

UNIVERSITYWIDE TASK FORCE ON AIDS
UNIVERSITY OF CALIFORNIA

AIDS RESEARCH PROGRAM 1984-1985

I. Title of Project: _____

II. Principal Investigator:

_____ Name (last, first, M.I.)	_____ Signature
Mailing address (use only those lines necessary, no abbreviations please):	
_____ Department/Division	_____ Phone numbers:
_____ Bldg./Room #	_____ Office:
_____ School	_____ Laboratory:
_____ Campus	_____ Dept.(messages):
_____ Zip	

III. Eligibility of Responsible Investigator:

Official UC title

IV. Co-investigators:

V. Responsible Administrator (please see instructions):

I verify that the information given by the applicant concerning his/her appointment and current grant status is correct and complete.

_____ Signature	_____ Name	_____ Title (chair, etc.)	_____ Dept.
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VI. Review & Approval:

A. Human Subjects

- ☐ 1. Does not apply
☐ 2. Approval attached
☐ 3. Review pending

B. Biohazards

- ☐ 1. Does not apply
☐ 2. Memorandum of Understanding
and Agreement attached
☐ 3. Review pending

C. Contracts & Grants: Information as required on the attached checklist is complete and detailed in the application.

Signature: _____ Date: _____

IX. Biographical Sketch

(give the following information for all professional personnel)

Name	Official Title
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Education (begin with the baccalaureate training and include postdoctoral)

Institution and location	Degree	Year	Scientific Field
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Honors

Major Research Interest	Role in Proposed Project
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Research and/or Professional Experience (Starting with present position, list pertinent professional background and employment)

Publications Pertinent to Proposed Research

IX. Biographical Sketch

(give the following information for all professional personnel)

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Honors

Major Research Interest	Role in Proposed Project
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Research and/or Professional Experience (Starting with present position, list pertinent professional background and employment)

Publications Pertinent to Proposed Research

X. Summary of Research Proposal for the AIDS Research Program

UNIVERSITYWIDE TASK FORCE ON AIDS: 1984-1985 Grant Awards

Name and Title of Principal Investigator

Department and Campus

Title of Project

Use this space to summarize concisely your proposed research. Outline objectives and methods. Underscore the KEY words (not to exceed 10) in your abstract.

If the AIDS Task Force awards you a research grant, copies of this page may be distributed to UC Contract and Grant Offices and to other interested individuals and groups.

IX. Biographical Sketch

(give the following information for all professional personnel)

Name	Official Title
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Troy E. Daniels

Professor

Education (begin with the baccalaureate training and include postdoctoral)

Institution and location	Degree	Year	Scientific Field
School of Dentistry, Univ. of CA, SF	D.D.S.	1967	Dentistry
USPHS Hosp., New Orleans, Louisiana	Intern	1967-68	Dentistry/Medicine
NCI Trainee, Univ. of CA, SF	-	1971-73	Oncology
Graduate Division, Univ. of CA, SF	M.S.	1973	Oral Biol./Immunol.

Honors

1967 Omicron Kappa Upsilon (National Dental Honor Society)
 1977 Distinguished Teaching Award, Academic Senate UCSF
 1981 NIH National Research Service Award for Senior Fellows
 1983 Fellowship, American College of Dentists

Major Research Interest	Role in Proposed Project
Immunological mechanisms of human oral mucosal diseases	Co-investigator

Research and/or Professional Experience (Starting with present position, list pertinent professional background and employment)

1981-Now University of California, San Francisco, School of Dentistry; Professor, Oral Medicine and Oral Pathology

1981-82 Royal Dental College, Copenhagen, Denmark; Visiting Professor

1977-81 University of California, San Francisco, School of Dentistry; Associate Professor, Oral Medicine and Oral Pathology

1973-77 University of California, San Francisco, School of Dentistry; Assistant Professor, Oral Biology

1970-71 University of California, San Francisco, School of Dentistry; Lecturer, Oral Diagnosis

1968-70 USPHS Alaska Native Health Center, Nome, Alaska; Chief, Dental Services

Publications Pertinent to Proposed Research

Daniels, T. and Newbrun, E. 1966. Measurement of protein and free and bound carbohydrates in human parotid saliva. Arch. Oral Biol. 11:1171-1180.

Daniels, T.E., Sylvester, R.A., Silverman, S., Polando, V. and Talal, N. 1974. Tubuloreticular structures within labial salivary glands in patients with Sjogren's syndrome. Arthritis Rheum. 17:593-597.

Greenspan, J.S., Daniels, T.E., Talal, N. and Sylvester, R.A. 1974. The histopathology of Sjogren's syndrome in labial salivary gland biopsies. Oral Surg. 37:217-229.

- Cremer, N.E., Daniels, T.E., Oshiro, L.S., Marcus, F., Claypool, R., Sylvester, R.A. and Talal, N. 1974. Immunologic and virologic studies of cultured labial biopsy cells from patients with Sjogren's syndrome. *Clin. Exp. Immunol.* 18:213-224.
- Daniels, T.E. and Silverman, S., Michalski, J.P., Greenspan, J.S., Sylvester, R.A. and Talal, N. 1975. The oral component of Sjogren's syndrome. *Oral Surg.* 39:875-885.
- Talal, N., Grey, H.M., Zvaifler, N., Michalski, J.P. and Daniels, T.E. 1975. Elevated salivary and synovial fluid B₂-microglobulin in Sjogren's syndrome and rheumatoid arthritis. *Science* 187:1196-1198.
- Michalski, J., Daniels, T.E., Talal, N. and Grey, H. 1975. Beta₂-microglobulin and lymphocytic infiltration in Sjogren's syndrome. *N. Engl. J. Med.* 293:1228-1231.
- Daniels, T.E., Powell, M.R., Sylvester, R.A. and Talal, N. 1979. An evaluation of salivary scintigraphy in Sjogren's syndrome. *Arthritis Rheum.* 22:809-814.
- Fischbach, M., Char, D., Christensen, M., Daniels, T.E., Whaley, K., Alspaugh, M. and Talal, N. 1980. Immune complexes in Sjogren's syndrome. *Arthritis Rheum.* 23:791-795.
- Fye, K.H., Daniels, T.E., Zulman, J., Michalski, J.P., Jaffe, R. and Talal, N. 1980. Aplastic anemia and lymphoma in Sjogren's syndrome. *Arthritis Rheum.* 23:1321-1325.
- Daniels, T.E. 1980. Immunopathology of recurrent aphthous ulcers and bullous diseases of the mouth. In O'Connor, G.R., ed. *Immunologic Diseases of the Mucous Membranes: Pathology, Diagnosis and Treatment.* Mason, N.Y., pp. 95-108.
- Miyasaka, N., Sauvezie, B., Pierce, D.A., Daniels, T.E. and Talal, N. 1980. Decreased autologous mixed lymphocyte reaction in Sjogren's syndrome. *J. Clin. Invest.* 66:928-933.
- Chudwin, D.S., Daniels, T.E., Wara, D.W., Ammann, A., Barrett, D.J., Whitcher, J.P. and Cowan, J.J. 1981. Spectrum of Sjogren syndrome in children. *J. Pediatr.* 98:213-217.
- Daniels, T.E. and Quadra-White, C. 1981. Direct immunofluorescence in oral mucosal disease: a diagnostic analysis of 130 cases. *Oral Surg.* 51:38-47.
- Tabbara, K.F., Ostler, H.B., Biswell, R. and Daniels, T.E. 1982. Chronic ulcerative conjunctivitis in a patient with alpha-1-antitrypsin deficiency. *Ann. Ophthalmol.* 14:280-282.
- Shillitoe, E.J., Daniels, T.E., Whitcher, J.P., Strand, C.V., Talal, N. and Greenspan, J.S. 1982. Antibody to cytomegalovirus in patients with Sjogren's syndrome as determined by an ELISA. *Arthritis Rheum.* 25:260-265.
- Bakhshi, A., Miyasaka, N., Kavathas, P., Daniels, T.E., Strand, C.V., Herzenberg, L.A. and Talal, N. 1983. Lymphocyte subsets in Sjogren's syndrome: A quantitative analysis using monoclonal antibodies and the fluorescence activated cell sorter. *J. Clin. Lab. Immunol.* 10:63-69.
- Hansen, L.S., Silverman, S. and Daniels, T.E. 1983. The differential diagnosis of pyostomatitis vegetans and its relation to bowel disease. *Oral Surg.* 55:363-373.
- Dabelsteen, E. and Daniels, T.E. 1983. Tumor associated antigens in oral cancer and premalignant lesions. *Dtsch. Z. Mund-Kiefer-Gesichts-Chir.* 7:87-92.
- Daniels, T.E. 1984. Labial salivary gland biopsy in Sjogren's syndrome: Assessment as a diagnostic criterion in 362 suspected cases. *Arthritis Rheum.* 27:147-156.
- Daniels, T.E. 1984. Human mucosal Langerhans cells: Post-mortem identification of regional variations in oral mucosa. *J. Invest. Dermatol.* 82:21-24.
- Chou, M.J., Daniels, T.E. 1984. Langerhans cell and T cell identification in oral mucosal lichen planus. *J. Dent. Res.* 63:192.
- Daniels, T.E., Schwartz, O., Larsen, V., Dabelsteen, E., Pindborg, J. 1984. Langerhans cells in candidal leukoplakia. *International Assoc. of Oral Pathologists - Amsterdam.*

UCSF BIOSAFETY COMMITTEE
INFECTIOUS AGENTS REVIEW FORM

This form is for work involving Class 1*, 2*, or 3 Agents. (See listing in Appendix A of the Biosafety Guidelines.) Submit 10 copies to C-116.

Principal Investigator John S. Greenspan Mailing Address 604-HSW Phone ext. 2943 Date 9/29/83

Agent(s):

UCSF
CLASSIFICATION:

AIDS "agent"

2*

Description of Operational Protocol:

UCSF AIDS Tissue and Serum Bank

Location of work:

Rooms(s): HSW-698

Will infected animals be housed in the
Animal Care Facility?

No X Yes

Classification: P2*

Animals: N/A

Personnel Involved:

Number involved: N/A

John S. Greenspan

Special problems? N/A

Yvonne de Souza

Sue Karg
Jennifer Kline

Deborah Daniels

Charles Hwang

Maha Abdel-Salam

TBA 2 or 3 more

Office Use:

Approval Number I6301
Approval Date 10/21/83
Expiration Date
Sent to ACF

9/30/83
Date
10/21/83
Date

[Signature]
Signature, Principal Investigator

[Signature]
Signature, Chairman, Biosafety Committee

BIOLOGICAL LABORATORY INSPECTION REPORT & CERTIFICATION

P.I. Dr. J. Greenspan Bldg./Rm. # HSE 698
 Biohazard AIDS
 Approved for P-2 Work
 Signed [Signature] Date 12/8/83

SUMMARY OF BIOLOGICAL SAFETY GUIDELINES FOR PHYSICAL CONTAINMENT LEVELS P1, P2, P3

<u>SAFETY MEASURES</u>	<u>P1</u>	<u>P2</u>	<u>P3</u>
Public Access	Not recommended	Not permitted while experiments requiring P2 containment are in progress <u>OK</u>	Prohibited; access to lab personnel is controlled
Access for Children	Not recommended	Not recommended <u>OK</u>	No one under 16 permitted
Laboratory Doors	Closed while experiments are in progress and posted with Biohazard sign	Closed while experiments are in progress and posted with Biohazard sign <u>OK</u>	Closed while experiments are in progress and posted with Biohazard sign
Incubators, Refrigerators, etc.	Biohazard sign	Biohazard sign <u>OK</u>	Biohazard sign
Laboratory Separated from General Traffic Patterns of Building	No	No <u>NA</u>	Yes
Laboratory Ventilation	Inflow from access corridor	Inflow from access corridor <u>OK</u>	Inflow from access corridor
Vacuum Lines	No special requirements	No special requirements <u>OK</u>	Shall be protected by filters and liquid traps
Eating, Drinking, Smoking and Storage of Food in Lab	Not permitted	Not permitted <u>OK</u>	Not permitted
Handwashing Facilities	Should be available	Required: staff encouraged to wash frequently and when leaving laboratory <u>OK</u>	Required: staff encouraged to wash frequently and when leaving laboratory
Outer Street Wear	May be kept in laboratory	May be kept in laboratory <u>OK</u>	Shall not be kept in laboratory

SAFETY MEASURESP1P2P3

Use of Laboratory Clothing	At the discretion of the laboratory supervisor	Required: should not be worn outside the building or to lunch room <i>OK</i>	Required: but should not be worn outside lab; must be decontaminated before going to laundry; gloves shall be worn; lab shoes recommended
Pipetting	Mechanical devices required	Mechanical devices required <i>OK</i>	Mechanical devices required
Use of Biological Safety Cabinets or Other Physical Containment Equipment	Recommended for procedures producing considerable aerosols	Recommended for procedures producing considerable aerosols <i>OK have</i>	Required to be available within controlled areas and must be used for ALL procedures
Working on Open Bench Top	Permitted	Permitted <i>OK</i>	Not permitted: Biological Safety Cabinet must be used
Decontamination of Work Surfaces	Daily and following spills	Daily and following spills <i>OK</i>	Following completion of experimental activity
Floors	Clear for mopping	Clear for mopping <i>OK</i>	Clear for mopping
Autoclave	Not required	Must be available within building <i>OK have HSW 604</i>	Required to be within controlled area
Infectious Liquid Wastes	Decontaminate before disposal	Autoclave <i>OK</i>	Autoclave
Infectious Solid Wastes	Decontaminate or package in leak-proof containers before removal from lab	Autoclave <i>OK</i>	Autoclave, plus packaged material shall be sterilized before disposal or incineration
Emergency Plan	Required	Required <i>OK</i>	Required
Training Program & Records	Required	Required <i>OK</i>	Required

REMARKS _____

cc: Biosafety Committee
P.I.

ABSTRACT OF RESEARCH PLAN

PROJECT NUMBER

CURRENT 83/84

NAME AND ADDRESS OF APPLICANT ORGANIZATION (Same as Item 11, page 1)

Department of Oral Medicine and Hospital Dentistry, School of Dentistry
University of California, San Francisco, CA 94143

TITLE OF APPLICATION (Same as Item 1, page 1)

Multiple factors in the etiology of oral cancer

Name, Title and Department of all professional personnel engaged on project, beginning with Principal Investigator/Program Director

Edward J. Shillitoe, Assistant Professor, Oral Medicine and Hospital Dentistry,
School of Dentistry

J. Michael Bishop, Professor, Microbiology, School of Medicine

* John S. Greenspan, Professor, Oral Medicine and Hospital Dentistry,
School of Dentistry

ABSTRACT OF RESEARCH PLAN: Concisely describe the application's specific aims, methodology and long-term objectives, making reference to the scientific disciplines involved and the health-relatedness of the project. The abstract should be self-contained so that it can serve as a succinct and accurate description of the application when separated from it. DO NOT EXCEED THE SPACE PROVIDED.

Cancer of the mouth is probably the result of the interaction of several factors. Epidemiological studies have shown that the disease is more common in cigarette smokers, and the risk increases with an increase in the amount smoked per day. Smokers have higher levels of antibody to Herpes simplex virus type-1 (HSV-1) than non-smokers, and antibody to HSV-1 is associated with oral cancer. We will use an animal model to test the hypothesis that constituents of cigarette smoke can reactivate latent herpes simplex virus, and that a co-carcinogenic interaction between them causes the tumor.

Mice will be infected on the skin with an oral isolate of HSV-1. After the infection has resolved and the virus has become latent, experiments will show if it can be reactivated with condensates of tobacco smoke and with various polycyclic hydrocarbons. If HSV is found to increase the rate of the tumor induction, the role of the virus will be studied to find if it is an initiator, a promotor, or both. The tumors will be examined for HSV antigens by a peroxidase-antiperoxidase method. The mice's serum antibodies to HSV will be measured by an enzyme-linked immunosorbent assay, and HSV DNA will be sought in the tumor cells by nucleic acid hybridization. Efforts will then be made to inhibit the co-carcinogenic effect with selected drugs.

Data obtained will be compared with existing data from human patients to find if the animal model is comparable with the human disease.

This project will accumulate evidence to support or refute a possible etiology of oral cancer, and it may provide a rational mechanism for the prevention of that disease.

LABORATORY ANIMALS INVOLVED. Identify by common names. If none, state "none"

Mice

X. Summary of Research Proposal for the AIDS Research Program

UNIVERSITYWIDE TASK FORCE ON AIDS: 1984-1985 Grant Awards

JOHN S. GREENSPAN PROFESSOR, ORAL BIOLOGY AND PATHOLOGY
Name and Title of Principal Investigator

Department of Stomatology (Division of Oral Biology) San Francisco
Department and Campus

Oral viral leukoplakia - a new lesion in association with AIDS.
Title of Project

Use this space to summarize concisely your proposed research. Outline objectives and methods. Underscore the KEY words (not to exceed 10) in your abstract.

We propose to investigate a new form of oral leukoplakia which we have recently described as part of the AIDS group of diseases. The lesion occurs ^{histologically} ~~in~~ male homosexuals with evidence of immunosuppression & has a distinctive ~~clinical~~ and histological appearance. There is preliminary evidence for the presence of papillomaviruses, antigen shown immunocytochemically, but not on electronmicroscopy. However, plentiful ~~stains~~ is found on EM, but apparently of the herpes group. [INSERT] We propose to characterize approximately 25 cases to assess risk factors, presence of immunosuppression & prognosis. Specimens will be taken at biopsy ^(from lesions & control tissues) & subjected to immunopathological & virological investigation to further elucidate the extent & nature of viral involvement. Evidence for the following viruses will be sought: Human papillomavirus [HPV], Herpes simplex I and II [HSV], cytomegalovirus [CMV], Epstein Barr virus [EBV]. The methods to be used will include culture, PAP and immunofluorescence with artificial antisera [HPV and EB], ABC and IF with monoclonal antibodies [HSV I and II, CMV] DNA probes - Southern blot or in situ hybridization [HPV, HSV I and II, CMV and EBV] and electron microscopy. Local immunosuppression will be examined by demonstrating numbers, allocation and Ia status of intraepithelial Langerhans cells and their relationship to the virus elements. As probes for HTLV III/LAV become available, these too will be applied.

If the AIDS Task Force awards you a research grant, copies of this page may be distributed to UC Contract and Grant Offices and to other interested individuals and groups.

* A high rate of development of the PCP form of AIDS is suggested by initial data.

X. Summary of Research Proposal for the AIDS Research Program

UNIVERSITYWIDE TASK FORCE ON AIDS: 1984-1985 Grant Awards

Name and Title of Principal Investigator

Department and Campus

Title of Project

Use this space to summarize concisely your proposed research. Outline objectives and methods. Underscore the KEY words (not to exceed 10) in your abstract.

If the AIDS Task Force awards you a research grant, copies of this page may be distributed to UC Contract and Grant Offices and to other interested individuals and groups.

XI. Explanation of ProjectTitle Oral viral leukoplakia - a new lesion in association with AIDSIntroduction/Background

Between October 1981 and July 1984, we have seen 37 cases of a previously unrecorded lesion of the tongue in male homosexuals in the San Francisco area. The lesions are on the lateral margins of the tongue and are of varying sizes (Fig 1 Appendix). They are usually poorly demarcated and show a corrugated or "hairy" surface. Although usually painless, the lesions have been noticed by the patients, several of whom report spontaneous regression and reappearance. We have provisionally named the lesion hairy leukoplakia (HL). (1)

Although all of our patients with HL have been in apparent good health at the time of diagnosis, all reported previous use of recreational drugs, most gave a history of hepatitis B and many had diffuse lymphadenopathy. Of a few cases tested for immunocompetence using skin tests and T_4/T_8 ratios as criteria, most showed some evidence of immunosuppression. None of our patients had AIDS at the time of diagnosis of HL, but 8/37 have developed Pneumocystis carinii pneumonia (PCP) in the 33 months of our preliminary study.

The lesion has a distinctive histopathology (Fig 2 a & b Appendix) and shows features suggestive of a human papillomavirus (HPV) lesion. These include the "hairs", which are due to localized acanthosis and parakeratosis, as well as marked koilocytosis - ballooning of prickle cells with pyknotic nuclei and perinuclear halos. In a very preliminary study, 74% of 23 biopsies showed evidence of papillomavirus antigen using an immunocytochemical technique. (Fig 3 a & b Appendix) Presuming the lesion to be a form of papillomavirus-induced flat oral wart, we initiated an electron microscopic search for papillomavirus particles. In the six cases examined to date, we have found no evidence of HPV virions. However, to our surprise, 5/6 cases showed plentiful evidence

of a herpes-type virus. (Fig 4 a & b Appendix) We do not yet know if this is cytomegalovirus (CMV), herpes simplex (HSV) type 1 or 2, Epstein-Barr virus (EBV) or varicella-zoster virus (VZV).

The identification of the viruses or virus elements present in HL is at a preliminary stage. Perhaps an HPV type is present and complete and represents a fairly straightforward example of opportunistic infection in a host tissue which is immunocompromised both systemically and also locally. Langerhans cells (LC) are known to be decreased in the skin in AIDS (2), while the keratinized surface of the tongue is known to have areas devoid of LC (3). A second virus may then simply colonize the hyperplastic epithelium of the wart-like lesion produced by the HPV. However, papillomavirus particles are not seen and the herpes-type virus is present in very large quantities. Perhaps the HPV is defective, produces antigen and cytopathic effects but not complete virus particles. Perhaps a herpes-type virus (CMV, HSV⁻¹ or 2, EBV or VZV) is present and contributes to the lesion. Even more speculative and exciting are the possibilities, firstly that only the herpes-type virus is present and is responsible for both the HPV antigen staining and production of the lesion, or secondly that a hybrid HPV/herpes-type virus is present. Such hybrids between the papavovirus SV40 and adenoviruses have been described (4).

The lesion is of interest for several other reasons. Recent reports suggest a previously unsuspected and possibly widespread role for HPV in oral epithelial hyperplasia, dysplasia and carcinoma (5-8). Of interest in this regard is the observation of several cases of oral carcinoma in young male homosexuals in San Francisco (9). Similar relationships have been studied extensively between HPV and carcinoma of the uterine cervix. (10,11). There is also thought to be an association, as yet not well understood, between HSV-2 and uterine carcinoma (12) as well as a possible analogous association between oral cancer and HSV-1 (13, 14). Papillomaviruses (15), herpes simplex virus (16), cytomegalovirus (17), and EB virus (18) can all transform cells and behave as oncogenic agents.

Specific Aims

1. To further define and investigate the etiology, pathogenesis and natural history of oral "hairy" leukoplakia (HL), a newly described lesion which is part of the AIDS group of diseases.
2. To identify the virus elements thought to be present in the lesion and to assess their incidence in a group of HL cases.
3. To explore local, intramucosal, immune mechanisms by identifying and counting Langerhans cells (LC), including enumerating those bearing Ia antigen and try to determine their morphological relationship to HPV and other viral antigen-bearing cells.
4. To follow HL cases prospectively and to attempt to define risk factors for the development of Pneumocystis carinii pneumonia (PCP) on other forms of AIDS. Further, to assess the risk, if any, of transformation of the lesion into oral squamous cell carcinoma.

Methods

Patients

Approximately 750 new patients are seen each year in the Oral Medicine Clinic at UCSF, among a total of almost 5,000 patient visits per year. At present, about 1 to 2 new cases of HL are being seen each week. We anticipate at least this rate of accession of new cases during the 8 months of the proposed grant period. The cases are referred by physicians and dentists in the city of San Francisco and adjoining areas but most are referred from the AIDS Clinical Research Center/KS Clinic. As the new UCSF AIDS clinic becomes functional, and as awareness of the lesion spreads among clinicians, the referral rate may increase. We therefore anticipate no difficulty in recruiting 30 to 50 new cases for some or all parts of the study. In addition, paraffin blocks are available in

35 existing biopsies from 30 cases, while frozen tissue from 15 cases and serum from 14 cases are stored in the UCSF AIDS Specimen Bank. EM blocks have been prepared in 8 cases in addition to those described in the Introduction. Thus a good deal of material awaits investigation and the project can undergo major expansion as soon as funding becomes available.

All new patients will be invited to participate and those who agree will give a detailed medical and social history using the UCSF AIDS Clinical Research Center protocols. Complete oral exam will be performed by Dr. D. Greenspan, backed-up by Dr. T.E. Daniels and Dr. J.S. Greenspan if needed. Physical examination will be performed by Dr. H. Hollander in the UCSF AIDS Clinic or by Dr. M. Conant and his nurse-practitioner staff in the UCSF AIDS Clinical Research Center/KS Clinic. Clinical data, and ultimately all laboratory data, will be stored in the UCSF AIDS Clinical Research Center computer.

Oral Biopsy/Histopathology

White lesions suspected of representing the lesion of HL will be subjected to incisional biopsy using local anesthesia. The specimen will be divided as follows:

1. A portion will be routinely fixed and processed for histopathology and PAS staining. These will be performed by the Oral Pathology service of the department, on which the Principal Investigator is an attending oral pathologist. The same paraffin blocks will be used for PAP staining for HPV antigen (see below).
2. A portion will be cut into 1 mm^3 fragments for electron microscopy. (see below)
3. A portion will be used for cultural virological studies (see below)
4. The remainder will be quick-frozen in liquid nitrogen and then stored in sealed vials in liquid nitrogen in the tissue bank of the UCSF AIDS Specimen Bank. Cryostat sections will be cut for some of the virus studies and for the identification of Langerhan's cells. This frozen tissue will also be the source of DNA for Southern blot studies.

Control Tissues

1. "Positive" controls will include skin and oral verrucae and condylomata (control for HPV), HSV-1 and -2, CMV, EBV, and VZV infected cell lines.
2. "NEGATIVE" controls will include specimens from other oral epithelial lesions undergoing diagnostic biopsy in our clinics. These will include hyperkeratosis, dysplasia and carcinoma, candidiasis and lichen planus. We will also use normal oral mucosa obtained during plastic procedures.
3. A further, most important, control will be non-lesional tissue adjoining HL lesions. This control tissue should help us to establish if the agents found are confined to the HL lesion or are more widely present in the epithelium of these patients.

Immunological Studies

Delayed cutaneous hypersensitivity will be tested using PPD, candida, mumps and trichophytin antigens. Assessment of peripheral blood lymphocyte subtype levels and T_4/T_8 ratios will be performed by Dr. Conrad Casavant in the Clinical Immunology laboratory at UCSF.

Ultrastructural Studies

Tissue fragments will be fixed for 12-48 hours in 3% glutaraldehyde (19), postfixed for 1 hour in 1% buffered osmic acid, (20) dehydrated in graded ethanol and propylene oxide and embedded in EPON 812 (21). Thin sections will be cut from areas selected by light microscope examination of semithin sections and will be double-stained on Formvar-coated grids with saturated uranylacetate and modified lead citrate (22); sections will be screened for HPV virions (23-24) and for other viruses by examining at least 100 cells from each case. The instrument to be used is the Siemens Elmiskop 1A, operating at 80KV, in the EM facility of the Division of Oral Medicine.

Virological Studies

Our intent is to:

1. Confirm or refute the presence of human papillomavirus (HPV).

2. To confirm the presence of the herpes-type virus, seen in 5/6 cases on electron microscopy in our preliminary study.
3. To attempt to identify the latter using techniques to demonstrate HSV-1 and 2, CMV, EBV and VZV. Details follow and the plan is summarized in Table I.

a) Culture (25)

← Fresh tissue specimens will be transported to Dr Drew's laboratory where attempts will be made to culture virus from them using host cells, notably human lung fibroblasts, suitable for members of the human herpesvirus group. Evidence of cytopathic effects will be sought.

b) Papillomavirus

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The search for papillomavirus will include attempts to further identify HPV antigen, electromicroscopy to find whole virus particles and hybridization with DNA probes.

HPV antigen

Paraffin sections will be cut and subjected to the peroxidase-antiperoxidase technique using antisera to papillomavirus antigen. We will employ two antisera. Both have been raised in rabbits using as antigen preparations of the core antigen of bovine papillomavirus. One is available commercially (DAKO) while the other is the gift of Dr. A.B. Jenson of Georgetown University. Control procedures will include the use of serum from non-immunized rabbits as well as incubations omitting each of the other stages. Control tissues will include oral verrucae (positive control) and oral hyperkeratosis, carcinoma, candidiasis and lichen planus (negative controls, as well as non-lesional tissue from HL patients.

←
HPV particles on EM (see above)

HPV-DNA

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Evidence for papillomavirus DNA will be sought from DNA extracted from frozen HL tissue sent by us to Dr. A.B. Jenson using the Southern blot technique (see below) and probes provided by Professor H. zur Hausen. Twenty five probes for that number of HPV types are available, but initial experiments will be under non-stringent conditions to determine if human papillomavirus DNA of any type is present. Subsequent work will be directed at determining the type or types of HPV-DNA if present.

c) Herpes Simplex Virus-1 and 2

Experiments will involve culture, attempts to identify antigen in tissue sections and hybridization with DNA probes. EM studies are described above.

HSV antigen

The battery of 9 monoclonal antibodies provided by Dr. M. Zweig will be used. We will apply the avidin-biotin-peroxidase (ABC) technique (26) with which we have 4 years of experience. This will permit identification and localization of most stages of HSV-1 and 2. The antibodies are: Anti ICP4, 58-S (HSV-1); Anti ICP5, 56-S (HSV-1 capsid protein); Anti ICP6, 2-S, (HSV-1); Anti ICP6, 105-S, (HSV-2); Anti ICP6, 48-S, (HSV-1 Cross reacting); Anti ICP8, 39-S, (HSV-1 DNA binding protein); Anti gB, 35-S, (HSV-1 glycoprotein); Anti gB, 3-S, (HSV-1); Anti gD, 4-S, (HSV-1).

HSV-DNA

The probes for HSV-1 and 2 DNA, provided by Dr. R. Hyman will be used by Dr. J. Felton in the Southern blot technique (27). The recombinant probes to be used will include EcoRI fragments B and F of HSV-1 (Patton strain) as well as BglII fragments C and N of HSV-2 (333 strain). Vector-only probes (e.g., pACYC184) will be used as negative controls. A further control will be a cloned, single-copy normal human gene used as a probe to demonstrate that the method is adequately sensitive. All the above probes, and also probes consisting of whole, intact HSV-1 and 2 DNA, will be made

radioactive and hybridized to nitrocellulose blots of electrophoretically separated DNA from HL lesions, uninvolved mucosa of HL patients, and the same patient's blood leukocytes. Other controls will include blood leukocytes of healthy individuals and from HSV-transformed cell lines and the untransformed parent cell lines from which they are derived. Blots of artificially constructed mixtures of various DNAs may also be used, for example HSV-I virion DNA and normal human DNA in a genome- equivalent ratio of 1:1. Blots will be prepared from DNAs cut with several different restriction endonucleases, and the hybridization reactions will be performed under conditions of various degrees of stringency.

d) Cytomegalovirus (CMV)

Experiments will involve culture and EM (see above) as well as attempts to identify CMV antigens and CMV-DNA

CMV Antigen

Cryostat sections will be reacted with pooled mouse monoclonal antibody to CMV (Immulog/Syva Labs) in a biotin-avidin FITC system (EY Labs).

CMV-DNA

In-situ hybridization, using cryostat sections, will be applied and will involve the ENZO Biochem. Inc. "Patho-Gene" kit. A biotinylated DNA probe is hybridized to CMV-DNA and is detected by a streptavidin-horseradish peroxidase-complex and DAB system.

e) Epstein-Barr virus (EBV)

Dr. Lennette will use human sera to identify CMV and EBV by IF. Cryostat sections will be reacted with at least two sets of reference human sera of specificities: EBNA+/CMV-, EBNA-/CMV+ and EBNA+/CMV+ (minimum 6 sera per cases). A guinea pig complement/anti-guinea pig C3 system (fluorescein-conjugated) will be used. (28)

EBV-DNA probes are available commercially and we will develop their use in the in situ hybridization assay.

f) Varicella-zoster-virus

Dr. Lennette will use human sera with antibodies to VZV. Dr. Lennette is developing the use of new anti-VZV monoclonals and we will apply these also, as well as VZV-DNA probes when they become available.

The field of in situ identification of viruses within tissues is developing rapidly. Most of the reagents and techniques are fairly new. Modifications and improvements can be expected to occur even during the period of this grant.

We will apply these to our problem as they become available.

Langerhans Cell Studies

These will be supervised by Dr. T.E. Daniels

Langerhans cells (LC) will be identified in 10 μ m cryostat sections using adenosine triphosphatase (ATPase) histochemistry (29, 3) and by immunohistochemistry using commercial monoclonal antibodies against the T6 antigen (30) and the Ia antigen (31) in the ABC technique (26). LC will be counted in standard fields and the results correlated with the nature and location of virus antigen or DNA in the immunohistochemical and in situ techniques. Our hypothesis is that selective loss of LC is related to colonization by HPV and/or the as-yet-unidentified herpes-type virus. The control biopsies from non-lesional tongue areas in our HL patients will clearly be very important in this aspect of our study.

Prospective Study

Our HL patients will be examined twice during the 8 months of this proposal and at intervals thereafter. We will record the development of features of AIDS, notably PCP, which has already occurred in 8 cases. We will attempt to assess risk factors for this complication and any others which develop. For this aspect of the study, we have enlisted the support of Dr. W. Winkelstein of the UCB School of Public Health who is the

PI of a substantial NIAIMD longitudinal study of cohorts of male homosexuals and controls. He has assigned a graduate student to our study and we are in the process of developing our joint protocol. No funds are requested for this part of our HL investigation except for a small sum to fund assessment of the presence and titer of antibodies to ARV (AIDS associated retrovirus) by Dr. J. Levy. This virus appears to have many of the properties of HTLV-III (32) and LAV (33). We are also in the process of negotiating with Professor Chermann at the Pasteur Institute, Paris, to have LAV titers performed.

TABLE I.

VIRUS	COMPONENT	TECHNIQUE	INVESTIGATOR	CONTROL TISSUE
HPV	Whole virus particles	EM	J. Greenspan	1. Oral warts/skin warts 2. Non-lesional tissue from HL cases 3. Other oral epithelial lesions
	Antigen DNA	PAP/antisera Southern blot	J. Greenspan A.B.Jenson	
Herpes Group	Whole virus particles Live virus	EM culture	J. Greenspan Drew	Non-lesional HL patient tissue
HSV1 & 2	Live virus Antigens	Culture ABC/monoclonals	Greenspan/ Hwang	1. Oral herpes, lesions, infected cells 2. Non-lesional HL patient tissue 3. Other oral lesions
	DNA	Southern blot	Felton/Hwang	
CMV	Antigens	IF/monoclonals	Lenette	1. Known positive CMV lesions/cells 2. Non lesional HL patients tissue 3. Other oral lesions
	DNA	<u>In situ</u> hybridization	Drew	
EBV	Antigens DNA	IF/antisera <u>In situ</u> hybridization (when reagents are available & reliable)	Lennette J. Greenspan/ Felton	1. Known positive EBV lesions/cells 2. as above 3. as above
VZV	Antigens DNA	IF/antisera and <u>in situ</u> hybridization (when both are available)	J. Greenspan/ Lennette	2/3 as above
ARV	Antibodies in serum	IF	Levy	1. Normal controls 2. ARV positive sera.

