ANTIOXIDANTS & REDOX SIGNALING
Volume 9, Number 1, 2007

News & Views

ICAM-1 Cross-Linking Stimulates Endothelial Glutathione Synthesis

HEATHER M. PRUITT,[1] WILL LANGSTON,[2]
CHRISTOPHER G. KEVIL,[2] and RAKESH P. PATEL[1]

ABSTRACT

What mechanisms regulate endothelial glutathione (GSH) during inflammation? Addressing this question is critical in understanding mechanisms leading to endothelial dysfunction and cardiovascular disease. Herein, the authors show data that support the hypothesis that the intercellular cell adhesion molecule-1 (ICAM-1) regulates GSH. Ligating either constitutive or induced ICAM-1 on the endothelial surface, or exposing endothelial cells to soluble ICAM-1, increases GSH concentrations. ICAM-1 is important in mediating leukocyte adhesion and modulates endothelial signaling pathways important in controlling transmigration. The present data underscore a novel function for ICAM-1 in modulating GSH metabolism and raise the hypothesis that this adhesion molecule controls endothelial redox status under basal and inflammatory conditions. *Antioxid. Redox Signal.* 9, 159–164.

GLUTATHIONE AND REDOX MODULATION OF ENDOTHELIAL FUNCTION

Located at the interface between the circulation and the vessel wall, the endothelium plays a critical role in vascular homeostasis. Indeed, endothelial function is a clinical indicator of vascular function, with dysfunction being an independent predictor of vascular diseases such as atherosclerosis (26). The general concept is that a dysfunctional endothelium provides a surface that is pro-inflammatory, pro-thrombotic, and pro-oxidative, leading initially to enhanced leukocyte adhesion and emigration that eventually culminates in development of an atherosclerotic lesion (12). At the molecular level, many factors and mechanisms have been identified that lead to the dysfunctional endothelial phenotype. This includes lower concentrations of cellular reductants (also referred to as antioxidants) including glutathione (GSH), and increased expression of pro-inflammatory adhesion molecules (1, 2, 12).

Glutathione is a thiol-containing tripeptide that is integral in the antioxidant network and serves to both protect cells from oxidative stress and regulate redox signaling (19). For example, recent studies suggest that an adaptive strategy by which the endothelium may protect itself from cytotoxic stimuli is to respond to low nontoxic levels of these stimuli (including NO, reactive nitrogen and oxygen species, and other electrophilic compounds) by increasing GSH synthesis (17, 19). Furthermore, lower endothelial GSH concentrations are associated with impaired vascular function in hypercholesterolemia and increased risk for myocardial infarction and cerebral infarction (cardiovascular disease) (1, 11). However, mechanisms that regulate glutathione during the course of inflammation remain unclear.

[1]Department of Pathology, Molecular and Cellular Division and Center for Free Radical Biology, University of Alabama at Birmingham, Birmingham Alabama.
[2]Department of Pathology, Louisiana State University Health Sciences Center, Shreveport, Louisiana.

EMERGING CONCEPTS IN ICAM-1 BIOLOGY: MODULATION OF ENDOTHELIAL FUNCTION

ICAM-1 (intercellular cell adhesion molecule-1) is an adhesion molecule expressed constitutively on the endothelial surface and that is also significantly induced by inflammatory cytokines. The most commonly ascribed function of inducible ICAM-1 is to mediate adhesive interactions with leukocytes expressing cognate ligands (CD11a/CD18 or CD11b/CD18), although clustering of constitutive ICAM-1 on the endothelial surface also mediates adhesion during the very initial stages of the inflammatory response (6). In addition to tethering a leukocyte physically, ligation of inducible ICAM-1 activates diverse signaling pathways, depending on the origin of the endothelial cell (28). These include activation of Rho kinases and modulation of the cytoskeleton, the functional consequences of which include facilitating leukocyte transmigration (5, 15, 27). Moreover, ligation of inducible ICAM-1 with fibrinogen activates endothelial MAP (mitogen activated protein) kinases, resulting in cytoprotection (23). These data underscore the concept that this adhesion molecule plays a critical role in diverse endothelial cell functions beyond physical capture and tethering of circulating leukocytes.

