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Comparative Biochemistry and Physiology, Part B 143 (2006) 107–115
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Production, characterization and applications of mouse anti-grass carp (*Ctenopharyngodon idellus*) growth hormone monoclonal antibodies

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Received 17 June 2005; received in revised form 25 October 2005; accepted 26 October 2005

Abstract

Mouse anti-grass carp growth hormone (gcGH) monoclonal antibody (MAb) secretors were produced by PEG-mediated fusion of NS-1 myeloma cells and splenic B-lymphocytes of gcGH hyper-immunized mice. Positive secretors were screened by direct ELISA and cloned by limiting dilution. Three positive secretors, 21D3, 22G5 and 23B3, were obtained in a single fusion trial. Anti-gcGH MAbs were produced by growing hybridomas in the peritoneal cavity of pristane-primed mouse. The three MAbs were isotyped to be IgG2a, IgG2b and IgM, respectively. IgG MAbs were purified from ascitic fluid by Hitrap protein G column and IgM MAb was purified by gel filtration chromatography. The purified MAbs were highly specific and had moderate binding affinity. The MAbs were successfully used for the purification of native gcGH from mature grass carp pituitary extract by one-step immunoaffinity chromatography, for the quantification of gcGH by competitive sandwich ELISA, and for the probing of somatotropes in grass carp pituitary by immunohistochemistry.

Keywords: Affinity chromatography; ELISA; Grass carp; Growth hormone; Hybridoma; Immunohistochemistry; Monoclonal antibody; Somatotropes

1. Introduction

Growth hormone (GH) or somatotropin is a polypeptide hormone of about 190 amino acids and is secreted by pituitary gland. It is present in all vertebrate species. The structure and function of GH are highly conserved during evolution (Chang et al., 1992; Chen et al., 1994). The major physiologic actions of GH are direct diabetogenic action and indirect somatogenic action (Adamson, 1981). Adipocytes are the target for diabetogenic effect of GH. The binding of GH to GH receptor of adipocytes triggers the breakdown of triglyceride and the suppression of the uptake and accumulation of circulating lipids (Goodman et al., 1991). The somatogenic action of GH is complex and is mediated through the intermediate; insulin-like growth factor-1 (IGF-1) (Isaksson, 2004). Liver is the primary site for the production of systemic IGF-1 upon GH stimulation. GH also stimulates target cells like cartilage to produce IGF-1 locally. Circulating IGF-1 together with locally produced IGF-1 stimulate the growth of target cells. Muscle and bone are the major targets for GH somatogenic effect. The secretion of GH is primarily controlled by two hypothalamic hormones (growth hormone-releasing hormone and somatostatin), one stomach hormone (ghrelin), and the IGF-1 feedback loop (Veldhuis and Bowers, 2003). GH secretion is enhanced by growth hormone-releasing hormone (Pombo et al., 2001; Kato et al., 2002) and ghrelin (Anderson et al., 2005; Unniappan and Peter, 2005), and is suppressed by somatostatin and IGF-1 negative feedback (Pombo et al., 2001; Kato et al., 2002). In fish, the regulation of GH secretion and the metabolic effects of GH are basically similar to that of mammals (Shepherd et al., 1997; Reinecke et al., 2005). The only major difference is fish GH also plays role in osmoregulation (Sakamoto et al., 1997). GH stimulates an increase in the Na+, K+ ATPase activity in the gill of fish (Shepherd et al., 1997), and such physiological change is essential for seawater adaptation.

The application of GH in aquaculture by transgenesis and exogeneous administration of recombinant GH is still experimental, though different fish GH cDNAs have been cloned and expressed in various expression systems like salmon GH in cyanobacterium (Kawata et al., 1991), yellow tail GH in the marine photosynthetic bacterium *Rhodobacter* SP NKPB 0021

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doi:10.1016/j.cbpb.2005.10.012

(Burgess et al., 1993), carp GH in *Escherichia coli* A1645 (Fine et al., 1993), rainbow trout GH in yeast (Tsai et al., 1993), trout GH in tobacco plant (Bosch et al., 1994), goldfish GH in *E. coli* (Mahmoud et al., 1998), flounder GH in *E. coli* (Jeh et al., 1998), grass carp GH in silkworm larvae *Bombyx mori* (Ho et al., 1998), in *E. coli* BL21 (Wang et al., 2001) and in yeast (Wang et al., 2003), dolphin fish GH in *E. coli* BL21 (Paduel et al., 1999), Indian major carp GH in *E. coli* and zebrafish (Venugopal et al., 2002), giant catfish GH in *E. coli* (Promdonkoy et al., 2004), and tilapia GH in transgenic tilapia (Caelers et al., 2005).

