The development of an HIV vaccine candidate using a phage display library
by Jon Morrell Jacobs

A dissertation submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Biochemistry
Montana State University

Abstract:
The development of an effective HIV vaccine is of immense importance in the long term control of HIV infection worldwide. A successful vaccine would help prevent the establishment of a chronic HIV infection which has been shown to be difficult if not impossible to eradicate from an individual. A number of neutralizing monoclonal antibodies (mAbs) are directed against the HIV glycoprotein gp120 and bind to highly conserved residues. Determining the nature of such binding epitopes would be useful in developing a vaccine candidate that would direct an immune response against these conserved residues of gp120. By using the technique of peptide phage display mapping, mAbs were used to screen a random peptide library to identify peptides which bind specifically to the mAb. A nonapeptide library was screened with six antibodies, and for three of these mAbs, sequences were determined that bound specifically to their corresponding mAbs. Synthetic peptides corresponding to two of these sequences were able to compete with gp120 for binding to the antibodies. These synthetic peptides were then coupled to carrier proteins and used to immunize mice. Sera from mice immunized with two of the peptide conjugates bound to gp120, indicating that this approach has potential for the design of novel HIV vaccine antigens. An additional consensus peptide sequence, QSYP, appeared as an artifact during the screening of one of the monoclonal antibodies. Phage bearing this peptide sequence were also selected by three other laboratories which screened the same phage library against three unrelated mAb preparations. It was determined that phage displaying the QSYP sequence were not bound by the mAb of interest, but rather bound to bovine IgG contaminating the mAb samples which was derived from the fetal calf serum present in the hybridoma growth media. Implications of this finding for interpretation of phage library screening results, and possible uses of the QSYP consensus peptide are discussed.

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APPROVAL

of a dissertation submitted by

Jon Morrell Jacobs

This dissertation has been read by each member of the dissertation committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Signature Jon M. Jones

Date Sept 16, 2002

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ABSTRACT

The development of an effective HIV vaccine is of immense importance in the long term control of HIV infection worldwide. A successful vaccine would help prevent the establishment of a chronic HIV infection which has been shown to be difficult if not impossible to eradicate from an individual. A number of neutralizing monoclonal antibodies (mAbs) are directed against the HIV glycoprotein gp120 and bind to highly conserved residues. Determining the nature of such binding epitopes would be useful in developing a vaccine candidate that would direct an immune response against these conserved residues of gp120. By using the technique of peptide phage display mapping, mAbs were used to screen a random peptide library to identify peptides which bind specifically to the mAb. A nonapeptide library was screened with six antibodies, and for three of these mAbs, sequences were determined that bound specifically to their corresponding mAbs. Synthetic peptides corresponding to two of these sequences were able to compete with gp120 for binding to the antibodies. These synthetic peptides were then coupled to carrier proteins and used to immunize mice. Sera from mice immunized with two of the peptide conjugates bound to gp120, indicating that this approach has potential for the design of novel HIV vaccine antigens. An additional consensus peptide sequence, QSYP, appeared as an artifact during the screening of one of the monoclonal antibodies. Phage bearing this peptide sequence were also selected by three other laboratories which screened the same phage library against three unrelated mAb preparations. It was determined that phage displaying the QSYP sequence were not bound by the mAb of interest, but rather bound to bovine IgG contaminating the mAb samples which was derived from the fetal calf serum present in the hybridoma growth media. Implications of this finding for interpretation of phage library screening results, and possible uses of the QSYP consensus peptide are discussed.

CHAPTER ONE

INTRODUCTION AND REVIEW OF THE STRUCTURE AND FUNCTION OF HIV

The History of AIDS and the HIV virus

Acquired Immune Deficiency Syndrome (AIDS) is the syndrome caused by the destructive effects of the Human Immunodeficiency Virus (HIV), which ranks as one of the most important infectious diseases in the history of mankind. It is commonly believed that HIV-1 subclass M, the most widespread form of the virus, initiated infection into the human population by a cross-species transmission event, in which a human was infected from a chimpanzee, the most likely source being subspecies *P. t. troglodytes*, carrying the Simian Immunodeficiency Virus (SIV) SIVcpz (reviewed by Hahn et al., 2000). The geography points to somewhere in west equatorial Africa, and the date, due to molecular mutational clock analysis, points to a last common ancestor around the early 1930's (1915-1945), with the actual crossover event mostly likely occurring sometime before this date (Korber et al., 2000). From that point on, HIV/AIDS was essentially undetected until 1981, when doctors in California, and then later in New York, began to see patients with an increase in opportunistic infections, mostly a rare form of pneumonia, *Pneumocystis carinii*, as well as other viruses, fungi, and skin cancer, most often seen in

