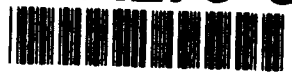

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A series of experiments have begun to examine the growth characteristics of HIV-1 infected T cells in vitro to assist us in identifying factors that may correspond to highly virulent strains of HIV-1 in vivo. In addition, certain in vitro growth characteristics may also correspond to less virulent forms of HIV-1.

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FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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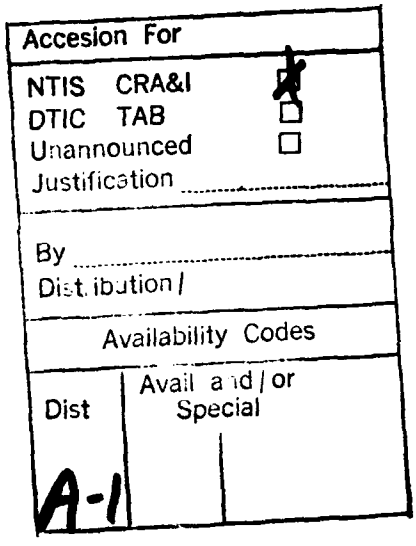

1993 Final Report

1993

A. *In vitro* growth characteristics of HIV-1 infected T cells.

1. Fluorescent staining.

We have begun a series of experiments to examine the growth characteristics of HIV-1 infected T cells *in vitro* to assist us in identifying factors that may correspond to highly virulent strains of HIV-1 *in vivo*. In addition, certain *in vitro* growth characteristics may also correspond to less virulent forms of HIV-1. For example, studies by others have suggested that HIV-1 isolates which readily form syncytia *in vitro* may be associated with a more rapid rate of disease progression *in vivo*. Using fluorescent intracellular vital stains, we have expanded upon these studies by examining SupT1 cells for changes in cellular makeup following infection with HIV-1. At present we are staining cells with probes for F-actin, microtubules, p24, and DNA. Our initial studies have indicated that 48 hrs after infection with HIV-1, syncytia no longer express CD4. Using FITC conjugated Leu 3a, we have observed that single cells stain intensely for CD4, while syncytia appear to be CD4 negative. We also observed that syncytia organize their nuclei into a blastula-like formation. In addition, we have observed the extension of pseudopods from the plasma membrane which extend several cell diameters in distance (Sylwester, et. al., 1993, *J. Cell. Sci.*, in press). We are currently examining what role these pseudopods may play in cell recruitment and HIV-1 transmission (Soll and Kennedy, submitted for publication).

2. Examination of cell interactions over prolonged periods of time by videotape.

The physical interaction of cells is difficult to examine visually, since these interactions sometimes extend over long periods of time. Thus, cell movements and interactions that last several hours in duration may be misinterpreted if examined visually for only several minutes. We have demonstrated that these complex interactions are best interpreted if videotaped and then examined during high speed playback. Our initial experiments utilized a video camera mounted to an inverted microscope with a hot air portable incubator attached. A flask containing HIV-1 infected SupT1 cells was then videotaped utilizing a video cassette recorder (VCR). These initial experiments examined cell interactions over periods of up to 30 minutes. We observed, in flasks containing HIV-1 infected SupT1 cells, the directed movement of single cells towards syncytia (Sylwester, et. al., 1993, *J. Cell. Sci.*, in press). These results suggest that chemotactic factors may be released from syncytia that result in the recruitment of single cells. This mechanism of cell recruitment by syncytia may play a role in the transmission of HIV-1 to uninfected cells *in vitro* and possibly *in vivo*.

Following these initial studies, we decided to examine HIV-1 infected cell cultures continuously over a period of several days. This was accomplished by placing the inverted microscope inside an incubator. A flask containing HIV-1 infected SupT1 cells could then be observed under conditions of constant temperature and $C0_2$ concentration. The video camera and VCR were again attached as described above. Analysis of videotapes spanning 72 continuous hours demonstrated a wide variety of cell interactions. These included the directed movement of syncytia towards either single cells or other syncytia. Syncytial movement was apparently the result of pseudopod extensions from the

forward area of the syncytia. These pseudopods appeared to grasp the flask and pull the syncytia in a directed movement. In addition, we observed that individual syncytia could migrate towards each other and eventually fuse, forming a single larger syncytial mass. The death of these syncytia was also observed, resulting from the rupture of the plasma membrane and the spilling of cytoplasmic contents. We are presently analyzing a number of the cell motility parameters both single cells and syncytia utilizing these videotapes and a 2-dimensional dynamic image analysis system (2-D-DIAS).

