Antioxidant regulation of cell adhesion

CHANDAN K. SEN and SASHWATI ROY

Departments of Surgery and Molecular and Cellular Biochemistry, Heart and Lung Research Institute, The Ohio State University Medical Center, Columbus, OH 43210; and Department of Physiology, University of Kuopio, FINLAND

ABSTRACT

SEN, C. K., and S. ROY. Antioxidant regulation of cell adhesion. *Med. Sci. Sports Exerc.*, Vol. 33, No. 3, 2001, pp. 377–381. Cell-cell and cell-matrix contacts are dependent on cell surface density, localization, and avidity state of surface-localized adhesion molecules. Cell adhesion represents a process that is centrally important in immune function and inflammation. This process is sensitive to various agonists including oxidants. Oxidants may directly as well as indirectly induce cell adhesion. In other cases, cytokines and related agents may induce cell adhesion by oxidant-dependent mechanisms. Various redox-sensitive sites in the signal transduction path leading to cell adhesion have been identified. Different chemical classes of nutritional antioxidants regulate cell adhesion by modulating specific signal transduction pathways. Numerous studies have confirmed that physical exercise influences the redox status of various cells and tissues. Recent evidences also show that physical exercise influences several cell adhesion related molecules. Whether such regulation has a redox component remains to be tested. Antioxidant supplementation studies testing the effect of exercise on cell adhesion should provide critical insight. **Key Words:** REDOX, OXIDATIVE STRESS, INFLAMMATION, LYMPHOCYTE, IMMUNE FUNCTION

Cell adhesion is a multi-step process including rolling, firm attachment, and transmigration of leukocytes (Fig. 1). Selectins and integrins are necessary for an initial tethering, triggering, firm attachment, and transendothelial migration of lymphocytes. Intercellular adhesion molecule-1 (ICAM-1, CD54) and vascular cell adhesion molecule-1 (VCAM-1, CD 106; Fig. 1) are inducible cell surface glycoproteins. The ligands for ICAM-1 and VCAM-1 on lymphocyte are lymphocyte function-associated antigen-1 (LFA-1, CD11a/CD18) and very late antigens-4 (VLA-4, CD49 d/CD29), respectively (Fig. 1). The inappropriate or abnormal sequestration of leukocytes at specific sites is a central component in the development of a variety of autoimmune diseases and pathologic inflammatory disorders (5,6).

Cell adhesion molecule expression and adhesive properties of cells are greatly modified by several conditions involving redox imbalances. The following two lines of evidences indicate that the overall cell adhesion process is redox regulated: i) direct activation of cell adhesion processes by oxidants (29,34), and ii) inhibitory action of antioxidants on cell adhesion molecule expression and function (29).

0195-9131/01/3303-0377/$3.00/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

Submitted for publication October 1999.
Accepted for publication May 2000.

Physical Exercise and Cell Adhesion

Numerous independent studies confirm that under certain conditions physical exercise causes redox changes in various tissues, including blood (38–40). Although the underlying mechanisms are yet unclear, various types of physical exercise have been shown to influence the adhesion molecule profile of peripheral blood lymphocytes (10,14,18,23,24,28,43). The pulmonary vascular bed is an important reservoir for the marginated pool of leukocytes that can be mobilized by exercise. During the first 10–30 min of exercise, an almost maximal increase of T and B lymphocytes, monocytes, and natural killer cells from the marginal pool into the blood circulation is hypothesized to be facilitated via decreased function of adhesion molecules (10,28). Indeed, moderate intensity endurance exercise decreases integrin expression in human lymphocytes (14). The effect of exercise on integrins appears to be largely dependent on exercise intensity and duration. Expression of CD11b on granulocytes increases with intense endurance exercise but not with moderate endurance training. Thus, exhaustive exercise may be one stimulus for the up-regulation of integrin adhesive receptors on granulocytes. This phenomenon could be in part responsible for increased adhesion of granulocytes to endothelial cells and could facilitate tissue infiltration after endurance exercise.

