EXHIBIT 1

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INTERNAL MEMO

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Memo #: SMT533

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Date: July 18, 1985

CONFIDENTIAL

From: J. Sninsky & S. Kwok

Subject: Detection of AIDS Associated Virus(es) as a Potential Target

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We propose to initiate a project involving the direct detection of AIDS associated viral DNA in peripheral blood cells using polymerase chain reaction (PCR) amplified DNA. Diagnostic tests to identify sera with antibodies to the AIDS associated virus(es) are currently being used in blood banks to screen out potentially contaminated blood. If the similarities of the AIDS associated viruses and lentiviruses, noted below, extend to the ability of the virus(es) to reside as a DNA copy either integrated in chromosomal DNA or as a proviral episome without producing significant quantities of virus particles, a direct immunological approach would by definition prove unsuccessful. A DNA probe approach would potentially have the ability to identify those individuals who were persistently infected but were not producing virus or individuals who were antibody negative but culture positive. Due to the complexity of DNA probe based tests (especially PCR), the diagnostic assay would be a research test and possibly a confirmatory for the antibody test and/or culturing. In addition, an assay that would have the ability to detect infected cells without having to culture virus would be exceedingly helpful in following patients using various therapeutic regimens to resolve the infection.

The acquired immune deficiency syndrome (AIDS) is a transmissable disorder of the cellular immune system resulting in frequently fatal opportunistic infections or neoplasms. In addition, AIDS is frequently complicated by central nervous system dysfunction. The groups at high risk for AIDS include homosexual or bisexual men and their partners, hemophiliacs, Haitians, central Africans, and intravenous drug abusers. In addition, children of high risk mothers and recipients of random blood products have developed AIDS. The aetiologic agent(s) for this disease has been identified as a human retrovirus and designated human T cell leukemia virus III (HTLV III), lymphadenopathy associated virus (LAV) or (LAVA), and AIDS associated virus (ARV-2). The isolates from the various laboratories represent identical or closely related viruses by numerous criteria (i.e., morphology, immunological cross-reactivities of envelope and nucleocapsid proteins, nucleotide sequence, and entry into helper T cells using the T4 antigen). A simian virus isolated from chimpanzees and macques suffering from symtoms indistinquishable from AIDS in humans is also closely related by these same criteria.

Perhaps one of the more intriguing observations about the viruses associated with the

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AIDS syndrome is their resemblance to the mature virion of the subfamily Lentivirinae. Members of this pathogenic but non-oncogenic viral group include visna virus, maedi virus, and equine infectious anemia virus (EIAV). The similarities between the AIDS associated viruses and lentiviruses include:

(1) VIRION MORPHOLOGY

Electron microscopy of thin sections of virus producing cells show three aspects that are strikingly similar with lentiviruses; (i) immature particles budding at the cell surface with a dense crescent in close contact with the plasma membranes, (ii) immature particles released from the cell surface with a ring- or crescent-like immature core, and (iii) mature particles with a small eccentric core.

(2) IMMUNOLOGICAL CROSS-REACTIVITY

The core protein of one of the AIDS viruses isolated, LAV, (and presumably by the others) can be precipitated with sera from horses infected with EIAV.

(3) NUCLEOTIDE SEQUENCE

Results obtained by molecular hybridization and heteroduplex analysis indicate that a greater extent of nucleotide sequence homology exists between the AIDS viruses and visna (a member of the Lentivirinae) than any other virus (e.g., type B; murine mammary tumor virus, MMTV or type C; Moloney murine leukemia virus, human T-cell lymphotropic virus type I and II, and Rous sarcoma virus). The homology observed under conditions of low stringency spanned the entire genome, but was strongest in the gag/pol region.

(4) BRAIN LOCALIZATION

AIDS associated viral DNA was detected in the brains of individuals with AIDS and unexplained debilitating dementia or encephalopathy. The relative abundance of AIDS associated viral DNA in the brain was equal to, and sometimes greater than that found in lymphoid tissues of infected individuals. DNA homologous to the AIDS associated virus has frequently not been detected by Southern hybridization (thought to have a sensitivity capable of detecting one viral DNA molecule for every ten cells) in DNA isolated from spleen, lymph node, liver, and peripheral blood cells. In addition, it has been noted that the relative abundance of AIDS associated viral RNA per cell as determined by _in situ_ hybridization is generally greater in brain than in lymph node, peripheral blood, or bone marrow.

