Biochemical and Biophysical Research Communications xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Human monoclonal antibodies derived from a patient infected with 2009 pandemic influenza A virus broadly cross-neutralize group 1 influenza viruses

Yang Pan [a], Tadahiro Sasaki [a,h], Ritsuko Kubota-Koketsu [a,b,h], Yuji Inoue [a,h], Mayo Yasugi [a,c,h], Akifumi Yamashita [a], Ririn Ramadhany [a], Yasuha Arai [a], Anariwa Du [a,h], Naphatsawan Boonsathorn [a,d,h], Madiha S. Ibrahim [a,e], Tomo Daidoji [a,f], Takaaki Nakaya [a,f,h], Ken-ichiro Ono [g,h], Yoshinobu Okuno [b], Kazuyoshi Ikuta [a,h,*], Yohei Watanabe [a,h,*]

[a] Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, Japan
[b] Kanonji Institute, The Research Foundation for Microbial Diseases of Osaka University, Kanonji, Kagawa, Japan
[c] Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Izumisano, Osaka, Japan
[d] Department of Medical Sciences, Ministry of Public Health, Muang, Nonthaburi, Thailand
[e] Department of Microbiology and Immunology, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt
[f] Department of Infectious Diseases, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto, Japan
[g] Ina Laboratory, Medical & Biological Laboratories Corporation, Ltd., Ina, Nagano, Japan
[h] JST/JICA, Science and Technology Research Partnership for Sustainable Development (SATREPS), Tokyo, Japan

ARTICLE INFO

Article history:
Received 2 May 2014
Available online xxxx

Keywords:
Influenza A virus
Human monoclonal antibody
Cross-neutralizing antibody
Global epitope

ABSTRACT

Influenza viruses are a continuous threat to human public health because of their ability to evolve rapidly through genetic drift and reassortment. Three human monoclonal antibodies (HuMAbs) were generated in this study, 1H11, 2H5 and 5G2, and they cross-neutralize a diverse range of group 1 influenza A viruses, including seasonal H1N1, 2009 pandemic H1N1 (H1N1pdm) and avian H5N1 and H9N2. The three HuMAbs were prepared by fusing peripheral blood lymphocytes from an H1N1pdm-infected patient with a newly developed fusion partner cell line, SPYMEG. All the HuMAbs had little hemagglutination inhibition activity but had strong membrane-fusion inhibition activity against influenza viruses. A protease digestion assay showed the HuMAbs targeted commonly a short α-helix region in the stalk of the hemagglutinin. Furthermore, Ile45Phe and Glu47Gly double substitutions in the α-helix region made the HA unrecognizable by the HuMAbs. These two amino acid residues are highly conserved in the HAs of H1N1, H5N1 and H9N2 viruses. The HuMAbs reported here may be potential candidates for the development of therapeutic antibodies against group 1 influenza viruses.

1. Introduction

Vaccination is currently considered the best option to control influenza. However, this approach has several limitations, mainly because of viral antigenic changes, a phenomenon known as antigenic drift [1]. Seasonal influenza vaccines normally induce a panel of anti-hemagglutinin (HA) antibodies. HA is synthesized as a precursor, HA0, and proteolytic cleavage into disulfide-linked HA1 and HA2 is required for the infectivity of progeny virions [2]. HA is divided into two distinct structural regions, the globular head responsible for virus binding to the cell receptor and the stem region containing the fusion domain. Influenza infection and vaccination produces antibodies that target predominantly antigenic sites in the globular head domain, thereby blocking virus-cell interaction [3]. Vaccine-induced antibody responses usually are strain-specific. In addition, the rapid acquisition of amino acids substitutions, predominantly in the globular head, allows influenza viruses to escape from neutralizing immune responses. Consequently, the WHO updates annually the influenza virus strains included in the current vaccine, on the basis of those currently in circulation and those predicted to circulate the next influenza

* Corresponding authors at: Department of Virology, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, Osaka, Japan. Fax: +81 6 6879 8310.
E-mail addresses: Ikuta@biken.osaka-u.ac.jp (K. Ikuta), nabe@biken.osaka-u.ac.jp (Y. Watanabe).

http://dx.doi.org/10.1016/j.bbrc.2014.05.060
0006-291X/

season [4,5]. A major limitation of current vaccine approach against a new pandemic is the time required to produce an appropriate quantity of antigenically matched vaccine [4,5]. In addition, the emergence of resistance to antiviral drugs in recent years further limits the options available for the control of influenza [6]. Therefore, many researchers are now examining the prospect of new prophylactic and therapeutic approaches, including antibody therapy.

Human monoclonal antibodies (HuMAbs) have advantageous properties as prophylactic and therapeutic reagents, including a long half-life in the serum and high specificity [7]. Several studies have reported cross-neutralizing HuMAbs against group 1 influenza A viruses (H1, H2, H5, H6, H8, H9, H11, H12, H13 and H16) [7–12], group 2 influenza A viruses (H3, H4, H7, H10, H14 and H15) [13], both group 1 and 2 influenza A viruses [14,15], and influenza B virus [16,17]. Most of these HuMAbs were prepared from human display libraries and bind to a conserved epitope in the stem region of HA. However, the diversity of combinational phage display libraries is sometimes many orders of magnitude greater than the diversity of the human variable region repertoire [18]. In addition, HuMAbs generated by this method are the products of random combination between immunoglobulin variable region of the heavy (VH) chain and light (VL) chain genes.

On the other hand, we have reported a cell-to-cell fusion method using a new fusion partner cell line, SPYMEG, and have generated naturally functioning HuMAbs against influenza viruses [17,19,20] and dengue viruses [21,22]. This method has been optimized to fuse SPYMEG with patient-derived peripheral blood mononuclear cells (PBMCs) [23]. Here, we report the characterization of three anti-influenza HuMAbs generated from the PBMCs of an influenza-infected patient.