Physical exercise also decreases both the relative presence (%CD62 L+) (24) as well as expression (43) of L-selectin in human peripheral blood lymphocytes. The possible mechanisms, yet unresolved, that may explain for such effect include i) shedding of L- selectin, ii) selective entry of L-selectin-negative subsets, and/or iii) selective removal of

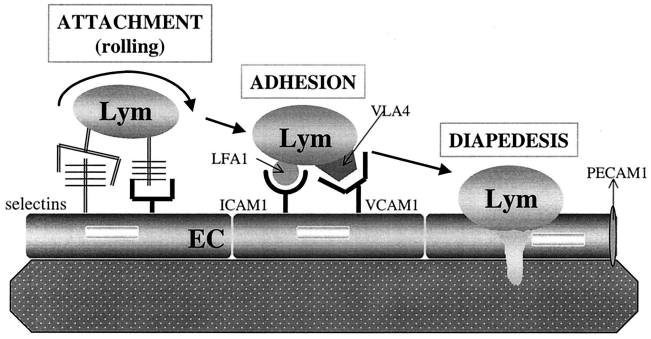

FIGURE 1—Multi-step model of leukocyte-endothelial cell adhesion. Leu, leukocyte; EC, endothelial cell. VLA-4, ligand for VCAM; LFA-1, ligand for ICAM-1.

L-selectin-positive subsets. In contrast, L-selectin expression in monocytes increased in response to exercise (21), suggesting that exercise down-regulates lymphocyte L-selectin expression by a cell-type specific mechanism. Fewer studies have examined the effect of exercise on ICAM expression, and at present there is no study that has reported the effect of exercise on ICAM expression in lymphocyte subsets. It has, however, been shown in humans that a single bout of exercise significantly increases the presence of soluble (shed from cells) ICAM-1 (CD54) in the circulation (28). Although it is clear that the expression of ICAM-1 is oxidant sensitive (Fig. 2), it is unknown whether the effect of exercise on ICAM-1 expression is oxidant-mediated.

Oxidants as Inducers of Cell Adhesion

Oxidants (e.g., H_2O_2, $O_2^{\cdot-}$ and $HO^{\cdot}$) are known to induce adherence of leukocytes to endothelial cells (31,34). Treatment of human umbilical vein endothelial cells (HUVEC) with oxidant generating systems such as hypoxanthine and xanthine oxidase ($O_2^{\cdot-}$ generating system) or oxidants such as H_2O_2, induced adherence of neutrophils to HUVEC (31,34). Catalase (200 $IU \cdot mL^{-1}$) completely abolished the increase in adherence, whereas superoxide dismutase (100 $IU \cdot mL^{-1}$) had no effect, suggesting that H_2O_2, and not $O_2^{\cdot}$, accounts for the effects of reactive oxygen species on cell adhesion (34). Direct activation of HUVEC with H_2O_2 also increased adherence of leukocytes to these cells (31). Overexpression of 15-lipoxygenase, a oxidant-generating enzyme, in human endothelial cells induces VCAM-1 expression by a NF-$\varkappa$B dependent antioxidant-sensitive mechanism (44). Evidence suggesting that oxidants regulate cell adhesion has been also obtained from the study of ionizing radiation, cigarette smoke, and ozone as well as ischemia-reperfusion.

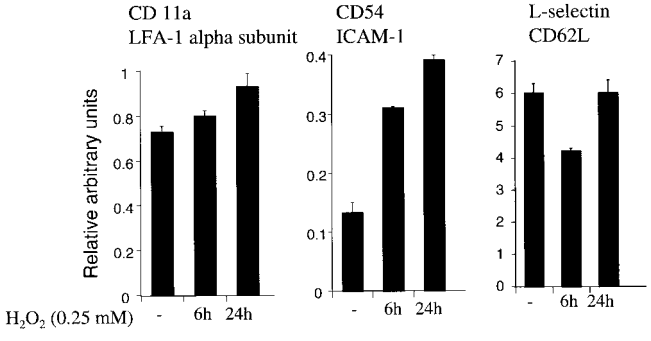

FIGURE 2—Oxidant-induced changes in adhesion molecule expression of human Jurkat T cells. Cells were treated with 0.25 mM hydrogen peroxide for the indicated time intervals. Adhesion molecule expression was determined using flow cytometry as described previously (13,31). Hydrogen peroxide potently enhanced ICAM-1 (CD54) expression, whereas the expression of leukocyte function associate complex LFA-1 (CD11a) was marginally influenced and the expression of L-selectin (CD62 L) was not influenced at all after 24 h. Differential regulation of various adhesion molecules was thus evident. Values are mean ± SD.