Lentiviruses cause a chronic degenerative neurologic disease in sheep and viral DNA has been detected in cells from the choroid plexus of infected animals.

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(5) REPLICATION

The AIDS associated viruses and lentiviruses display an accumulation of linear double-stranded proviral DNA. The other retroviruses do not accumulate such an intermediate; these viruses only produce similar proviral forms as transient intermediates prior to incorporation of the DNA copy of the viral genome into cellular chromosomal DNA.

(6) MARKED HETEROGENEITY

Both the AIDS associated viruses and lentiviruses display a marked heterogeneity in viral DNA, most notably in the env gene that encodes the viral coat protein. Proposed mechanisms of persistence by the lentiviruses include continued variability of env encoded polypeptides in order to avoid recognition by the immune surveillance system. For example, visna is able to persist for periods as long as eight years (AIDS associated virus persistent infection has been recorded as long as five years) in an animal with demonstrable neutralizing antibody.

SIGNIFICANCE OF AIDS ASSOCIATED VIRUS AND LENTIVIRUS CLOSE TAXONOMIC RELATIONSHIP

Lentivirus virion production is known to be suppressed in infected animals. While as much as 18% of cells can be identified as containing visna viral DNA and 14% capable of induced virus production, only 0.02% of the cells could be stained by immunofluorescence using antibody to the principle nucleocapsid structural polypeptide of the virus. The conclusion from these data was that only 1/1000 of the cells that contain viral DNA were synthesizing detectable amounts of the major viral gene product <u>in vivo</u>. The noted restriction of virus production has been proposed as a mechanism for persistence analogous to the lysogenic relationship between bacteriophage and their bacterial hosts. Virus genetic information is therefore conserved and the virus survives to perpetuate infection because the infected cell is neither detected nor destroyed by the immune surveillance system of the host animal.

If the similarities of the AIDS associated viruses and lentiviruses in general, or visna specifically, extend to the ability of the virus to reside in cells without producing virus, the direct detection of the AIDS associated viruses may prove problematic in persistently infected asymptomatic individuals.

INTRAMURAL STUDIES TO DATE PERTINENT TO DETECTION OF AIDS ASSOCIATED VIRUSES

(1) The application of the polymerase chain reaction to reconstruction experiments using the B-globin gene as a model system indicate that as few as 5000 molecules of DNA of an agent can be detected in the presence of a vast excess (one microgram) of human chromosomal DNA in 16 hours. This essentially means that PCR should be capable of allowing the detection of one to two molecules of

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nucleic acid of a pathogenic agent in every 100 cells. The detection protocol used involved electrophoretic fractionation of an endonuclease cleaved radioactive (^{32}-P) end-labelled oligonucleotide (oligomer restriction).

(2) RNA viruses show high mutation frequencies (error frequency per genome doubling of 10^{-3} to 10^{-4} at a given base position compared to 10^{-8} to 10^{-11} per incorporated nucleotide for chromosomal genes) partly because of the lack of the proofreading enzymes that assure fidelity of DNA replication. This high mutation frequency is coupled with high rates of replication reflected in rates of RNA genome evolution which can be more than 10^{6}-fold greater than the rates of the DNA chromosomal evolution of their hosts. As expected, the AIDS associated viral genome in general and the envelope gene specifically is known to undergo considerable variability. Are there regions of the viral genome that remain constant? We have used computer algorithms to identify regions of the genome that are identical among all the isolates sequenced and reported to date. Most notable are regions within the gag gene which are conserved; one region is almost 200 residues in length.

PRINCIPAL QUESTIONS

1. Do the AIDS associated viruses persist as DNA copies in the absence of virus production in peripheral blood cells prior to the onset of symptomatic AIDS?

2. Can PCR be used to amplify viral DNA in order to identify individuals which are persistently infected by this class of viruses but are asymptomatic?

PROPOSED ORDER OF TASKS

1. Obtain either cloned AIDS viral DNA or DNA from an infected cell line to serve as a positive control model system.

2. Determine if PCR can be used to amplify AIDS associated viral DNA.

3. Obtain clinical material from AIDS patients (blood would be best initially).

4. Determine if PCR can be used to identify viral genomic sequences in individuals with pre-AIDS, AIDS, or in a high risk group. Compare PCR score to culturing virus, seropositivity, and/or direct antigen detection (direct antigen detection not presently possible).

YOUR COMMENTS AND SUGGESTIONS ARE REQUESTED.