Fish with GH transgene exhibits growth enhancement is well reported. The fold of weight gain as a result of GH transgene expression was documented as 3–17 (Du et al., 1992; Rahman et al., 1998; Pitkanen et al., 1999; Devlin et al., 2001). Besides growth enhancement, GH overexpression also improves feed-conversion efficiency up to nearly 2 fold (Nam et al., 2001). The carcass composition of transgenic fish is also affected by the high level of GH. Dunham et al. demonstrated the muscle of transgenic common carp had a lower percentage of moisture and lipids but higher percentage of protein than wild type (Dunham et al., 2002). In most of the studies, GH transgene was driven to overexpress ectopically by constitutive promoter like human cytomegalovirus (CMV) immediate-early promoter (Pitkanen et al., 1999) or β-actin promoter (Nam et al., 2001). The enhancement of growth is not only determined by the level of expression of the GH transgene, but also the genetic background of the host (Devlin et al., 2001). The application of GH carrying transgenic fish in aquaculture is mainly resisted by the fear that escaping transgenic species can have irreversible negative impact on native species and environment (Muir, 2004).

The production of monoclonal antibodies against fish GHs for the purification, the quantification and the study of the GHs is lagging behind their expression studies, and only a few reports are available (Furuya et al., 1987; Farbridge et al., 1990, 1991). We have reported the cloning of grass carp cDNA (Ho et al., 1991) and the expression of gcGH in silkworm larva (Ho et al., 1998). We here report the production of monoclonal antibodies against grass carp growth hormone and its potential applications.

2. Materials and methods

2.1. Hyper-immunization

Female albino BALB/c mice of 6–8 weeks old were hyper-immunized as described in Monoclonal Antibodies (Baumgarten et al., 1992) with slight modifications. Water in oil emulsion was prepared by mixing equal volumes of ngcGH at 40 μg/mL phosphate-buffered saline (PBS) and complete Freund's adjuvant (cFA). Mouse was primed by intraperitoneal (i.p.) injection of 200 μL of the emulsion on day 1. On day 27, booster injection, which was prepared by mixing equal volumes of ngcGH and incomplete Freund's adjuvant (iFA), was injected i.p. at a dose of 200 μΛ per mouse. Second booster of 200 μL was delivered on day 34 through i.p. route. Immunized mouse was finally hyperimmunized with 200 μL of ngcGH at 20 μg/mL PBS intravenously on days 68, 69 and 70, respectively.

2.2. Cell fusion

Log growing phase NS-1 myeloma cells (ATCC, Manasses, VA) were fused with splenic lymphocytes of hyperimmunized mice at 1:5 ratio. Two milliliters of NS-1 myeloma cell of 5.6×10^7 cells was added into 10 mL splenic lymphocytes of 5.6×10^7 cells. The cells were mixed gently in a 50-mL Falcon centrifuge tube and were pelleted by centrifugation (5 min; 200×*g*, 25 °C). Cell pellet was collected and was dissociated by flicking the bottom of the tube. PEG 4000 (Invitrogen, Carlsbad, CA) of 1.5 mL at 37 °C was added into the cell suspension slowly. The mixture was pipetted gently up and down for several times and then rocked gently for 1 min at room temperature (RT). PBS equilibrated at 37 °C was then added drop-by-drop (1 mL in 30 s, 3 mL in 30 s and 16 mL in 60 s). The fusion cocktail was centrifuged (5 min; 200×*g*, RT) and supernatant was discarded. Cell pellet was incubated at RT for 5 min. After fusion reaction, cells were suspended in 30 mL RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and recombinant murine interleukin 6 (Invitrogen, Carlsbad, CA, USA) of 10 units per mL. The cell suspension was distributed into three 96 well plates (100 μL/well). Twenty-four hours after plating out, 100 μL of 2×HAT medium was added to select hybridoma. At 10–15 days after plating out, conditioned medium of 100 μL was removed for direct ELISA screening. Goat-anti-mouse HRP conjugate was the second antibody and ABTS (Sigma-Aldrich, St. Louis, MO, USA) was the chromogenic substrate. A second screening was performed on 20 days after fusion to ensure no slow growing positive clones were ignored. The number of cell colonies produced was counted through microscope.

2.3. Cloning of hybridoma

Limiting dilution cloning was used to enrich positive colonies. Positive colonies were removed and cell number was counted with a hemacytometer. Cell concentration was adjusted to 10 cells/mL with supplemented medium (RPMI-1640 with 10% FBS). Splenic/feeder cells were prepared aseptically from non-immunized female albino BALB/c mice and were resuspended in supplemented medium at 5×10^6 cells/mL. Equal volumes of hybridoma cells and feeder cells were mixed, and were then distributed to 96-well plate at one hybridoma cell/well (200 μL). Ten days after plating out, 100 μL of medium was removed from well with observable colony, for ELISA screening. Cloning was repeated until all the colonies obtained, after limiting dilution, were positive. Male albino BALB/c mice of 6–8 weeks old were injected i.p. with 0.5 mL of pristane (Sigma-Aldrich, St. Louis, MO). Thirteen days after pristane priming, mouse was injected i.p. with 0.5 mL of hybridoma cells at 3.6×10^6 cells/mL PBS. Ascitic fluid was collected as peritoneal lavage on 15–30 days after hybridoma injection.