

**TISSUE COMMITTEE; ADDITIONAL
REQUESTS FOR SPECIMENS
1/84-3/85**

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January 6, 1984

Dr. Luan Eng Lie Injo
UCSF, Dept. Epidemiology
and International Health

Dear Dr. Lie Injo,

This letter is to certify that I am happy to continue the collaboration with have established and will continue to make blood from diagnosed AIDS patients available to you for your studies of hepatitis B virus. I think your studies are potentially important and I am happy to continue this collaboration. Thank you very much.

Sincerely,

A handwritten signature in cursive script, appearing to read "Paul Volberding".

Paul Volberding, M.D.
Dir. AIDS/KS Clinic
San Francisco General



January 13, 1984

Eugene L. Gottfried, M.D.
Director, Clinical Laboratories
San Francisco General Hospital

Dear Gene:

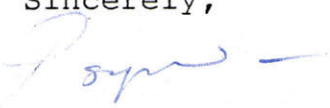
I received your memo concerning the E-B Virus serologies for our chemotherapy protocol, and appreciate your concerns. In brief, the study is a prospective analysis of the use of the experimental chemotherapy agent, ICRF-159 (Razoxone) in the treatment of patients with Kaposi's sarcoma in AIDS. This is an NCOG study, which is not supported by a drug company, and therefore unfunded.

We anticipate treating approximately 25 patients with before and after EBV serology from each patient. We anticipate that the duration of the project should be no more than 4 months, and any questions about records should be directed to me in the Oncology Clinic at ext. 8830.

No specific account number has been set up for these laboratories and we are currently simply freezing sera for a batch run after the study is completed. If you are unable to perform these through your laboratory budget, I would plan to set up a separate fund, although I anticipate that Judy Wilber in the City Labs will do them free of charge.

Thank you very much for your attention to our study. I hope that this clarifies our purposes.

Sincerely,


Paul Volberding, M.D.
Chief, Medical Oncology, SFGH

City and County of San Francisco

Department of
Public Health

San Francisco General Hospital
Medical Center



November 25, 1983

MEMORANDUM

TO: Paul A. Volberding
Chief, Medical Oncology

FROM: Eugene L. Gottfried, M.D. *ELG*
Director, Clinical Laboratories

SUBJ: Request for Information: E-B Virus Study

We have received a request for laboratory tests in connection with your research project. The policy of the Clinical Laboratories is to provide assistance with research projects whenever possible, provided that such assistance does not interfere with our primary responsibility of clinical testing for patient care. (This topic is discussed in the Clinical Laboratory Manual, 1983 edition, page 5; a copy is attached.)

In order to be sure that we can meet your needs for this project, I would like a note from you with the following information:

1. The name of the study (to identify requisitions)
2. Approximate total number and frequency of specimens and tests
3. Duration of the project
4. Person in charge of records - name, mailing address, extension
5. Account number to be charged *

*Since this is a "send-out" procedure, it will be best if arrangements are made for direct billing to you by the outside laboratory.

Please feel free to call me at x 8588 if you have any questions or comments, or if I can be of further assistance with your project.

EG/lh

INTRODUCTION

The Clinical Laboratory Manual of the San Francisco General Hospital Medical Center is a compact guide to the services of the Clinical Laboratories. Included are lists of available services and tests, reference values ("normal limits"), and comments to help you interpret test results.

For timely, efficient service, please follow the recommended procedures for collecting specimens and ordering tests. Consult the manual first if you have a question about the availability of a test or the kind of specimen needed. Special approval is required for any procedure not listed.

Although this manual answers the questions most often asked about laboratory services, you are invited to call on the specialized resources of the Laboratory Medicine Service for more detailed information or assistance with complex problems. We believe that good laboratory service depends upon a close working relationship between the clinical services and the laboratory staff. A Laboratory Medicine Resident is always on call for consultation and assistance. The Laboratory Director and Section Chiefs are available for consultation on both general problems and individual cases. (See list on page 8.)

"Laboratory error" may be suspected if test results are inconsistent with the clinical picture or with previous results. In such cases, it is important to call the Laboratory Medicine Resident IMMEDIATELY to check for possible clerical and/or technical problems. If necessary, the test will be repeated on the same specimen or a fresh specimen. Samples received by the Clinical Laboratories are usually retained for one week. Even so, any delay in checking a questioned result makes it harder to identify the problem and may jeopardize patient care.

We receive test requests from time to time in connection with research protocols. Our policy is to provide assistance with research projects when possible, as long as the project does not interfere with our primary responsibility of clinical testing for patient care. Advance approval is required. Project requests should be sent to the Laboratory Director's office and should include the following information: specific tests required; total number of specimens and tests; proposed schedule of testing; and source of funding. The cost of research procedures is the responsibility of the investigator and cannot be absorbed by the laboratory or billed to patients.

This manual will be updated annually. Supplemental information, revisions and new procedures will be announced throughout the year in laboratory newsletters and bulletins.

We hope you will find this handbook useful, and we welcome your comments for future editions.



Eugene L. Gottfried, M.D.
Director, Clinical Laboratories



January 13, 1984

Alfred J. Saah, M.D., M.P.H.
Medical Epidemiologist
Epidemiology and Biometry Section
National Institute of Allergy
and Infectious Diseases

Dear Al:

I enjoyed receiving the information from you concerning the health care worker epidemiology study you are conducting at the NIH. We are in the final phase of putting together a pilot study here in San Francisco and have taken the liberty of using your instrument in our discussions of one that we might adapt here.

It was interesting to hear how you resolved the question of highly confidential questions, like sexual activity and drug use. I suspect we will be forced to do much the same here. I look forward to talking about these studies in more detail with you. I expect to be at the NIH for an investigator's meeting on the 23rd and 24th of February. Perhaps we can set aside some time then to go over this material.

Again, thank you very much for your generosity in sharing your material with me. I look forward to seeing you.

Sincerely,

Signed

Paul Volberding, M.D.
Director, AIDS/KS Clinic, SFGH



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20205

November 11, 1983

Paul Volberding, M.D.
Medical Service, 5H22
San Francisco General Hospital
San Francisco, California 94110

Dear Paul:

It was a pleasure meeting with you at the Epidemiology Workshop in Rockville. Our study of health care workers is about to begin and I remembered your interest in collaborating.

We have decided that we will defer asking about sexual orientation and intravenous drug use until a need arises. While you may wish to include such questions now, one needs to consider the harm that may be done in recruiting participants. We will make enquiries about health status and will do a baseline physical exam on those with percutaneous or mucus membrane exposure.

The protocol and questionnaire are enclosed; the questionnaire may need touching up. If you have any suggestions that might better quantitate or evaluate exposure, please call me. We have struggled a great deal with the approach to collect this type of information.

Because we expect a very low number of outcome events, an analysis of combined data will almost certainly be preferable to studying our results individually. So we are interested in a real collaborative effort, and hope that you agree that we can be more effective by working together on this.

Let's keep in touch.

Sincerely,

A handwritten signature in cursive script, appearing to be "al", is written below the word "Sincerely,".

Alfred J. Saah, M.D., M.P.H.
Medical Epidemiologist
Epidemiology and Biometry Section
National Institute of Allergy
and Infectious Diseases

Enclosure

cc: Dr. Henderson

NUMBER: _____

NAME: _____
(Last, First, M.I.)

Phone: Office/Lab _____

Home _____

Address: Building _____ /Room Number _____

THERE ARE THREE (3) QUESTIONNAIRES ATTACHED. THEY ARE:

1. CLINICAL STAFF (COLOR CODED YELLOW) -- Pages 2 thru 7
2. HOSPITAL LABORATORY (COLOR CODED BLUE) -- Pages 8 thru 11
3. RESEARCH LABORATORY (COLOR CODED GREEN) -- Pages 12 thru 14

IF YOU WORK WITH OR CARE FOR PATIENTS WHO MAY HAVE ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS) THEN COMPLETE THE YELLOW CLINICAL STAFF QUESTIONNAIRE.

IF YOU WORK IN THE HOSPITAL LABORATORY AND DO NOT WORK WITH PATIENTS, THEN COMPLETE THE BLUE QUESTIONNAIRE.

IF YOU WORK IN A RESEARCH LABORATORY THAT HAS PROCESSED SPECIMENS FROM PATIENTS WITH AIDS, THEN COMPLETE THE GREEN QUESTIONNAIRE.

IF YOU ARE A CLINICIAN OR WORK WITH PATIENTS AND ALSO WORK IN A LABORATORY, PLEASE COMPLETE EACH APPROPRIATE QUESTIONNAIRE, I.E., THE YELLOW ONE AND EITHER THE BLUE OR GREEN ONE.

Office use

Codes _____

Clinical StaffNumber: _____
1-6Today's Date: _____
7-12

1. Job Title: ¹Nurse _____, ²Physician _____, ³Aide _____, ⁴Respiratory Therapist _____,
⁵Physical Therapist _____, ⁶I.V. Team _____, ⁷Venipuncture Staff _____,
⁸Radiology Technician _____, ⁹Other _____
2. In what month and year did you begin working at NIH or elsewhere in this capacity
(count the year that you started to have daily patient contact)? _____
14-15 16-17
3. Birthdate: _____ (mo/da/yr) 4. Sex: M _____ F _____
18-23 24
5. Race: ¹Black _____ ³Hispanic _____ 6. Ethnic Background: _____
²⁵ ²White _____ ⁴Oriental _____ ²⁶⁻²⁷ (e.g., German, Irish, African)
7. Social Security Number: _____ - _____ - _____
28-36
8. IN YOUR PAST MEDICAL HISTORY: (Please check all that you may have had at any time). IF NONE, CHECK HERE _____.
- | | |
|---|--|
| _____ Diabetes mellitus, ³⁷
insulin dependent | _____ Hepatitis, chronic ⁴¹ |
| _____ Chronic anemia ³⁸ | _____ Tuberculosis (active) ⁴² |
| _____ Chronic renal failure ³⁹ | _____ Bleeding disorder/clotting factor deficiency ⁴³ |
| _____ Malignancy ⁴⁰ | _____ Other infections (specify) ⁴⁴ |
9. WERE YOU HOSPITALIZED IN THE PAST 5 YEARS? _____ Yes ¹ _____ No ²
45

IF YES, please give diagnosis and date:

Month/Year

Diagnosis _____
46-47

52-53

58-59

Date _____
48-51

Date _____
54-57

Date _____
60-63

Number: 1-6

10. HAVE YOU HAD ANY TRANSFUSIONS OF BLOOD OR PACKED RED BLOOD CELLS WITHIN THE PAST 5 YEARS? ₆₄ Yes ¹ No ² Unknown ³

IF YES: Number of units: 65-66 Date: 67-72

11. HAVE YOU HAD ANY TRANSFUSIONS OF OTHER BLOOD PRODUCTS (e.g., platelets, plasma, cryoprecipitate, Factor IX concentrate) WITHIN THE PAST 5 YEARS? ₇₃

 Yes ¹ No ² Unknown ³ Date: 74-79

12. PLEASE CHECK ALL MEDICATIONS TAKEN WITHIN THE PAST 5 YEARS.
IF NONE, CHECK HERE .

	<u>From</u>	<u>To</u>
<u>80</u> Systemic corticosteroids	Dates: <u>7-12</u>	<u>13-18</u>
<u>19</u> Cytotoxic chemotherapy or other immunosuppressive therapy	Dates: <u>20-25</u>	<u>26-31</u>
<u>32</u> Hepatitis B immune globulin	Dates: <u>33-38</u>	<u>39-44</u>
<u>45</u> Immune serum globulin	Dates: <u>46-51</u>	<u>52-57</u>

13. Where do you spend your time working in the Clinical Center? (e.g., MICU, 11 E, X-ray department)

99% in 58 59-61
 90% in 62 63-65
 80% in 66 67-69
 70% in 70 71-73
 60% in 74 75-77
 50% in 78 7-9
 40% in 10 11-13
 30% in 14 15-17
 20% in 18 19-21
 10% in 22 23-25

(continued on next page)

Number: 1-6

All others less than 10% each 26 27-29
30 31-33

14. As far as you know, have you touched a patient with AIDS in a clinical setting or handled their specimens?

¹ Yes 34 ² No 34 IF NO, go to Question 17.

NURSES AND AIDES - CONTINUE WITH QUESTION 15. ALL OTHERS, GO TO QUESTION 17.

15. What is the approximate total number of AIDS patients that were assigned to you during the last 6 months? 35-37

16. Of these patients, how many required the following average number of hours of your care per shift? Care means tending to the physical or psychological needs of the patient.

		<u>Number of Patients</u>	
A.	less than 1 hour/shift 38-40	<u> </u>	
B.	1-3 hours/shift 41-43	<u> </u>	
C.	3-5 hours/shift 44-46	<u> </u>	
D.	5-8 hours/shift 47-49	<u> </u>	
Total: 50-52		<u> </u>	TOTAL NUMBER OF PATIENTS IN THIS COLUMN SHOULD EQUAL YOUR ANSWER TO QUESTION 15.

Of the patients in each of the above groups (A,B,C,D) how many did you take care of for more than 10 shifts?

	<u>Group</u>	<u>No. of patients cared for more than 10 shifts in last 6 months</u>
53-55	A	<u> </u>
56-58	B	<u> </u>
59-61	C	<u> </u>
62-64	D	<u> </u>

Number: _____

1-6

- 17.A. In the last 6 months, how many of the following procedures have you assisted with or performed in AIDS patients. Also, please describe any unexpected or accidental exposure that occurred during a procedure, e.g., soiling of bare hands with blood, urine, feces, or sputum and when it occurred.

<u>Procedures</u>	<u>Total Number</u>	<u>Type of Unexpected or Accidental Exposure</u>	<u>Date Month</u>
Bone marrow aspiration 7	8-9	10-11	12-13
Bone marrow biopsy 14	15-16	17-18	19-20
Lumbar puncture 21	22-23	24-25	26-27
Procto/sigmoidoscopy 28	29-30	31-32	33-34
Placing central venous line 35	36-37	38-39	40-41
Placing arterial line 42	43-44	45-46	47-48
Skin biopsy 49	50-51	52-53	54-55
Lung biopsy 56	57-58	59-60	61-62
Tracheostomy 63	64-65	66-67	68-69
Cystoscopy 70	71-72	73-74	75-76
Bronchoscopy 77	78-79	7-8	9-10
Esophago/gastro/duodenoscopy 11	12-13	14-15	16-17
Colonoscopy 18	19-20	21-22	23-24
Cardiopulmonary Resuscitation 25	26-27	28-29	30-31
Other (specify) 32	33-34	35-36	37-38

Number: 1-6

- B. This part is the same as above, except count total procedures in an average week during the last 6 months.

<u>Procedures</u>	<u>Total Number</u>	<u>Unexpected or Accidental Exposure</u>	<u>Date Month</u>
Changing bed 39	<u>40-41</u>	<u>42-43</u>	<u>44-45</u>
Help at toilet 46	<u>47-48</u>	<u>49-50</u>	<u>51-52</u>
Starting or changing IVs 53	<u>54-55</u>	<u>56-57</u>	<u>58-59</u>
Induced sputum 60	<u>61-62</u>	<u>63-64</u>	<u>65-66</u>
I.M. injection 67	<u>68-69</u>	<u>70-71</u>	<u>72-73</u>
Suctioning airway 74	<u>75-76</u>	<u>77-78</u>	<u>79-80</u>

18. In the last 6 months, were you splashed in your eyes, mouth, or face with blood, urine, feces, or sputum from a patient with AIDS?

Yes 7 No 8-9 IF YES, which material(s)? 8-9

How many times? 10-11 (Total)

Please circle month(s) of occurrence within the last 6 months: 12-13 14-15 16-17

Jan Feb Mar Apr May June Jul Aug Sep Oct Nov Dec

19. In the last 2 years, including the last 6 months, were you splashed in your eyes, mouth, or face with blood, urine, feces, or sputum from a patient with AIDS?

Yes 18 No 19

IF YES, which material(s) 19-20 How many times? 21-22 (total)

20. In the last 2 years, including the last 6 months, have you been stuck with a needle or scalpel that had been used on an AIDS patient? Yes 23 No 24

Total number of times 24-25

Number: 1-6

21. Have you had diarrhea during the past year that kept you from going to work?

1 Yes 26 2 No 27-28

IF YES, how many days did it last? 27-28

22. Have you had an illness that lasted for at least 2 weeks (14 days) in the past 6 months?

Yes 29 No 30

IF YES, please check each of the following symptoms that you had at any time during the illness:

30 Fever greater than 101°F

31 Enlarged or tender lymph nodes ("glands")

32 Unintentional weight loss of 15 or more pounds, or 10% of your normal weight.

23. Have you been involved in processing clinical specimens from patients with

AIDS in research laboratories? Yes 33 No 34

in clinical laboratories? Yes 34 No 35

IF YES to either, please complete the corresponding GREEN or BLUE questionnaire.