One mechanism by which oxidants influence cell adhesion is by inducing certain protein tyrosine phosphorylation reactions (29). $pp125^{FAK}$ (focal adhesion kinase; FAK) is one such oxidant-sensitive kinase (29,33). Tyrosine phosphorylation of the proteins of focal adhesions (FAK, paxillin, and p130cas) is an early event that regulate the interaction of integrins with the cytoskeleton and/or with the extracellular matrix (33). The other major proteins identified with increased phosphorylation following exposure to $O_2^{\cdot-}$ were paxillin (PAX) and p130cas (29). Both of these are focal adhesion proteins and serve as substrates of FAK (42). Increase in phosphorylation of FAK, paxillin, and p130cas was evident as early as 1 min after $O_2^{\cdot-}$ exposure. The phosphorylation levels continued to increase at least up to 15 min after the exposure to $O_2^{\cdot-}$. The $O_2^{\cdot-}$ treatment of cells did not affect the total protein levels of FAK, paxillin or p130cas (29). Other possible mechanisms by which oxidants regulate cell adhesion have not been characterized at present.

Antioxidant Inhibition of Induced Cell Adhesion

Several classes of antioxidants (e.g., thiol, phenolic, and flavonoid) down-regulate inducible cell adhesion molecule expression as well as cell-cell adhesion. The specific mechanism for each chemical class of antioxidant seems to be unique.

Thiol antioxidants

Several studies have shown that thiol antioxidants (35–37) down-regulate cytokine- or oxidant-induced expression of adhesion molecules (1,22,25,29–31,45). At a concentration as high as 20 mM, N-acetylcysteine (NAC) has been shown to strongly inhibit ICAM-1 expression induced by H_2O_2 or cytokines in keratinocytes, whereas under the same conditions pyrrolidine dithiocarbamate (PDTC) was less effective in preventing inducible ICAM-1 expression (12). In HUVEC, however, interleukin-1β-activated VCAM-1 gene expression has been observed to be repressed approximately 90% by the both antioxidants PDTC (50 μM) as well as NAC (30 mM) (22). NAC is a clinically safe drug. In humans, NAC supplementation has been shown to prevent exercise-induced oxidative stress in peripheral blood as measured by glutathione oxidation (40). Pharmacokinetic studies of NAC in humans show that only up to 25 μM of NAC is available in human plasma after oral intake. How-