ICAM-1 REGULATES ENDOTHELIAL GSH

Despite many studies documenting interplay between endothelial redox status and inflammation, little is known about the direct mechanisms involved. Using endothelial cells deficient in ICAM-1, our recent studies suggested that under basal (*i.e.*, noninflammatory) conditions, a reciprocal and inverse relationship between ICAM-1 and GSH levels exists (9). Specifically, deletion of ICAM-1 increases cellular GSH, and conversely, increasing GSH decreases constitutive ICAM-1 expression. The mechanism underlying this relationship between ICAM-1 and GSH does not involve reactive species but occurs via a post-translational mechanism that stabilizes the rate-limiting enzyme in GSH synthesis. The functional consequences of increased GSH by ICAM-1 deletion may include tempering subsequent endothelial responses to inflammatory stimuli, and modulation of endothelial migration and angiogenic responses (8). Importantly, these effects on GSH concentrations were mediated by constitutive ICAM-1, distinguishing it from ICAM-1-dependent signaling discussed above, the latter of which has been elucidated predominantly from ligation of inducible ICAM-1. To better understand how ICAM-1 regulates GSH under basal and inflammatory conditions, we tested whether ICAM-1 ligation modulates endothelial GSH.

Cross-linking of constitutive ICAM-1 increases endothelial GSH

Addition of first an anti-ICAM-1 antibody followed by a corresponding secondary antibody ligates and then clusters ICAM-1 into signaling domains such as caveolae and presumably thereby initiates diverse signaling pathways (5, 15, 23, 27). Using a similar approach, Figs. 1 and 2 show that ICAM-1 ligation also increases GSH and thereby may modulate endothelial redox signaling pathways. Figure 1 shows that cross-linking constitutive ICAM-1 in human umbilical vein endothelial cells (HUVEC) increases GSH levels about twofold. Addition of either anti-ICAM-1 or secondary antibody alone had no effect on GSH. Moreover, addition of cross-linking antibodies to ICAM-1-/- mouse aortic endothelial cells (MAEC) or ligation of ICAM-2 had no effect, demonstrating specificity of responses to ICAM-1 ligation (Fig. 1). The biological role of basally expressed ICAM-1 remains poorly defined with potential functions including immune surveillance under noninflammatory conditions and mediation of early (minutes) leukocyte adhesion upon administration of an inflammatory stimulus being described (6). Our recent work with ICAM-1-deficient endothelial cells suggests that constitutive ICAM-1 regulates endothelial GSH (9), which in turn modulates endothelial migration and responses to angiogenic stimuli (Langston and Kevil, unpublished observations). Results shown in Fig. 1 extend these observations to include ICAM-1 ligation as a stimulus for regulating basal endothelial GSH concentrations. The biological importance for basal ICAM-1 regulation of GSH remains unclear. An emerging concept in endothelial and redox biology in general is that cellular responses to low nontoxic concentrations of stimuli (that at higher concentrations would induce cell death and that include a host of reactive species) is to increase GSH; and this may serve as an 'adaptation' strategy that allows the cell to survive upon a subsequent exposure to higher concentrations of the stimulus (3, 17). This may play a critical role, for example, in phenomena such as

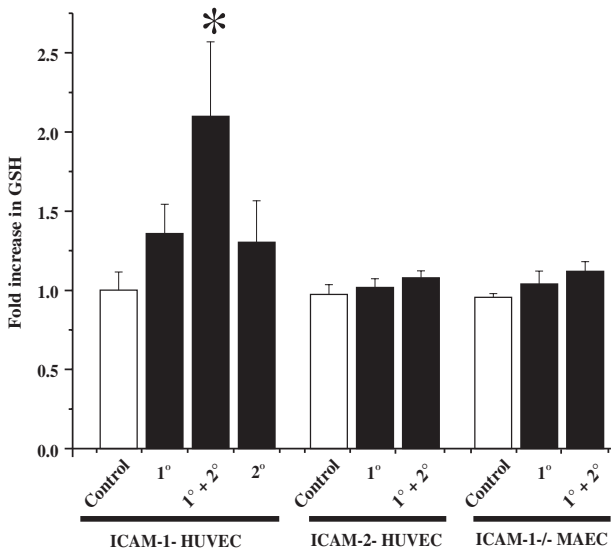

FIG. 1. Cross-linking of constitutive ICAM-1 increases endothelial GSH. Constitutive ICAM-1 or ICAM-2 was cross-linked in HUVEC or MAEC deficient in ICAM-1 (ICAM-1-/-) and GSH measured as described in Methods. Data show fold changes relative to respective controls and are mean ± SEM, $n = 3–5$, $*p = < 0.05$.

