Available online at www.sciencedirect.com
SCIENCE DIRECT®
Experimental Gerontology 39 (2004) 165–171
Experimental Gerontology
www.elsevier.com/locate/expgero

Mini Review

Aging and the endothelium

P. d'Alessio[a,b,*]

[a]*University Paris XI, Orsay Cedex, France*
[b]*Inserm U506, Hôpital P. Brousse, 12, ave. Paul Vaillant Couturier, 94807 Villejuif, France*

Received 3 July 2003; received in revised form 15 October 2003; accepted 22 October 2003

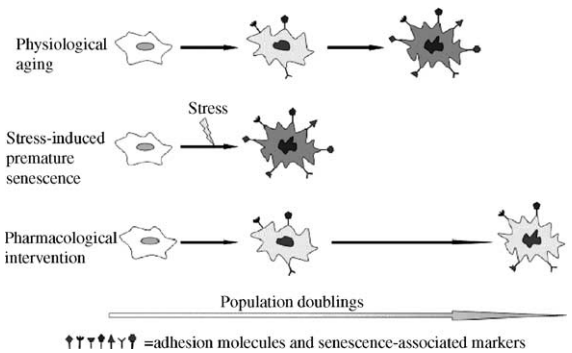
Abstract

One link between aging and endothelial function is the inflammatory response. On one hand, the latter shortens the biological engaged by activated leukocytes against invaders or stressing agents. On the other hand, the surveyed tissues become targets of the toxicity of reactive oxygen species, ROS. The ensuing regeneration is source of transcriptional infidelity, leading to the alteration of the repaired tissue. Hence, the toll of inflammatory stress consists in premature senescence of cell and tissues. This hypothesis is discussed in the present review, which focuses on the molecular targets relevant for cancer and degenerative diseases, both tributary to an inflammatory environment and taking advantage from the consequences of cell and tissue dysfunctions characteristic of aging. Eventually, adaptation to stress, whatever its origin - inflammatory and/or psychosocial – is discussed. Basal nitric oxide (NO) release, such as provided through moderate exercise, seems to be the most potent guardian against immune, nervous and cardiovascular over-stimulation. Tissue regeneration is also obtained by circulating endothelial progenitors able to recognize the damaged tissue. To avoid post-inflammatory alterations resulting in detrimental changes of tissues and organs, the pharmacological protection of endothelium by agents able to modulate its activation seems crucial to us.
© 2003 Elsevier Inc. All rights reserved.

Keywords: Endothelium-dependent aging; Inflammation–Senescence Links; Stress adaptation; Cyto-protection by NO; Endothelial progenitor's repair

The endothelium is a singular organ existing in two forms, as monolayer lining vascular walls and as a circulating cell community bearing progenitor characteristics. To what extent should it be concerned by aging and how distinctively from any other organ or cell type? The hypothesis that we will try to infer, is that the endothelium is peculiarly linked to aging through the inflammatory reaction that constantly keeps the body on alert.

Aging is a visible phenomenon reflecting a plethora of mostly invisible changes in the animal or human being. It is associated with modifications in many biochemical and architectural components witnessing alterations of physiological statuses, such as protein and tissue change, cell cycle setback or forth, unbalancing of the oxidant–antioxidant equilibrium, increasing vulnerability towards the environmental impact and increasing complexity in organizing a response to environmental challenges. We will try to describe the role that the endothelium may play in the change of life pattern that is aging.



1. Endothelial compliance

The term *compliance* is used to indicate the taking of the correct amount of the prescribed medicine at the proper time. Still within the medical syllabus, the term defines

* Tel.: +33-1-45595146; fax: +33-1-45595359.
E-mail address: dalessio@vjf.inserm.fr (P. d'Alessio).

0531-5565/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.exger.2003.10.025

Table 1
Organ system changes with age adapted from ASA (American Society of Anesthesiologists, Critical Care of the Elderly Patient, by H. Russel Harvey and Douglas B. Coursin)

Circulatory	↓vascular compliance, ↓ resistance. Subsequent ↓ systolic **BP (blood pressure)** ↓ effective circulating volume.
Cardiac	↓ afterload leads to ↓ **LV (left ventricle)** wall stress and leads to **LVH (left ventricular hypertrophy)** and ↓ **LV (left ventricle)** compliance.
Pulmonary	↓chest wall compliance: ↓ **TLC (total lung capacity)**, ↓ **VC (vital capacity)**, ↓ **FRC (functional residual capacity)**, ↓ lung elastic recoil, ↓ lung compliance, ↓ closing capacity.
Renal	Fewer cortical nephrons ↓ **GFR (glomerular filtration rate)**.
Nervous	↓ responsiveness to autonomic nervous system, ↓response to exogenous α and ß agonists (mix of receptor and second messenger defects) response to stress.

the conformity of the body's organs to fulfill the functional demand of the subject. Table 1 is intended to illustrate the link between specific tissue and/or organ compliance and (decading) organ function in the course of time.