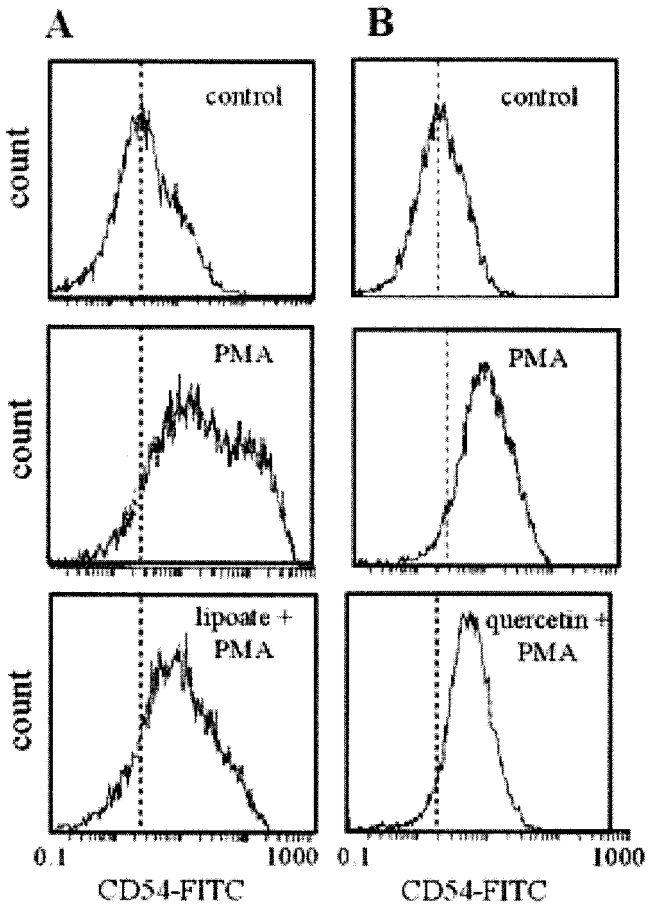

FIGURE 3—Antioxidant nutrients α-lipoic acid and quercetin suppress phorbol ester-induced expression of ICAM-1 in human endothelial cells. Cells were treated with A) racemic lipoic acid 100 μM for 48 h or B) quercetin 50 μM for 24 h before phorbol ester challenge. PMA, treated with phorbol myristate acetate 100 nM for 24 h after lipoate or no treatment. ICAM-1 expression was determined using a flow cytometer as described previously (13,31).

ever, in all of the studies showing the efficacy of NAC to inhibit agonist-induced ICAM-1 or VCAM-1 expression, high millimolar range (5–30 mM) of the drug was necessary. PDTC, the other thiol antioxidant used widely in these studies, has never been tested for safety in humans.

We investigated the effects of α-lipoic acid on agonist (phorbol ester and TNF-α)-induced cell adhesion processes in human endothelial cells (Fig. 3). α-Lipoate has been safely used for human therapy to treat complications associated with diabetes. Pharmacokinetic studies of α-lipoate have shown that after a single orally administered dose (10 mg·kg body weight), the plasma concentration may reach up to 60–70 μM. Higher concentrations of α-lipoate can be achieved if the drug is administered intravenously (27). Dietary lipoic acid supplementation has been recently shown to be available to tissues and favorably influence exercise-induced oxidative stress (15,16). At clinically relevant doses (50–100 μM), lipoate down-regulated agonist induced adhesion of T-cells to endothelial cells, and the agonist induced ICAM-1 and VCAM-1 expression on endothelial cells (29–31).

Vitamin E

In addition to decreasing low-density lipoprotein (LDL) oxidation, α-tocopherol has been suggested to exert intracellular effects on adhesive properties of cells (e.g., monocytes and platelets) that are crucial in atherogenesis (7). Pretreatment of HUVEC with α-tocopherol has been shown to down-regulate agonist (interleukin-1, thrombin, or PMA)-induced monocytic adhesion to HUVEC (9). Such inhibition correlates with decreased steady state levels of E-selectin mRNA and cell surface expression of E-selectin (9). Probucol also showed a similar effect on cell adhesion as that of α-tocopherol (9). Co-treatment of lipoate and α-tocopherol has been observed to be more effective in down-regulating agonist-induced adhesion of leukocytes to endothelial cells compared with treatment with either of the antioxidants alone (30).

Vitamin C

Cigarette smoke-induced leukocyte adhesion to micro- and macro-vascular endothelium and leukocyte-platelet aggregate formation is prevented by dietary or intravenous pretreatment with the water-soluble antioxidant ascorbate (20). Restoration of reduced plasma vitamin C concentrations in smokers by oral supplementation has been shown to decrease cigarette smoke-induced monocyte adhesion (46).

Flavonoids

Treatment of human endothelial cells with certain hydroxyflavones and flavanols inhibit cytokine-induced ICAM-1, VCAM-1, and E-selectin expression in human endothelial cells (11). Apigenin is a potent flavone that has been reported to inhibit adhesion molecule expression in endothelial cells in a dose- and time-dependent manner by regulating transcription (11). Apigenin is also known to inhibit TNF-α induced ICAM-1 expression *in vivo* (26). Cell adhesion regulatory effects of flavonoids have been evident in other independent studies. The flavonoid delphinidin chloride (CAS 528–53–0, IdB 1056) inhibited acetylcholine and sodium nitroprusside-induced adherence of leukocytes to the venular endothelium in diabetic hamsters (2). 5-Methoxyflavanone, and more potently 5-methoxyflavone, down-regulated indomethacin-induced leukocyte adherence to mesenteric venules (4). The flavonoid 2-(3-amino-phenyl)-8-methoxy-chromene-4-one (PD 098063) selectively blocks TNF-α induced VCAM-1 expression in endothelial cells in a concentration-dependent manner but had no effect on ICAM-1 expression. This selective inhibition of agonist-induced VCAM-1 protein and gene expression by PD 098063 was through NF-$\varkappa$B-independent mechanism(s) (47). We have recently observed that quercetin, a potent antioxidant, suppressed agonist-induced ICAM-1 protein (Fig. 3) and gene expression (Fig. 4) (17). Herbal extracts containing mixtures of bioflavonoids, e.g., pine bark extract have shown potent inhibitory effect on interferon γ induced adhesion of T cells to human