XII Relation of the Project to the AIDS Problem

We believe that further investigation of the nature, etiology, pathogenesis and progress of this new entity within the AIDS spectrum, oral hairy leukoplakia, is appropriate for the following reasons:

1. Such information may improve understanding of the role of viruses and local immunosuppression in the pathogenesis of AIDS.
2. Identification of the viruses present will contribute to an understanding of which organisms cause opportunistic infections in AIDS. The elucidation of the relationship between the viruses present could lead to increased understanding of virus-virus interactions, hybrid viruses and their roles in infection, immunosuppression and oncogenesis.
3. Determining which cases progress to the full syndrome, with PCP or KS, may help in earlier diagnosis of AIDS and ultimately contribute, in part, to its prevention and control.
4. Finally, investigation of the potential of this virally induced hyperplasia to undergo dysplastic and neoplastic transformation may contribute to elucidating the problem of neoplasia in AIDS.

XIII Progress Reports

The Principal Investigator is not currently a direct recipient of Task Force monies. However, he has received such funds in two ways. He is responsible for the UCSF AIDS Specimen Bank which is supported by the Task Force grant to the UCSF AIDS Clinical Research Center. Secondly, he has been the co-investigator on a Task Force grant to Jay Beckstead M.D. Therefore, relevant portions of the progress reports of those two projects are attached. However, neither is directly related to the current proposal. The specimen bank will be used to accept and store HL specimens and sera, just as it is used to store tissue and serum for many other projects, and no funds are requested in the present proposal for such support activities provided by the specimen bank.

LEGENDS FIGURES

Fig 1

Fig 2

REFERENCES

1. Greenspan, D., Greenspan, J.S., Conant, M., et al. Oral "Hairy" Leukoplakia in male homosexuals. 1984 submitted.
2. Belsito, D., Sanchez, M.R., Baer, R.L., et al. Reduced Langerhan's cell Ia antigen and ATPase activity in patients with the aquired immunodeficiency syndrome. *N. Engl. J. Med.* 1984 310:1279-1282.
3. Daniels, T.E. Human mucosal Langerhan's cells: postmortem identification of regional variation in oral mucosa. *J. Invest. Derm.* 1984 21-24.
4. Grodzicker, T., Adenovirus - SV40 hybrids. Chap. II in *DNA Tumor Viruses* ed Tooze, J. 1980 Cold Spring Harbor Laboratory 577-614.
- 5.. Jenson, A.B., Lancaster, W.D., Hartmann, D.P. and Shaffer, E.L. Frequency and distribution of papillomavirus structural antigens in verrucae, multiple papillomas and condylomata of the oral cavity. *Am. J. Path.* 1982 107:212-218.
6. Loning, T., Reichart, P., Staquet, M.J., et al. Occurence of papillomavirus structural antigens in oral papillomas and leukoplakias. *J. Oral. Path.* 1984 13:155-165.
7. Syrjanen, K.J., Syrjanen, S.M., Lamberg, M.A. and Pyrhonen, S. Human papillomavirus (HPV) involvement in squamous cell lesions of the oral cavity. *Proc. Finn. Dent. Soc.* 1983 79:1-8.
8. Jarrett, W.F.H., McNiel, P.E., Grimshaw, W.R., et al. High incidence of cattle cancer with a possible interaction between an environmental carcinogen and a papilloma virus. *Nature* 1978 274:215-217.
9. Lozada-Nur, F., Silverman, S., Migliorati, C., et al. Oral manifestations of tumor and opportunistic infections in the aquired immunodeficiency syndrome (AIDS). *Oral Surg.* 1983 56:491-494.
10. Reid, R., Crum, C.P., Herschman, B.R., et al. Genital warts and cervical cancer. *Cancer* 1984 53:943-953.
11. Gissman, L., Boshart, M., Durst, M., et al. Presence of human papillomavirus in genital tumors. *J. Invest. Derm.* 1984 83:26s-28s.
12. McDougall, J.K., Crum, C.P., Fenoglio, C.M., et al. Herpesvirus-specific RNA and protein in carcinoma of the uterine cervix. *Proc. Natl. Acad. Sci. USA* 1982 79:3858-3857.
13. Eglin, R.P., Scully, C., Lehner, T., et al. Detection of RNA complementary to herpes simplex virus in human oral squamous cell carcinoma. *Lancet.* 1983 ii:766-768.
14. Shillitoe, E.J., Greenspan, D., Greenspan, J.S., and Silverman, S. Antibody to early and late antigens of herpes simplex virus type I in patients with oral cancer. *Cancer* 1984 54:266-273.
15. Howley, P.M. The molecular biology of papillomavirus transformation. *Am. J. Path.* 1983 113:414-421.
16. Rapp, F., Duff, R. Transformation of hamster-embryo fibroblasts by herpes simplex viruses types 1 and 2. *Cancer Res.* 1973 33:1527-1534.
17. Geder, L., Kreider, J., Rapp, F. Human cells transformed in vitro by human cytomegalovirus: tumorigenicity in athymic nude mice. *J. Natl. Cancer Inst.* 1977 58:1003-1007.
18. Wolf, H., and Seibl, R. Benign and malignant disease caused by EBV. *J. Invest. Derm.* 1984 83:88s-95s.
19. Karnovsky, M.A. F formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. *J. Cell Biol.* 1965 27:#2, 137A-138A.
20. Palade, G.E. A study of fixation for electron microscopy. *J. Exp. Med.* 1952 95:285-297.
21. Luft, J.H. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol* 1961 9:409-414.

22. Reynolds, E.S. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 1963 17:208-212.
23. Williams, M.G., Howatson, A.F. and Almeida J.D. Morphological characterization of the virus of the human common wart (verruca vulgaris). *Nature* 1961 189:895-897.165
24. Frithiof, L. and Wersall, J. Virus-like particles in papillomas of the human oral cavity. *Arch. Virusforschung* 1967 21:31-44.
25. Benyesh-Melnick, M. Cytomegaloviruses. In: Lennette, E. H., Schmidt, N.J. eds. Diagnostic procedures for viral and rickettsial infections (4th ed). New York American Public Health Association 1969 701-732.
26. Hsu, S.M., Raine, L. and Fanger, H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques. *J. Histochem. Chtochem.* 1981 29:577-580.
27. Southern, E.M. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 1975 98:503-517.
28. Reedman, B.M., Klein, G. Cellular localization of Epstein-Barr virus (EBV) associated complement-fixing antigen in producer and non-producer lymphoblastoid cell lines. *Int. J. Cancer.* 1973 11:499-520.
29. Wolf, K. and Winkelmann, R.K. Quantitative studies on the Langerhan's cell population of guinea pig epidemics. *J. Invest. Derm.* 1967 48:504-513.
30. Fithian, E., Kung, P., Goldstein, G., et al. Reactivity of Langerhan's cells with hybridoma antibody. *Proc. Natl. Acad. Sci.* 1981 78:2541-2544.
31. Rowden, G., Lewis, M.G., and Sullivan, A.L. Ta antigen on human epidermal Langerhan's cells. *Nature* 1977 268:247-248.
32. Popovic, M., Sarngadharan, M.G., Read, E. and Gallo, R.C. Detection, isolation and continuous production of cytopathic retroviruses (HTLIII) from patients with AIDS and pre-AIDS. *Science* 1984 224:497-500.
33. Barre-Sinoussi, F., Chermann, J.C., Rey, F., et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for aquired immunodeficiency syndrome (AIDS). *Science* 1983 220:868-871.

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XI. Explanation of Project

Title Oral viral leukoplakia - a new lesion in association with AIDS

Introduction/Background

Between October 1981 and July 1984, we have seen 37 cases of a previously unrecorded lesion of the tongue in male homosexuals in the San Francisco area. The lesions are on the lateral margins of the tongue and are of varying sizes. They are usually poorly demarcated and show a corrugated or "hairy" surface. Although usually painless, the lesions have been noticed by the patients, several of whom report spontaneous regression and reappearance. We have provisionally named the lesion hairy leukoplakia (HL).

Although all of our patients with HL have been in apparent good health at the time of diagnosis, all reported previous use of recreational drugs, most gave a history of hepatitis B and many had diffuse lymphadenopathy. Of a few cases tested for immunocompetence using skin tests and T_4/T_8 ratios as criteria, all showed some evidence of immunosuppression. None of our patients had AIDS at the time of diagnosis of HL, but 8/37 have developed PCP ^{defined previously?} in the 33 months of our preliminary study.

The lesion has a distinctive histopathology and shows features suggestive of a human papillomavirus (HPV) lesion. These include the "hairs", which are due to localized acanthosis and parakeratosis, as well as marked koilocytosis - ballooning of prickle cells with pyknotic nuclei and perinuclear halos. In a very preliminary study, 74% of 23 biopsies showed evidence of papillomavirus antigen using an immunocytochemical technique. Presuming the lesion to be a form of papillomavirus-induced flat oral wart, we initiated an electronmicroscopic search for papillomavirus particles. In ~~only~~ ^{the} six cases examined to date, we have found no evidence of HPV. ^{virions.} However, to our surprise, 5/6 cases showed plentiful evidence of a herpes-type virus. We do not yet know if this is cytomegalovirus (CMV), herpes simplex (HSV) type 1 or 2, Epstein-Barr virus (EBV) or

? from other studies?
varicella-zoster virus (VZV).

The identification of the viruses or virus elements present in HL is at a preliminary stage. Perhaps an HPV type is present and complete and represents a fairly straightforward example of opportunistic infection in a host tissue which is immunocompromised both systemically and also locally. Langerhans cells (LC) are known to be decreased in the skin in AIDS (...), while the keratinized surface of the tongue is known to have areas devoid of LC (...). The second virus may then simply colonise the hyperplastic epithelium of the wart-like lesion produced by the HPV. However, papillomavirus particles are not seen and the herpes-type virus is present in very large quantities. Perhaps the HPV is defective, produces antigen and cytopathic effects but not complete virus particles. Perhaps a herpes-type virus (CMV, HSV^{-1 2} or ²EBV or VZV) is present and contributes to the lesion. Even more speculative and exciting are the possibilities, firstly that the herpes-type virus only is present and produces both the HPV antigen staining and causes the lesion, or secondly that a hybrid HPV/herpes-type virus is present. Such a hybrid between SV40 and an adenovirus has been described (...).

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The lesion is of interest for several further reasons. Thus, recent reports suggest a previously unsuspected and possibly widespread role for HPV in oral epithelial hyperplasia, dysplasia and carcinoma (...). Of interest in this regard is the observation of several cases of oral carcinoma in young male homosexuals in San Francisco (...). Similar relationships have been studied extensively between HPV and carcinoma of the uterine cervix. (...). There is also thought to be an association, as yet not well understood, between HSV⁻¹ and uterine carcinoma as well as a possible analogous association between oral cancer and HSV⁻¹ (...). Papillomaviruses (...), herpes simplex virus (...), cytomegalovirus (...), and EB virus (...) can all transform cells and behave as oncogenic agents.

Specific Aims

1. To further define and investigate the etiology, pathogenesis and natural history of oral "hairy" leukoplakia (HL), a newly described lesion which is part of the AIDS group of diseases.
2. To identify the virus elements thought to be present in the lesion and to assess their incidence in a group of HL cases.
3. To explore local, intramucosal, immune mechanisms by identifying and counting Langerhans cells (LC), including ^{the enumeration of} ~~enumerating~~ those bearing Ia antigen.
4. To follow HL cases prospectively and to attempt to define risk factors for the development of Pneumocystis carinii pneumonia (PCP). Further, to assess the risk, if any, of transformation of the lesion into oral squamous cell carcinoma.

Methods

Patients

Approximately 750 new patients are seen each year in the Oral Medicine Clinic at UCSF, among a total of almost 5,000 patient visits per year. At present, about 1 to 2 new cases of HL are being seen each week. We anticipate at least this rate of accession of new cases during the 8 months of the proposed grant period. The cases are referred by physicians and dentists in the city of San Francisco and adjoining areas, but most are referred from the AIDS Clinical Research Center/KS Clinic. As the new UCSF AIDS clinic becomes functional, and as awareness of the lesion spreads among clinicians, the referral rate may increase. We therefore anticipate no difficulty in recruiting 30 to 50 new cases for some or all parts of the study. In addition, paraffin blocks are available in 35 existing biopsies from 30 cases, while frozen tissue from 15 cases and serum from 14 cases are stored in the UCSF AIDS Specimen Bank. EM blocks have been prepared in 8 cases in addition to those described in the Introduction. Thus a good deal of material awaits investigation and the project can undergo major expansion as soon as funding becomes available.

All new patients will be invited to participate and those who agree will give a detailed medical and social history using the UCSF AIDS Clinical Research Center protocols. Complete oral exam will be performed by Dr. D. Greenspan, backed-up by Dr. T.E. Daniels and Dr. J.S. Greenspan if needed. Physical examination will be performed by Dr. H. Hollander in the UCSF AIDS Clinic or by Dr. M. Conant and his nurse-practitioner staff in the UCSF AIDS Clinical Research Center/KS Clinic. Clinical data, and ultimately all laboratory data, will be stored in the UCSF AIDS Clinical Research Center computer.

Oral Biopsy/Histopathology

White lesions suspected as representing the lesion of HL will be subjected to incisional biopsy using local anesthesia. We have not found suturing to be useful and healing is usually uneventful. The specimen will be divided as follows:

1. A portion will be routinely fixed and processed for histopathology and PAS staining. ^{is} ~~There~~ will be performed by the Oral Pathology service of the department, on which the Principal Investigator is an attending oral pathologist. The same paraffin blocks will be used for PAP staining for HSV antigen (see below).
2. A portion will be cut into 1mm^3 fragments for electron microscopy. (see below)
3. A portion will be used for cultural virological studies (see below)
4. The remainder will be quick-frozen in liquid nitrogen and then stored in sealed vials in liquid nitrogen in the tissue bank of the UCSF AIDS Specimen Bank. Cryostat sections will be cut for some of the virus studies and for the identification of Langerhan's cells.

Control Tissues

1. "POSITIVE" controls will include skin and oral verrucae and condylomata (control for HPV) HSV⁻¹ and 2 infected cells lines, CMV infected cell lines, EBV infected lymphoblasts and VZV infected cell lines.

no mention of samples for DNA work

2. "NEGATIVE" controls will be specimens from other oral epithelial lesions undergoing diagnostic biopsy in our clinics. These will include hyperkeratosis, dysplasia and carcinoma, candidiasis and lichen planus. We will also use normal oral mucosa obtained at biopsy and during plastic procedures.
3. A further, most important, control will be non-lesional tissue adjoining HL lesions. This control tissue should help us to establish if the agents found are confined to the HL lesion or are more widely present in the epithelium of these immunosuppressed homosexual patients.

Immunological Studies

Delayed cutaneous hypersensitivity will be tested using PPD, candida, mumps and trichophytin antigens. Assessment of peripheral blood lymphocyte subtype ~~numbers~~ ^{levels?} and T_4/T_8 ratios will be performed by Dr. Conrad Casavant in the Clinical Immunology laboratory at UCSF.

Ultrastructural Studies

Tissue fragments will be fixed for 12-48 hours in 3% glutaraldehyde ^{aldehyde} (...), postfixed for 1 hour in 1% buffered osmic acid, (...) dehydrated in graded ethanol and propylene oxide and embedded in EPON 812 (...). Thin sections will be cut from areas selected by examining semithin sections and will be double-stained on Formvar-coated grids with saturated uranyl acetate and modified lead citrate (...); sections will be screened for HPV ^{virus} and ^{for} other viruses by examining at least 100 cells from each case. The instrument to be used is the Siemens Elmiskop 1A, operating at 80KV, in the EM facility of the Division of Oral Medicine.

Virological Studies

Our intent is to:

1. Confirm or refute the presence of human papillomavirus (HPV).
2. ~~To determine if the herpes-type virus, seen in 5/6 cases on electron microscopy~~ ^{confirm the presence of} in our preliminary study, is indeed present.

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-1
3. To attempt to identify the latter using techniques to demonstrate HSV⁻¹ and 2, CMV, EBV and VZV. Details follow and the plan is summarized in Table 1.

a) Culture

Fresh tissue specimens will be transported to Dr. Drew's laboratory where attempts will be made to culture virus ^{from them} using host cells, notably human lung fibroblasts, suitable for members of the human herpesvirus group. Evidence of cytopathic effects will be sought.

b) Papillomavirus

The search for papillomavirus will include attempts to further identify HPV antigen, electromicroscopy for whole virus particles and ~~the use of~~ DNA probes. ^{hybridization with}

HPV antigen

Paraffin sections will be cut and subjected to the peroxidase-antiperoxidase technique using antisera to papillomavirus antigen. The technique to be used is that of Sternberger (....) and we will employ two antisera. Both ~~are~~ ^{have been} raised in rabbits using as antigen preparations of the core antigen of bovine papillomavirus. One is available commercially (DAKO) while the other is the gift of Dr. A.B. Jenson of Georgetown University. Control procedures will include the use of serum from non-immunized rabbits as well as incubations omitting each of the other stages. Sections will be preincubated with H_2O_2 to block endogenous peroxidase and then with normal swine serum to block existing activity against human antigens in the active secondary swine anti-rabbit antiserum. The final reaction product will be developed using aminoethylcarbazole (AEC) which produces a red ^e homogenous dye. This is more readily distinguished from the hematoxylin counterstain than is the brown reaction product of DAB. Control tissues will include oral verrucae (positive control) and oral hyperkeratosis,

carcinoma, candidiasis and lichen planus (negative controls, as well as non-lesional tissue from HL patients.

HPV particles on EM (see above)

HPV-DNA

Evidence for papillomavirus DNA will be sought by Dr. A.B. Jenson using probes provided by Professor H. zur Hausen. Twenty five probes for that number of HPV types are available, but initial experiments on DNA extracted from frozen HL tissue sent by us will be under non-stringent conditions to determine the presence of the entire HPV genome. Subsequent work will be directed at determining the type or types of HPV-DNA if present. Dr. Jenson will use the Southern blot technique (see below).

c) Herpes Simplex Virus 1 and 2

Experiments will involve culture, attempts to identify antigen in tissue sections and ~~the use of~~ ^{hybridization with} DNA probes. EM studies are also described above.

HSV antigen

The battery of 9 monoclonal antibodies provided by Dr. M. Zweig will be used. We will apply the avidin-biotin-peroxidase (ABC) technique (...) with which we have 4 years of experience. This will permit identification and localization of most stages of HSV ⁻¹ and 2. The monoclonals are:

Anti ICP4	58-S	HSV- ¹ ₁
Anti ICP5	56-S	HSV- ¹ ₁ Capsid protein
Anti ICP6	2-S	HSV- ¹ ₁
Anti ICP6	105-S	HSV-2
Anti ICP6	48-S	HSV- ¹ ₁ Cross reacting
Anti ICP8	39-S	HSV- ¹ ₁ DNA binding protein

? *Question*
if papilloma
virus DNA of
any type is
present
?

Anti gB	35-S	HSV-1 glycoprotein
Anti gB	3-S	HSV-1
Anti gD	4-S	HSV-1

will include
EcoRI fragments
B and F of

HSV-DNA

SUBSTITUTE

The probes for HSV-1 and 2 DNA, provided by Dr. R. Hyman will be used by Dr. J.

- recombinant be
- Patton
- will be hybridized to nitrocellulose blots of electrophoretically separated DNA from HL lesions, uninvolved mucosa from HL patients, and the same patient's blood leukocytes. Other controls will include blood leukocytes from healthy individuals and HSV-transformed cell lines as well as the untransformed parent cell lines from which they are derived. Blots of artificially constructed mixtures of various DNAs may also be used, including HSV-1 virion DNA and normal human DNA in a genome-equivalent ratio of 1:1. A further control will be a cloned, single copy normal human gene used as a probe to demonstrate that the method is adequately sensitive. Vector only probes (e.g. PACYC184) will be used as negative controls. Blots will be prepared from DNAs cut with several different restriction endonucleases, and the hybridization reactions will be performed under conditions of various degrees of stringency.

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Dr. Lennette will use human sera to identify CMV and EBV ^{by} in IF. Cryostat sections will be reacted with at least two sets of reference human sera of specificities: EBNA+/CMV-, EBNA-/CMV+ and EBNA+/CMV+ (minimum 6 sera per ^S cat~~e~~). A guinea pig complement/anti-guinea pig C3 system (fluorescein-conjugated) will be used.

EBV-DNA probes are available commercially and we will develop their use in the ~~in-situ~~ ^b hybridization assay.

f) Varicella-zoster~~x~~ virus

Dr. Lⁿennette will use human sera with antibodies to VZ^VA. Dr. Lennette is developing the use of new anti-VZV monoclonals and we will apply these also, as well as VZV-DNA probes when they become available.

The field of in-situ identification of viruses within tissues is developing rapidly. Most of the reagents and techniques are fairly new. Modifications and improvements can be expected to occur even during the period of this grant. We will apply these to our problem as they become available.

Langerhans Cell Studies

These will be supervised by Dr. T.E. Daniels

Langerhans cells (LC) will be identified in 10 ^M cryostat sections using adenosine triphosphatase (ATPase) histochemistry (...) and by immunohistochemistry using commercial monoclonal antibodies against the T6 antigen (...) and the Ia antigen (...) in the ABC technique (...). LC will be counted in standard fields and the results correlated with the nature and location of virus antigen or DNA in the immunohistochemical and in-situ techniques. Our hypothesis is that selective loss of LC is related to colonization by HPV and/or the as-yet-unidentified herpes-type virus. The control tongue biopsies from

non-lesional areas in our HL patients will clearly be very important in this aspect of our study.

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TABLE 1.

VIRUS	COMPONENT	TECHNIQUE	INVESTIGATOR	CONTROL TISSUE
HPV	Whole virus particles	EM	J. Greenspan	1. Oral warts/skin warts 2. Non-lesional tissue from HL cases 3. Other oral epithelial lesions Non-lesional HL patient tissue
	Antigen DNA	PAP/antisera Southern blot	J. Greenspan A.B. Jensen	
Herpes Group	Whole virus particles Live virus	EM culture	J. Greenspan Drew	1. Oral herpes, lesions, infected cells 2. Non-lesional HL patient tissue 3. Other oral lesions
HSV1 & 2	Live virus Antigens	Culture ABC/monoclonals	Greenspan/ Hwang	
	DNA	Southern blot	Felton/Hwang	
CMV	Antigens DNA DNA	IF/monoclonals In situ In situ hybridization	Lenette Drew Drew	1. Known positive CMV lesions/cells 2. Non-lesional HL patients tissue 3. Other oral lesions
EBV	Antigens DNA	IF/antisera In situ hybridization (when reagents are available & reliable)	Lenette Greenspan/ Felton	1. Known positive EBV lesions/cells 2. as above 3. as above
VZV	Antigens DNA	IF/antisera and in situ hybridization (when both are available)	Greenspan/ Lenette	2/3 as above
ARV	Antibodies in serum	IF	Levy	1. Normal controls 2. ARV positive sera.

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We believe that further investigation of the nature, etiology, pathogenesis and progress of this new entity within the AIDS spectrum, oral hairy leukoplakia, is appropriate for the following reasons:

1. Such information may improve understanding of the role of viruses and local immunosuppression in the pathogenesis of AIDS.
2. Identification of the viruses present will contribute to an understanding of why certain organisms cause opportunistic infections in AIDS. The elucidation of the relationship between the viruses could lead to increased understanding of defective oncogenic viruses, virus-virus interactions and hybrid viruses and their roles in infection, immunosuppression and oncogenesis.
3. Determining which cases progress to the full syndrome, with PCP, may help in earlier diagnosis of AIDS and ultimately contribute, in part, to its prevention and control.
4. Finally, investigation of the potential of this virally induced hyperplasia to undergo dysplastic and neoplastic transformation may contribute to elucidating the problem of neoplasia in AIDS.

XIII Progress Reports

The Principal Investigator is not currently a direct recipient of Task Force monies. However, he has received such funds in two ways. He is responsible for the UCSF AIDS Specimen Bank which is supported by the Task Force grant to the UCSF AIDS Clinical Research Center. Secondly, he has been the co-investigator on a Task Force grant to Jay Beckstead, M.D. Therefore, relevant portions of the progress reports of those two projects are attached. However, neither is directly related to the current proposal. The specimen bank will be used to accept and store HL specimens and sera, just as it is used to store tissue and serum for many other projects, and no funds are requested in the present proposal for such support activities provided by the specimen bank.

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7/24/84 ✓ DC
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XI. Explanation of Project

Title Oral viral leukoplakia - a new lesion in association with AIDS

Introduction/Background

Between October 1981 and July 1984, we have seen 37 cases of a previously unrecorded lesion of the tongue in male homosexuals in the San Francisco area. The lesions are on the lateral margins of the tongue and are of varying sizes. They are usually poorly demarcated and show a corrugated or "hairy" surface. Although usually painless, the lesions have been noticed by the patients, several of whom report spontaneous regression and reappearance. We have provisionally named the lesion hairy leukoplakia (HL).

Although all of our patients with HL have been in apparent good health at the time of diagnosis, all reported previous use of recreational drugs, most gave a history of hepatitis B and many had diffuse lymphadenopathy. Of a few cases tested for immunocompetence using skin tests and T_4/T_8 ratios as criteria, all showed some evidence of immunosuppression. None of our patients had AIDS at the time of diagnosis of HL, but 8/37 have developed PCP in the 33 months of our preliminary study.

The lesion has a distinctive histopathology and shows features suggestive of a human papillomavirus (HPV) lesion. These include the "hairs", which are due to localized acanthosis and parakeratosis, as well as marked koilocytosis - ballooning of prickle cells with pyknotic nuclei and perinuclear halos. In a very preliminary study, 74% of 23 biopsies showed evidence of papillomavirus antigen using an immunocytochemical technique. Presuming the lesion to be a form of papillomavirus-induced flat oral wart, we initiated an electronmicroscopic search for papillomavirus particles. In only six cases examined to date, we have found no evidence of HPV. However, to our surprise, 5/6 cases showed plentiful evidence of a herpes-type virus. We do not yet know if this is cytomegalovirus (CMV), herpes simplex (HSV) type 1 or 2, Epstein-Barr virus (EBV) or

varicella-zoster virus (VZV).

The identification of the viruses or virus elements present in HL is at a preliminary stage. Perhaps an HPV type is present and complete and represents a fairly straightforward example of opportunistic infection in a host tissue which is immunocompromised both systemically and also locally. Langerhans cells (LC) are known to be decreased in the skin in AIDS (...), while the keratinized surface of the tongue is known to have areas devoid of LC (...). ^(?) The second virus may then simply colonise the hyperplastic epithelium of the wart-like lesion produced by the HPV. However, papillomavirus particles are not seen and the herpes-type virus is present in very large quantities. Perhaps the HPV is defective, produces antigen and cytopathic effects but not complete virus particles. Perhaps a herpes-type virus (CMV, HSVI or II, EBV or VZV) is present and contributes to the lesion. Even more speculative and exciting are the possibilities, firstly that the herpes-type virus only is present and produces both the HPV antigen staining and causes the lesion, and secondly that a hybrid HPV/herpes-type virus is present. Such a hybrid between SV40 and an adenovirus has been described (...).

The lesion is of interest for several further reasons. Thus, recent reports suggest a previously unsuspected and possibly widespread role for HPV in oral epithelial hyperplasia, dysplasia and and carcinoma (...). Of interest in this regard is the observation of several cases of oral carcinoma in young male homosexuals in San Francisco (...). Similar relationships have been studied extensively between HPV and carcinoma of the uterine cervix. (...). There is also thought to an association, as yet not well understood, between HSV2 and uterine carcinoma as well as a possible analogous association between oral cancer and HSVI (...). Papillomaviruses (...), herpes simplex virus (...), cytomegalovirus (...), and EB virus (...) can all transform cells and behave as oncogenic agents.

Specific Aims

1. To further define and investigate the etiology, pathogenesis and natural history of oral "hairy" leukoplakia (HL), a newly described lesion which is part of the AIDS group of diseases.
2. To identify the virus elements thought to be present in the lesion and to assess their incidence in a group of HL cases.
3. To explore local, intramucosal, immune mechanisms by identifying and counting Langerhans cells (LC), including enumerating those bearing Ia antigen, *and try to observe their morphological relationships to HIV antigen-containing cells.*
4. To follow HL cases prospectively and to attempt to define risk factors for the development of Pneumocystis carinii pneumonia (PCP). Further, to assess the risk, if any, of transformation of the lesion into oral squamous cell carcinoma.

Methods

Patients

Approximately 750 new patients are seen each year in the Oral Medicine Clinic at UCSF, among a total of almost 5,000 patient visits per year. At present, about 1 to 2 new cases of HL are being seen each week. We anticipate at least this rate of accession of new cases during the 8 months of the proposed grant period. The cases are referred by physicians and dentists in the city of San Francisco and adjoining areas but most are referred from the AIDS Clinical Research Center/KS Clinic. As the new UCSF AIDS clinic becomes functional, and as awareness of the lesion spreads among clinicians, the referral rate may increase. We therefore anticipate no difficulty in recruiting 30 to 50 new cases for some or all parts of the study. In addition, paraffin blocks are available in 35 existing biopsies from 30 cases, while frozen tissue from 15 cases and serum from 14 cases are stored in the UCSF AIDS Specimen Bank. EM blocks have been prepared in 8 cases in addition to those described in the Introduction. Thus a good deal of material awaits investigation and the project can undergo major expansion as soon as funding becomes available.

All new patients will be invited to participate and those who agree will give a detailed medical and social history using the UCSF AIDS Clinical Research Center protocols. Complete oral exam will be performed by Dr. D. Greenspan, backed-up by Dr. T.E. Daniels and Dr. J.S. Greenspan if needed. Physical examination will be performed by Dr. H. Hollander in the UCSF AIDS Clinic or by Dr. M. Conant and his nurse-practitioner staff in the UCSF AIDS Clinical Research Center/KS Clinic. Clinical data, and ultimately all laboratory data, will be stored in the UCSF AIDS Clinical Research Center computer.

Oral Biopsy/Histopathology

*more or
eliminate
in CKP
protocol* White lesions suspected as representing the lesion of HL will be subjected to incisional biopsy using local anesthesia. (We have not found suturing to be useful and healing is usually uneventful.) The specimen will be divided as follows:

1. A portion will be routinely fixed and processed for histopathology and PAS staining. ^SThere will be performed by the Oral Pathology service of the department, on which the Principal Investigator is an attending oral pathologist. The same paraffin blocks will be used for PAP staining for HSV ^(P)antigen (see below). >
^
2. A portion will be cut into 1mm³ fragments for electronmicroscopy. (see below)
3. A portion will be used for cultural virological studies (see below)
4. The remainder will be quick-frozen in liquid nitrogen and then stored in sealed vials in liquid nitrogen in the tissue bank of the UCSF AIDS Specimen Bank. Cryostat sections will be cut for some of the virus studies and for the identification of Langerhan's cells.

Control Tissues

1. "POSITIVE" controls will include skin and oral verrucae and condylomata (control for HPV) HSV1 and 2 infected cells lines, CMV infected cell lines, EBV infected lymphoblasts and VZV infected cell lines.

2. "NEGATIVE" controls will be specimens from other oral epithelial lesions undergoing diagnostic biopsy in our clinics. These will include hyperkeratosis, dysplasia and carcinoma, candidiasis and lichen planus. We will also use normal oral mucosa obtained ~~at biopsy and~~ during plastic procedures.
3. A further, most important, control will be non-lesional tissue adjoining HL lesions. This control tissue should help us to establish if the agents found are confined to the HL lesion or are more widely present in the epithelium of these ~~(immunosuppressed homosexual)~~ patients.

Immunological Studies

Delayed cutaneous hypersensitivity will be tested using PPD, candida, mumps and trichophyton antigens. Assessment of peripheral blood lymphocyte subtype numbers and T_4/T_8 ratios will be performed by Dr. Conrad Casavant in the Clinical Immunology laboratory at UCSF.

Ultrastructural Studies

Tissue fragments will be fixed for 12-48 hours in 3% glutaraldehyde (...), postfixed for 1 hour in 1% buffered osmic acid, (...) dehydrated in graded ethanol and propylene oxide and embedded in EPON 812 (...). Thin sections will be cut from areas selected by examining semithin sections and will be double-stained on Formvar-coated grids with saturated uranylacetate and modified lead citrate (...); sections will be screened for HPV and other viruses by examining at least 100 cells from each case. The instrument to be used is the Siemens Elmiskop 1A, operating at 80KV, in the EM facility of the Division of Oral Medicine.

Virological Studies

Our intent is to:

1. Confirm or refute the presence of human papillomavirus (HPV).
2. To determine if the herpes-type virus, seen in 5/6 cases on electronmicroscopy in our preliminary study, is indeed present.

- making too wide*
3. To attempt to identify the latter using techniques to demonstrate HSV1 and 2, CMV, EBV and VZV. Details follow and the plan is summarized in Table 1.

a) Culture

Fresh tissue specimens will be transported to Dr Drew's laboratory where attempts will be made to culture virus using host ^{cells} notably human lung fibroblasts, suitable for members of the human herpesvirus group. Evidence of cytopathic effects will be sought.

b) Papillomavirus

← The search for papillomavirus will include attempts to further identify HPV antigen, electromicroscopy ^{to find} for whole virus particles and the use of DNA ^{HPV-} probes. ?

HPV antigen

Paraffin sections will be cut and subjected to the peroxidase-antiperoxidase technique using antisera to papillomavirus antigen. The technique to be used is that of Sternberger (...) and we will employ two antisera. Both are raised in rabbits using as antigen preparations of the core antigen of bovine papillomavirus. One is available commercially (DAKO) while the other is the gift of Dr. A.B. Jenson of Georgetown University. ~~Control procedures will include the use of serum from non-immunized rabbits as well as incubations omitting each of the other stages. Sections will be preincubated with H_2O_2 to block endogenous peroxidase and then with normal swine serum to block existing activity against human antigens in the active secondary swine anti-rabbit antiserum. The final reaction product will be developed using aminoethylcarbazole (AEC) which produces a red homogenous dye. This is more readily distinguished from the hematoxylin counterstain than is the brown reaction product of DAB.~~ Control tissues will include oral verrucae (positive control) and oral hyperkeratosis,

carcinoma, candidiasis and lichen planus (negative controls, as well as non-lesional tissue from HL patients.