We have chosen to transfer the term *compliance* at the cellular level, referring to the capacity of a cell to accomplish specific functions within a specific cell community. Dividing the body into blood and tissues, vascular endothelium is required to modulate vascular tone and impeach bleeding. It also intervenes in the catabolism of lipoproteins and nutrients and is involved in the recruitment of activated immune cells, as well as of circulating cancer metastasis. During the inflammatory reaction against microorganisms invading tissues and organs, the endothelium is exposed to various reactive oxygen species (ROS) and inflammatory mediators resulting in an enhanced peroxide tone. Alteration of cellular redox levels leads to phenotypic and functional changes in endothelial cells, due to modifications in nuclear transcription factors activation. Experimental procedures that are finalized, either in vitro or in vivo to challenge vascular endothelium with inflammatory mediators, such as specific cytokines, ROS or bacterial lipopolysaccharide. Most of the results quoted are based on in vitro research on primary cultures of endothelial cells obtained either by dissection of the umbilical cord or isolated from organs (organ-specific endothelial cells). The proof of concept of the link between inflammation and aging is also deduced from in vitro results, illustrating the tendency towards irreversible expression of inflammatory markers by senescent endothelial cell populations, as the senescent phenotype of endothelial cells can be accelerated by inflammatory stress (Toussaint et al., 2002).

2. Endothelium and vascular dysfunction

A part from the inflammatory reaction, the vascular endothelium also regulates the post-inflammatory fibroproliferative process. The clinical importance of endothelial compliance (Vapaatalo and Mervaala, 2001) has been shown in atherosclerosis and cardiovascular disease (Egashira, 2002; Asai et al., 2000; Lakatta, 2003) and gender differences (Jensen-Urstad and Johansson, 2001) corroborate endothelium as a dynamic endocrine organ relevant for aging.

Recent reports (Cepinskas et al., 2003) illustrate the significant auto-regulatory capacity of the endothelium (Altieri, 1999), consisting in the development of a consequential resistance to apoptosis within an oxidative and pro-inflammatory microenvironment. This could prove all the more important, as it has been shown that endothelial CD95-mediated disseminated apoptosis precedes extensive lesions of the underlying tissue (Janin et al., 2002). However, the simple contact between endothelial cells and polymorphonuclear (PMN) leukocytes down-regulates their three known membrane-initiated PMN apoptotic pathways Fas, TNF, and TRAIL (Tennenberg et al., 2002) showing that complex mechanisms preside over the cross-talk between endothelium and activated leukocytes.

Aging by itself enhances the sensitivity of endothelial cells towards apoptotic stimuli (Hoffmann et al., 2001). This is mainly due to a reduced nitric oxide (NO) production by endothelial NOS (NOS-III), hence to a decreased blockade of caspase activity through the diminution of the S-nitrosylation of the cystein in their active site. In term, this contributes to the development of

endothelial dysfunction relevant for atherogenesis, plaque erosion and acute coronary syndromes (Dimmeler et al., 2002). The Fas/FasL system may be involved in the development of Myocardial Infarction/Restenosis injury (Shiraishi et al., 2002), as it favours the onset of pro-inflammatory conditions. However, endothelial cell specific over-expression of Fas ligand seems to attenuate ischaemia-reperfusion injury in the heart through anti-inflammatory actions such as the reduction of myocardial neutrophil infiltration (Yang et al., 2003).

Furthermore, over-expression of Fas-L in endothelial cells leads or not to increased apoptosis of these cells according to the species, in relationship with the subcellular compartment where it is expressed and linked to the level of FLIP (Flice inhibitory protein) expression. This differential sensitivity of endothelial cells to Fas-mediated apoptosis could represent an effector mechanism in the regulation of the animal life-span (Bouchet et al., 2002).

Environmental factors could be considered as a relevant item in endothelial function, as irradiation-damaged endothelial cells persist in the vasculature of irradiated tissue for a prolonged period after the initial insult (Oh et al., 2001).

3. Pro-inflammation and tissue degeneration

An essential nexus between endothelium and inflammation important for tissue and organ dysfunction can hardly be denied. In fact, pro-inflammatory pattern is common to various diseases (Esch and Stefano, 2002). This is because inflammation shortens biological battles fighting against invasion of microorganisms via activated leukocytes. By doing so, it introduces high rates of ROS into the surveyed tissue, destructive for microorganisms as well as for the underlying tissue components, sort of innocent bystanders. This represents a toll of the inflammatory reaction remaining unpaid and requiring tissue repair by de novo protein synthesis. In fact, in response to injury, the endothelium integrates a variety of triggered pathways displaying cyto-protection from pro-inflammatory effectors. This may delay its own dysfunction beyond the resulting tissue dysfunction, which in turn is just what matters for aging.