24. If you have any comments regarding this questionnaire or exposure, please write them here: 35-37

Hospital Laboratory
(No routine direct patient contact)

Number: 1-6

Today's Date: 7-12

1. Job title: Laboratory technician 13, Laboratory assistant 14, Scientist 15-16
(Circle degree(s): MS, MA, PhD, MD), Technologist 17,

Other: 18 : _____
(Describe)

2. Check lab in which you work:

Chemistry 19-21

Hematology _____

Coagulation _____

Microbiology _____

Blood bank _____

Surgical pathology _____

Pathology _____

3. List the lab assignments that you have had in the last 6 months:

22-23

4. Birthdate: _____ (mo/da/yr) 5. Sex: M 30 F _____

6. Race: ¹ Black _____ ³ Hispanic _____ 7. Ethnic background: 32-33 (e.g., German, Irish, African)
³¹ ² White _____ ⁴ Oriental _____

8. Social Security Number: _____ - _____ - _____
34-42

Number: 1-6

9. IN YOUR PAST MEDICAL HISTORY: (Please check all that you may have had at any time.) IF NONE, CHECK HERE _____.

☐ Diabetes mellitus ⁴³ insulin dependent	☐ Hepatitis, chronic ⁴⁷
☐ Chronic anemia ⁴⁴	☐ Tuberculosis (active) ⁴⁸
☐ Chronic renal failure ⁴⁵	☐ Bleeding disorder/clotting factor deficiency ⁴⁹
☐ Malignancy ⁴⁶	☐ Other infections (specify) ⁵⁰

10. WERE YOU HOSPITALIZED IN THE PAST 5 YEARS? ⁵¹ ☐ Yes¹ ☐ No²

IF YES, please give diagnosis and date:

Month/Year

Diagnosis <u>52-53</u>	Date <u>54-57</u>
Diagnosis <u>58-59</u>	Date <u>60-63</u>
Diagnosis <u>64-65</u>	Date <u>66-69</u>

11. HAVE YOU HAD ANY TRANSFUSIONS OF BLOOD OR PACKED RED BLOOD CELLS WITHIN THE PAST 5 YEARS? ⁷⁰ ☐ Yes¹ ☐ No² ☐ Unknown³

IF YES: Number of units: 71-72 Date: 73-78

12. HAVE YOU HAD ANY TRANSFUSIONS OF OTHER BLOOD PRODUCTS (e.g., platelets, plasma, cryoprecipitate, Factor IX concentrate) WITHIN THE PAST 5 YEARS? ⁷⁹

☐ Yes¹ ☐ No² ☐ Unknown³ Date: 7-12

Number: 1-6

13. MEDICATIONS TAKEN WITHIN THE PAST 5 YEARS:
IF NONE, CHECK HERE _____.

	<u>From</u>	<u>To</u>
<u>13</u> Systemic corticosteroids	Dates: <u>14-19</u>	<u>20-25</u>
<u>26</u> Cytotoxic chemotherapy or other immunosuppressive therapy	Dates: <u>27-32</u>	<u>33-38</u>
<u>39</u> Hepatitis B immune globulin	Dates: <u>40-45</u>	<u>46-51</u>
<u>52</u> Immune serum globulin	Dates: <u>53-58</u>	<u>59-64</u>

14. In what year did you first work in a laboratory at or outside of NIH that processed human specimens? 65-66 _____
15. In what month and year did you first work in a laboratory at or outside of NIH that processed specimens from patients with AIDS? 67-70 _____
16. Please check all the types of specimens that you have processed in the last 6 months.

Blood	<u>71</u>
Urine	<u>72</u>
Feces	<u>73</u>
Sputum	<u>74</u>
Tissue	<u>75</u>
Spinal fluid	<u>76</u>

17. In the last 6 months, how many times (total) have you had direct contact with patient tissue, secretions, or blood as a result of:

Broken containers	<u>77</u>
Centrifuge accidents	<u>78</u>
Cuts or scratches in lab	<u>79</u>
Needle sticks	<u>80</u>
A splash	<u>7</u>

Number: _____
1-6

18. In the last 6 months, have you had excessive or unusual exposure to clinical specimens from AIDS patients? Yes _____, No _____
8

IF YES, please describe the exposure(s): _____
9-10, 11-12, 13-14

19. Do you process surgical pathology specimens? Yes _____, No _____
15

20. In the last 2 years, have you done or assisted in autopsies on patients who had AIDS? Yes _____, No _____ IF YES, how many? _____
16 17-19

21. Have you had diarrhea during the last year that kept you from going to work?
1 Yes _____ 2 No _____
20

IF YES, how many days did it last? _____
21-22

22. Have you had an illness that lasted for at least 2 weeks (14 days) in the last 6 months? Yes _____ No _____
23

IF YES, please check each of the following symptoms that you had at any time during the illness:

_____ Fever greater than 101°F
24

_____ Enlarged or tender lymph nodes ("glands")
25

_____ Unintentional weight loss of 15 or more pounds, or 10% of your normal weight.
26

Research LaboratoryNumber: 1-6Today's Date: 7-121. Job Title: Laboratory technical staff 13 Scientist 14

2. What types of human specimens does your lab process? (Check all that apply.)

Tissue 15 (surgical pathology 16; pathology 17)
 Blood 18
 Sputum 19
 Spinal fluid 20
 Urine 21
 Feces 22

3. Birthdate: 23-28 (mo/da/yr) 4. Sex: M 29 F 30

5. Race: ¹ Black 30 ³ Hispanic 31
² White 32 ⁴ Oriental 33

6. Ethnic background: 31-32 (e.g., German, Irish, African)7. Social Security Number: 33-41 - 42 - 438. IN YOUR PAST MEDICAL HISTORY: (Please check all that you may have had at any possible time.) IF NONE, CHECK HERE 44.45 Diabetes mellitus, ⁴² insulin dependent46 Hepatitis, chronic47 Chronic anemia48 Tuberculosis (active)49 Chronic renal failure50 Bleeding disorder/clotting factor deficiency51 Malignancy ⁴⁵52 Other infections (specify) ⁴⁹53

Number: 1-6

9. WERE YOU HOSPITALIZED IN THE PAST 5 YEARS? ¹ Yes ² No

IF YES, please give diagnosis and date:

Month/Year

Diagnosis 51-52 Date 53-56

Diagnosis 57-58 Date 59-62

Diagnosis 63-64 Date 65-68

10. HAVE YOU HAD ANY TRANSFUSIONS OF BLOOD OR PACKED RED BLOOD CELLS WITHIN THE PAST 5 YEARS? ⁶⁹

¹ Yes ² No ³ Unknown

IF YES: Number of units: 70-71 Date 72-77

11. HAVE YOU HAD ANY TRANSFUSIONS OF OTHER BLOOD PRODUCTS (e.g., platelets, plasma, cryoprecipitate, Factor IX concentrate) WITHIN THE PAST 5 YEARS? ⁷⁸

¹ Yes ² No ³ Unknown Date 7-12

12. PLEASE CHECK ALL MEDICATIONS TAKEN WITHIN THE PAST 5 YEARS.
IF NONE, CHECK HERE _____.

	From	To
<u>13</u> Systemic corticosteroids	Dates: <u>14-19</u>	<u>20-25</u>
<u>26</u> Cytotoxic chemotherapy or other immunosuppressive therapy	Dates: <u>27-32</u>	<u>33-38</u>
<u>39</u> Hepatitis B immune globulin	Dates: <u>40-45</u>	<u>46-51</u>
<u>52</u> Immune serum globulin	Dates: <u>53-58</u>	<u>59-64</u>

13. In what year did you first work in a laboratory at or outside of NIH that processed human specimens? 65-66

Number: 1-6

14. In what month and year, as far as you know, did you first work in a laboratory at or outside of NIH that processed specimens from patients with AIDS? 67-70

15. Do you now process specimens from patients with AIDS?

1 Yes 71 2 No 3 Don't Know

IF YES, on approximately how many of the 120 work days in the last 6 months did you process specimens from AIDS patients? 72-74

16. Have you had diarrhea during the last year that kept you from going to work?

1 Yes 75 2 No

IF YES, how many days did it last? 76-77

17. Have you had an illness that lasted for at least 2 weeks (14 days) in the last 6 months? Yes 78 No

IF YES, please check each of the following symptoms that you had at any time during this illness:

79 Fever greater than 101⁰F

80 Enlarged or tender lymph nodes ("glands")

7 Unintentional weight loss of 15 or more pounds, or 10% of your normal weight

18. Do you have clinical (patient care) responsibilities? Yes 8 No

IF YES, please complete the "Clinical Staff" questionnaire.

Clinical Project No. _____

To: Chairman, Clinical Research Subpanel, NIAID

Recommended by: _____
Deputy Director, Clinical Center

Project Title: Evaluation of exposure to AIDS Patients Among NIH Personnel

Identifying Words: Employee Health, Acquired Immunodeficiency Syndrome (AIDS), Epidemiology

Principal Investigator: David K. Henderson, M.D., CC, NIH

Associate Investigators: Richard A. Kaslow, M.D., NIAID, MIDP
Alfred J. Saah, M.D., NIAID, MIDP
Anthony S. Fauci, M.D., NIAID, LCI
H. Clifford Lane, M.D., NIAID, LI
Henry Masur, M.D., CC, CCM

Estimated Duration of Study: 5 years

Number of Patients Needed: N/A

Kind of Patients Needed: NIH Employees

Precis: This study is designed to assess the potential for nosocomial (i.e. patient to staff) transmission of the newly described Acquired Immunodeficiency Syndrome (AIDS). The populations to be studied include: 1) clinical patient-care-givers, 2) clinical laboratory staff involved in processing and testing laboratory specimens from these patients, and 3) laboratory staff in research labs who receive specimens from these patients and work with blood and blood products from these patients.

There are three major aspects of this study:

1. An epidemiologic questionnaire will be distributed to all participants in the study.
2. Sera will be banked from all employees working in the areas outlined above in anticipation of the development of a test which detects an identifying antigen or antibody for the syndrome.
3. Any employee who sustains a needlestick (or any percutaneous or mucosal splash injury) with a device contaminated with blood, serum, excreta, or secretions from these patients will be evaluated immunologically for baseline function and then will be studied every six months for the appearance of the nonspecific markers (i.e. lymphocytopenia, decreased OKT_4/OKT_8 ratio) of the syndrome.

Narrative Description:

A. Background

The Centers for Disease Control (CDC) reported a marked increase in the number of cases of Kaposi's Sarcoma in the United States in the 7-3-81 issue of the Morbidity and Mortality Weekly Report. These cases and the original MMWR report (6/4/81) heralded the onset of the Acquired Immunodeficiency Syndrome (AIDS). Since then more than 2,000 of these cases have been identified.

Although no agent has been identified as causing AIDS, a large body of epidemiologic data suggests that the syndrome is transmissible and may be transmitted either venereally or by percutaneous inoculation of "infected" blood or blood products.

Since virtually every patient who develops AIDS as we know it eventually becomes critically ill, these patients have required extensive hospitalization, and frequently have required numerous invasive procedures.

Thus far, there have been no documented instances of patient-to-staff nosocomial transmission; however, little is known regarding the incubation period for AIDS or the period during which AIDS may be communicable. Since the epidemiology of AIDS appears to be similar to the epidemiology of hepatitis B, the potential for nosocomial transmission would appear to exist.

B. Objectives

The objectives of this study are threefold:

1. To collect demographic data regarding NIH personnel involved in the care of AIDS patients and in the handling of specimens from AIDS patients.
2. To collect baseline serum samples from these employees (and semi-annual follow-up samples) in anticipation of the development of a serologic test for AIDS.
3. To obtain baseline immunologic function on all employees who sustain needlestick injuries or mucosal splashes with material from an AIDS patient.

C. Eligibility and Exclusions

All NIH employees, especially those involved in the care of AIDS patients or involved in the handling, processing or assessment of specimens from these patients will be eligible for inclusion in this study.

There are no exclusions from this study.

D. Specific Aims

1. To obtain epidemiologic and exposure information from NIH employees involved in the care or evaluation of AIDS patients.

2. To obtain baseline and follow-up serum samples on employees in anticipation of being able to use these samples to define the nosocomial epidemiology of AIDS.
3. To collect baseline immunologic data on any employee who has a documented mucosal splash or percutaneous injury with a needle or other sharp item that was contaminated with blood, serum, secretions or excreta from an AIDS patient; and to perform an "immunologic profile" on these employees at six month intervals.

E. Rationale

Since AIDS apparently represents a new syndrome, the risk of transmission to hospital personnel is unknown. Studies such as the ones described above may shed some initial light on the magnitude of risk and on risk factors associated with the potential for transmission of this syndrome.

Since the epidemiology of AIDS appears to be similar to hepatitis B, it is reasonable to expect the eventual detection of a blood or serum-borne antigen for the syndrome. Banking serum on employees who have exposure to AIDS patients or to clinical specimens from these patients would seem a reasonable plan for eventually evaluating in-hospital exposure.

Since virtually every patient with AIDS has had dramatic immunologic abnormalities, particularly with respect to cell-mediated immunity, performing baseline immunologic profiles on employees with potential blood-to-blood exposure to AIDS is currently the only way to follow our employees.

F. Procedure

Employees working in each of the settings described above will receive a memorandum describing the protocol and asking for participation.

All personnel desiring to participate will fill out the appropriate epidemiologic questionnaire and will then have 10 cc. of venous blood drawn for serum storage. Repeat serum samples will be obtained every six months. An interim questionnaire will also be administered (see below).

Employees who have documented instances of mucosal splashes or of percutaneous inoculation with contaminated needles will have an additional 60 cc. of venous blood drawn. Lymphocytes will be separated from this blood and will be utilized in the immunologic assays described below. These patients will be reassessed by these same procedures every six months for the duration of the study.

G. Methods and Materials

1. Study Population

All personnel involved in the care of AIDS patients or involved in the handling and/or processing of clinical or research specimens from AIDS patients will be invited to participate in the study.

2. Epidemiologic Questionnaire

Data will be collected from each participant at the time of entry into the study regarding type of work done, contact with AIDS patients, contact serum, blood, or other clinical samples from these patients. These questionnaires will be administered regularly at approximately six month intervals.

A purpose of the questionnaire is to measure exposure to patients with AIDS and/or to clinical specimens. Enquiries regarding health status and medicines are designed to account for other potential reasons for immunosuppression. Enquiries regarding risk factors for AIDS were deliberately excluded at this time because of the highly sensitive nature of the information, and the lack of relevance of these data at the time of initial enrollment. A need for such information would exist at the time of outcome, i.e., clinical AIDS or serological evidence of infection with the "AIDS agent". In either case, the information regarding risk factors can be sought from some or all of the study participants when a more tangible reason exists for its collection. The consent form does specify that enquiries regarding risk factors (e.g., male homosexuality, intravenous drug use) for AIDS may be made at a future time if it is deemed necessary.

Employees leaving the NIH will be followed by mail on at least a yearly basis.

3. Serologic Testing

Since no test is currently available to identify a marker for AIDS in serum, these sera will be stored at -80° C until such a test is perfected.

4. Immunologic Profile

Any staff member who sustains a mucosal splash with blood or serum from an AIDS patient, or who sustains a percutaneous injury with a needle or other sharp object contaminated with blood or serum from an AIDS patient will be asked to allow us to draw 60 cc. of venous blood. This blood will be utilized to assess the employee's baseline immunologic function. Assessments included in the immunologic profile are:

- A. Enumeration of time-zero plaque-forming cells.
- B. In vitro antibody production in response to pokeweed mitogen (PWM).
- C. Fluorescence-activated cell sorting to determine numbers of T-cells, B-cells, OKT₄ positive T cells (T helper/inducer cells) and OKT₈ positive T cells (suppressor/cytotoxic cells).
- D. Measurement of the in vitro blastogenic responses to the mitogens Concanavalin A, Phytohemmagglutinin, Pokeweed mitogen and to the antigen tetanus toxoid.

These tests are now standardized in Dr. Lane's and Dr. Fauci's laboratories.

Employees with potential transcutaneous inoculation of contaminated serum or blood will be serially assessed at six month intervals for changes in immunologic function for the duration of the study.

H. Endpoint

1. This study will be conducted for a period of five years. If during that period of time, a serologic test is identified which is a clear marker for AIDS, the study could be completed prior to the five year period.

I. Significance

The potential significance of this study lies 1) in the collection of demographic and epidemiologic data which may help identify risk factors and estimate their relative importance for the nosocomial transmission of AIDS; 2) in the potential for protecting Clinical Center and NIH employees by documenting intact immune function at the time of a needlestick injury or mucosal splash.

J. Anticipated Hazards

1. Venipuncture

- A. Local discomfort at time of venipuncture.
- B. Local bruising.
- C. Fainting.
- D. (Rare) infection at venipuncture site.

2. Phlebotomy

- A. Total volume of blood to be drawn at every six month interval is 10 cc.
- B. If transcutaneous or mucus membrane contamination occurs, total volume of blood to be drawn every six months would increase to 70cc to allow for immunologic profile.



January 31, 1984

Neil Cooper, M.D.
Scripps Clinic and Research
Foundation
10666 N. Torrey Pines Road
La Jolla, CA 92037

Dear Dr. Cooper:

We reviewed your request for collaborative studies of complement pathways in patients with AIDS. As Dr. Conant may have mentioned, all requests for tissue are handled through the Tissue Committee of the NIH/AIDS Working Group at UCSF. We were very interested in your proposal and would be happy to assist you by providing you with required specimens.

As you can appreciate, we receive many requests for collaborative studies on material from AIDS patients, and have thus drafted an agreement form for prospective collaborations. This agreement form is enclosed, and if you could return it to me I would appreciate it. Dr. John Greenspan at the address below can be contacted directly to provide the specimens you require.

We are excited about your proposal and look forward to assisting you with your work and in seeing your results. Thank you very much for your interest.

Sincerely,

A handwritten signature in dark ink, appearing to read "Paul Volberding".