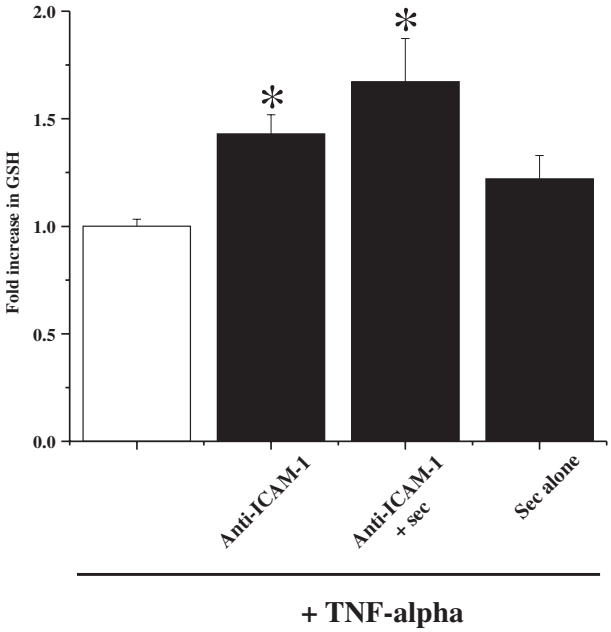

FIG. 2. Cross-linking of inducible ICAM-1 increases endothelial GSH. TNF-α (10 ng/ml) was added to HUVEC for 4–5 h to induce ICAM-1. ICAM-1 was then cross-linked and GSH measured as described in Methods. Data show fold changes relative to respective controls and are mean ± SEM, $n = 3$. *$p = < 0.05$.

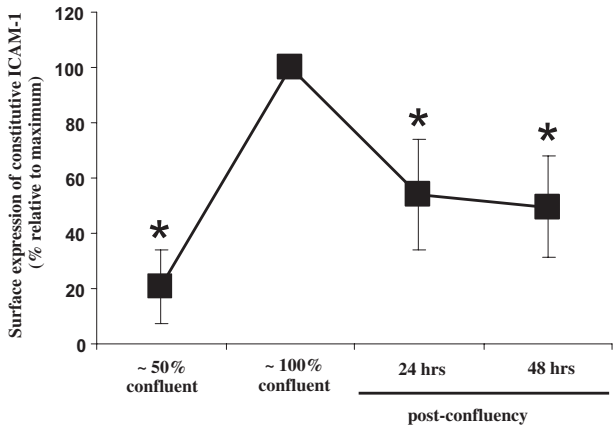

FIG. 3. Surface expression of constitutive ICAM-1 changes with endothelial cell confluency. Surface expression of ICAM-1 was measured by ELISA and paralleled cell growth being maximum as cells became confluent. However, postconfluency ICAM-1 levels decrease reaching a steady state close to 50% of maximum at 24 h. Data show levels of ICAM-1 relative to maximum and are mean ± SEM ($n = 3$), *$p = < 0.05$ relative to maximum at confluent state.

the exercise paradox in which oxidative stress induced by exercise yields cardioprotective effects (14). Given that circulating leukocytes are also activated during exercise (16), it is interesting to speculate that ligation of constitutive ICAM-1 may provide a stimulus for adaptive responses in the endothelium, resulting in increased GSH.

Cross-linking of inducible ICAM-1 increases endothelial GSH

As discussed above, ICAM-1 signaling is better characterized with the inducible isoform. Figure 2 shows that ligation of ICAM-1 on TNFα-activated HUVEC also increases GSH. Whereas both primary and secondary antibodies were required with constitutive ICAM-1, cross-linking with an anti-ICAM-1 antibody alone increased GSH, which was not affected by further cross-linking/clustering with a secondary antibody. Why cross-linking of inducible ICAM-1 with an anti-ICAM-1 antibody alone was sufficient to increase GSH compared to a requirement of both anti-ICAM-1 and secondary antibody with constitutive ICAM-1 remains unclear.