HPV particles on EM (see above)

HPV-DNA

Evidence for papillomavirus DNA will be sought ^{to} by Dr. A.B. Jenson using probes provided by Professor H. zur Hausen. Twenty five probes for that number of HPV types are available but initial experiments ^{from} ~~on~~ DNA extracted from frozen HL tissue sent by us will be under non-stringent conditions to determine the presence of the entire HPV genome. Subsequent work will be directed at determining the type or types of HPV-DNA if present. Dr. Jenson will use the Southern blot technique (see below).

c) Herpes Simplex Virus 1 and 2

Experiments will involve culture, attempts to identify antigen in tissue sections and the use of DNA probes. EM studies are ^{also} ~~also~~ described above.

HSV antigen

The battery of 9 monoclonal antibodies provided by Dr. M Zweig will be used. We will apply the avidin-biotin-peroxidase (ABC) technique (...) with which we have 4 years of experience. This will permit identification and localization of most stages of HSV1 and 2. The ^{antibodies} ~~monoclonals~~ are:

Anti ICP4	58-S	HSV-1
Anti ICP5	56-S	HSV-1 Capsid protein
Anti ICP6	2-S	HSV-1
Anti ICP6	105-S	HSV-2
Anti ICP6	48-S	HSV-1 Cross reacting
Anti ICP8	39-S	HSV-1 DNA binding protein

Anti gB	35-S	HSV-I glycoprotein
Anti gB	3-S	HSV-I
Anti gD	4-S	HSV-I

HSV-DNA

The probes for HSVI and 2 DNA, provided by Dr. R. Hyman will be used by Dr. J. Felton in the Southern blot technique (...). The probes to ^{take? include} used HSV-I (strain Patton) as well as ^{Bgl II} ~~Bgl II~~ fragments ^{C and N} ~~and N~~ of HSV-2 (strain 333). There, the whole viral DNA probes, will be hybridized to nitrocellulose blots of electrophoretically separated DNA from HL lesions, uninvolved mucosa~~x~~ from HL patients and the same patient's blood leukocytes. Other controls will include blood leucocytes from healthy individuals, HSV-transformed cell lines as well as the untransformed parent cell lines from which they are derived. Blots of artifically constructed mixtures of various DNAs may also be used, including HSV-I virion DNA and normal human DNA in a genome equivalent ratio of 1:1. A further control will be a cloned, single copy normal human gene used as a probe to demonstrate that the method is adequately sensitive. Vector only probes (e.g. PACYC184) will be used as negative controls. Blots will be prepared from DNAs cut with several different restriction endonucleases, and the hybridization reactions will be performed under conditions of various degrees of stringency.

← d) Cytomegalovirus (CMV)

Experiments will involve culture and EM (see above) as well as attempts to identify CMV antigens and CMV-DNA

← CMV Antigen

Cryostat sections will be reacted with pooled ^{mouse} ~~mouse~~ monoclonal antibody to CMV (Immuglog/Syva Labs) in a biotin-avidin FITC system (EY Labs).

CMV-DNA

In-situ hybridization, using cryostat sections, will be applied and will involve the ENZO Biochem. Inc. "Patho-Gene" kit. A biotinylated DNA

probe is hybridized to CMV-DNA and is detected by a streptavidin-horseradish peroxidase-complex and DAB system.

e) Epstein-Barr virus (EBV)

Dr. Lennette will use human sera to identify CMV and EBV in IF. Cryostat sections will be reacted with at least two sets of reference human sera of specificities: EBNA+/CMV-, EBNA-/CMV+ and EBNA+/CMV+ (minimum 6 sera per ^Scate). A guinea pig complement/anti-guinea pig C3 system (fluorescein conjugated) will be used.

EBV-DNA probes are available commercially and we will develop their use in the in-situ hybridization assay.

f) Varicella-zoster-virus

Dr. Lennette will use human sera with antibodies to VZ^VA. Dr. Lennette is developing the use of new anti-VZV monoclonals and we will apply these also, as well as VZV-DNA probes when they become available.

The field of in-situ identification of viruses within tissues is developing rapidly. Most of the reagents and techniques are fairly new. Modifications and improvements can be expected to occur even during the period of this grant. We will apply these to our problem as they become available.

Langerhans Cell Studies

These will be supervised by Dr. T.E. Daniels

Langerhans cells (LC) will be identified in 10 μ m cryostat sections using adenosine triphosphatase (ATPase) histochemistry (...) and by immunohistochemistry using commercial monoclonal antibodies against the T6 antigen (...) and the Ia antigen (...) in the ABC technique (...). LC will be counted in standard fields and the results correlated with the nature and location of virus antigen or DNA in the immunohistochemical and in-situ techniques. Our hypothesis is that selective loss of LC is related to colonization by HPV and/or the as-yet-unidentified herpes-type virus. The control tongue biopsies from

non-lesional areas in our HL patients will clearly be very important in this aspect of our study.

Prospective Study

Our HL patients will be examined twice during the 8 months of this proposal and at intervals thereafter. We will record the development of features of AIDS, notably PCP, which has already occurred in 8 cases. We will attempt to assess risk factors for this complication and any others which develop. For this aspect of the study, we have enlisted the support of Dr. W. Winkelstein of the UCB School of Public Health who is the PI of a substantial NIAID longitudinal study of cohorts of male homosexuals and controls. He has assigned a graduate student to our study and we are in the process of developing our joint protocol. No funds are requested for this part of our HL investigation except for a small sum to fund assessment of the presence and titer of antibodies to ARV (AIDS associated retrovirus) by Dr. J. Levy. This virus appears to have many of the properties of HTLVIII (...) and LAV (...). We are also in the process of negotiating with Professor Chermann at the Pasteur Institute, Paris, to have LAV titers performed.

TABLE I.

VIRUS	COMPONENT	TECHNIQUE	INVESTIGATOR	CONTROL TISSUE
HPV	Whole virus particles	EM	J. Greenspan	1. Oral warts/skin warts
	Antigen DNA	PAP/antisera Southern blot	J. Greenspan A.B. Jensen	2. Non-lesional tissue from HL cases 3. Other oral ep-epithelial lesions
Herpes Group	Whole virus particles Live virus	EM culture	J. Greenspan Drew	Non-lesional HL patient tissue
HSV1 & 2	Live virus Antigens	Culture ABC/monoclonals	J. Greenspan/ Hwang	1. Oral herpes, lesions, infected cells
	DNA	Southern blot	Felton/Hwang	2. Non-lesional HL patient tissue 3. Other oral lesions
CMV	Antigens DNA DNA	IF/monoclonals In situ In situ hybridization	Lenette Drew Drew	1. Known positive CMV lesions/cells 2. Non lesional HL patients tissue 3. Other oral lesions
EBV	Antigens DNA	IF/antisera In-situ hybridization (when reagents are available & reliable)	Lenette J. Greenspan/ Felton	1. Known positive EBV lesions/cells 2. as above 3. as above
VZV	Antigens DNA	IF/antisera and in-situ hybridization (when both are available)	J. Greenspan/ Lenette	2/3 as above
ARV	Antibodies in serum	IF	Levy	1. Normal controls 2. ARV positive sera.

XII Relation of the Project to the AIDS Problem

We believe that further investigation of the nature, etiology, pathogenesis and progress of this new entity within the AIDS spectrum, oral hairy leukoplakia, is appropriate for the following reasons:

1. Such information may improve understanding of the role of viruses and local immunosuppression in the pathogenesis of AIDS.
2. Identification of the viruses present will contribute to an understanding of ~~why~~ ^{which?} certain ~~organisms~~ ^{too present} cause opportunistic infections in AIDS. The elucidation of the relationship between the ~~viruses~~ ^{too present} could lead to increased understanding of ~~(defective oncogenic viruses)~~ ^{or KS?} virus-virus interactions, and hybrid viruses and their roles in infection, immunosuppression and oncogenesis. ~~which?~~
3. Determining which cases progress to the full syndrome, with PCP, may help in earlier diagnosis of AIDS and ultimately contribute, in part, to its prevention and control.
4. Finally, investigation of the potential of this virally induced hyperplasia to undergo dysplastic and neoplastic transformation may contribute to elucidating the problem of neoplasia in AIDS.

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The Principal Investigator is not currently a direct recipient of Task Force monies. However, he has received such funds in two ways. He is responsible for the UCSF AIDS Specimen Bank which is supported by the Task Force grant to the UCSF AIDS Clinical Research Center. Secondly, he has been the co-investigator on a Task Force grant to Jay Beckstead M.D. Therefore, relevant portions of the progress reports of those two projects are attached. However, neither is directly related to the current proposal. The specimen bank will be used to accept and store HL specimens and sera, just as it is used to store tissue and serum for many other projects, and no funds are requested in the present proposal for such support activities provided by the specimen bank.

REFERENCES

1. GREEN SPAN D, GREEN SPAN JS, CONANT M et al. ORAL 'HAIRY' LEUKOPLAKIA IN MALE HOMOSEXUALS. 1984 Submitted
2. Ref # 18 of manuscript [attached]
BELSITO
3. Ref # 19 of ms. DANIELS.
4. Ref # 22 of ms. GRODZICKER
5. Ref # 24 " JENSON
6. Ref # 25 " LONING
7. Ref # 26 " STRJANEN
8. Ref # 27 " JARRETT
9. Ref # 28 " LOZADA-NOR
10. Ref # 29 " REID
11. Ref # 30 " GISSMAN
12. Ref # 31 " McDougall

A 10 GRANT reference

13 Ref # 32 from ms EGLIN

14 Ref # 33 from ms SHILLITOE

15 Ref # 34 " " HOWLEY

16 Ref # 35 " " RAPP

17 Ref # 36 " " GEDER

18 Ref # 37 " " WOLF

19 Ref # 1 of ms KARNOWSKY

20 Ref # 2 of ms PALADE

21 Ref # 3 of ms LUFT

22 Ref # 4 of ms REYNOLDS

23 Ref # 5 of ms WILLIAMS

24 Ref # 6 of ms FRITH OF

25 BENYESH - MELNICK M. Cytomegaloviruses.
In: LENNETTE E.H., SCHMIDT NT eds

Diagnostic procedures for viral and rickettsial infections [4th ed]. New York. American Public Health Association 1969. 701-732.

26 Hsu SM, RAINE Lord FANGER H. Use of avidin-biotin-peroxidase complex [ABC] in immunoperoxidase techniques. ~~1981~~. J. Histochem. Cytochem. 1981. 29 577-580

27 SOUTHERN EM; Detection of specific sequences ~~of~~ among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 1975. 98 503-517

28 REEDMAN BM, KLEING. Cellular localization of Epstein-Barr virus [EBV] associated complement-fixing antigen in producer and non-producer lymphoblastoid cell lines. Int. J. Cancer. 1973. 11 499-520

29 WOLF K and WINKELMANN RK. Quantitative studies ~~of~~ on the Langerhans' cell population of guinea pig epidermis. J. invest. Derm. 1967. 42 504-513

30. FITHIAN E, KUNG P, GOLDSTEIN, G et al.
Reactivity of Langerhans cells with
hybridoma antibody. Proc. Natl. Acad.
Sci. ~~78~~ 1981. 78 2541-2544

31 ROWDEN G, LEWIS MG, and SULLIVAN
A L. Ia antigen on human
epidermal Langerhans cells.
Nature. 1977. 268 247-248.

32 Ref # 15 of ms POPOVIC

33 Ref # 16 of ms BARRE-SINOUSSE.

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22-99

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The lesion has a distinctive histopathology and shows features suggestive of a human papillomavirus (HPV) lesion. These include the "hairs", which are due to localized acanthosis and parakeratosis, as well as marked koilocytosis - ballooning of prickle cells with pyknotic nuclei and perinuclear halos. In a very preliminary study, 74% of 23 biopsies showed evidence of papillomavirus antigen using an immunocytochemical technique. (FIG 3a-h APPENDIX) Presuming the lesion to be a form of papillomavirus-induced flat oral wart, we initiated an electronmicroscopic search for papillomavirus particles. In ^{the} only six cases examined to date, we have found no evidence of HPV. ^{virions} However, to our surprise, 5/6 cases showed plentiful evidence of a herpes-type virus. (FIG 4a-h APPENDIX) We do not yet know if this is cytomegalovirus (CMV), herpes simplex (HSV) type 1 or 2, Epstein-Barr virus (EBV) or

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Delayed cutaneous hypersensitivity will be tested using PPD, candida, mumps and trichophytin antigens. Assessment of peripheral blood lymphocyte subtype ^{levels} ~~number~~ and T_4/T_8 ratios will be performed by Dr. Conrad Casavant in the Clinical Immunology laboratory at UCSF.

Ultrastructural Studies

Tissue fragments will be fixed for 12-48 hours in 3% ^{glutaraldehyde} ~~glutared~~ (...), postfixed for 1 hour in 1% buffered osmic acid, ⁽²⁰⁾ ~~(...)~~ dehydrated in graded ethanol and propylene oxide and embedded in EPON 812 ⁽²¹⁾ ~~(...)~~. Thin sections will be cut from areas selected by ^{light} ~~microscope~~ examining ^{section of} ~~semithin~~ sections and will be double-stained on Formvar-coated grids with saturated uranyl acetate and modified lead citrate ⁽²²⁾ ~~(...)~~; sections will be screened for HPV ^{virus} ~~virus~~ ⁽²³⁻²⁴⁾ ~~(...)~~ and ^{for} ~~other~~ viruses by examining at least 100 cells from each case. The instrument to be used is the Siemens Elmiskop 1A, operating at 80KV, in the EM facility of the Division of Oral Medicine.

Virological Studies

Our intent is to:

1. Confirm or refute the presence of human papillomavirus (HPV).
2. To ^{confirm the presence of} ~~determine if~~ the herpes-type virus, seen in 5/6 cases on electron microscopy in our preliminary study, ~~is indeed present~~.

3. To attempt to identify the latter using techniques to demonstrate HSV⁻¹ and 2, CMV, EBV and VZV. Details follow and the plan is summarized in Table 1.

a) Culture (25)

Fresh tissue specimens will be transported to Dr Drew's laboratory where attempts will be made to culture virus ^{from them} using host cells, notably human lung fibroblasts, suitable for members of the human herpesvirus group. Evidence of cytopathic effects will be sought.

b) Papillomavirus

The search for papillomavirus will include attempts to further identify HPV antigen, electromicroscopy ^{to find} for whole virus particles and ^{HPV} the use of DNA probes. hybridization with

HPV antigen

Paraffin sections will be cut and subjected to the peroxidase-antiperoxidase technique using antisera to papillomavirus antigen. ~~The technique to be used is that of Sternberger (....) and we will employ two antisera. Both are~~ ^{have been} raised in rabbits using as antigen preparations of the core antigen of bovine papillomavirus. One is available commercially (DAKO) while the other is the gift of Dr. A.B. Jenson of Georgetown University. Control procedures will include the use of serum from non-immunized rabbits as well as incubations omitting each of the other stages. ~~Sections will be preincubated with H₂O₂ to block endogenous peroxidase and then with normal swine serum to block existing activity against human antigens in the active secondary swine anti-rabbit antiserum. The final reaction product will be developed using aminoethylcarbazole (AEC) which produces a red homogenous dye. This is more readily distinguished from the hematoxylin counterstain than is the brown reaction product of DAB.~~ Control tissues will include oral verrucae (positive control) and oral hyperkeratosis,

The Southern blot technique (see below) and

carcinoma, candidiasis and lichen planus (negative controls, as well as non-lesional tissue from HL patients.

HPV particles on EM (see above)

HPV-DNA

Evidence for papillomavirus DNA will be sought ^{to} by Dr. A.B. Jenson using probes provided by Professor H. zur Hausen. Twenty five probes for that number of HPV types are available, but initial experiments ^{from} on DNA

extracted from frozen HL tissue sent by us will be under non-stringent conditions to determine ~~the presence of the entire HPV genome.~~ ^{if human papillomavirus DNA of any type is present.}

Subsequent work will be directed at determining the type or types of HPV-DNA if present. ~~Dr. Jenson will use the Southern blot technique (see below).~~

c) Herpes Simplex Virus ⁻¹ and 2

Experiments will involve culture, attempts to identify antigen in tissue sections and ^{hybridization with} ~~the use of~~ DNA probes. EM studies are ~~also~~ described above.

HSV antigen

The battery of 9 monoclonal antibodies provided by Dr. M. Zweig will be used. We will apply the avidin-biotin-peroxidase (ABC) technique ²⁶ with which we have 4 years of experience. This will permit identification and

localization of most ⁻¹ ~~stages~~ of HSV ^{antibodies} and 2. The ~~monoclonals~~ are: ^{Anti-ICP4, 58S}
[HSV-1]; ^{Anti-ICP5, 56S} [HSV-1 capsid protein] - 70 on

Anti ICP4	58-S	HSV- ⁻¹
Anti ICP5	56-S	HSV- ⁻¹ Capsid protein
Anti ICP6	2-S	HSV- ⁻¹
Anti ICP6	105-S	HSV-2
Anti ICP6	48-S	HSV- ⁻¹ Cross reacting
Anti ICP8	39-S	HSV- ⁻¹ DNA binding protein

Reenter
one after
another
to save
space

Substitute

HSV-DNA

The probes for HSV-1 and 2 DNA, provided by Dr. R. Hyman will be used by Dr. J. Felton in the Southern blot technique (21). The recombinant probes to be used will include EcoRI fragments B and F of HSV-1 (Patton strain) as well as BglIII fragments C and N of HSV-2 (333 strain). Vector-only probes (e.g., pACYC184) will be used as negative controls. A further control will be a cloned, single-copy normal human gene used as a probe to demonstrate that the method is adequately sensitive. All the above probes, and also probes consisting of whole, intact HSV-1 and 2 DNA, will be ^{made radioactive and} hybridized to nitrocellulose blots of electrophoretically separated DNA from HL lesions, uninvolved mucosa of HL patients, and the same patient's blood leukocytes. Other controls will include DNA from blood leukocytes of healthy individuals and from HSV-transformed cell lines ^{and} ~~as well as~~ the untransformed ^{parent} cell lines from which they are derived. Blots of artificially constructed mixtures of various DNA's [?] may also be used, for example HSV-1 virion DNA and normal human DNA mixed in a genome-equivalent ratio of 1:1. Blots will be prepared from DNAs [?] cut with several different restriction endonucleases, and the hybridization reactions will be performed under conditions of various degrees of stringency.

Carol
see previous page

Anti gB	35-S	HSV-1 glycoprotein
Anti gB	3-S	HSV-1
Anti gD	4-S	HSV-1

HSV-DNA

see attached substitute

The probes for HSV1 and 2 DNA, provided by Dr. R. Hyman will be used by Dr. J. Felton in the Southern blot technique (...). The probes to be used HSV-1 (strain Patton) as well as ~~BglII~~ fragments ^{be include} ~~card~~ ^{card N} of HSV-2 (strain 333). There, the whole viral DNA probes, will be hybridized to nitro-cellulose blots of electrophoretically separated DNA from HL lesions, uninvolved mucosa, from HL patients and the same patient's blood leukocytes. Other controls will include blood leukocytes from healthy individuals, HSV-transformed cell lines as well as the untransformed parent cell lines from which they are derived. Blots of artifically constructed mixtures of various DNAs may also be used, including HSV-1 virion DNA and normal human DNA in a genome equivalent ratio of 1:1. A further control will be a cloned, single copy normal human gene used as a probe to demonstrate that the method is adequately sensitive. Vector only probes (e.g. PACYC184) will be used as negative controls. Blots will be prepared from DNAs cut with several different restriction endonucleases, and the hybridization reactions will be performed under conditions of various degrees of stringency.

d) Cytomegalovirus (CMV)

Experiments will involve culture and EM (see above) as well as attempts to identify CMV antigens and CMV-DNA

CMV Antigen

Cryostat sections will be reacted with pooled ^{mouse} ~~mouse~~ monoclonal antibody to CMV (Immuglog/Syva Labs) in a biotin-avidin FITC system (EY Labs).

CMV-DNA

In-situ hybridization, using cryostat sections, will be applied and will involve the ENZO Biochem. Inc. "Patho-Gene" kit. A biotinylated DNA

probe is hybridized to CMV-DNA and is detected by a streptavidin-horseradish peroxidase-complex and DAB system.

e) Epstein-Barr virus (EBV)

Dr. Lennette will use human sera to identify CMV and EBV in IF. Cryostat sections will be reacted with at least two sets of reference human sera of specificities: EBNA+/CMV-, EBNA-/CMV+ and EBNA+/CMV+ (minimum 6 sera per case). A guinea pig complement/anti-guinea pig C3 system (fluorescein conjugated) will be used. (28)

EBV-DNA probes are available commercially and we will develop their use in the in-situ hybridization assay.

f) Varicella-zoster-virus

Dr. Lennette will use human sera with antibodies to VZA. Dr. Lennette is developing the use of new anti-VZV monoclonals and we will apply these also, as well as VZV-DNA probes when they become available.

The field of in-situ identification of viruses within tissues is developing rapidly. Most of the reagents and techniques are fairly new. Modifications and improvements can be expected to occur even during the period of this grant. We will apply these to our problem as they become available.

Langerhans Cell Studies

These will be supervised by Dr. T.E. Daniels

Langerhans cells (LC) will be identified in 10 μ m cryostat sections using adenosine triphosphatase (ATPase) histochemistry (29, 30) and by immunohistochemistry using commercial monoclonal antibodies against the T6 antigen (30) and the Ia antigen (31) in the ABC technique (26). LC will be counted in standard fields and the results correlated with the nature and location of virus antigen or DNA in the immunohistochemical and in-situ techniques. Our hypothesis is that selective loss of LC is related to colonization by HPV and/or the as-yet-unidentified herpes-type virus. The control tongue biopsies from non-lesion

^{tongue}
non-lesional areas in our HL patients will clearly be very important in this aspect of our study.

Prospective Study

Our HL patients will be examined twice during the 8 months of this proposal and at intervals thereafter. We will record the development of features of AIDS, notably PCP, which has already occurred in 8 cases. We will attempt to assess risk factors for this complication and any others which develop. For this aspect of the study, we have enlisted the support of Dr. W. Winkelstein of the UCB School of Public Health who is the PI of a substantial NIAID longitudinal study of cohorts of male homosexuals and controls. He has assigned a graduate student to our study and we are in the process of developing our joint protocol. No funds are requested for this part of our HL investigation except for a small sum to fund assessment of the presence and titer of antibodies to ARV (AIDS associated retrovirus) by Dr. J. Levy. This virus appears to have many of the properties of HTLV^{-III}_{III} (32) and LAV (33). We are also in the process of negotiating with Professor Chermann at the Pasteur Institute, Paris, to have LAV titers performed.

TABLE I.

VIRUS	COMPONENT	TECHNIQUE	INVESTIGATOR	CONTROL TISSUE
HPV	Whole virus particles	EM	J. Greenspan	1. Oral warts/skin warts 2. Non-lesional tissue from HL cases 3. Other oral epithelial lesions Non-lesional HL patient tissue
	Antigen DNA	PAP/antisera Southern blot	J. Greenspan A.B. Jenson	
Herpes Group	Whole virus particles Live virus	EM culture	J. Greenspan Drew	Non-lesional HL patient tissue
HSV1 & 2	Live virus	Culture	J. Greenspan/ Hwang	1. Oral herpes, lesions, infected cells 2. Non-lesional HL patient tissue 3. Other oral lesions
	Antigens	ABC/monoclonals		
	DNA	Southern blot	Felton/Hwang	
CMV	Antigens	IF/monoclonals	Lenette	1. Known positive CMV lesions/cells 2. Non lesional HL patients tissue 3. Other oral lesions
	DNA DNA	In situ In situ hybridization	Drew Drew	
EBV	Antigens	IF/antisera	Lenette	1. Known positive EBV lesions/cells 2. as above 3. as above
	DNA	In situ hybridization (when reagents are available & reliable)	J. Greenspan/ Felton	
VZV	Antigens	IF/antisera	J. Greenspan/ Lenette	2/3 as above
	DNA	and in situ hybridization (when both are available)		
ARV	Antibodies in serum	IF	Levy	1. Normal controls 2. ARV positive sera.

XII Relation of the Project to the AIDS Problem

We believe that further investigation of the nature, etiology, pathogenesis and progress of this new entity within the AIDS spectrum, oral hairy leukoplakia, is appropriate for the following reasons:

1. Such information may improve understanding of the role of viruses and local immunosuppression in the pathogenesis of AIDS.
2. Identification of the viruses present will contribute to an understanding of ~~why~~ ^{which} ~~certain~~ organisms cause opportunistic infections in AIDS. The elucidation of the relationship between the viruses ~~present~~ ^{present} could lead to increased understanding of ~~defective oncogenic viruses~~, virus-virus interactions, ~~and~~ ^{works} hybrid viruses and their roles in infection, immunosuppression and oncogenesis.
3. Determining which cases progress to the full syndrome, with PCP, ^{works} may help in earlier diagnosis of AIDS and ultimately contribute, in part, to its prevention and control.
4. Finally, investigation of the potential of this virally induced hyperplasia to undergo dysplastic and neoplastic transformation may contribute to elucidating the problem of neoplasia in AIDS.

15

XIII Progress Reports

The Principal Investigator is not currently a direct recipient of Task Force monies. However, he has received such funds in two ways. He is responsible for the UCSF AIDS Specimen Bank which is supported by the Task Force grant to the UCSF AIDS Clinical Research Center. Secondly, he has been the co-investigator on a Task Force grant to Jay Beckstead M.D. Therefore, relevant portions of the progress reports of those two projects are attached. However, neither is directly related to the current proposal. The specimen bank will be used to accept and store HL specimens and sera, just as it is used to store tissue and serum for many other projects, and no funds are requested in the present proposal for such support activities provided by the specimen bank.

LEGENDS TO FIGURES

Fig 1 Clinical appearance of the lesion

Fig 2a H & E stained section
original mag x 16
surface projections (large arrow)
K Koilocytosis (small arrow)

Fig 2b H & E stained section
original mag x 100
K Koilocytotic ^{prickle} ~~prickle~~ cells (arrow)

PRICKLE

Fig 3a PAP reaction for papillomavirus antigen
original mag x 160
Note positive staining in nuclei (arrows)

Fig 3b control procedure-normal rabbit serum
original mag x 160
nuclei are not stained (arrows)

Fig 4 ~~HERPES-TYPE VIRUS~~ Herpes-type virus

Fig 4a ^{mag} ~~Mag~~ x 50,000
^{is} ~~in~~tranuclear virions 100 nm diameter (hollow arrows)
^{is} ~~in~~uclear-enveloped virions, 175 nm diameter (solid arrows)

Fig 4b Mag x 60,000
virions 250 nm diameter in intercellular spaces
~~DESMISOME (D)~~ desmosome (D)

① single space
② no gaps between
ref. please

REFERENCES

1. Greenspan, D., Greenspan, J.S., Conant, M., et al. Oral "Hairy" Leukoplakia in male homosexuals. 1984 submitted.
2. Belsito, D., Sanchez, M.R., Baer, R.L., et al. Reduced Langerhan's cell Ia antigen and ATPase activity in patients with the aquired immunodeficiency syndrome. N. Engl. J. Med. 1984 310:1279-1282.
3. Daniels, T.E. Human mucosal Langerhan's cells: postmortem identification of regional variation in oral mucosa. J. Invest. Derm. 1984 21-24.
4. Grodzicker, T., Adenovirus - SV40 hybrids. Chap. II in: DNA Tumor Viruses, ed Tooze, J. 1980 Cold Spring Harbor Laboratory 577-614.
- 5.. Jenson, A.B., Lancaster, W.D., Hartmann, D.P. and Shaffer, E.L. Frequency and distribution of papillomavirus structural antigens in verrucae, multiple papillomas and condylomata of the oral cavity. Am. J. Path. 1982 107:212-218.
6. Loning, T., Reichart, P., Staquet, M.J., et al. Occurence of papillomavirus structural antigens in oral papillomas and leukoplakias. J. Oral. Path. 1984 13:155-165.
7. Syrjanen, K.J., Syrjanen, S.M., Lamberg, M.A. and Pyrhonen, S. Human papillomavirus (HPV) involvement in squamous cell lesions of the oral cavity. Proc. Finn. Dent. Soc. 1983 79:1-8.
8. Jarrett, W.F.H., McNiel, P.E., Grimshaw, W.R., et al. High incidence of cattle

cancer with a possible interaction between an environmental carcinogen and a papilloma virus. Nature 1978 274:215-217.

9. Lozada-Nur, F., Silverman, S., Migliorati, C., et al. Oral manifestations of tumor and opportunistic infections in the acquired immunodeficiency syndrome (AIDS). Oral Surg. 1983 56:491-494.
10. Reid, R., Crum, C.P., Herschman, B.R., et al. Genital warts and cervical cancer. Cancer 1984 53:943-953.
11. Gissman, L., Boshart, M., Durst, M., et al. Presence of human papillomavirus in genital tumors. J. Invest. Derm. 1984 83:26s-28s.
12. McDougall, J.K., Crum, C.P., Fenoglio, C.M., et al. Herpesvirus-specific RNA and protein in carcinoma of the uterine cervix. Proc. Natl. Acad. Sci. USA 1982 79:3858-3857.
13. Eglin, R.P., Scully, C., Lehner, T., et al. Detection of RNA complementary to herpes simplex virus in human oral squamous cell carcinoma. Lancet. 1983 ii:766-768.
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14. Shillitoe, E.J., Greenspan, D., Greenspan, J.S., and Silverman, S. Antibody to early and late antigens of herpes simplex virus type 1 in patients with oral cancer. Cancer 1984 54:266-273.
15. Howley, P.M. The molecular biology of papillomavirus transformation. Am. J. Path. 1983 113:414-421.

16. Rapp, F., Duff, R. Transformation of hamster-embryo fibroblasts by herpes simplex viruses types 1 and 2. *Cancer Res.* 1973 33:1527-1534.
17. Geder, L., Kreider, J., Rapp, F. Human cells transformed in vitro by human cytomegalovirus: tumorigenicity in athymic nude mice. *J. Natl. Cancer Inst.* 1977 58:1003-1007.
18. Wolf, H., and Seibl, R. Benign and malignant disease caused by EBV. *J. Invest. Derm.* 1984 83:88s-95s.
19. Karnovsky, M.A. F formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. *J. Cell Biol.* 1965 27:#2, 137A-138A.
20. Palade, G.E. A study of fixation for electron microscopy. *J. Exp. Med.* 1952 95:285-297.
21. Luft, J.H. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol* 1961 9:409-414.
22. Reynolds, E.S. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 1963 17:208-212.
23. Williams, M.G., Howatson, A.F. and Almeida J.D. Morphological characterization of the virus of the human common wart (verruca vulgaris). *Nature* 1961 189:895-897.165

24. Frithiof, L. and Wersall, J. Virus-like particles in papillomas of the human oral cavity. Arch. Virusforschung 1967 21:31-44.
25. Benyesh-Melnick, M. Cytomegaloviruses. In: Lennette, E. H., Schmidt, N.J. eds. Diagnostic procedures for viral and rickettsial infections (4th ed). New York American Public Health Association 1969 701-732.
26. Hsu, S.M., ^{Raine}RAine, L. and Fanger, H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques. J. Histochem. Chtochem. 1981 29:577-580.
27. Southern, E.M. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 1975 98:503-517.
28. Reedman, B.M., Klein, G. Cellular localization of Epstein-Barr virus (EBV) associated complement-fixing antigen in producer and non-producer lymphoblastoid cell lines. Int. J. Cancer. 1973 11:499-520.
29. Wolf, K. and Winkelmann, R.K. Quantitative studies on the Langerhorn's cell population of guinea pig epidemics. J. Invest. Derm. 1967 48:504-513.
30. Fithian, E., Kung, P., Goldstein, G., et al. Reactivity of Langerhan's cells with hybridoma antibody. Proc. Natl. Acad. Sci. 1981 78:2541-2544.
31. Rowden, G., Lewis, M.G., and Sullivan, A.L. Ia antigen on human epidermal Langerhan's cells. Nature 1977 268:247-248.
32. Popovic, M., Sarngadharan, M.G., Read, E. and Gallo, R.C. Detection, isolation and

continuous production of cytopathic retroviruses (HTLIII) from patients with AIDS and pre-AIDS. Science 1984 224:497-500.

33. Barre-Sinoussi, F., Chermann, J.C., Rey, F., et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immunodeficiency syndrome (AIDS). Science 1983 220:868-871.

References

1. Karnovsky, M.A. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. *J. Cell Biol.* 1965 27:#2, 137A-138A.
2. Palade, G.E. A study of fixation for electron microscopy. *J. Exp. Med.* 1952 95:285-297.
3. Luft, J.H. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol* 1961 9:409-414.
4. Reynolds, E.S. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 1963 17:208-212.
5. CDC. AIDS update. *MMWR* 1983 309-311.
6. Jenson, A.B., Sommers, S., Payling-Wright, C., et al. Human papillomavirus: frequency and distribution in plantan and common warts. *Lab. Invest.* 1982 47:491-497.
7. Crum, C., Ikenberg, H., Richart, R.M. and Gissman, L. Human papillomavirus type 16 and early cervical neoplasia. *N. Eng. J. Med.* 1984 310:880-883.
8. Williams, M.G., Howatson, A.F. and Almeida J.D. Morphological characterization of the virus of the human common wart (verruca vulgaris). *Nature* 1961 189:895-897.
9. Frithiof, L. and Wersall, J. Virus-like particles in papillomas of the human oral cavity. *Arch. Virusforschung* 1967 21:31-44.
10. Biggs, P.M., de The', G., Payne, L.N. Oncogeneis and herpes viruses. IARC Scientific Publications No 2. IARC Lyons 1972
11. WHO Collaborating Centre for Oral Precancerous Lesions. Definition of leukoplakia and related lesions: as aid to studies in oral precancer. *Oral Surg.* 1978 46:581-539.
12. Gross, G., Pfister, H., Hagedorn, M. and Gissmann, L. Correlation between human papillomavirus (HPV) type and histology of warts. *J. Invest. Drms.* 1982 78:160-164.
13. Oguchi, M., Komura, J., Tagami, H. and Ofuji, S. Ultrastructural studies of spontaneously regressing plane warts. *Arch. Dermatol. Res.* 1981 270:403-411.
14. Murray, H.W., Rubin, B.Y., Masur, H. and Roberts, R.R. Impaired production of lymphokines and immune (gamma) interferon in the aquired immunodeficiency syndrome. *N. Engl. J. Med.* 1984 310:883-889.
15. Popovic, M., Sarngadharan, M.G., Read, E. and Gallo, R.C. Detection, isolation and continuous production of cytopathic retroviruses (HTLIII) from patients with AIDS and pre-AIDS. *Science* 1984 224:497-500.
16. Barre-Sinoussi, F., Chermann, J.C., Rey, F., et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for aquired immunodeficiency syndrome (AIDS). *Science* 1983 220:868-871.