This view is corroborated by a new twist within the free radical theory of aging, recently introduced by Dukan. In contrast to the classical view (Harman, 1956), this work (Dukan et al., 2000; Dukan and Nystrom, 1998) provides evidence that oxidative damage to proteins seems to rely more on a susceptibility to ROS rather than on a modification of the balance between oxidative species and antioxidant defense mechanisms. Thus, ordinary transcriptional or translational infidelity would lead to an accumulation of structurally imperfect proteins more susceptible to ROS. As these misfolded proteins accumulate, the amount of oxidative damage measured in the cell increases. A vicious circle characterized by altered proteins eliciting the synthesis of further altered proteins, is lanced.

In endothelial cells, inflammatory challenge and replicative senescence are associated to the over-expression of the inducible adhesion molecule Vascular Cell Adhesion Molecule-1 (VCAM-1) (Marconi and D'Alessio, 2003) resulting in an over-recruiting propensity for leukocytes, as well as of circulating cancer cells, amplifying the tissue destruction and fibrotic repair process. Age-related endothelial changes also involve cell morphology. Phenotypic changes linked to oxidative stress have been shown to impair coronary arteriolar function (Csiszar et al., 2002) and endothelial cytoskeletal alterations following ROS and inflammatory cytokine stimulation are able to dramatically modify cell signaling.

4. Endothelium and signal mecanotransduction

Cell cytoskeleton contributes quite fundamentally to signal transduction. Recent work (Huang and Ingber, 1999) discloses the importance of actin cytoskeleton binding to extracellular matrix components for cell signaling during normal tissue patterning in embryogenesis and cancer formation in adult life. Hence, mechanical properties of cytoskeleton structure, considered as an information processing network (Ingber, 2003a,b) influence cell signaling.

In endothelial cells, actin cytoskeleton is at the base of morphological modifications observed during functional change. At the basal level, a molecular bridge constituted by the cellular actin cytoskeleton, the intracellular actin partner α-actinin and the membrane-based Focal Adhesion Complex is linked with several extracellular matrix proteins. Several structures, localized or bound at the membrane level, such as the small GTPase Rho (Chen et al., 1999; Miao et al., 2001) and caveolae (Boyd et al., 2003; Ratajczak et al., 2003) are profoundly linked to endothelial actin cytoskeleton (Su et al., 2003; Ratajczak et al., 2003) and have been shown to actively participate in cell signaling in endothelial cells.

Moreover, blood flow patterns induced shear stress is able by itself to activate the functional bridge of signal mecano-transduction and participate in the regulation of adhesive molecule and integrin expression both in health (Yoshida et al., 1996; Chen et al., 1999; Li et al., 1999) and disease (Elrayess et al., 2003).

This functional mecanotransduction unit from whom endothelial cell signaling mostly depends is destinated to be altered in the course of oxidative stress dependent senescence, as well as during altered blood flow patterns, such as in ischemia-reperfusion or developing atherothrombosis, compromising efficiency in cell signaling. In fact, in endothelial cells, morphological changes are known to be a hallmark of senescence related cell alterations (Wagner et al., 2001). Otherwise, during angiogenesis,

the cytoskeletal tension has been shown to display a crucial role, alternatively regulating growth, proliferation or apoptosis (Dike et al., 1999).

5. Endothelium and cancer

The link between endothelium and aging, well established by endothelial senescence markers in age-related vascular diseases (Muller et al., 2002b) also emanates from the relevance of endothelium for cancer (Singh et al., 2002). Among other markers, endothelial Fas ligand expression contributes to the regulation of cancer cell transmigration through endothelium in secondary sites of metastasis (Osanai et al., 2002).

Furthermore, endothelial apoptosis is a homeostatic factor regulating angiogenesis-dependent tumor growth (Garcia-Barros et al., 2003). More generally, cancer is characterized by an enhanced pro-inflammatory environment, encouraging tumor cell dissemination through endothelial diapedesis (Orr and Wang, 2001; Orr et al., 2000). Hence, a great variability of endothelia belonging to different organs and vascular districts can be recognized (Lafrenie et al., 1992). Recently, we have shown (D'Anna et al., 2001) the capacity of HPV-16 derived oncoprotein E7 to produce IL-6 and IL-8 in organ-specific endothelial cells, including the macro and microvascular phenotypes. Interestingly, our studies on endothelial cells from the microvasculature of the skin and the cardiac auricle, identified as sites of cervical cancer metastasis (Shimotsu et al., 1998), have shown a greater response of CrMVEn to E7 treatment as compared to that of other endothelia, suggesting a possible selectivity of E7 oncoprotein for organ-specific microvascular endothelial cells. Thus, knowledge about organ-specific endothelial features may prove precious in endothelial funded therapeutic strategies devoted to inhibit cancer metastasis dissemination and also by analogy in infectious disease resistant to antibiotic treatment (Muller et al., 2002a).

6. Endothelium and psycho-social stress

Individual sensitivity to cellular stress plays an important role for the rate of aging and seems linked to the expression of an adaptor protein (Migliaccio et al., 1999). Hence, a poorer stress response predicts a longer life-span.