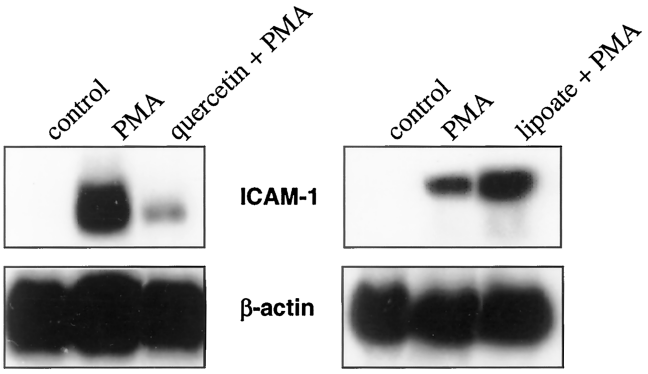

FIGURE 4—Northern blot showing the effect of quercetin and α-lipoic acid on phorbol ester-induced ICAM-1 mRNA expression. Left panel, human endothelial ECV cells were either not treated (control) or treated with 100 nM phorbol myristate acetate for 6 h (PMA). Quercetin treatment (50 μM) was for 3 h before PMA treatment. Right panel, HUVEC cells were either not treated (control) or treated with 100 nM phorbol myristate acetate for 6 h (PMA). Lipoate treatment (R-lipoic acid, 100 μM) was for 48 h before PMA treatment as shown in Figure 3. β-Actin mRNA expression was studied as reference.

keratinocytes. Interferon γ induced ICAM-1 expression was inhibited in herbal extract treated keratinocytes (3).

Molecular Basis of Antioxidant Action

Because of diverse chemical structure of the antioxidants, it is rather difficult to understand the exact mechanisms and sites of action of antioxidants. Some antioxidants appear to regulate adhesion molecule expression at the transcriptional level, whereas others may modulate later events (Fig. 4). Among the thiol antioxidants studied, PDTC, and high concentrations (mM) of NAC suppress inducible adhesion molecule expression at least in some cells through NF-κB dependent mechanisms (22,32,45). In astrocytoma cells, PDTC- and NAC-inhibited IL-1α induced adhesion molecule expression but failed to block IL-1α mediated activation of NF-κB (25). The exact regulatory mechanisms of these thiol compounds on NF-κB activation are not yet known. Studies related to the understanding of mechanisms underlying the inhibitory effect of lipoate on inducible cell adhesion molecule expression clearly indicate that the mechanism of action is independent of NF-κB activity (29–31).

α-Tocopherol inhibit protein kinase C (PKC) activity in some cell lines (41). PKC plays a major role in the expression of cell adhesion (19). Faruqui et al. (9) reported that inhibition of inducible cell adhesion by tocopherol was not dependent on the effects of tocopherol on PKC or NF-κB. In contrast, tocopherol-succinate has been observed to prevent monocytic cell adhesion to cytokine-stimulated endothelial cells by inhibiting the activation of NF-κB (8).

In most cases, the mechanisms that mediate the suppressive effect of flavonoids on cell adhesion have been reported not to be dependent on the activation of NF-κB (11,17). Recently, we have observed that the flavonoid quercetin significantly and dose dependently down-regulated agonist-induced AP-1, but not NF-κB, activation in human endothelial cells. The decrease in inducible AP-1 activation was observed to be associated with the inhibitory effects of quercetin on c-Jun NH2-terminal kinase (JNK) pathway (17). In another study, we observed that a pine bark extract inhibited interferon γ-induced ICAM-1 expression in human keratinocytes by blocking inducible STAT1 activation (3).