Paul Volberding, M.D.
Chief, Medical Oncology
Director, AIDS/KS Clinic, SFGH

cc: John Greenspan, M.D.
Professor and Vice Chairman
OMHD
S-653
University of California
San Francisco, CA 94143

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SCHOOL OF MEDICINE
Department of Microbiology
and Immunology

SAN FRANCISCO, CALIFORNIA 94143

February 1, 1984

Dr. Judith Luce
Department of Medicine
San Francisco General
Wd 86, Bldg. 80

Dear Judy:

It was nice to see you last week. I would like to pursue your offer to collect cells from any pleural effusions that you tap in patients with breast cancer. Ed Liu, one of the people here interested in breast cancer, will give you a call next week to discuss the best means for storing and transporting the samples, hoping to optimize our chances of recovering intact nucleic acid.

I also enclose two papers describing recent work on the putative oncogene associated in mouse mammary cancers.

With best regards,

A handwritten signature in blue ink, which appears to read "Harold".

Harold E. Varmus, M.D.
American Cancer Society
Research Professor

HEV/jm
Enclosures

Mode of proviral activation of a putative mammary oncogene (*int-1*) on mouse chromosome 15

Roel Nusse*, Albert van Ooyen*, David Cox†, Yuen Kai T. Fung‡ & Harold Varmus†

* Department of Virology, Netherlands Cancer Institute, Antoni van Leeuwenhoekhuis, Plesmanlaan 121, Amsterdam, The Netherlands
† Department of Pediatrics and ‡ Department of Microbiology, University of California, San Francisco, California 94143, USA

*Most mammary carcinomas induced in C3H mice by the mouse mammary tumour virus (MMTV) bear a new proviral insertion within a highly conserved locus on chromosome 15 called *int-1*. A transcriptional unit within this locus is inactive in all tested normal tissues but expressed at low levels in mammary tumours with proviral insertions positioned on either the 5' and 3' sides of the gene. Transcription of the proviruses proceeds away from *int-1*; thus an indirect mechanism appears to activate expression of this putative oncogene.*

THE proviruses of retroviruses can integrate at many sites in host genomes; thus, like other movable genetic elements, they act as insertion mutagens¹. Two classes of proviral insertion mutations have been described: recessive mutations that disrupt and prevent expression of genes²⁻⁶ and presumably dominant mutations that activate gene expression, leading to induction of tumours by retroviruses without their own oncogenes⁷⁻¹³. For example, during the induction of B-cell lymphoma by the avian leukosis virus (ALV), proviral insertion mutations augment transcription of *c-myc*, the cellular progenitor of a retroviral oncogene⁷, either by provision of a retroviral promoter⁷⁻¹⁰ or by an indirect effect on a host promoter¹⁰.

The proposal that retroviruses lacking oncogenes induce tumours by insertion mutation of host genes suggests a strategy for the isolation of novel oncogenes. By molecular cloning of a single new mouse mammary tumour virus (MMTV) provirus from a C3H mouse mammary tumour, we have isolated a region of the mouse genome that contains an MMTV proviral insertion in the majority of C3H mammary tumours¹³. Although this region lacks homology with any of the available retroviral oncogenes, we have previously argued that it harbours an oncogene, called *int-1*, instrumental in mammary tumorigenesis because (1) MMTV proviruses can integrate at many sites in the host genome (implying that insertions near *int-1* confer a selective growth advantage) and (2) because the insertions are accompanied by the appearance of transcriptional products from *int-1*, a silent domain in non-neoplastic mammary tissue.

We demonstrate here that insertion mutations in the *int-1* locus usually stimulate transcriptional activity by an indirect mechanism: MMTV proviruses are located either upstream from transcribed *int-1* sequences in the opposite orientation or downstream in the same orientation. We also assign the *int-1* gene to mouse chromosome 15 and show that *int-1*, like the progenitors of viral oncogenes, has been conserved during evolution.

Locating and orienting MMTV proviruses

We reported previously¹³ that 18 of 26 C3H mouse mammary tumours contain MMTV proviral DNA within a common region of approximately 20 kilobases (kb). The insertions are distributed with roughly equal frequency on both sides of a portion of the locus that is represented in tumour-specific, 2.6-kb polyadenylated RNA. To assess the mechanisms involved in the activation of the *int-1* transcriptional unit, we have now determined the sites of proviral insertion more precisely and ascertained the transcriptional directions of the proviruses and the *int-1* gene. These studies involved physical mapping of the interrupted *int-1* loci with restriction endonucleases, using the

DNA transfer method and a collection of molecular annealing reagents specific for portions of *int-1* and for the 5' and 3' halves of MMTV proviral DNA. The *int-1* probes were derived by plasmid subcloning of unique sequence DNA from recombinant λ bacteriophages containing overlapping inserts of the *int-1* domain from a library of normal BALB/c mouse DNA. Probe C detects *int-1* RNA in mammary tumours; probe D represents a region located leftward of probe C on the *int-1* map as it is conventionally drawn (see Fig. 3). The probes used to orientate proviral DNA [MMTV-*env* and MMTV-*gag* (Fig. 1)] represent portions of MMTV DNA that reside near the 5' and 3' ends of the provirus but exclude sequences from the long terminal repeats (LTRs). In general, our strategy was to use enzymes that cleaved *int-1* at widely separated sites and divided MMTV proviral DNA into two portions distinguishable with MMTV-*env* and MMTV-*gag* probes.

Proviruses on the 5' side of *int-1*

Two enzymes, *Bgl*II and *Kpn*I, proved to be particularly useful for the analysis of MMTV proviruses present to the left of the *int-1* transcriptional unit. (We will show below that transcription of *int-1* proceeds from left to right on the physical map; thus, proviruses inserted to the left of the region represented by probe C can be considered to lie 5' to at least a portion of the transcriptional template.) *Bgl*II cleaves the normal C3H mouse DNA at a leftward site (position -21 on the map in Fig. 3) to generate a 14-kb fragment that anneals with probe C. In addition, *Bgl*II cleaves the MMTV provirus at two sites, one of them near the middle of the element (Fig. 1). Proviral DNA in eight tumours was found to disrupt the normal 14-kb *Bgl*II fragment from one of the two *int-1* alleles. As illustrated for six of these tumours in Fig. 1a and b, the novel fragments also annealed with the MMTV-*gag* probe, indicating that the proviruses are positioned either to the right of the probe C homology in the same transcriptional orientation as *int-1* or to the left of the probe C region in the opposite orientation. In all eight tumours, additional mapping, illustrated here for a few tumours with *Kpn*I and probe D, showed that the latter possibility was correct (Fig. 1c-d). *Kpn*I cleaves the *int-1* domain at the most leftward site we have mapped (position -23) and at position -11, to generate a fragment of 12 kb that hybridizes with probe D. The MMTV provirus is cut once by *Kpn*I. Novel *Kpn*I fragments annealing with probe D (Fig. 1c) were also detected by the MMTV-*env* probe (Fig. 1d), demonstrating that the proviruses are inserted upstream from the probe C homology region in orientations opposite to the transcriptional direction of *int-1*.

Table 1 Segregation of mouse *int-1* and mouse chromosome in Chinese hamster-mouse somatic cell hybrids

Chromosome	Marker enzyme	<i>Int-1</i> /chromosome (no. of clones)				Discordance %
		+/+	+/-	-/+	-/-	
1	PEP-3	5	4	1	2	42
2	AK-1	15	2	8	4	34
3	—	2	7	1	2	67
4	PGD	13	5	4	8	30
5	—	2	7	1	2	67
6	TPI-1	3	6	7	5	62
7	GPI-1	4	5	2	1	58
8	GR-1	4	5	1	2	50
9	MOD-1	1	8	1	2	75
10	PEP-2	2	7	2	1	75
11	—	0	9	0	3	75
12	ACP-1	5	4	1	2	42
13	—	4	5	0	3	42
14	NP-1	10	6	5	7	39
15	AS-2	17	0	1	10	4
16	SOD-1	3	6	2	0	73
17	GLO-1	2	7	1	2	67
18	PEP-1	7	2	5	8	32
19	GOT-1	12	6	1	11	23
X	HPRT	15	3	12	0	50
Y	—	0	9	0	3	75

Thirty clones of hybrids formed by polyethylene glycol fusion of mouse spleen cells or macrophages with an established Chinese hamster cell line (380-6), deficient for hypoxanthine phosphoribosyl transferase (HPRT), were analysed for mouse *int-1* and enzyme markers, although not all clones were analysed for every enzyme marker. Twelve of the hybrid clones were also analysed by karyotype analysis as described previously²². The symbols for the marker enzymes, their chromosome assignments and the electrophoretic procedures used to separate the Chinese hamster and mouse enzymes have been previously described²². In general, the presence or absence of a given mouse chromosome, as determined by karyotypic analysis, agreed with the presence or absence, respectively, of the enzyme marker for that chromosome. However, in several instances, the enzyme marker for a given chromosome was present in a clone that karyotypically lacked that mouse chromosome, indicating chromosome breakage and/or rearrangement. The presence of mouse chromosomes 3, 5, 11, 13 and Y was based on karyotype analysis alone.

Proviruses on the 3' side of *int-1*

Similar analytical strategies were applied to DNA from 10 tumours previously shown to have insertions between positions 0 and -8 on the *int-1* map; an 11th tumour (number 24), previously thought to lack an *int-1* insertion, was also found to have a provirus within this region during the extended tests. Comparative annealings with *int-1* and MMTV probes (ref. 13 and data not shown) revealed that all 11 proviruses were oriented in the same direction, with transcription proceeding from left to right on the *int-1* map.

A particularly interesting part of this analysis, pertinent to the mechanism of expression of *int-1*, is illustrated in Fig. 2. When DNA from tumour 53 was cleaved with *Bam*HI and annealed with probe C, two novel fragments were detected in digests of each sample, indicating that the insert is positioned within the region detected by the probe. Similar results were obtained with DNA from tumour 11 (not shown). Proviruses in three other tumours (numbers 55, 12 and 15) were found to be positioned at very similar sites, about 0.6–0.8 kb to the right of the insertion site in tumour 53 (Fig. 2 and unpublished data). The inserts in these tumours lie within the transcriptional unit, and affect the size of resulting transcripts (see below).

We conclude that 19 of 26 C3H-mammary tumours carry MMTV proviruses in the *int-1* locus (Fig. 3). Eight proviruses are distributed between positions -11 and -18; each of these is oriented so that transcription of the provirus proceeds from right to left. Eleven proviruses are located within the region

extending from position -8 to 0, and all are oriented so that transcription proceeds from left to right. Mapping with several endonucleases provides no evidence for deletions within either proviral DNA or *int-1*; the several atypical restriction maps of proviral DNA we encountered are best explained by restriction site polymorphisms (data not shown).

int-1 transcripts

We have previously shown that tumours bearing proviruses in the *int-1* region contain polyadenylated transcripts that anneal with probe C, whereas no RNA homologous to probe C is detectable in normal mammary glands¹³. The abundance of the *int-1* transcripts is not great; less than 10 copies per cell have been estimated by three independent methods (unpublished results).

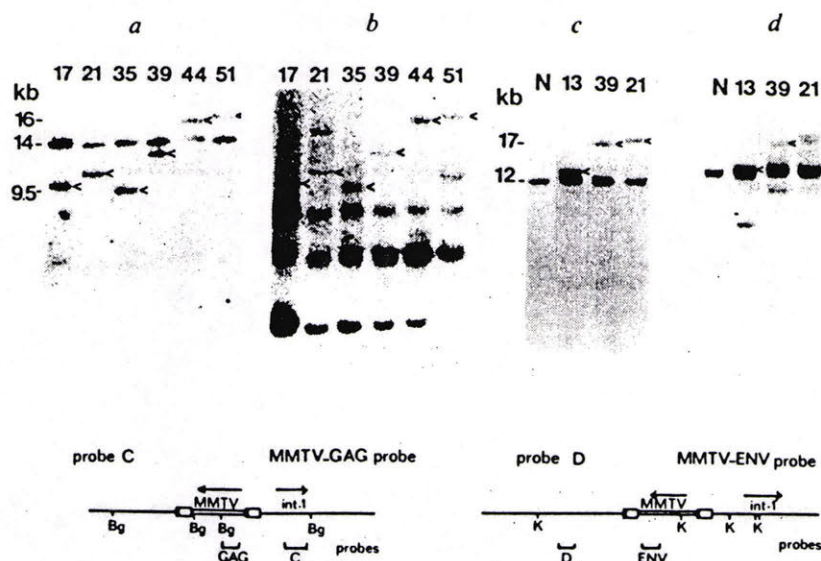
To judge the mechanism by which the insertion mutations activate expression of *int-1*, it was necessary to determine the direction of transcription of the cellular gene. The *Bam*HI-*Eco*RI fragment that constitutes probe C was recloned in both orientations in the bacteriophage vector M13. Restriction mapping of the replicative forms established the orientation of each insert and hence the polarity of strands present in single-stranded virion DNA (data not shown). Radioactive probes were synthesized by random priming on templates provided by two phages, each bearing one of the two strands. Only one of these probes was found to anneal to RNA from tumour 44, previously shown to contain an *int-1* transcript 2.6 kb in length (Fig. 4a); the composition of the probe established the direction of transcription to be from left to right on the *int-1* map as presented in Fig. 3.

The *int-1* transcripts characterized in our earlier report were, like those in tumour 44, approximately 2.6 kb long¹³. In a wider survey of all those tumours from which undegraded polyadenylated RNA could be obtained, we discovered atypical *int-1* transcripts of 3.2 kb in tumour 11 and of 3.8 kb in tumours 12 and 15 (Fig. 4b). In these three tumours, no 2.6-kb *int-1* RNA was found. The conventional 2.6 kb *int-1* RNA species was present in all the other tested tumours with *int-1* insertions (for example, in tumours 24 and 13 in Fig. 4b) and in one tumour (number 14) in which an *int-1* mutation has not been found (unpublished data).

The probable composition of the larger, atypical RNA species was suggested by an intriguing correlation with the site of insertion: in tumours 11, 12 and 15, integration of proviral DNA occurred within or near the right-hand boundary of the region represented by probe C (see Fig. 2). (Suitable RNA samples were not available from the other tumours, 53 and 55, with insertions in this region.) If the proviruses were located within the transcribed domain, upstream from the natural polyadenylation site for *int-1* RNA, the 5' LTR would be appropriately positioned to provide a surrogate polyadenylation site. The enlarged size of the *int-1* transcript could then be ascribed to the length of the U3 region of the MMTV LTR (~1.2 kb)^{14–16}. A similar situation has been encountered in an avian leukosis virus (ALV) insertion mutant affecting the chicken *c-myc* locus¹⁰.

It was not possible to test this explanation by attempting to anneal a labelled probe for the MMTV LTR directly to gel fractionated RNA, because virus-specific RNA transcribed in a normal fashion from proviral DNA was vastly more abundant than transcripts from *int-1*. We attempted to circumvent this problem by using the 'sandwich hybridization' procedure^{10,16} to test for RNA molecules containing MMTV U3 sequences covalently linked to *int-1* sequences. Restriction fragments of phage DNA containing a portion of the *int-1* domain were fractionated in an agarose gel, transferred to nitrocellulose filters, and annealed with unlabelled RNA from tumour 12 (containing a 3.2 kb transcript) or from tumour 13 (with a conventional 2.6 kb transcript). After removal of unannealed RNA, the filter was incubated with a ³²P-labelled *Pst*I fragment containing virtually all of the MMTV LTR. One autoradio-

Fig. 1 MMTV proviruses on the 5' side of the *int-1* transcriptional unit are in the transcriptional orientation opposite to that of *int-1*. DNA samples (10 µg) from mammary tumours (numbers indicated on top) were digested with *Bgl*III (Bg, panels a and b) or *Kpn*I (K, panels c and d), electrophoresed in 0.8% agarose gels, and transferred to nitrocellulose¹³. The filters were first incubated with probe C (a) or probe D (c), washed and exposed to X-ray film. Subsequently, the probes were removed from the filters and the filters were incubated with MMTV-*gag* probe (b) or with MMTV-*env* probe (d). The origin of the probes is indicated in the diagrams at the bottom of the figure and in Fig. 3; MMTV-*gag* (3.2 kb) is a *Pvu*II-*Bgl*III fragment obtained from a clone of proviral DNA endogenous to GR mice (ref. 56); MMTV-*env* (1.8 kb) is a *Pst*I fragment derived from MMTV (C3H) DNA and cloned into pBR322 (ref. 57). Arrows indicate tumour-specific bands that show rearrangements of the *int-1* locus detected with probes C and D and bands in the same position detected with the MMTV probes.



graphic signal, at the position of the *int-1* fragment homologous to probe C, was observed in the experiment conducted with RNA from tumour 12; no reaction was detected with the sample from tumour 13 (Fig. 4c). From this experiment we conclude that *int-1* and MMTV sequences are covalently joined in transcripts from tumour 12; we presume, but have not proved, that the MMTV sequences are located at the 3' end of the 3.2-kb transcript in this tumour.

Conservation of the *int-1* locus

We have proposed that the *int-1* domain harbours an oncogene that contributes to mammary tumorigenesis following transcriptional activation by proviral integration. However, the locus lacks homology to all tested retroviral oncogenes including *sis*, *ski*, *fos*, *fms* and *yes* (data not shown), as well as the 11 examined previously¹³, and it does not anneal with the NIH/3T3 cell transforming gene detected in mammary tumour DNA by Lane *et al.* (ref. 17 and our unpublished results with M. A. Lane and G. M. Cooper). Moreover, we have been unable to measure *int-1* RNA in a variety of tissues from mature mice (liver, spleen or brain), in cultured mouse cells, or in normal mammary glands from pregnant or lactating females (data not shown). Since no protein product of *int-1* has been identified, we sought support for the idea that the *int-1* region contains a coding domain by testing for conserved *int-1* sequences, homologous to probe C, in the DNA of other vertebrates. As illustrated in Fig. 5, *Bam*HI fragments that anneal effectively with probe C in conditions of only moderately reduced stringency are present in digests of DNA from fish, birds and several mammals, including man. These results are similar to those observed with conserved cellular genes, including the known oncogenes¹⁸. In addition, as found for the progenitors of some retroviral oncogenes¹⁹, sequences homologous to *int-1* can be detected in the genome of *Drosophila melanogaster* (lane 1).

