Short Paper

Preparation of a Monoclonal Antibody Specific to Chum Salmon Growth Hormone

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(Received June 2, 1994)

Key words: monoclonal antibody, chum salmon, growth hormone

Growth hormone (GH) is synthesized in and secreted from the pituitary gland to regulate postnatal somatic growth and metabolism in vertebrates. In fish, the mechanism of GH release from the pituitary gland has been studied using organ culture pituitary systems^{1,2)} and GH content measured by radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) using polyclonal antibodies. In these systems, besides GH, high levels of other pituitary hormones were found in the media. Polyclonal antibodies may cross-react with the GH-related hormone such as prolactin and somatolactin. To avoid this problem, a highly specific GH antibody is required. Monoclonal antibodies (MAb) against salmon GH have been obtained.^{3,4)} However, there is no information with regards to the cross-reactivity with somatolactin. The aim of the present study is to prepare a MAb specific to chum salmon GH, which is suitable for use on the determination of the GH content in media from organ culture pituitary system.

Preparation of MAb was performed according to the method described previously.⁵⁾ Four-week-old female BALB/c mice were immunized with chum salmon GH suspended in Freund's complete adjuvant. Four immunizations were made at seven day intervals. After the last immunization, the mice were sacrificed and spleen cells were fused with myeloma cells (sp2/0-Ag9-K13) in the presence of 50% polyethylene glycol 4000. Fused spleen-myeloma cells were selected by culture in RPMI 1640 medium containing hypoxanthine, aminopterin, and thymidine. Antibodies produced by hybridoma cells in the culture medium were screened by ELISA using chum salmon GH as the antigen. The hybridoma cells secreting antibody against GH were cloned by limiting dilution. The most specific clone was implanted intraperitoneally into BALB/c mice, and ascites fluid was collected. The MAb, designated sGHM1 was purified from the ascites by ammonium sulfate precipitation, and then redissolved in PBS and stored at -80°C until use.

Reactivity and specificity of sGHM1 were determined by use of a non-competitive ELISA method. Each 50 μ l of an antigen solution was fixed to the wells of a polystyrene microtiter plate by an overnight incubation at 4°C and then treated with 200 μ l of 3% skim milk in PBS. Fifty μ l of sGHM1 (1:300) was added to each well and incubated at

37°C for 1 h. After three were washed with PBS containing 0.05% Tween 20 (PBST), 50 μ l of 1:1000 diluted alkaline phosphatase conjugated goat anti-mouse IgM (TAGO Inc.) was added and incubated at 37°C for 1 h. After washing three times with PBST, 100 μ l of 1 mg/ml of *p*-nitrophenylphosphate (Sigma Chemical) solution in 1 M of diethanolamine buffer (pH 9.8) was added to each well, and incubated at 37°C for 15-30 min. The absorbance of the yellow color developed in each well was measured at 405 nm.

The MAb subclass obtained in this study, sGHM1, was an IgM possessing a κ light chain, which was determined by a typing test system (Amersham, UK). The MAb reacted with chum salmon GH in a dose-dependent manner, the minimum measurable concentration of chum salmon GH being 20 ng/well (approximately 1 pmol) in the non-competitive ELISA system. To test the specificity of the sGHM1, other chum salmon pituitary hormones, as are prolactin, somatolactin, gonadotropin I, gonadotropin II, endorphin II, N-terminal peptide of proopiomelanocortin I, α - and β -melanotropin, and melanin-concentrating hormone were used. Among these hormones, the sGHM1 only reacted with GH (Fig. 1). Furthermore, the cross-reactivity of the sGHM1 with the GHs from other species (nine teleosts, one elasmobranch, and seven tetrapods) was also tested (Fig. 2). sGHM1 reacted with GHs from salmon, bonito and tuna, but not with GHs from other teleosts and tetrapods at equimolar concentrations. These results may suggest that sGHM1 recognized a common epitope of GH molecules among salmon, bonito, and tuna. Further study may clarify the epitope structure of GH of these species.

We have prepared a MAb specific to salmon GH which is a useful tool for the measurement of GH in media from organ-cultured pituitary system and therefore, will allow us to study the mechanism of GH release from the pituitary gland of salmonid.

References

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Fig. 1. Specificity of sGHM1 for chum salmon pituitary hormones in a non-competitive ELISA.

Each value represents the mean of duplicate determinations. Pituitary hormones are abbreviated as follows: GTH I (gonadotropin I), GTH II (gonadotropin II), α -MSH (α -melanotropin), β -MSM (β -melanotropin), EP II (endorphin II), NPP (N-terminal peptide of proopiomelanocortin) and MCH (melanin-concentrating hormone).

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Fig. 2. Cross-reactivities of sGHM1 for nine teleost, one elasmobranch, and seven tetrapod GHs in a non-competitive ELISA.

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