But also psychological stress arising from pain due to local inflammation (Brain, 2000) in rheumatoid arthritis has been put into a dynamical perspective with the age-related pathology (Braz et al., 2001).

In the etiopathogenesis of atherosclerosis, endothelial injury has been considered an initiating event. Psycho-social stress activating the sympathetic nervous system, would induce an inflammatory reaction (Esch et al., 2002a), rendering endothelium the final target, even in the absence of dietary cholesterol (Skantze et al., 1998). In fact, stress derived from social status (Kaplan and Manuck, 1999) indeed induces endothelial dysfunction (Plante, 2002), and this effect is mediated via adrenoceptor activation (Strawn et al., 1991). However, it is uncertain whether social disruption and its consequences associated with both sympathetic nervous system arousal and indexes of endothelial dysfunction may be prevented by treatment with an adrenergic blocking agent (Rozanski et al., 1999). In addition, although social stress compromises the endothelial integrity of various vascular segments relevant for the development of atherosclerosis, it remains questionable if these factors can influence initial lesion formation. The answers to the many questions that this topic opens, will most probably emerge from a better knowledge of the mechanisms of adaptation to stress.

7. Endothelium and adaptation to stress

Moderate exercise is known to stimulate NO release. Nitric Oxide (NO) has been shown to be critical as to the adaptation to stress and the stress related disease process (Pshennikova et al., 2001; Esch et al., 2002b; Hambrecht et al., 1998). Basal NO production seems to be the most potent guardian to immune, nervous and cardiovascular stimulation (Stefano et al., 2000). We and others have documented the beneficial role of NO for endothelial *compliance* in ischemic disease (Marconi et al., 2003), inflammatory models (Zadeh et al., 2000) and endothelial senescence (Matsushita et al., 2001; Gewaltig and Kojda, 2002). Reactive nitrogen species are indeed related to aging (Drew and Leeuwenburgh, 2002) and so are prostanoid dilator pathways.

Furthermore, consumption of fresh fruits, rich in vitamins and antioxidant enzymes, specifically counteract endothelial cell apoptosis in congestive heart failure, supporting the idea of a link between oxidative stress and cardiovascular pathology (Rossig et al., 2001). Likewise, the pleasant sensation of taste inducing vagus nerve stimulation, is able to turn down the adrenal pro-inflammatory response (Borovikova et al., 2000).

Hence, the delay in aging, meant as endothelial-mediated tissue dysfunction, can be supported by appropriate physical and nutritional habits.

8. Endothelium and tissue regeneration

If stress renders vascular endothelium dysfunctional, supporting age-related disease, it also bears the opposite tendency of tissue regeneration by circulating progenitors (Deutsch et al., 2002; Grisaru et al., 2001).

In particular, two populations of endothelial cells are concerned by this potential function, marrow derived

angiocompetent hematopoietic cells (EPCs) and circulating endothelial progenitors (CEPs). In fact, co-recruitment of hemtopoietic stem cells and hematopoietic progenitor cells, along with EPCs and CEPs, may contribute to the initiation and sustain of neoangiogenesis. Following tissue injury signals, regeneration responds enabling adult marrow to lance its tissue specific stem and progenitor cell reservoir for localized tissue repair. Indeed, vascular trauma and organ regeneration result in the release of chemokines that mobilize and recruit EPCs and CEPs. In particular, exposure to organ-specific angiogenic and matrix factors may be necessary to program EPCs and CEPs to home and incorporate into a particular tissue (Donovan et al., 2000; LeCouter et al., 2001).

Different types of cell therapies have been envisaged focusing on the clinical arena of age related diseases.

In ischemic limb disease, blood and lymphatic vessels impairment leads to edema formation that contributes to non-healing ulcers. Supplementation of VEGF-C (Rafii and Lyden, 2003) enhances lymphangiogenesis and angiogenesis and decreases edema formation. Moreover, delivery of Syk + and SLP-76 + hematopoietic cells have been shown to contribute to the separation of lymphatic and blood vessels, promoting a balanced revascularization in ischemic limbs (Szuba et al., 2002).

Endothelial dysfunction due to long-lasting pro-inflammatory stimulation has been shown to be of relevance in thrombogenicity and atherosclerosis (Sata et al., 2002): one therapeutic use of autologous EPCs and CEPs is to generate non-thrombogenic vascular cells to coat decellularized or biodegradable surfaces (Noishiki et al., 1996).

The question remains open, why the mobilization of progenitor cells does not restore automatically tissue vascularization, and why cell mediated therapeutical assistance may be required. Two possible explanations are thinkable: one is that the inflammatory environment of altered endothelium characteristic in age related pathologies, sacrifices CEPs; alternatively, a lack of blood supply such as experimented in athero-thrombosis or limb ischemia, hinders the ability of EPCs and CEPs to recognize damaged vasculature.