Mouse chromosome 15

Specific karyotypic anomalies, some involving putative oncogenes, have recently been found to be associated with a number of different neoplasms²⁰. Trisomy of mouse chromosome 13, for example, is frequently observed in MMTV-induced mammary tumours²¹. It was therefore of interest to determine the chromosomal location of *int-1*. To assign *int-1* to a specific mouse chromosome, we used probe C to detect restriction fragments containing *int-1* in digests of genomic DNA from 30 Chinese hamster-mouse somatic cell hybrid clones segregating

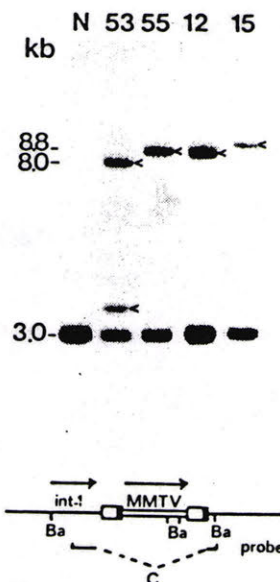
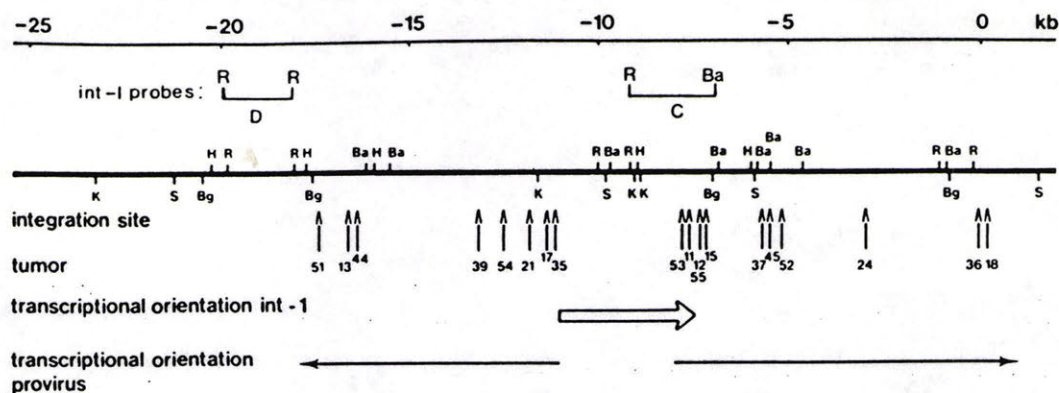


Fig. 2 Proviruses on the 3' side of *int-1* are in the same transcriptional orientation as *int-1*. Tumour DNA (number indicated on top, N: normal DNA) was digested with *Bam*HI (Ba), transferred to nitrocellulose filters and annealed with probe C. The sizes of the arrowed, novel restriction fragments and additional mapping data (not shown) show that the proviruses were integrated as drawn at the bottom of the figure. This illustration shows the provirus from tumour 53, providing an explanation for the two novel *int-1* fragments detected with probe C. Proviruses in the other tumours are located further rightward (see Fig. 3).

mouse chromosomes. These hybrids together contain the full complement of murine chromosomes except for chromosomes 11 and Y (ref. 22). The mouse chromosome complement of each hybrid clone was determined by isozyme and/or karyotype analyses as previously described²². The single *int-1*-specific *Eco*RI fragment of 8.6 kb present in digests of genomic mouse DNA could be easily distinguished from the *int-1*-specific *Eco*RI fragment of 2.8 kb derived from Chinese hamster DNA (not shown). An analysis of the segregation of murine *int-1* and enzyme markers for each of 16 mouse chromosomes (all but 3, 5, 11, 13 and Y) revealed 96% concordant segregation of *int-1* and arylsulphatase A (AS-1), an enzyme marker for mouse chromosome 15, but discordant segregation of *int-1* and the

Fig. 3 A map of the proviral integration sites in the *int-1* locus in C3H mice. Restriction enzyme sites were mapped using C3H and BALB/c chromosomal DNA and DNA from several clones of recombinant bacteriophage containing overlapping inserts from normal BALB/c DNA. Position 0 is defined as the insertion site of the MMTV provirus in tumour 18; this provirus was originally cloned with its flanking DNA to obtain



DNA from the *int-1* locus. Other molecular clones, encompassing positions -22 to +20 on the *int-1* map, were obtained by sequential screening of a Charon 4A bacteriophage library of normal BALB/c mouse DNA with probes from the *int-1* region, as described previously¹³. R, *EcoRI*; Ba, *BamHI*; Bg, *BglII*; K, *KpnI*; S, *SacI*; H, *HindIII*. Probes C and D are fragments of single copy DNA positioned as indicated on the map. Vertical arrows above tumour numbers designate sites of integration. The wide horizontal arrow denotes transcriptional orientation of *int-1* and the approximate extent of the transcribed domain. The narrow horizontal arrows show the direction of transcription of proviruses integrated in the regions demarcated by the arrows.

other enzyme markers (Table 1). When probe C was removed from the DNA blots and the samples reannealed with a probe for the *c-myc* oncogene (data not shown), we observed perfect concordance between *int-1* and the mouse *EcoRI* DNA fragment derived from the *c-myc* locus previously assigned to mouse chromosome 15 (refs 23, 24). This finding, consistent with the isoenzyme results, provided further evidence for the assignment of *int-1* to mouse chromosome 15. Karyotype analyses of 12 selected hybrid clones ruled out an association between *int-1* and chromosomes 3, 5, 11, 13 or Y and were also consistent with the assignment of *int-1* to chromosome 15 (Table 1). The single discordant clone, which was weakly positive for AS-2 but negative for *int-1*, revealed no evidence of a recognizable mouse chromosome 15. Presumably, this hybrid contains a small, non-recognizable fragment of chromosome 15 which includes AS-2 but not *int-1*. Taken together, these data indicate that *int-1* is located on mouse chromosome 15.

Implications

The results described here support our previous contention that tumour induction by MMTV involves insertion mutations that activate a previously unknown gene called *int-1*. At the same time they promote confidence in the general proposition that oncogenic viruses lacking viral oncogenes may initiate tumour growth by insertional mechanisms. This hypothesis fosters an experimental strategy for isolation of oncogenes that is formally analogous to what has been termed 'transposon tagging' in other systems²⁵: identification of the mutagenic provirus in tumour DNA should lead directly to the isolation of a linked, mutated cellular oncogene. These ideas now receive support from studies of avian B-cell lymphomas induced by at least two different retroviruses^{7,11}, erythroleukaemia induced by an avian virus that also causes B-cell lymphoma²⁶, T-cell lymphoma induced by murine leukaemia virus²⁷ and mammary tumours induced by MMTV^{13,28}. At present only circumstantial evidence implicates the affected cellular genes in the oncogenic process: (1) mutation of the same gene in several tumours of the same type; (2) augmented expression and (3) in some cases homology to progenitors of retroviral oncogenes.

Application of the transposon tagging method has led to the discovery of at least two potential mammary oncogenes: the *int-1* gene commonly implicated in tumours arising in C3H mice and the *int-2* domain recently found by Peters *et al.*²⁸ to be interrupted by proviral insertions in about half of the mammary tumours arising in the BR6 mouse strain. These two targets lack evidence of homology with known retroviral oncogenes or with each other, and they are located on different chromosomes (C. Dickson and R. N., unpublished results). In addition, since

substantial numbers of tumours lack insertions in either domain, there may be yet other targets for MMTV insertion mutations.

The case for oncogenic roles for *int-1* and *int-2* rests primarily on the argument that MMTV proviral DNA appears capable of integration into many different regions in the mouse genome; hence the repeated finding of proviruses at these two loci implies that the tumours are clonal outgrowths of cells²⁹ with a growth advantage conferred at least in part by the insertion mutations. The case for *int-1* also rests on the evidence that the locus is transcriptionally active in tumours bearing *int-1* mutations but not in normal tissues. Although a protein product of *int-1* has not been identified, a coding function is implied by the conservation of at least part of the transcribed domain during vertebrate evolution (Fig. 5); these findings conform to those obtained with the cellular genes accepted as potential oncogenes by virtue of their homology with retroviral oncogenes.

Our assignment of *int-1* to mouse chromosome 15 does not offer additional insight into its role as an oncogene: karyotypic abnormalities involving this chromosome have not been reported in mouse mammary tumours, and no endogenous MMTV proviruses have been assigned to chromosome 15 (ref. 30). However, an unrelated oncogene, *c-myc*, also present on mouse chromosome 15, recombines with an immunoglobulin locus on chromosome 12 to produce the translocations found in several plasmacytoma lines^{24,31}. Although the distance between *int-1* and *c-myc* on mouse chromosome 15 is not known, mammary tumours with proviral insertions at *int-1* do not display abnormal expression of *c-myc* (unpublished data of authors).

The core of the present report concerns the mechanisms by which MMTV proviruses activate expression of *int-1*. In ALV-induced bursal lymphomas, augmented expression of *c-myc* is most commonly achieved by a mechanism referred to as promoter insertion: ALV proviral DNA on the 5' side of *c-myc* exons in the same transcriptional orientation provides an efficient transcriptional promoter in the form of an LTR⁷⁻¹⁰. However, in the 19 MMTV-induced mammary tumours studied here we have found no instance in which a promoter insertion mechanism activates expression of *int-1*. Instead, MMTV proviruses are positioned either on the 5' side of the transcriptional template for *int-1* in the opposite orientation or on the 3' side in the same orientation. (This rule, however, is not inviolable: in one tumour recently obtained from a BALB/cC3H mouse, a provirus is present on the 5' side of the *int-1* transcriptional domain in the same orientation (R.N., unpublished).)

Previous examples of the two arrangements observed here, in which an LTR cannot serve as promoter for an activated gene, have been described in ALV-induced bursal lymphomas¹⁰. In these few instances, the effect on expression of *c-myc* was ascribed to a retroviral 'enhancer' element, akin to similar elements first identified in papovavirus genomes³²⁻³⁸. Using

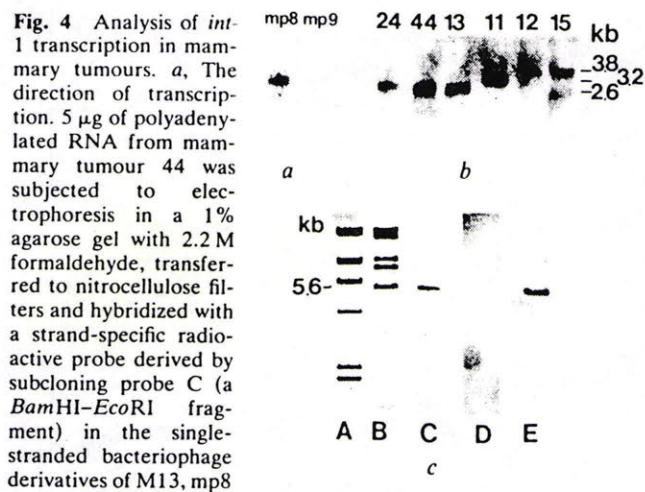


Fig. 4 Analysis of *int-1* transcription in mammary tumours. *a*, The direction of transcription. 5 µg of polyadenylated RNA from mammary tumour 44 was subjected to electrophoresis in a 1% agarose gel with 2.2 M formaldehyde, transferred to nitrocellulose filters and hybridized with a strand-specific radioactive probe derived by subcloning probe C (a *Bam*HI-*Eco*RI fragment) in the single-stranded bacteriophage derivatives of M13, mp8 and mp9, in both orientations. The autoradiogram shows the hybridization to 2.6-kb RNA with probes made by transcribing the recombinant M13mp8 DNA with reverse transcriptase in the presence of 32 P-dCTP with oligomers of calf thymus DNA as random primers. A probe made similarly from the recombinant M13mp9 DNA did not detect a transcript. The direction of transcription of the *Eco*RI-*Bam*HI fragment must proceed from the *Eco*RI site to the *Bam*HI site or from left to right on the *int-1* map as shown in Fig. 1. *b*, The length of *int-1* polyadenylated RNA in mammary tumours varies from 2.6 to 3.8 kb. Polyadenylated RNA (5 mg) from mammary tumours with MMTV proviral integrations at the *int-1* locus was subjected to electrophoresis in 1% agarose gels containing 2.2 M formaldehyde¹³ and hybridized with probe C. Tumour numbers are indicated on top (see Fig. 3 for the positions of the proviruses within *int-1*). Tumours 24, 44 and 13 (and also tumours 17, 18, 21, 35, 36, 37, 39, 45) contain a 2.6-kb RNA hybridizing with probe C. Tumour 11 has a 3.2-kb RNA and tumours 12 and 15 have a 3.8-kb RNA species. *c*, Sandwich hybridization demonstrates covalent linkage of *int-1* sequences to MMTV LTR sequences in tumour 12. DNA from a bacteriophage clone covering the normal *int-1* region from positions -20 to -3.4 was digested with *Eco*RI and subjected to electrophoresis (lane B), next to a marker lane (A) containing wild-type λ DNA digested with *Hind*III. DNA was transferred to nitrocellulose filters (several identical filters were prepared from one gel). Direct annealing to radioactive probe C identified a 5.6-kb *Eco*RI fragment (arrow) that contained the *int-1* transcriptional domain (lane C). (This fragment maps from the *Eco*RI site at position -9 in *int-1* to an *Eco*RI linker at position -3.4.) Other filters were first incubated with unlabelled RNA from tumour 13 (lane D) or tumour 12 (lane E). These filters were washed and then hybridized to a radioactive probe from the MMTV LTR (a 1.4-kb *Pst*I fragment containing all but 10 bp of the LTR). Only the filter that was annealed first with the RNA from tumour 12 shows hybridization with the *Eco*RI fragment that also hybridized with probe C. Lanes A and B are photographs of the ethidium-bromide stained gel, the other lanes are autoradiograms.

Methods: 20 µg of poly(A)⁺ RNA was resuspended in 2 ml of an annealing mix consisting of 50% formamide, 0.5 M NaCl, 20 mM PIPES pH 6.8, 5 mM Na₂EDTA, 0.4% SDS, 250 µg ml⁻¹ poly(A), 2× Denhardt's solution, and 200 µg ml⁻¹ carrier yeast RNA. This solution was applied to the filter-bound DNA and incubated for 24 h at 41 °C. The filter was washed twice at 53 °C for 15 min with 0.1×SSC, 0.1% SDS. The filter was blotted dry, annealed to MMTV-LTR probe (2×10⁶ c.p.m.), washed twice for 1 h at 37 °C with 0.1×SSC, 0.1% SDS, dried and autoradiographed.

DNA transformation techniques, retroviral³⁹⁻⁴² and cellular⁴³ sequences with enhancer activity have been shown to act in *cis*, over variable distances and independent of polarity, to improve the efficiency of heterologous promoters.

Our evidence that MMTV DNA can activate the expression of a previously silent gene in tumours has several of the characteristics of enhancement: the mechanism of activation operates in a manner independent of orientation, over distances of at least 10 kb, and in *cis* rather than *trans*. The basis for claiming *cis* activation is our observation that insertions between positions -8 and -6 on the *int-1* map are associated only with

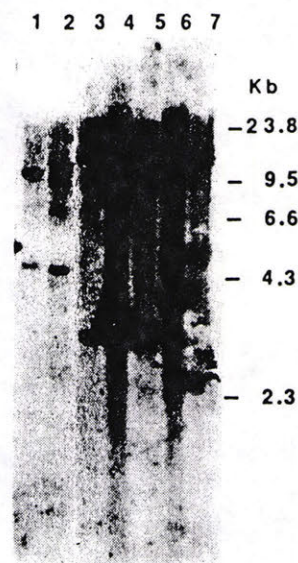


Fig. 5 Sequences homologous to *int-1* are present in all vertebrates and *Drosophila*. Cellular DNA (10–15 µg) from the indicated sources was digested with *Bam*HI, subjected to electrophoresis in an 0.8% agarose gel, transferred to nitrocellulose filters, and annealed with probe C in 37.5% formamide, 3×SSC, at 42 °C. After washing in 2×SSC at 50 °C, the filter was exposed to X-ray film for 6 days. Lane 1, adult *Drosophila melanogaster* (Oregon-S strain); lane 2, fish (*X. helleri*); lane 3, chicken erythrocytes (strain 15×7); lane 4, mouse liver (BALB/c strain); lane 5, rat hepatoma cells (H4IIE line, ATCC); lane 6, domestic cat liver; lane 7, human mammary epithelial cells (HBL100 line; ATCC). Numbers at the right (in kb) refer to the positions of *Hind*III fragments of λ phage DNA present in the same gel.

transcripts of atypically large size (Fig. 4b), despite the persistence of the chromosome bearing a normal *int-1* allele; we conclude that only the physically rearranged locus has been activated. Other aspects of the situation may also be germane to a consideration of mechanism. (1) The observed effect is conversion of an ostensibly silent gene to one expressed at low levels (less than 10 RNA molecules per cell), rather than to amplify expression of a gene normally transcribed. (2) Enhancer functions are likely to be cell specific⁴⁴⁻⁴⁹ and perhaps target gene specific, so it is plausible that the MMTV enhancer works preferentially in mammary cells and upon *int-1*. (3) Initiation of MMTV transcription is greatly stimulated by glucocorticoid hormones⁵⁰, and the hormonal response displays some of the properties of enhancement⁵¹⁻⁵³; hence it is possible that activation of *int-1* is also affected by physiological doses of hormone. This final point should be amenable to direct test by growing mouse mammary tumour cells with *int-1* insertions in controlled conditions in culture.

