Active

Project #: E-19-X04
Center #: 10/24-6-R725-2A0
Contract#: 5 R29 HL44960-02
Subprojects ?: N

Cost share #: Center shr 
Rev #: 3
Center shr #: OCA file 
Mod #: MEMO OF 4/20/93
Document : GRANT

Prime #: Work type : RES

Main project #: Contract entity: GTRC

Project unit: CHEM ENGR Unit code: 02.010.114
Project director(s): CHEM ENGR
WICK T M (404)894-8795

Sponsor/division names: DHHS/PHS/NIH
Sponsor/division codes: 108

Award period: 920701 to 930630 (performance) 930930 (reports)

Sponsor amount New this change Total to date
Contract value 0.00 103,324.00
Funded 0.00 103,324.00

Cost sharing amount 0.00

Does subcontracting plan apply ?: N

Title: MECHANISM OF SICKLE ERYTHROCYTE/ENDOTHELIAL ADHESION

PROJECT ADMINISTRATION DATA

OCA contact: Kathleen R. Ehlinger 894-4820
Sponsor technical contact Sponsor issuing office

DR. CLARICE REID
(301)496-6931 TIJUANA DECOSTER
(301)496-7257

NATIONAL HEART, LUNG, AND BLOOD INST NATIONAL HEART, LUNG, & BLOOD INST
NATIONAL INSTITUTES OF HEALTH NATIONAL INSTITUTES OF HEALTH
9000 ROCKVILLE PIKE 9000 ROCKVILLE PIKE
BETHESDA, MD 20892 BETHESDA, MD 20892

Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : N/A NIH supplemental sheet
Equipment title vests with: Sponsor GIT X

Administrative comments - ISSUED TO REVISE DELIVERABLE SCHEDULE.
GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date: 09/24/93

Project No. E-19-X04

Project Director: WICK T M

Center No. 10/24-6-R7254-2A0

School/Lab: CHEM ENGR

Sponsor: DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH

Contract/Grant No. 5 R29 HL44960-02

Contract Entity: GTRC

Prime Contract No.

Title: MECHANISM OF SICKLE ERYTHROCYTE/ENDOTHELIAL ADHESION

Effective Completion Date: 930630 (Performance) 930930 (Reports)

Closeout Actions Required:

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Y/N</th>
<th>Date Submitted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final Invoice or Copy of Final Invoice</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>Final Report of Inventions and/or Subcontracts</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>Government Property Inventory &amp; Related Certificate</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Classified Material Certificate</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Release and Assignment</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>

Comments: EFFECTIVE DATE 7-1-92. CONTRACT VALUE $103,324.

Subproject Under Main Project No.________

Continues Project No. E-19-660

Distribution Required:

<table>
<thead>
<tr>
<th>Department</th>
<th>Y/N</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Director</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>Administrative Network Representative</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>GTRI Accounting/Grants and Contracts</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>Procurement/Supply Services</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>Research Property Management</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>Research Security Services</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Reports Coordinator (OCA)</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>GTRC</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>Project File</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>Other: CARL BAXTER-FMD</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>FRED CAIN-ODD</td>
<td>Y</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Final Patent Questionnaire sent to PDPI.
DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
APPLICATION
FOR CONTINUATION GRANT

REVIEW GROUP TYPE ACTIVITY GRANT NUMBER
TOTAL PROJECT PERIOD
From: 07/25/91 Through: 06/30/96
REQUESTED BUDGET PERIOD
From: 07/01/93 Through: 06/30/94

To be verified by applicant. Check information in Items 1 through 6. If incorrect, furnish correct information in Item 13.

1. TITLE OF PROJECT
MECHANISM OF SICKLE ERYTHROCYTE/ENDOTHELIAL ADHESION

2a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR
(Wick, Timothy M)
GEORGIA INST. OF TECHNOLOGY
778 ATLANTIC DR

2b. DEPARTMENT, SERVICE, LABORATORY OR EQUIVALENT
SCHOOL OF CHEMICAL ENGINEERING

2c. MAJOR SUBDIVISION
COLLEGE OF ENGINEERING

3. ORGANIZATIONAL COMPONENT TO RECEIVE CREDIT FOR
BIOMEDICAL RESEARCH SUPPORT GRANT (See instructions)

20 OTHER

BITNET/INTERNET ADDRESS

2a. BITNET/INTERNET ADDRESS
timothy.wick@che.gatech.edu

4. APPLICANT ORGANIZATION (Name and address, street, city, state, zip code)

GEORGIA TECH RES CORP
OCA/PID RM 246 CRB
GEORGIA INST. OF TECHNOLOGY
ATLANTA, GA 30332-0420

5. ENTITY IDENTIFICATION NUMBER
1580603146A1

6. TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIAL
CONTRACTING OFFICER
OCA/PID, RM 246 CRB
GEORGIA INST. OF TECHNOLOGY
ATLANTA, GA 30332-0420

Complete the following (see instructions)

7. HUMAN SUBJECTS
If "YES" 

8. VERTEBRATE ANIMALS
If "YES"

9. PERFORMANCE SITE(S) (Organizations and addresses)
Georgia Institute of Technology
Cellular Biomechanics Laboratory
Space Science and Technology Building A
Room 217
Atlanta, GA 30332-0405

10. COSTS REQUESTED FOR NEXT BUDGET PERIOD
10a. DIRECT $ 10b. TOTAL $

11. INVENTIONS AND PATENTS (See instructions)

12a. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Item 2a)

12b. NAME OF ADMINISTRATIVE OFFICIAL (Item 6)
Janis L. Goddard

12c. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (Item 15)
Janis L. Goddard
Contracting Officer

13. USE THIS SPACE FOR CORRECTIONS TO ITEMS 1 THROUGH 6. INDICATE THE NUMBER(S) WHERE ANSWERS APPLY.

None

14. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application. Willful provision of false information is a criminal offense (U.S. Code, Title 18, Section 1001). I am aware that any false, fictitious, or fraudulent statement may, in addition to other remedies available to the Government, subject me to civil penalties under the Program Fraud Civil Remedies Act of 1986 (45 CFR 79).

SIGNATURE OF PERSON NAMED IN 2a
Automation "Per" signature not acceptable.

DATE

15. CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with the Public Health Service terms and conditions if a grant is awarded as the result of this application. A willfully false certification is a criminal offense (U.S. Code, Title 18, Section 1001). I am aware that any false, fictitious, or fraudulent statement may, in addition to other remedies available to the Government, subject me to civil penalties under the Program Fraud Civil Remedies Act of 1986 (45 CFR 79).

SIGNATURE OF PERSON NAMED IN 12c
Automation "Per" signature not acceptable.