Endothelium is thus linked to aging not only promoting tissue degeneration by the import of activated leukocytes and circulating tumor cells, but also by its remnant capacity of regeneration through circulating progenitor cells, of therapeutic relevance. At length, as brought up by the regenerative potential of CEPs (Rafii and Lyden, 2003), the link of endothelium to aging is established by its capacity to reverse it, once it appears phenotypically as endothelial dysfunction.

9. Summary

In a life-time scenario, endothelium's features (adhesive tendency, cytokine sensitivity, pro- and anti-apoptotic mechanisms, number and 'vitality' of circulating progenitors) govern its relevance for aging. Vascular endothelium integrity depends on the subject's nutritional tradition, his or her propensity to exercise and ability to deal with emotions. Hence, the combinatorial degeneracy of environmental and genetic components (Edelman and Gally, 2001) will modulate the shift either to disease, be it cancer, cardiovascular dysfunction, degenerative disease or healthy endurance. Endothelium is thus an organ that should be highly assisted pharmacologically to avoid detrimental changes associated to post-inflammatory dysfunction implicating cell senescence and tissue degeneration, promoting tumor microenvironment (Carpenito et al., 2002).

In principle protection of endothelium from stress-linked pro-inflammatory disease would depend on the tenure of the subject's general life-style, whereby care for dietary habits and body exercise are the best *trainers* for the endothelium to face challenge coming from in and outside stressors. On that account, the chance to prevent age-related disease is closely linked to the ongoing practice of a basic *discipline*, the ideal procedure for the attainment of psychological fulfilling activities, these latter in turn being essential for endothelium's *compliance*.

References

Altieri, D.C., 1999. Paracrine control of endothelial cell survival. J. Clin. Invest. 104, 845.

Asai, K., Kudej, R.K., Shen, Y.T., 2000. Peripheral vascular endothelial dysfunction and apoptosis in old mokeys. Arterioscler. Thromb. Vasc. Biol. 20, 1493–1499.

Borovikova, L.V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G.I., Watkins, L.R., Wang, H., Abumrad, N., Eaton, J.W., Tracey, K.J., 2000. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 405, 458–462.

Bouchet, D., Tesson, L., Menoret, S., Charreau, B., Mathieu, P., Yagita, H., Duisit, G., Anegon, I., 2002. Differential sensitivity of endothelial cells of various species to apoptosis induced by gene transfer of fas ligand: role of flip levels. Mol. Med. 8, 612–623.

Boyd, N.L., Park, H., Yi, H., Boo, Y.C., Sorescu, G.P., Sykes, M., Jo, H., 2003. Chronic shear induces caveolae formation and alters ERK and akt responses in endothelial cells. Am. J. Physiol. Heart Circ. Physiol. 285(3), H1113–22.

Brain, S.D., 2000. New feelings about the role of sensory nerves in inflammation. Nat. Med. 6, 134–135.

Braz, J., Beaufour, C., Coutaux, A., Epstein, A.L., Cesselin, F., Hamon, M., Pohl, M., 2001. Therapeutic efficacy in experimental polyarthritis of viral-driven enkephalin overproduction in sensory neurons. J. Neurosci. 21, 7881–7888.

Carpenito, C., Davis, P.D., Dougherty, S.T., Dougherty, G.J., 2002. Exploiting the differential production of angiogenic factors within the tumor microenvironment in the design of a novel vascular-targeted gene therapy-based approach to the treatment of cancer. Int. J. Radiat. Oncol. Biol. Phys. 54, 1473–1478.

Cepinskas, G., Savickiene, J., Ionescu, C.V., Kvietys, P.R., 2003. PMN transendothelial migration decreases nuclear NFkappaB in IL-1beta-activated endothelial cells: role of PECAM-1. J. Cell Biol. 161, 641–651.

Chen, K.D., Li, Y.S., Kim, M., Li, S., Yuan, S., Chien, S., Shyy, J.Y., 1999. Mechanotransduction in response to shear stress. Roles of

receptor tyrosine kinases, integrins, and Shc. J. Biol. Chem. 274, 18393–18400.

Csiszar, A., Ungvari, Z., Edwards, J.G., Kaminski, P., Wolin, M.S., Koller, A., Kaley, G., 2002. Aging-induced phenotypic changes and oxidative stress impair coronary arteriolar function. Circ. Res. 90, 1159–1166.

D'Anna, R., Le Buanec, H., Alessandri, G., Caruso, A., Burny, A., Gallo, R., Zagury, J.F., Zagury, D., D'Alessio, P., 2001. Selective activation of cervical microvascular endothelial cells by human papillomavirus 16-e7 oncoprotein. J. Natl Cancer Inst. 93, 1843–1851.

Deutsch, V.R., Pick, M., Perry, C., Grisaru, D., Hemo, Y., Golan-Hadari, D., Grant, A., Eldor, A., Soreq, H., 2002. The stress-associated acetylcholinesterase variant AChE-R is expressed in human CD34(+) hematopoietic progenitors and its C-terminal peptide ARP promotes their proliferation. Exp. Hematol. 30, 1153–1161.