patients having immune deficiencies (CDC, 1981; reviewed by Grmek, 1990). These symptoms would persist in the individual until their death, which would ultimately be caused by opportunistic infections allowed to thrive, due to the patient's severe immunosuppression. Cases during this time primarily came from the male homosexual population in the U.S. but once the syndrome was reported, cases were diagnosed elsewhere in the world. In 1982 the term AIDS was coined to describe the immune deficient condition common in all patients but it was not until 1983 that the pathogenetic factor was discovered to be a previously undetermined retrovirus (Barre-Sinoussi et al., 1983; Gallo et al., 1984). After a race to isolate and characterize the previously unknown virus and a spirited debate over who was first to discover it, the virus was finally given the name Human Immunodeficiency Virus or HIV in 1986 (Grmek, 1990; Levy, 1998). HIV has since spread into the heterosexual population and has become the world-wide epidemic that we know today.

At the end of 2001, there were approximately 40 million people world-wide that were living infected with HIV, many of them unaware of their infection (WHO, 2001). Approximately 5 million people were newly infected in 2001 and 3 million deaths were associated with AIDS related diseases. In North America approximately 940,000 people now live with HIV with about 45,000 new infections per year (WHO, 2001). Out of the 40 million individuals infected with HIV, a vast majority of those cases (28 million) live in Sub-Saharan Africa. HIV/AIDS is now the leading cause of death is this area and the fourth leading cause of death worldwide. In Sub-Saharan Africa, the life expectancy is now only 47 years, compared to approximately 62 years were it not for HIV/AIDS. In the

worst-affected countries, life expectancy is even less than 40 years, due to the widespread impact of HIV/AIDS. The epidemic has risen faster in Eastern Europe and Central Asia than in any other region in 2001. In the Russian Federation, reported cases of HIV/AIDS have doubled annually since 1998, with more than 130,000 total cases now reported. Such exponential growth is also seen in most of the Central Asian republics (Kyrgyzstan, Kazakhstan, Tajikistan, and Uzbekistan) as well as parts of Central Europe (Ukraine and Estonia). These numbers are on a smaller scale compared to other areas involved in the epidemic, but considering the exponential increase in cases reported, the widespread intravenous drug use in this area (in the Ukraine, three-quarters of the current HIV infections are caused by intravenous drug use), the large population, and the disintegration of public health services, it is in Central Asia that the potential for an explosion in HIV/AIDS cases is the greatest (WHO, 2001).

HIV is divided into two main branches, HIV-1 and HIV-2, which differ in the SIV strain from which they originated (Levy,1998). The HIV-2 epidemic has been comparatively small, and has been essentially contained within Africa. The nucleotide sequence of HIV-2 is actually more closely related to SIV than to HIV-1 (Hahn et al., 2000; Levy, 1998). HIV-1 itself is divided into three groups: M, O, and N (Main, Outlier, and Non-outlier respectively), shown in Fig.1.1. HIV-1 groups N and O have also been mostly contained within Africa, with group N almost exclusively found in Cameroon. It is HIV-1 , subclass M, that is responsible for the world-wide epidemic that is seen today. Each branch, HIV-1 and HIV-2, as well as each group, M, O, and N, are believed to have originated from separate species cross-over events originating from various strains of SIV

found in either sooty mangabeys or chimpanzees. The primate natural host of SIV is not affected by infection of the virus. This is seen with all SIV primate natural hosts in regard to their respective strains of SIV, even though some natural hosts carry viral loads high enough to cause AIDS in humans (Kurth and Norley 1996). This suggests that factors other than the virus itself are involved in disease progression and it is believed that the host species response to the viral infection and genetic host factors play an important role in containment of the virus in the host (Dalgleish et al., 1999).

Fig. 1.1 shows evolution of SIV within its natural host in black, with each black/red color change representing a zoonotic transmission event. These transmission events are believed to be the result of close contacts between humans and SIV infected primates in many regions of Africa. The hunting and consumption of primates by humans is very common in these regions and it is believed that this process would provide numerous avenues for a transmission event (Hahn et al., 2000). Red lines represent evolution of HIV-1 or HIV-2 in humans. Group M is further divided into 11 clades, denoted A through K, that represent the diverse genetic sequences of HIV-1 seen throughout the world. It is clade B that is primarily found in the U.S. and within this clade, strains IIIB, MN, and SF2 are some of the more commonly used virus isolates utilized in research.

