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SHORT COMMUNICATION

8-1A, a Human Monoclonal Antibody that Reacts with Intact Human Chorionic Gonadotropin

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The incidence of choriocarcinoma has decreased over time and therapeutic results have improved about 90% complete remission in patients without extensive metastasis. However, some choriocarcinomas metastasize to other organs and show resistance to chemotherapy, having a poor prognosis despite multidisciplinary treatment. Better methods of early diagnosis for recurrence or micrometastasis, and treatment against cases with intractable gestational trophoblastic neoplasia (GTN) are needed to improve the prognosis.

Human chorionic gonadotropin (hCG) is a glycoprotein hormone composed of two dissimilar subunits and a tumor marker to make a diagnosis and monitor therapeutic effect in GTN. Even when hCG levels in the serum become too low to measure with the hCG β-CTP system which is the most sensitive assay, there are estimated to be approximately 10,000 trophoblastic cells in the body. Residual trophoblast cells may cause symptoms such as bleeding or undergo malignant transformation to choriocarcinoma.

Since most monoclonal antibodies developed so far are murine, administration creates human anti-mouse antibodies, resulting in clinical failure. More recent mouse/human chimeric antibodies or humanized antibodies still possess substantial immunogenicity that makes repeated administration difficult. In the present study, KM mice that can produce completely human monoclonal antibodies were used to prepare hCG-specific human monoclonal antibody. This yielded 8-1A, a human monoclonal antibody capable of reacting with intact hCG. In the future, new diagnostic techniques and treatments for chorionic diseases may be developed using this kind of human monoclonal antibody.
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INTRODUCTION

Human chorionic gonadotropin (hCG) is a glycoprotein consisting of two dissimilar subunits (α and β) that bind solely by charge interactions. Upon following up of gestational trophoblastic neoplasia (GTN), the hCG β-CTP system is currently the most sensitive assay and it measures blood hCG levels as low as 0.5 mIU/mL. It is thought that approximately 200,000 trophoblastic cells can produce 10,000 mIU of hCG per day. Therefore, when hCG levels become too low to be measured with the hCG β-CTP system, there may still be approximately 10,000 residual trophoblast cells in the body. Such residual cells are invisible clinically, but may undergo malignant transformation to choriocarcinoma. Therefore, elimination of residual trophoblast cells is important to prevent recurrence and allow withdrawal from chemotherapy.

Use of monoclonal antibodies as both diagnostic and therapeutic tools is appealing because these antibodies are highly specific, but clinical application has been limited by the immunogenicity of antibodies created in animals. Accordingly, development of human antibodies has been awaited because of presumed higher specificity, lower immunogenicity, and longer half-life. We have used cross-bred trans-chromosomic mice, designated as KM mice [1,2] to create a human monoclonal

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antibody for hCG. KM mice was established by cross-breeding trans-chromosomic mice containing a germline transmittable human chromosome 14 fragment that covers the entire human Ig heavy chain loci and transgenic mice containing the YAC-transgene that covers 50% of the human Ig kappa light chain loci [3]. KM mice possess the entire human Ig heavy chain gene locus, express all subclasses of heavy chain γ, and show a pattern of Ig expression identical to that seen in humans. These mice have already been successfully used to develop human antibodies for various target antigens, i.e., fully human monoclonal antibodies. Employing KM mice, we have previously established human monoclonal antibodies (HMMC-1 and HMOCC-1) that were highly specific for endometrial carcinoma and ovarian carcinoma, respectively [4,5], while another group has reported on the use of KM mice to establish a human monoclonal antibody for CEA that shows antitumor activity [6], and an antibody for TRAIL-R2 that shows an apoptotic effect [7].

GTN including choriocarcinoma is one of the most sensitive to chemotherapy than other gynecologic cancers, and responds dramatically to chemotherapeutic agents, with a complete remission rate of up to approximately 90% in the absence of extensive metastasis [8]. However, there are still choriocarcinomas with metastases to other organs such as the brain and liver that show resistance to chemotherapy. In such cases, even multidisciplinary treatment achieves a poor outcome. To improve the prognosis of intractable GTN, better methods of early diagnosis and treatment are necessary. In the present study, we created a fully human monoclonal antibody for hCG using KM mice. When administered to patients, this antibody should be useful for diagnosing and treating intractable GTN.