Dike, L.E., Chen, C.S., Mrksich, M., Tien, J., Whitesides, G.M., Ingber, D.E., 1999. Geometric control of switching between growth, apoptosis, and differentiation during angiogenesis using micropatterned substrates. In vitro Cell. Dev. Biol. Anim. 35, 441–448.

Dimmeler, S., Haendeler, J., Zeiher, A.M., 2002. Regulation of endothelial cell apoptosis in atherothrombosis. Curr. Opin. Lipidol. 13, 531–536.

Donovan, M.J., et al., 2000. Brain derived neurotrophic factor is an endothelial survival factor required for intramyocardial vessel stabilization. Development 127, 4531–4540.

Drew, B., Leeuwenburgh, C., 2002. Aging and the role of reactive nitrogen species. Ann. N. Y. Acad. Sci. 959, 66–81.

Dukan, S., Nystrom, T., 1998. Bacterial senescence: stasis results in increased and differential oxidation of cytoplasmic proteins leading to developmental induction of the heat shock regulon. Genes Dev. 12, 3431–3441.

Dukan, S., Farewell, A., Ballesteros, M., Taddei, F., Radman, M., Nystrom, T., 2000. Protein oxidation in response to increased transcriptional or translational errors. Proc. Natl Acad. Sci. USA 97, 5746–5749.

Edelman, G.M., Gally, J.A., 2001. Degeneracy and complexity in biological systems. Proc. Natl Acad. Sci. USA 98, 13763–13768.

Egashira, K., 2002. Clinical importance of endothelial function in arteriosclerosis and ischemic heart disease. Circ. J. 66, 529–533.

Elrayess, M.A., Webb, K.E., Flavell, D.M., Syvanne, M., Taskinen, M.R., Frick, M.H., Nieminen, M.S., Kesaniemi, Y.A., Pasternack, A., Jukema, J.W., Kastelein, J.J., Zwinderman, A.H., Humphries, S.E., 2003. A novel functional polymorphism in the PECAM-1 gene (53G > A) is associated with progression of atherosclerosis in the LOCAT and REGRESS studies. Atherosclerosis 168, 131–138.

Esch, T., Stefano, G., 2002. Proinflammation: a common denominator or initiator of different pathophysiological disease processes. Med. Sci. Monit. 8, HY1–HY9.

Esch, T., Stefano, G.B., Fricchione, G.L., Benson, H., 2002a. Stress in cardiovascular diseases. Med. Sci. Monit. 8, RA93–RA101.

Esch, T., Stefano, G.B., Fricchione, G.L., Benson, H., 2002b. Stress-related diseases—a potential role for nitric oxide. Med. Sci. Monit. 8, RA103–RA118.

Garcia-Barros, M., Paris, F., Cordon-Cardo, C., Lyden, D., Rafii, S., Haimovitz-Friedman, A., Fuks, Z., Kolesnick, R., 2003. Tumor response to radiotherapy regulated by endothelial cell apoptosis. Science 300, 1155–1159.

Gewaltig, M.T., Kojda, G., 2002. Vasoprotection by nitric oxide: mechanisms and therapeutic potential. Cardiovasc. Res. 55, 250–260.

Grisaru, D., Deutsch, V., Shapira, M., Pick, M., Sternfeld, M., Melamed-Book, N., Kaufer, D., Galyam, N., Gait, M.J., Owen, D., Lessing, J.B., Eldor, A., Soreq, H., 2001. ARP, a peptide derived from the stress-associated acetylcholinesterase variant, has hematopoietic growth promoting activities. Mol. Med. 7, 93–105.

Hambrecht, R., Fiehn, E., Weigl, C., Gielen, S., Hamann, C., Kaiser, R., Yu, J., Adams, V., Niebauer, J., Schuler, G., 1998. Regular physical exercise corrects endothelial dysfunction and improves exercise capacity in patients with chronic heart failure. Circulation 98, 2709–2715.

Harman, D., 1956. Aging: a theory based on free radical and radiation chemistry. J. Gerontol., 298–300.

Hoffmann, J., Haendeler, J., Aicher, A., Rossig, L., Vasa, M., Zeiher, A.M., Dimmeler, S., 2001. Aging enhances the sensitivity of endothelial cells toward apoptotic stimuli: important role of nitric oxide. Circ. Res. 89, 709–715.

Huang, S., Ingber, D.E., 1999. The structural and mechanical complexity of cell-growth control. Nat. Cell Biol. 1, E131–E138.

Ingber, D.E., 2003a. Tensegrity. I. Cell structure and hierarchical systems biology. J. Cell. Sci. 116, 1157–1173.

Ingber, D.E., 2003b. Tensegrity. II. How structural networks influence cellular information processing networks. J. Cell. Sci. 116, 1397–1408.