2. Materials and methods

2.1. Ethics statement

Human materials were collected using protocols approved by the Institutional Review Boards of Osaka University (Approval number 19-8-6).

2.2. HuMAb preparation

Hybridomas producing anti-influenza HuMAbs were prepared using the fusion partner cell line, SPYMEG (Medical & Biological Laboratories), as described previously [17,19]. Briefly, 10 ml blood was drawn in February 2011 from a patient (35 years old, male, Japanese) infected with H1N1pdm. H1N1pdm infection was diagnosed by the Prime Check Flu (H1N1) 2009 kit (Alfresa Pharma), which specifically detects H1N1pdm. PBMCs were isolated from blood samples by density gradient centrifugation through Ficoll-Paque Plus (GE Healthcare). The PBMCs were fused with SPYMEG using polyethylene glycol 1500 (Roche) and the fused cells were selectively cultured in Dulbecco's modified Eagle medium (Life Technologies) supplemented with 15% fetal bovine serum and hypoxanthine–aminopterin–thymidine. Cell supernatants were subjected to screening for influenza virus specific antibodies by immunofluorescence assay (IFA). The cells in the positive wells were cloned by limiting dilution and screened again. The hybridoma cell clones were then cultured and expanded in Hybridoma-SFM (Life Technologies). MAbs were purified from 100 ml hybridoma culture supernatant by affinity chromatography using HiTrap Protein G HP Columns (GE Healthcare) and then dialyzed against PBS.

2.3. IgG isotyping

ELISA microplates (MaxiSorp; ThermoFisher Scientific Nunc) were coated with goat anti-human IgG (Jackson ImmunoResearch Laboratories) in 0.05 M sodium bicarbonate buffer (pH 8.6) overnight at 4 °C. After washing with PBS containing 0.1% Tween-20 (PBS-T), the wells were blocked with 0.5% bovine serum albumin (BSA) in PBS for 1 h at 37 °C. The wells were then washed with PBS-T and incubated with hybridoma supernatants or control serum for 2 h at 37 °C. After washing with PBS-T, the wells were incubated with HRP-conjugated anti-human IgG_1, IgG_2, IgG_3 or IgG_4 antibodies (SouthernBiotech) for 1 h at 37 °C. The wells were then washed five times with PBS-T and incubated with 3,3′,5,5′-tetramethylbenzidine peroxidase substrate (Kirkegaard & Perry Laboratories) at room temperature (RT) in the dark. After 20 min, the reaction was stopped with 2 N H_2SO_4. Color development was measured at 450 nm in ELISA Photometer (BioTek Instruments).

2.4. Sequencing of the HuMAb variable regions

Total RNA was extracted from the hybridoma cells using an RNeasy Mini kit (Qiagen) and subjected to RT-PCR using PrimeScript RT reagent kit (Takara Bio) and oligo (dT) primer. The regions encoding the HuMAb H- and L-chains were amplified by PCR using the following primer pairs: 5′-ATGGAGTTTGGGCTGAGCTGGGTT-3′ (H-chain forward) and 5′-CTCCCGCGGCTTTGTCTTGGCATTA-3′ (H-chain reverse) or 5′-ATGGCCTGGRYCYCMYTCYWCCTM-3′ (L-chain forward) and 5′-TGGCAGCTGTAGCTTCTGTGGGACT-3′ (L-chain reverse). The PCR products were purified using a QIAquick PCR Purification kit (Qiagen). After electrophoresis, a discrete band was extracted using QIAquick Gel Extraction kit (Qiagen) and the nucleotide sequence was determined using a BigDye Terminator v3.1 Cycle Sequencing kit and ABI Prism 3100 Genetic Analyzer (Applied Biosystems). The sequences were used to search the NCBI database using IgBLAST website (http://www.ncbi.nlm.nih.gov/igblast/).

2.5. Viruses

Six H1N1pdm virus strains (A/Suita/1/2009, A/Osaka/168/2009, A/California/07/2009, A/Suita/117/2011, A/Suita/104/2011 and A/Suita/105/2011), two seasonal H1N1 strains (A/Brisbane/59/2007 and A/PR8/1934), one H2N2 virus strain (A/Izumi/5/1965), two H3N2 virus strains (A/Aichi/2/1968 and A/Uruguay/716/2007), two H5N1 virus strains (A/Duck/Egypt/D1Br12/2007 and A/Chicken/Egypt/RIMD12-3/2008), one H7N7 virus strain (A/Tufted duck/Shimane/124R/1980), one H9N2 virus strain (A/Turkey/Wisconsin/1/1966) and two influenza B virus strains (B/Florida/4/2006 and B/Malaysia/2506/2004) were used in the study. Viruses were propagated in either Madin-Darby canine kidney (MDCK) cells or 9-day-old embryonated chicken eggs.

2.6. Construction of HA-expressing plasmids

Wild and truncated HA gene sequences from A/Suita/1/2009 were generated by one step RT-PCR and inserted into the pGEM-T Easy Vector (Promega). Mutant HA genes were generated by site-directed mutagenesis PCR (GeneTailor Site-Directed Mutagenesis System; Life technologies). Each gene was subcloned into the expression vector pCAGGS [17]. The expression plasmids were transfected into human embryonic kidney 293T cells using Lipofectamine 2000 (Life technologies) according to the manufacturer's instructions.

Please cite this article in press as: Y. Pan et al., Human monoclonal antibodies derived from a patient infected with 2009 pandemic influenza A virus broadly cross-neutralize group 1 influenza viruses, Biochem. Biophys. Res. Commun. (2014), http://dx.doi.org/10.1016/j.bbrc.2014.05.060