In the course of the current studies, it was noticed that if cross-linking of constitutive ICAM-1 was initiated under identical experimental conditions but with cells that were confluent for ≥24 h, no changes in GSH were observed (results not shown). Since ICAM-1 expression may change with cell growth and confluence and the requirement for stimuli to be above a threshold concentration for activation of signaling, we measured constitutive ICAM-1 levels at different stages of cell growth. Figure 3 shows that constitutive ICAM-1 surface expression was significantly lower in cells confluent ≥24 h compared to cells confluent <24 h. We speculate that for ICAM-1 ligation to initiate signaling and increase GSH concentrations there is a requirement for a threshold surface ICAM-1 concentration, below which ligation does not initiate cell signaling. Such a requirement for a threshold concentration may explain why cross-linking alone of inducible ICAM-1, present in higher concentrations compared to constitutive ICAM-1, was sufficient to stimulate GSH. A requirement for threshold levels of constitutive ICAM-1 would also allow regulation of GSH concentrations in specific vascular compartments. *In vivo* studies have demonstrated constitutive ICAM-1 expression varies depending on the vascular bed in question (18). To our knowledge, no such systematic analysis of GSH concentrations has been performed. Based on the data shown herein, however, we suggest that an important determinant of basal GSH levels in the endothelium will be constitutive ICAM-1.

GSH synthesis changes with cell growth, and recently was shown to be down regulated during apoptosis (4, 10, 20). However, the dynamic changes in GSH concentration during inflammation are poorly defined. Our studies suggest that GSH concentrations will increase in later stages of the inflammatory response, secondary to leukocyte adherence. Potential functions for this increase in GSH include cytoprotection and mediating resolution of inflammation. Indeed, increasing GSH concentrations decrease adhesion molecule expression and may allow for optimal generation of other species that exert anti-inflammatory functions, for example, nitric oxide (1).

An important determinant of the effects of increasing GSH is the GSH:GSSG ratio. ICAM-1 deletion increases this ratio in addition to increasing total GSH (9). A similar effect by ICAM-1 cross-linking would lead to a greater reductive capacity and increased ability of cells to withstand oxidative

stimuli, consistent with the proposal that ligation of ICAM-1 would be cytoprotective. Ongoing studies are evaluating this hypothesis. In summary, these data support a role for ICAM-1 as regulator of GSH in endothelial cells under basal and inflammatory stress conditions.

sICAM-1 and GSH

Our previous data have shown that genetic deletion of ICAM-1 results in increased GSH levels (9). How both deletion and ligation of ICAM-1 lead to increased GSH concentrations is at first glance contradictory. In the aforementioned study, ICAM-1-deficient endothelial cells were derived from knockout mice in which exon 4 of the ICAM-1 gene was deleted (29). A more detailed characterization of these mice demonstrated that this deletion in fact resulted in expression of alternatively spliced isoforms of ICAM-1 that are readily cleaved and secreted, resulting in increased levels of sICAM-1 in the circulation (24). Data shown in Fig. 4A confirms this observation, demonstrating increased levels of sICAM-1 in conditioned media from ICAM-1-deficient endothelial cells compared to corresponding wild-type cells. ICAM-1 has a short 28-amino acid cytoplasmic tail, which importantly remains in the cell upon cleavage of the extracellular IgG domains (22). The cytoplasmic domain lacks characterized signaling motifs, although a weak immunoreceptor tyrosine-based inhibitory motifs (ITIM)-like motif has been described (23) and recent data support a role in transmitting intracellular signals upon ICAM-1 ligation to mediate leukocyte transmigration (5, 30). We speculate therefore that in our experimental models and under basal conditions, ICAM-1 deletion mimics ICAM-1 ligation, with both activating pathways that stimulate GSH synthesis via the cytoplasmic domain. The lack of clear signaling motifs on the cytoplasmic domain of ICAM-1 renders speculation of potential mechanisms difficult. However, our previous studies suggest an increased activity of the rate-limiting enzyme in GSH biosynthesis, γ-glutamate cysteine ligase, plays a role (9). Moreover, this occurs via post-translational mechanisms, suggesting that the cytoplasmic tail of ICAM-1 could affect protein turnover and stability.