DATE
### Detailed Budget for Next Budget Period

**Direct Costs Only**

**From:** 15 July 1993  
**Through:** 30 June 1994  
**Grant Number:** HL44960-03

<table>
<thead>
<tr>
<th>Name</th>
<th>Role on Project</th>
<th>Type of APPT (months)</th>
<th>Effort on Proj.</th>
<th>Inst. Salary</th>
<th>Dollar Amount Requested (Omit Cents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timothy M. Wick</td>
<td>Principal Investigator</td>
<td>12</td>
<td>20</td>
<td>64,690</td>
<td>12,938 3,520</td>
</tr>
<tr>
<td>James R. Eckman*</td>
<td>Co-Investigator</td>
<td>12</td>
<td>5</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Anjali Kumar</td>
<td></td>
<td>12</td>
<td>100</td>
<td>15,000</td>
<td>15,000</td>
</tr>
</tbody>
</table>

Total Personel: 27,938 + 3,520 = 31,458

**Consultant Costs**

None.

**Equipment (Itemize)**

None.

**Supplies (Itemize by category)**

- Tissue culture media, fetal bovine serum endothelial cell growth factors, adhesive proteins, buffers, antibiotics, EC mitogen: 4,400
- Disposable plasticware (LabTek chambers, pipets, flasks, filters, gloves): 2,370
- Electrophoresis supplies, ELISA reagents: 1,985
- Monoclonal antibodies, synthetic peptides: 1,500

**Travel**

To attend 2 scientific meetings: 1,654

**Consultant Costs**

None.

**Other Expenses (Itemize by category)**

- Machine shop, glass shop, electronics shop fees: 250
- Publication fees, artwork, photography: 853

**Subtotal Direct Costs for Next Budget Period**

**Consolidation/Contractual Costs**

<table>
<thead>
<tr>
<th>Direct Costs</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>$</td>
<td></td>
</tr>
</tbody>
</table>

**Total Direct Costs for Next Budget Period**

(Enter on Page 1, Item 10a)  
$ 44,470
Personnel: Fringe benefits are 27.2% of salary. Salary and fringe benefits for Dr. Wick have been reduced $20,670 to eliminate the funding overlap between this project and the Georgia Comprehensive Sickle Cell Center (see Other Support). All other costs reflect a 5% annual increase as funded in the original application.

Principal Investigator - Dr. Timothy M. Wick, Ph.D.: Funding is requested for the Principal Investigator to provide time to organize the study, coordinate in vitro investigations with clinical studies, perform experiments, analyze data, prepare manuscripts, hold regular laboratory meetings of the investigators, and develop progress reports. It is estimated that 50% of Dr Wick's time will be devoted to these tasks.

Graduate Student - Anjali Kumar: Ms. Kumar has been working in the laboratory since September 1991. She has recently begun doing sickle cell adherence studies and is responsible for most of the new data presented in the Results section. Ms. Kumar will devote 100% of her effort to this project. Ms. Kumar is (and will continue to be) responsible for the adhesion assays related to α4β1/VCAM-1 mediated adherence, the red cell activation with phorbol ester, and the mechanism of thrombospondin-mediated adherence.

Supplies: Tissue culture costs are based upon current performance of 3 flow experiments per week as well as current usage and costs. Media, serum, growth factors, buffers, and other chemicals as well as plasticware, glassware, and gloves are required for cell cultures and adhesion assays. Monoclonal antibodies to adhesion receptors will be used to identify receptors that are necessary for sickle erythrocyte adherence to endothelium.

Travel: Funds are requested for Dr. Wick to attend ASH and the annual Meeting of the Sickle Cell Disease Program to present research and interact with colleagues interested in similar and related areas of hematology and sickle cell anemia.

Other Expenses: Funds are requested to cover the cost photocopying and postage related to the transfer of data and data forms between Emory, Grady and Georgia Tech, medical illustrations and page costs. Machine shop charges are required to construct new adhesion systems.
BIOPGRAPHICAL SKETCH

Give the following information for the key personnel and consultants listed on page 2. Begin with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME

Wick, Timothy M.

POSITION/TITLE

Assistant Professor

BIRTHDATE (Mo., Day, Yr.)

July 9, 1961

EDUCATION

(Including baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION

University of Colorado, Boulder CO

Rice University, Houston, Texas

Rice University, Houston, Texas

DEGREE

B.S.

Ph.D.

Post Doc

YEAR

1983

1988

1988

FIELD OF STUDY

Chemical Engineering

Chemical Engineering

Biochemistry and Chemical Engineering

RESEARCH AND PROFESSIONAL EXPERIENCE:

Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Experience

9/88-present

Assistant Professor, School of Chemical Engineering, Georgia Institute of Technology, Atlanta, GA

4/93-present

Adjunct Assistant Professor, School of Mechanical Engineering, Georgia Tech, Atlanta, GA

2/88-9/88

Post-doctoral Research Associate, Department of Chemical Engineering, Rice University, Houston, TX

2/88-9/88

Post-doctoral Research Associate, Department of Chemical Engineering, Rice University, Houston, TX

Honors and Awards

1992

Lilly Foundation Teaching Fellowship

1991

Young Investigator Award Finalist. The 1991 World Congress on Medical Physics and Biomedical Engineering (Kyoto, Japan)

1991

American Heart Association-Georgia Affiliate, Grant-In-Aid

1991

The Whitaker Foundation, Biomedical Engineering Research Grant

1991

NIH-First Independent Research and Transition (FIRST) Award

1990, 91

Du Pont Young Faculty Award

1989

American Heart Association-Georgia Affiliate, Grant-In-Aid

1987

Beecham Award for outstanding original research presented at annual meeting of the Southern Society for Clinical Investigation, the Southern Section of the AFCR and the Southern Society for Pediatric Research

1986

Omega Chi Epsilon (National Chemical Engineering Honor Society)

Original Articles


Indicates publications directly arising from currently funded research (HL44960)

Published Abstracts (selected)


GRANT NUMBER: HL44906-03

BIOGRAPHICAL SKETCH

Give the following information for all new key personnel, consultants, and collaborators.
Copy this page for each person.

NAME
James Robert Eckman

POSITION TITLE
Associate Professor of Medicine

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE</th>
<th>YEAR CONFERRED</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of Minnesota,</td>
<td>B.A.</td>
<td>1965</td>
<td>Zoology</td>
</tr>
<tr>
<td>University of Minnesota</td>
<td></td>
<td>1966-67</td>
<td>MD/PhD</td>
</tr>
<tr>
<td>University of Minnesota</td>
<td>M.D.</td>
<td>1970</td>
<td>Physiology</td>
</tr>
<tr>
<td>University of Minnesota</td>
<td></td>
<td></td>
<td>Medicine</td>
</tr>
</tbody>
</table>

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individual who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

APPOINTMENTS:
Internship: Univ. of Minnesota Hospitals, Straight Medicine, 1970-1971
Residency: Univ. of Minnesota Hospitals, Internal Medicine, 1973-1973
Fellowship: Univ. of Minnesota Hospitals, Hematology 1974-1976
Instructor of Medicine, Univ. of Minnesota Medical School, 1973-1976
Assistant Professor of Medicine, Univ. of Minn. Med. School, 1976-1978
Assistant Professor of Medicine, Emory Univ. School of Medicine, 1978-1980
Associate Professor of Medicine, Emory Univ. School of Medicine, 1980-
Assistant Professor of Pediatrics, Emory Univ. School of Medicine, 1984-

HONORS:
Phi Beta Kappa, Alpha Omega Alpha; Diplomate of the American Boards of Internal Medicine General Internal Medicine Boards, October 1974; Subspecialty of Hematology, June 1978.