MATERIALS AND METHODS

Mice

KM mice were supplied by Kirin Brewery (Tokyo, Japan). All animals were handled in accordance with the protocol established by the Animal Care Committee of Keio University School of Medicine.

Chemicals

The following agents were used: intact hCG (1st WHO Reference Reagent 2001, NIBSC, Code No. 99/688, United Kingdom), hCG (Teikoku Hormone MFG, Tokyo, Japan), hCG (Sigma, St. Louis, MO, USA), recombinant hCG (rhCG: Sigma, St. Louis, MO, USA), recombinant hCG-α (rhCG-α: Sigma, St. Louis, MO, USA), recombinant hCG-β (rhCG-β: Sigma, St. Louis, MO, USA), recombinant LH (rLH: Sigma, St. Louis, MO, USA), hCG beta core fragment (hCG-βcf: 1st WHO Reference Reagent 2001, NIBSC, Code No. 99/708, United Kingdom), hCG nicked beta subunit (hCG-βn: 1st WHO Reference Reagent 2001, NIBSC, Code No. 99/692, United Kingdom), hCG nicked (hCG-n: 1st WHO Reference Reagent 2001, NIBSC, Code No. 99/642, United Kingdom), nicked hCG (nhCG: kindly provided by Dr. Nishimura) [9].

Immunization of KM mice and screening of hybridomas

A dose of 500 μg of hCG (kindly provided by Mochida Pharmaceutical, Tokyo, Japan) was injected into KM mice every two weeks. Spleen cells from immunized cross-bred TC mice were fused with Sp 2/0-Ag 14 mouse myeloma cells (ATCC No. CRL-1581) using S-clone cloning medium (Sanko Junyaku, Tokyo, Japan), hybridoma cloning factor (Igen International, Gaithersburg, MD, USA), HAT (ICN Biochemicals, Costa Mesa, CA, USA), and HT (ICN Biochemicals, Costa Mesa, CA, USA). Fused hybridoma cells were seeded onto 96-well plates and clones positive for hCG were selected by screening culture supernatants with ELISA. In brief, hCG (Mochida Pharmaceutical, Tokyo, Japan) was immobilized on the 96-well plates (Nalgen Nunc International, Rochester, NY, USA) at a concentration of 0.1 μg/well and blocked with 1% BSA at 250 μL/well. Then 100 μL of hybridoma culture supernatant was added to every well and reacted with peroxidase-conjugated rabbit anti-human IgG specific for the gamma chain (Dako, Glostrup, Denmark) as the secondary antibody, after which a plate reader (Bio Rad Laboratories, Hercules, CA, USA) was used to measure the absorbance at 490 nm. Initial screening with hCG (Mochida Pharmaceutical, Tokyo, Japan) yielded 51 positive hybridomas. These clones were further selected by using the supernatant of hybridomas directed against intact hCG (1st WHO Reference Reagent 2001, NIBSC, Code No. 99/688, United Kingdom), hCG (Teikoku Hormone MFG, Tokyo, Japan), hCG (Sigma, St. Louis, MO, USA), recombinant hCG (Sigma, St. Louis, MO, USA), rhCG-α (Sigma, St. Louis, MO, USA), rhCG-β (Sigma, St. Louis, MO, USA), and rLH (Sigma, St. Louis, MO, USA). We selected clone 8-1A based upon the results of ELISA and carried out limiting dilution to isolate a single clone producing a monoclonal antibody.

Detection of the human monoclonal antibody

Acrylamide uniform 12.5% gel (Bio Rad Laboratories, Hercules, CA, USA) was used to perform sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of hybridoma supernatants. After blotting onto a membrane (Millipore, Billerica, MA, USA), incubation was done overnight at 4 °C with skim milk (Dainippon Pharmaceutical, Osaka, Japan) in 0.1% Tween 20 containing 10 mM Tris—HCl with 0.15 M NaCl (pH 7.6) for blocking. Then bound and free (HRP-conjugated) goat anti-human kappa light chain antibody (Bethyl Laboratories, Montgomery, TX, USA), mouse lambda bound and free antibody for HRP (Bethyl Laboratories, Montgomery, TX, USA), peroxidase-conjugated rabbit anti-human IgG specific for gamma chains (Dako, Glostrup, Denmark), peroxidase-conjugated affinity-purified goat anti-mouse IgM, μ chain specific (Jackson ImmunoResearch Laboratories, West Grove, PA, USA), and