Finally, these results suggest a general interplay between ICAM-1 and endothelial GSH metabolism during both basal and inflammatory conditions. sICAM-1 is also produced during inflammation and is largely used as a marker to assess risk for inflammatory diseases. However, it has also been investigated as a potential anti-inflammatory effector by binding to circulating leukocytes (13). Moreover, an emerging concept is that soluble factors associated with inflammation are not only markers but may modulate inflammatory responses (*e.g.*, C-reactive protein) (25). We therefore tested whether sICAM-1 could modulate endothelial GSH levels. Interestingly, Fig. 4B shows that sICAM-1 increases GSH in a dose-dependent manner that saturates ~1 ng/ml, and reveals a potentially novel anti-inflammatory mechanism for sICAM-1 by enhancing the antioxidant status in endothelial cells. The observation that sICAM-1 effects are saturable also indicates a role for sICAM-1 interactions with a cell-surface receptor or enzyme.

In summary, this study further clarifies the relationship between ICAM-1 and GSH in endothelial cells. Ligation of ICAM-1 under basal or inflammatory conditions increases GSH concentrations. Given the anti-inflammatory/antioxidant and general vasculo-protective effects of GSH, this activity of basal ICAM-1 may be viewed as a mechanism to ensure vascular homeostasis or an adaptive response to 'low' levels of inflammatory stimuli. In the context of inflammation, increasing GSH may limit or begin to resolve the inflammatory response. Similarly, sICAM-1 also stimulates GSH, further indicating that GSH metabolism is integrated and regulated by ICAM-1 during the inflammatory response. Although the precise mechanisms by which ICAM-1 regulates GSH remain to be elucidated, the data presented herein indicate that in addition to modulating leukocyte adhesion and transmigration, ICAM-1 is a critical regulator of endothelial redox status via GSH.

ACKNOWLEDGMENTS

This work was supported from Grants HL70146 (RPP) and HL80482 (CGK) from the National Institutes of Health.

ABBREVIATIONS

ICAM-1, intercellular adhesion molecule-1; ICAM-2, intercellular adhesion molecule-2; sICAM-1, soluble intercellular adhesion molecule-1; GSH, glutathione (oxidized + reduced); HUVEC, human umbilical vein endothelial cells;

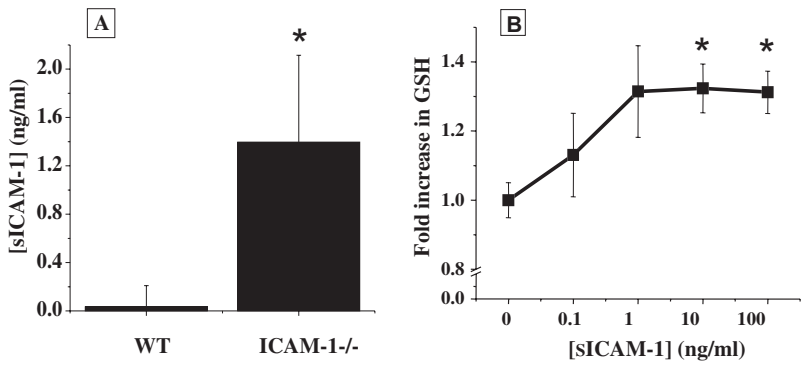

FIG. 4. sICAM-1 and endothelial GSH. (**A**) sICAM-1 was measured in conditioned media from either WT or ICAM-1-/- MAEC. Data show mean ± SEM $n = 6$, $*p = < 0.05$. (**B**) sICAM-1 stimulates GSH synthesis in HUVEC. sICAM-1 was incubated with HUVEC for 14 h, and GSH measured. Data show fold change relative to control and are mean ± SEM, $n = 3$, $*p = < 0.01$.

MAEC, mouse aortic endothelial cells; TNFα, tumor necrosis factor-α.

APPENDIX

Materials

All materials were obtained from Sigma Chemical Co (St. Louis, MO) unless otherwise stated. Human Umbilical Vein Endothelial Cells (HUVEC) were purchased from Cambrex (Rockland, MD) and cultured as described below. Mouse aortic endothelial cells (MAEC) from wild-type (WT MAEC) or ICAM-1 deficient (ICAM-1 −/− MAEC) were isolated as previously described (7). Monoclonal antibodies used in cross-linking of ICAM-1 on HUVEC were obtained from R&D Systems, Minneapolis, MN (mouse anti-human, clone 11C81), from Bender Med Systems, Burlingame, CA (BMS109) for ICAM-2, and YN-1 (ATCC) for ICAM-1 on MAEC. Corresponding secondary goat anti-mouse isotype matched antibodies were obtained from R&D Systems. Recombinant soluble ICAM-1 (sICAM-1) corresponding to a form truncated at the ectodomain side of the beginning of the transmembrane region (aa.472) was purchased from Bender Med Systems.