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17. Lutzner, M., Croissant, O., Ducasse, M.F., et al. A potentially oncogenic human papillomavirus (HPV 5) found in two renal allograft recipients. *J. Invest. Derm.* 1980 75:353-356.
 18. Belsito, D., Sanchez, M.R., Baer, R.L., et al. Reduced Langerhan's cell Ia antigen and ATPase activity in patients with the acquired immunodeficiency syndrome. *N. Engl. J. Med.* 1984 310:1279-1282.
 19. Daniels, T.E. Human mucosal Langerhan's cells: postmortem identification of regional variation in oral mucosa. *J. Invest. Derm.* 1984 21-24.
 20. Toews, G.B., Bergstressen, P.R., Streilein, J.W. Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. *J. Immunol.* 1980 124:445-453.
 21. Sixbey, J.W., Nedrud, J.G., Raab-Traub, H., et al. Epstein-Barr virus replication in oropharyngeal epithelial cells. *N. Engl. J. Med.* 1984 310:1225-30.
 22. Grodzicker, T., Adenovirus - SV40 hybrids. Chap. II in *DNA Tumor Viruses* ed Tooze, J. 1980 Cold Spring Harbor Laboratory 577-614.
 23. Mathur-Waugh, U.L., Enlow, R.W., Spigland, I., et al. Longitudinal study of persistent generalized lymphadenopathy in homosexual men: relation to acquired immunodeficiency syndrome. *Lancet* 1984 i:1033-1038.
 24. Jenson, A.B., Lancaster, W.D., Hartmann, D.P. and Shaffer, E.L. Frequency and distribution of papillomavirus structural antigens in verrucae, multiple papillomas and condylomata of the oral cavity. *Am. J. Path.* 1982 107:212-218.
 25. Loning, T., Reichart, P., Staquet, M.J., et al. Occurrence of papillomavirus structural antigens in oral papillomas and leukoplakias. *J. Oral. Path.* 1984 13:155-165.
 26. Syrjanen, K.J., Syrjanen, S.M., Lamberg, M.A. and Pyrhonen, S. Human papillomavirus (HPV) involvement in squamous cell lesions of the oral cavity. *Proc. Finn. Dent. Soc.* 1983 79:1-8.
 27. Jarrett, W.F.H., McNeil, P.E., Grimshaw, W.R., et al. High incidence of cattle cancer with a possible interaction between an environmental carcinogen and a papilloma virus. *Nature* 1978 274:215-217.
 28. Lozada-Nur, F., Silverman, S., Migliorati, C., et al. Oral manifestations of tumor and opportunistic infections in the acquired immunodeficiency syndrome (AIDS). *Oral Surg.* 1983 56:491-494.
 29. Reid, R., Crum, C.P., Herschman, B.R., et al. Genital warts and cervical cancer. *Cancer* 1984 53:943-953.
 30. Gissman, L., Boshart, M., Durst, M., et al. Presence of human papillomavirus in genital tumors. *J. Invest. Derm.* 1984 83:26s-28s.
 31. McDougall, J.K., Crum, C.P., Fenoglio, C.M., et al. Herpesvirus-specific RNA and protein in carcinoma of the uterine cervix. *Proc. Natl. Acad. Sci. USA*

1982 79:3858-3857.

- 13 32. Eglin, R.P., Scully, C., Lehner, T., et al. Detection of RNA complementary to herpes simplex virus in human oral squamous cell carcinoma. *Lancet*. 1983 ii:766-768.
- 14 33. Shillitoe, E.J., Greenspan, D., Greenspan, J.S., and Silverman, S. Antibody to early and late antigens of herpes simplex virus type I in patients with oral cancer. *Cancer* 1984 54:266-273.
- 15 34. Howley, P.M. The molecular biology of papillomavirus transformation. *Am. J. Path.* 1983 113:414-421.
- 16 35. Rapp, F., Duff, R. Transformation of hamster-embryo fibroblasts by herpes simplex viruses types I and 2. *Cancer Res.* 1973 33:1527-1534.
- 17 36. Geder, L., Kreider, J., Rapp, F. Human cells transformed in vitro by human cytomegalovirus: tumorigenicity in athymic nude mice. *J. Natl. Cancer Inst.* 1977 58:1003-1007.
- 18 37. Wolf, H., and Seibl, R. Benign and malignant disease caused by EBV. *J. Invest. Derm.* 1984 83:88s-95s.

BUDJET JUSTIFICATION

I. Personnel

Principal Investigator

Dr. J. S. Greenspan will plan, supervise and review all aspects of the study. He will liaise with coinvestigators and consultants and maintain all data. He will prepare reports, abstracts and papers. He will present the results at meetings and symposia.

Co-Investigators:

1. Dr. D. Greenspan will be responsible for all clinical aspects of the study including seeing the patients, obtaining informed consent, performing biopsies and providing appropriate specimens for the PI, other co-investigators and consultants. She will collate clinical and laboratory data and analyse it to establish correlations. She will participate in the preparation of reports, will prepare abstracts, give presentations at meetings and prepare manuscripts.
2. Dr. J. Felton will be responsible for the herpes simplex virus DNA studies. He will supervise technical staff who perform Southern blot and other assays and will be involved in the day-to-day performance of the assays. He will contribute to reports, prepare abstracts, give presentations and prepare manuscripts.
3. Dr. T.E. Daniels will provide clinical back-up to Dr. D. Greenspan. He will be responsible for the Langerhan's cell studies including supervising technical staff, performing counts and analysing data. He will contribute to reports, prepare abstracts, give presentations and prepare manuscripts.

Other UCSF Participants (see letters)

1. Dr. W.L. Drew will be responsible for the cytomegalovirus studies including viral culture, immunofluorescence and immunoperoxidase using monoclonal antibodies, and in-situ hybridization using DNA probes.
2. Dr. S. Silverman will consult on clinical aspects of the project.
3. Dr. J. Levy will detect antibody to the AIDS associated retrovirus (ARV) in sera from HL patients. He will initiate studies to culture ARV from HL biopsy tissue.

Consultants

(see letters)

1. Dr. E. Lennette will investigate the presence of Epstein Barr virus, cytomegalovirus, and varicella zoster virus. She will use immunofluorescence with both antisera and monoclonal antibodies.
2. Dr. R. Hyman has agreed to provide his battery of probes for HSV 1 and 2 DNA. He will consult with the PI and Dr. Felton on interpretation of the results obtained with there reagents.
3. Dr. A.B. Jenson will investigate the presence of human papillomavirus DNA

using the Southern blot technique.

4. Dr. M. Zweig has donated a panel of monoclonal antibodies to antigens of HSV 1 and 2. He will consult on the use of these and on interpretation of data on HSV.

Staff:

1. C.B.C. Hwang is a PhD student, in the Experimental Pathology program, working as a research assistant in Dr. J. Greenspan's laboratory. He has worked on herpes simplex virus for about 3 years and is adept with the use of Dr. Zweig's monoclonal antibodies in the ABC technique. He will perform the assay and will also do the Southern blot work for HSV-DNA using Dr. Hyman's probes and guided by Dr. Felton.
2. Ilse Sauerwald is a very experienced histology and histochemistry technician. She will accept and divide up HL tissue, freeze and store it in the AIDS Specimen Bank as appropriate, and cut sections using the biohazard cryostat in the P2* room. She will send material to co-investigations and consultants. She will perform the PAP technique for HPV antigen and the procedure for Langerhan's cells. In addition, she will screen slides, perform photography and prepare draft reports.
3. Vibeke Petersen is an experienced electronmicroscopist. She will perform all laboratory phases of the ultrastructural work including tissue preparation, EM viewing and photography, preparation of data reports and contribution to manuscripts.
4. Carol Fiuren. Support is requested for 1/2 day per week office support for the project.

2. Supplies and Expense

- i Dr. J. Greenspan's lab.
 - a. Cytochemistry. Substrates, ABC and PAP kits, slides, buffers, cryostat supplies, materials and processing for photography. = \$2,500
 - b. E.M. Fixatives, embedding material, glass knives, slides, grids, photography and maintenance of the EM (40% of \$2000 contract). = \$4,000
 - c. Microbiology. Support is requested for candida culture and identification, for sending samples to consultants Drew, Lennette and Jenson and for new antiviral antisera and monoclonals as these become available. = \$1,500
- ii Laboratory Immunology
T₄/T₈ ratio, 30 cases X \$75 = \$1,500
(special research rate for members of the UCSF AIDS research group). To be performed by Dr. C. Cassavant. (see letter)
- iii Dr. Drew
Thirty cases X 200 = \$6,000

iv Dr. Lennette

Thirty cases X 100 = \$3,000

v Dr. Jenson

Thirty cases X 50 = \$1,500

vi Dr. Levy

Thirty cases X 25 = \$ 750

vii Travel

Two investigators to one national
meeting each (ASM, AACR or IADR) = \$2,000

XI. Explanation of Project

Title Oral viral leukoplakia - a new lesion in association with AIDS

Introduction/Background

Between October 1981 and July 1984, we have seen 37 cases of a previously unrecorded lesion of the tongue in male homosexuals in the San Francisco area. The lesions are on the lateral margins of the tongue and ^{are} of varying sizes. They are usually poorly demarcated and show a corrugated or "hairy" surface. Although usually painless, the lesions have been noticed by the patients, several of whom report spontaneous regression and reappearance. We have provisionally named the lesion hairy leukoplakia (HL).

Although all of our patients with HL have been in apparent good health at the time of diagnosis, all reported previous use of recreational drugs, most gave a history of hepatitis B and many had diffuse lymphadenopathy. Of a few cases tested for immunocompetence using skin tests and T_4/T_8 ratios as criteria, all showed some evidence of immunosuppression. None of our patients had AIDS at the time of diagnosis of HL, but 8/37 have developed PCP in the 33 months of our preliminary study.

The lesion has a distinctive histopathology and shows features suggestive of a human papillomavirus (HPV) lesion. These include the "hairs", which are due to localized acanthosis and parakeratosis, as well as marked koilocytosis - ballooning of prickle cells with pyknotic nuclei and perinuclear halos. In a very preliminary study, 74% of 23 biopsies showed evidence of papillomavirus antigen using an immunocytochemical technique. Presuming the lesion to be a form of papillomavirus-induced flat oral wart, we initiated an electronmicroscopic search for papillomavirus particles. In only six cases examined to date, we have found no evidence of HPV. However, to our surprise, 5/6 cases showed plentiful evidence of a herpes-type virus. We do not yet know if this is cytomegalovirus (CMV), herpes simplex (HSV) type 1 or 2, Epstein Barvirus (EBV) or

Epstein-Barr virus

varicella-zoster virus (VZV).

The identification of the viruses or virus elements present in HL is ~~incomplete~~. *at a preliminary stage.* Perhaps an HPV type is present and complete and represents a fairly straightforward example of opportunistic infection in a host tissue which is immunocompromised both systemically and also locally. Langerhans cells (LC) are known to be decreased in the skin in AIDS (...), while the keratinized surface of the tongue is known to have areas devoid of LC (...). The second virus may then simply colonise the hyperplastic epithelium of the wart-like lesion produced by the HPV. However, papillomavirus particles are not seen and the herpes-type virus is present in very large quantities. Perhaps the HPV is defective, produces antigen and cytopathic effects but not complete virus particles. Perhaps a herpes-type virus (CMV, HSVI or II, EBV or VZV) is present and contributes to the lesion. Even more speculative and exciting are the possibilities, firstly that the herpes-type virus only is present and produces both the HPV antigen staining and causes the lesion, and secondly that a hybrid HPV/herpes-type virus is present. Such a hybrid between SV40 and an adenovirus has been described (...).

The lesion is of interest for several further reasons. Thus, recent reports suggest a previously unsuspected and possibly widespread role for HPV in oral epithelial hyperplasia, dysplasia and and carcinoma (...). Of interest in this regard is the observation of several cases of oral carcinoma in young male homosexuals in San Francisco (...). Similar relationships have been studied extensively between HPV and carcinoma of the uterine cervix. (...). There is also thought to an association, as yet not well understood, between HSV2 and uterine carcinoma as well as a possible *analogous* association between oral cancer and HSV1 (...). Papillomaviruses (...), herpes simplex virus (...), cytomegalovirus (...), and EB virus (...) can all transform cells and behave as oncogenic agents.

Specific Aims

1. To further define and investigate the etiology, pathogenesis and natural history of oral "hairy" leukoplakia (HL), a newly described lesion which is part of the AIDS group of diseases.
2. To identify the virus elements thought to be present in the lesion and to assess their incidence in a group of HL cases.
3. To explore local, intramucosal, immune mechanisms by identifying and counting Langerhans cells (LC), including enumerating those bearing Ia antigen.
4. To follow HL cases prospectively and to attempt to define risk factors for the development of Pneumocystic carinii pneumonia (PCP). Further, to assess the risk, if any, of transformation of the lesion into oral squamous cell carcinoma.

Methods

Patients

Approximately 750 new patients are seen each year in the Oral Medicine Clinic at UCSF, among a total of almost 5,000 patient visits per year. At present, about 1 to 2 new cases of HL are being seen each week. We anticipate at least this rate of accession of new cases during the 8 months of the proposed grant period. The cases are referred by physicians and dentists in the city of San Francisco and adjoining areas but most are referred from the AIDS Clinical Research Center/KS Clinic. As the new UCSF AIDS clinic becomes functional, and as awareness of the lesion spreads among clinicians, the referral rate may increase. We therefore anticipate no difficulty in recruiting 30 to 50 new cases for some or all parts of the study. In addition, paraffin blocks are available in 35 existing biopsies from 30 cases, while frozen tissue from 15 cases and serum from 14 cases are stored in the UCSF AIDS Specimen Bank. EM blocks have been prepared in 8 cases in addition to those described in the Introduction. Thus a good deal of material awaits investigation and the project can undergo major expansion as soon as funding becomes available.

All new patients will be invited to participate and those who agree will give a detailed medical and social history using the UCSF AIDS Clinical Research Center protocols. Complete oral exam will be performed by Dr. D. Greenspan, backed-up by Dr. T.E. Daniels and Dr. J.S. Greenspan if needed. Physical examination will be performed by Dr. H. Hollander in the UCSF AIDS Clinic or by Dr. M. Conant and his nurse-practitioner staff in the UCSF AIDS Clinical Research Center/KS Clinic. Clinical data, and ultimately all laboratory data, will be stored in the UCSF AIDS Clinical Research Center computer.

Oral Biopsy/Histopathology

White lesions suspected as representing the lesion of HL will be subjected to incisional biopsy using local anesthesia. We have not found suturing to be useful and healing is usually uneventful. The specimen will be divided as follows:

1. A portion will be routinely fixed and processed for histopathology and PAS staining. There will be performed by the Oral Pathology service of the department, on which the Principal Investigator is an attending oral pathologist. The same paraffin blocks will be used for PAP staining for HSV antigen (see below).
2. A portion will be cut into 1mm³ fragments for electronmicroscopy. (see below)
3. A portion will be used for cultural virological studies (see below)
4. The remainder will be quick-frozen in liquid nitrogen and then stored in sealed vials in liquid nitrogen in the tissue bank of the UCSF AIDS Specimen Bank. Cryostat sections will be cut for some of the virus studies and for the identification of Langerhan's cells.

Control Tissues

1. "POSITIVE" controls will include skin and oral verrucae and condylomata (control for HPV), HSV1 and 2 infected Vero cells, CMV infected fibroblasts, EBV infected lymphoblasts and VZV infected cell lines.

2. "NEGATIVE" controls will be specimens from other oral epithelial lesions undergoing diagnostic biopsy in our clinics. These will include hyperkeratosis, dysplasia and carcinoma, candidiasis, ^{and} lichen planus. We will also use normal oral mucosa obtained at biopsy and during plastic procedures.
3. A further, most important, control will be non-lesional tissue adjoining HL lesions. This control tissue should help us to establish if the agents found are confined to the HL lesion ^{or} are more widely present in the epithelium of these immunosuppressed homosexual patients.

Immunological Studies

Delayed cutaneous hypersensitivity will be tested using PPD, candida, mumps and trichophytin antigens. Assessment of ^{peripheral blood} lymphocyte subtype numbers and T_4/T_8 ratios will be performed by Dr. Conrad Casavant in the Clinical Immunology laboratory at UCSF.

Ultrastructural Studies

Tissue fragments will be fixed for 12-48 hours in 3% glutaraldehyde (...), postfixed for 1 hour in 1% buffered osmic acid, (...) dehydrated in graded ethanol and propylene oxide and embedded in EPON 812 (...). Thin sections will be cut from areas selected by examining semithin sections and will be double-stained on Formvar-coated grids with saturated uranylacetate and modified lead citrate (...); sections will be screened for HPV and other viruses by examining at least 100 cells from each case. The instrument to be used is the Siemens Elmiskop 1A, operating at 80KV, in the EM facility of the Division of Oral Medicine.

Virological Studies

Our intent is to:

1. Confirm or refute the presence of human papillomavirus (HPV).
2. To determine if the herpes-type virus, seen in 5/6 cases on electronmicroscopy in our preliminary study, is indeed present.
3. To attempt to identify the latter using techniques to demonstrate HSV1 and 2,

CMV, EBV and VZV. Details follow and the plan is summarized in Table 1.

a) Culture

Fresh tissue specimens will be transported to Dr Drew's laboratory where attempts will be made to culture virus using host cells, notable human lung fibroblasts, suitable for members of the human herpesvirus group. Evidence of ^(cytopathic) effects will be sought.

b) Papillomavirus

The search for ^{one word} papilloma virus will include attempts to further identify HPV antigen, electromicroscopy for whole virus particles and the use of DNA probes.

HPV antigen

Paraffin sections will be cut and subjected to the peroxidase-antiperoxidase technique using antisera to papillomavirus antigen. The technique to be used is that of Sternberger (....) and we will employ two antisera. Both are raised in rabbits using as antigen preparations of the core antigen of bovine papillomavirus. One is available commercially (DAKO) while the other is the gift of Dr. A.B. Jenson of Georgetown University. Control procedures will include the use of serum from non-immunized rabbits as well as incubations omitting each of the other stages. Sections will be preincubated with H_2O_2 to block endogenous peroxidase and then with normal swine serum to block existing activity against human antigens in the active secondary swine anti-rabbit antiserum. The final reaction product will be developed using aminoethylcarbazole (AEC) which produces a red homogenous dye. This is more readily distinguished from the hematoxylin counterstain than is the brown reaction product of DAB. Control tissues will include oral verrucae (positive control) and oral hyperkeratosis,

carcinoma, candidiasis and lichen planus. (negative controls), as well as non-lesional tissue from HCL patients.

HPV particles on EM (see above)

HPV-DNA

Evidence for papillomavirus DNA will be sought by Dr. A.B. Jenson using probes provided by Professor H. zur Hausen. Twenty five probes for that number of HPV types are available but initial experiments on DNA extracted from frozen HL tissue sent by us will be under non-stringent conditions to determine the presence of the entire HPV genome. Subsequent work will be directed at identifying parts of the genome and at determining the type or types of HPV-DNA if present. Dr. Jenson will use the Southern blot technique (see below).

c) Herpes Simplex Virus 1 and 2

Experiments will involve culture, attempts to identify antigen in tissue sections and the use of DNA probes. EM studies are also described above.

HSV antigen

The battery of 9 monoclonal antibodies provided by Dr. M Zweig will be used. We will apply the avidin-biotin-peroxidase (ABC) technique (...) with which we have 4 years of experience. This will permit identification and localization of most stages of HSV1 and 2. The monoclonals are:

Anti ICP4	58-S	HSV-1
Anti ICP5	56-S	HSV-1 Capsid protein
Anti ICP6	2-S	HSV-1
Anti ICP6	105-S	HSV-2
Anti ICP6	48-S	HSV-1 Cross reacting
Anti ICP8	39-S	HSV-1 DNA binding protein
Anti gB	35-S	HSV-1 glycoprotein
Anti gB	3-S	HSV-1

Anti gD 4-S HSV-1

EBV-DNA probes are available commercially and we will develop their use in the in-situ hybridization assay.

d) Cytomegalovirus (CMV)

Experiments will involve culture and EM (see above) as well as attempts to identify CMV antigens and CMV-DNA

CMV Antigen

Cryostat sections will be reacted with pooled mouse monoclonal antibody to CMV (Immuglog/Sygn Labs) in a biotin-avidin FITC system (EY Labs).

CMV-DNA

In-situ hybridization, again using cryostat sections, will be applied and will involve the ENZO Biochem. Inc. "Patho-Gene" kit. A biotinylated DNA probe is hybridized to CMV-DNA and is detected by a streptavidin-horse radish peroxidase-complex and DAB system.

e) Epstein-Barr virus (EBV)

Dr. Lennette will use human sera to identify EBV in IF. Cryostat sections will be reacted with at least two reference human sera of specificities: EBNA+/CMV-, EBNA-/CMV+ and EBNA+/CMV+. A guinea pig complement/anti-guinea pig C3 system (fluorescein conjugated) will be used. She will also investigate CMV antigen using anti-CMV monoclonal antibodies in an indirect IF system.

f) Varicella-zoster-virus

Dr Lennette will use human sera with antibodies to VZV.

Dr. Lennette is developing the use of new anti-VZV monoclonals and we will apply these also, as well as VZV-DNA probes when they become available.

The field of in-situ identification of viruses within tissues is developing rapidly. Most of the reagents and techniques are fairly new. Modifications and improvements can be expected to occur even during the period of this grant. We will apply these to our problem as they become available.

HSV-DNA

The probes for HSV1 and 2 DNA, provided by Dr. R. Hyman will be used by Dr. J.

Felton in the Southern blot technique (...). Details follow

PREVIOUS
The probes to be used will include EcoRI fragments B and F of HSV-1 (strain Patton) as well as BglII fragments C and N of HSV-2 (strain 333). These, and the whole viral DNA probes, will be hybridized to nitrocellulose blots of electrophoretically separated DNA from HL lesions ~~lesions~~ uninvolved mucosa from HL patients and the ~~lesions~~ same patients' blood leukocytes. Other controls will include blood leukocytes from healthy individuals, HSV-transformed cell lines as well as the untransformed parent cell lines from which they are derived. Blots may also be used, including mixtures of various DNAs and normal human DNA in a genome equivalent ratio of 1:1. A further control will be a cloned single copy normal human gene used as a probe to demonstrate that the method is adequately sensitive. Vector and probes (e.g. pAEXC134) will be used as negative controls. Blots will be prepared from DNAs cut with several different restriction endonucleases, and the hybridization reaction will be performed under conditions of various degrees of stringency.

Langerhans Cell Studies

These will be supervised by Dr. T.E. Daniels

Langerhans cells (LC) will be identified in 10 μ m cryostat sections using adenosine triphosphatase (ATPase) histochemistry (...) and by immunohistochemistry using

commercial monoclonal antibodies against the T6 antigen (...) and the Ia antigen (...) in the ABC technique (...). LC will be counted in standard fields and the results correlated with the nature and location of virus antigen or DNA in the immunohistochemical and in-situ techniques. Our hypothesis is that selective loss of LC is related to colonization by HPV and/or the as-yet-unidentified herpes-type virus. The control tongue biopsies from non-lesional areas in our HL patients will clearly be very important in this aspect of our study.

Prospective Study

Our HL patients will be examined twice during the 8 months of this proposal and at intervals thereafter. We will record the development of features of AIDS, notably PCP, which has already occurred in 8 cases. We will attempt to assess risk factors for this complication and any others which develop. For this aspect of the study, we have enlisted the support of Dr. W. Winkelstein of the UCB School of Public Health who is the PI of a substantial NIAID longitudinal study of cohorts of male homosexuals and controls. He has assigned a graduate student to our study and we are in the process of developing our joint protocol. No funds are requested for this part of our HL investigation except for a small sum to fund assessment of the presence and titer of antibodies to ARV (AIDS associated retrovirus)S by Dr. J. Levy. This virus appears to have many of the properties of HTLVIII (...) and LAV (...). We are also in the process of negotiating with Professor Chermann at the Pasteur Institute, Paris, to have LAV titers performed.

TABLE 1.

VIRUS	COMPONENT	TECHNIQUE	INVESTIGATOR	CONTROL TISSUE
HPV	Whole virus particles	EM	J. Greenspan	1. Oral warts/skin warts
	Antigen DNA	PAP/antisera Southern blot	J. Greenspan A.B. Jensen	2. Non-lesional tissue from HL cases 3. Other oral epithelial lesions
Herpes Group	Whole virus particles <i>Live virus</i>	EM <i>Culture</i>	J. Greenspan <i>Drew</i>	Non-lesional HL patient tissue
HSV 1 & 2	Live virus Antigens	Culture ABC/monoclonals	Drew Greenspan/ <i>Hwang</i>	1. Oral herpes, lesions, infected cells
	DNA	Southern blot	Hwang Felton/Hwang	2. Non-lesional HL patient tissue 3. Other oral lesions
CMV	Live virus Antigens	Culture IF/monoclonals	Drew Lennette	1. Known positive CMV lesions/cells
	DNA	In situ hybridization	Drew	2. Non lesional HL patients tissue 3. Other oral lesions
EBV	Antigens	IF/antisera	Lennette	1. Known positive EBV lesions/cells
	DNA	In-situ hybridization (when reagents are available & reliable)	Greenspan/ Felton	2. as above 3. as above
VZV	Antigens <i>DNA</i>	IF/antisera and in-situ hybridization (when both are available)	Greenspan/ Lennette	2/3 as above
ARZ	Antibodies in serum	IF	Levy	1. Normal controls 2. ARV positive sera

XII Relation of the Project to the AIDS Problem

We believe that further investigation of the nature, etiology, pathogenesis and progress of this new entity within the AIDS spectrum, oral hairy leukoplakia, is appropriate for the following reasons:

1. Such information may improve understanding of the role of viruses and local immunosuppression in the pathogenesis of AIDS.
2. Identification of the viruses present will contribute to an understanding of why certain organisms cause opportunistic infections in AIDS. The elucidation of the relationship between the viruses could lead to increased understanding of defective oncogenic viruses, virus-virus interactions and hybrid viruses and their roles in infection, immunosuppression and oncogenesis.
3. Determining which cases ^{progress} transform to the full syndrome, with PCP, may help in earlier diagnosis of AIDS and ultimately contribute, in part, to its prevention and control.
4. Finally, investigation of the potential of this virally induced hyperplasia to undergo dysplastic and neoplastic transformation may contribute to elucidating the problem of neoplasia in AIDS.

HYBRID

XIII Progress Reports

The Principal Investigator is not currently a direct recipient of Task Force monies. However, he has received such funds in two ways. He is responsible for the UCSF AIDS Specimen Bank which is supported by the Task Force grant to the UCSF AIDS Clinical Research Center. Secondly, he has been the co-investigator on a Task Force grant to Jay Beckstead M.D. Therefore, relevant portions of the progress reports of those two projects are attached. However, neither is directly related to the current proposal. The specimen bank will be used to accept and store HL specimens and sera, just as it is used to store tissue and serum for many other projects, and no funds are requested in the present proposal for such support activities provided by the specimen bank.

Michela Reichman, Assistant Chancellor

Source: Andy Evangelista (415) 666-2557

EMBARGOED FOR RELEASE:

7:00 AM (PDT), WEDNESDAY, MAY 9

UC-SAN FRANCISCO STUDIES CONFIRM FRENCH FINDING OF RETROVIRUS IN AIDS
AND DEMONSTRATE A RETROVIRUS COULD BE PASSED THROUGH BLOOD CLOTTING FACTOR

TORONTO -- A UC-San Francisco virologist today reported results of two studies that add further support to the theory that a retrovirus plays a major role in acquired immune deficiency syndrome.

A study by Jay Levy, MD, UCSF associate professor of medicine and an investigator in the Cancer Research Institute, confirms findings by French scientists that a retrovirus -- lymphadenopathy associated virus (LAV) -- could be the primary cause of AIDS. So far, Levy has found evidence of LAV or a LAV-like agent in blood cells of 28 of 50 AIDS patients in San Francisco. In three of the 28, Levy's laboratory has direct proof of the similarity to LAV.

The study was reported here today at a joint meeting of the American Society of Clinical Oncology and the American Association for Cancer Research.

Last year, researchers (F. Barre-Sinoussi, L. Montagnier, J.C. Chermann) at the Pasteur Institute in Paris discovered LAV in blood of AIDS and pre-AIDS patients in France. The suspected virus grows preferentially in certain lymphocytes (white blood cells), which become damaged or diminished in AIDS patients. The loss of these cells appears to be responsible for their inability to fight off opportunistic infections and cancer. Levy's research group found retroviral enzymes and proteins in white blood cells, as well as antibodies to LAV, which indicate infection, in over 50 percent of the patients studied. Evidence of the virus was not detected in any of 21 healthy controls. For the study, Levy used reagents supplied by the Pasteur Institute researchers to compare his isolates with theirs. Electron micrographs also have confirmed the similarities, Levy reported.

The relationship of the UCSF LAV-like agent and LAV to the HTLV-III retrovirus, recently described by Robert Gallo of the National Cancer Institute, is not yet known, says Levy. Recent reports, however, indicate strong

(more)

similarities between LAV and HTLV-III. Although they are major steps in AIDS research, Levy cautioned that these new findings do not yet establish absolute proof that either virus or a similar one is the cause of AIDS. He is conducting experiments, infecting animals with the UCSF isolates, in an attempt to determine whether or not they are responsible for AIDS.

In a separate presentation at the ASCO and AACR meetings, Levy today reported results showing retroviruses can survive procedures previously used to produce the clotting factor (Factor VIII) used by hemophiliacs. Levy, working with researchers from Cutter Laboratories in Berkeley (Milton M. Mozen, PhD, and George Mitra, PhD), tested the stability of a mouse retrovirus, resembling LAV, through the clotting factor production process. The process did not substantially inactivate the retrovirus, Levy reported.

This is counter to previous beliefs that a retrovirus could not survive such a process, and the study possibly explains why some 30 hemophiliacs in the United States have developed AIDS. The factor, used by hemophiliacs to prevent uncontrollable bleeding, is usually prepared from blood contributed by thousands of donors. The study by Levy and his coworkers did indicate that the heating procedure used now by Cutter eliminates the retrovirus effectively.

The process is now being used by manufacturers of Factor VIII to further minimize the chances of hemophiliacs contracting AIDS, says Levy.

Taken together, Levy's studies -- indicating presence of LAV or a LAV-like agent in the blood of AIDS patients in San Francisco and demonstration that a retrovirus can be transmitted in blood clotting factors -- lend further support to the theory that a retrovirus is involved in AIDS.

Levy's research is supported by grants from the state of California and the National Institutes of Health. His UCSF research team includes Susan Kramer, PhD, Lyndon Oshiro, PhD, Anthony Hoffman, Jill Landis and Joni Shimabukoro.

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① ENZ BIOCHEM

EB/VZV

DNA probe

Bwtm

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1 (2m
② VZV monoclonal
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JUDITH C. WILBER, Ph.D.
VIROLOGIST

SAN FRANCISCO DEPARTMENT OF PUBLIC HEALTH
101 GROVE STREET, RM. 419
SAN FRANCISCO, CALIFORNIA 94102

558.3004

J.I.D. Sympo

P18s Ep. verr. $\rightarrow$ malignant

P26s HPV in genital tumour

P76s #37 Southern

UCSF AIDS CLINICAL RESEARCH CENTER

INTERIM FINAL REPORT

1983 - 1984

For HLGant

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interim final report covers the dif-
ferent expenditures in different stages and, therefore, a full year's final
report cannot be made until November, 1984 or one year after the actual ap-
propriations.

* II. JULY THROUGH NOVEMBER, 1983 -- ESTABLISHMENT OF TISSUE-SERUM BANK

As identified in the proposal to the State of California, the most im-
mediate need for support for the AIDS Clinical Research Center was the establish-
ment of a centralized tissue and serum bank.

Tissue and Serum Bank

1. Facilities

The tissue/serum storage facility was moved into a special P₂*
biohazard room in November of 1983. New equipment used exclusively
for the bank includes a -70°F Revco upright freezer with inventory
system, alarms, recorder and liquid nitrogen backup, a large liquid N₂
storage container and liquid N₂ storage refrigerator, a Baker bio-
hazard hood, a binocular microscope, a Slee cryostat, a Beckman centri-
fuge, and associated accessories. An IBM computer is on order.

2. Accessions

Specimen acquisition has accelerated from the rate established be-
fore State funding and we now accept approximately 20 sera and 3 tissue
specimens each week. Total specimens at 5/18/84 were:

Serum 542

Tissue 180

Arrangements ~~are being negotiated~~ ^{have been made} for the acquisition and storage of
autopsy specimens.

UCSF AIDS CLINICAL RESEARCH CENTER

INTERIM FINAL REPORT

1983 - 1984

~~P.~~ INTRODUCTION

The UCSF AIDS Clinical Research Center was conceived approximately two years ago and, in collaboration with University of California physicians and scientists at all five campuses, a proposal was made to the State of California for a special legislative appropriation. This appropriation was made and monies were allocated to the different university campuses for a wide variety of AIDS related research projects. One of the largest single items funded by this appropriation was the establishment of the UCSF AIDS Clinical Research Center. Initial funding in the amount of approximately \$125,000 was made available in August, 1983 and a subsequent appropriation in the approximate amount of \$365,000 was received in November, 1983. Therefore, because the monies were ~~actually~~ available only in stages, some of which were made available only six months ago, this ~~interim~~ final report covers the different expenditures in different stages and, therefore, a full year's final report cannot be made until November, 1984 or one year after the actual appropriations.

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Serum 542

Tissue 180

Arrangements ~~are being negotiated~~ ^{have been made} for the acquisition and storage of autopsy specimens.

3. Withdrawals

Batches of specimens are released to collaborators following procedures approved by the Tissue Committee.

Serum Nine collaborations with investigators outside the UCSF AIDS Clinical Center Investigators group have been entered into. Five of these are with other U.C. investigators, one with an out-of-state colleague, and two with Federal agencies. ~~Attachment #1~~ AA

Tissue Seven new collaborations are in progress. Three are outside the UC system including one outside the USA, while four are with UC colleagues, all in northern California.

These serum and tissue collaborations are in addition to 12 projects using our specimens within the UCSF AIDS Clinical Center Investigators Group. ~~Attachment #2~~ AA

4. Systemwide Aspects

A UC Systemwide AIDS Task Force Specimen Banking Committee was established at the direction of the Task Force and includes Dr. J.S. Greenspan, Chair (UCSF), Dr. M. Gottlieb (UCLA), Dr. P. Volberding, Dr. M. Conant, Dr. A. MacCutcheon (UCSD), Dr. T. Cesario (UCI), Dr. N. Sun (UCLA), Dr. M. Gardner (UCD) and support staff personnel. Systemwide guidelines, based on UCSF experience, have been proposed. The two main AIDS Specimen Banks, at UCSF and UCLA, are directed by Drs. Greenspan and Gottlieb. We have consulted frequently. Dr. Gottlieb has come up to UCSF to review our methods and equipment, and Dr. Greenspan will go to UCLA in the next month to reciprocate. We have begun to standardize techniques and records.

In order to provide for the immediate establishment of this tissue and serum bank, the entire initial appropriation was made available to Dr. Greenspan for this purpose.