B. Examination of antibody fine specificity to gp160 epitopes in sera from infected individuals from Argentina.

The recent identification of at least five families of HIV-1 throughout the world has increased the need to characterize viral isolates from a variety of geographic regions. These studies are necessary to better understand which formulations of potential HIV-1 vaccines and therapeutics may have the greatest chance of being effective on a worldwide basis. Serologic studies of HIV-1 infected individuals have frequently been conducted in the United States and Europe while other areas have received less attention. For example, Central and South American countries have had few published studies concerning HIV-1 infections. We examined sera from HIV-1 infected individuals from Argentina (n=63) for antibody reactivity to a panel of V3 based synthetic peptides. Our results indicate that the HIV-1 isolates MN, SF2, and NY5 (or phenotypically similar ones) are commonly found in Argentina. HIV-1 isolates LAI and RF appear to be less frequently found among the Argentinean population. In addition, we observed that a lower percentage of sera from infected individuals from Argentina contained antibodies reactive with the gp160 epitope 600-611 than sera from the United States (67% and 94%, respectively). To our knowledge, this represents the first detailed study involving the prevalence of particular HIV-1 isolates in South America. Determining the prevalence of HIV-1 isolates is particularly important for the design of potential HIV-1 vaccines and possible immunotherapeutics.

C. Longitudinal analysis of the humoral immune response to HIV-1 gp160.

Antibody responses to conserved HIV-1 IIIB gp160 epitopes was longitudinally examined in HIV-1 infected individuals. Twelve hundred individuals were evaluated and sequential sera from 25 rapidly progressing (RP) and 30 nonprogressing (NP) patients collected over an average of 4 years were examined. Initial sera from the RP group contained greater reactivity to a gp120 epitope defined by peptide 503-528 than the NP counterparts ($p < .001$). Reactivity declined with sequential serum for the RP group, paralleling disease progression. Conversely, antibody recognition to this site developed in 23% of the NP group with time. However, 60% of the NP group never developed a response to an epitope. This suggests sequential examination of antibody response to an epitope within the gp120 carboxyl-terminus may have prognostic significance. No association between antibodies directed against the gp160 epitopes and *in vitro* neutralizing activity utilizing the IIIB laboratory isolate was observed (Wong, et. al., 1993, *J. Infect. Dis.*, in press).

1992

A. Correlation between sera containing antibodies which recognize HIV-1 V3 based peptides in infected humans and neutralizing activity *in vitro*.

Since the V3 region of gp120 has been designated the principal neutralizing determinant (PND) of HIV-1, determining the importance of antibodies to this epitope in human sera is critical. This is particularly true, since antibody recognition of this epitope is frequently measured following immunization with potential HIV-1 vaccines. We examined sera from HIV-1 infected individuals from the United States (n=37) and Tanzania (n=74) for antibody reactivity to a panel of V3-based synthetic peptides. We observed that the majority of sera from both countries were reactive against the MN based peptide (84% and 87%, respectively). This data suggests that the MN isolate, or a phenotypically similar one, is commonly found in both countries. When sera were examined for neutralizing activity, a smaller percentage of sera from both countries neutralized the MN isolate *in vitro* (16% and 47%, respectively). In addition, we were unable to establish a correlation between anti-V3 antibody titers and neutralization titers. We were therefore unable to establish a correlation between the presence of anti-V3 antibodies in human sera and neutralizing activity *in vitro* (Warren, et. al., *J. Virol.* 66:5210, 1992). These results suggest that neutralizing epitopes outside of the PND appear to be important in the neutralization of HIV-1 *in vitro*.