Summary and Conclusion

Cell adhesion represents a process that is centrally important in immune function and inflammation. This process is sensitive to various agonists including oxidants. Oxidants may directly induce cell adhesion. In other cases, cytokines and related agents may induce cell adhesion by oxidant-dependent mechanisms. Various redox-sensitive sites in the signal transduction path leading to cell adhesion have been identified. Numerous studies have confirmed that physical exercise influences the redox status of various cells and tissues. Recent evidences also show that physical exercise influences several cell adhesion related molecules. Whether such regulation has a redox component remains to be tested. Antioxidant supplementation studies testing the effect of exercise on cell adhesion should provide critical insight.

Work supported by NIH GM27345, Finnish Ministry of Education and Juho Vainio Foundation, Helsinki to CKS.

Address for correspondence: Chandan K. Sen, Ph.D., FACSM, Laboratory of Molecular Medicine, 512 Heart & Lung Institute, The Ohio State University Medical Center, 473 West 12th Avenue, Columbus, OH 43210-1252; E-mail: sen-1@medctr.osu.edu.

REFERENCES

1. Aoki, T., Y. Suzuki, K. Suzuki, et al. Modulation of ICAM-1 expression by extracellular glutathione in hyperoxia-exposed human pulmonary artery endothelial cells. *Am. J. Respir. Cell Mol. Biol.* 15:319–327, 1996.
2. Bertuglia, S., S. Malandrino, and A. Colantuoni. Effects of the natural flavonoid delphinidin on diabetic microangiopathy. *Arzneimittelforschung* 45:481–485, 1995.
3. Bito, T., S. Roy, C. K. Sen, and L. Packer. Pine bark pycnogenol inhibits IFN-gamma induced adhesion of T cells to human keratinocytes by inhibiting inducible ICAM-1 expression. *Free Rad. Biol. Med.*, in press.
4. Blank, M. A., B. L. Ems, L. M. O. Brien, et al. Flavonoid-induced gastroprotection in rats: role of blood flow and leukocyte adherence. *Digestion* 58:147–154, 1997.
5. Collins, T. L., P. D. Kassner, B. E. Bierer, and S. J. Burakoff. Adhesion receptors in lymphocyte activation. *Curr. Opin. Immunol.* 6:385–393, 1994.
6. Dailey, M. O. Expression of T lymphocyte adhesion molecules: regulation during antigen-induced T cell activation and differentiation. *Crit. Rev. Immunol.* 18:153–184, 1998.
7. Devaraj, S., and I. Jialal. The effects of alpha-tocopherol on critical cells in atherogenesis. *Curr. Opin. Lipidol.* 9:11–15, 1998.
8. Erl, W., C. Weber, C. Wardemann, and P. C. Weber. alpha-Tocopheryl succinate inhibits monocytic cell adhesion to endothelial cells by suppressing NF-kappa B mobilization. *Am. J. Physiol.* 273:H634–640, 1997.
9. Faruqi, R., C. de la Motte, and P. E. Dicorleto. Alpha-tocopherol inhibits agonist-induced monocytic cell adhesion to