ADVISORY COMMITTEES:

ORIGINAL ARTICLES (Selected from a list 68 original articles and chapters):
1-10. 1984.


FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to significant delays in the review and/or funding of the application.

Other support is defined as all funds or resources, whether Federal, non-Federal, or institutional, available to the principal investigator/program director (and other key personnel named in the application) in direct support of their research endeavors through research or training grants, cooperative agreements, contracts, fellowships, gifts, prizes, and other means.

Reporting requirements are: For each of the key personnel, describe (1) all currently active support and (2) all applications and proposals pending review or award, whether related to this application or not. If the support is part of a larger project, identify the principal investigator/program director and provide the data for the relevant subproject(s). If an individual has no active or pending support, check “None.” Use continuation pages as needed to provide the required information in the format as shown below. Key personnel are defined as all individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project.

<table>
<thead>
<tr>
<th>Name</th>
<th>Active</th>
<th>Pending</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timothy M. Wick</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>a. Source and identifying no.</th>
<th>The Whitaker Foundation</th>
<th>P.I. T.M. Wick</th>
</tr>
</thead>
<tbody>
<tr>
<td>b. Title</td>
<td>Endothelial Cell Activation and Blood Cell Adhesion in Atherosclerosis</td>
<td></td>
</tr>
<tr>
<td>c. Your role on project</td>
<td>Principal Investigator</td>
<td>% Effort 25%</td>
</tr>
<tr>
<td>d. Dates and costs of entire project</td>
<td>1 July 1991 - 30 June 1994</td>
<td>($179,999)</td>
</tr>
<tr>
<td>d. Dates and costs of current year</td>
<td>1 July 1992 - 30 June 1993</td>
<td>($48,444 direct)</td>
</tr>
<tr>
<td>e. Specific aims of project</td>
<td>To elucidate the mechanism of adherence of monocytes to endothelial cells under physiologically relevant conditions.</td>
<td></td>
</tr>
<tr>
<td>f. Describe scientific and budgetary overlap</td>
<td>No scientific or budgetary overlap.</td>
<td></td>
</tr>
<tr>
<td>g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)</td>
<td>None.</td>
<td></td>
</tr>
</tbody>
</table>
2.

a. **Source and Identifying Number:** NIH 1-P01-HL48482-01

P.I. James R. Eckman, M.D., Associate Professor of Medicine in Hematology/Oncology, Assistant Professor of Pediatrics in Human Genetics, Emory University School of Medicine, Atlanta, Georgia.

**Title:** Georgia Comprehensive Sickle Cell Center

c. **Your role on project:** Collaborating Investigator

d. **Dates and costs of entire project:** 1 April 1993 - 31 March 1998 $5,085,570 (direct)

d. **Dates and costs current year:** 1 April 1993 - 31 March 1994 $867,809 (direct)

e. **Specific aims of project:** The primary goal of the Georgia Comprehensive Sickle Center is to provide basic and clinical research, education, laboratory diagnosis, counseling and patient care in sickle cell syndromes. Research projects will address issues of pathophysiology and treatment for important complications in patients with these disorders. Dr. Wick's role in the Center is to develop and execute investigations into the effects of sickle red blood cells on endothelial cell morphology and function. These important studies will provide insight into the mechanism of sickle red cell induced endothelial cell damage and the related clinical complications (such as stroke), not necessarily related to microvascular occlusion. We are testing the hypothesis that sickle erythrocytes alter endothelial cell morphology, metabolic processes, and function. Specifically, we have data that sickle red blood cells inhibit endothelial cell responses to arterial levels of shear stress, sickle cells induce endothelial cell adhesion molecule expression, and stimulation of endothelial cells with sickle red cells increases the affinity of the endothelium for sickle erythrocytes. The SCORE research that involves Dr. Wick is complementary to the erythrocyte adherence studies related to microvascular occlusion ongoing under the current R29 award.

f. **Describe budgetary and scientific overlap:** The budgetary overlap is limited to the PI's salary.

g. **Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.):** The PI's salary on this project will be reduced from 50% to 20%. This reduction eliminates the funding overlap between the R29 and Dr. Wick's funding from the SCORE which arose because the projects are complementary, and some of Dr. Wick's efforts benefit both projects simultaneously. For example, study design, sample collection, data analysis, research group meetings with collaborators, report writing, etc. for the projects are interrelated and Dr. Wick, as PI, actively participates in each of these areas. In addition, Dr. Wick is heavily involved in the design and execution of experiments related to both projects. The 20% effort on the R29 and the 30% effort on the SCORE and the related revised budgets accurately reflect Dr. Wick's commitment to and participation in both projects. Note that with these budget revisions, Dr. Wick still devotes 50% effort to his studies in the area of sickle cell/endothelial adherence and the related effects of sickle cells on endothelial cell biology. With this revision, the PI's salary and fringe benefits have been reduced by $20,670 in the R29 for years 3-5.

The aims will be adjusted slightly as indicated in the report (see Plans). This adjustment is made solely on the basis of our results to date as reported. We will focus our efforts on identifying the adherence mechanism(s) utilized when sickle cells are suspended in autologous plasma. This emphasis is based on the knowledge that plasma is the milieu *in vivo* and our belief that integrin receptors account for a significant fraction of the plasma-mediated adherence. We are interested in investigating anti-integrin receptor peptides for their ability to inhibit or reverse adherence in a plasma environment in order to provide data of use in the development of anti-adhesion therapies.

With this budget adjustment, NIH will be funding 1 graduate student and Dr. Wick for 20% effort on the R29 and 2 graduate students and Dr. Wick for 30% effort on the SCORE research. Clearly our preliminary data as detailed in the proposals justify this level of funding for sickle cell/endothelial cell interactions.
3. 
   a. Source and Identifying Number: NIH T32GM08433-02    P.I. Robert M. Nerem, Ph.D.

   Title: Cellular Engineering Training Program

   b. Your role on project: Collaborating Faculty   % Effort: 5%


   e. Specific aims of project: This project provides funds for predoctoral students studying Cellular Engineering.

   f. Describe budgetary and scientific overlap: None.

   g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.): None.

4. 
   Source and Identifying Number: The Whitaker Foundation    P.I. Robert M. Nerem, Ph.D.

   Title: Biomedical Engineering Education: An Interdisciplinary Tissue Engineering Education and Research Program

   b. Your role on project: Participating faculty   % Effort: 0%

   c. Dates and costs of entire project:  $3,000,000 (1 September 1993 - 31 August 1996)

   d. Dates and costs current year:  $1,500,000 (1 September 1993 - 31 August 1994)

   e. Specific aims of project: This grant provides funds for laboratory space renovation, the hiring of six new faculty in Tissue Engineering, and a limited number of graduate student stipends.

   f. Describe budgetary and scientific overlap: None.

   g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.): None.