Cell culture

WT and ICAM-1 deficient (-/-) MAEC were cultured as previously described (9) in MCDB-131 complete media + 10% FBS (Atlanta Biologicals, Atlanta, GA). Cells between passages 3–5 and 3–4 days post-confluency were used in the studies reported here. HUVEC were cultured by seeding at a density of 12,500 cells/cm^2 onto gelatin/fibronectin coated tissue culture in EGM complete media + 2% FBS (Clonetics, Rockland, MD) and used between passages 3–8, and within 1 day of reaching confluency.

ICAM-1 and -2 cross-linking

HUVEC were grown in 12-well tissue culture plates, washed 2X with sterile HBSS, and primary antibodies added (5 μg/ml) in HBSS (0.5 ml) for 20 min. Cells were then gently washed 2X with HBSS and secondary antibody (2.5–5 μg/ml) added for 20 min. After a final wash (2X HBSS), cells were incubated in EGM + 2% FBS for 18–22 h, and GSH concentrations measured. For TNFα studies, HUVEC were first treated with TNFα (5–10 ng/ml) for 4–5 h. HUVEC were then washed (2X HBSS), rinsed, and ICAM-1 cross-linking according to the protocol described above.

ELISA detection of surface ICAM-1 expression

HUVEC were grown in 96-well dishes in EGM + 2% FBS. At various times after splitting to achieve different confluent states, cells were washed 3X with sterile PBS and fixed with 1% paraformaldeyde, 20 min, 25°C. For detection of surface ICAM-1, cells were first blocked with PBS + 3% BSA, 30–45 min, 25°C, and then washed 2X PBS + 1% BSA. Cells were then treated with PBS + 1% BSA alone or containing either anti-ICAM-1 antibody (0.1 μg/ml), 30 min, 25°C, followed by washing 2X PBS + 1% BSA, and then incubation with corresponding HRP-linked secondary antibody, 30 min, 25°C. After a final wash, TMB (supersentive for ELISA, Sigma) was diluted 1:1 in water and added to cells. Absorbance increase at 590 nm was monitored continuously every 2 min for 30 min using a Victor^2 plate reader (Perkin Elmer, Shelton, CT). The initial rate of absorbance is increase was calculated and is proportional to concentration of ICAM-1. In all experiments, control incubations with secondary antibody alone were included to determine background rate of absorbance increase at 590 nm due to nonspecific interactions. These rates were subtracted from rates obtained with anti-ICAM-1 antibody.

ELISA detection of sICAM-1

Conditioned media from WT or ICAM-1-/- MAEC were collected and concentrated using centricons (MW cutoff 10 KDa), and then sICAM-1 was measured using a commercially available ELISA kit (Bender Med Systems). Concentrations were calculated after subtracting media blanks and by comparison to a standard curve generated using recombinant sICAM-1.

GSH measurement

Total (oxidized + reduced) GSH was measured as previously described in cell lysates (9, 21). Cell lysates were obtained after rinsing 2X in ice cold PBS + 10 μ*M* DTPA, and then lysis in PBS + 0.1% Triton X-100 followed by centrifugation (12,000 rpm, 20 min, 5°C). GSH concentrations were determined on the lysates and normalized to total protein content (determined using BioRad protein assay with reference to BSA as a standard, Biorad, Hercules, CA).

Statistical analysis

Data were analyzed by Students *t*-test with significance set at 0.05. All data were compared to corresponding controls. Corresponding number of replicates are indicated in figure legends.