III. DEAN'S COMMITTEE

Beginning in the Fall of 1983, the Dean's Advisory Committee for the AIDS Clinical Research Center, under the chairmanship of Dr. John Ziegler, began to meet monthly to provide advice, consultation, and overall policy direction for the proposed Clinical Research Center. At the initial meetings of the Dean's Committee, the concept of the center as outlined in the initial proposal was endorsed and supported and deliberations began regarding the most appropriate and effective use of the database being generated through the tissue and serum bank. Of particular importance to the Dean's Committee was the issue of access to specimens. The Committee sought to avoid conflicts and confrontations among and between researchers who were contributing tissue to the bank and those who wished to draw upon it for research and study. By providing a forum for open discussion, the Dean's Committee has been able to avoid such conflicts and has maintained a harmonious relationship and dialogue among the various participants and users.

Library
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QR372

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M 64

part 2

1980

→ DNA Tumor Viruses
ed. by John Tooze

published 1980

by Cold Spring Harbor Laboratory

Chapter 11

"Adenovirus-SV40 Hybrids"

written by T. Grodzicker

p. 577-614.

This book is also referred to as:

Molecular Biology of Tumor Viruses,
Second Edition

Part 2

2d	15%	57.50	No	1200	3 mo
3d	30%		salary		3
			10		6.7

Consult:

- Drew	20 x 300	6	6	4
- Lennette	20 x 400	8	8	6
- Jenson	20 x 300	6	6	0
- Hyman	0			

Felton	30%	8	5.3
--------	-----	---	-----

Hwang	50%	9	6
-------	-----	---	---

de Souza	50%	9	6
----------	-----	---	---

Viche	50%	14	9.7
-------	-----	----	-----

Ilse	20%	13	8.7
------	-----	----	-----

Supplies	500 mo	6	4
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Tel/Ts	20 x 75	1.5	1.5
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Travel		2	2
--------	--	---	---

Other	200 mo	2.4	1.6
-------	--------	-----	-----

62.8

6	1.2	} 44
5.2	1.04	
8	1.7	
8.7	5.4	
8.7		
		12
		65

JH Barent

52.9
1.5
13.4

90 Bank. 76.2 - 66.2 = 10

Memo from
JOHN S. GREENSPAN

University of California, San Francisco 94143
Telephone (415) 666-2220

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

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SANTA BARBARA • SANTA CRUZ

SCHOOL OF MEDICINE
Department of Microbiology
and Immunology

SAN FRANCISCO, CALIFORNIA 94143

Fri 13 July

Dr. Greenspan -

Thought this might be of
interest to you. It seems clear that Southern
blots of your material should be screened with
probes from multiple of the known HPV subtypes.

Best regards,

Don Ganem

P.S. In thinking it over, it would seem best for
Girish to do the HPV blots if his lab is also doing
the in situ work. Both Linda Stowring and
Hubert Blum in his lab have extensive experience
with the method.

Project

Title

Specific Aims

Background / Introduction

Methods

- 1 Patients
- 2 ~~Biosps~~ / H'pata
- 3 Immunological studies
- 4 EM
- 5 Virus culture & identification

1 To further define ~~eried~~, a ~~metc~~th
2 To determine the incidence & nature of viruses present in the HCL lesion

3 To assess competency of local intramucosal immune mechanisms by identifying a cont. IC & identify cont. that IC being Ia Ag

4 To follow HL cases prospectively & attempt to define risk factors for trans. to PCP. Further, to assess risk of an. of transformat. to oral squamous cell

6. Prognosis
- PCP
- Oral Ca
Incidence & Risk factors

HPV ← EM
DNA
Jensen

HSV 1 & 2

Culture - Drew

Ag - Monoclonal ABC

- Charlie / Zweip
- Lennette

DNA

Felton / Hwang / Hyman

CMV

Culture - Drew

Ag - IF / ABC Lennette

DNA - In situ Drew

EB

~~Culture~~ Culture - Drew

Ag - IF. Lennette

? DNA

VZV

- to be organised

Also later - ① GM on to ~~Demist~~^{Levy}, ② ARV ~~Levy~~^{Levy}

Viral Cultures

- 1) The tissues are minced well in MEM using scalpel and forceps.
- 2) This preparation is inoculated into culture tubes containing Human Epithelial Lung fibroblasts.
- 3) The media on the tubes are changed regularly and observed for viral cytopathic effects.

CMV Fluorescence

- 1) The tissue sections are incubated with pooled mouse monoclonal antibody to CMV. (Immulok Labs & Syva Labs)
- 2) After washing, the slides are then incubated with goat anti-mouse antibody which has been bioninylated. (E.Y. Labs)
- 3) After washing, the slides are reincubated with an avidin-FITC conjugate. (E.Y. Labs)
- 4) The slides are then washed and read.

In Situ Detection of CMV-DNA

The detection of CMV-DNA is done using the Patho-Gene kit produced by ENZO Biochem, Inc.

In this method, a biotinylated DNA probe is hybridized to CMV-DNA and detection is by use of a streptavidin-horseradish peroxidase complex followed by diaminobenzidine tetrahydrochloride.

used
7/24
D

References on E. M. Method:

- 1) KARNOVSKY, M. A. "A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy." J. CELL BIOL. 27: 137A, 1965
- 2) PALADE, G. E. "A Study of fixation for electron microscopy" J. Exp. MED. 95: 285, 1952
- 3) LUFT, J. H. "Improvements in epoxy resin embedding methods." J. Biophys. Biochem. Cytol. 9: 409, 1961
- 4) REYNOLDS, E. S. "The use of lead citrate at high pH as an electron-opaque stain in electron microscopy." J. Cell Biol. 17: 208, 1963



UNIVERSITY OF CALIFORNIA
AIDS SPECIMEN BANK
HSW-604
(415) 666-2943

SAN FRANCISCO, CALIFORNIA 94143

UCSF AIDS CLINICAL RESEARCH CENTER

AIDS SPECIMEN BANK

John S. Greenspan

Progress Report at 5/18/84

1. Facilities

The tissue/serum storage facility was moved into a special P₂* biohazard room in November of 1983. New equipment used exclusively for the bank includes a -70°F Revco upright freezer with inventory system, alarms, recorder and liquid nitrogen backup, a large liquid N₂ storage container and liquid N₂ storage refrigerator, a Baker biohazard hood, a binocular microscope, a Slee cryostat, a Beckman centrifuge, and associated accessories. An IBM computer is on order.

2. Accessions

Specimen acquisition has accelerated from the rate established before State funding and we now accept approximately 20 sera and 3 tissue specimens each week. Total specimens at 5/18/84 were:

Serum 542

Tissue 180

Arrangements are being negotiated for the acquisition and storage of autopsy specimens.

3. Withdrawals

Batches of specimens are released to collaborators following procedures approved by the Tissue Committee.

Serum Nine collaborations with investigators outside the UCSF AIDS Clinical Center Investigators group have been entered into. Five of these are with other U.C. investigators, one with an out-of-state colleague, and two with Federal agencies.

Tissue Seven new collaborations are in progress. Three are outside the UC system, including one outside the USA, while four are with UC colleagues, all in northern California.

These serum and tissue collaborations are in addition to about 12 projects using our specimens within the UCSF AIDS Clinical Center Investigators Group.

Plans for 84/85

Continued growth in both accessions and withdrawals will occur. Serum bank expansion will require a further -70° Revco freezer. Use of the AIDS Bank biohazard room for skin, mucosal and conjunctival immunodiagnosis mandates the purchase of a fluorescence microscope. Appropriate increases in personnel support are anticipated commensurate with current and expected workload. However, equipment and renovation costs will be lower than in the start-up year.

Systemwide Aspects

A UC Systemwide AIDS Task Force Specimen Banking Committee was established at the direction of the Task Force and includes Dr. J.S. Greenspan, Chair (UCSF), Dr. M. Gottlieb (UCLA), Dr. P. Volberding, Dr. M. Conant, Dr. A. MacCutcheon (UCSD), Dr. T. Cesario (UCI), Dr. N. Sun (UCLA), Dr. M. Gardner (UCD) and support staff personnel. Systemwide guidelines, based on UCSF experience, have been proposed. The two main AIDS Specimen Banks, at UCSF and UCLA, are directed by Drs. Greenspan and Gottlieb. We have consulted frequently. Dr. Gottlieb has come up to UCSF to review our methods and equipment, and Dr. Greenspan will go to UCLA in the next month to reciprocate. We have begun to standardize techniques and records.

DETAILED BUDGET FOR FIRST 12-MONTH PERIOD

FROM

July 1, 1984

THROUGH

June 30, 1985

DESCRIPTION (Itemize)		TIME OR EFFORT %/HRS.	AMOUNT REQUESTED (Omit cents)		
PERSONNEL			SALARY	FRINGE BENEFITS	TOTAL
NAME	TITLE OF POSITION				
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
* 20% Salary, 30% Effort					
					78,725
CONSULTANT COSTS Nil					
EQUIPMENT					
Microscope - Fluorescence/photomicroscope			23,000		
Revco-70 Freezer with back-up and accessories			8,000		31,000
SUPPLIES					
Liquid nitrogen, dry ice			1,250		
Cryostat accessories, knives, slides, etc.			2,250		
Gloves, masks, gowns			2,000		
Film, processing chemicals, solutions			1,000		
Computer supplies			500		
					7,000
TRAVEL					
DOMESTIC 1 national, 1 local			1,500		1,500
FOREIGN Nil					
PATIENT COSTS (See instructions)					
Nil					
ALTERATIONS AND RENOVATIONS					
Nil					
OTHER EXPENSES (Itemize)					
Servicing equipment			1,250		
Xerox, mail, phone			1,100		
Charts, slides, printing			1,150		3,500
TOTAL DIRECT COST (Enter on Page 1, Item 5)					121,725
INDIRECT COST (See Instructions)		DATE OF DHEW AGREEMENT:			
Nil % S&W*				☐ WAIVED	
Nil % TDC* Nil				☐ UNDER NEGOTIATION WITH:	
*IF THIS IS A SPECIAL RATE (e.g. off-site), SO INDICATE.				Nil	

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HOSPITALS AND CLINICS

SAN FRANCISCO, CALIFORNIA 94143

May 7, 1984

TO: AIDS Clinical Research Center
Principal Investigators

FR: Marcus A. Conant, M.D.
Director, AIDS Clinical Research Center

RE: Site Visit and Progress Report

The University-Wide Task Force on AIDS conducted their previously announced site visit on Monday, April 23. The site visit team was comprised of Dr. Broude from UC San Diego and Dr. Cesario from UC Irvine. We reviewed for them the structure of the AIDS Clinical Research Center which is presented schematically on the diagram attached.

The major purpose of the AIDS Clinical Research Center is to collect and study patients who voluntarily agree to answer questions about their personal lives and who volunteer to donate blood and tissue for scientific study. Initially, we plan to collect specimens from UCSF, San Francisco General Hospital and the VA Hospital. If funds are available, we anticipate expanding our activities to include other hospitals in the Bay Area and other physicians in the community. At the core of this investigation is a computer based data system on individuals with AIDS, gay men with ARC, gay men at risk for acquiring one of these diseases, gay men free of those risks and heterosexual controls. Specimens for study are being placed in a Tissue Bank for investigations at this hospital and with collaborators in the University system elsewhere and collaborators throughout the world.

Two hundred thousand dollars of our grant has been set aside for this computer based network. Nine IBM personal computers, which will serve as terminals for the hospitals involved and for the investigators at this center, have already been ordered through University Purchasing. There has been a delay in their delivery because of the usual delay in getting equipment through the University system, however, the order is now with IBM and we are promised delivery of the first IBM-PC computer on May 23.

Mr. Frank Becker has been hired as a computer consultant to analyze our project and advise us on the type of mainframe system that we need to purchase and the software programs that we should utilize. Mr. Becker will be calling and meeting with each of you over the next two weeks.

One hundred thousand dollars of our AIDS Clinical Research Center funds were spend last July for the Tissue Bank, under the direction of Dr. John Greenspan. The Tissue Bank is now the largest tissue repository in the nation and is functioning extremely smoothly. The site visitors toured Dr. Greenspan's laboratory and were favorably impressed with the equipment and procedures that he has established.

Most of the specimens in the Tissue Bank were collected at the Kaposi's Sarcoma Clinic over the past two years. For the last six months, increasing numbers of specimens have been collected at San Francisco General Hospital and given the number of patients being seen at that facility at the present time we would anticipate that this number will continue to grow. San Francisco General Hospital was extremely fortunate in receiving a grant from the City of San Francisco to initiate an AIDS Clinic. At our urging, the University of California San Francisco has decided to establish an AIDS Clinic, which should relieve the burden already being perceived by the physicians at San Francisco General. Dr. Harry Hollander has been chosen to run the AIDS Clinic and \$100,000.00 of our AIDS Clinical Research funds have been set aside as seed money for his salary and for salary for his staff. His staff will include a nurse practitioner, a secretary and a social worker.

Funds in the AIDS Clinical Research Center continue to support the Kaposi's Sarcoma Clinic. That Clinic continues to stage patients with histologically proved Kaposi's Sarcoma and then refer them to San Francisco General Hospital for protocol treatment. Patients who agree donate specimens for the Tissue Bank during the staging workup. In addition to this group, we are following lovers of people with Kaposi's Sarcoma, men who choose alternative hollistic care and patients with traditional Kaposi's Sarcoma. The Kaposi's Sarcoma Clinic is also conducting a small study on immunological status of homosexual men who have never engaged in receptive anal or oral sex. Approximately \$80,000.00 of our AIDS Clinical Research grant is used to support this activity; \$40,000.00 is used for salaries and \$40,000.00 is used to defray the costs of studies performed on individuals who are not ill.

Education is a major component of the AIDS Clinical Research Center. Initially, we had viewed this educational activity as being directed primarily to physicians and health care providers. As we became aware of the fact that significant behavior modification would be necessary to prevent the spread to a larger number of gay men we have shifted the focus of the education program to study behavior modification and ways of disseminating and implementing health positive measures. The AIDS Clinical Research Center sponsored a major national behavior modification seminar on May 4-5 in an effort to reach a consensus on what the message should be to the gay community and how best to present that message.

We pointed out to the site visitors that the state funds were released to us in two pulses. One-fourth of the funds, approximately \$100,000.00 were released in August of 1983. Since we felt that the Tissue Bank was the most important component of the Clinical Research Center, we spent virtually 100% of the money on that facility within the first two months. The second pulse of money was released in November.

May 7, 1984

AIDS Clinical Research Center - Principal Investigators

Page 3

That money was used to hire an administrative assistant and to order computers. Those computers were ordered in February of this year. A committee, chaired by Dr. Stobo of the Department of Medicine, has been extremely cooperative in studying and setting up the AIDS Clinic. The AIDS Clinic will begin functioning on July 1, 1984. On balance, we feel that we have accomplished a tremendous amount in the five months since the bulk of the state funds were released and we are proud of our achievement. The site visitors voiced their admiration for what we had accomplished and it is my perception that they will recommend continued funding in the next year. Selected ones of you were invited to present material at the site visit which I thought would help the visitors understand our mission and how we all work together. I would like to again thank you for your presentations which were extremely positive and beneficial. We have been notified by the University-Wide Task Force that they would like our budget for 1984-85 by May 31. The AIDS Clinical Research Center administrative Assistant, Ernesto Hinojos, is putting together that proposal. I would like to invite each of you to submit to Ernesto a budget for those activities which fall under the AIDS Clinical Research Center. You should use a standard NIH format. Ernesto can assist you with this process, if necessary. Since we are facing a May 31 deadline, I would like to ask that you have those proposals to us no later than May 20.

Dr. Volberding

Dr. Ziegler

Dr. Abrams

Dr. Moss

Dr. Levy

Dr. Drew

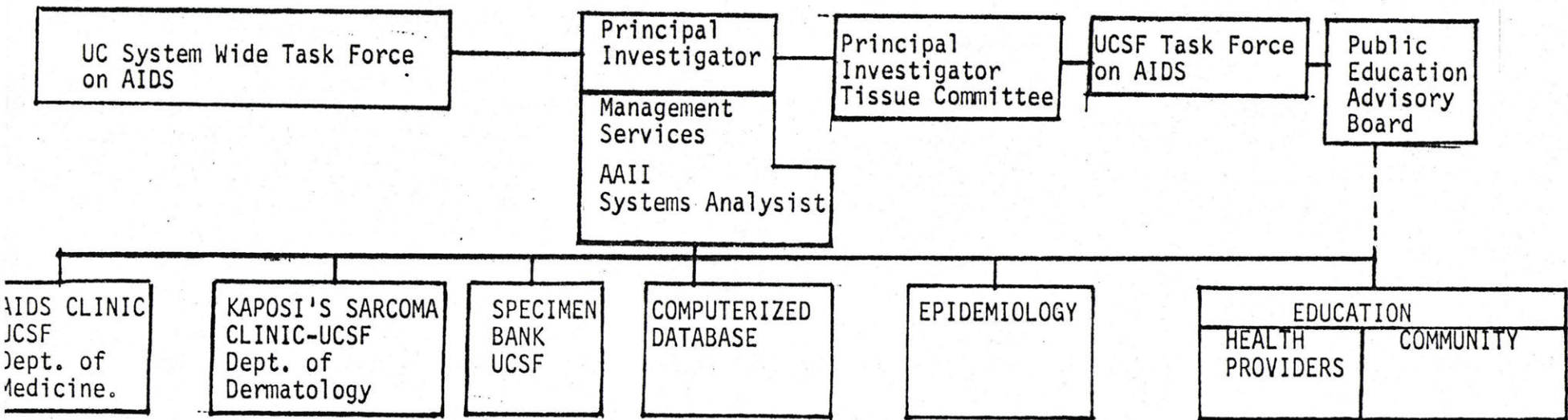
Dr. Casavant

Dr. Ammann

Dr. Stites

Dr. Greenspan

AIDS CLINICAL RESEARCH CENTER AT UCSF



ec Wanda

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SAN FRANCISCO, CALIFORNIA 94143

AIDS Clinical Research Center

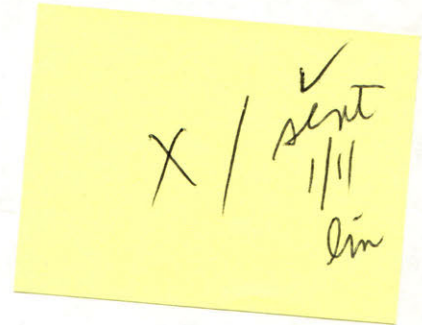
December 27, 1984

MEMORANDUM

TO: Teddi Johnson, UCSF, A 342

FROM: Marcus A. Conant, M.D.
Director, AIDS Clinical Research Center

RE: Tissue Bank funding



Dr. Greenspan has requested that all of the funds allocated for the tissue bank be transferred to him. Dr. Greenspan was awarded \$69,220. by the University Task Force on AIDS. \$34,611, which represented 1/2 of his annual allocation, was transferred on Oct. 26.

Would you be kind enough to transfer the remaining \$34,611. to Dr. Greenspan's account.

Dr. Greenspan understands that these funds are for the periods Feb.-April and May-July. Unless a supplemental budget is approved by the University Task Force on AIDS, the tissue bank will be unfunded from Aug. 1, 1985 until Nov. 1, 1986.

MAC/ds

cc: Dr. John Greenspan, UCSF, HSW 604

AIDS tissue totals

Total: 180

Breakdown:

AIDS - 31

KS - 84

LNS - 21

Heavy leukoplakia - 6

AIDS? - 1

KS, HSV - 4

KS, OI - 2

KS, CA - 2

KS, PCP - 1

KS, LNS - 6

Hodgkin's - 1

KS, LNS, OI - 1

R/o autoimmune dermatitis - 2

R/o KS - 1

Leukoplakia - 1

PCP, BL - 1

AIDS Bank Totals as of 18 May 84

Serum -

Total = 542

Breakdown:

KS - 101
MOSS - 323
LNS - 35
PCP - 14
ITP - 10
INS - 7
CON - 13
? - 11
BL - 1
R/O AIDS - 12

KS, LNS - 3
OI - 3
KS, PCP - 1
KS, CA - 1
LNS, VZV - 1
EBV - 1
R/O PCP, LNS - 1
LNS, ITP - 1
KS, LNS, CRY - 1
KS, CRY - 1
MAR - 1

UNIVERSITYWIDE TASK FORCE ON AIDS
UNIVERSITY OF CALIFORNIA

AIDS RESEARCH PROGRAM 1983-1984

I. Title of Project: UCSF AIDS TISSUE/SERUM BANK

II. Principal Investigator:

Greenspan, John S.

Name (last, first, M.I.)

Signature

Mailing address (use only those lines necessary, no abbreviations please):

Stomatology/Oral Biology

Phone numbers:

Department/Division

Office: (415) 666-2220

HSW 604

Laboratory: (415) 666-2220

Bldg./Room #

Dept.(messages): (415) 666-2220

Dentistry

School

San Francisco

94143

Campus

Zip

III. Eligibility of Responsible Investigator:

Professor

Official UC title

IV. Co-investigators:

See Part XI

V. Responsible Administrator (please see instructions):

I verify that the information given by the applicant concerning his/her appointment and current grant status is correct and complete.

Signature

Paul B. Robertson, Acting Chair

Name

Title (chair, etc.)

Stomatology

Dept.

VI. Review & Approval:

A. Human Subjects

- ☐ 1. Does not apply
☐ 2. Approval attached

☒ 3. Review pending

B. Biohazards

- ☐ 1. Does not apply
☐ 2. Memorandum of Understanding and Agreement attached

☒ 3. Review pending P₂* 698 HSW

C. Contracts & Grants: Information as required on the attached checklist is complete and detailed in the application.

Signature: _____

Date: _____

VII. Budget:

1. Personnel (list all professional personnel to be associated with project)

On a separate page, provide justification of listed personnel for whom support is requested. (See Section XI)

<u>Name</u>	<u>Official University Title of Position</u>	<u>% Time</u>	<u>Salary Requested</u>
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

Note: No fringe benefits are included.

*30% time, 20% salary

Total personnel \$ 49,290.

2. Supplies & Expense (list general categories)

Liquid nitrogen, dry ice	2,000	
Sterile disposables	1,000	
Cryostat supplies, plastic disposables	1,000	
Film, processing, chemicals	1,500	
Equipment service	1,000	
Mail, xerox, charts, slides	1,000	
Phone	350	
Travel (to consult with UCLA and other groups and to attend meetings)	1,500	
	Total S&E \$	<u>9,350.</u>

3. Equipment (list items over \$200 and on a separate page provide justification for items over \$500) (See Section XI)

Freezers, Revco -70 with backup inventory & recorder 2 @ 7,000	14,000
Liquid N ₂ apparatus Almac	2,500
Holders, racks, boxes	1,500
Binocular microscope A.O.	2,500
Bench centrifuge IEC	3,700
IBM PC XT with peripherals, software	9,600

4. Renovations. For P2* room

8,000

Total Equipment \$ 33,800.

TOTAL REQUESTED \$ 100,440.

VIII. Additional Financial Support (see instructions): List all program projects and all sources of funds which support your research. For each item list (a) grant number, (b) granting agency, (c) project title, (d) grant period, (e) length of grant commitment, (f) amount (both total period and overlap period), and (g) relationship to proposed AIDS grant. Attach the abstract of each grant application. Use continuation sheets as required.

It is the responsibility of the applicant to clearly document that there is no overlap between other grants and the proposed AIDS grant.

1. Other grants available during the requested grant period:

1. State Lupus Commission 10/1/82-9/30/83 \$ 10,000
No overlap - unrelated project "Role of lpr gene
in murine lupus"
2. NIDR DE05330 "Herpes virus antibodies in oral cancer" 1983-1986 \$173,000
No overlap - unrelated project. Co-investigator
5%.

2. Other grant applications pending:

1. Supplement to 1 U01 CA/A1 34980-1 "Acquired Immunodeficiency Syndrome" Proposal is for support of the AIDS Tissue & Serum Bank July 1, 1984 - June 30, 1987 to commence when funding from this application for State funding are used up. \$138,650 direct costs first year; \$402,846 total direct costs over 3 years.
2. NIDR R02 "Multiple factors in the etiology of oral cancer." 1983-1986. \$160,000 over three years; \$56,000 direct costs first year.
No overlap.

ABSTRACT OF RESEARCH PLAN

CURRENT

Support

NAME AND ADDRESS OF APPLICANT ORGANIZATION (Same as Item 11, page 1)

Dept. of Oral Medicine and Hospital Dentistry, School of Dentistry,
University of California, San Francisco, CA 94143

TITLE OF APPLICATION (Same as Item 1, page 1)

Herpes virus antibodies in patients with oral cancer

Name, Title and Department of all professional personnel engaged on project, beginning with Principal Investigator/Program Director

Edward J. Shillitoe, Assistant Professor, Dept. of Oral Medicine and Hospital
Dentistry, School of Dentistry.

Sol Silverman Jr., Professor, Dept. of Oral Medicine and Hospital Dentistry,
School of Dentistry.

John S. Greenspan, Professor, Dept. of Oral Medicine and Hospital Dentistry,
School of Dentistry.

William M. Wara, Associate Professor, Dept. of Radiation Oncology, School of
Medicine.

ABSTRACT OF RESEARCH PLAN: Concisely describe the application's specific aims, methodology and long-term objectives, making reference to the scientific disciplines involved and the health-relatedness of the project. The abstract should be self-contained so that it can serve as a succinct and accurate description of the application when separated from it. DO NOT EXCEED THE SPACE PROVIDED.

We will study patients with oral squamous cell carcinoma, in order to find why that disease is associated with antibody to herpes simplex virus type-1 (HSV-1).

A prospective study of patients with oral cancer will be continued to find if their survival after treatment is associated with their levels of IgG, IgA or IgM antibody to HSV-1 at the time of diagnosis. Repeated serum samples will continue to be obtained from each patient to find if rising levels of IgG antibody or falling levels of IgA or IgM antibody to HSV-1 can be used to predict recurrence of the tumor. Repeated serum samples will also be obtained from patients with oral leukoplakia to find if antibodies to HSV-1 change significantly before, or at the time of malignant transformation.

Efforts will be made to isolate a putative tumor-associated HSV-1 antigen. The protein will be identified at the various stages of its purification by showing that it reacts with serum antibody from cancer patients to a greater extent than with serum antibody from control subjects. This should reveal more specific information regarding the association between oral cancer and antibody to HSV-1 than can be gained when whole virus is used as an antigen. Once the tumor-associated antigen has been purified to homogeneity, antisera to it will be raised in guinea pigs, and a monoclonal antibody to it will be raised in a mouse hybridoma system. The antisera and monoclonal antibody will be used to stain oral cancer tissue by an avidin-biotin peroxidase method, in an effort to locate the protein in the malignant cells. This may allow classification of patients with oral cancer into those whose tumor is associated with HSV-1, and those whose tumor is not. This classification will be examined to find if it is associated with significant clinical differences, such as prognosis, of the patients.

If an HSV-1 antigen is found in cells of oral leukoplakia or carcinoma, future efforts would be directed towards elucidation of its function in the initiation and maintenance of malignancy, and eventually methods to inhibit these activities might be developed.

LABORATORY ANIMALS INVOLVED. Identify by common names. If none, state "none"

Mice, Guinea pigs.

Current Support Seaman - State Lupus Grant
3. Short Summary of the Project: Ends 10/31/83

In this proposed second year of our work on this topic, we intend to further investigate the lymphoproliferative and autoimmune disease in lpr-bearing strains of mice. In particular, we will attempt to confirm and further explore our observation (see progress report) that the lymphoproliferative and autoimmune features of the murine disease, induced by the gene lp, have separate manifestations and respond differently to experimental intervention.

4. Background Information and Significance of the Project

Our recent work (see progress report) has indicated that the immunopathological features of murine disease, induced by the gene lpr, which is responsible for interleukin-2 deficiency, may have two components. Thus, the massive lymphoid proliferation which occurs in C57 Black/lpr mice is due to an influx and proliferation of Thy 1.2 bearing lymphocytes, while the few infiltrates of chronic inflammatory cells which we found in salivary glands, kidney, and other organs were predominantly negative for this antigen. Similarly, in our experiments involving attempts to treat the disease with monoclonal antibodies and other agents, clinical and serological features of autoimmune disease were reduced by anti-Thy 1.2 antibody, while histological and immunopathological features of autoimmunity were not significantly affected. We propose to further investigate this phenomenon by performing serial histopathological and immunopathological examinations of mice of other lpr-bearing strains at a range of ages from birth to beyond maturity. In addition, we intend to continue to support the work of Dr. Seaman's group by providing histopathological and immunopathological examinations of animals in his experiments (see Dr. Seaman's proposal).

Methods

Please see progress report for description of pathological and immunopathological examination procedures. In addition to the antibodies used during this current year, as further antibodies against murine lymphocyte and other mononuclear cell types become available, we intend to use these also. We will use these methods on mice of the strains MRL/lpr, AKR/lpr and C3h/lpr. These studies of the natural history and sequential development of the disease in these mice will be complementary to Dr. Seaman's experimental interventions, using a variety of agents to manipulate the disease, as models for the treatment of human lupus and allied conditions. It should be emphasized that the salivary gland and other lymphoinfiltrative disease offer a model in some respects for human Sjogren's syndrome. This disease is closely allied to lupus erythematosus and is probably part of the same disease complex or group of diseases. Current concepts regarding its etiology, pathogenesis and approach to treatment closely parallel, or are identical with those concerning human systemic lupus erythematosus. It is therefore anticipated that the findings from our studies will also have an eventual impact on our understanding of and management of Sjogren's syndrome.

Summary.

Principal Investigators P. Volberding/J. Zeigler

AIDS TISSUE AND SERUM BANK

1. Introduction

It has been our intent from the inception of the UCSF AIDS research program to establish an extensive, well documented collection of tissue and serum from AIDS cases, individuals at risk and selected controls. The uses of these specimen collections fall into two broad categories:

i) Collection of sufficient specimens, in a relatively short period, to perform our current and planned studies and to supply collaborators.

ii) Build up, over a longer period, extensive specimen collections for prospective studies, as part of existing plans and also to permit testing of new hypothesis as new information on AIDS becomes available, but on well documented cases, the progress and outcome of which is recorded.

2. Present arrangements

a) Tissue Committee

Proposed studies are reviewed by this committee, at regularly scheduled meetings, for appropriateness and quality and to avoid overlap and conflict with other studies.

Members: Volberding (Chair), Zeigler, Conant, Greenspan, Levy, Ammann, Stites, Drew, Abrams.

The Tissue Committee uses the following criteria in reviewing proposals requesting tissue or serum specimens:

- i) Relevance to the problems of etiology, pathogenesis, diagnosis and treatment of AIDS.
- ii) Compliance with human subjects and biohazard regulations
- iii) Evidence that specimens will be used economically and appropriately
- iv) Retention of sufficient material for long term port-hoc studies and for retesting.
- v) Avoidance of overlap between investigations in our core/collaboration group.

Greenspan
7/1/84-6/30/87
Submitted
4/17/83
PENDING

NIH

Pending
NHL

B) Tissue collection and distribution

Biopsy and autopsy tissue specimens are collected, stored and distributed according to the protocol outlined in Figure 2.

C) Tissue bank

Over the last 16 months, we have collected 201 tissue specimens which are currently frozen and stored in liquid nitrogen. These comprise:

Kaposi's Sarcoma Skin (AIDS)	70
Kaposi's Sarcoma - skin (Traditional)	3
Kaposi's Sarcoma - Lymphnode form	40
Normal Skin (KS Patients)	31
Normal Skin (Controls)	6
Lymphadenopathy nodes	35
Control nodes	7
AIDS lymphomas	3
Other (Lung, prostate, salivary gland)	6

Sections have been cut for the following studies:

Dr. Drew - CMV in KS and LNS
Dr. Greenspan - Lymphocyte subsets in KS and LNS
Dr. Lennette - EBV in KS and LNS
Dr. Beckstead - Histogenesis of KS
Dr. Kramer - Basement membrane and extra-cellular matrix components in KS.
Drs. Rutter/Altmann/Zeimer - Hepatitis B virus in KS

Several other studies will be commenced if funding is provided. It must be mentioned that cryosectioning of fresh frozen unfixed AIDS material is regarded as potentially quite hazardous because of the danger of microtome knife cuts and contamination with tissue. Highly trained and dedicated personnel are required, as is a dedicated cryostat and a room used only for AIDS work. The cryostat cannot be used for other studies without laborious decontamination.

D) Serum Collection and distribution

Blood is collected and distributed according to the protocol outlined in Figure 3. A 5ml serum specimen is obtained at each blood collection and is divided into 5-ml aliquots. These are frozen and stored at -70C.

E) Serum Bank

We have now accumulated approximately 700 frozen serum samples from AIDS patients, individuals from high risk groups and controls. This collection has been extensively used by Drs. Drew and Levy as well as by non-UCSF collaborators. In addition, Dr. Drew has a further 900 specimens frozen and documented which will be transferred to the specimen bank if we are funded.

ABSTRACT OF RESEARCH PLAN

NAME AND ADDRESS OF APPLICANT ORGANIZATION (Same as Item 11, page 1)
Department of Oral Medicine and Hospital Dentistry, School of Dentistry
University of California, San Francisco, CA 94143

TITLE OF APPLICATION (Same as Item 1, page 1)
Multiple factors in the etiology of oral cancer

Name, Title and Department of all professional personnel engaged on project, beginning with Principal Investigator/Program Director

Edward J. Shillitoe, Assistant Professor, Oral Medicine and Hospital Dentistry,
School of Dentistry

J. Michael Bishop, Professor, Microbiology, School of Medicine

John S. Greenspan, Professor, Oral Medicine and Hospital Dentistry,
School of Dentistry

ABSTRACT OF RESEARCH PLAN: Concisely describe the application's specific aims, methodology and long-term objectives, making reference to the scientific disciplines involved and the health-relatedness of the project. The abstract should be self-contained so that it can serve as a succinct and accurate description of the application when separated from it. DO NOT EXCEED THE SPACE PROVIDED.

Cancer of the mouth is probably the result of the interaction of several factors. Epidemiological studies have shown that the disease is more common in cigarette smokers, and the risk increases with an increase in the amount smoked per day. Smokers have higher levels of antibody to Herpes simplex virus type-1 (HSV-1) than non-smokers, and antibody to HSV-1 is associated with oral cancer. We will use an animal model to test the hypothesis that constituents of cigarette smoke can reactivate latent herpes simplex virus, and that a co-carcinogenic interaction between them causes the tumor.

Mice will be infected on the skin with an oral isolate of HSV-1. After the infection has resolved and the virus has become latent, experiments will show if it can be reactivated with condensates of tobacco smoke and with various polycyclic hydrocarbons. If HSV is found to increase the rate of the tumor induction, the role of the virus will be studied to find if it is an initiator, a promotor, or both. The tumors will be examined for HSV antigens by a peroxidase-antiperoxidase method. The mice's serum antibodies to HSV will be measured by an enzyme-linked immunosorbent assay, and HSV DNA will be sought in the tumor cells by nucleic acid hybridization. Efforts will then be made to inhibit the co-carcinogenic effect with selected drugs.