   PENDING

   None.

   PLANNED

   None.
5.
Source and Identifying Number: NSF BCS9111761

Title: Reconstitution of a blood vessel in culture

b. Your role on project: Co-investigator

% Effort: 5%

c. Dates and costs of entire project: $443,740 (1 September 1991 - 28 February 1995)

d. Dates and costs current year: $178,461 (1 September 1992 - 31 August 1993) > ($10,000 annual direct costs for Dr. Wick)

e. Specific aims of project: Dr. Wick will evaluate the thrombogenicity of tissue engineered blood vessels developed in Dr. Nerem's lab.

f. Describe budgetary and scientific overlap: None.

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.): None.
OTHER SUPPORT
(Use continuation pages if necessary)

FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to significant delays in the review and/or funding of the application.

Other support is defined as all funds or resources, whether Federal, non-Federal, or institutional, available to the principal investigator/program director (and other key personnel named in the application) in direct support of their research endeavors through research or training grants, cooperative agreements, contracts, fellowships, gifts, prizes, and other means.

Reporting requirements are: For each of the key personnel, describe (1) all currently active support and (2) all applications and proposals pending review or award, whether related to this application or not. If the support is part of a larger project, identify the principal investigator/program director and provide the data for the relevant subproject(s). If an individual has no active or pending support, check “None.” Use continuation pages as needed to provide the required information in the format as shown below. Key personnel are defined as all individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project.

Name  James R. Eckman  Active  X  Pending  None

a. Source and identifying no.  NIH/NHLBI, 1P60 HL48482-01  P.I.  James Eckman

Title  Georgia Comprehensive Sickle Cell Center

b. Your role on project  Principal Investigator  % Effort  32%

c. Dates and costs of entire project  4/1/93 - 3/31/98: $8,299,001

d. Dates and costs of current year  4/1/93 - 3/31/94: $1,237,856

e. Specific aims of project  The primary goal of the grant is to provide basic and clinical research, education, laboratory diagnosis, counseling and patient care in sickle cell syndromes. Research projects will address issues of pathophysiology and treatment for important complications in patients with these disorders.

f. Describe scientific and budgetary overlap  None.

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)  None.
OTHER SUPPORT
(Use continuation pages if necessary)

FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to significant delays in the review and/or funding of the application.

Other support is defined as all funds or resources, whether Federal, non-Federal, or institutional, available to the principal investigator/program director (and other key personnel named in the application) in direct support of their research endeavors through research or training grants, cooperative agreements, contracts, fellowships, gifts, prizes, and other means.

Reporting requirements are: For each of the key personnel, describe (1) all currently active support and (2) all applications and proposals pending review or award, whether related to this application or not. If the support is part of a larger project, identify the principal investigator/program director and provide the data for the relevant subproject(s). If an individual has no active or pending support, check “None.” Use continuation pages as needed to provide the required information in the format as shown below. Key personnel are defined as all individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project.

Name  James R. Eckman  Active  X  Pending  None  Fulton-Dekalb
   b. Title  Treatment of Sickle Cell Anemia
   c. Your role on project  Supervision of clinical care  % Effort  70%
   d. Dates and costs of entire project  7/1/86 - 6/30/93: $605,000 per year
   e. Dates and costs of current year  7/1/92 - 6/30/93: $605,000
   f. Specific aims of project  Provision of clinical care of sickle cell patients.
   f. Describe scientific and budgetary overlap  None.
   g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)
      None.
FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to significant delays in the review and/or funding of the application.

Other support is defined as all funds or resources, whether Federal, non-Federal, or institutional, available to the principal investigator/program director (and other key personnel named in the application) in direct support of their research endeavors through research or training grants, cooperative agreements, contracts, fellowships, gifts, prizes, and other means.

Reporting requirements are: For each of the key personnel, describe (1) all currently active support and (2) all applications and proposals pending review or award, whether related to this application or not. If the support is part of a larger project, identify the principal investigator/program director and provide the data for the relevant subproject(s). If an individual has no active or pending support, check “None.” Use continuation pages as needed to provide the required information in the format as shown below. Key personnel are defined as all individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project.

<table>
<thead>
<tr>
<th>Name</th>
<th>Active</th>
<th>Pending</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>James R. Eckman</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source and identifying no. H.H.S. Block Grant Fulton-Dekalb P.I. Hosp. Authority

Title Cord Blood Screening for Hemoglobinopathies

Your role on project Supervision of newborn screening program % Effort 5%

dates and costs of entire project 10/1/85 - 9/30/93: $58,156 per year

dates and costs of current year 10/1/92 - 9/30/93: $58,156

e. Specific aims of project To provide newborn screening for sickle cell disease and other hemoglobinopathies for all newborn babies at Grady Memorial Hospital.

Describe scientific and budgetary overlap None.

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)

None.
OTHER SUPPORT  
(Use continuation pages if necessary)  

GRANT NUMBER  
HL44960-03  

FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to significant delays in the review and/or funding of the application.

Other support is defined as all funds or resources, whether Federal, non-Federal, or institutional, available to the principal investigator/program director (and other key personnel named in the application) in direct support of their research endeavors through research or training grants, cooperative agreements, contracts, fellowships, gifts, prizes, and other means.

Reporting requirements are: For each of the key personnel, describe (1) all currently active support and (2) all applications and proposals pending review or award, whether related to this application or not. If the support is part of a larger project, identify the principal investigator/program director and provide the data for the relevant subproject(s). If an individual has no active or pending support, check "None." Use continuation pages as needed to provide the required information in the format as shown below. Key personnel are defined as all individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project.

<table>
<thead>
<tr>
<th>Name</th>
<th>Active</th>
<th>Pending</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>James R. Eckman</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>a. Source and identifying no.</th>
<th>P.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIH/NHLBI, 1U01 45692-01</td>
<td>Samuel Charache</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>b. Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyurea in Sickle Cell: Subcontract to Johns Hopkins</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>c. Your role on project</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local Principal Investigator</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>d. Dates and costs of entire project</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/1/91 - 4/30/96: $346,864 per year entire project</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>e. Dates and costs of current year</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/1/92 - 4/30/93: $67,370</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>f. Specific aims of project</th>
</tr>
</thead>
<tbody>
<tr>
<td>This is a multi-center study of hydroxyurea in sickle cell anemia to determine whether or not treatment with hydroxyurea titrated to maximum tolerated doses will reduce to at least 50% the frequency of vaso-occlusive (painful) crisis. The secondary objectives are to establish the relationship of fetal hemoglobin levels and other patients or treatment characteristics to the occurrence of vaso-occlusive (painful) crises, and the effect of treatment on the quality of patients' lives.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>g. Describe scientific and budgetary overlap</th>
</tr>
</thead>
<tbody>
<tr>
<td>None.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None.</td>
</tr>
</tbody>
</table>


FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to significant delays in the review and/or funding of the application.