REFERENCES

1. Adachi T and Cohen RA. Decreased aortic glutathione levels may contribute to impaired nitric oxide-induced relaxation in hypercholesterolaemia. *Br J Pharmacol* 129: 1014–1020, 2000.
2. Biswas SK, Newby DE, Rahman I, and Megson IL. Depressed glutathione synthesis precedes oxidative stress and atherogenesis in Apo-E(-/-) mice. *Biochem Biophys Res Commun* 338: 1368–1373, 2005.
3. Dickinson DA, Moellering DR, Iles KE, Patel RP, Levonen AL, Wigley A, Darley–Usmar VM, and Forman HJ. Cytoprotection against oxidative stress and the regulation of glutathione synthesis. *Biol Chem* 384: 527–537, 2003.
4. Franklin CC, Krejsa CM, Pierce RH, White CC, Fausto N, and Kavanagh TJ. Caspase-3-dependent cleavage of the glutamate-L-cysteine ligase catalytic subunit during apoptotic cell death. *Am J Pathol* 160: 1887–1894, 2002.
5. Greenwood J, Amos CL, Walters CE, Couraud PO, Lyck R, Engelhardt B, and Adamson P. Intracellular domain of brain endothelial intercellular adhesion molecule-1 is essential for T lymphocyte-mediated signaling and migration. *J Immunol* 171: 2099–2108, 2003.
6. Javaid K, Rahman A, Anwar KN, Frey RS, Minshall RD, and Malik AB. Tumor necrosis factor-alpha induces early-onset endothelial adhesivity by protein kinase Czeta-dependent activation of intercellular adhesion molecule-1. *Circ Res* 92: 1089–1097, 2003.
7. Kevil CG and Bullard DC. *In vitro* culture and characterization of gene targeted mouse endothelium. *Acta Physiol Scand* 173: 151–157, 2001.
8. Kevil CG, Orr AW, Langston W, Mickett K, Murphy–Ullrich J, Patel RP, Kucik DF, and Bullard DC. Intercellular adhesion molecule-1 (ICAM-1) regulates endothelial cell motility through a nitric oxide-dependent pathway. *J Biol Chem* 279: 19230–19238, 2004.
9. Kevil CG, Pruitt H, Kavanagh TJ, Wilkerson J, Farin F, Moellering D, Darley–Usmar VM, Bullard DC, and Patel RP. Regulation of endothelial glutathione by ICAM-1: implications for inflammation. *FASEB J* 18: 1321–1323, 2004.
10. Kobayashi SD, Voyich JM, Somerville GA, Braughton KR, Malech HL, Musser JM, and DeLeo FR. An apoptosis-differentiation program in human polymorphonuclear leukocytes facilitates resolution of inflammation. *J Leukoc Biol* 73: 315–322, 2003.
11. Koide S, Kugiyama K, Sugiyama S, Nakamura S, Fukushima H, Honda O, Yoshimura M, and Ogawa H. Association of polymorphism in glutamate-cysteine ligase catalytic subunit gene with coronary vasomotor dysfunction and myocardial infarction. *J Am Coll Cardiol* 41: 539–545, 2003.
12. Libby P. Inflammation in atherosclerosis. *Nature* 420: 868–874, 2002.
13. Martin S, Heidenthal E, Schulte B, Rothe H, and Kolb H. Soluble forms of intercellular adhesion molecule-1 inhibit insulitis and