The use of an MMTV enhancer function to activate expression of *int-1* could explain the uniform orientations of proviruses on each side of the proposed transcriptional unit (see Fig. 3). Enhancers are thought to act only or preferentially upon proximal promoters^{54,55}; if the MMTV enhancer is located on the 5' side of the initiation site for viral RNA, within the 1.2-kb U3 sequence of the LTR, then proviruses would require the orientations observed to avoid interposing an MMTV promoter between the enhancer and its *int-1* target.

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1. Varmus, H. E. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) 411-503 (Academic, New York, 1983).
2. Varmus, H. E., Quintrell, N. & Ortiz, S. *Cell* **25**, 23-36 (1981).
3. Jenkins, N. A., Copeland, M. G., Taylor, B. A. & Lee, B. K. *Nature* **293**, 370-374 (1981).
4. Copeland, N. G., Jenkins, N. A. & Lee, B. K. *Proc. natn. Acad. Sci. U.S.A.* **80**, 247-249 (1983).
5. Jaenisch, R. *et al. Cell* **32**, 209-216 (1983).
6. Kuff, E. L. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 1992-1996 (1983).
7. Hayward, W. S., Neel, B. G. & Astrin, S. M. *Nature* **290**, 475-480 (1981).
8. Neel, B. G., Hayward, W. S., Robinson, H. L., Fang, J. & Astrin, S. M. *Cell* **23**, 323-324 (1981).
9. Payne, G. S. *et al. Cell* **23**, 311-322 (1981).
10. Payne, G. S., Bishop, J. M. & Varmus, H. E. *Nature* **295**, 209-213 (1982).
11. Noori-Daloii, M. R., Swift, R. A., Kung, H. J., Crittenden, L. B. & Witter, R. L. *Nature* **294**, 574-575 (1981).
12. Fung, Y. K. T., Fadly, A. M., Crittenden, L. B. & Kung, K. J. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3418-3422 (1981).
13. Nusse, R. & Varmus, H. E. *Cell* **31**, 99-109 (1982).
14. Shank, P. R., Cohen, J. C., Varmus, H. E., Yamamoto, K. R. & Ringold, G. M. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2112-2116 (1978).
15. Donehower, L. A., Huang, A. L. & Hager, G. L. *J. Virol.* **37**, 226-238 (1981).
16. Dunn, A. R. & Hassel, J. A. *Cell* **12**, 23-36 (1977).
17. Lane, M. A., Saiten, A. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5185-5189 (1981).
18. Bishop, J. M. & Varmus, H. E. in *The Molecular Biology of Tumor Viruses, Pt III* (eds Weiss, R. A., Teich, N., Varmus, H. E. & Coffin, J. M.) 999-1109 (Cold Spring Harbor Laboratory, New York, 1982).
19. Shilo, B. Z. & Weinberg, R. A. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6789-6797 (1981).
20. Rowley, J. D. *Nature* **301**, 290-291 (1982).
21. Dofuku, R., Utakoji, T. & Matsuzawa, A. *J. natn. Cancer Inst.* **63**, 651-656 (1979).
22. Cox, D. R., Sawicki, J. A., Yee, D., Appella, E. & Epstein, C. J. *Proc. natn. Acad. Sci. U.S.A.* **79**, 1930-1934 (1982).
23. Crews, S., Barth, R., Hood, L., L., Prehn, J. & Calame, K. *Science* **218**, 1319-1321 (1982).
24. Sakaguchi, A. Y., Lalley, P. A. & Naylor, S. L. *Somat. Cell Genet.* **9**, 391-406 (1983).
25. Bingham, P. M., Levis, R. & Rubin, G. M. *Cell* **25**, 693-704 (1981).
26. Fung, Y. K., Lewis, W. G., Crittenden, L. B. & Kung, H.-J. *Cell* **33**, 357-368 (1983).
27. Tschlis, P. N., Gunter-Strauss, P. & Hu, L. F. *Nature* **302**, 445-449 (1983).
28. Peters, G., Brooke, S., Smith, R. & Dickson, C. *Cell* **33**, 369-377 (1983).
29. Cohen, J. C., Shank, P., Morris, V. L., Cardiff, R. & Varmus, H. E. *Cell* **16**, 333-345 (1978).
30. Coffin, J. M. in *The Molecular Biology of Tumor Viruses, Pt III* (eds Weiss, R. A., Teich, N., Varmus, H. E. & Coffin, J. M.) 1109-1203 (Cold Spring Harbor Laboratory, New York, 1982).
31. Sheng Ong, G. L., Keath, B. J., Piccoli, S. P. & Cole, M. D. *Cell* **31**, 443-452 (1982).
32. Banerji, J., Rusconi, S. & Schaffner, W. *Cell* **27**, 299-308 (1981).
33. Moreau, P., Hen, R., Everett, R. & Gaub, M. P. *Nucleic Acids Res.* **9**, 6047-6069 (1981).
34. DeVilliers, J. & Schaffner, W. *Nucleic Acids Res.* **9**, 6251-6264 (1981).
35. Capecchi, M. R. *Cell* **22**, 479-488 (1980).
36. Weiher, H., Konig, M. & Gruss, P. *Science* **219**, 626-631 (1983).
37. Lusky, M., Berg, L., Weiher, H. & Botchan, M. *Molec. Cell Biol.* **3**, 1108-1122 (1983).
38. Fromm, M. & Berg, P. *Molec. cell Biol.* **3**, 991-999 (1983).
39. Levinson, B., Khoury, G., Vande Woude, G. & Gruss, P. *Nature* **295**, 568-572 (1982).
40. Kriegler, M. & Botchan, M. *Molec. cell Biol.* **3**, 325-339 (1983).
41. Jolly, P. J., Esty, A. C., Sbramanti, S., Friedmann, T. & Verma, I. M. *Nucleic Acids Res.* **11**, 1855-1872 (1983).
42. Luciw, P., Bishop, J. M., Varmus, H. E. & Capecchi, M. *Cell* **33**, 705-716 (1983).
43. Conrad, S. E. & Botchan, M. *Molec. cell Biol.* **2**, 949-965 (1982).
44. Fujimura, F. K., Deininger, P. L., Friedman, T. & Linney, E. *Cell* **23**, 809-814 (1981).
45. Katinka, M., Vasseur, M., Montreau, N., Yaniv, M. & Blangy, D. *Nature* **290**, 720-722 (1981).
46. Sekikawa, K. & Levine, A. J. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1100-1104 (1981).
47. Laimins, L. A., Khoury, G., Gorman, C., Howard, B. & Gruss, P. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6453-6456 (1982).
48. Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. *Cell* **33**, 717-728 (1983).
49. Banerji, J., Olson, L. & Schaffner, W. *Cell* **33**, 729-740 (1983).
50. Varmus, H. E., Ringold, G. & Yamamoto, K. R. in *Glucocorticoid Hormone Action* (eds Baxter, J. D. & Rousseau, G. G.) 253-289 (Springer, New York, 1979).
51. Chandler, V. L., Maler, B. A. & Yamamoto, K. R. *Cell* **33**, 489-499 (1983).
52. Hynes, N., Van Ooyen, A. J. J., Kennedy, N., Herrlich, P., Ponta, H. & Groner, B. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3637-3641 (1983).
53. Majors, J. E. & Varmus, H. E. *Proc. natn. Acad. Sci. U.S.A.* **80**, 5866-5870 (1983).
54. Wasylyk, B., Wasylyk, C., Augereau, P. & Chambon, P. *Cell* **32**, 503-514 (1983).
55. DeVilliers, J., Olson, L., Banerji, J. & Schaffner, W. *Cold Spring Harbor Symp. Quant. Biol.* **47**, 911-920 (1982).
56. Hynes, N. E., Kennedy, N., Rahmsdorf, V. & Groner, B. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2038-2041 (1981).
57. Majors, J. E. & Varmus, H. E. *Nature* **289**, 253-258 (1981).

Correlation between segmental flexibility and effector function of antibodies

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Mouse monoclonal anti-dansyl antibodies with the same antigen-binding sites but different heavy chain constant regions were generated. The extent of segmental flexibility in times of nanoseconds and the capacity to fix complement were greatest for IgG2b, intermediate for IgG2a, and least for IgG1 and IgE. Hence, the effector functions of immunoglobulin isotypes may be controlled in part by the freedom of movement of their Fab arms.

IMMUNOGLOBULIN G is a flexible Y-shaped molecule. The two antigen-binding Fab units of IgG are joined to an Fc unit at a hinge that allows the angle between the Fab parts to vary over a broad angular range, as shown by hydrodynamic¹, electron microscopic^{2,3}, and X-ray crystallographic studies⁴⁻⁶. Nanosecond fluorescence polarization measurements have demonstrated that immunoglobulin molecules exhibit segmental flexibility in the nanosecond time range⁷⁻⁹. Segmental flexibility is likely to be important in enabling immunoglobulins to bind optimally to multivalent antigens and to carry out certain effector functions. Studies of polyclonal antibody populations have suggested that immunoglobulin classes differ in their degree of segmental flexibility¹⁰⁻¹¹. We have explored the relationship between hinge motion and effector function in specially constructed families of homogeneous immunoglobulins. Mouse monoclonal anti-dansyl antibodies with the same antigen-combining sites but different heavy chain constant regions (Fig. 1) were generated by selecting somatic variants in hybridoma cell lines. The extent of segmental flexibility in times of nanoseconds (measured by fluorescence spectroscopy) and the capacity to fix complement were greatest for IgG2b, intermediate for IgG2a, and least for IgG1 and IgE. Hence, the effector functions of immunoglobulin isotypes may be controlled in part by the freedom of movement of their Fab arms.

In most antibody-producing hybridoma cell lines, variant cells arise which produce antibody containing a different heavy chain¹². For example, cells producing IgG2b sometimes arise from a cell line producing IgG1. The frequency of these heavy-chain switch variants is ordinarily low (10^{-5} - 10^{-6} per generation). These rare cells can be separated by fluorescence-activated cell sorting on the basis of the appearance of a different heavy chain on their cell surface^{13,14}. In these switch variants, the light chain remains the same, whereas V_H becomes joined to a different C_H. Consequently, the antigen-combining site is the same as in the parent, despite the change in the heavy-chain constant region. Many of these switch variants are stable and can be cloned.

We chose to generate a family of homogeneous mouse anti-dansyl (DNS) antibodies for two reasons. First, the fluorescence emission spectrum of the bound dansyl chromophore is very responsive to the polarity of its environment¹⁵, making it a convenient and sensitive indicator of whether the antigen-combining site of the switch variant antibody is in fact the same as that of the parent line. Second, the bound dansyl chromophore has an excited state lifetime suitable for determining the segmental flexibility of immunoglobulin isotypes by nanosecond fluorescence polarization spectroscopy¹⁶. Variant hybridoma cell lines producing different immunoglobulin isotypes were



February 3, 1984

Luan Eng Lie-Injo, M.D.
Department of Epidemiology
and International Health
HSW 1699
UCSF

Dear Dr. Lie-Injo:

This letter is to certify that I am happy to continue the collaboration we have established and will continue to make blood from diagnosed AIDS patients available to you for your studies of hepatitis B virus. I think your studies are potentially important and I am happy to continue this collaboration. Thank you very much.

Sincerely

A handwritten signature in dark ink, appearing to read "Paul Volberding".

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

Memorandum

To : Health and Welfare Agency
Committee for Protection of Human Subjects
714 P Street, Room 1290
Sacramento, CA 95814

Date : February 6, 1984

Subject: Project No. 83-11-04

ATTN: Dr. Charles Gardipee
Chairman

From : Richard W. Emmons, M.D., Ph.D.
Chief, Viral and Rickettsial
Disease Laboratory Section
Berkeley ATSS 571-2573

RWE
Emmons

Following up on your meeting of November 4, 1983, and your letter to me of November 29, 1983, I have made the change requested in the consent form of our proposed project, "Infectious Etiology of Acquired Immune Deficiency Syndrome (AIDS) and Kaposi's Sarcoma". The change is in Item 5 and an original and 11 copies of the retyped and corrected "Informed Consent" form are attached.

I have been informed by telephone that the project has received approval by the review committee at NIH, but has not been ranked at a fundable level. Therefore it appears likely that we will not be able to proceed. Nevertheless, in case an appeal is successful or we can resubmit a (revised) proposal which might be accepted, I would like to keep this file open. Essentially the same protocol should be useable for a future revised project.

RWE/d1

Attachments: 1) Informed Consent Form
2) Letter of 11/29/83

cc: All co-investigators and Collaborators
on Grant NIH-NCI-DCCP-BCB-83-3

Informed Consent to Participate as a Research Subject in a Study of The Infectious Etiology of Acquired Immune Deficiency Syndrome (AIDS) and Kaposi's Sarcoma.

1. I have read and understand the attached description of the research project entitled "Infectious Etiology of Acquired Immune Deficiency Syndrome (AIDS) and Kaposi's Sarcoma" which is supported by the National Cancer Institute and the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, U.S. Public Health Service.
 2. I understand that the purpose of this study is to discover the cause of acquired immune deficiency syndrome (AIDS) and Kaposi's sarcoma (KS), that my participation might help in this discovery, and that if the cause can be found, then it may be possible to more easily detect, prevent or cure the conditions.
 3. I therefore agree to be a research subject in this project and to undergo interviews, physical examinations, and laboratory tests. I understand that I have the right to withdraw from this study at any time and can decide to withhold information about myself and withdraw permission to use my specimens for laboratory tests, all without any adverse effect to myself, and without any prejudice or adverse actions by the institutions or medical personnel who are providing care and treatment to me.
 4. I agree to allow previous medical records and results of laboratory tests, and previously taken laboratory specimens, to also be used in this present study if they are needed.
 5. I agree to allow single or multiple specimens to be taken from me for laboratory tests and to be used in attempts to find the cause of AIDS and KS. Such specimens may include: blood, urine, feces, saliva, throat swab, rectal swab, semen (ejaculate). If biopsy specimens of my tissues, or other specimens which require surgical procedures to obtain them, are needed for the study I understand that I will be asked to sign separate consent forms for each procedure. These biopsy specimens may consist of cerebrospinal fluid, bone marrow, lymph nodes, rectal mucosa, Kaposi's sarcoma tissue, transtracheal lung aspirate or lung tissue, and other tumor tissue.
- I understand that there is some risk in providing such specimens, and that the process of obtaining specimens may cause me some adverse effects, such as slight pain or risk of bleeding after collection of blood samples; pain following biopsy procedures; reactions to the anesthesia used during surgical biopsy procedures; and special risks of surgical procedures which will be explained to me before I agree to undergo such surgical procedures. I understand that it is not possible to guarantee that there will be no adverse effects, but that efforts will be made to prevent or minimize these, and that I will be assured of and assisted in obtaining proper medical care for any adverse effects that occur.

6. I understand that I have the right and can know the information recorded about me and the results of laboratory tests done on my specimens as part of this study.

7. I understand that my records, laboratory tests results, and specimens will be identified by my name and other personal identifying information, but that this information will be kept confidential, for use only by the research study personnel in order to carry out the purposes of the study. I understand that my privacy and identity will be protected and not revealed to the public, news media, or to unauthorized persons.

8. I understand that any medical condition or test results discovered during this study which might affect my health will be promptly brought to the attention of my personal physician or medical care designate, or to me, so that I can seek help in dealing with it.

9. I understand that if I wish to get any more information about the study or about myself and my role in the study, I can do so by contacting the office of the Principal Investigator:

Richard W. Emmons, M.D.
Viral and Rickettsial Disease Laboratory
California Department of Health Services
2151 Berkeley Way
Berkeley, CA 94704 (415) 540-2573

Signed:

Study subject name _____ Date _____

Witness name _____ Date _____

Street and house # _____

City _____ State _____ Zip _____

Telephone _____

OFFICE OF STATEWIDE HEALTH PLANNING AND DEVELOPMENT

1600 9TH STREET

SACRAMENTO, CA 95814

(916) 322-3212



November 29, 1983

Committee for the Protection
of Human Subjects
1600 - 9th Street, Room 430
Sacramento, CA 95814

Richard Emmons, M.D., Ph.D., Chief
Viral and Rickettsial Disease
Laboratory Section
Department of Health Services
2151 Berkeley Way, Room 452
Berkeley, CA 94704

Dear Doctor Emmons:

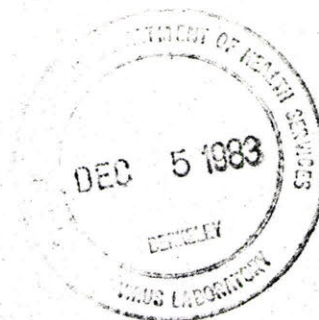
RE: Project Entitled "Infectious Etiology of Acquired Immune Deficiency
Syndrome (AIDS) and Kaposi's Sarcoma"
Project No. 83-11-04

The State Committee for the Protection of Human Subjects at its regular
meeting on November 4, 1983 in Berkeley reviewed the above-entitled
project.