Other support is defined as all funds or resources, whether Federal, non-Federal, or institutional, available to the principal investigator/program director (and other key personnel named in the application) in direct support of their research endeavors through research or training grants, cooperative agreements, contracts, fellowships, gifts, prizes, and other means.

Reporting requirements are: For each of the key personnel, describe (1) all currently active support and (2) all applications and proposals pending review or award, whether related to this application or not. If the support is part of a larger project, identify the principal investigator/program director and provide the data for the relevant subproject(s). If an individual has no active or pending support, check "None." Use continuation pages as needed to provide the required information in the format as shown below. Key personnel are defined as all individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project.

Name: James R. Eckman          Active: X  Pending:    None
a. Source and identifying no.: National Cancer Institute  P.I.: Melvin Moore
Title: Grady Memorial Hospital Clinical Oncology Program
b. Your role on project: Co-Investigator  % Effort: 5%
c. Dates and costs of entire project: 7/1/90 - 6/30/93: $24,959 per year
d. Dates and costs of current year: 7/1/92 - 6/30/93: $24,959
e. Specific aims of project: To enroll minority patients in cancer chemotherapy trials.

f. Describe scientific and budgetary overlap: None.
g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)

   Effort is to be reduced to 0%.
OTHER SUPPORT
(Use continuation pages if necessary)

FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to significant delays in the review and/or funding of the application.

Other support is defined as all funds or resources, whether Federal, non-Federal, or institutional, available to the principal investigator/program director (and other key personnel named in the application) in direct support of their research endeavors through research or training grants, cooperative agreements, contracts, fellowships, gifts, prizes, and other means.

Reporting requirements are: For each of the key personnel, describe (1) all currently active support and (2) all applications and proposals pending review or award, whether related to this application or not. If the support is part of a larger project, identify the principal investigator/program director and provide the data for the relevant subproject(s). If an individual has no active or pending support, check “None.” Use continuation pages as needed to provide the required information in the format as shown below. Key personnel are defined as all individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project.

Name: James R. Eckman

a. Source and identifying no. Georgia D.H.R.

Title: Treatment of Sickle Cell Anemia

b. Your role on project: Supervision of clinical care

c. Dates and costs of entire project: 7/1/93 - 6/30/94: $575,000 per year

d. Dates and costs of current year: 7/1/93 - 7/30/94: $575,000

e. Specific aims of project: Provision of clinical care of sickle cell patients.

f. Describe scientific and budgetary overlap: None.

g. Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)

Dr. Eckman’s previously proposed clinical effort and salary on this grant will be reduced to 50% because of the greater effort devoted to research.
FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to significant delays in the review and/or funding of the application.

Other support is defined as all funds or resources, whether Federal, non-Federal, or institutional, available to the principal investigator/program director (and other key personnel named in the application) in direct support of their research endeavors through research or training grants, cooperative agreements, contracts, fellowships, gifts, prizes, and other means.

Reporting requirements are: For each of the key personnel, describe (1) all currently active support and (2) all applications and proposals pending review or award, whether related to this application or not. If the support is part of a larger project, identify the principal investigator/program director and provide the data for the relevant subproject(s). If an individual has no active or pending support, check "None." Use continuation pages as needed to provide the required information in the format as shown below. Key personnel are defined as all individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project.

Name: James R. Eckman
Active: X
Pending: None

Source and identifying no.: H.H.S. Block Grant

Title: Cord Blood Screening for Hemoglobinopathies

Your role on project: Supervision of newborn screening program
Effort: 5%

Dates and costs of entire project: 10/1/93 - 9/30/94: $58,156 per year

Dates and costs of current year: 10/1/93 - 9/30/94: $58,156

Specific aims of project: To provide newborn screening for sickle cell disease and other hemoglobinopathies for all newborn babies at Grady Memorial Hospital.

Describe scientific and budgetary overlap: None.

Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.):
None.
1. Specific Aims
The tendency for hemoglobin SS to polymerize at low oxygen tension is assumed to be the dominant factor in sickle cell pathology. Since morphological sickling is delayed after hemoglobin deoxygenation, factors which delay red cell microcirculatory transit are likely antecedents to microvascular occlusion, ischemic tissue damage, and pain episodes characteristic of sickle cell anemia. Our central hypothesis is that sickle erythrocyte adherence to microvascular endothelium delays erythrocyte microcirculatory transit. This partial obstruction allows for intracapillary red cell sickling leading to complete occlusion (1). Our data clearly indicate that plasma, red cell and endothelial cell factors as well as local hemodynamics all likely contribute to adherence and occlusion in vivo (2-7). Our specific aims for this project are to (i) characterize differences in sickle red blood cell adherence to phenotypically diverse endothelium (veins, arteries, microvessels); (ii) identify specific plasma factors, red cell membrane abnormalities, and endothelial ligands which promote sickle red blood cell (SRBC) adherence, and (iii) characterize the interpatient and intrapatient adherence mechanisms and degree of adherence during asymptomatic periods and pain episodes. The long-term goal of this research is to discover the mechanisms and extent of sickle red cell adherence to different vascular sites during pain episodes and asymptomatic periods. These studies will be invaluable to the development of effective anti-adhesion therapies to eliminate or reduce the ischemic tissue damage associated with blood vessel occlusion in sickle cell anemia.

2. Studies and Results
Methods
Adherence of sickle red blood cells (SRBC) to cultured human umbilical vein (HUVEC) and microvascular (MEC) endothelial cells was quantified under dynamic flow conditions in vitro essentially as described in the original grant application (6).

Thrombospondin-Mediated Sickle Red Cell Adherence to Microvascular Endothelium
We have previously reported that thrombospondin (TSP), possibly released from activated sickle platelets in vivo, promotes sickle red cell adherence to MEC under physiological flow conditions (2). A main goal for this budget period was further elucidation of the mechanism of TSP-mediated adherence. TSP binds to CD36 and the vitronectin receptor on MEC (2). However, it is not known whether TSP receptors are expressed on SRBC. We (Figure 1 & ref. 3) and others (8) have recently demonstrated that sickle reticulocytes express CD36, a TSP receptor (9). Normal reticulocytes also express CD36, but to a much lesser degree (data not shown - see reference 25). As shown in Figure 2, preincubation of SRBC with anti-CD36 antibody quantitatively blocks TSP-mediated SRBC adherence to MEC.

When either SRBC or MEC are incubated with TSP, TSP promotes SRBC adherence (Fig 3). However separate incubation of both the SRBC and MEC with TSP prior to a flow adhesion assay does not promosimultaneous occupation of TSP receptors on both the SRBC and MEC inhibits adherence.