cultured human endothelial cells. *J. Clin. Invest.* 94:592–600, 1994.
10. GABRIEL, H. H., and W. KINDERMANN. Adhesion molecules during immune response to exercise. *Can. J. Physiol. Pharmacol.* 76: 512–523, 1998.
11. GERRITSEN, M. E., W. W. CARLEY, G. E. RANGES, et al. Flavonoids inhibit cytokine-induced endothelial cell adhesion protein gene expression. *Am. J. Pathol.* 147:278–292, 1995.
12. IKEDA, M., K. K. SCHROEDER, L. B. MOSHER, C. W. WOODS, and A. L. AKESON. Suppressive effect of antioxidants on intercellular adhesion molecule-1 (ICAM-1) expression in human epidermal keratinocytes. *J. Invest. Dermatol.* 103:791–796, 1994.
13. JANSSEN, Y. M., and C. K. SEN. Nuclear factor kappa B activity in response to oxidants and antioxidants. *Methods Enzymol.* 300: 363–374, 1999.
14. JORDAN, J., R. BENEKE, M. HUTLER, A. VEITH, H. HALLER, and F. C. LUFT. Moderate exercise leads to decreased expression of beta1 and beta2 integrins on leucocytes. *Eur. J. Appl. Physiol.* 76:192–194, 1997.
15. KHANNA, S., M. ATALAY, D. E. LAAKSONEN, M. GUL, S. ROY, and C. K. SEN. Alpha-lipoic acid supplementation: tissue glutathione homeostasis at rest and after exercise. *J. Appl. Physiol.* 86:1191–1196, 1999.
16. KHANNA, S., M. ATALAY, J. K. LODGE, et al. Skeletal muscle and liver lipoyllysine content in response to exercise, training and dietary alpha-lipoic acid supplementation. *Biochem. Mol. Biol. Int.* 46:297–306, 1998.
17. KOBUCHI, H., S. ROY, C. K. SEN, H. G. NGUYEN, and L. PACKER. Quercetin inhibits inducible ICAM-1 expression in human endothelial cells through the JNK pathway. *Am. J. Physiol.* 277:C403–C411, 1999.
18. KUROKAWA, Y., S. SHINKAI, J. TORII, S. HINO, and P. N. SHEK. Exercise-induced changes in the expression of surface adhesion molecules on circulating granulocytes and lymphocytes subpopulations. *Eur. J. Appl. Physiol.* 71:245–252, 1995.
19. KVALE, D., and R. HOLME. Intercellular adhesion molecule-1 (ICAM-1; CD54) expression in human hepatocytic cells depends on protein kinase C. *J. Hepatol.* 25:670–676, 1996.
20. LEHR, H. A., B. FREI, and K. E. ARFORS. Vitamin C prevents cigarette smoke-induced leukocyte aggregation and adhesion to endothelium in vivo. *Proc. Natl. Acad. Sci. USA* 91:7688–7692, 1994.
21. MALM, C., R. LENKEI, and B. SJODIN. Effects of eccentric exercise on the immune system in men. *J. Appl. Physiol.* 86:461–468, 1999.
22. MARUI, N., M. K. OFFERMANN, R. SWERLICK, et al. Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. *J. Clin. Invest.* 92:1866–1874, 1993.
23. MASTRO, A. M., D. A. SCHLOSSER, D. S. GROVE, et al. Lymphocyte subpopulations in lymphoid organs of rats after acute resistance exercise. *Med. Sci. Sports Exerc.* 31:74–81, 1999.
24. MILES, M. P., S. K. LEACH, W. J. KRAEMER, K. DOHI, J. A. BUSH, and A. M. MASTRO. Leukocyte adhesion molecule expression during intense resistance exercise. *J. Appl. Physiol.* 84:1604–1609, 1998.
25. MOYNAGH, P. N., D. C. WILLIAMS, and O. N. LA. Activation of NF-kappa B and induction of vascular cell adhesion molecule-1 and intracellular adhesion molecule-1 expression in human glial cells by IL-1: modulation by antioxidants. *J. Immunol.* 153:2681–2690, 1994.
26. PANES, J., M. E. GERRITSEN, D. C. ANDERSON, M. MIYASAKA, and D. N. GRANGER. Apigenin inhibits tumor necrosis factor-induced intercellular adhesion molecule-1 upregulation in vivo. *Microcirculation* 3:279–286, 1996.
27. PETER, G., and H. O. BORBE. Absorption of 7,8-^{14}C-rac-a-lipoic acid from *in situ* ligated segments of gastroinestinal tract of the rat. *Arzneimittelforschung* 45:293–229, 1995.
28. REHMAN, J., P. J. MILLS, S. M. CARTER, J. CHOU, J. THOMAS, and A. S. MAISEL. Dynamic exercise leads to an increase in circulating ICAM-1: further evidence for adrenergic modulation of cell adhesion. *Brain Behav. Immun.* 11:343–351, 1997.
