chains (77 and 72 kd) and two light chains (28 and 26 kd). MAbs were produced by fusion of myeloma cells (SP2/0) with Balb/c mouse spleen cells previously primed with the flounder Igs. Finally, three hybridoma clones, FIM 511, FIM 519 and FIM 562 were established to recognize both 2 heavy chains, 26 kd of light chain and 28 kd of light chain, respectively. On the other hand, the flounder immune sera collected on the weekly basis were tested on ELISA and immunoblot analysis whether boosting effect is present in flounder humoral immune system. As a result, the secondary immune response in flounder was ascertained on ELISA, but not on immunoblot analysis. Further, we observed an alteration of serum protein levels following immunization. Our MAbs and basic information on flounder humoral immune system obtained in this study will be helpful to control and monitor the efficiency of fish vaccines and therapeutic process of flounder diseases.