B. Examination of anti-V3 antibodies in various mouse strains receiving multiple immunizations with recombinant gp160.

We examined the immunogenicity of a bacculovirus produced gp160 in a variety of mouse strains. We were particularly interested in whether group specific neutralizing antibodies could be produced in these animals following repeated immunizations. Sera from the rgp160 immunized mice (BALB/c, A/J, C57BL, CBA, DBA, and SJL) were initially screened for antibody reactivity to a panel of V3-based synthetic peptides. Of the six strains of mice examined, only the A/J mice produced antibodies which cross-reacted with each of the V3-based synthetic peptides. Sera from the other five strains contained antibodies which remained type specific for the V3 epitope (Wolf, et. al., *Mol. Immunol.* 29:989, 1992). These sera were then examined for their ability to neutralize HIV-1 IIIB and MN *in vitro*. We were unable to detect neutralizing activity of any sera when measured by reduction in p24 levels (Warren, et. al., *Exp. Clin. Immunogenet.* 26:630, 1993). These results suggest that a bacculovirus produced rgp160 may have limited ability to induce group specific neutralizing antibodies in animals following repeated immunization.

C. Comparison of the humoral immune response to HIV-1 gp160 epitopes in sera from infected individuals from the United States and Tanzania.

Regional isolates of HIV-1 have been reported throughout the world. Due to the amino acid sequence variability in the envelope glycoprotein, these regional isolates may potentially differ in their levels of virulence *in vivo*. We compared sera from HIV-1 infected individuals from Tanzania and the United States for anti-gp160 antibody fine specificity. Sera from each country was reacted against a panel of gp160 based synthetic peptides. Several

differences in antibody reactivity was observed between sera from these two geographically distinct populations. The greatest difference was observed in antibody reactivity to the 600-611 epitope of gp160. While greater than 90% of the sera from the United States were reactive with this epitope, fewer than 50% of the Tanzanian sera were reactive (Nkya, et. al., *J. Med. Virol.* 37:61, 1992). Significant differences in seroreactivity were also observed against two other gp160 epitopes. These observations suggest that regional isolates of HIV-1 may exist in Tanzania which differ from those commonly found in the United States. This may be particularly important since potential AIDS vaccines which are comprised of common HIV-1 isolates from the United States may not be as effective against African isolates (Warren, et. al., *J. Clin. Micro.* 30:126, 1992).

D. Isolation of HIV-1 from infected individuals from Tanzania.

The isolation and characterization of HIV-1 from infected individuals in Africa could be important for better understanding the epidemiologic differences observed between HIV infections in Africa and the United States. We have isolated HIV-1 from the peripheral blood lymphocytes of two infected individuals from Tanzania (designated NKYA-1 and NKYA-2). Sera from these two individuals were subsequently screen by ELISA against a panel of gp160 based synthetic peptides. We observed that sera from NKYA-1 was unreactive against V3 peptides corresponding to HIV-1 isolates MN and IIIB. In addition, this sera was also unreactive against a peptide corresponding to amino acids 600-611, an epitope which is highly conserved among United States and European isolates. In contrast, sera from NKYA-2 contained antibodies reactive against peptides corresponding to the V3 region of MN but not IIIB. This sera was also reactive against the 600-611 peptide. These two isolates also had different *in vitro* growth characteristics. We observed that NKYA-1 isolate formed few syncytia and was not cytotoxic to SupT1 cells. In contrast, NKYA-2 formed many large syncytia and lead to the death of SupT1 cells in culture. These HIV-1 isolates from Tanzania will be useful reagents in testing sera for neutralizing activity *in vitro*.

1991

A. Fine specificity of the humoral immune response to gp160 in HIV-1 infected humans.

Sera from HIV-1 infected USAF personnel was examined for antibody reactivity to a panel of gp160 based synthetic peptides. These individuals were placed into three groups according to their levels of CD4+ cells. The three groups consisted of individuals with: (i) greater than 400 CD4+ cells; (ii) between 200-400 CD4+ cells; and (iii) less than 200 CD4+ cells/mm^3. We observed that antibody reactivity to several weakly immunogenic epitopes (i.e. a.a. 425-448 and 846-860) declined in the groups as CD4 levels declined. We also observed that antibody reactivity to the 503-528 epitope declined in the groups as CD4 levels declined. This loss of antibody reactivity to gp160 epitopes suggests that the humoral immune response to gp160 narrows with disease progression (Warren, et. al., *J. Clin. Immunol.*, 11:10, 1991). The loss of antibodies to gp160 epitopes may potentially lead to the loss of neutralizing antibodies and may contribute to an increased rate of disease progression.