Data obtained will be compared with existing data from human patients to find if the animal model is comparable with the human disease.

This project will accumulate evidence to support or refute a possible etiology of oral cancer, and it may provide a rational mechanism for the prevention of that disease.

LABORATORY ANIMALS INVOLVED. Identify by common names. If none, state "none"

Mice

IX. Biographical Sketch

(give the following information for all professional personnel)

Name	Official Title		
John S. Greenspan	Professor of Oral Biology and Oral Pathology		
Education (begin with the baccalaureate training and include postdoctoral)			
Institution and location	Degree	Year	Scientific Field
Royal Free Hosp.Med.School(London)	B.Sc.	1959	Anatomy, 1st Class Honors
Royal Dental Hosp. of London, School of Dental Surgery (Univ.of London)	B.D.S.	1963	Honors-Surgery & Dent. Surgery
	Ph.D.	1967	Experimental Pathology
Member, Royal College of Pathologists		1971	Oral Pathology
Honors			
1982 Fellow, American College of Dentists			
1983 Fellow, Royal College of Pathologists			
Major Research Interest		Role in Proposed Project	
Oral Pathology/Immunopathology		Co-investigator	
Tissue and Serum Bank		Director	
Research and/or Professional Experience (Starting with present position, list pertinent professional background and employment)			
1982 - Chairman, Division of Oral Biology, Department of Stomatology.			
1977-82 - Vice Chairman for Research and Graduate Programs. Department of Oral Medicine and Hospital Dentistry, U.C.S.F. School of Dentistry.			
1976 - Professor of Oral Biology and Oral Pathology, School of Dentistry and School of Medicine, University of California, San Francisco, CA.			
1972-76 - Senior Lecturer with Consultant Status (full time) in Oral Pathology, Royal Dental Hospital of London, School of Dental Surgery; Consultant Dental Surgeon to the Royal Dental Hospital, St. George's Hospital; and Consultant Oral Pathologist, St. John's Hospital for Diseases of the Skin, London, England.			
1968-72 - Senior Lecturer Oral Pathology, Royal Dental Hospital of London, School of Dental Surgery.			
1969-71 - Fellow (part time), Dept. of Pathology, St. George's Hospital Medical School.			
1963-68 - Lecturer (full time) Oral Pathology, Royal Dental Hospital of London, School of Dental Surgery.			
1963 - Research Fellow, Dept. of Pathology, Royal Postgraduate Medical School, London.			
1962 - Assistant House Surgeon, Royal Dental Hospital, London.			
Publications Pertinent to Proposed Research			
Barth C, White C, Dawson C, and Greenspan JS and Schachter J, 1983, Lymphocyte subsets in conjunctival smears from cases of endemic trachoma. (Submitted for publication).			
Greenspan JS, and Shillitoe EJ, 1983, Microbial pathogenesis in oral soft tissue diseases. J. Dent. Res. (In press).			

- Armitage GC, Banks TA, Newbrun E, Greenspan JS, Hoover CI and Anderson JH, 1983, Immunologic observations in Rhesus monkeys with Shigella - associated periodontal disease. *J. Periodont. Res.* (In press).
- Seaman W, Wofsy D, Greenspan JS, and Ledbetter JA, 1983, Monoclonal antibody to Thy-1 reduces lymphoproliferation and renal disease in autoimmune MRL/lpr mice. *J. Immunol.* 130:1713-1718.
- Shillitoe EJ, Greenspan D, Greenspan JS and Silverman S Jr., 1983, Immunoglobulin class of antibody to herpes simplex virus in patients with oral cancer. *Cancer* 51:65-71.
- Greenspan JS, Daniels TE, Shillitoe ES, and Olson JA, 1982, Mononuclear cell subpopulations in recurrent aphthous stomatitis using monoclonal antibodies, flow cytometry and the ABC technique. *J. Dent. Res.* 61:346, Abstract 1507.
- Shillitoe EJ, Daniels TE, Whitcher JP, Strand CV, Talal N, and Greenspan JS, 1982, Antibody to cytomegalovirus in patients with Sjogren's syndrome, as determined by ELISA. *Arthritis Rheum.* 25:331-336.
- Shillitoe EJ, Greenspan D, Greenspan JS, Hansen LS, Silverman S Jr., 1982, Neutralizing antibody to Herpes simplex virus type 1 in patients with oral cancer. *Cancer* 49:2315-2320.
- Greenspan JS, Gadol N, Talal N and Olson JA, 1981, Antibody-dependent cellular cytotoxicity in recurrent aphthous ulceration. *Clin. Exp. Immunol.* 44:603-610.
- Jensen RH, Greenspan JS, Moore D, Talal N and Roubinian JR, 1981, Quantitative immunofluorescence microscopy of renal glomeruli from mice exhibiting murine lupus erythematosus. *J. Immunol. Meth.* 42:343-353.
- Gindhart TD, and Greenspan JS, 1980, Pre-neoplastic lymphoid hyperplasia in autoimmune New Zealand mice. Animal model of human disease: Angio-immunoblastic lymphadenopathy with dysproteinemia. *Am. J. Pathol.* 99:805-808.
- Roubinian J, Talal N, Greenspan JS, Goodman JR and Nussensweig V, 1979, Danazol's failure to suppress autoimmunity in NZB/NZW F1 mice. *Arthritis Rheum.* 22:1399-1402.
- Dockrell HM and Greenspan JS, 1979, Histochemical identification of T-cells in oral lichen planus. *Oral Surg.* 48:42-46.
- Roubinian JR, Talal N, Greenspan JS and Goodman JR, 1979, Delayed androgen treatment prolongs survival in murine lupus. *J. Clin. Invest.* 63:902-911.
- Oberle LK, Fukuyama K, Daniels TE and Greenspan JS, 1979, Immuno-histochemistry of-keratin in human oral epithelium. *Arch. Oral Biol.* 24:321-326.
- Zetter BR, Daniels TE, Quadra-White C and Greenspan JS, 1979, LETS protein in normal and pathological human oral epithelium. *J. Dent. Res.* 58:484-488.
- Seymour GJ and Greenspan JS, 1979, The phenotypic characterization of lymphocyte subpopulation in established human periodontal disease. *J. Periodont. Res.* 14:39-46.
- Seymour GJ, Dockrell HM and Greenspan JS, 1978, Enzyme differentiation of lymphocyte subpopulations in sections of human lymph nodes, tonsils and periodontal disease. *Clin. Exp. Immunol.* 32:169-178.
- Roubinian J, Talal N, Greenspan JS, Goodman J and Siiteri P, 1978, The effects of castration and sex hormone treatment on survival, anti-nucleic acid antibodies and glomerulonephritis. *J. Exp. Med.* 147:1568-1583.
- Dockrell HM, Seymour GJ, Playfair JHL and Greenspan JS, 1978, Cytochemical identification of T and B cells in situ in mouse lymphoid tissue and lymph nodes from the rat, gerbil and cat. *Ann. Immunol. (Inst Pasteur)* 129C:617-633.
- Siegel R, Chen KH, Aguilar JM and Greenspan JS, 1978, Biochemical and immunochemical study of lysyl oxidase in experimental hepatic fibrosis in the rat. *Proc. Natl. Acad. Sci. USA* 75:2945-2949.

X. Summary of Research Proposal for the AIDS Research Program

UNIVERSITYWIDE TASK FORCE ON AIDS: 1983-1984 Grant Awards

John S. Greenspan, Professor

Name and Title of Principal Investigator

Stomatology, San Francisco

Department and Campus

UCSF AIDS Tissue and Serum Bank

Title of Project

Use this space to summarize concisely your proposed research. Outline objectives and methods. Underscore the KEY words (not to exceed 10) in your abstract.

It is proposed to continue to establish an extensive, well-documented collection of tissue and serum from AIDS cases, individuals at risk and selected controls. These will be made available to collaborators at UCSF, other UC campuses and outside the UC system. This will permit economical multiple use of specimens in both retrospective & prospective studies.

The bank has been initiated, procedures for reviewing requests are in place and several collaborative projects are underway. This has been done without allocated resources and cannot continue unless new funding is made available.

If the AIDS Task Force awards you a research grant, copies of this page may be distributed to UC Contract and Grant Offices and to other interested UC individuals and groups.

XI. Explanation of Project

Principal Investigator John S. Greenspan

UCSF AIDS TISSUE AND SERUM BANK

1. Introduction

It has been our intent from the inception of the UCSF AIDS research program to establish an extensive, well documented collection of tissue and serum from AIDS cases, individuals at risk and selected controls. The uses of these specimen collections fall into two broad categories:

i) Collection of sufficient specimens, in a relatively short period, to perform our current and planned studies and to supply collaborators.

ii) Build up, over a longer period, extensive specimen collections for prospective studies, as part of existing plans and also to permit testing of new hypotheses as new information on AIDS becomes available, but on well documented cases, the progress and outcome of which is recorded.

2. Present arrangements

A) Tissue Committee

Proposed studies are reviewed by this committee, at regularly scheduled meetings, for appropriateness and quality and to avoid overlap and conflict with other studies.

Members: Volberding (Chair), Zeigler, Conant, Greenspan, Levy, Armann, Stites, Drew, Abrams.

The Tissue Committee uses the following criteria in reviewing proposals requesting tissue or serum specimens:

- i) Relevance to the problems of etiology, pathogenesis, diagnosis and treatment of AIDS.
- ii) Compliance with human subjects and biohazard regulations
- iii) Evidence that specimens will be used economically and appropriately
- iv) Retention of sufficient material for long term post-hoc studies and for retesting.
- v) Avoidance of overlap between investigations in our core/collaboration group.

B) Tissue collection and distribution

Biopsy and autopsy tissue specimens are collected, stored and distributed according to the protocol outlined in Figure 2.

C) Tissue bank

Over the last 18 months, we have collected 263 tissue specimens which are currently frozen and stored in liquid nitrogen. These comprise:

Kaposi's Sarcoma Skin (AIDS)	75
Kaposi's Sarcoma - skin (Traditional)	3
Kaposi's Sarcoma - Lymphnode form	45
Normal Skin (KS Patients)	40
Normal Skin (Controls)	6
Lymphadenopathy nodes	70
Control nodes	15
AIDS lymphomas	3
Other (Lung, prostate, salivary gland)	6

Sections have been cut for the following studies:

Dr. Drew - CMV in KS and LNS
Dr. Greenspan - Lymphocyte subsets in KS and LNS
Dr. Lennette - EBV in KS and LNS
Dr. Beckstead - Histogenesis of KS
Dr. Kramer - Basement membrane and extra-cellular matrix components in KS.
Drs. Rutter/Altmann/Zeimer - Hepatitis B virus in KS

Several other studies will be commenced if funding is confirmed. It must be mentioned that cryosectioning of fresh frozen unfixed AIDS material is regarded as potentially quite hazardous because of the danger of microtome knife cuts and contamination with tissue. Highly trained and dedicated personnel are required, as is a dedicated cryostat and a room used only for AIDS work. The cryostat cannot be used for other studies without laborious decontamination.

D) Serum Collection and distribution

Blood is collected and distributed according to the protocol outlined in Figure 3. A 5ml serum specimen is obtained at each blood collection and is divided into 1 ml aliquots. These are frozen and stored at -70C.

E) Serum Bank

We have now accumulated approximately 700 frozen serum samples from AIDS patients, individuals from high risk groups and controls. This collection has been extensively used by Drs. Drew and Levy as well as by non-UCSF collaborators. In addition, Dr. Drew has a further 900 specimens frozen and documented which will be transferred to the specimen bank if we are funded.

F) Other specimens

We are reviewing proposals to store semen and sputum. Please see figure 4 for data on the rate of acquisition of specimens.

3. Biohazard aspects

Specimens are collected, handled stored and processed according to CDC guidelines (MMWR 31 577 1982). The room in which the blood and tissue are processed and stored has been assigned to this function on a temporary basis but must be allowed to return to routine use as soon as possible. Another room is available (see below) and will become dedicated to the AIDS specimen bank. Specimens must be taken to and from this facility in appropriate containers and by personnel aware of precautions and of procedures to be used in cases of spillage. Plentiful supplies of disposable gloves, masks and gowns are essential.

4. Personnel

A) Director, specimen bank.

Dr. John Greenspan is an immunopathologist with over 20 years of experience in tissue cryosectioning and histochemistry. His PhD training was with A.G. Everson Pearse, one of the developers of the cryostat, and his graduate student project was commenced using the prototype Pearse-Slee cryostat. He has 11 years experience of tissue immunopathology and cellular immunology involving studies of Sjogren's syndrome, murine autoimmunity, mucosal diseases and lymphoma, and 1 1/2 years involvement in the UCSF AIDS project. Dr. Greenspan will be responsible for all aspects of the specimen bank including establishing and supervising procedures, hiring and training staff, liason with the PIs, member of the UCSF AIDS group, University administration (Office of Environmental Health and Safety; Committee on Human Research), outside collaborators and appropriate NCI personnel. He will be responsible for the specimen data base, specimen selection for studies, periodic reports and contributions to publications. He will devote 30% of his time to this project and 20% salary is requested.

B) Staff Research Associate II

A fully trained medical laboratory technician will be appointed with experience in clinical and research laboratory work involving handling blood and blood products and in data handling using computer technology. This person will retrieve specimens from on-campus sites, San Francisco General Hospital and the VA Hospital and record specimen details in the computer. He/she will perform CBC and differential counts, centrifuge blood to obtain serum, aliquot samples, freeze and store them, retrieve specimens for study and maintain the data base. He/she will also prepare lymphocyte suspensions and stain them using monoclonal antibodies and immunofluorescence for counting in the FACS. This individual will, under the supervision of the director, set up and maintain procedures for receiving, handling, processing and storing tissue and serum. He/she will cut the sections and collaborate in immunohistochemical work where appropriate. At present, this job is performed half-time by a technician paid from other sources available to Dr. Greenspan. A new full-time person will be kept fully occupied and will release the existing individual for her normal tasks.

C) Administrative Assistant II

A experienced word processing operator with organizing skills will be appointed. He/she will: tabulate and prepare lists of specimens, liaise with clinic and laboratory staff and provide office support for the director. He/she will be available, when possible, to support Dr. Conant and the AIDS research group in the preparation of manuscripts and reports. This individual will also perform fiscal functions including ordering equipment, renovations and supplies and will make travel arrangements. He/she will deal with receiving requests for specimens and sending specimens to collaborators.

5. Laboratory

The specimen bank is located in the facilities of the Division of Oral Biology of the Department of Stomatology, UCSF. These are on the 6th floor of the Health Sciences West research tower. These laboratories of the Division occupy 4000 ft². A room of approximately 120 ft² with its own access off the main laboratory (see map attached) has been assigned for the AIDS specimen bank. When the alterations, for which funds are requested in this application, are completed and equipment is in place, this room will qualify as a P2* biohazard facility and will be used solely for storage and handling of AIDS specimens.

Funds are requested for these alterations, to include benches, services, air conditioning, new doors and windows.

6. Equipment

The AIDS specimen bank has been allocated funds, to purchase a new cryostat and a biohazard hood, through a UCSF Academic Senate multiple user equipment grant awarded in competition to Drs. Greenspan, Beckstead and Zeigler. These items have been ordered. The additional equipment requested here will complete the requirements of the bank and will permit exclusive use, in the AIDS specimen bank, of all the apparatus required.

The -70 Freezer is to store serum. The liquid nitrogen freezer and accessories are for the storage of frozen tissue specimens. The microscope is requested for use in examining cryostat specimens cut for collaborative investigations or in our own AIDS projects. The bench centrifuge will be used in the separation of serum samples from whole blood. A second freezer will be required towards the end of the year as specimens accrue in Dr. Moss' studies.

7. Computer

The AIDS investigations group, led by Drs. Zeigler, Conant and Volberding, are about to decide from among several options concerning data storage and processing systems. The major choice is between the use of one of the existing mainframes on campus or aquisition of our own system. Either choice will make available to us a sophisticated system capable of handling all of our needs. Our current urgent need is for an IBM Personal Computer with monitor, printer and graphics capabilities. Funds are requested to purchase this equipment and for software and training of personnel. The computer facilities will be used, for the purposes of the specimen bank, to record serum and tissue aquisitions, including selected data on each specimen and patient, to record disposition of samples from each specimen, to prepare lists of specimens of each type, and to retrieve data on material sent to each investigator. The computer will also be used for word processing, data analysis, and electronic mail within the group.

8. Office

The office of the Division of Oral Biology will serve the AIDS specimen bank. There is sufficient space and office equipment for the additional administrative assistant requested. The division has access to an NBI word processor which is currently being used to record information on tissue and serum specimens. This data will be transferred to the AIDS computer system when it becomes operational.

9. Supplies

Liquid nitrogen and dry ice are for freezing, transport and storage of specimens. Disposables are requested for biohazard protection of personnel. Supplies are also requested for the preparation of cryostat section of tissue and aliquots of serum.

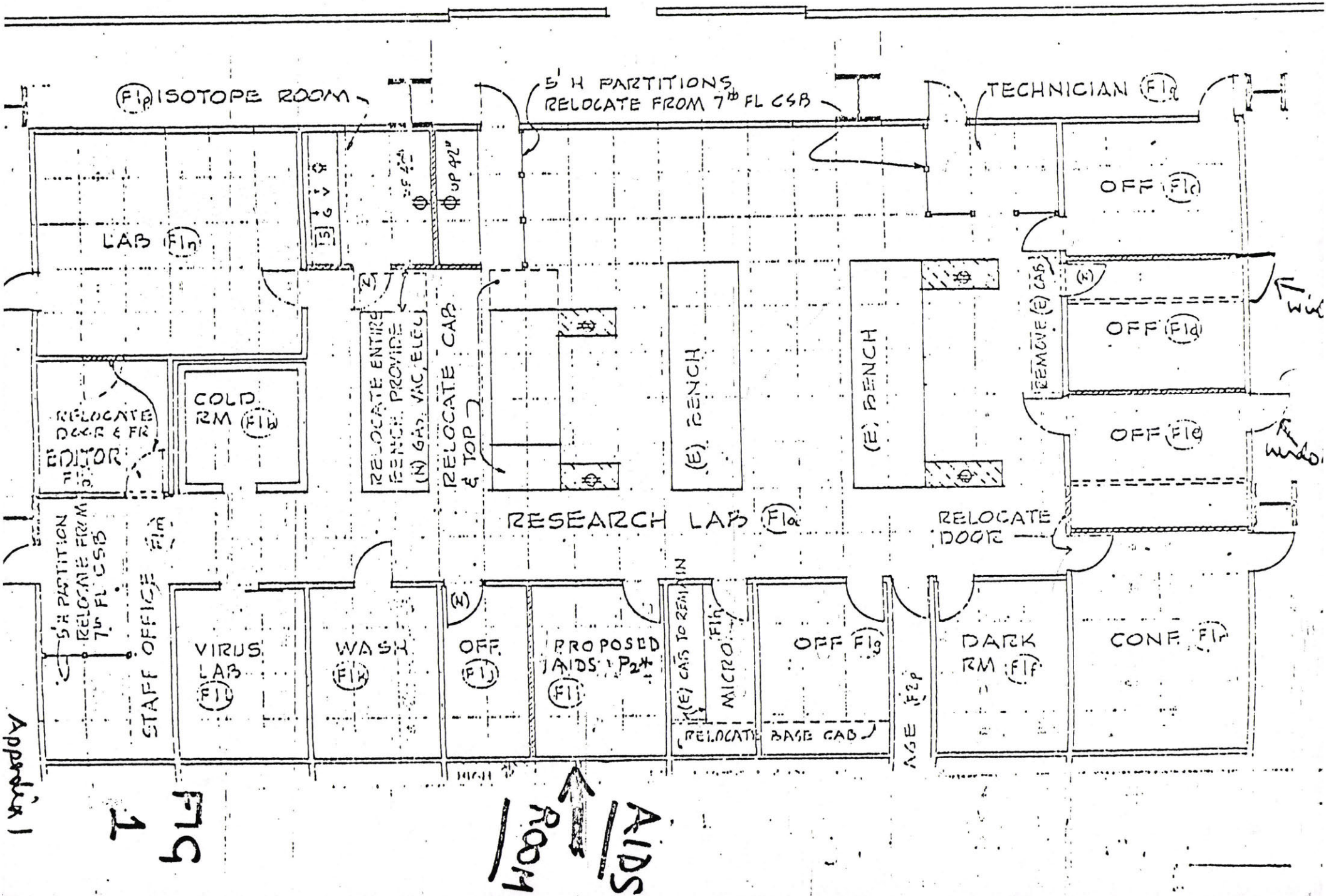
10. Travel

The specimen bank director (Dr. Greenspan) is involved in several of the studies outlined by other members of the UCSF AIDS Group and intends to attend scientific/professional meetings and to read papers. He and the SRA II will also travel in order to consult with colleagues in the UCLA group and other collaborators.

Note: The sum of \$75,821 has been assigned, from the emergency distribution by UCCRCC, for the specimen bank. The number of specimens being accepted has increased more than anticipated however, as has the number of requests for tissue and serum. Thus one year funding of \$100,400 total is now requested (this includes the \$75,821).

XII. Relation to the AIDS problem

We believe that the establishment of the bank is a key event in the UCSF AIDS research program. It has begun and will continue to be a priceless resource in the process of investigating the etiology and pathogenesis of the AIDS group of diseases.



BIOPSY OR BLOOD SPECIMEN INFORMATION FORM
AIDS RESEARCH PROJECT

LEAVE BLANK

BIRTHDATE _____

AGE _____

PATIENT NAME _____

SEX _____

CLINICAL DIAGNOSIS

KS

LYMPHADENOPATHY

PCP

OTHER _____

CURRENT TREATMENT NONE

CHEMOTHERAPY

TYPE, DURATION _____

INTERFERON

DURATION _____

OTHER _____

IF NONE, HAS PT. EVER BEEN TREATED FOR KS, AND WITH WHAT?

SPECIMEN
BIOPSY

SKIN: SITE _____

AMOUNT TISSUE _____

TYPE TISSUE

NORMAL SKIN

TUMOR

NODE: SITE _____

OTHER: (BE SPECIFIC) _____

HAS TISSUE BEEN SENT ELSEWHERE?

YES

NO

IF "YES", WHERE? _____

TYPE OF
BLOOD SPECIMEN

WHOLE
BLOOD

SERUM

PLASMA

NUMBER OF TUBES _____

TYPE OF TUBES _____

DATE OBTAINED: _____

RESULTS:

PATH Dx _____

BLOOD WORK _____

COMMENT: _____

Appendix 2

CURRENT TISSUE PROTOCOL

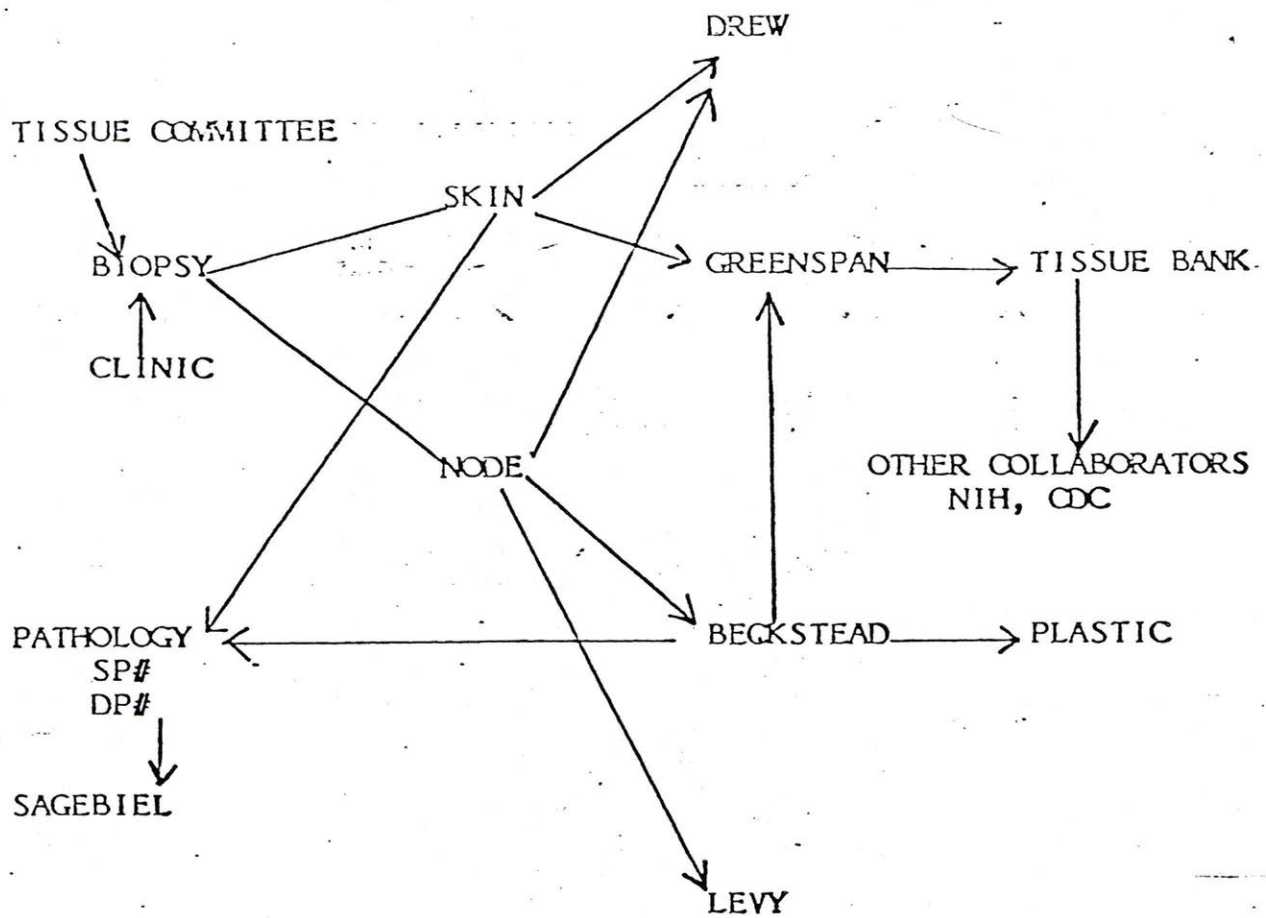


Fig 2

CURRENT BLOOD PROTOCOL

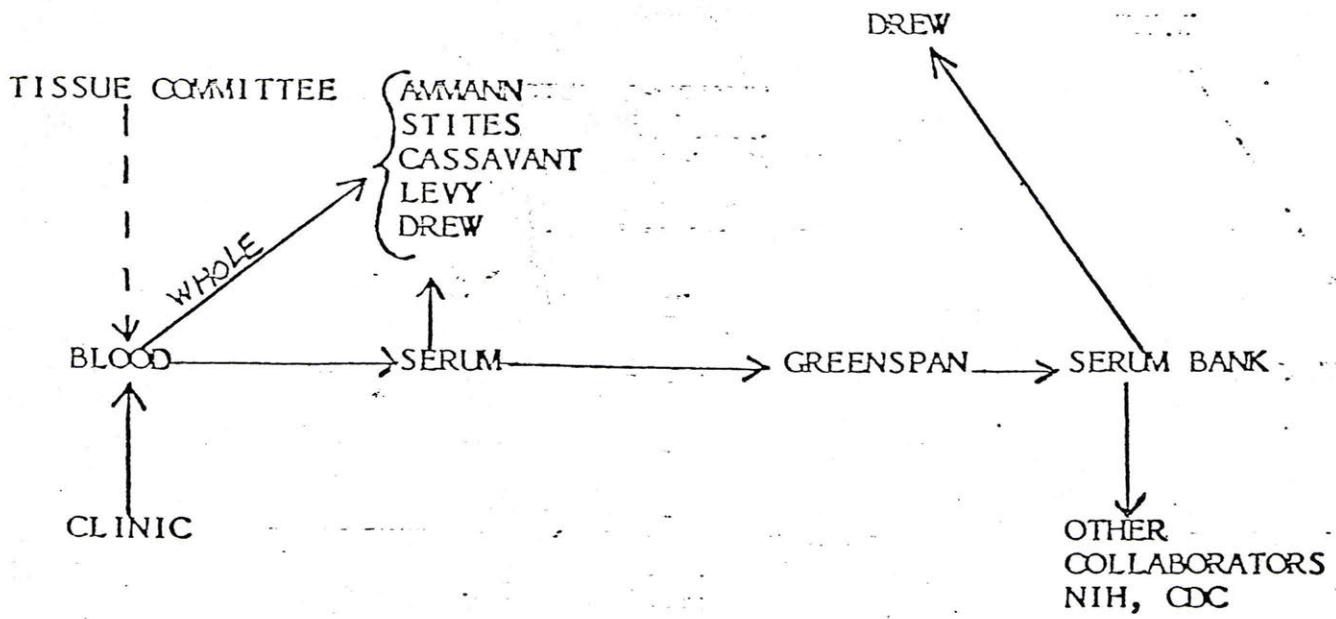


Fig 3

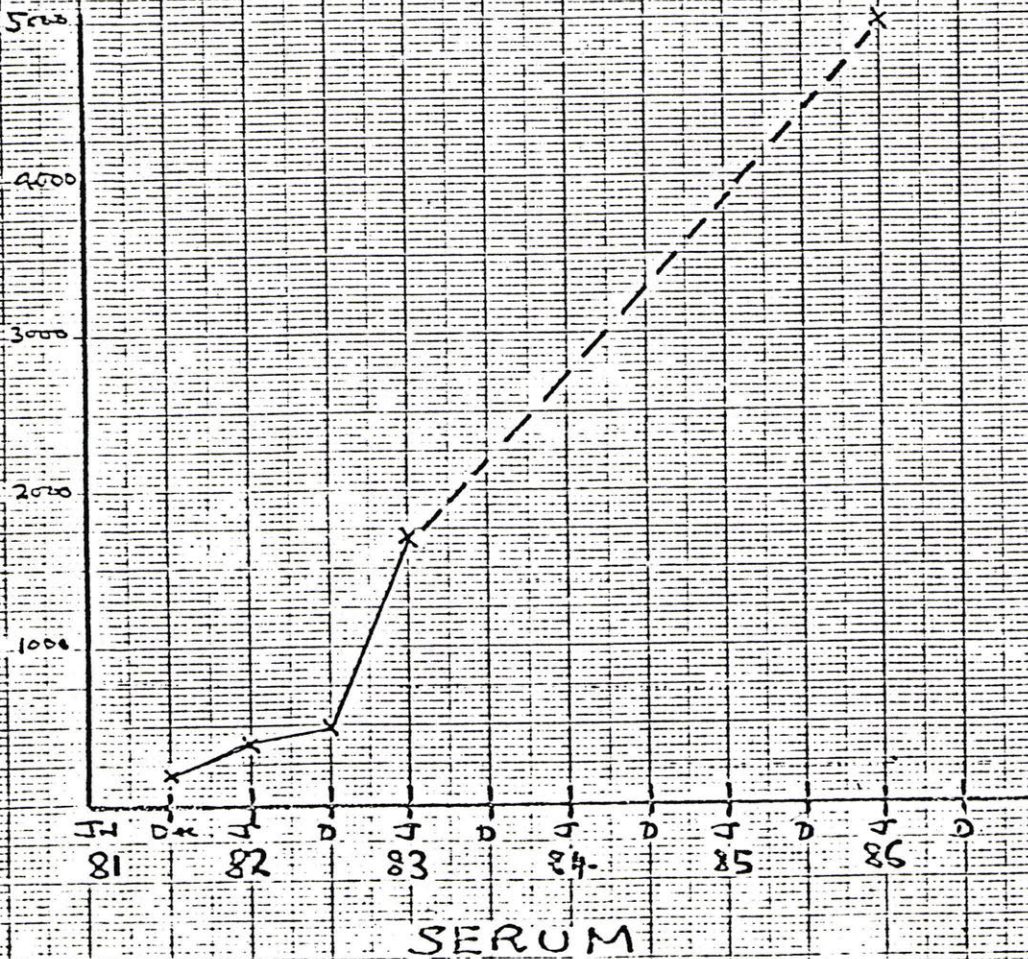
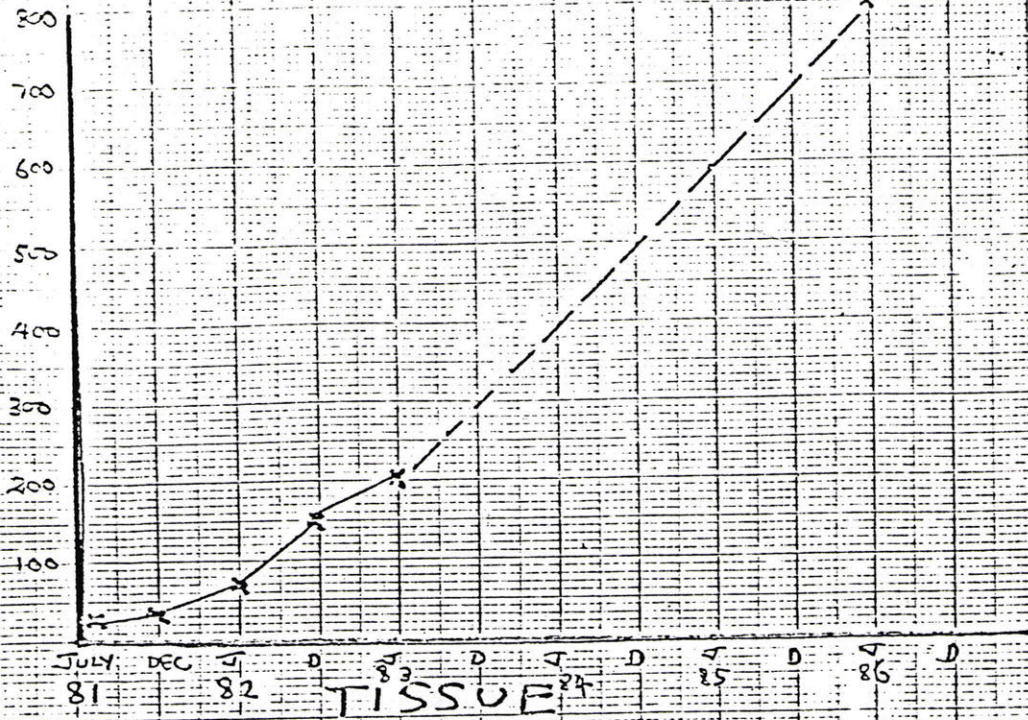


FIGURE A

Appendix 5

Mike Gottlieb

UCLA

4/26/84

Site Visit - Task Force

Not measuring up to us.

Can Re visit? ← ^{Paul} Mark

? Is there one form - get these from him

MEMORANDUM OF CALL

TO:

☐ YOU WERE CALLED BY— ☐ YOU WERE VISITED BY—

OF (Organization)

☐ PLEASE CALL → PHONE NO.
CODE/EXT.....

