Recombinant Human IgG antibodies against Human Cytomegalovirus

TAO DUAN[#,1], XIAO-FANG WANG[#,1], SHU-YUAN XIAO[+], SHU-YAN GU[+], AND MI-FANG LIANG[#,2]

[#]Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 100052, China; [+]Department of Pathology and Center for Biodefence and Emerging Infectious Diseases, University of Texas Medical Branch, Galveston, Texas 77555-0609, USA

Objective To study the passive immunization with human monoclonal antibodies as for prophylaxis of human cytomegalovirus (HCMV) infection. **Methods** Fab monoclonal antibodies to HCMV were recovered by repertoire cloning of mRNA from a HCMV infected individual. Antigen binding specificity, CDR sequence of V_H and V_L and neutralizing activity on HCMV AD_{169} stain were analyzed *in vitro*. The light and heavy chain Fd fragment genes of Fab antibodies were further cloned into a recombinant baculovirus expression vector pAC-κ-Fc to express intact IgG. Secreted products were purified with affinity chromatography using protein G. **Results** SDS-PAGE and Western blot confirmed the expression of the intact IgG. Immuno-blotting and -precipitation were used to identify HCMV proteins. One Fab monoclonal antibody recognized a conformational HCMV protein. **Conclusion** IgG antibodies can neutralize the HCMV AD_{169} strain efficiently at a titer of 2.5 μg/mL and may prove valuable for passive immunoprophylaxis against HCMV infection in humans.

Key words: Human cytomegalovirus; Human engineering antibody; Phage display; Recombinant baculovirus expression

REFERENCES

1. Plummer G (1973). Cytomegaloviruses of man and animals. *Prog Med Virol* **15**, 92-125.
2. Smith M G (1954). Propagation of salivary gland virus of the mouse in tissue cultures. *Proc Soc Exp Biol Med* **86**, 435-440.
3. Li C R, Greenberg P D, Gilbert M J, *et al*. (1994). Recovery of HLA-restricted cytomegalovirus (CMV)-specific T-cell responses after allogeneic bone marrow transplant: correlation with CMV disease and effect of gancicrovir prophylaxis. *Blood* **83**, 1971–1979.
4. Conti D J, Shen G, Singh T, *et al*. (1997). Ganciclovir prophylaxis of cytomegalovirus disease. *Transplant Proc* **29**, 804-806.
5. Gonczol E, Berencsi K, Pincus S, *et al*. (1995). Preclinical evaluation of an ALVAC (canarypox): human cytomegalovirus glycoprotein B vaccine candidate. *Vaccine* **13**, 1080-1085.
6. Gonczol E, Plotkin S (2001). Development of a cytomegalovirus vaccine: lessons from recent clinical trials. *Expert Opin Biol Ther* **1**, 401-412.
7. Snydman D R, Falagas M E, Avery R, *et al*. (2001). Use of combination cytomegalovirus immune globulin plus ganciclovir for prophylaxis in CMV-seronegative liver transplant recipients of a CMV-seropositive donor organ: a multicenter, open-label study. *Transplant Proc* **33**, 2571-2575.
8. Snydman D R, Werner B G, Dougherty N N, *et al*. (1994). A further analysis of the use of cytomegalovirus immune globulin in orthotopic liver transplant patients at risk for primary infection. Boston Center for Liver Transplantation CMVIG-Study Group. *Transplant Proc* **26**(5 Suppl 1), 23-27.
9. Snydman D R, Werner B G, Dougherty N N, *et al*. (1993). Cytomegalovirus immune globulin prophylaxis in liver transplantation. A randomized, double-blind, placebo-controlled trial. The Boston Center for Liver Transplantation CMVIG Study Group. *Annals of Internal Medicine* **119**, 984-991.
10. Snydman D R (1990). Cytomegalovirus immunoglobulins in the prevention and treatment of cytomegalovirus disease. *Rev Infect Dis* **12**(Suppl 7), S839-848.
11. Borucki M J, Spritzler J, Asmuth D M, *et al*. (2004). A phase II, double-masked, randomized, placebo-controlled evaluation of a human monoclonal anti-Cytomegalovirus antibody (MSL-109) in combination with standard therapy versus standard therapy alone in the treatment of AIDS patients with Cytomegalovirus retinitis. *Antiviral Res* **64**, 103-111.
12. Pollard R, Nokta M, Pappas P, *et al*. (1992). A phase I/IIA study of a human monoclonal anti-cytomegalovirus antibody in patients with AIDS. *Antiviral Res* **17**, 111.
13. Lakeman F, Blevins C, Whitley R, *et al*. (1991). In vitro neutralization of cytomegalovirus strain by a human monoclonal antibody, MSL-109 (abstract). *Antiviral Res* **15**, (Suppl. 1), 77.
14. Barbas C F III, Hu D, Dunlop N, *et al*. (1994). *In vitro* evolution of a neutralizing human antibody to human immunodeficiency virus type 1 to enhance affinity and broaden strain cross-reactivity. *Proc Natl Acad Sci* **91**, 3809-3813.