HIV Structure and Function

HIV-1 is a member of one of the five major primate lineages of the lentivirus family of animal retroviruses. Lentiviruses are capable of long-term latent infections, as

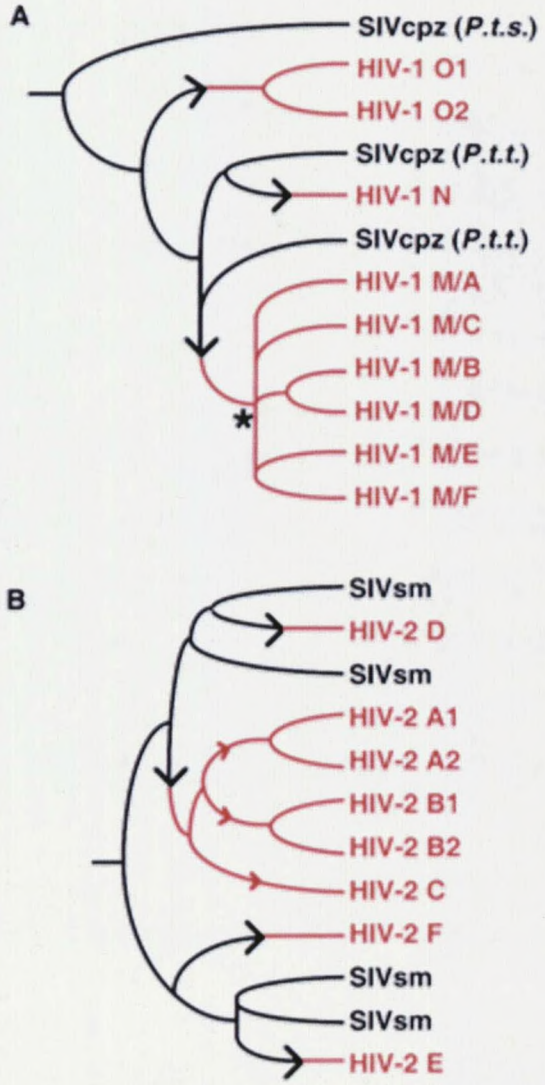

Figure 1.1 **Schematic trees of HIV-1 and HIV-2 showing the multiple independent zoonotic transmissions of SIVcpz and SIVsm to humans.** Branches in black show evolution of SIV within its natural host with black arrows indicating points of cross-species transmission into human hosts (red) and the subsequent evolution of HIV. (**A**) SIVcpz and the three main groups of HIV-1: O, N, and M. (**B**) SIVsm and HIV-2, which is comprised of six subtypes, A through F. (Reprinted with permission from Hahn et al., copyright 2000 American Association for the Advancement of Science.)

well as short term cytopathic effects, which produce slow-progressing, yet fatal diseases.

HIV particles consist of two identical strands of RNA, approx. 9.2 kb long, contained within a cone-shaped core composed of the p24 gag capsid protein and the p6 gag nucleocapsid protein (reviewed by Levy, 1998). Also contained inside the core and closely associated with the strands of RNA are the enzymes reverse transcriptase (RT), integrase, and protease. The core is surrounded by a phospholipid bilayer which is supported by a viral matrix composed of the p17 gag protein inside of the bilayer (Fig.1.2). The phospholipid bilayer envelope of the virus contains up to 72 trimers of the envelope glycoprotein gp120 non-covalently attached to the transmembrane glycoprotein gp41 in the mature virus (Levy, 1998). The RNA of HIV (Fig.1.3) has the gag, pol, and env genes characteristic of retroviruses, as well as a range of unique accessory genes labeled tat, rev, nef, vif, vpu, and vpr. The gag gene encodes for a 55 Kd protein that is cleaved to form the p24, p17 and p15 structural proteins of the virus. The pol gene products include RT, protease, and integrase enzymes. The env gene codes for the surface glycoprotein gp160, which is later cleaved by a host cell protease into gp120 and gp41 before assembly of the virion.