The Committee approved the project subject to the following conditions:

1. Change the consent form to reflect the discussion during the
meeting. The issue was in item five of the consent form which reads,
"If biopsy specimens of my tissues or other specimens which require
surgical procedures to obtain them, are needed for the study, I will
show my willingness to provide them by signing a separate permission
form for each procedure." It was the members concern that it be
clear to participants that by signing this document they are not
signing a consent form for all the procedures in this one form.
Wording suggested by the members was "I will be asked to sign
separate consent forms for each procedure."

Please advise us promptly in writing of your compliance with these
conditions, sending us copies of appropriate documents or other revised
materials.



Richard Emmons, M.D., Ph.D., Chief
Project No. 83-11-04
Page 2
November 29, 1983

The number assigned this project is 83-11-04. It would be helpful if in future correspondence concerning this protocol you would refer to this number. The expiration date for this approval is November 3, 1984. If the project is to continue beyond that date, a continuation protocol including a brief progress report, must be submitted for Committee review and approval at least one month in advance of the expiration date.

Any significant changes in the use of human subjects from that described in your approved protocol must be brought to the attention of the Committee.

Should you have any questions regarding human subjects requirements, please contact the committee at (916) 322-3212.

Sincerely,

Charles R. Gardipee, M.D.

Charles R. Gardipee, M.D.,
Chairperson
Committee for the Protection
of Human Subjects

cc: Members, Committee for the
Protection of Human Subjects

UNIVERSITY OF CALIFORNIA, DAVIS

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



SANTA BARBARA • SANTA CRUZ

Please reply to:

DEPARTMENT OF PATHOLOGY
SCHOOL OF MEDICINE
DAVIS, CALIFORNIA 95616
(916) 752-2710

DIVISION OF PATHOLOGY
UC DAVIS MEDICAL CENTER
2315 STOCKTON BLVD.
SACRAMENTO, CALIFORNIA 95817
(916) 453-2525

July 31, 1984

provisional OK 5 ES spec.

John S. Greenspan, Ph.D., FRCPATH
Chairman, Specimen Banking Committee
U.C. Systemwide Taskforce on AIDS
Room HSW 604
U.C.S.F.
San Francisco, CA 94143

Dear Dr. Greenspan:

We will be submitting a grant proposal to the University of California AIDS Taskforce to study the distribution of AIDS associated retroviruses in human and monkey tissues. Preliminary studies have shown the presence of proviral DNA SAIDS virus sequences in certain rhesus tissues at the California Regional Primate Center. We are proposing to study the distribution of AIDS associated retroviruses in humans with AIDS, through DNA southern blot analysis and in situ hybridization following the SAIDS examples.

We are seeking to obtain examples of Kaposi's Sarcoma in skin, lymph node and spleen. In addition we would like to study lymph nodes, spleen and salivary gland from patients with pre-AIDS and without Kaposi's Sarcoma. We currently have DNA probes to several AIDS associated retroviruses. Our laboratory has considerable experience in molecular biology and retroviruses under the direction of Marty Bryant, M.D., Ph.D. The curriculum vitae of our investigators are included for your review.

Having recently completed a survey of various tissues from monkeys with SAIDS using CMV viral probes with an in situ technique we are confident a similar procedure will work using AIDS virus probes on human material.

Assistance from the U.C. Tissue Bank is crucial to the success of this project and we greatly appreciate your consideration of this proposal.

Sincerely,

Steve Hinrichs, M.D.
Assistant Professor
Anatomic Pathology

SH/br

Enclosures

LX. Biographical Sketch

(give the following information for all professional personnel)

Name	Official Title
Hinrichs, Steven H.	Assistant Professor

Education (begin with the baccalaureate training and include postdoctoral)

Institution and location	Degree	Year	Scientific Field
University of North Dakota	B.S.	1976	
University of North Dakota	B.S.	1978	Medicine
University of North Dakota	M.D.	1980	Medicine
UC Davis	Intern	1980-81	Pathology
UC Davis	Resident	1981-84	Pathology

Honors

Special Alumni Award, 1977
 Outstanding Paper Presentation, the North Dakota Medical Assoc Award, May 1979
 National Fund for Medical Education, Medical Perspectives Fellowship, 1978-79
 Landgraf Research Award, 1983

Major Research Interest	Role in Proposed Project
Mechanisms of malignant transformation and role of oncogenes in embryogenesis	Principle Investigator

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

July 1984 - present; Assistant Professor; University of California, Davis, Medical Center; Department of Pathology

Publications Pertinent to Proposed Research

1. Gardner, M.B., W.F. Sullivan, and S.H. Hinrichs. Pathology of virus infection. In: Human Virology. Oxford University Press. 1983.
2. Hinrichs, S.H., M.A. Jaramillo, P.H. Gumerlock, M.B. Gardner, J.P. Lewis, and A.E. Freeman. Myxoid chondrosarcoma with a translocation involving chromosomes 9 and 22. Cancer Genetics and Cytogenetics. 1984.
3. Bryant, M., G. Heidecker, J. Lund, S. Hinrichs, D. Myerson and M. Gardner. Detection of Simian-Aids related CMV infection by in situ hybridization. 1984. (In Press).

IX. Biographical Sketch

(give the following information for all professional personnel)

Name	Official Title		
Bryant, Martin L.	Research Fellow		
Education (begin with the baccalaureate training and include postdoctoral)			
Institution and location	Degree	Year	Scientific Field
Duke University, Durham, NC	B.S.	1971	Chemistry
Calif State Univ, San Diego,CA	M.S.	1973	Chemistry & Biochemistry
Univ of So Calif, LA, CA	Ph.D.	1977	Experimental Pathology
Univ of So Calif, LA, CA	M.D.	1982	Medicine
Duke University, Durham, NC	Resident	1983	Pediatrics

Honors

Edmondson Fellowship, 1974-75; Veterans of Foreign Wars National Research Scholarship, 1975; NIH, NCI Training Grant, 1976-77; Student Fellowship in Oncology, 1980, 1981; Hematology-Oncology Clinical Clerkship, NIH, 1981; National Health Service Corps Scholarship, 1978-82.

Major Research Interest	Role in Proposed Project
Diagnostic Applications of Molecular Biology	Co-investigator

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

- 1971-73 Maintenance of normal and tumorous human and colon cells in vitro. Electron microscopic examination of mouse alveolar type II adenoma and human lung cells with emphasis on membrane integrity and resolution, University of CA, San Diego and California State University, San Diego.
- 1972-73 Staff Research Associate, Dept of Medicine, University of CA, San Diego, CA
- 1973-77 Biological and biochemical characterization of lymphomagenic and paralytogenic type C RNA tumor viruses: clonal isolation, antigenic characterization, molecular hybridization and protein analysis; in vivo pathogenesis and immunological prevention of disease, Pediatrics and Microbiology, Univ of Southern CA.
- 1975-76 Research Scholar, Department of Pathology, Univ of Southern CA.
- 1977-78 Molecular biology of endogenous primate retroviruses, NCI, NIH.
- 1977-78 Research Fellow, Meloy Laboratories, Springfield, VA.
- 1978-82 Immunopathology of lymphomas, Dept of Pathology, Univ of Southern CA.

Publications Pertinent to Proposed Research

1. Bryant, M.L., Stoner, G.D. and Metzger, R.P.: Protein-bound carbohydrate content of normal and tumorous human lung tissue. Biochem. Biophys. Acta 343:226234, 1974.
2. Bryant, M.L. and Kelment, V.: Clonal heterogeneity of wild mouse leukemia viruses; host range and antigenicity. Virology 73:532-536, 1976.
3. Elder, J.H., Jensen, F.C., Bryant, M.L. and Lerner, R.A.: Polymorphism of the major envelope glycoprotein (gp70) of murine C-type viruses: virion-associated and differentiation antigens encoded by a multigene family. Nature 267:23-28, 1977.

Biographical Sketch
Bryant, Martin L.

4. Gardner, M.B., Klement, V., Henderson, B.E., Casagrande, J., Bryant, M.L., Dougherty, M.F. and Estes, J.D.: Paralysis and oncornaviruses of wild mice. In Tumours of Early Life in Man and Animals L. Severi (ed.), Perugia University Press, Montelucente, Italy, pp. 343-356, 1978.
5. Bryant, M.L., Pal, B.K., Gardner, M.B., Elder, J.H., Jensen, F.C. and Lerner, R.A.: Structural analysis of the major envelope glycoprotein (gp70) of the amphotropic and ecotropic type C viruses of wild mice. Virology 84: 348-358, 1978.
6. Bryant, M.L., Roy-Burman, P., Gardner, M.B. and Pal, B.K.: Genetic Relationship of wild mouse amphotropic virus to murine ecotropic and xenotropic viruses. Virology 88:389-393, 1978.
7. Pal, B.K., Bryant, M.L., and Roy-Burman, P.: RNA tumor virus phosphoproteins: primary structural analysis and identification of phosphopeptides. J. Virol. 25:928-932, 1978.
8. Gardner, M.B., Klement, B., Rasheed, S., Estes, J.D., Rongey, R.W., Dougherty, M.F. and Bryant, M.L.: Persistent MuLV infection in wild mice. In Persistent Viruses, J.G. Stevens, G.J. Todaro and C.F. Fox (eds.), Academic Press, New York, pp. 115-131, 1978.
9. Bryant, M.L., Sherr, C.J., Sen, A. and Todaro, G.J.: Molecular diversity among five different endogenous primate retroviruses. J. Virol. 28:300-313, 1978.
10. Bryant, M.L., Nalewaik, R.P., Tibbs, V.C. and Todaro, G.J.: Comparison of two techniques for protein isolation and radioiodination by tryptic peptide mapping. Anal. Biochem. 96:84-89, 1979.
11. Todaro, J.G., DeLarco, J.E., Marquardt, H., Bryant, M.L., Sherwin, S.A. and Sliski, A.H.: Polypeptide growth factors produced by tumor cells and virus-transformed cells: a possible growth advantage for the producer cells. In Sato, G.H. and Ross, H. (Editors): Hormones and Cell Culture, Cold Spring Harbor Conferences on Cell Proliferation, New York, Cold Spring Harbor Lab. 1979, pp. 113-127.
12. Bryant, M.L., Scott, J., Pal, B.K. and Gardner, M.B.: Immunopathology of natural and experimental lymphomas induced by wild mouse leukemia virus. Am. J. Pathol. 104:272-278, 1981.

LX. Biographical Sketch

(give the following information for all professional personnel)

Name	Official Title
------	----------------

Osborn, Kent G.	Postgraduate Research Pathologist
-----------------	-----------------------------------

Education (begin with the baccalaureate training and include postdoctoral)

Institution and location	Degree	Year	Scientific Field
Univ of Calif, San Diego, CA	B.S.	1973	Zoology
Univ of Calif, Davis, CA	D.V.M.	1981	Veterinary Medicine
Univ of Calif, Davis, CA	Ph.D.	present	Comparative Pathology
(pending graduation 1985)			

Honors

ARCS Foundation, Inc., Scholarship Award, 1980
Jacob Traum Scholarship, 1984

Major Research Interest

Role in Proposed Project

Management of Infectious Diseases

Co-investigator

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

1981-present	Postgraduate research pathologist, Calif Primate Research Center and Veterinary Medical Teaching Hospital, Univ of Calif, Davis.
1978-81	Laboratory assistant, Respiratory Disease Morphology, Calif Primate Research Center, Univ of Calif, Davis.
1973-77	Special projects coordinator, senior laboratory technician and senior animal care specialist, Department of Animal Care, Sea World, Inc., 1720 South Shores Road, San Diego, CA 92109.
1973	Independent research project studying parasitism of two species of clams. University of Calif Bodega Marine Lab, Bodega, CA.
1972-73	Research assistant in Zoology. Department of Zoology, Univ of Calif, Davis.

Publications Pertinent to Proposed Research

1. Lerche, N.W., Osborn, K.G., Marx, P.A., Prahalada, S., Maul, D.H., Henrichson, R.V., Arthur, L.O., Gilden, R.V., Munn, R.J., Bryant, M.L., Heidecker-Fanning, G., Gardner, M.B.: An inapparent carrier of simian AIDS and evidence for natural transmission through saliva. (Submitted 1984).
2. Osborn, K.G., Prahalada, S., Lowenstine, L.J., Gardner, M.B., Maul, D.H., Henrickson, R.V.: The pathology of an epizootic of acquired immunodeficiency in rhesus macaques. Am J Pathol 1984, 114:94-103.

Biographical Sketch
Osborn, Kent G.

3. Gravell, M., London, W.T., Houff, S.A., Madden, D.L., Dalakas, M.C., Sever, J.L., Osborn, K.G., Maul, D.H., Henrickson, R.V., Marx, P.A., Lerche, N.W., Prahalada, S., Gardner, M.B.: Transmission of simian acquired immunodeficiency syndrome (SAIDS) with blood or filtered plasma. *Science* 1984, 223:74-76.
4. Marx, P.A., Maul, D.H., Osborn, K.G., Lerche, N.W., Moody, P., Lowenstine, L.J., Henrickson, R.V., Arthur, L.O., Gravell, M., London, W.T., Sever, J.L., Levy, J.A., Munn, R.J., Gardner, M.B.: Simian AIDS: Isolation of a type D retrovirus and disease transmission. *Science* 1984, 223:1083-1086.
5. Henrickson, R.V., Maul, D.H., Lerche, N.W., Gardner, M.B., Sever, J.L., Madden, D.L., Osborn, K.G., Lowenstine, L.J., Prahalada, S.: Clinical features of acquired immunodeficiency in rhesus monkeys. *Lab Anim Sci* 1984, 34:140-145.
6. Meyer, P.R., Ormerod, L.D. Osborn, K.G., Henrickson, R.V., Modlin, R., Lowenstine, L.J., Gardner, M.B., Smith, R.E., Taylor, C. R.: The evolution of lymph node based acquired immune deficiency syndrome (AIDS) in monkey and man: an evaluation using monoclonal antibodies in frozen section. (Submitted 1984).
7. London, W.T., Sever, J.L., Madden, D.L., Henrickson, R.V., Gravell, M., Maul, D.H., Dalakas, M.C., Osborn, K.G., Houff, S. A., Gardner, M.B.: Experimental transmission of simian acquired immunodeficiency syndrome (SAIDS) and Kaposi's-like skin lesions. *Lancet* 1983, Oct 15:869-873.
8. Henrickson, R.V., Maul, D.H., Osborn, K.G., Sever, J.L., Madden, D.L., Ellingsworth, L. R., Anderson, J.H., Lowenstine, L.J., Gardner, M.B.: Epidemic of acquired immunodeficiency in rhesus monkeys. *Lancet* 1983, Feb 19:388-390.
9. Lynn-Johnson, M.H., Osborn, K.G., Gillespie, J.R., Schwartz, L.W.: A comparison of lung volumes in rats before and after fixation for morphologic studies. *Am Rev Res Dis* 1980, 121(4):374 (abstract).
10. Cornell, L.H., Osborn, K.G., Antrim, J.E., Simpson, J.G.: Coccidioidomycosis in a California sea otter (Enhydra lutris). *J. Wildl Dis* 1979, 15(3):373-378.
11. Cornell, L.H., Asper, E.D., Osborn, K.G., White, M.H.: Investigations on Cryogenic Marking Procedures for Marine Mammals. US Department of Commerce National Technical Information Service 1979, Feb:24 pp.
12. Cornell, L.H., Asper, E.D., Antrim, J.E., Osborn, K.G., Gurevich, V.S.: Experiences of Sea World from 1963 to present with Tursiops species reproduction and some plans for the future. In: *Breeding Dolphins: Present Status, Suggestions for the Future*. US Department of Commerce National Technical Information Service 1977, Nov:66-70.
13. Cornell, L.H., Golden, B.J., Osborn, K.G.: Pseudopersistent urachus in a baby walrus. *J Am Vet Med Ass* 1975, 167(7):548-549.

X. Summary of Research Proposal for the AIDS Research Program

UNIVERSITYWIDE TASK FORCE ON AIDS: 1984-1985 Grant Awards

Steven H. Hinrichs, Assistant Professor of Pathology

Name and Title of Principal Investigator

Pathology, University of Calif, Davis, Medical Center

Department and Campus

Tissue and Cell Distribution of Retroviruses Associated
with Acquired Immunodeficiency of Humans and Non-Human Primates.

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.

Recent seroepidemiologic and virus isolation studies reported by the National Institutes of Health, the Pasteur Institute and others have specifically implicated human T-lymphotropic retroviruses in the pathogenesis of AIDS. Similarly, a viral etiology has been established for the Simian acquired immunodeficiency syndrome (SAIDS) occurring in rhesus monkeys at the California Primate Research Center (CPRC). In addition to selective abnormalities in immune regulation, a wide variety of unusual lesions has been discovered in both AIDS and SAIDS. In humans, Kaposi's sarcoma, lymphoma, lymphadenopathy and oral leukoplakia have been reported. In non-human primates lymphoma, fibrosarcoma, Kaposi's sarcoma, and retroperitoneal fibrosis have been described. It remains to be demonstrated whether these various disease entities are a result of the compromised immune system or a consequence of infection by retroviruses associated with AIDS and SAIDS. Considerable insight into the pathogenesis of AIDS and its related neoplasms would come from the demonstration of integrated retrovirus proviral DNA in the affected tissues, including lymphoma and Kaposi's sarcoma. We propose to search for the presence of AIDS associated retroviruses in diseased and neoplastic tissues from subjects with AIDS and SAIDS by Southern blot analysis using virus specific gene probes. Then, we will determine the type of cell infected, whether endothelium, lymphocyte, or fibroblast using in situ hybridization. Our experimental approach should uncover the natural cellular reservoir of the AIDS and SAIDS retroviruses associated with immune deficiency in humans and monkeys.

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.

