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Original article

Recombinant human monoclonal antibodies to human cytomegalovirus glycoprotein B neutralize virus in a complement-dependent manner

Akane Ohta [a,1], Ayano Fujita [a,c], Tsugiya Murayama [d], Yoshitaka Iba [a,b], Yoshikazu Kurosawa [a,b], Tetsushi Yoshikawa [a,c,*], Yoshizo Asano [a,c]

[a] *21st Century COE Research Center, Toyoake, Aichi, Japan*
[b] *Division of Immunology, Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi, Japan*
[c] *Department of Pediatrics, Fujita Health University School of Medicine, Toyoake, Aichi, Japan*
[d] *Department of Microbiology and Immunology, Faculty of Pharmaceutical Sciences, Hokuriku University, Kanazawa, Japan*

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Abstract

Human antibodies specific for HCMV are currently considered as potential anti-HCMV therapeutic agents. In this study, we used a combinatorial human antibody library to isolate and characterize complete human monoclonal antibodies that effectively neutralize HCMV in a complement-dependent manner. One hundred and six clones were isolated in two independent screens using HCMV virions and recombinant glycoprotein B, gB654, as antigens. All of the clones recognized the same molecule gB and were classified into 14 groups based on the amino acid sequence of the V_H region. Seven representative clones from these 14 groups had a strong gB654 binding affinity by surface plasmon resonance (SPR). A pairwise binding competition analysis suggested that there were three groups based on differences in the gB recognition sites. Although Fab fragments of the seven groups showed strong affinity for gB, none of the Fab fragments neutralized HCMV infectivity *in vitro*. In contrast, complete human IgG_1 antibodies of at least three groups neutralized HCMV in a complement-dependent manner. These data suggest that potent therapeutic antibodies can be obtained from a human antibody library, including most of the functional antibodies that mediate humoral immunity to the selected pathogen.
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1. Introduction

Primary human cytomegalovirus (HCMV) infection generally occurs in infancy or early childhood but is asymptomatic in immunocompetent hosts. However, HCMV is a major cause of morbidity and mortality in immunocompromised individuals, including transplant recipients, AIDS patients and newborn infants [1]. Additionally, congenital HCMV infection can cause severe neurological and visceral manifestations and in rare instances is fatal. Due to the number and severity of these infections, HCMV infection is associated with a large economical burden. Antiviral drugs (Ganciclovir, GCV; Foscarnet, PFA; Cidofovir, CDV; etc.) have been used to treat patients with HCMV-related diseases [2]. Although these drugs reduce the risk of HCMV-related diseases in patients, they can induce serious side effects, including myelosuppression and renal toxicity [3]. Another treatment strategy is cytomegalovirus-hyper immune globulin (CMVIG), which is used as a pre-emptive treatment for post-transplantation patients [4,5]. A meta-analysis performed by Bonaros et al. suggested that prophylactic administration of CMVIG effectively prevents CMV disease and CMV-associated deaths in patients after solid organ transplantation [6]. Moreover, it was

* Corresponding author. Department of Pediatrics, Fujita Health University School of Medicine, Toyoake, Aichi, 4701192 Japan. Tel.: +81 562 939251; fax: +81 562 952216.
E-mail address: tetsushi@fujita-hu.ac.jp (T. Yoshikawa).
[1] Present address: Department of Virology, Graduate School of Medicine, Nagoya University, Nagoya, Japan.

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suggested that treating pregnant women with CMV-hyperimmune globulin is an effective treatment and prevention strategy for congenital CMV infection [7]. These data suggest that administering anti-HCMV immunoglobulin is a promising treatment option for HCMV infection. However, as immunoglobulin is obtained from donated blood, the possibility of using contaminated or infectious blood is not completely eliminated. Thus, effective monoclonal antibodies that prevent HCMV infection and are free from the risk of infection may be useful to treat patients.