Janin, A., Deschaumes, C., Daneshpouy, M., Estaquier, J., Micic-Polianski, J., Rajagopalan-Levasseur, P., Akarid, K., Mounier, N., Gluckman, E., Socie, G., Ameisen, J.C., 2002. CD95 engagement induces disseminated endothelial cell apoptosis in vivo: immunopathologic implications. Blood 99, 2940–2947.

Jensen-Urstad, K., Johansson, J., 2001. Gender difference in age-related changes in vascular function. J. Intern. Med. 250, 29–36.

Kaplan, J.R., Manuck, S.B., 1999. Status, stress, and atherosclerosis: the role of environment and individual behavior. Ann. N. Y. Acad. Sci. 896, 145–161.

Lafrenie, R., Shaughnessy, S.G., Orr, F.W., 1992. Cancer cell interactions with injured or activated endothelium. Cancer Metastasis 11, 377–388.

Lakatta, E.G., 2003. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises. Part III. Cellular and molecular clues to heart and arterial aging. Circulation 107, 490–497.

LeCouter, J., Kowalski, J., Foster, J., Hass, P., Zhang, Z., Dillard-Telm, L., Frantz, G., Rangell, L., DeGuzman, L., Keller, GA., Peale, F., Gurney, A., Hillan, KJ., Ferrara, N. 2001. Identification of an angiogenic mitogen selective for endocrine gland endothelium. Nature 412, 877–884.

Li, S., Chen, B.P., Azuma, N., Hu, Y.L., Wu, S.Z., Sumpio, B.E., Shyy, J.Y., Chien, S., 1999. Distinct roles for the small GTPases Cdc42 and Rho in endothelial responses to shear stress. J. Clin. Invest. 103, 1141–1150.

Marconi, A., D'Alessio, P., 2003. Oxidative stress and endothelial senescence. Proc. 11th Biennial Meet. Free Radic. Res. Int. Soc., 125–128.

Marconi, A., Darquenne, S., Boulmerka, A., Mosnier, M., D'Alessio, P., 2003. Naftidrofuryl-driven regulation of endothelial ICAM-1 involves nitric oxide. Free Radic. Biol. Med. 34, 616–625.

Matsushita, H., Chang, E., Glassford, A.J., Cooke, J.P., Chiu, C.P., Tsao, P.S., 2001. eNOS activity is reduced in senescent human endothelial cells: preservation by hTERT immortalization. Circ. Res. 89, 793–798.

Miao, L., Calvert, J.W., Tang, J., Parent, A.D., Zhang, J.H., 2001. Age-related RhoA expression in blood vessels of rats. Mech. Ageing Dev. 122, 1757–1770.

Migliaccio, E., Giorgio, M., Mele, S., Pelicci, G., Reboldi, P., Pandolfi, P.P., Lanfrancone, L., Pelicci, P.G., 1999. The p66shc adaptor protein controls oxidative stress response and life span in mammals. Nature 402, 309–313.

Muller, A.M., Cronen, C., Muller, K.M., Kirkpatrick, C.J., 2002a. Heterogeneous expression of cell adhesion molecules by endothelial cells in ARDS. J. Pathol. 198, 270–275.

Muller, A.M., Skrzynski, C., Nesslinger, M., Skipka, G., Muller, K.M., 2002b. Correlation of age with in vivo expression of endothelial markers. Exp. Gerontol. 37, 713–719.

Noishiki, Y., Tomizawa, Y., Yamane, Y., Matsumoto, A., 1996. Autocrine angiogenic vascular prosthesis with bone marrow transplantation. Nat. Med. 2, 90–93.

Oh, C.W., Bump, E.A., Kim, J.S., Janigro, D., Mayberg, M.R., 2001. Induction of a senescence-like phenotype in bovine aortic endothelial cells by ionizing radiation. Radiat. Res. 156, 232–240.

Orr, F.W., Wang, H.H., 2001. Tumor cell interactions with the microvasculature: a rate-limiting step in metastasis. Surg. Oncol. Clin. N. Am. 10, 357–381.see also pages ix–x.

Orr, F.W., Wang, H.H., Lafrenie, R.M., Scherbarth, S., Nance, D.M., 2000. Interactions between cancer cells and the endothelium in metastasis. J. Pathol. 190, 310–329.

Osanai, M., Chiba, H., Kojima, T., Fujibe, M., Kuwahara, K., Kimura, H., Satoh, M., Sawada, N., 2002. Hepatocyte nuclear factor (HNF)-4alpha induces expression of endothelial fas ligand (FasL) to prevent cancer cell transmigration: a novel defense mechanism of endothelium against cancer metastasis. Jpn. J. Cancer Res. 93, 532–541.

Plante, G.E., 2002. Vascular response to stress in health and disease. Metabolism 51, 25–30.

Pshennikova, M.G., Bondarenko, N.A., Shimkovich, M.V., 2001. Nitric oxide as a factor of genetically determined resistance to stress damages and adaptive protection. Bull. Exp. Biol. Med. 132, 1048–1050.