B. Immunogenicity studies utilizing gp160 synthetic peptides.

In collaborative studies, we have also examined the effects of various adjuvants on inducing antibody responses to gp160 based synthetic peptides. We examined the ability of alumina hydroxide (alum) and various derivatives of stearyl tyrosine to induce antibodies reactive to peptide 503-535 in experimental animals. Previous studies have indicated that both alum and stearyl tyrosine can induce comparable anti-peptide and ant-gp160 titers in mice, rabbits, and baboons. However, groups of mice and baboons immunized with stearyl tyrosine also produced neutralizing antibodies, whereas alum did not (Nixon, et. al., *J. Virol.* 5:141, 1992). Previous studies have also indicated that peptide 503-535 can induce neutralizing antibodies when used to immunize experimental animals. The neutralizing antibodies induced by peptide 503-535/stearyl tyrosine were assessed by an *in vitro* cell cytotoxicity assay using the IIIB isolate. The observation that stearyl tyrosine can alter the quality of the antibody response when compared to alum (i.e. neutralizing versus non-neutralizing) suggests that this adjuvant may be useful in studies involving immune responses to HIV-1 gp160.

C. Generation and characterization of anti-idiotypic antibodies reactive with an anti-503-535 Ab1.

Three monoclonal anti-idiotypic antibody (anti-Id, Ab2) preparations specific for a rabbit anti-503-535 peptide have been generated. This peptide comprises the carboxyl terminus of gp120 along with the amino terminus of gp41. Several studies have indicated that antibodies reactive with this peptide possess HIV-1 neutralizing activity *in vitro*. These Ab2 preparations each appear to recognize an interspecies cross-reactive Id expressed on anti-503-535 antibodies. This interspecies Id was observed to be antigen combining site related and was expressed on Ab1 from rabbits, mice, and baboons. Immunization of rabbits with the three murine monoclonal Ab2 preparations induced Ab3 responses which recognized HIV-1 gp120 (Zaghouani, et. al., *Proc. Natl. Acad. Sci.* USA, 88:5645, 1991). In addition, these Ab3 responses have been demonstrated to possess HIV-1 neutralizing activity *in vitro*.

D. Molecular basis for monoclonal anti-Id.

MC1 is a murine monoclonal anti-Id which is specific for a rabbit antibody which recognizes a peptide based on HIV-1 gp160 amino acids 735-752. We have previously identified the 735-752 epitope as one which is recognized by neutralizing antibodies. The cDNA which encodes for the V region of MC1 was cloned and sequenced. The V regional heavy chain (V_H) sequence was found to share homology with a group of mouse monoclonal antibodies specific for the 2-phenyl-oxazalone hapten. This group of antibodies expressed a V_H region encoded by a gene designated V_HOx-1 which is a member of the murine V_HQ52 gene family. In addition, the D_H and J_H regions appear to be encoded by DSP2.8 and J_{H2} gene segments, respectively. The V region kappa chain (V_k) of MC1 is encoded by the V_{k21} gene family and the J_k region is derived from the J_{k4} germline gene family (Lohman, et. al., *Gene* 105:283, 1991). This data represents our preliminary characterization of the molecular basis on murine monoclonal anti-Id. Since no primary amino acid sequence homology exists between the V region sequence of MC1 and peptide 735-752, the mechanism of how this non-internal image anti-Id induced anti-gp41 Ab3 responses specific for peptide 735-752 in mice remains to be determined. We propose to sequence

additional murine monoclonal anti-Id V regions as they become available and assess whether non-internal image versus internal image utilize similar or different V region genes. In addition, we will determine whether V region gene usage by murine monoclonal anti-Id in the HIV-1 gp160 system is biased or represents a random event reflecting V region gene frequencies. The biased utilization of V region genes may be associated with proximity of V genes to the constant region genes or the possibility that genetic mechanisms, such as gene conversion, select particular V regions for association with a given population of anti-Id subclass.

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