14:48:11 OCA PAD AMENDMENT - PROJECT HEADER INFORMATION 01/26/95

Active

Project #: E-25-T30 Cost share #: Rev #: 1
Center # : 10/24-6-R7695-2A0 Center shr #: OCA file #:
Work type : RES
Contract#: AGR DTD 11/25/92 Mod #: ADMIN Document : SUBCONT
Prime #: 5 P01 HL48667-03 Contract entity: GTRC

Subprojects ? : N CFDA: N/A
Main project: PE #: N/A

Project unit: MECH ENGR Unit code: 02.010.126
Project director(s):
NEREM R M MECH ENGR (404)894-2768

Sponsor/division names: EMORY UNIVERSITY / ATLANTA, GA
Sponsor/division codes: 400 / 012

Award period: 940901 to 950831 (performance) 950831 (reports)

Sponsor amount	New this change	Total to date
Contract value	0.00	59,135.00
Funded	0.00	59,135.00
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: MECHANISMS OF ENDOTHELIAL-MONOCYTE ADHESION MOLECULAR REGULATION

PROJECT ADMINISTRATION DATA

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Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : NA NA supplemental sheet
Equipment title vests with: Sponsor X GIT
NONE PROPOSED
Administrative comments -
ADMIN MOD TO CORRECT BILLING ADDRESS.

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

(2)

NOTICE OF PROJECT CLOSEOUT

SR-254

Closeout Notice Date 08/31/95

Project No. E-25-T30 Center No. 10/24-6-R7695-2A0

Project Director NEREM R M School/Lab MECH ENGR

Sponsor EMORY UNIVERSITY/ATLANTA, GA

Contract/Grant No. AGR DTD 11/25/92 Contract Entity GTRC

Prime Contract No. 5 P01 HL48667-03

Title MECHANISMS OF ENDOTHELIAL-MONOCYTE ADHESION MOLECULAR REGULATION

Effective Completion Date 950831 (Performance) 950831 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	
Final Report of Inventions and/or Subcontracts	Y	
Government Property Inventory & Related Certificate	N	
Classified Material Certificate	N	
Release and Assignment	N	
Other	N	

Comments

Subproject Under Main Project No.

Continues Project No.

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other	N
	N

NOTE: Final Patent Questionnaire sent to PDPI.

E-25-T30 #1

(New)

FLOW AND THE ASSOCIATED SHEAR STRESS REGULATES VCAM-1 GENE EXPRESSION AND TRANSCRIPTION IN HUMAN VASCULAR ENDOTHELIAL CELLS

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Atherosclerosis is a disease with a focal pattern, one where there is a higher predilection for lesion development at sites characterized hemodynamically by low, oscillatory shear stress and prolonged residence times of macromolecular particles and cells. An important characteristic of the disease is the oxidation-reduction (redox) sensitive expression of the cell adhesion molecule VCAM-1 by the vascular endothelium.

To test the hypothesis that chronic exposure to laminar shear stress has a "protective" effect on the endothelium, inhibiting cytokine induced VCAM-1 gene expression, confluent human umbilical vein endothelial cells (HUVECs) were grown to confluency, exposed to a laminar shear stress of 5 dynes/cm^2 for 24 hours in parallel plate flow chambers, and then statically incubated in fresh media with IL-1ß (10 U/ml) for 4 hours. Total cellular RNA was isolated and analyzed by Northern filter hybridization using human adhesion molecule specific cDNA probes as previously described (Marui et al. 1993). Shear preconditioned HUVECs were markedly inhibited (80%) in their ability to activate VCAM-1 gene expression at the mRNA level, while ICAM-1 expression was increased and E-selectin gene expression did not appear to be significantly changed. To further investigate the effect of shear stress on VCAM-1 transcription, cells were transfected with a VCAM-1 promoter region fused to the reporter gene chloramphenicol acetyltransferase (CAT). Enzymatic activity was determined using ^{14}C-chloramphenicol and thin layer chromatography. The results indicate that for cells exposed to a shear stress for 24 hours and stimulated with IL-1ß, CAT enzyme activity was almost completely inhibited, as compared to statically incubated control cells, suggesting a shear stress modulation of the VCAM-1 promoter.

In a similar manner, HUVEC monolayers, subjected to shear preconditioning followed by immunofluorescent

flow cytometric analysis of VCAM-1 and ICAM-1 cell surface expression, showed that for IL-1ß stimulated monolayers, the imposition of shear stress reduced the level of VCAM-1 by approximately 85%. In contrast, for ICAM-1 the level of expression was approximately equal for both statically maintained and flow preconditioned monolayers.

Application of a suspension of the human monocyte cell line, THP-1 (previously treated with anti-LFA-1 in order to prevent ICAM-1 mediated binding), to stimulated and non-treated flow preconditioned monolayers resulted in the respective reduction in VCAM-1 mediated binding by 79 ± 4% and 90 ± 2% relative to that of stimulated HUVECs maintained in static culture. These results support the hypothesis that for VCAM-1 flow preconditioning desensitizes the endothelium to the action of stimulatory agents, such as IL-1ß, and thus has a protective effect. These effects of shear stress are strikingly similar to those of thiol antioxidant pyrrolidine dithiocarbamate (PDTC) and suggest that it is through a redox sensitive signal transduction mechanism that shear stress may control VCAM-1 expression.

For unstimulated HUVEC monolayers and in contrast to the effect of a steady shear stress where there is little effect on the level of VCAM-1 expressed, oscillatory flow studies have indicated an upregulation of VCAM-1 expression. For confluent HUVEC monolayers subjected to an oscillatory shear stress (0 ± 5 dynes/cm^2) for 24 hours, followed by immunofluorescent flow cytometric analysis, VCAM-1 expression exhibited 9-fold increase and ICAM-1 an 11-fold increase, this relative to the levels present on statically maintained endothelial cells. Comparisons of these levels with those determined for the IL-1ß stimulated cells in static culture indicated that, in both instances, the levels elicited by the oscillatory flow were approximately 50% that of the statically maintained, stimulated monolayers.

These results thus indicate that adhesion molecule expression by endothelial cells is sensitive to the exact nature of the flow environment, being different for different flow conditions. The exposure of endothelial cells to steady shear stress was found to have little effect on VCAM-1 expression for the unstimulated case, but to reduce both VCAM-1 expression and VCAM-1 mediated monocyte binding as induced by IL-1ß. This is in contrast to oscillatory flow, a prominent feature of the hemodynamics in branched regions of the vasculature, where for the unstimulated case there is an increase in adhesion molecule expression, thereby providing for enhanced monocyte binding.

REFERENCE:

Marui, N., MK Offerman, R Swerlick, C Kunsch, CA Rosen, M Ahmad, RW Alexander, and RM Medford (1993). "VCAM-1 Gene Transcription and Expression is Regulated Through an Antioxidant Sensitive Mechanism in Human Vascular Endothelial Cells." J. Clin. Invest.92: 1866-1874.