Because of recent developments in antibody engineering, a number of antibody preparations are currently used as clinical treatments [8]. There are two major technologies used to isolate human or humanized monoclonal antibodies: hybridoma technology used to produce mouse-human chimeric antibodies and phage display antibody library technology [9]. The anti-HCMV human monoclonal antibody, MSL-109 (Protein Design Laboratories, CA, USA), is a product of hybridoma technology that uses a non-producing murine-human hybrid myeloma fused to human B-lymphocytes that are stimulated *in vitro* [10]. However, when the efficacy of MSL-109 was evaluated in several clinical trials, this monoclonal antibody showed no significant reduction in CMV infection or CMV disease [11,12]. It remains unclear why MSL-109 was ineffective in clinical trials despite the robust neutralizing activity *in vitro*. By contrast, phage-displayed library technology enables various antibody clones to be selected [9]. We previously established a human antibody library that contains a large number of clones from surgically resected tonsillar tissues and obtained over three hundred antibody clones against carcinoma-specific antigens, and also obtained antibodies against rotavirus and varicella zoster virus [13–15].

It is widely thought that establishing a safe and effective antibody treatment may be important for improving treatment strategies for HCMV infections. In order to produce effective human monoclonal antibodies, we thought that a phage library established from lymphocytes from a healthy individual with a high anti-HCMV antibody titer would be suitable. Therefore, in the present study, we established a large antibody library derived from a pediatrician with a high anti-HCMV antibody titer. Thus, it is expected that comprehensively screening this large antibody library would yield many suitable types of anti-HCMV-neutralizing antibodies.

2. Materials and methods

2.1. Cells and viruses

The human embryonic lung fibroblasts cell line MRC-5 was maintained in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum. The human embryonic kidney cell line HEK293T was maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum. HCMV laboratory strain AD169 was distributed from the Research Institute for Microbial Diseases (Osaka, Japan) and propagated in MRC-5 cells. HCMV strain Towne was propagated in HEL cells. 91S and 93R were clinically isolated viruses propagated from previously reported viruses [16].

2.2. Construction of gB654 expression plasmids

gB654 is a truncated form of glycoprotein B, encoded by HCMV UL55. gB654 consists of 654 amino acids and excludes the N-terminal 24 amino acids that encode the signal peptide and amino acids 680 to 906. The gB654 expression plasmid was constructed using standard methods. The UL55 gene was directly amplified by pfu DNA polymerase (Fermentus) following the manufacturer's instructions, using the HCMV AD169 genome as a template. We used the following primer set, which contains enzyme recognition site (italicized), (5′-primer, GGGCCC*AAGCTT*GTCTTCTAGTACTT CCCATGC; and 3′-primer, CGCCGC*GGATCC*GAGAGG TCAAAAACGTTGCTGG). The amplified fragments were digested with HindIII and BamHI enzymes and then cloned into the pSecTag2A (Invitrogen, Carlsbad, CA) vector. The pSecTag2A vector contained a 6×His-tag and was used for soluble protein expression.

2.3. Expression and purification of gB654 6×His-tagged protein

The gB654 expression plasmid, pgB654-SecTag2A, was extracted from transformed *Escherichia coli*, competent cells JM109 (Promega) using the Plasmid Maxi Kit (QIAGEN, Germantown, MD). 27 μg of purified plasmid was transfected into 293 T cells in 15-cm dishes using Lipofectoamine 2000 reagent (Invitrogen). After a 48-h incubation with serum-free medium, the supernatant of transfected cells was harvested, and affinity-purified using 6×His-tag with Ni-NTA agarose (QIAGEN) following the manufacturer's instructions. The purified gB654-myc-His protein was detected by Western blotting with a rabbit polyclonal anti-myc antibody (MBL, Nagoya) diluted 1:2000, a mouse monoclonal anti-Histidine-tag antibody (MBL) diluted 1:5000, and a goat polyclonal anti-HCMV antibody (Biogenesis, England) diluted 1:2000.

2.4. Antibody library

The human antibody library "A-library" was used as the antibody source. The library was constructed using the lymphocyte-rich fraction of peripheral blood donated by a volunteer who had a high HCMV-neutralizing antibody titer. Mononuclear cells were collected by apheresis from the equivalent of 3 L of blood from an HCMV-seropositive pediatrician. The resulting cell population contained approximately 10^6 B-lymphocytes. This library is a combinatorial antibody library, which was made from V_H and V_L DNAs reverse-transcribed using total RNAs from lymphocytes as described previously [13, 15]. An Fab antibody fused to cp3 (Fab-cp3) was expressed on the phage surface. The plasmid, which includes the V_H, C_H1, V_L and C_L coding regions and the

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