We have previously reported that antibodies to either the vitronectin receptor (αvβ3) or CD36 quantitatively inhibit TSP-mediated SRBC adherence to MEC (2). Considerable ongoing debate does not clarify whether both of these receptors are TSP receptors or whether CD36 and αvβ3 are closely opposed such that antibody occupation of one receptor sterically inhibits TSP access to the other receptor (10-12). To address this issue, we have utilized the sextapeptide (CSVTCG) sequence that is the purported CD36-binding domain of TSP (13) and the RGD sequence that binds to integrin receptors (14) to investigate further the role of CD36 and VnR in TSP-mediated adherence.
Preincubation of either SRBC or MEC with CSVTCG also abolishes TSP-mediated adherence (Fig 4). Similarly, preincubation of MEC with RGD abolished adherence (Fig 5).

Fig 1: Sickle RBC express CD36, a TSP receptor. SRBC were labeled with both thiazole orange (FL1) and anti-CD36 antibody (FL2) and analyzed by fluorescence activated cell sorting (FACS) (24). The population of RBC in quadrant 2 represent 8% of the patients RBC that are CD36-expressing reticulocytes.

Fig 2: Anti-CD36 antibody inhibits TSP-mediated adherence to MEC. Preincubation of either SRBC or MEC with OKM5 antibody inhibits SRBC adherence to a similar extent (shown in parentheses). Unbound antibody was washed away prior to the adherence assay. Data are mean±SEM for the number of experiments in this and all plots (except where indicated).

Fig 3: TSP mediates adhesion of SRBC to TSP. Preincubation of either SRBC or MEC with TSP promotes SRBC adherence; incubation of both MEC and SRBC with TSP does not promote adherence.

Fig 4: TSP-mediated SRBC adherence is blocked by the antagonist peptide CSVTCG. Incubation of either MEC of SRBC with CSVTCG peptide quantitatively inhibits SRBC adherence to MEC.
Fig 5: Preincubation of MEC with RGDS peptide inhibits TSP-mediated SRBC adhesion to MEC 92%.

**Endothelial Stimulation**

Using monoclonal antibodies and fluorescence activates cell sorting (FACS) we have recently demonstrated that a subpopulation of young reticulocytes in sickle blood express the $\alpha_4\beta_1$ (VLA-4) integrin receptor (Fig 6). Normal reticulocytes also express VLA-4, but to a lesser degree (data not shown). Sickle reticulocytes do not express the $\alpha_1$, $\alpha_2$, $\alpha_3$, $\alpha_5$, $\alpha_6$, $\alpha_v$, $\beta_2$, $\beta_3$ integrin receptors (Fig 6). The ligand for $\alpha_4\beta_1$ is VCAM-1 (15) which is expressed by HUVEC (16) and MEC (17) after stimulation with cytokines such as tumor necrosis factor (16,17). SRBC, but not NRBC, adhere to TNF-\(\alpha\) stimulated HUVEC (Fig 7) and this adherence is inhibited greater than 70% by incubating SRBC with an anti-$\alpha_4\beta_1$ antibody or the HUVEC with an anti-VCAM-1 antibody (Fig 8). TNF-\(\alpha\) also induces VCAM-1 expression on MEC (17). In one experiment, SRBC also adhered to cytokine activated MEC via a $\alpha_4\beta_1$/VCAM-1 dependent adherence pathway (Fig 9) similar to that observed for HUVEC.

Fig 6: SRBC were labeled with thiazole orange (to identify reticulocytes) (FL1) and an anti-$\alpha_4$ or anti-$\beta_1$ (FL2) antibody and analyzed by FACS as described in Fig 1. The cells stained positive for both $\alpha_4$ and $\beta_1$. 
Sickle Erythrocyte Stimulation

VLA-4 is constitutively expressed on a subpopulation of sickle reticulocytes (Fig 6) and promotes adherence to activated HUVEC via VCAM-1 (Fig 7). Recent data suggest that β1 integrins, including VLA-4, can be stimulated to exhibit higher affinity for their ligands (e.g., activated) (18) by phorbol esters and other agonists. In order to test the hypothesis that erythrocyte activation elevates sickle cell adherence to endothelium, we stimulated SRBC with phorbol dibutyrate (PDBu) prior to the flow adherence assays. As seen in Figure 10, PDBu activated sickle, but not normal, RBC are more adherent to cultured endothelium as compared to unstimulated SRBC. These adherence assays were conducted in the absence of any adherence proteins and without endothelial cell stimulation. Thus, the phorbol ester induced SRBC adherence appears to be via a pathway not dependent upon exogenous proteins or endothelial cell activation.

Fig 7: TNF-α stimulated HUVEC support SRBC Adherence. Sickle, but not normal, RBC adhere to TNF-α stimulated HUVEC.

Fig 8: SRBC adhere to cytokine stimulated HUVEC via a VLA-4/VCAM-1 dependent mechanism. Anti-VCAM-1 or anti-VLA-4 antibody inhibited SRBC adherence to TNF-α stimulated HUVEC by 80% and 74%, respectively. Neither incubation of HUVEC with anti-ICAM-1 or SRBC with anti-glycophorin inhibited SRBC adherence.

Fig 9: Sickle RBC adhere to TNF-α stimulated MEC. MEC were activated with TNF, washed SRBC suspended in SFM (containing no adhesive proteins) were perfused over the monolayer and adherent sickle cells were enumerated as described in Figs 7 & 8. Adherence was partially blocked by anti-α4β1 antibody on SRBC or by anti-VCAM-1 antibody on the MEC.

Fig 10: Phorbol ester stimulation of SRBC elevates SRBC adherence to HUVEC. SRBC suspended in SFM were pretreated with phorbol ester (PDBu) and adherence to unstimulated HUVEC was quantified. In some experiments, HUVEC were pretreated with anti-VCAM-1 or SRBC were pretreated with anti-VLA-4. The percent antibody inhibition is shown above each bar.
3. Significance

Effect of Endothelial Cell Phenotype

We have recently reported that SRBC adherence to microvasculature (19). Notably, high molecular weight von Willebrand factor multimers promote adherence to HUVEC but not MEC (19). In contrast, autologous plasma promotes very high SRBC adherence to MEC, but only low levels of SRBC adherence to HUVEC (19). The MEC are likely predominately of capillary origin, whereas the HUVEC may closely mimic the post-capillary venule endothelium (19). Thus, these data indicate that multiple and different pathways exist for SRBC adherence - and that (for example) plasma-mediated adherence in the capillaries and adherence to post-capillary venule endothelium via high molecular weight vWF possibly act in tandem to provide greater blood flow impediment in the microcirculation.