29. ROY, S., C. K. SEN, A. GOZIN, V. ANDRIEU, and C. PASQUIER. Redox regulation of adhesion processes. In: *Antioxidant and Redox Regulation of Genes*, C. K. Sen, H. Sies, and P. A. Baeuerle (Eds.). San Diego: Academic Press, pp. 265–295, 1999.
30. ROY, S., C. K. SEN, H. KOBUCHI, and L. PACKER. Antioxidant regulation of phorbol ester-induced adhesion of human jurkat T-cells to endothelial cells. *Free Rad. Biol. Med.* 25:229–241, 1998.
31. ROY, S., C. K. SEN, and L. PACKER. Determination of cell-cell adhesion in response to oxidants and antioxidants. *Methods Enzymol.* 300:395–401, 1999.
32. SATO, M., T. MIYAZAKI, T. NAGAYA, Y. MURATA, N. IDA, K. MAEDA, and H. SEO. Antioxidants inhibit tumor necrosis factor-alpha mediated stimulation of interleukin-8, monocyte chemoattractant protein-1, and collagenase expression in cultured human synovial cells. *J. Rheumatol.* 23:432–438, 1996.
33. SCHALLER, M. D., C. A. BORGMAN, B. S. COBB, R. R. VINES, A. B. REYNOLDS, and J. T. PARSONS. pp125fak a structurally distinctive protein-tyrosine kinase associated with focal adhesions. *Proc. Natl. Acad. Sci. USA* 89:5192–5196, 1992.
34. SELLAK, H., E. FRANZINI, J. HAKIM, and C. PASQUIER. Reactive oxygen species rapidly increase endothelial ICAM-1 ability to bind neutrophils without detectable upregulation. *Blood* 83:2669–2677, 1994.
35. SEN, C. K. Nutritional biochemistry of cellular glutathione. *J. Nutr. Biochem.* 8:660–672, 1997.
36. SEN, C. K. Redox signaling and the emerging therapeutic potential of thiol antioxidants. *Biochem. Pharmacol.* 55:1747–1758, 1998.
37. SEN, C. K., and L. PACKER. Antioxidant and redox regulation of gene transcription [see comments]. *FASEB J.* 10:709–720, 1996.
38. SEN, C. K., L. PACKER, and O. HANNINEN (Eds.). *Exercise and Oxygen Toxicity*. Amsterdam: Elsevier Science, 1994, pp. 1–536.
39. SEN, C. K., L. PACKER, and O. HANNINEN (Eds.). *Handbook of Oxidants and Antioxidants in Exercise*. Amsterdam: Elsevier, 2000.
40. SEN, C. K., T. RANKINEN, S. VAISANEN, and R. RAURAMAA. Oxidative stress after human exercise: effect of N-acetylcysteine supplementation. *J. Appl. Physiol.* 76:2570–2577, 1994.
41. TASINATO, A., D. BOSCOBOINIK, G. M. BARTOLI, P. MARONI, and A. AZZI. d-alpha-tocopherol inhibition of vascular smooth muscle cell proliferation occurs at physiological concentrations, correlates with protein kinase C inhibition, and is independent of its antioxidant properties. *Proc. Natl. Acad. Sci. USA* 92:12190–12194, 1995.
42. TURNER, C. E., J. R. GLENNEY, Jr., and K. BURRIDGE. Paxillin: a new vinculin-binding protein present in focal adhesions. *J. Cell Biol.* 111:1059–1068, 1990.
43. van EEDEN, S. F., J. GRANTON, J. M. HARDS, B. MOORE, and J. C. HOGG. Expression of the cell adhesion molecules on leukocytes that demarginate during acute maximal exercise. *J. Appl. Physiol.* 86:970–976, 1999.
44. VIITA, H., C. K. SEN, S. ROY, T. SIJAMAKI, T. NIKKARI, and S. YLA-HERTTUALA. High expression of human 15-lipoxygenase induces NF-kappaB mediated expression of vascular cell adhesion molecule 1, and T-cell adhesion on human endothelial cells. *Antiox. Redox. Signal.* 1:83–96, 1999.
45. WEBER, C., W. ERL, A. PIETSCH, M. STROBEL, H. W. ZIEGLER-HEITBROCK, and P. C. WEBER. Antioxidants inhibit monocyte adhesion by suppressing nuclear factor-kappa B mobilization and induction of vascular cell adhesion molecule-1 in endothelial cells stimulated to generate radicals. *Arterioscler. Thromb.* 14:1665–1673, 1994.
46. WEBER, C., W. ERL, K. WEBER, and P. C. WEBER. Increased adhesiveness of isolated monocytes to endothelium is prevented by vitamin C intake in smokers. *Circulation* 93:1488–1492, 1996.
47. WOLLE, J., R. R. HILL, E. FERGUSON, et al. Selective inhibition of tumor necrosis factor-induced vascular cell adhesion molecule-1 gene expression by a novel flavonoid: lack of effect on transcription factor NF-kappa B. *Arterioscler. Thromb. Vasc. Biol.* 16: 1501–1508, 1996.