onset of autoimmune diabetes. *Diabetologia* 41: 1298–1303, 1998.
14. Meilhac O, Ramachandran S, Chiang K, Santanam N, and Parthasarathy S. Role of arterial wall antioxidant defense in beneficial effects of exercise on atherosclerosis in mice. *Arterioscler Thromb Vasc Biol* 21: 1681–1688, 2001.
15. Millan J, Hewlett L, Glyn M, Toomre D, Clark P, and Ridley AJ. Lymphocyte transcellular migration occurs through recruitment of endothelial ICAM-1 to caveola- and F-actin-rich domains. *Nat Cell Biol* 8: 113–123, 2006.
16. Mills PJ, Hong S, Redwine L, Carter SM, Chiu A, Ziegler MG, Dimsdale JE, and Maisel AS. Physical fitness attenuates leukocyte–endothelial adhesion in response to acute exercise. *J Appl Physiol* 101: 785–788, 2006.
17. Moellering DR, Levonen AL, Go YM, Patel RP, Dickinson DA, Forman HJ, and Darley–Usmar VM. Induction of glutathione synthesis by oxidized low-density lipoprotein and 1-palmitoyl-2-arachidonyl phosphatidylcholine: protection against quinone-mediated oxidative stress. *Biochem J* 362: 51–59, 2002.
18. Panes J, Perry MA, Anderson DC, Manning A, Leone B, Cepinskas G, Rosenbloom CL, Miyasaka M, Kvietys PR, and Granger DN. Regional differences in constitutive and induced ICAM-1 expression *in vivo*. *Am J Physiol* 269: H1955–1964, 1995.
19. Patel RP, Levonen A, Crawford JH, and Darley–Usmar VM. Mechanisms of the pro- and anti-oxidant actions of nitric oxide in atherosclerosis. *Cardiovasc Res* 47: 465–474, 2000.
20. Poot M, Teubert H, Rabinovitch PS, and Kavanagh TJ. De novo synthesis of glutathione is required for both entry into and progression through the cell cycle. *J Cell Physiol* 163: 555–560, 1995.
21. Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Anal Biochem* 27: 502–522, 1969.
22. Tsakadze NL, Sen U, Zhao Z, Sithu SD, English WR, and D'Souza SE. Signals mediating cleavage of intercellular adhesion molecule-1. *Am J Physiol Cell Physiol* 287: C55–63, 2004.
23. Tsakadze NL, Zhao Z, and D'Souza SE. Interactions of intercellular adhesion molecule-1 with fibrinogen. *Trends Cardiovasc Med* 12: 101–108, 2002.
24. van Den Engel NK, Heidenthal E, Vinke A, Kolb H, and Martin S. Circulating forms of intercellular adhesion molecule (ICAM)-1 in mice lacking membranous ICAM-1. *Blood* 95: 1350–1355, 2000.
25. Venugopal SK, Devaraj S, Yuhanna I, Shaul P, and Jialal I. Demonstration that C-reactive protein decreases eNOS expression and bioactivity in human aortic endothelial cells. *Circulation* 106: 1439–1441, 2002.
26. Vita JA. Endothelial function and clinical outcome. *Heart* 91: 1278–1279, 2005.
27. Wang Q and Doerschuk CM. The signaling pathways induced by neutrophil-endothelial cell adhesion. *Antioxid Redox Signal* 4: 39–47, 2002.
28. Wang Q, Pfeiffer GR, 2nd, Stevens T, and Doerschuk CM. Lung microvascular and arterial endothelial cells differ in their responses to intercellular adhesion molecule-1 ligation. *Am J Respir Crit Care Med* 166: 872–877, 2002.
29. Xu H, Gonzalo JA, St Pierre Y, Williams IR, Kupper TS, Cotran RS, Springer TA, and Gutierrez–Ramos JC. Leukocytosis and resistance to septic shock in intercellular adhesion molecule 1-deficient mice. *J Exp Med* 180: 95–109, 1994.
30. Yang L, Froio RM, Sciuto TE, Dvorak AM, Alon R, and Luscinskas FW. ICAM-1 regulates neutrophil adhesion and transcellular migration of TNF-alpha-activated vascular endothelium under flow. *Blood* 106: 584–592, 2005.

Address reprint requests to:
Rakesh P. Patel
Department of Pathology
901 19th Street South
BMR-2, Room 307
University of Alabama at Birmingham
Birmingham, AL 35216

E-mail: patel@path.uab.edu

Date of first submission to ARS Central, August 18, 2006; date of final revised submission, August 31, 2006; date of acceptance, September 1, 2006.

This article has been cited by:

1. A.S. Bala Bhaskar, Nimesh Gupta, P.V. Lakshmana Rao. 2012. Transcriptomic profile of host response in mouse brain after exposure to plant toxin abrin. *Toxicology* **299**:1, 33-43. [CrossRef]
2. Shyamal C. Bir, Gopi K. Kolluru, Kai Fang, Christopher G. Kevil. 2012. Redox balance dynamically regulates vascular growth and remodeling. *Seminars in Cell & Developmental Biology* **23**:7, 745-757. [CrossRef]
3. Arshad Rahman , Fabeha Fazal . 2009. Hug Tightly and Say Goodbye: Role of Endothelial ICAM-1 in Leukocyte Transmigration. *Antioxidants & Redox Signaling* **11**:4, 823-839. [Abstract] [Full Text PDF] [Full Text PDF with Links]