The life cycle of HIV begins when bodily fluids such as semen or blood from an infected individual come in contact with naive cells from another individual. The initial contact between virus particle and cell begins with the recognition by the viral gp120 glycoprotein of the CD4 molecule expressed on either T-cells, macrophages, or dendritic cells, as shown in Fig.1.2 (Dalgleish et al., 1986; Sattentau, 1998). The affinity between monomeric gp120 and CD4 is strong, between 1 and 10nM, but the affinity of soluble

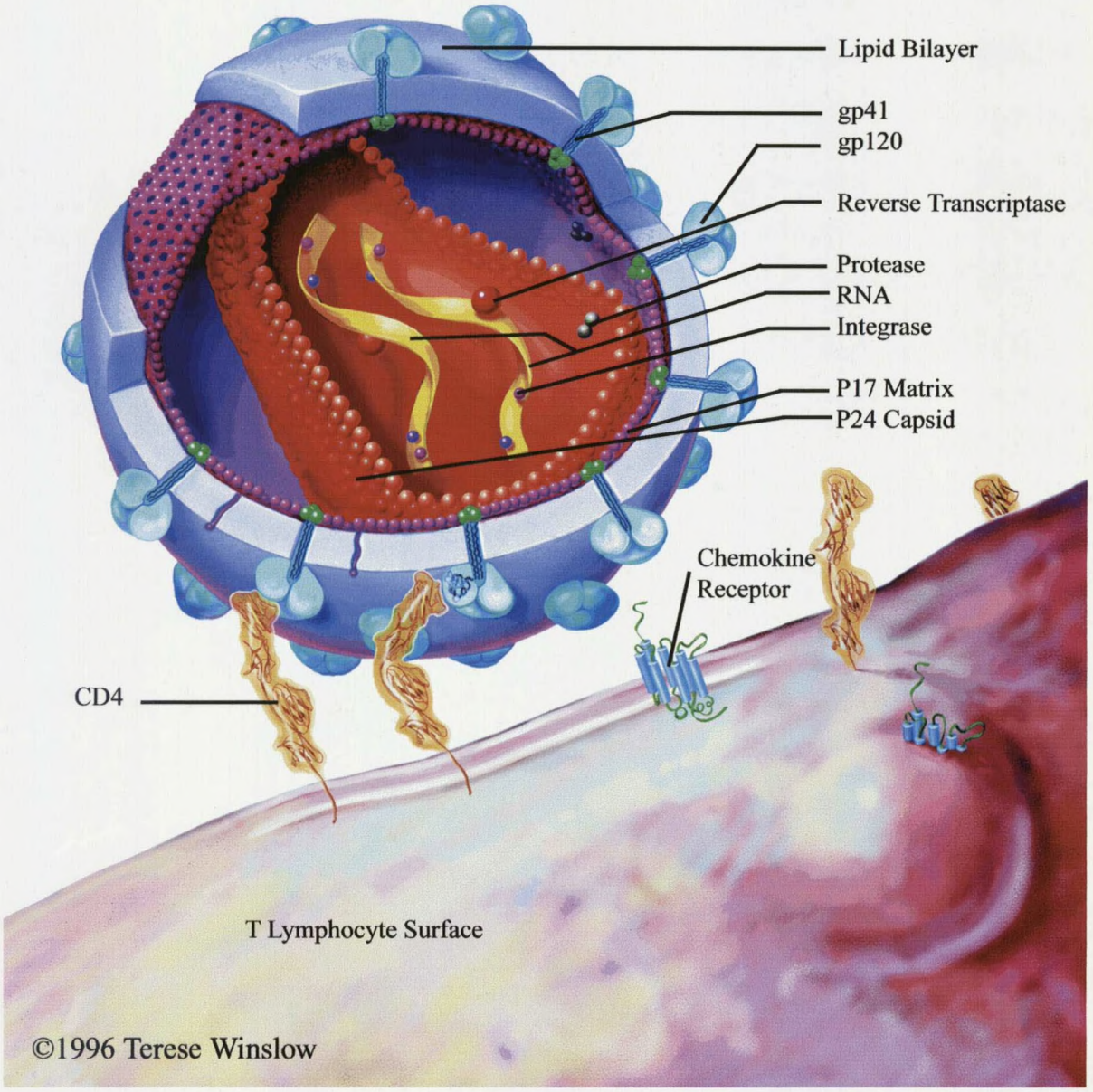

Fig.1.2 **Picture of an HIV virion.** (Used with permission from Teresa Winslow, Medical Illustration ©1996 Teresa Winslow)