☐ WILL CALL AGAIN

☐ IS WAITING TO SEE YOU

☐ RETURNED YOUR CALL

☐ WISHES AN APPOINTMENT

MESSAGE

(213) 825

3/7/8

1257

RECEIVED BY

DATE

TIME

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO



SANTA BARBARA • SANTA CRUZ

SCHOOL OF MEDICINE
DEPARTMENT OF MEDICINE

SAN FRANCISCO, CALIFORNIA 94143

April 16, 1984

Walter L. Way, M.D.
Chairman, Committee on Human Research
UCSF
Clinics 116

Dear Dr. Way:

Enclosed is a short form application, consent form, and a brief protocol for the prospective collection and preservation of serum and tissue specimens from patients with AIDS. As we have identified more and more patients suffering from AIDS in San Francisco, and especially as research monies have become available for studies in this disease, the number of collaborators asking for specimens has increased dramatically. To accomodate these collaborations and to minimize the inconvenience to the patient we have been collecting serum and Kaposi's sarcoma skin biopsies at the time of diagnosis. These specimens have been frozen in a centralized facility under the direction of Dr. John Greenspan at UCSF. Initially these specimens were collected under a study protocol approved by CHR in 1982. This approval, initially just for patients with Kaposi's sarcoma, has since expired and the purpose of the attached protocol and consent form is to again obtain approval for this prospective collection of specimens for collaborative research and to include patients with other AIDS diagnoses including opportunistic infections and the AIDS related complex including lymphadenopathy syndrome and idiopathic thrombocytopenic purpura. In our application and consent form we have taken, we think, necessary precautions to insure the subject's confidentiality which is done by coding specimens to the San Francisco Department of Health AIDS registry. By this prospective collection of specimens from patients with AIDS, we feel we are minimizing the inconvenience and confusion caused by this research endeavor. Also, by collecting specimens in advance, we are able to aliquot blood in smaller than phlebotomy in a case-by-case basis.

I appreciate the somewhat unique aspects of the attached protocol but feel that it meets the needs of this research in a way than minimized the impact of these studies on our patient care.

Thank you very much for your consideration.

Sincerely,

Paul A. Volberding, M.D.
Director, AIDS/KS Clinic
San Francisco General Hospital

PAV/slh

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
COMMITTEE ON HUMAN RESEARCH

INITIAL SHORT-FORM APPLICATION
Submission Date 4/16/84

Principal Investigator University Assistant Prof. Med. UCSF
(UCSF Faculty) Paul A. Volberding, M.D. Title Chief Onc. SFGH Dept. Medicine/SFGH
P.I. Mailing Address Oncology Service, Ward 86, SFGH P.I. 821-8830
(campus if possible) Phone No.
Co-Investigator Is the principal investigator
and Title Marcus Conant, M.D. the sponsor/advisor only? Yes No
Co-P.I. Mailing Co-P.I.
Address UCSF Utilization Review M 181 Phone No. 666-3261
Project Collection and Preservation of Serum and Tissue Specimens from Patients with AIDS
Title for Multiple Collaborative Studies.

INSTRUCTIONS: PLEASE TYPE; SUBMIT

-4 COPIES OF THIS TWO-PAGE FORM

-4 COPIES OF ANY ATTACHMENTS (CONSENT FORMS, QUESTIONNAIRES, ETC.)

To be safe, allow 3 weeks for processing.

A) The point of this project is (Explain background, rationale, hypothesis, basic design, etc.): Patients with AIDS in San Francisco are currently the focus of multiple collaborative research studies both at UCSF and other institutions. The purposes of these research projects are varied but include an understanding of the etiology, immunology, epidemiology and therapy of this syndrome. Because there are so many requests for collaborative studies on specimens, specially including blood and biopsies of Kaposi's sarcoma from patients with AIDS, in this project these specimens will be collected on a regular basis both at time of diagnosis and at infrequent but regular intervals following diagnosis and preserved in a centralized facility which will insure the proper handling of these specimens with regards to bio-hazard potentials and which will insure the confidentiality of the patients from whom these specimens were collected.

In this project approximately 20 cc's of serum will be frozen at the time of diagnosis and every 3-6 months following diagnosis of AIDS. In addition, biopsies of Kaposi's sarcoma lesions and, in some case, normal skin for comparison will also be obtained at time of diagnosis and preserved.

B) The subject population(s) will be selected (or excluded) on the following criteria (Discuss how access will be gained as well as any problems relevant to special subject populations): Subjects will be selected for this project based on diagnosed acquired Immunodeficiency Syndrome or AIDS related complex (ARC) which includes, the syndrome of effused reactive lymphadenopathy and idiopathic thrombocytopenic purpura. Patients will be chosen from clinic populations at S.F.G.H., UCSF, and the San Francisco VA Hospital. The nature of the project will be discussed with the potential subject and consent signed before specimens are collected.

C) The following procedures involving humans will be done for purposes of the study (If applicable, include interview themes and questionnaires if not commonly known):

The expedited review category number from Consent Forum, Issue 5, is .

For the purposes of this study, no questionnaires or interviews will be required. Blood will be collected at time of diagnosis and every 3-6 months but at no time will amount to more than 20 cc's. Biopsies of Kaposi's sarcoma lesions and adjacent normal skin will be performed at initial diagnosis. Subsequent skin biopsies will be performed only if clinically needed for other management purposes. The principal indication for subsequent biopsies is generally to assess response or lack of response to therapy.

Investigator: Paul A. Volberding, M.D.

D) The risks involved in these procedures and the methods of minimizing the risks, inconveniences, or discomforts are (Include any potential for loss of privacy):
The risks involved with these studies are limited to those of venipuncture and skin biopsies. The risks of venipuncture are bruising or discomfort at the venipuncture site and occasionally temporary bruising. The risks of skin biopsy are discomfort at the biopsy site, minor bleeding, and rarely, infection of the biopsy.

The other major risk involved in any studies with AIDS revolves around questions of confidentiality. In this study patients' specimens will be coded at the site they are obtained by a code number which does not identify the patient by name or by hospital record number. Instead patients are identified by an AIDS registry code number generated by the San Francisco Department of Health and the key to this code is kept separate from medical records. Thus, questions of confidentiality should be minimized and the subjects are so informed in the attached consent form.

E) Describe the anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result:

There are no immediate benefits to the subjects who have agreed to participate in this project. However, the information obtained by collaborative studies of AIDS material obtained in this way is significant and may be of real value in identifying more clearly the nature of this disease. In addition, the patients who agree to participate in this project of prospective specimen preservation for collaborative research will be spared the otherwise inevitable multiple requests for blood and/or tissue specimens at later times. By having a centralized repository of AIDS specimens, patients will not need to be approached for these subsequent collaborations.

F) Describe the consent process and attach all consent documents. If waiver from use of written consent is requested, give the justification.

Consent will be obtained by signature of the attached consent form if approved by the Committee on Human Research. Patients will be asked to sign this consent before specimens are collected for preservation.

UNIVERSITY OF CALIFORNIA
CONSENT TO BE A RESEARCH SUBJECT
OVERALL CONSENT FOR AIDS SPECIMEN COLLECTION

Because many facts about AIDS remain unknown and because more cases are being diagnosed at a rapid rate, my physician, Dr. _____ and other investigators are interested in learning as much as possible about this syndrome.

Since I have been diagnosed as having AIDS, I have been asked to participate in a study to further assist their investigation. I have been asked to allow certain tests and procedures, i.e. blood drawing, lung function test and possible biopsies be performed.

By signing this consent I agree that blood will be drawn and the amount drawn from time to time will vary, but no more than 450cc (15 ounces) will be drawn in an eight (8) week period and no more than one (1) time a week. I am aware of the risk when having blood drawn, such as discomfort from needle puncture, possible bruising and, very rarely, infection. I agree that lung function tests such as chest x-rays and measurement of lung capacity be performed and realize these particular tests may be performed as frequently as every four (4) months.

A cancer known as Kaposi's sarcoma can accompany the acquired immune deficiency which produces lesions of the skin. If I am found to have any cancerous lesions I agree that skin biopsies be performed for evaluation of the lesions. I have been informed of the risk that may accompany biopsies such as minimal pain at site of biopsy, minimal bleeding and rarely, infection.

By signing this consent I am not agreeing to participate in any therapy program and retain the right to discontinue as a participant at any time without fear that further alternative treatments will be denied me. I agree to participate in this study and have been informed of the risk and benefit both orally and in writing and understand that I may refuse any test at any time.

All medical information such as medical records, test procedures and their results that pertain to me are totally confidential. My name will be kept separate from lists of tests and test results, my name will be replaced by a code number and any entry of my test(s) and test results into a computer will be entered by code number only.

If at any time during this study I am physically injured as a result of this study, treatment will be available. The cost of such treatment will be covered by the University, depending on the number and/or nature of the factors involved. For further information concerning compensation I may call (415) 666-1814.

Subject Name _____

Date _____

PROTOCOL FOR THE CLINICAL STUDY OF AIDS AT UCSF, FOR THE
PROSPECTIVE COLLECTION OF SPECIMENS FOR RESEARCH PURPOSES

INTRODUCTION

The Acquired Immune Deficiency Syndrome or AIDS is a serious new disease in which the cellular immune system regulation appears to be altered increasing dramatically the rate of malignancies, including Kaposi's sarcoma and non-Hodgkin's lymphoma and a variety of severe life threatening opportunistic infections, especially pneumocystis carinii pneumonia.

Because of the serious nature of this illness and the rapid spread of the epidemic, and because of the probability that the study of AIDS will result in advancement in our knowledge of the relationship between the immune system and cancers, extensive research projects are underway. Numerous investigators at UCSF, other UC campuses, and other medical centers across the country are interested in studying the epidemiology, etiology and immunology of this disease.

Because San Francisco represents a major focus of this epidemic and because of the coordination of AIDS activities at UCSF, numerous requests are received to provide patient material for these studies. To coordinate the collection and distribution of these specimens a Tissue Committee of the UCSF AIDS Working Group has been established. The chair of this committee is Dr. Paul Volberding.

In addition, a tissue and serum bank has been organized under the direction of Dr. John Greenspan to coordinate the optimum storage and processing of these specimens with appropriate biohazard precautions. To minimize the inconvenience of multiple studies to patients with AIDS and to prevent confusion among collaborating investigators, this protocol is intended to serve as a method for the centralization of this collection process. This protocol is not intended to give permission for any therapy studies. Additionally, any invasive tests that are not specifically outlined in this proposal will have separate protocols and consents approved through the Human Research Committee.

PROTOCOL

At the time of initial evaluation patients with AIDS or AIDS related conditions as defined by the CDC, will be asked to participate in an ongoing research effort. This will consist of collection of approximately 45cc's of blood on the first clinic visit and if the person has Kaposi's sarcoma will also include a biopsy of a Kaposi's lesion and adjacent normal skin. These specimens will be preserved by the Tissue and Serum Bank with code numbers to preserve patient confidentiality. In addition, at the initial clinic visit baseline, chest x-ray and pulmonary function tests will be obtained. Any other studies indicated for the patient's clinical condition will also be collected.

Patients will be followed as clinically indicated for their illness and will have additional blood collected for preservation in the Tissue Bank every 3 to 6 months. At no time will the amount of blood collected for this purpose exceed 30cc's. In addition, 10 follow-up patients will receive serial pulmonary function testing every 4 months. Subsequent biopsies of tumors may be performed, but only if clinically indicated, to assess response to therapy.

Any other invasive testing that is not of routine clinical utility will require a separate consent from each patient.

CONFIDENTIALITY

Because of the nature of AIDS and the risk groups involved in the epidemic, confidentiality concerns have been raised by a number of groups. To insure confidentiality of specimens collected on this protocol, no specimen will be identified by the patient's name. Code numbers will be used for this purpose as provided by the City Health Department AIDS Registry. This system of coding has been in place for the past several months and has been deemed acceptable by the Centers for Disease Control.

SUMMARY OF AIDS CLINICAL RESEARCH PROTOCOL

A DIAGNOSIS ON FIRST VISIT

	<u>AMOUNT</u>
BLOOD:	
STUDIES AS INDICATED CLINICALLY (CBC, CHEM PANELS ETC)	approx 20cc
BLOOD (SERUM) FOR PRESERVATION IN SPECIMEN BANK	approx 20cc

BIOPSY:

SKIN BIOPSY FOR DIAGNOSIS OF SUSPICIOUS LESION	- 4mm punch
SKIN BIOPSY OF LESION FOR PRESERVATION/ RESEARCH	- 4mm punch
BIOPSY OF NORMAL SKIN FOR PRESERVATION/RESEARCH	- 4mm punch
BIOPSY OF LYMPH NODE FOR DIAGNOSIS AS CLINICALLY INDICATED	
PORTION OF NODE TO BE PRESERVED FOR RESEARCH (NO BIOPSY OF NODE FOR RESEARCH ONLY)	

PULMONARY FUNCTION TESTING: BASELINE TESTING

TESTING IN FOLLOW-UP PERIOD

	<u>AMOUNT</u>	<u>FREQUENCY</u>
BLOOD:		
BLOOD (SERUM) FOR PRESERVING/RESEARCH	20 cc	3-6 months
BIOPSY:		
KS LESIONS BIOPSIED FOR CLINICAL INDICATIONS:	- 4 mm punch	clinically as indicated
LYMPH NODE BIOPSIED FOR CLINICAL INDICATIONS:	portion of biopsy	as indicated
PULMONARY FUNCTION TESTS:		4 months

AIDS CLINICAL RESEARCH GROUP

Form: Biopsy or Specimen Information Form

Leave Blank

Date _____
Acct# _____
R#-Tissue _____
AS#-Serum _____

Date Specimen Obtained _____
Contributing M.D. _____
Facility _____

Patient Code # _____
Birth Date _____
Age _____ Sex _____
Risk Group _____

Clinical Diagnosis:

KS _____
PCP _____
Lymphadenopathy _____
ITP _____
Other Infection _____
Other Malignancy _____
Coded Study _____

Study # _____

Current Therapy:

Therapy for Malignancy _____
Therapy for Infection _____
Ever Received Chemotherapy yes _____ no _____

Specimen: Type of Blood Specimen _____
 Blood Whole Blood _____ Plasma _____ Serum _____ Separated Cells _____

 Number of Tubes _____
 Tube Type _____

Biopsy:

Skin _____ Node _____ KS _____ Normal Skin _____ Other _____
Amount of Tissue _____ gms (estimation)
Location Tissue Sent (if elsewhere) _____

SP or DP # _____
Patient Unit # _____

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SANTA BARBARA • SANTA CRUZ

SCHOOL OF MEDICINE
DEPARTMENT OF MEDICINE

SAN FRANCISCO, CALIFORNIA 94143

April 16, 1984

Walter L. Way, M.D.
Chairman, Committee on Human Research
UCSF
Clinics 116

Dear Dr. Way:

Enclosed is a short form application, consent form, and a brief protocol for the prospective collection and preservation of serum and tissue specimens from patients with AIDS. As we have identified more and more patients suffering from AIDS in San Francisco, and especially as research monies have become available for studies in this disease, the number of collaborators asking for specimens has increased dramatically. To accomodate these collaborations and to minimize the inconvenience to the patient we have been collecting serum and Kaposi's sarcoma skin biopsies at the time of diagnosis. These specimens have been frozen in a centralized facility under the direction of Dr. John Greenspan at UCSF. Initially these specimens were collected under a study protocol approved by CHR in 1982. This approval, initially just for patients with Kaposi's sarcoma, has since expired and the purpose of the attached protocol and consent form is to again obtain approval for this prospective collection of specimens for collaborative research and to include patients with other AIDS diagnoses including opportunistic infections and the AIDS related complex including lymphadenopathy syndrome and idiopathic thrombocytopenic purpura. In our application and consent form we have taken, we think, necessary precautions to insure the subject's confidentiality which is done by coding specimens to the San Francisco Department of Health AIDS registry. By this prospective collection of specimens from patients with AIDS, we feel we are minimizing the inconvenience and confusion caused by this research endeavor. Also, by collecting specimens in advance, we are able to aliquot blood in smaller than phlebotomy in a case-by-case basis.

I appreciate the somewhat unique aspects of the attached protocol but feel that it meets the needs of this research in a way than minimized the impact of these studies on our patient care.

Thank you very much for your consideration.

Sincerely,

Paul A. Volberding, M.D.
Director, AIDS/KS Clinic
San Francisco General Hospital

PAV/slh

Principal Investigator University Assistant Prof. Med. UCSF
(UCSF Faculty) Paul A. Volberding, M.D. Title Chief Onc. SFGH Dept. Medicine/SFGH
P.I. Mailing Address Oncology Service, Ward 86, SFGH P.I. Phone No. 821-8830
(campus if possible)
Co-Investigator Marcus Conant, M.D. Is the principal investigator
and Title Marcus Conant, M.D. the sponsor/advisor only? Yes No
Co-P.I. Mailing Address UCSF Utilization Review M 181 Co-P.I. Phone No. 666-3261
Project Collection and Preservation of Serum and Tissue Specimens from Patients with AIDS
Title for Multiple Collaborative Studies.

INSTRUCTIONS: PLEASE TYPE; SUBMIT

-4 COPIES OF THIS TWO-PAGE FORM

-4 COPIES OF ANY ATTACHMENTS (CONSENT FORMS, QUESTIONNAIRES, ETC.)

To be safe, allow 3 weeks for processing.

A) The point of this project is (Explain background, rationale, hypothesis, basic design, etc.): Patients with AIDS in San Francisco are currently the focus of multiple collaborative research studies both at UCSF and other institutions. The purposes of these research objects are varied but include an understanding of the etiology, immunology, epidemiology and therapy of this syndrome. Because there are so many requests for collaborative studies on specimens, especially including blood and biopsies of Kaposi's sarcoma from patients with AIDS, in this project these specimens will be collected on a regular basis both at time of diagnosis and at infrequent regular intervals following diagnosis and preserved in a centralized facility which will insure proper handling of these specimens with regards to bio-hazard potentials and which will insure confidentiality of the patients from whom these specimens were collected.

In this project approximately 20 cc's of serum will be frozen at the time of diagnosis and every 3-6 months following diagnosis of AIDS. In addition, biopsies of Kaposi's sarcoma lesions and some case, normal skin for comparison will also be obtained at time of diagnosis and preserved.

B) The subject population(s) will be selected (or excluded) on the following criteria

(Discuss how access will be gained as well as any problems relevant to special subject populations): Subjects will be selected for this project based on diagnosed acquired Immunodeficiency Syndrome or AIDS related complex (ARC) which includes, the syndrome of effused reactive lymphadenopathy and idiopathic thrombocytopenic purpura. Patients will be chosen from clinic populations at S.F.G.H., UCSF, and the San Francisco VA Hospital. The nature of the project will be discussed with the potential subject and consent signed before specimens are collected.

C) The following procedures involving humans will be done for purposes of the study (If applicable, include interview themes and questionnaires if not commonly known):

The expedited review category number from Consent Forum, Issue 5, is _____.

For the purposes of this study, no questionnaires or interviews will be required. Blood will be collected at time of diagnosis and every 3-6 months but at no time will amount to more than 20 cc's. Biopsies of Kaposi's sarcoma lesions and adjacent normal skin will be performed at initial diagnosis. Subsequent skin biopsies will be performed only if clinically needed for other management purposes. The principal indication for subsequent biopsies is generally to assess response or lack of response to therapy.

Investigator: Paul A. Volberding, M.D.

D) The risks involved in these procedures and the methods of minimizing the risks, inconveniences, or discomforts are (Include any potential for loss of privacy):
The risks involved with these studies are limited to those of venipuncture and skin biopsies. The risks of venipuncture are bruising or discomfort at the venipuncture site and occasionally temporary bruising. The risks of skin biopsy are discomfort at the biopsy site, minor bleeding, and rarely, infection of the biopsy.

The other major risk involved in any studies with AIDS revolves around questions of confidentiality. In this study patients' specimens will be coded at the site they are obtained by a code number which does not identify the patient by name or by hospital record number. Instead patients are identified by an AIDS registry code number generated by the San Francisco Department of Health and the key to this code is kept separate from medical records. Thus, questions of confidentiality should be minimized and the subjects are so informed in the attached consent form.

E) Describe the anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result:

There are no immediate benefits to the subjects who have agreed to participate in this project. However, the information obtained by collaborative studies of AIDS material obtained in this way is significant and may be of real value in identifying more clearly the nature of this disease. In addition, the patients who agree to participate in this project of prospective specimen preservation for collaborative research will be spared the otherwise inevitable multiple requests for blood and/or tissue specimens at later times. By having a centralized repository of AIDS specimens, patients will not need to be approached for these subsequent collaborations.

F) Describe the consent process and attach all consent documents. If waiver from use of written consent is requested, give the justification.

Consent will be obtained by signature of the attached consent form if approved by the Committee on Human Research. Patients will be asked to sign this consent before specimens are collected for preservation.

UNIVERSITY OF CALIFORNIA
CONSENT TO BE A RESEARCH SUBJECT
OVERALL CONSENT FOR AIDS SPECIMEN COLLECTION

Because many facts about AIDS remain unknown and because more cases are being diagnosed at a rapid rate, my physician, Dr. _____ and other investigators are interested in learning as much as possible about this syndrome.

Since I have been diagnosed as having AIDS, I have been asked to participate in a study to further assist their investigation. I have been asked to allow certain tests and procedures, i.e. blood drawing, lung function test and possible biopsies be performed.

By signing this consent I agree that blood will be drawn and the amount drawn from time to time will vary, but no more than 450cc (15 ounces) will be drawn in an eight (8) week period and no more than one (1) time a week. I am aware of the risk when having blood drawn, such as discomfort from needle puncture, possible bruising and, very rarely, infection. I agree that lung function tests such as chest x-rays and measurement of lung capacity be performed and realize these particular tests may be performed as frequently as every four (4) months.

A cancer known as Kaposi's sarcoma can accompany the acquired immune deficiency which produces lesions of the skin. If I am found to have any cancerous lesions I agree that skin biopsies be performed for evaluation of the lesions. I have been informed of the risk that may accompany biopsies such as minimal pain at site of biopsy, minimal bleeding and rarely, infection.

By signing this consent I am not agreeing to participate in any therapy program and retain the right to discontinue as a participant at any time without fear that further alternative treatments will be denied me. I agree to participate in this study and have been informed of the risk and benefit both orally and in writing and understand that I may refuse any test at any time.

All medical information such as medical records, test procedures and their results that pertain to me are totally confidential. My name will be kept separate from lists of tests and test results, my name will be replaced by a code number and any entry of my test(s) and test results into a computer will be entered by code number only.

If at any time during this study I am physically injured as a result of this study, treatment will be available. The cost of such treatment will be covered by the University, depending on the number and/or nature of the factors involved. For further information concerning compensation I may call (415) 606-1814.

Subject Name _____

Date _____

PROTOCOL FOR THE CLINICAL STUDY OF AIDS AT UCSF, FOR THE
PROSPECTIVE COLLECTION OF SPECIMENS FOR RESEARCH PURPOSES

INTRODUCTION

The Acquired Immune Deficiency Syndrome or AIDS is a serious new disease in which the cellular immune system regulation appears to be altered increasing dramatically the rate of malignancies, including Kaposi's sarcoma and non-Hodgkin's lymphoma and a variety of severe life threatening opportunistic infections, especially pneumocystis carinii pneumonia.

Because of the serious nature of this illness and the rapid spread of the epidemic, and because of the probability that the study of AIDS will result in advancement in our knowledge of the relationship between the immune system and cancers, extensive research projects are underway. Numerous investigators at UCSF, other UC campuses, and other medical centers across the country are interested in studying the epidemiology, etiology and immunology of this disease.

Because San Francisco represents a major focus of this epidemic and because of the coordination of AIDS activities at UCSF, numerous requests are received to provide patient material for these studies. To coordinate the collection and distribution of these specimens a Tissue Committee of the UCSF AIDS Working Group has been established. The chair of this committee is Dr. Paul Volberding.

In addition, a tissue and serum bank has been organized under the direction of Dr. John Greenspan to coordinate the optimum storage and processing of these specimens with appropriate biohazard precautions. To minimize the inconvenience of multiple studies to patients with AIDS and to prevent confusion among collaborating investigators, this protocol is intended to serve as a method for the centralization of this collection process. This protocol is not intended to give permission for any therapy studies. Additionally, any invasive tests that are not specifically outlined in this proposal will have separate protocols and consents approved through the Human Research Committee.

PROTOCOL

At the time of initial evaluation patients with AIDS or AIDS related conditions as defined by the CDC, will be asked to participate in an ongoing research effort. This will consist of collection of approximately 45cc's of blood on the first clinic visit and if the person has Kaposi's sarcoma will also include a biopsy of a Kaposi's lesion and adjacent normal skin. These specimens will be preserved by the Tissue and Serum Bank with code numbers to preserve patient confidentiality. In addition, at the initial clinic visit baseline, chest x-ray and pulmonary function tests will be obtained. Any other studies indicated for the patient's clinical condition will also be collected.

Patients will be followed as clinically indicated for their illness and will have additional blood collected for preservation in the Tissue Bank every 3 to 6 months. At no time will the amount of blood collected for this purpose exceed 30cc's. In addition, 10 follow-up patients will receive serial pulmonary function testing every 4 months. Subsequent biopsies of tumors may be performed, but only if clinically indicated, to assess response to therapy.

Any other invasive testing that is not of routine clinical utility will require a separate consent from each patient.

CONFIDENTIALITY

Because of the nature of AIDS and the risk groups involved in the epidemic, confidentiality concerns have been raised by a number of groups. To insure confidentiality of specimens collected on this protocol, no specimen will be identified by the patient's name. Code numbers will be used for this purpose as provided by the City Health Department AIDS Registry. This system of coding has been in place for the past several months and has been deemed acceptable by the Centers for Disease Control.

SUMMARY OF AIDS CLINICAL RESEARCH PROTOCOL

A DIAGNOSIS ON FIRST VISIT

	<u>AMOUNT</u>
BLOOD:	
STUDIES AS INDICATED CLINICALLY (CBC, CHEM PANELS ETC)	approx 20cc
BLOOD (SERUM) FOR PRESERVATION IN SPECIMEN BANK	approx 20cc

BIOPSY:

SKIN BIOPSY FOR DIAGNOSIS OF SUSPICIOUS LESION	- 4mm punch
SKIN BIOPSY OF LESION FOR PRESERVATION/ RESEARCH	- 4mm punch
BIOPSY OF NORMAL SKIN FOR PRESERVATION/RESEARCH	- 4mm punch
BIOPSY OF LYMPH NODE FOR DIAGNOSIS AS CLINICALLY INDICATED	
PORTION OF NODE TO BE PRESERVED FOR RESEARCH (NO BIOPSY OF NODE FOR RESEARCH ONLY)	

PULMONARY FUNCTION TESTING: BASELINE TESTING

TESTING IN FOLLOW-UP PERIOD

	<u>AMOUNT</u>	<u>FREQUENCY</u>
BLOOD:		
BLOOD (SERUM) FOR PRESERVING/RESEARCH	20 cc	3-6 months
BIOPSY:		
KS LESIONS BIOPSIED FOR CLINICAL INDICATIONS:	- 4 mm punch	clinically as indicated
LYMPH NODE BIOPSIED FOR CLINICAL INDICATIONS:	portion of biopsy	as indicated
PULMONARY FUNCTION TESTS:		4 months

Form: Biopsy or Specimen Information Form

Leave Blank

Date _____
Acct# _____
R#-Tissue _____
AS#-Serum _____

Date Specimen Obtained _____
Contributing M.D. _____
Facility _____

Patient Code # _____
Birth Date _____
Age _____ Sex _____
Risk Group _____

Clinical Diagnosis:

KS _____
PCP _____
Lymphadenopathy _____
ITP _____
Other Infection _____
Other Malignancy _____
Coded Study _____

Study # _____

Current Therapy:

Therapy for Malignancy _____
Therapy for Infection _____
Ever Received Chemotherapy yes _____ no _____

Specimen: _____
Type of Blood Specimen _____
Blood _____ Whole Blood _____ Plasma _____ Serum _____ Separated Cells _____
Number of Tubes _____
Tube Type _____

Biopsy:

Skin _____ Node _____ KS _____ Normal Skin _____ Other _____
Amount of Tissue _____ gms (estimation)
Location Tissue Sent (if elsewhere) _____

SP or DP # _____
Patient Unit # _____

Gottlieb

May '31 Deadline for renewal
application. Probably 2 centers only

Standardize collection / storage / data acquisition

4/23/84

Additional Collaboration

serum	Gallo	-	HTLV
S+T	Francis	-	LAV
T	Zur Hausen	-	HPV
T	Lie Injo	-	HBV
S	Cooper	-	I. complexes
S	Michalski	- pending -	Acute phase protein
T	Kramer	-	Fibronectin, lamin, invasion
T	Bechtold	-	Hepatitis 4S, LNS.
S+T	Kron	-	Anti Ab.
S	Havel	-	β Lipoprotein.
S	Bernik	-	
S	Hadley	-	Fungi
T	Modlin	-	IL ₂ + NK cells in LNS
S	Rifer	-	Diagnosis of PCP.

S - 9

T - 7

(24)

14S
10T

15 in long exp
7 outside uc
2 uc outside
uc

Tissue Committee
Facilities

Serum protocol

Tissue protocol

Collaboration

Status of collection

System (state) work

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UNIVERSITY OF CALIFORNIA
AIDS SPECIMEN BANK
HSW-604
(415) 666-2943

SAN FRANCISCO, CALIFORNIA 94143

20 April 1984

SERUM BANK INVENTORY

Total number of samples to date: 479
Total number of samples received in 1984: 249

189 out 23

Breakdown of serum bank:

Kaposi's sarcoma: 93
Moss control sera: 276
Lymphadenopathy: 31
Pneumocystis carinii: 9
R/O Pneumocystis & Lymphadenopathy: 1
Lymphadenopathy & ITP: 1
ITP: 11
Kaposi's sarcoma, Lymphadenopathy, and Cryptococcus: 1
Kaposi's sarcoma & Cryptococcus: 1
Insemination study: 8
Contact study: 12
Kaposi's sarcoma & Pneumocystis: 1
Kaposi's sarcoma & Candidiasis: 2
Epstein Barr Virus: 1
Lymphadenopathy & Herpes simplex virus: 1
Kaposi's sarcoma, Herpes simplex virus, & Lymphadenopathy: 1
Opportunistic infections: 3
Burkitt's lymphoma: 1
Kaposi's sarcoma, Lymphadenopathy, & Candidiasis: 1
Unknowns: 11
R/O AIDS: 10
Kaposi's sarcoma and Lymphadenopathy: 3

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UNIVERSITY OF CALIFORNIA
AIDS SPECIMEN BANK
HSW-604
(415) 666-2943

SAN FRANCISCO, CALIFORNIA 94143

20 April 1984

TISSUE BANK INVENTORY

Total number of samples to date: 162
Total number of samples received in 1984: 25

780483

Breakdown of tissue bank:

Kaposi's sarcoma: 83
AIDS: 32
Lymphadenopathy: 24
Kaposi's sarcoma & Lymphadenopathy: 5
Kaposi's sarcoma & Opportunistic infections: 4
Kaposi's sarcoma & Pneumocystis: 1
Kaposi's sarcoma, Lymphadenopathy, & Opportunistic infection: 1
Hodgkin's : 1
R/O Auto immune dermatitis: 2
Kaposi's sarcoma & Candidiasis: 2
R/O Kaposi's sarcoma: 2
Kaposi's sarcoma & Herpes simplex virus: 5
Leukoplakia (AIDS related): 1
Pneumocystis & Burkitt's Lymphoma: 1

1/23/84

CODES FOR AIDS DATA BANK:

KS = Kaposi's sarcoma
PCP = Pneumocystis carinii pneumonia
LNS = Lymphadenopathy
CON = Contact study
MOS = Moss study
OI = Opportunistic infection NOS
CAN = Candidiasis
VZV = Herpes zoster
HSV = Herpes simplex
EBV = Epstein Barr virus

TOTAL TISSUE SAMPLES: 140

Breakdown of tissue samples:

KS tumors: 31
KS "normal" skin: 7
KS nodes: 29
KS bowel: 1
KS lung: 1
KS prostate: 1
AIDS lymph nodes: 37 [LNS]
AIDS salivary glands: 1
AIDS tonsils: 1
LNS nodes: 10
LNS tonsils: 2
LNS adenoid: 1
Hodgkin's node: 1
OI: 3

DISPOSITION CODES:

1 = L. Drew
2 = E. Lennette
3 = J. Levy
4 = J. Greenspan
5 = Rutter/Ziemer

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HOSPITALS AND CLINICS

SAN FRANCISCO, CALIFORNIA 94143

AIDS CLINICAL RESEARCH CENTER UCSF

Site Visit - Monday, April 23, 1984 - 10:00 a.m. - 3:00 p.m.

AGENDA

- 10:00 am - 10:20 am - Overview of the Mission, Structure and Function of the AIDS Clinical Research Center - Dr. Marcus A. Conant
- 10:20 am - 10:30 am - How the AIDS Clinical Research Center has Contributed to Finding the Cause of AIDS. - Dr. Jay Levy
- 10:30 am - 10:40 am - AIDS Tissue Banks - Dr. John Greenspan
- 10:40 am - 11:00 am - The Role of the Kaposi's Sarcoma Clinic in Defining the Natural History of K.S. and Tissue Acquisition - Dr. Paul Volberding
- 11:00 am - 11:15 am - Coffee Break
- 11:15 am - 11:20 am - Establishment of AIDS Clinic at UCSF - Dr. Jack Stobo
- 11:20 am - 11:30 am - Multi-User Computer System - Mr. Franklin Jacobson
- 11:30 am - 11:40 am - Recommendations for Epidemiological Surveillance of Health Care Workers - Dr. Andrew Moss
- 11:40 am - 11:50 am - Behavior Modification Program - Mr. Leon McKusick
- 11:50 am - 12:10 pm - Future Plans for the AIDS Clinical Research Center - UCSF Dr. Marcus Conant
- 12:10 pm - 1:30 pm - Executive Lunch Session

April 19

John -

Frank from K.S. clinic called re:

Site Visit (Monday)

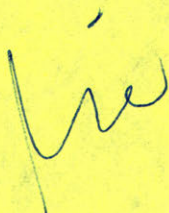
some changes in agenda:

two visitors:

Abe Broude from San Diego

Tom Casserow from Irvine

will start at 10:00 with Dr. Kanter and you
will speak from 10:30 to 10:40 on the Tissue
Bank. Please stress the need for the bank
and the number of researchers who depend on
the bank for their tissue specs.

A handwritten signature in blue ink, appearing to be 'Pie' or a similar stylized name, located in the lower right quadrant of the page.