[1]Both Tao DUAN and Xiao-Fang WANG are the first authors.
[2]Correspondence should be addressed to Mi-Fang LIANG. Tel: 86-10-63583153. Fax: 86-10-63572877. E-mail: mifangl@vip.sina.com
Biographical note of the first author: Xiao-Fang WANG, female, born in 1971, assistant professor at Institute for Viral Disease Control and Prevention, China CDC, majoring in Virology.

0895-3988/2008
CN 11-2816/Q

15. Burioni R, Williamson R A, Sanna P P, *et al*. (1994). Recombinant human Fab to glycoprotein D neutralizes infectivity and prevents cell-to-cell transmission of herpes simplex viruses 1 and 2 *in vitro*. *Proc Natl Acad Sci* **91**, 355-359.
16. Sanna P P, Williamson R A, De L A, *et al*. (1995). Directed selection of recombinant human monoclonal antibodies to herpes simplex virus glycoproteins from phage display libraries. *Proc Natl Acad Sci* **92**, 6439-6443.
17. Robinson R F, Nahata M C (2000). Respiratory sgucytial virus (RSV) immune globulin and palivizumab for prevention of RSV infection. *Am J Health Syst Pharm* **57**, 259-264.
18. Goncalvez A P, Men R, Wernly C, *et al*. (2004). Chimpanzee Fab fragments and a derived humanized immunoglobulin G1 antibody that efficiently cross-neutralize dengue type 1 and type 2 viruses. *J Virol* **78**, 12910-12918.
19. Men R, Yamashiro T, Goncalvez A P, *et al*. (2004). Identification of chimpanzee Fab fragments by repertoire cloning and production of a full-length humanized immunoglobulin G1 antibody that is highly efficient for neutralization of dengue type 4 virus. *J Virol* **78**, 4665-4674.
20. Liang M, Mahler M, Koch J, *et al*. (2003). Generation of an HFRS patient-derived neutralizing recombinant antibody to Hantaan virus G1 protein and definition of the neutralizing domain. *J Med Virol* **69**, 99-107.
21. Koch J, Liang M, Queitsch I, *et al*. (2003). Human recombinant neutralizing antibodies against hantaan virus G2 protein. *Virology* **308**, 64-73.
22. Duan T, Liang M, Gu S (2003). Human anti-HCMV neutralizing Fab antibody generated by phage display library. *Chinese Journal of Experimental and Clinical Virology* **17**, 247-250. (In Chinese)
23. Nejatollahi F, Hodgetts S J, Vallely P J, *et al*. (2002). Human recombinant antibodies to human cytomegalovirus glycoproteins gB and gH. *FEMS Immunol Med Microbiol* **15**, 237-244.
24. Takekoshi M, Maeda F, Tachibana H, *et al*. (1998). Human monoclonal anti-HCMV neutralizing antibody from phage display libraries. *J Virol Methods* **74**, 89-98.
25. Williamson R A, Burioni R, Sanna P P, *et al*. (1993). Human monoclonal antibodies against a plethora of viral pathogens from single combinatorial libraries. *Proc Natl Acad Sci* **90**, 4141-4145.
26. Liang M, Dübel S, Li D, *et al*. (2001). Baculovirus expression cassette vectors for rapid production of complete human IgG from phage display selected antibody fragments. *J Immunol Meth* **247**, 119-130.
27. Liang M, Dübel S (2001). Expression of antibody fragments using the Baculovirus system. In: Antibody Engineering, ed: Kontermann, R. and Dübel, S. Springer Verlag, Heidelberg.
28. Britt M J, Mach M (1996). Human cytomegalovirus glycoproteins. *Intervirology* **39**, 401-412.
29. Spaete R R, Gehrz R C, Landini M P (1994). Human cytomegalovirus structural proteins. *J Gen Virol* **75**, 3287-3308.
30. Rasmussen L E, Nelson R M, Kelsall D C, *et al*. (1984). Murine monoclonal antibody to a single protein neutralizes the infectivity of human cytomegalovirus. *Proc Natl Acad Sci* **81**, 876-880.
31. Barbas C F III, Burton, Dennis R (1991). Monoclonal antibodies from combinatorial libraries. Cold Spring Harbor Laboratory Cousre.
32. Zhou G Y, Bernard R (2002). Truncated forms of glycoprotein D of herpes simlex virus 1 capable of blocking apotosis and of low-efficiency entry into cell form a heterodimer dependent on the presence of a cysteine located in the shared transmembrane domains. *J Virol* **76**, 11469-11475.
33. Joassin L, Somze A, Register M (1989). Detection by enzyme-linked immunosorbent assay of specific immunoglobulin G isotypes in primary and established cytomegalovirus infections. *J Clin Microbiol* **27**, 139-144.
34. Vey M, Schafer W, Reis B, *et al*. (1995). Proteolytic processing of human cytomegalovirus glycoprotein B (gpUL55) is mediated by the human endoprotease furin. *Virology* **206**, 746-749.
35. Lopes F B, Colaco S, May J S, *et al*. (2004). Characterization of Murine Gammaherpesvirus 68 Glycoprotein B. *J Virol* **78**, 13370-13375.

(Received December 26, 2007 *Accepted July* 2, 2008*)*