8/22/88

Memo from
JOHN S. GREENSPAN

To:

Paul Volberding ✓

Marcus Conant

From: John Greenspan

Re: Stites Request

Lets put this on
the agenda for the
Tissue Committee

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

JK

RECEIVED	
AUG 6 1984	
ORAL BIOLOGY & PERIODONTOLOGY	
AM ☐	PM ☐

August 3, 1984

To: Dr. John Greenspan
Professor and Chairman
Oral Biology

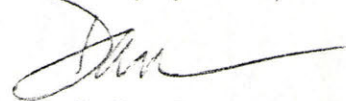
Chairman,
AIDS Serum and Tissue Bank
HSW 605

Dear John:

I would like to request 8-10 sera from AIDS patients that are likely to be anti-HTLV-3+. The purpose of this request is to provide these sera to Dr. Noel Warner who is Scientific Director of Becton Dickinson Monoclonal Center and incidentally Adjunct Professor of Laboratory Medicine at UCSF. Noel and his colleagues are developing an ELISA assay for antibodies to HTLV-3 and would like to check some of their current findings with some allegedly positive sera. We need approximately 0.5 ml from each specimen. I would appreciate it if you could institute the emergency request procedures you discussed with me today in order to obtain the sera. There seems to be some urgency in this matter.

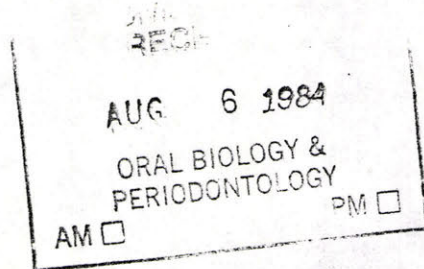
Thank you.

Sincerely yours,



Daniel P. Stites, M.D.
Professor of Laboratory Medicine
and Medicine
Director, Immunology Laboratory

DPS:ca



August 3, 1984

To: John Greenspan, B.D.D.S., Ph.D.
Professor of Oral Medicine
Director,
UCSF AIDS Tissue and Serum Bank
HSW 604

From: Daniel P. Stites, M.D. 
Professor of Laboratory Medicine
and Medicine
Director, Immunology Laboratory
M-523

Re: AIDS Virus Clinical Immunology Testing

Our laboratory has been approached independently by two companies, Genetic Systems of Seattle, Washington and Becton Dickinson Monoclonal Antibody Center at Mountain View, California to become involved in large scale clinical trials of assays for HTLV-3 and LAV. I am enclosing copies of letters from the two companies briefly stating their positions. Our laboratory is extremely interested in participating with both of these companies in an attempt to perfect an assay which could be used at bench for clinical detection of antibody to the viruses or virus. An additional benefit of this study will allow direct comparison between the LAV antigen provided by Institute Pasteur and the Gallo or HTLV-3 antibody provided through the NIH contracting mechanism for Becton Dickinson. Obviously, details of support and other arrangements would have to be worked out but before we can proceed we need approval from the Tissue Bank to proceed. We very much solicit your input and other members of the Committee in helping us develop a proposal which would be mutually beneficial to all of the members of the AIDS research groups here at UCSF. I would appreciate your dealing with this at the earliest possible date for a meeting of the Committee.

DPS:ca
Enc.

Becton Dickinson Monoclonal Center, Inc.
2375 Garcia Avenue
Mountain View, California 94043
Telephone: (415) 968-7744

**BECTON
DICKINSON**

August 1, 1984

Daniel P. Stites, M. D.
Department of Laboratory Medicine
Clinical Immunology
HSE593
University of California, San Francisco
San Francisco, California 94143

Dear Dr. Stites:

We are currently exploring anti-HTLV-3 antibody testing, and we understand you have samples from patients who might have been exposed to this virus. We would be very interested in your evaluation of our antibody test on samples from such patients. Could you find out what arrangements are necessary, and let us know if you would be able to perform an evaluation?

Thank you for your consideration in this matter.

Sincerely,



Christopher Snow, Ph.D.
Manager, External R&D Projects

CS:jf



Genetic Systems

July 24, 1984

Daniel Stites, M.D.
M523-Immunology
Department of Laboratory Medicine
University of California San Francisco
San Francisco, California 94143

Dear Dr. Stites:

We are interested in participating in a clinical study with your group of investigators to evaluate the use of our diagnostic test for the AIDS virus.

Genetic Systems Corporation has formed a collaboration with the scientists at the Pasteur Institute in Paris to develop a serology test for lymphadenopathy-associated virus (LAV) which is a T-lymphotrophic retrovirus isolated from a homosexual man with lymphadenopathy. This virus has been linked with AIDS since: (1) the virus has been isolated from patients with AIDS and lymphadenopathy syndrome, (2) antibodies to the virus have been detected in patients with AIDS and lymphadenopathy, and (3) the virus has been isolated from a blood donor-recipient pair of AIDS patients. The virus is very similar to HTLV III, another T-lymphotrophic virus isolated under similar circumstances.

We will be developing an automated immunoassay to detect antibody to whole disrupted LAV in blood specimens. The format of the assay will be oriented to screening large numbers of samples.

The clinical studies would involve two different populations. The first would be a prevalence study on blood samples from the blood bank. Approximately 10,000 donor specimens would be screened for antibody to the virus. A limited study on approximately 1,000 specimens would be performed to compare our assay to confirmatory assays such as immunoprecipitation or infectivity. The confirmatory tests could either be performed in San Francisco or in Seattle. The second study would investigate a high risk population such as that seen at a

Daniel Stites, M.D.

July 24, 1984

Page Two

sexually-transmitted disease or gay clinic. This study would test 500 specimens from patients with lymphodenopathy, AIDS, and matched controls to screen for antibody and perform confirmatory tests. We would perform all of the data analysis needed for these studies at Genetic Systems Corporation.

I hope that you are interested in collaborating with us on these clinical trials and am looking forward to hearing from you.

Sincerely,



Lynn Goldstein, M.D.
Director, Microbiology

LG/hc
1965H

Becton Dickinson Monoclonal Center, Inc.
2375 Garcia Avenue
Mountain View, California 94043
Telephone: (415) 968-7744

**BECTON
DICKINSON**

August 3, 1984

Dr. Daniel P. Stites
Department of Laboratory Medicine
Clinical Immunology
HSE593
University of California, San Francisco
Third and Parnassus Avenues
San Francisco, California 94143

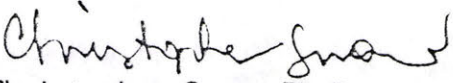
Dear Dr. Stites:

We are in the process of developing a test to measure anti-HTLV antibody in serum samples, and we understand that you have specimens from patients who might have been exposed to this virus. We would be very interested in your evaluation of our nonisotopic, serum based anti-HTLV-3 antibody assay, and are therefore writing to explore the possibility of providing materials for testing by University of California under Becton Dickinson's IND.

Could you find out what arrangements are necessary and let us know if you would be willing to perform such an evaluation?

Thank you for your consideration in this matter.

Sincerely,


Christopher Snow, Ph.D.
Manager, External R&D Projects

CS:jf

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

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SANTA BARBARA • SANTA CRUZ

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

SAN FRANCISCO, CALIFORNIA 94143

July 31, 1984

To: Melvin B. Heyman, M.D.
Assistant Professor of Pediatrics
M 650

From: John S. Greenspan, Ph.D., F.R.C.Path.
Director, UCSF AIDS Specimen Bank

Re: Serum Antibodies from AIDS Patients

Dear Dr. Heyman:

Thank you for your letter of July 27, 1984, regarding the Specimen Bank last week, we requested. The decision to form a subcommittee of the UCSF AIDS Specimen Bank, with Volberding, Chairman; Greenspan, Secretary; and Andrew Moss who will be the liaison to the Specimen Bank.

There are several items that we will ask you to adhere to in the draft of this agreement.

We wish you success in your efforts and assistance to you.

Yours sincerely,


John S. Greenspan
Director UCSF AIDS Specimen Bank

JSG/cf

cc: Arthur Ammann, M.D.
Paul Volberding, M.D.
Marcus Conant, M.D.
Andrew Moss, Ph.D.

File in test tissue committee shelf.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

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SANTA BARBARA • SANTA CRUZ

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

SAN FRANCISCO, CALIFORNIA 94143

July 31, 1984

To: Melvin B. Heyman, M.D.
Assistant Professor of Pediatrics
M 650

From: John S. Greenspan, Ph.D., F.R.C.Path.
Director, UCSF AIDS Specimen Bank

Re: Serum Antibodies from AIDS Patients

Dear Dr. Heyman:

Thank you for your letter of July 12, 1984. As we discussed when you came to visit the Specimen Bank last week, we are happy to provide you with the material you have requested. The decision to make these specimens available to you was made by a subcommittee of the UCSF Tissue Committee. That subcommittee consisted of Paul Volberding, Chairman; myself, Director of the Specimen Bank; and Marc Conant and Andrew Moss who will be providing most of the specimens which we are giving you.

There are some conditions attached to the use of these specimens which we will ask you to adhere to, and indeed you will be asked to sign a formal agreement. A draft of this agreement will be sent to you by Dr. Paul Volberding shortly.

We wish you success in your important research and hope that we continue to be of assistance to you.

Yours sincerely,

A handwritten signature in dark ink, appearing to read "John S. Greenspan", is written over a horizontal line.

John S. Greenspan
Director UCSF AIDS Specimen Bank

JSG/cf

cc: Arthur Ammann, M.D.
Paul Volberding, M.D. ✓
Marcus Conant, M.D.
Andrew Moss, Ph.D.

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

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SANTA BARBARA • SANTA CRUZ

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

SAN FRANCISCO, CALIFORNIA 94143

July 31, 1984

Marcus A. Conant, M.D.
350 Parnassus Avenue, Suite 808,
San Francisco, Ca. 94117

Dear Marc:

Thank you for reminding me about the general applicability of the new UCSF AIDS Specimen Bank Rules dated 7-1-1984. I will indeed bring these to the attention to all members of the UC Systemwide Task Force on AIDS Specimen Banking Committee at our next meeting. If you have no objections, I may send a copy down to Mike Gottlieb before our next meeting so that he can know what is going on. He runs the only other significant Specimen Bank in the UC system.

Yours sincerely,

A handwritten signature in dark ink, appearing to read "John", is written over a horizontal line.

John S. Greenspan
B.Sc., B.D.S., Ph.D., F.R.C.Path.
Professor of Oral Biology and Pathology
Director UCSF AIDS Specimen Bank

JSG/cf

cc: Merle Sande, M.D.
Paul Volberding, M.D. ✓

Marcus Augustine Conant M.D.

A PROFESSIONAL CORPORATION
DERMATOLOGIST
August 13, 1984
(415) 661-2613

350 Parnassus Avenue, Suite 808,
San Francisco 94117

TO: Paul Volberding
Chairman, Tissue Committee

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

Mac

RE: Study of Dr. Dennis J. Haubrich

Dennis Haubrich is a professor of social work at the Ryerson Polytechnical Institute in Toronto, Canada. He visited our Kaposi's Sarcoma Clinic last spring and met with Lydia Tomasoc, Tom Coates, and Jeff Mendell. During that time he formulated the proposal which is attached and he is asking for access to some of our Kaposi's Sarcoma patients to conduct his study. I have check with Lydia, Tom, and Jeff and his work in no way interfere with the psychological assessment which they have already initiated.

I think that the work will be valuable and has the added advantage of showing that the AIDS Clinical Research Center is cooperating fully with investigators from other institutions and other countries.

If you feel that the committee will agree to Dr. Haubrich's proposal please have your secretary call me and I will indicate to him that he can plan to conduct the study next fall.

P.S. This is the proposal I spoke about
last week. Would you please
send him the necessary
group

Bobbi

Susan - P.S. Send
the guy in Toronto
our Tissue Committee
approved form.

W

Marcus Augustine Conant M.D.

A PROFESSIONAL CORPORATION
DERMATOLOGIST
August 13, 1984
(415) 661-2613

350 Parnassus Avenue, Suite 808,
San Francisco 94117

TO: Paul Volberding
Chairman, Tissue Committee

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

Mac

RE: Study of Dr. Dennis J. Haubrich

Dennis Haubrich is a professor of social work at the Ryerson Polytechnical Institute in Toronto, Canada. He visited our Kaposi's Sarcoma Clinic last spring and met with Lydia Tomasoc, Tom Coates, and Jeff Mendell. During that time he formulated the proposal which is attached and he is asking for access to some of our Kaposi's Sarcoma patients to conduct his study. I have checked with Lydia, Tom, and Jeff and his work in no way interfere with the psychological assessment which they have already initiated.

I think that the work will be valuable and has the added advantage of showing that the AIDS Clinical Research Center is cooperating fully with investigators from other institutions and other countries.

If you feel that the committee will agree to Dr. Haubrich's proposal please have your secretary call me and I will indicate to him that he can plan to conduct the study next fall.

P.S. This is the proposal I spoke about
last week. Would you please have Bobbi
send him the necessary agreement to our
group

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San Francisco, CA 94114
(415) 621-1408

June 29, 1984

Dr. Marcus A. Conant
350 Parnassus Avenue
Suite 808
San Francisco, CA 94117

Dear Dr. Conant:

I am writing in response to your letter of May 14 to address the questions raised by the Principal Investigators Group pertaining to my rationale for selecting the AIDS population and elaborate on my research methodology.

Rationale

The aim of the study is to identify the sequence of psychosocial issues and resulting adaptive tasks posed by the illness for the patient and his personal social network (e.g., friends, lover, family) for the purpose of social work intervention. As a conceptual framework for social work practice, the study will be concerned with the specific application of person-environment transactional theory as a means of achieving this aim. Use of this theoretical model in other studies of life-threatening illnesses has identified the concrete nature of the disease as the single most important factor that determines the adjustments an individual must make to an illness (Mages and Mendelsohn, 1979; Weisman and Worden, 1977). Also, when a person is afflicted with a chronic, fatal disease, the occurrence of psychosocial problems, in the broadest sense, must be anticipated as a part of the natural history of the disease (Koenig, 1968). Because AIDS is a relatively new phenomenon that affects potentially vulnerable minority populations, it is assumed that the psychosocial impact of the disease is both profound and widely ramified. As such, in order to understand the psychosocial impact of AIDS, what is needed is a clear and detailed picture of the specific realities that AIDS patients face, and how these realities are perceived and dealt with by the AIDS patient and those within his social network. While AIDS patients present variations in symptomatology, personality, age, social status, and systems of support, it is hypothesized that, to some extent, all AIDS patients must come to grips with a common set of circumstances and psychosocial issues during the course of their illness. In other words, the AIDS patient is faced with a unique set of person-environment transactions

(psychosocial issues and adaptive tasks) that merit identification through research and intervention by the helping professions.

My interest in the psychosocial effects of AIDS stems from my professional experience as a social work practitioner and educator in the health care field. From a social work viewpoint, the general goal of the health care system is to minimize the negative psychosocial impact of a disease, and to maximize the patient's self-determination, self-esteem, and systems of support. However, any mode of intervention must first of all take into account the specific nature of the disease, the personal, social, and cultural characteristics of those affected, and the social supports available to them in their everyday environment. It is this contribution that I wish to make to the quality of life of AIDS patients.

Methodology

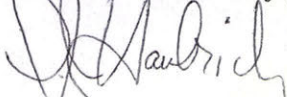
The proposed study views the patient's response to AIDS as encompassing a series of events or psychosocial transitions in an individual life history whose course is substantially altered by the realities of the disease, and that, at any one time, the individual must adapt to and master the personal and environmental stresses imposed by the disease. To understand and predict these alterations, it is first of all necessary to define the sequence of issues raised by the disease and the common modes of handling them. Second, it is necessary to identify the situational factors (the particularities of the disease, individual differences, and interpersonal situations) that affect an individual's adaptation to AIDS. Third, it is necessary to get some sense of what the illness and its consequences mean to individual patients. In order to comprehend both the general and the unique in patient's reactions to a life-threatening illness, the most suited method of study is the intensive analysis of a series of individual cases with a subsequent search for commonalities among them. Therefore, it is the individual patient within the context of his impinging social environment which constitutes the basic unit of analysis, not a set of variables or specific behaviors. (See Mages and Mendelsohn [1979] for a full description of this approach.)

The data will be collected by means of intensive, semi-structured interviews, employing both fixed-choice and open-ended questions, on a sample of approximately 15 patients. Given a retrospective sample, i.e., those who have been diagnosed and treated for the disease for an extended period of time, the interview schedule would consist of approximately three to four sessions with the patient and at least one interview with the patient's collateral (friend, lover, or family member). The patient interviews will focus on AIDS-related events, intra- and inter-personal coping mechanisms, and personal history. The collateral interview will focus on the collateral's perceptions of the patient's illness, their psychosocial problems and needs, and the

role of the collateral as a potential helping agent. I am currently developing the interview protocols based upon existing studies. In addition, I am also planning to incorporate an instrument that measures person-environment fit to be adapted from the work of Coulton (1979) and Wetzel (1978). This instrument is designed to assess psychosocial problems and needs resulting from illness, and to inform strategies for intervention and program planning.

Given the current status of my preliminary work, I would not anticipate beginning to collect data until late September. Should you have any further questions or points of clarification, please do not hesitate to contact me. In closing, I would like to thank you for considering my research proposal. I hope that I can look forward to undertaking this work at the University of California.