Here we report of two additional adherence pathways; one mediated by TSP and its receptors on SRBC (CD36) and microvascular endothelium (CD36 and α4β3) and the other mediated by SRBC VLA-4 (α4β1) binding to VCAM-1 expressed on TNF-α stimulated endothelium. Recently, Sugihara, et al. (8) reported that TSP also mediates SRBC adherence to HUVEC via CD36 on the SRBC and unidentified receptor(s) on HUVEC. However, an RGDS peptide inhibited TSP-mediated binding (8) indicating that HUVEC integrin receptors are involved in adherence. Thus, the mechanism of TSP mediated SRBC adherence to MEC and HUVEC is apparently analogous. Similarly, the α4β1/VCAM-1 adherence is significant for both TNF-α stimulated HUVEC (Fig 8) and MEC (Fig 9). Thus, these two pathways support adherence of SRBC to both large vessel and microvascular endothelium and may participate in adherence in vivo in both the capillaries and post-capillary venules.

Endothelial Cell Activation

One of our most significant observations is that SRBC express the α4β1 integrin receptor and adhere to TNF-α activated HUVEC and MEC. Cytokine levels are elevated in sickle patients during asymptomatic periods and during acute illness (20). It is possible, especially in light of our recent data (Figs 6-9), that cytokine production may induce endothelial cell VCAM-1 expression and SRBC adherence in vivo, leading to microvascular occlusion. These data suggest that anti-cytokine therapy, to inhibit VCAM-1 expression, may be an alternative therapeutic strategy to prevent, minimize, or reverse sickle cell adherence to vascular endothelium and the accompanying necrotic tissue damage. We will further explore this hypothesis by monitoring patient cytokine levels in order to attempt correlations between plasma cytokine level, in vitro endothelial adherence, and disease severity.

Sickle Cell Activation

The data of Figure 10 suggest that sickle red blood cells can exhibit higher affinity for endothelial cell receptors when stimulated with agonists such as phorbol ester. These data suggest an intriguing hypothesis that the SRBC can become 'activated' in vivo, exhibit greater affinity for the endothelium, and lead to increased endothelial adherence and vaso-occlusion in the absence of changes in plasma concentrations of adhesive proteins or endothelial cell activation. Since these data are only preliminary, we do not know if the increased adherence is due solely to α4β1 'activation' as we originally hypothesized or if other receptors are involved under these stimulation conditions. However, it is unlikely that PDBu is exerting its effect through endothelial cell activation since the phorbol ester is incubated with the red cells and the red cells are washed prior to the adherence assay. Obviously, we will continue to explore this hypothesis and preliminary data to further determine whether an 'activation' state exists for SRBC.

4. Plans

In general, we do not anticipate significant deviation from the original proposal. However, the future work will be focused on the data of most promise in elucidation of the mechanisms of sickle red cell/endothelial cell adherence in vivo, in order to provide data that is of use to developing anti-adhesion therapies. We will focus on identifying the plasma components responsible for sickle cell adherence and will identify which adhesion pathway or pathways are most prominent in the plasma milieu. Thus, we will investigate adherence when sickle red cells are suspended in autologous plasma.
(as opposed to incubated with purified proteins) since this mimics the *in vivo* milieu. Also, we will continue to identify and characterize the sickle red cell membrane receptor(s) and their ligands on endothelial cells.

**The Role of Integrin Receptors in Plasma-Mediated SRBC Adherence**

We have characterized several adherence pathways involving integrin receptors, including: VCAM-1/α4β1 (Fig 8), TSP/αvβ3 (ref 2), high molecular weight vWF/integrin receptor (ref 7). Others have shown that fibrinogen (21) and fibronectin (4) also promote SRBC, possibly through endothelial integrin receptors (22). We hypothesize that adherence to integrin receptors (possibly mediated by a variety of proteins) accounts for a significant fraction of the adherence *in vivo*. We will test this hypothesis by examining SRBC adherence when erythrocytes are suspended in autologous plasma. We will identify specific plasma factors which promote adherence, especially integrin receptor agonist proteins (e.g. TSP, vWF, fibrinogen, fibronectin). In parallel, we will investigate peptides based on the RGD motif (which competes with RGD-containing proteins for integrin receptors [23]) for their ability to inhibit plasma-mediated adherence. In general, these peptides are commercially available. These experiments will be of interest because the experimental system we utilize closely mimics the microvascular milieu *in vivo*. That is, our SRBC adhesion experiments will be performed in autologous plasma on human microvascular endothelium under shear conditions typical of that in the microcirculation. Successful identification of RGD-containing peptides that inhibit plasma-mediated adherence could form the basis for anti-adhesion therapies for patients.

"Activated' Sickle Red Cells"

The data of Figure 9 indicate that sickle red cell affinity for endothelial cell ligands can be significantly increased after stimulation with phorbol ester. This suggests that the red cell may periodically exhibit greater adhesivity for the endothelium *in vivo*. We plan to further explore this hypothesis with the following experiments. The fact that VLA4 on the SRBC accounts for only approximately half of the adherence when SRBC are stimulated with PDBu, suggests that other endothelial receptors are involved. Thus, using our monoclonal antibodies to a variety of adhesion receptors (VCAM-1, ELAM-1, ICAM-1, CD36, GPIb, α4β3, etc.) we will determine whether the stimulated RBC adhere via a known SRBC adherence pathway. In addition, since we have not directly demonstrated that the α4β1 positive RBC are the adherent RBC in response to phorbol ester we will test α4β1 positive and α4β1 negative cells subfractions of RBC to establish whether α4β1 on SRBC is involved in adherence. These subfractions will be generated by density gradient centrifugation (25) (since most of the α4β1 positive cells are reticulocytes which have low density) or fluorescence activated cell sorting (24). We have utilized both of these technologies and do not anticipate any difficulty.
Literature Cited


5. Human Subjects

a. General Guidelines

i. Proposed Use

Patients with sickle-cell syndromes (HbSS, HbSC, HbS β-thalassemia) not receiving anticoagulant therapy and without evidence of pregnancy, obvious infection, thromboembolic disease or liver disease will be eligible for this study. Patients will be studied once in pain crisis and twice during asymptomatic periods. An age and sex matched population of normal black individuals will serve as a control population. Approximately twenty patients and twenty control subjects, aged eighteen or older, will be studied annually. Ten milliliters of blood will be drawn by venipuncture for each experiment.

ii. Specimen Usage

None of the data from the experiments will be used for diagnosis or treatment of specific individuals.

iii. Patient Recruitment

Patients from the Sickle Cell Center or the in-patient service at Grady Memorial Hospital, Atlanta, GA and hospital staff will be recruited by Dr. James R. Eckman. Subjects will agree to participate in this study by signing a consent form approved by Georgia Tech and Emory University School of Medicine IRBs. The consent form explains the nature of the study, the details of blood collection, risks associated with drawing blood, the availability of personnel to discuss the results of the study, the assurance of anonymity, and the ability to withdraw from the study at any time without penalty or loss of benefits.

iv Potential Risks

The risks of drawing blood are minimal and include slight pain, bruising, and infection at the site of puncture. No viable alternative for drawing human blood exists.

v. Procedures to Minimize Risk

Patient confidentiality will be ensured by assigning a code to each patient studied (SS1, AA1 for sickle and normal donor, respectively) to be used when all data is reported. Blood will be drawn at Grady Hospital under the supervision of Dr. James R. Eckman, director of the Sickle Cell Clinic. Dr. Eckman will be available to answer questions and to arrange for emergency medical care if a medical problem develops during the course of this study.

vi. Justification

The risk of blood drawing is minimal compared to potential benefits of a better understanding of clotting abnormalities in sickle cell syndromes and their relationship to pain crisis.

b. Gender and Minority Inclusions

Study subjects will be patients diagnosed with sickle cell syndromes as defined above. These patients will primarily be of African descent, however no patients will be included or excluded on the basis of race. The study population will consist of approximately equal numbers of men and women. Exclusion criteria will be solely based on medical criteria as described above. Control subjects (volunteers without hemoglobinopathies) will be age, sex, and race-matched. These volunteers are recruited from the hospital staff at Grady Memorial Hospital in Atlanta.
6. Vertebrate Animals

None.

