Isolation of Monoclonal Antibodies Against a Chimeric Epitope on gp120/CD4 complex

Introduction

Extensive studies on Human Immunodeficiency Virus (HIV) surface proteins and its corresponding receptors as well as co-receptors continue to be highly-researched topics. The complex nature of the mechanism of the virus leads to difficulties in discovering an effective vaccine. One of the main focuses in developing an effective vaccine targets the mechanism of virus cell entry. HIV uses several methods of cell entry, one of which requires viral surface envelope glycoprotein, gp120, to bind to CD4. Receptor binding is followed by membrane fusion of the viral membrane to the host cell membrane, allowing viral genome entry to the cell. It has been shown that binding of gp120 to CD4 results in a conformational change in the structure of gp120, which exposes conserved regions of gp120 that are antigenic and are involved in chemokine receptor binding, which aids in virus cell entry[1]. This has lead to studies aimed at discovering the nature of these antigenic regions as well as isolation of monoclonal antibodies directed against them. One of the more commonly known monoclonal antibody against a CD4 induced conformational epitope on gp120 is 17b[2] and a x-ray crystal structure of gp120/CD4 complex bound to 17b can seen in figure 1[3]. Moreover, monoclonal antibody recognizing a linear protein of gp120 fused to D1D2 domain of CD4 by an amino acid linker has been shown to exist, although non-neutralizing[4]. Furthermore, anti-CD4 monoclonal antibody isolated from human CD4 transgenic mice has been discussed to be a possible explanation for CD4 autoimmune disease[5]. All of these studies have shown that there are conformational epitopes on gp120 and CD4 when they are bound, however, there has not been any report of a monoclonal antibody directed against native gp120/CD4 heterodimer that does not bind to gp120 and CD4 alone.

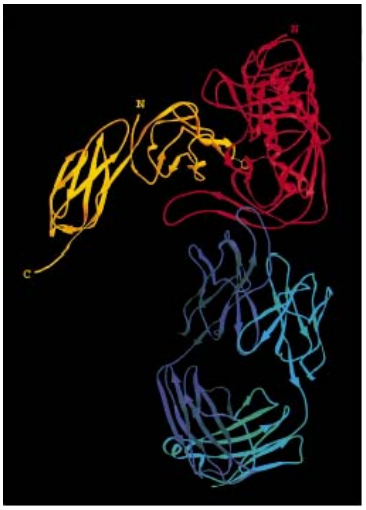

Figure 1. X-ray crystal structure of gp120(red)/CD4(yellow) bound to 17b(purple+blue)[3]

Proposal

Although neutralizing monoclonal antibodies against CD4 induced conformational epitope on gp120 have been isolated as well as anti-CD4 antibodies from human CD4 transgenic mice immunized with gp120/CD4 complex, none have been effective enough to be considered possible candidates for an HIV vaccine. An unexplored possibility on this topic is the existence of a chimeric eptiope on gp120/CD4 complex that includes both a region of gp120 and a region of CD4. Therefore, we hypothesize that a possible novel conformational chimeric epitope may exists on native gp120/CD4 heterodimer that is antigenic and that will lead to the generation of a monoclonal antibody that recognizes this complex epitope, but would not recognize either gp120 or CD4 alone. Moreover, we predict that such an antibody will participate in either neutralizing activity, induction of ADCC, or both.

To test this hypothesis, human CD4 transgenic mice will be used as a model for development of antibodies against gp120/CD4 heterodimer in its native form. Immunization of the mice will be done using gp120 and soluble CD4 conjugate with use of an adjuvant to boost antibody production. After infection, serum from the mice will be screened by enzyme linked immunosorbent assay (ELISA) for reactivity against gp120/CD4 conjugate; in order to screen for sera that only react with the conjugate (and not with either gp120 or CD4 alone), sera will be pre-absorbed with gp120 and CD4. Mice that contain anti-conjugate antibodies will be used to make monoclonal antibodies and those antibodies will be tested against gp120, CD4, and the conjugate in Western blot analysis. Monoclonal antibodies will then be tested for their anti-viral function in neutralizing and ADCC assay. Immunoprecipitation will also be performed to precipitate the chimeric eptiope, which can later be used to visualize the complex. In the long term, we will attempt to crystallize monoclonal antibody-gp120-CD4 complexes for x-ray crystallography in order to solve their tertiary structure.

The significance of these series of experiments would be that conjugate antigens that elicit antibody that has either neutralizing ability or one that will induce ADCC activity can be considered as a possible candidate for vaccine development. Furthermore, a x-ray crystal structure of the gp120/CD4 conjugate bound to this mAb can give us more insight into the structure of the complex and will contribute to the ongoing research on the mechanism of HIV.

My responsibility

My responsibility in this whole project would be to first prepare the gp120/sCD4 conjugate that will be injected into mice. Secondly, I will be performing the ELISA's to screen for antibodies and western blot analysis to confirm the results. I will also participate in isolating monoclonal antibodies of interest and, eventually, crystallizing immune complexes.

Timeline

December-January: Immunization of mice and obtaining serum
February: ELISA to screen for antibodies that recognize gp120/CD4 conjugate
March-May: Isolating monoclonal antibodies

Itemized Budget

ELISA reagents and consumable supplies -$500
Western blot reagents-$350
Chromium -$250
ADCC consumable supplies(pipette tips, flasks)-$200
Monoclonal antibody isolation reagents-$1000

Total amount: $2800

Reference

1. Dimitrov, D.S., Broder, C.C. 1997. *HIV and Membrane Receptors*. Chapman & Hall, NY, pp.70.
2. Thali, M., Moore, J.P., et al. 1993. Characterization of conserved human immunodeficiency virus type I gp120 neutralization epitopes exposed upon gp120-CD4 binding. J. Virol. 67: 3978-88.
3. Kwong, P.D., Wyatt, R., et al. 1998. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature 393: 648-59.
4. He, Y., Agostino, P., et al. 2003. Analysis of the immunogenic properties of a single-chain polypeptide analogue of the HIV-1 gp120-CD4 complex in transgenic mice that produce human immunoglobulins. Vaccine 21: 4421-4429.
5. Denisova, G., Lideman, L., et al. 2003. Characterization of new monoclonal antibodies that discriminate between soluble and membrane CD4 and compete with human anti-CD4 autoimmune sera. Mol. Immunol. 40: 231-239.