Yours sincerely,



Dennis J. Haubrich,
Professor,
School of Social Work
Ryerson Polytechnical Institute (Toronto)

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References

- Coulton, C. J. (1979) "Developing an Instrument to Measure Person-Environment Fit," Journal of Social Service Research, 3 (Winter), 159-73.
- _____ (1979) "A Study of Person-Environment Fit Among the Chronically Ill," Social Work in Health Care, 5 (1), 5-17.
- Koenig, R. R. (1968) "Fatal Illness: A Survey of Social Services Needs," Social Work, 13 (4), 85-90.
- Mages, N. L. and Mendelsohn, G. A. (1979) "Effects of Cancer on Patient's Lives: A Personalogical Approach," G. C. Stone, F. Cohen, N. E. Adler and Associates, Health Psychology - A Handbook, San Francisco: Jossey-Bass, 255-84.
- Weisman, A. D., and Worden, J. W. (1977) "Coping and Vulnerability in Cancer patients," Unpublished research report, Project Omega, Department of Psychiatry, Harvard Medical School, Massachusetts General Hospital.
- Wetzel, J. (1978) "Depression and Dependence Upon Unsustaining Environments," Clinical Social Work Journal, 6 (2), 75-89.

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HOSPITALS AND CLINICS

SAN FRANCISCO, CALIFORNIA 94143

AIDS Clinical Research Center

August 27, 1984

To: Dr. Paul Volberding

From: Dr. Marcus A. Conant *MC*

Re: Request for Sera for Antibody Tests to HTLV III

As you know John Greenspan has received from Dan Stites a number of requests from drug companies for sera to use to test for antibodies to HTLV III.

As we decided at last tissue meeting on August 15th, we would furnish coded specimens to any investigator who requested them from us at a standard charge. We would furnish the same specimens to UC investigators at no charge. We all agreed that a contract was necessary to be certain that the companies did not use the sera for purposes other than those approved by the Tissue Committee. I have spoken with Frank Jacobson and he tells me that this contract must be developed by Mr. Joe Cowan, the legal counsel for the university. I would suggest that you, as chairman of the Tissue Committee, jot down a rough draft of the contract we want to develop and send it to Mr. Cowan for his input.

cc Dr. John Ziegler, VAMC 141
Dr. John Greenspan, HSW 604
Dr. Dan Stites, M-523
Mr. Joe Cowan, 145 Irving St.

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SAN FRANCISCO, CALIFORNIA 94143

October 22, 1984

Steve Hinrichs, M.D.
Assistant Professor
University of California, Davis
Department of Pathology
School of Medicine
Davis, California 95616

Dear Dr. Hinrichs,

Your request (July 31, 1984) for human KS skin, nodes and spleen, "pre-AIDS" nodes, spleen and salivary gland was reviewed by the UCSf AIDS Tissue Committee on 10/3/84.

It was decided to provide material for your project as follows:

1. We can send you up to five frozen biopsies of skin KS with adjoining non-lesional skin from the same individuals. We can provide large numbers of paraffin sections of KS for your in situ technique if you can use such material.
2. We also agreed to provide a small number of lymph nodes with KS. We may not be able to supply other types of lymph node because of the small number available in the bank.
3. We can provide one or two specimens of KS spleen but again, provision of other types of spleen specimens will depend on the demands made by other studies.
4. Our specimens of salivary glands are fully committed at present, but we will endeavor to obtain further samples for you.

We hope that this material will enable you to get the project moving if you are funded.

JSG/Hinrichs
October 22, 1984
Page 2

Some conditions:

1. Please do not use the material for projects other than those defined in your letter of 7/31/84 and the accompanying proposal.
2. Please do not pass the material on to any other investigator.
3. You will of course want to include the names of the providers of the specimens, as is deemed appropriated by negotiation, on abstracts and publications.
4. Similarly, please acknowledge and identify the UCSF AIDS Specimen Bank in publications.
5. Currently, you will incur no costs for the specimens but we may ask you to contribute later towards the expenses of transportation to Davis.

Please contact me to discuss arrangements.

Good luck with your very important project.

Sincerely,



John S. Greenspan, B.Sc., B.D.S., Ph.D., F.R.C.Path.
Director, UCSF AIDS Specimen Bank

JSG/cf

cc: ☒ Paul Volberding M.D.
Marcus Conant M.D.

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SAN FRANCISCO, CALIFORNIA 94143

December 11, 1984

Donald H. Gilden, M.D.
Department of Neurology
Hospital of the University of Pennsylvania
3400 Spruce Street
Pennsylvania, PA 19104

Dear Dr. Gilden:

I am happy to tell you that the UCSF AIDS Tissue Committee has approved your project "VZV in AIDS." We will be sending frozen KS and non-lesion control skin and other AIDS tissue within 2 weeks. We will also send you some hairy leukoplakia specimens and non-lesion biopsies from the same HL patients.

Please confine use of these specimens to the VZV project and please do not pass any material on. We know that you will wish to include those who provide these specimens in publications and abstracts.

We appreciate the opportunity to collaborate with you.

Sincerely,


John S. Greenspan

JSG/lm

cc: M. Conant, MD
P. Volberding, MD
D. Greenspan, BDS

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SAN FRANCISCO, CALIFORNIA 94143

February 15, 1985

Kathelyn S. Steimer, Ph.D.
Chiron Corporation
4560 Horton Street
Emeryville, CA 94608

Dear Dr. Steimer,

I write in response to your letter of December 31, 1984 to Dr. Conant and in confirmation of our telephone conversation of 2/14/85.

Your proposal has been reviewed according to the expedited procedure of the UCSF AIDS Tissue Committee. Approval has been granted subject to the conditions listed below. Other conditions may be added, by mutual agreement, during your discussions with Dr. Conant.

The conditions:

1. The financial aspects are to be negotiated between your company and Dr. M. Conant.
2. The specimens are to be used only for the purposes of the study outlined in your proposal, with minor technical modifications to be permitted where appropriate.
3. Please do not pass the serum on to other investigators.
4. You will of course receive coded specimens and we will break the code together when your data is ready for analysis.
5. I know that you will wish to include the names of those clinicians who have cared for the patients and have provided the specimens in abstracts and publications. This is best negotiated well ahead and I would be happy to advise you. The question of my own role in abstracts and publications will depend on the extent to which I become involved in the work and is best left open at this time.

I will arrange for the aliquots to be prepared in the next few days and will release them to you on the completion of your negotiations with Dr. Conant.

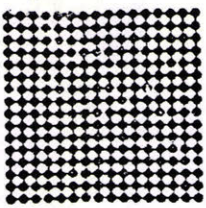
The members of the UCSF AIDS investigations group welcome this opportunity to assist in this important study.

Sincerely,

John S. Greenspan
BSc., BDS, PhD., FRCPATH.
Director, UCSF AIDS
Specimen Bank

JSG/cf

cc: Dr. M.A. Conant, Dr. J.A. Levy
Dr. P.A. Volberding, ✓ Dr. J.L. Zeigler
Ms. Y. de Souza



4560 HORTON STREET
EMERYVILLE CA 94608
(415) 655-8730

CHIRON
CORPORATION

December 31, 1984

Marcus Conant, M.D.
Kaposi Sarcoma Clinic
400 Parnassus Room A-310
San Francisco, California 94143

Dear Dr. Conant,

Enclosed is a brief proposal for the first phase of the studies that we discussed during our meeting on December 14. We are requesting sera from your serum bank to allow us to test our recombinant-based immunoassays for AIDS virus antibodies. Naturally, Chiron expects to make a donation to offset the costs in providing these materials.

Having used these serum samples to refine and validate our assays, we expect in the near future to begin an extensive epidemiological study of the antibody response to AIDS virus exposure. We should very much like to discuss with you the design of this study. I will be contacting you after the first of the year to arrange to meet with you at your convenience to discuss our request and future collaborations.

Sincerely,

Kathy Steimer

Kathelyn S. Steimer, Ph.D.
Senior Scientist

Hospitals and Clinics
San Francisco, California 94143

University of California, San Francisco . . . A Health Sciences Campus
AIDS Clinical Research Center

UCSF

March 5, 1985

Dr. Lacy R. Overby, Ph.D.
Vice-President
Chiron Corporation
4560 Horton St.
Emeryville, CA 94608

Dear Dr. Overby:

The Tissue Committee of the AIDS Clinical Research Center at UCSF has favorably reviewed your request for specimens currently in our tissue bank to test your new subunit antigen. I have spoken to Dr. John Greenspan, the Chief of the AIDS Tissue Bank, and he will release the requested specimens immediately so that your research will not be delayed. We are pleased to learn that Chiron can make a contribution of \$20,000 to the AIDS Clinical Research Center for these specimens, and we will use these funds to continue our efforts to collect specimens from individuals exposed to the AIDS retrovirus to assist research similar to your important project.

We are delighted with the excellent working relationship that you have developed with our colleague, Dr. Jay Levy, and we trust that work such as yours will soon identify a neutralizing antibody which will eventually lead to an effective vaccine against this wretched new disease.

Sincerely yours,

Marcus A. Conant, M.D.

MAC/ds

cc: Dr. Paul Volberding, UCSF, SFGH, 5H22
cc: Dr. John Ziegler, UCSF, VAMC 141
cc: Dr. John Greenspan, UCSF, HSW 604
cc: Dr. Jay Levy, UCSF, M 1282
cc: Mr. Dick Pabich, 4963 17th St., SF CA 94117

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March 14, 1985

To: ~~Paul~~ Volberding, MD - Chairman of the Tissue Committee
Marcus Conant, MD - Director AIDS Clinical Research Center

From: John S. Greenspan - Director, AIDS Specimen Bank. 

RE: Proposal from Robert E. Smith, MD, Ph. D

Robert Smith is the incoming President of the Histochemical Society and is a faculty member of the University located at the Lawrence Livermore Laboratory. He is also a consultant to Enzymes Systems Produces. He proposes a simple and quite exciting investigation which would require white cells from five people with AIDS, five disease free gay controls, and five sex partners of AIDS patients. Blood would be drawn in batches and brought over here where the white cells would be prepared in the AIDS specimen bank facility (we are doing this now). The enzyme test would be performed in the facility (which is, of course, aP2* bio-hazard facility) as a collaborative project with us being a co-investigator with Dr. Smith. We felt it appropriate to put the proposal to you as coming from him to avoid any appearance of conflict of interest on my part. Dr. Smith, and his company, would be prepared to negotiate an appropriate sum of money for the 15 specimens and also for the use of our facility and our staff. I believe that the 15 individuals could be recruited from a combination of Paul's SFGH clinics, and Marcus's CRC clinics and practice. If I am wrong in this please let me know because we would then involve Andrew Moss.

When you have had a chance to read this letter and the proposal please telephone me at 666-2220 to let me know of your decision to approve, to disapprove, or if we need to discuss the proposal further.

If we all agree to do the study I would be happy to negotiate the financial aspects or to arrange for all three of us to meet with Dr. Smith. He is, by the way, a highly respected scientist.

Attachments

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SAN FRANCISCO, CALIFORNIA 94143

March 14, 1985

To: Dr. Robert E. Smith

From: John S. Greenspan

RE:

Dear Robert,

We have received your proposal and I have circulated it and a recommendation for approval to the executive group of the UCSF AIDS Tissue Committee. You should hear from me within a week.

With kind personal regards,

Sincerely,

A handwritten signature in black ink, appearing to read "John", with a stylized flourish extending from the end.

John S. Greenspan

cc Yvonne de Suza
Mark Conant
Paul Volberding



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February 27, 1985

Dr. John S. Greenspan
Professor and Chairman
Dept. of Stomatology
Univ. of CA
San Francisco, CA 94143

Dear John,

Please find enclosed an outline for the research project entitled "Identification of the Proteinase DPP-IV in T-helper cells from Human Subjects believed to be Predisposed to AIDS". I look forward to hearing from you once you have reviewed this proposal and hope that it will be enacted with expediency.

Sincerely yours,

Robert E. Smith, M.D., Ph.D

RES/jk
Enclosure

It only takes a little ESP in your laboratory for qualitative biochemistry.

Identification of the Proteinase DPP-IV in T-helper cells
from Human Subjects believed to be predisposed to AIDS

The proteinase DPP-IV was discovered by Hopsu-Have and Glenner in 1966 in rat liver and subsequently isolated by Hopsu-Have and Sarimo from liver and pig kidney in 1967. McDonald and Smith observed the enzyme histochemically in 1968 using the synthetic substrate Gly-pro-4-Methoxy-beta-Naphthamide. In 1971 McDonald et.al. demonstrated the kidney localization of this enzyme to be lysosomal using electron microscopic histochemistry. The observations were further substantiated by Smith and Van Frank in 1975 in a histochemical study of the dipeptidyl aminopeptidases. Oya et.al. found the enzyme in the serum of various animals and humans but not until the studies of Lodja published in 1977 that the enzyme was identified to be in lymphocytes. In further studies published by Lodja in 1980 in the Journal of Histochemistry and Cytochemistry it was established that the enzyme was limited to T-lymphocytes and not found in beta-lymphocytes. Subsequent studies using the availability of monoclonal surface antibodies OK T-3, OK T-4, OK T-8 established that the enzyme was limited to the OK T-4 monoclonal antibody staining cells. The T-helper cell specificity of the enzyme was first described by Feller in 1982 and has been established by several different laboratories since that time. The identification of the active enzyme with the synthetic substrate Gly-pro-MNA has been the only substrate used until our most recent work. Feller recently, at the protease meetings in London on July 13, 1984, revealed the first use of a monoclonal antibody to this enzyme which correlated completely with the histochemical synthetic substrate identification of its activity.

At Enzyme Systems Products we have developed a new substrate for identifying the activity of this enzyme in T-helper cells. The substrate Lys-pro-AFC was synthesized in order to take advantage of the unique properties of the AFC

leaving group in either for the assay of the enzyme assay or for the identification of its isomers (isozymes). Enzyme assay studies reveal a shifting of blue fluorescence of substrate to the green fluorescence of the liberated product (1). Using a centrifugal fast analyzer, the Multistat III by Instrumentation Laboratories, we have studied over 100 serum and plasma samples from normal individuals as well as individuals with breast and colon cancer for the activity of DPP-IV. We have found that the enzyme is higher in serum than in plasma suggesting a release of the enzyme by clot forming mechanisms from white cells. We recently have studied the isoenzyme patterns of lymphocytes isolated by Ficol hypaque gradient and using the enzyme overlay technique recently published by myself in the December issue of the Journal of Histochemistry and Cytochemistry (2). The study of lymphocyte preparations has established certain parameters: First that there are four to five distinct isoenzymes which prior to electrophoresis but after digestion with neuramididase reduces the number of bands to three. The patterns of isoenzymes are no different between age matched males and females and there is apparently no difference in age groupings from 15 to 50 years of age for individuals who have no established pathology.

Present data establishes that T-helper cells are deficient in individuals with AIDS and Karposi's sarcoma; in lymph nodes as well as in circulating blood. It is our desire to acquire lymphocyte preparations from the following groups and to compare the isoenzyme patterns with that of presumed nondiseased homosexual individuals.

- 1) White cell lymphocyte preparation from five individuals with AIDS.
- 2) Lymphocyte preparations from five known homosexual individuals without AIDS.
- 3) Five samples from sex partners without AIDS in a homosexual situation.

From these three groups of five samples each we would also like to obtain plasma samples and serum samples. Plasma and serum samples would be assayed for total enzyme activity whereas the lymphocyte preparations would be used in isoelectric focusing an identification of isoenzyme patterns. We wish to establish if there is any difference, either presence or loss, in isoelectric points of the isozyme bands from those individuals with HTLV-III as opposed to those without AIDS whether homosexual or not. These studies have never been conducted and it is an approach I feel will gain a great deal of information possibly whether or not this enzyme is involved in the replication of the AIDS virus as has been suggested. I hope this proposal will be accepted by the committee with expediency.



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SAN FRANCISCO, CALIFORNIA 94143

March 18, 1985

Merle Sande, MD
Chairman
UC Systemwide Taskforce on AIDS
SFGH - 5H22
Campus Mail

Dear Dr. Sande:

UCSF AIDS Specimen Bank

We have received several requests for peripheral blood mononuclear cells (PBMC) obtained from at-risk individuals, those with AIDS and those with AIDS associated conditions. The requests seek cells on which to perform viral DNA studies, viral culture and enzyme assays. Drs. Conant and Volberding, among others, have offered to arrange to obtain the blood samples.

We are prepared to start this additional important aspect of the work of the bank at once. We will use a ficoll-hypaque preparation from whole blood and will aliquot freeze and store the PBMC thus obtained. We propose to process about 5 samples weekly to begin with.

I am writing to request a supplement to the budget of the specimen bank for the current year (84/85) to cover equipment and start-up costs of this new activity and to support it April - June 30, 1985. The budget for 1985/86 will include a request for funding for that year.

Supplement 1984/85

- | | |
|---|---------|
| 1. Liquid nitrogen freezer for cells
(including racks and boxes) | \$3,500 |
|---|---------|

This new freezer will be used for PBMC only.
The existing nitrogen freezer is for tissue
and will soon be full. The freezer proposed is
specifically designed for use for storing cells.

JSG/Sande
March 18, 1985
Page 2

2. Supplies - centrifuge tubes, disposables, ficoll-hypaque, culture media, vials and tubes, bags, liquid nitrogen.	\$1,800
Total	<u>\$5,300</u>

NOTE No personnel costs are requested for 1984/85 - the existing staff of the specimen bank will perform this work. The budget for 1985/86 will reflect our experience of the increased workload during April-June, 1985.

I would be happy to answer questions or provide further information.

Sincerely,



John S. Greenspan

JSG/lm

cc: Vice President Hooper
Dr. Phyllis Blair
~~Dr. Paul Volberding~~
Dr. John Zeigler
Dr. Jay A. Levy