7. Publications (from this project)

a. Journal Articles


Swerlick RA, JR Eckman, A Kumar, M Jettler, and TM Wick. Reticulocytes from patients with sickle cell anemia express the α4β1 integrin complex and bind to TNF-α stimulated endothelial cells via a VCAM-1/α4β1 dependent mechanism," Blood, in review (October 1992).

b. Abstracts and Meeting Presentations


Wick, TM, JR Eckman, A Kumar, M Jettler, RA Swerlick. Reticulocytes from patients with sickle cell anemia express the α4β1 integrin complex and bind to TNF-α activated endothelial cells via a VCAM-1/α4β1 dependent mechanism," Blood, 80:11a; 1992.


Wick TM, A Kumar, JR Eckman, and RA Swerlick. “Sickle Reticulocytes Express the α4β1 Integrin Complex and Bind to TNF-α Activated Endothelial Cells via a VCAM-1/α4β1 Dependent Mechanism,” 18th Annual Meeting of the National Sickle Cell Disease Program, Philadelphia, PA (May 1993).

7. Inventions and Patents

None.
PROGRESS REPORT (Personnel and Study Subjects)

All Personnel for the Current Budget Period
and Any Planned Changes in Personnel for the Next Budget Period

Use two sections. In the first section list All Current Personnel. In the second section list Planned Personnel Changes.

<table>
<thead>
<tr>
<th>Name</th>
<th>Degree(s)</th>
<th>SSN</th>
<th>Role on Project (e.g., PI, Res. Assoc.)</th>
<th>Date of Birth (MM/DD/YY)</th>
<th>Annual % Effort</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Current Personnel</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Timothy M. Wick</td>
<td>B.S., Ph.D.</td>
<td>505-94-2891</td>
<td>PI</td>
<td>07/09/61</td>
<td>20%</td>
</tr>
<tr>
<td>James R. Eckman</td>
<td>B.A., M.D.</td>
<td>471-48-8946</td>
<td>Co-Investigator</td>
<td>08/25/43</td>
<td>5%</td>
</tr>
<tr>
<td>Henri A. Brittain*</td>
<td>B.S., M.S., Ph.D.</td>
<td>264-53-1153</td>
<td>Graduate Student</td>
<td>01/18/59</td>
<td>100%</td>
</tr>
<tr>
<td>Anjali Kumar</td>
<td>B.S.</td>
<td>253-81-0772</td>
<td>Graduate Student</td>
<td>09/15/68</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Planned Changes**

None.

Mr. Brittain graduated in August 1992 and is no longer working on the project. Ms. Kumar is his replacement and has been working on the project since January 1993.

Provide the number of subjects enrolled in the study to date according to the following categories. (See Page 8 for definitions.)

<table>
<thead>
<tr>
<th></th>
<th>American Indian or Alaskan Native</th>
<th>Asian or Pacific Islander</th>
<th>Black, not of Hispanic Origin</th>
<th>Hispanic</th>
<th>White, not of Hispanic Origin</th>
<th>Other or Unknown</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>68</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>91</td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>159</td>
</tr>
</tbody>
</table>

PHS 2590 (Rev. 9/91) (Form Page 7) Page 29 (Use continuation page if necessary)
Check the appropriate boxes and provide the information requested. Make this page the last page of the signed original of the application.

1. ASSURANCES/CERTIFICATIONS
The following assurances/certifications are made by checking the appropriate boxes and verified by the signature of the OFFICIAL SIGNING FOR APPLICANT ORGANIZATION on the FACE PAGE of the application. Descriptions of individual assurances/certifications begin on page 9 of Specific Instructions.

<table>
<thead>
<tr>
<th>Assurance/Certification</th>
<th>Option 1</th>
<th>Option 2</th>
<th>Option 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Subjects</td>
<td>[X]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vertebrate Animals</td>
<td></td>
<td>[X]</td>
<td></td>
</tr>
<tr>
<td>Inventions and Patents</td>
<td>[X]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Debarment and Suspension</td>
<td>[X]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lobbying</td>
<td>[X]</td>
<td>[X]</td>
<td>[X]</td>
</tr>
<tr>
<td>Misconduct in Science</td>
<td>[X]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Civil Rights</td>
<td></td>
<td>[X]</td>
<td></td>
</tr>
<tr>
<td>Handicapped Individuals</td>
<td>[X]</td>
<td>[X]</td>
<td>[X]</td>
</tr>
<tr>
<td>Sex Discrimination</td>
<td>[X]</td>
<td>[X]</td>
<td>[X]</td>
</tr>
<tr>
<td>Age Discrimination</td>
<td>[X]</td>
<td>[X]</td>
<td>[X]</td>
</tr>
</tbody>
</table>

2. PROGRAM INCOME
All applications must indicate (Yes or No) whether program income is anticipated during the period(s) for which grant support is requested. [X] No [ ] Yes. If “Yes” use the format below to reflect the amount and source(s) of anticipated program income.

<table>
<thead>
<tr>
<th>Budget Period</th>
<th>Anticipated Amount</th>
<th>Source(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. INDIRECT COST
Indicate the applicant organization’s most recent indirect cost rate established with the appropriate DHHS Regional Office, or, in the case of nonprofit organizations, the rate established with the appropriate PHS Agency Cost Advisory Office. Indirect costs will not be paid on foreign grants, construction grants, grants to Federal organizations and grants to individuals, and usually not on conference grants. Follow any additional instructions provided for Research Career Development Awards, Institutional National Research Service Awards, and specialized grant applications.

- [ ] DHHS Agreement Dated: ____________________ [ ] No Indirect Costs Requested
- [X] No DHHS Agreement, but rates established with ________________ Date 8/26/92
- Office of Naval Research

CALCULATION*
Enter proposed budget period:
Amount of base $44,470 × Rate applied 0.449 = Indirect costs $19,967

*Check appropriate box(es):
- [X] Salary and wage base
- [ ] Modified total direct costs base
- [ ] Other base (Attach explanation)

PHS 2590 (Rev. 991) (Form Page 8) Page 30