University of California San Francisco
School of Dentistry
Division of Oral Biology
HSW-604
San Francisco, California 94143
(415) 666-2943
John S. Greenspan
BSc, BDS, PhD, FRCPath
Professor and Chairman

28 March 1984

SUMMARY OF AIDS TISSUE AND SERUM BANK

Total number of specimens in tissue bank: 157
Total number of specimens received in January-March 1984: 25

Total number of serum samples in serum bank: 399
Total number received in January-March 1984: 169

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SANTA BARBARA • SANTA CRUZ

HOSPITALS AND CLINICS

SAN FRANCISCO, CALIFORNIA 94143

AIDS CLINICAL RESEARCH CENTER UCSF

Site Visit - Monday, April 23, 1984 - 10:00 am - 3:00 pm

AGENDA

- 10:00 am - 10:20 am - Overview of the Mission, Structure and Function of the AIDS Clinical Research Center - Dr. Marcus Conant
- 10:20 am - 10:40 am - How the AIDS Clinical Research Center has contributed to finding the cause of AIDS - Dr. Jay Levy
- 10:40 am - 11:00 am - The role of the Kaposi's Sarcoma Clinic in defining the natural history of K.S. and tissue acquisition - Dr. Paul Volberding
- 11:00 am - 11:15 am - Coffee Break
- 11:15 am - 11:30 am - Establishment of AIDS Clinic at UCSF - Dr. Jack Stobo
- 11:30 am - 11:45 am - Multi-User computer system - Mr. Franklin Jacobson
- 11:45 am - 12:00 am - Recommendations for epidemiological surveillance of health care workers - Dr. Andrew Moss
- 12:00 am - 12:30 pm - AIDS Tissue and Serum Bank - Dr. John Greenspan
- 12:30 pm - 1:30 pm - Executive Lunch Session
- 1:30 pm - 2:00 pm - Behavior Modification Program - Mr. Leon McKusick
- 2:00 pm - 2:30 pm - Future plans for the AIDS Clinical Research Center - UCSF - Dr. Marcus Conant

The purpose of this site visit will be to answer the 3 following questions:

1. What has been done since July 1, 1983?
2. How has the money received been utilized?
3. How much funding do you anticipate needing for the next fiscal year (7/1/84 - 6/30/85)?

AGENDA PREPARATION: The Universitywide Task Force on AIDS office requests that you prepare and submit an agenda to them for the site visitors by April 13. The first speaker should be yourself, to present a cohesive summary of the Center's functions and its various working units, and how their functions interact with each other to create the AIDS Clinical Research Center. Also relevant at this time is how the use of state funds has helped the University of California system to contribute towards finding an answer for the AIDS problem.

Following your opening remarks, the Principal Investigators of the separate units (tissue bank, clinics, etc.) should be prepared to give concise presentations, detailing their unit's role in the Center, how they have utilized the state funds, and with what results. In addition, the presentors should elucidate as to what in the October 16, 1983 proposal has been accomplished, what is in the organizational stage, what is still pending, what has been altered and anticipated problems. Approximately one-half of the presentation time should be allotted for discussion and questions.

Following this the visitors will reconvene with the Director of the Center and the Chairman of the Dean's Committee to clarify any unanswered questions and to discuss the overall budget for the current fiscal year and the anticipated budget of the forthcoming fiscal year for the continued establishment and functioning of the AIDS Clinical Research Center.

LOGISTICS: The site visit should begin at 10:00 AM and be concluded by 3:00 PM. Plan on a 45 minute lunch period for a closed executive session for the site visit committee. At this time the site visit panel will discuss the morning presentations. If the site visitors are not getting the information they need this will be brought to your attention before the afternoon session begins. The assistance of your office in making simple arrangements for lunch (sandwiches, coffee, etc.) is necessary.

PLEASE SEND THE AGENDA, AND INFORMATION ON HOW THE SITE VISITORS CAN GET TO THE SITE FROM THE AIRPORT TO THE UNIVERSITYWIDE TASK FORCE ON AIDS OFFICE AT THE ADDRESS BELOW:

Universitywide Task Force on AIDS
c/o Office of Health Affairs
785 B University Hall
University of California
Berkeley, CA 94720
(415) 642-0732

4/16

Congress
Boxer
Fair

W. H. H. H.
NY A. O. F. I.
Susa
Stemmetz

4/23

State A. O. F. I.
Stemmetz

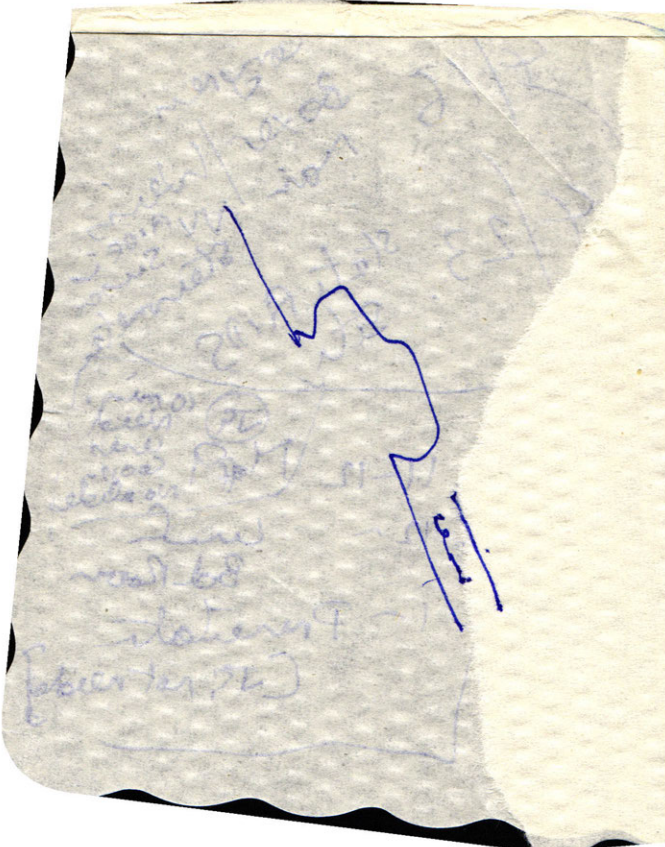
11-12
12-
1- Presentation
[JS not needed]

JS

11-12

10 min.
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No slides

Lunch
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UNIVERSITY OF CALIFORNIA

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO



SANTA BARBARA • SANTA CRUZ

UNIVERSITYWIDE TASK FORCE ON AIDS
OFFICE OF HEALTH AFFAIRS
785 UNIVERSITY HALL
UNIVERSITY OF CALIFORNIA
BERKELEY, CA 94720

March 26, 1984

Dr. Marcus Conant
Department of Dermatology
A-312
University of California
San Francisco, CA 94143

Dear Dr. Conant,

As you may remember, it was announced at the December 12 Workshop that there would be site visits to the AIDS Clinical Research Centers. The date of Monday, April 23, 1984 has been selected by the University-wide Task Force on AIDS for a site visit to the San Francisco AIDS Clinical Research Center.

Since the site visit will result in an official written report by the Task Force to the California State Assembly, some guidelines are outlined on the attached sheet regarding your preparation for the site visitors and what they will require from you and the other individuals involved in the Center.

In general, the purpose of this site visit is to allow for a scholarly exchange of information and to enable the Universitywide Task Force on AIDS to provide information to the California State Assembly on how the state funds they allocated to the University of California are contributing to the urgent need for a resolution of the AIDS problem.

Sincerely,

Merle A. Sande, M.D.
Chairman
Universitywide Task Force
on AIDS

Attachments

MAS/lbd

Autopsy - Mark C.

JG 11-11-20 a.m.
Pers
Equip If mch
Other

The purpose of this site visit will be to answer the 3 following questions:

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3. How much funding do you anticipate needing for the next fiscal year (7/1/84 - 6/30/85)?

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PLEASE SEND THE AGENDA, AND INFORMATION ON HOW THE SITE VISITORS CAN GET TO THE SITE FROM THE AIRPORT TO THE UNIVERSITYWIDE TASK FORCE ON AIDS OFFICE AT THE ADDRESS BELOW:

Universitywide Task Force on AIDS
c/o Office of Health Affairs
785 B University Hall
University of California
Berkeley, CA 94720
(415) 642-0732

Oct 1 - Sept 30

$$\begin{array}{rclcl} \text{Henry} & 23 & +8.45 & = 26.45 & \text{Total} \\ & & & - 15 & \text{Profit} \\ & & & \hline & & & 11.45 & \end{array}$$

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CANCER RESEARCH COORDINATING COMMITTEE

REVIEW OF APPLICATIONS FOR THE PROPOSED 1983-1984 AIDS (ACQUIRED IMMUNE DEFICIENCY SYNDROME) RESEARCH AWARD PROGRAM

To expedite this new grant application and review process, we are adapting the forms and procedures used by the Cancer Research Coordinating Committee in its review of applications for cancer research monies.

For the first review cycle of the proposed AIDS research program, we will be able to consider only those applications received in the CRCC Secretariat office by Thursday, June 16, 1983. Applications received after that time will be evaluated at a later review meeting.

Please submit THREE (3) copies of your proposal to the Secretariat by the June 16 deadline. The original, with appropriate signatures, should be sent at the same time to your campus Contract & Grants Office for later processing and transmission to us.

Send the 3 copies of your application to:

CRCC Secretariat
c/o Department of Microbiology & Immunology
University of California
Berkeley, CA 94720

If you have questions, please call our Administrative Assistant, Laura Driemeyer, at Berkeley campus (415) 642-2923.

DEADLINE: JUNE 16, 1983

(continued on reverse side)

CANCER RESEARCH COORDINATING COMMITTEE

GENERAL INFORMATION AND INSTRUCTIONS FOR GRANT APPLICATION

The funds are awarded by the Cancer Research Coordinating Committee, which is a systemwide University of California committee composed of faculty members chosen to include representatives from all campuses and from many areas of scientific expertise. The applications are reviewed by the Committee and judged on the basis of (1) scientific merit and (2) relevance to the AIDS problem. Here are a few guidelines which may be helpful in the preparation of the application.

ELIGIBLE APPLICANTS: Members of the Academic Senate are eligible to apply. In addition, applications will be accepted from appointees on UC administered payrolls on one of the nine UC campuses, in the Clinical Professor and Adjunct Professor series, the Professional Research series, or equivalent titles in the agricultural series. An individual may submit only one application.

The Committee expects that the project will be personally and directly supervised by the applicant in his/her campus laboratories; any proposed deviation from this must be fully justified in the grant application.

DURATION OF A GRANT: A grant is made for the fiscal year (7/1/83 to 6/30/84).

USE OF GRANT FUNDS:

Allowable grant expenses:

- a. salaries for technical assistance and for postdoctoral scholars
- b. supplies and services
- c. equipment items not readily available, providing that adequate justification is given for each item costing over \$500

Upon completion of a grant, all material purchased by the grant awardee becomes the property of the department or equivalent administrative unit of the awardee.

Restricted expenses:

The following expenses are generally disallowed. If an investigator wishes to request an exemption from this policy, a complete explanation of exceptional and justifying circumstances must be submitted:

- a. salaries for: the Principal Investigator, individuals eligible to apply for AIDS funds, regular or visiting academic staff, clerical help, and consultants
- b. costs for patient care
- c. office, duplication and communication expenses
- d. equipment maintenance and service contracts
- e. recharges for University-provided services or equipment usage
- f. travel expenses

CANCER RESEARCH COORDINATING COMMITTEE

INSTRUCTIONS FOR RESEARCH GRANT APPLICATIONS

THE CANCER RESEARCH COORDINATING COMMITTEE REQUIRES THE ORIGINAL AND THREE (3) COPIES (XEROX OR EQUIVALENT).

The following numbered paragraphs contain specific instructions pertaining to the similarly-numbered sections of the application form.

- I. TITLE OF PROJECT: The title should briefly describe the project.
- II. & PRINCIPAL INVESTIGATOR: Enter all requested information. The listed principal investigator is expected to be an active participant in and personally responsible for the project. Approved grants will be awarded in the name of the principal investigator and assigned to the administrative unit of the listed responsible administrator.
- V. RESPONSIBLE ADMINISTRATOR: The signature of the Chairperson or Director of your administrative unit must appear on the application.
- VI. HUMAN SUBJECTS AND BIOHAZARDS REVIEW: Check the appropriate statements and attach notices of Committee approval if required.
- VII. BUDGET: Identify all personnel who will be associated with the project by name and by title as listed in the University Staff Personnel Manual or Academic Salary Scales. Indicate the percent of annual salary requested and give a brief justification for listed personnel who will receive salary support. Do not include employee benefits, or overhead; employee benefits are added administratively and overhead is not applicable for these funds. Include names and titles of all professional personnel who will work on the project even if no salary is requested. Names of consultants should be included on a separate page.
- VIII. FINANCIAL SUPPORT: The principal investigator must supply a complete statement of financial support available and pending approval. Prepare a complete statement for all projects. Grant number, granting agency, project title, and dollar amount should be given. Effective dates should be listed whenever possible. In addition, include the abstract for each grant listed as available or pending, and clearly indicate the relationship of each grant to the proposed CRCC project. Use continuation pages as required.

It is the applicant's responsibility to provide evidence that there is no overlap between other research support and the grant request to the CRCC; the Committee will not request additional information, and in the absence of adequate evidence will assume that overlap exists.

(continued on reverse side)

IX. BIOGRAPHICAL SKETCH: Complete a biographical sketch for all professional personnel who will work on the project using the sheet provided. ONE additional page of pertinent publications may be included for each individual. Please do not include reprints of publications in this section.

X. SUMMARY OF RESEARCH PROPOSAL: Limit your synopsis to the space provided on page 5.

SECTIONS XI, XII AND XIII SHOULD BE TYPED, SINGLE-SPACE ON PLAIN WHITE BOND. NUMBER PAGES CONSECUTIVELY AT THE BOTTOM OF EACH PAGE. LEAVE 1" MARGINS. MATERIAL OTHER THAN FIVE PAGES FOR XI, ONE PAGE FOR XII MAY BE SUBMITTED AS AN APPENDIX IF NECESSARY, BUT THIS IS NOT ENCOURAGED.

XI. EXPLANATION OF PROJECT: The Committee must pass judgement on your project on the basis of information contained in this section. The detailed explanation must be preceded by a short (100 word) abstract of the proposed project. The detailed explanation of the project should emphasize what is expected to be accomplished with the funds, if awarded. Be sure that the explanation contains 1) a clear statement of the problem to be solved; 2) sufficient background material to orient those not familiar with the problem; 3) a summary of the methods to be used, and 4) essential references.

RESTRICT THE DETAILED EXPLANATION, INCLUDING THE ABSTRACT, TO NOT MORE THAN FIVE (5) PAGES OF SINGLE-SPACED TYPEWRITTEN MATERIAL. SEPARATE APPENDIXES MAY BE SUBMITTED IF CONSIDERED ESSENTIAL, BUT THIS IS NOT ENCOURAGED.

XII. RELATION OF THE PROJECT TO THE AIDS PROBLEM:

These funds are intended to support meritorious research clearly related to the AIDS problem. They are not intended to be used merely to increase support for biological research. The Cancer Research Coordinating Committee will consider carefully the relevance of projects to the AIDS problem; for this reason applicants should provide well documented evidence of relevance.

A NOTE ON FORMAT: Leave a 1" left margin on all pages for binding. DO NOT SEND DUPLEXED PAGES. We cannot return material sent with the application, such as pictures, graphs, and reprints. Please paper clip the original application and staple separately the three copies. Appendixes, if included, should not be attached to the applications. A cover letter is not necessary. PLEASE OBSERVE OUR PAGE LIMITS. WE CANNOT GUARANTEE THAT EXCESS MATERIAL WILL BE INCLUDED IN THE APPLICATION REVIEW PROCESS.

CANCER RESEARCH COORDINATING COMMITTEE
UNIVERSITY OF CALIFORNIA

AIDS RESEARCH PROGRAM

I. Title of Project: _____

II. Principal Investigator:

_____ Name (last, first, M.I.)	_____ Signature
_____ Mailing address (use only those lines necessary, no abbreviations please):	
_____ Department/Division	_____ Phone numbers:
_____ Bldg./Room #	_____ Office:
_____ School	_____ Laboratory:
_____ Campus	_____ Dept.(messages):
_____ Zip	

III. Eligibility of Responsible Investigator:

_____ Official UC title	_____ % on UC payroll	Member of Academic Senate: ☒ Yes ☒ No
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IV. Co-investigators:

V. Responsible Administrator (please see instructions):

I verify that the information given by the applicant concerning appointment,
% time employed, and current grant status is correct and complete.

_____ Signature	_____ Name	_____ Title (chair, etc.)	_____ Dept.
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VI. Review & Approval:

A. Human Subjects:

- ☒ 1. Does not apply
☒ 2. Approval attached
☒ 3. Review pending

B. Biohazards

- ☒ 1. Does not apply
☒ 2. Memorandum of Understanding
and Agreement attached
☒ 3. Review pending

C. Contracts & Grants: Information as required on the attached checklist
is complete and detailed in the application.

Signature: _____ Date: _____

VII. Budget:

1. Personnel (list all professional personnel to be associated with project):

<u>Name</u>	<u>Official University</u> <u>Title of Position</u>	<u>Salary</u> <u>Requested</u>
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Justification of listed personnel for whom support is requested:

Total personnel \$ _____

2. Supplies & Expense (list general categories):

Total S&E \$ _____

3. Equipment (list items over \$200 and provide justification
for items over \$500)

Total Equipment \$ _____

TOTAL REQUESTED \$ _____

VIII. Additional Financial Support (see instructions): List all program projects and all sources of funds which support your research. For each item list (a) grant number, (b) granting agency, (c) project title, (d) grant period, (e) length of grant commitment, (f) amount (both total period and overlap period), and (g) relationship to proposed AIDS grant. Attach the abstract of each grant application. Use continuation sheets as required.

It is the responsibility of the applicant to clearly document that there is no overlap between other grants and the proposed AIDS grant.

1. Other grants available during the requested grant period:

2. Other grant applications pending:

IX. Biographical Sketch

(give the following information for all professional personnel)

Name

Official Title

Education (begin with the baccalaureate training and include postdoctoral)

Institution and location

Degree

Year

Scientific Field

Honors

Major Research Interest

Role in Proposed Project

Research and/or Professional Experience (Starting with present position, list pertinent professional background and employment)

Publications Pertinent to Proposed Research

X. Summary of Research Proposal for the AIDS Research Program

CANCER RESEARCH COORDINATING COMMITTEE: 1983-1984 Grant Awards

Name and Title of Principal Investigator

Department and Campus

Title of Project

Use this space to summarize concisely your proposed research. Outline objectives and methods. Underscore the KEY words (not to exceed 10) in your abstract.

If the Cancer Research Coordinating Committee awards you a research grant, copies of this page may be distributed to UC Contract and Grant Offices and to other interested UC individuals and groups.

Dr. John S. Greenspan
May 6, 1983

Specimen Bank

John S. Greenspan

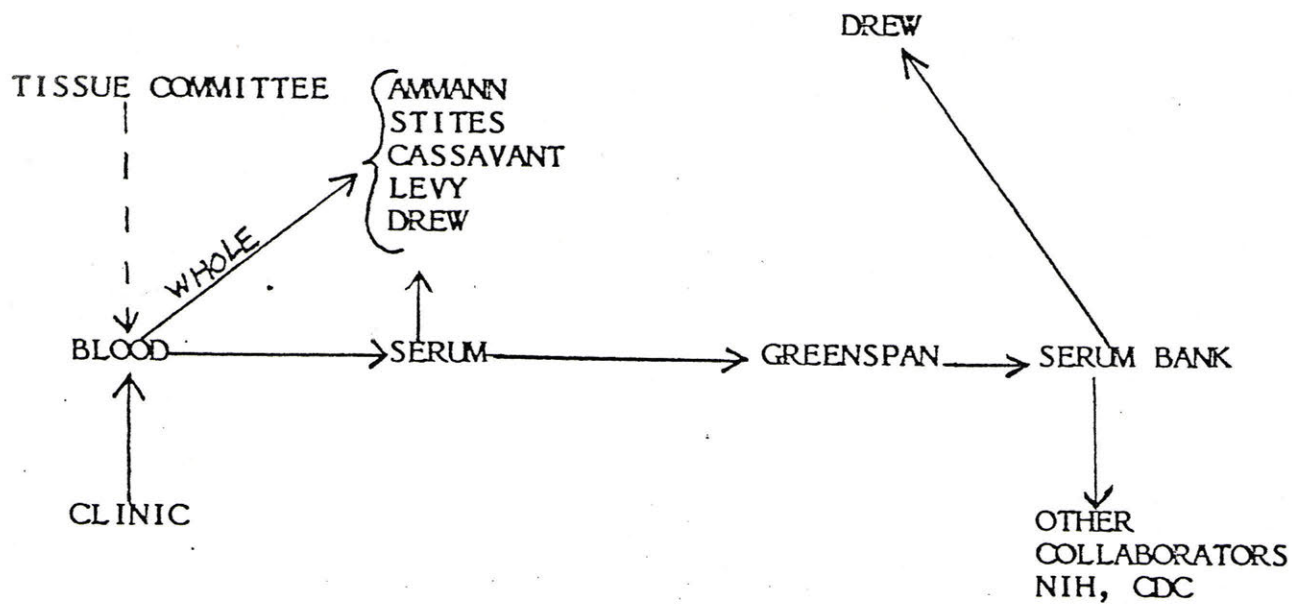
We will serve as the site for storage, handling, processing and retrieval of tissue specimens, semen and serum samples. About 1000 serum samples and 200 tissue samples per year are anticipated. Tissue will be cryosectioned or otherwise prepared for other investigators. Personnel costs are for these aspects. Computer facilities are essential and will service other investigators.

Campus Environmental Health and Safety Committee regulations now mandate alteration and additional facilities at the P₂ level. They are essential to all aspects of the AIDS project.

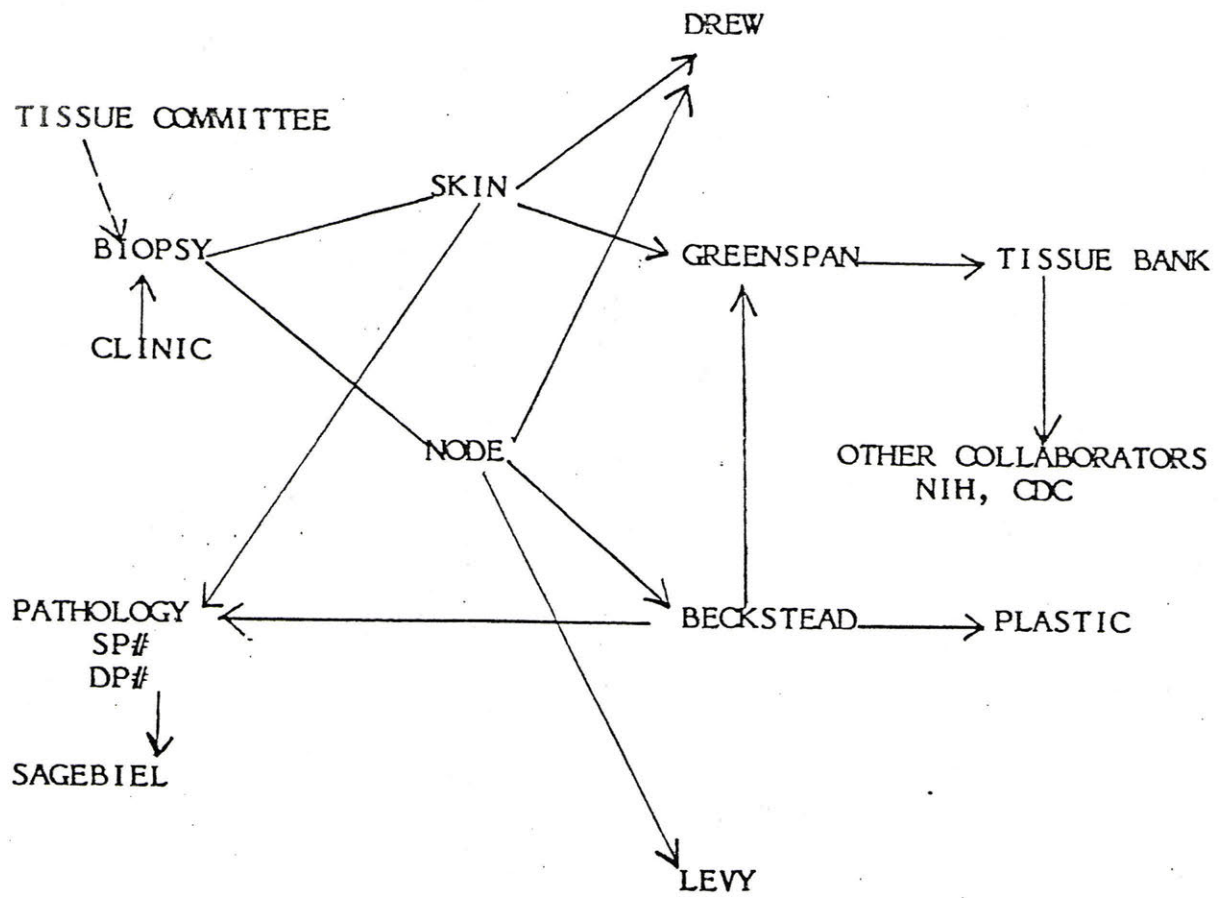
The Specimen Bank is a crucial part of the UCSF AIDS research effort. Specimens will be available to all investigators within the group and will also be made available to experts elsewhere. Such intragroup and collaborative studies have already been initiated (see attached).

cc: Dr. M. Conant

CURRENT BLOOD PROTOCOL



CURRENT TISSUE PROTOCOL



DETAILED BUDGET FOR FIRST 12-MONTH PERIOD: AIDS Specimen Bank Tissue, Serum and Semen

(1) SALARIES: LIST ALL SALARIED, NON-SALARIED PERSONNEL. PROJECT MERIT, PROMOTIONAL INCREASES IN COMPUTATION. USE TIME FOR STAFF EMPLOYEES ONLY — USE EFFORT FOR ACADEMICS.

John S. Greenspan
Division of Oral Biology
HSW-604 666-2220

FRINGE BENEFITS ARE CALCULATED AT 29 % ACADEMICS, 28 % STAFF.

Name and Title	Step	Time or Effort %	Based on Annual Rate	Actual Gross	Fringe Benefits	Total
(PI) [REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
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[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
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[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
Total Personnel						35,271

(2) CONSULTANT COSTS (ITEMIZE): Computer Center and Statistics

2,000

(3) EQUIPMENT (ITEMIZE) LIST ONLY ITEMS OVER \$500 UNLESS DESIGNATED THEFT-SENSITIVE:

Deep Freeze - 70 Revco

6,000

Deep Freeze - 20 Revco

4,000

Liquid N₂ equipment Almac

1,100

Holders/serum racks

1,500

Computer (IBM, PCXT + peripherals and software)

9,600

22,200

(4) SUPPLIES (ITEMIZE BY CATEGORY):

Liquid N₂/Dry ice

1,000

Cryostat supplies, disposables, slides

2,000

Film and processing, disks, chemicals

1,500

4,500

(5) TRAVEL DOMESTIC: 1 person, 1 local, 1 national

1,500

FOREIGN:

(6) PATIENT COSTS (INPATIENT): Nil

(OUTPATIENT):

(7) ALTERATIONS AND RENOVATIONS: P₂* facility alterations

8,000

(8) CONTRACTUAL OR THIRD PARTY COSTS: Nil

(9) OTHER EXPENSES (ITEMIZE): Servicing equipment

1,000

Mailroom, xeroxing, charts

1,000

Phone

350

2,350

(10) TOTAL DIRECT COST

75,821

(11) _____ % INDIRECT COST

(12) TOTAL REQUESTED FOR EXTRAMURAL SUPPORT

IF MATCHING FUNDS ARE REQUIRED, INDICATE AMOUNT \$ _____ SOURCE OF FUNDS _____

FACULTY EFFORT: LIST ALL ACTIVE OR PENDING GRANTS BY AGENCY, GRANT NUMBER OR TITLE AND PERCENT OF EFFORT DEVOTED TO EACH BY THE PRINCIPAL INVESTIGATOR (CONTINUE ON SEPARATE SHEET IF NECESSARY):

ACTIVE

PENDING

IMMUNOPATHOLOGY

Jay H. Beckstead and John S. Greenspan

Kaposi's sarcoma is a prominent problem among the populations of patients with acquired immunodeficiency. This unique population provides an opportunity to study a tumor the histogenesis and nature of which has been controversial for many years. There are conflicting reports in the literature regarding Factor VIII-related antigen in these tumors (1,2) in addition there are a few "ancient" manuscripts utilizing histochemistry (3). None of the studies presently available gives definitive information. We propose to embed specimens of Kaposi's sarcoma in plastic for combined enzyme and histochemical examination (4). Tissues will likewise be frozen for parallel experiments utilizing frozen section techniques (5). Our lab is also capable of localizing by enzyme and immunohistochemical markers at the ultrastructural level should this prove desirable. Recent work in our lab (6) has shown well-defined histochemical differences between different classes of vascular epithelium. We also have been successful in applying Anti-Factor VIII antiserum by an immunoperoxidase technique. The ability to explore all of these modalities in 2 micron sections with superb histology should solve many of the technical problems inherent in studies of heterogeneous tumors. This combination of approaches should allow an enzyme histochemical and immunohistochemical profile of Kaposi's sarcoma which will improve the diagnostic reproducibility and accuracy. It should also provide definitive information on the cell or origin and the reactive components within the tumor.

The population with acquired immunodeficiency presents other unusual clinicopathologic problems. Our group has recently described an increased incidence of malignancy, particularly malignancies likely to involve lymph nodes (7), while several centers report an unusual propensity to develop lymphadenopathy. This combination of circumstances results in clinicians taking a large number of lymph node biopsies in this patient population. This raises three major questions: 1) are biopsies necessary? 2) can examination of the lymph node provide prognostic information in individual patients? 3) can a clinical subpopulation that does benefit be identified? We propose to investigate these possibilities by a combination of enzyme and immunohistochemistry on plastic embedded lymph nodes (4) and immunohistochemistry on frozen lymph nodes (5). Recent work in our laboratory has shown that enzyme histochemistry can provide additional parameters useful in evaluating lymph node structure (6). This data combined with detailed study of the immunohistochemical distribution of cell types may provide a tool for understanding the lymph node changes in these patients.

The immunohistochemical approach will be directed towards the identification of mononuclear cell subpopulations. Thus identification and enumeration of T cells, B cells, and macrophages, not to mention T helper and T suppressor/cytotoxic subpopulations, should help characterize the lymph node abnormality. These results will be correlated both with those from the plastic embedded material and with other laboratory and clinical data including the results of peripheral blood immunological studies and the virological work. Frozen sections will be reacted with monoclonal antibodies T₁₁ (all T cells) T₄ (helper T cells) T₈ (suppressor/cytotoxic T cells) B₁ (B cells) and I₂ (Ia-like antigen on several subpopulations including activated T cells) obtained from Coulter Inc. and with OKM₁ obtained from Ortho. The avidin-biotin-peroxidase technique will be utilized (5) and cells will be identified and counted using techniques which are well-established in our laboratory (8). The results will be analyzed both qualitatively and quantitatively. Thus, morphological patterns will help to identify the nodes as normal, hyperplastic, or lymphomatous, while cell counts may allow the establishment of possible sequences in the progress from one of these stages to the next. The effects of treatment will be assessed in serial biopsies and the value of the procedure assessed.

References

1. Guarda, L.G., Silva, E.G., Ordonez, N.G., Smith, J.L. Jr. Factor VIII in Kaposi's sarcoma. Am. J. Clin. Pathol. 76: 197-220.
2. Burgdorf, W.H.C., Mukai, K. and Rosai, J. Immunohistochemical identification of factor VIII-related antigen in endothelial cells of cutaneous lesions of alleged vascular nature. Am. J. Clin. Pathol. 75: 167-171.
3. Pepler, W.J. The origin of Kaposi's haemangiosarcoma: A histochemical study. J. Path. Bact. 78: 553-557.
4. Beckstead, J.H., Halverson, P.S., Ries, C.A., and Bainton, D.F. Enzyme histochemistry in biopsy specimens of pathologic human bone marrow. Blood 57: 1088-1098, 1981.
5. Hsu, S.M., Raine, L. and Fanger, H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques. J. Histochem. Cytochem. 29: 577-580, 1981.
6. Beckstead, J.H. The evaluation of human lymph nodes using plastic sections and enzyme histochemistry. Am. J. Clin. Pathol. (submitted).
7. Ziegler, J.L., Drew, W.L., Miner, R.C., Mintz, L., Rosenbaum, E., Gershow, J., Lenette, E., Greenspan, J.S., Shillitoe, E.J., Beckstead, J., Cassavant, C., Yamamoto, K. An outbreak of Burkitt's-like lymphoma in homosexual men. Lancet Vol. II No. 8299, p. 631-633, September 18, 1981.
8. Greenspan, J.S., White, C., Daniels, T.E. Mononuclear cell populations in Sjogren's syndrome. J. Dent. Res. (in press).

cc: Dr. M. Conant

DETAILED BUDGET FOR FIRST 12-MONTH PERIOD: Immunopathology

(1) SALARIES: LIST ALL SALARIED, NON-SALARIED PERSONNEL. PROJECT MERIT, PROMOTIONAL INCREASES IN COMPUTATION. USE TIME FOR STAFF EMPLOYEES ONLY — USE EFFORT FOR ACADEMICS.

FRINGE BENEFITS ARE CALCULATED AT _____% ACADEMICS, _____% STAFF.

Name and Title	Step	Time or Effort %	Based on Annual Rate	Actual Gross	Fringe Benefits	Total
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
Total						33.820

(2) CONSULTANT COSTS (ITEMIZE): Nil

(3) EQUIPMENT (ITEMIZE) LIST ONLY ITEMS OVER \$500 UNLESS DESIGNATED THEFT-SENSITIVE:
Nil

(4) SUPPLIES (ITEMIZE BY CATEGORY):	Disposables, slides, film	2,000	
	Monoclonals, substrates	3,000	
	Plastic, chemicals, buffers	2,000	7,000

(5) TRAVEL DOMESTIC: <u>1 person, 1 national, 1 local</u>	1,500
FOREIGN: _____	

(6) PATIENT COSTS (INPATIENT): Nil
(OUTPATIENT): _____

(7) ALTERATIONS AND RENOVATIONS: Nil

(8) CONTRACTUAL OR THIRD PARTY COSTS: Nil

(9) OTHER EXPENSES (ITEMIZE):	Photography	1,000	
	Editing	750	
	Phone, mail, xerox	1,000	
	Journals, books	950	3,700

(10) TOTAL DIRECT COST,	46,020
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(11) _____% INDIRECT COST

(12) TOTAL REQUESTED FOR EXTRAMURAL SUPPORT	46,020
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IF MATCHING FUNDS ARE REQUIRED, INDICATE AMOUNT \$_____ SOURCE OF FUNDS _____

FACULTY EFFORT: LIST ALL ACTIVE OR PENDING GRANTS BY AGENCY, GRANT NUMBER OR TITLE AND PERCENT OF EFFORT DEVOTED TO EACH BY THE PRINCIPAL INVESTIGATOR (CONTINUE ON SEPARATE SHEET IF NECESSARY):

ACTIVE

PENDING