Rafii, S., Lyden, D., 2003. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. Nat. Med. 9, 702–712.

Ratajczak, P., Damy, T., Heymes, C., Oliviero, P., Marotte, F., Robidel, E., Sercombe, R., Boczkowski, J., Rappaport, L., Samuel, J.L., 2003. Caveolin-1 and -3 dissociations from caveolae to cytosol in the heart during aging and after myocardial infarction in rat. Cardiovasc. Res. 57, 358–369.

Rossig, L., Hoffmann, J., Hugel, B., Mallat, Z., Haase, A., Freyssinet, J.M., Tedgui, A., Aicher, A., Zeiher, A.M., Dimmeler, S., 2001. Vitamin C inhibits endothelial cell apoptosis in congestive heart failure. Circulation 104, 2182–2187.

Rozanski, A., Blumenthal, J.A., Kaplan, J., 1999. Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. Circulation 99, 2192–2217.

Sata, M., Saiura, A., Kunisato, A., Tojo, A., Okada, S., Tokuhisa, T., Hirai, H., Makuuchi, M., Hirata, Y., Nagai, R., 2002. Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis. Nat. Med. 8 (4), 403–409.

Shimotsu, Y., Ishida, Y., Fukuchi, K., Hayashida, K., Toba, M., Hamada, S., Takamiya, M., Satoh, T., Nakanishi, N., Nishimura, T., 1998. Fluorine-18-fluorodeoxyglucose PET identification of cardiac metastasis arising from uterine cervical carcinoma. J. Nucl. Med. 39 (12), 2084–2087.

Shiraishi, H., Toyozaki, T., Tsukamoto, Y., Saito, T., Masuda, Y., Hiroshima, K., Ohwada, H., Kobayashi, N., Hiroe, M., 2002. Antibody binding to fas ligand attenuates inflammatory cell infiltration and cytokine secretion, leading to reduction of myocardial infarct areas and reperfusion injury. Lab. Invest. 82, 1121–1129.

Singh, N., Prasad, S., Singer, D.R., MacAllister, R.J., 2002. Ageing is associated with impairment of nitric oxide and prostanoid dilator pathways in the human forearm. Clin. Sci. (Lond.) 102, 595–600.

Skantze, H.B., Kaplan, J., Pettersson, K., Manuck, S., Blomqvist, N., Kyes, R., Williams, K., Bondjers, G., 1998. Psychosocial stress causes endothelial injury in cynomolgus monkeys via beta1-adrenoceptor activation. Atherosclerosis 136, 153–161.

Stefano, G.B., Goumon, Y., Bilfinger, T.V., Welters, I.D., Cadet, P., 2000. Basal nitric oxide limits immune, nervous and cardiovascular excitation: human endothelia express a mu opiate receptor. Prog. Neurobiol. 60, 513–530.

Strawn, W.B., Bondjers, G., Kaplan, J.R., Manuck, S.B., Schwenke, D.C., Hansson, G.K., Shively, C.A., Clarkson, T.B., 1991. Endothelial dysfunction in response to psychosocial stress in monkeys. Circ. Res. 68, 1270–1279.

Su, Y., Edwards-Bennett, S., Bubb, M.R., Block, E.R., 2003. Regulation of endothelial nitric oxide synthase by the actin cytoskeleton. Am. J. Physiol. Cell Physiol. 284, C1542–C1549.

Szuba, A., Skobe, M., Karkkainen, MJ., Shin, WS., Beynet, DP., Rockson, NB., Dakhil, N., Spilman, S., Goris, ML., Strauss, HW., Quertermous, T., Alitalo, K., Rockson, SG., 2002. Therapeutic Lymphangiogenesis with human recombinant VEGF-C. Faseb J. 16(14), 1985–1987.

Tennenberg, S.D., Finkenauer, R., Wang, T., 2002. Endothelium down-regulates Fas, TNF, and TRAIL-induced neutrophil apoptosis. Surg. Infect. (Larchmt) 3, 351–357.

Toussaint, O., Dumont, P., Remacle, J., Dierick, J.F., Pascal, T., Frippiat, C., Magalhaes, J.P., Zdanov, S., Chainiaux, F., 2002. Stress-induced premature senescence or stress-induced senescence-like phenotype: one in vivo reality, two possible definitions? Sci. World J. 2, 230–247.

Vapaatalo, H., Mervaala, E., 2001. Clinically important factors influencing endothelial function. Med. Sci. Monit. 7, 1075–1085.

Wagner, M., Hampel, B., Bernhard, D., Hala, M., Zwerschke, W., Jansen-Durr, P., 2001. Replicative senescence of human endothelial cells in vitro involves G1 arrest, polyploidization and senescence-associated apoptosis. Exp. Gerontol. 36, 1327–1347.

Yang, J., Jones, S.P., Suhara, T., Greer, J.J., Ware, P.D., Nguyen, N.P., Perlman, H., Nelson, D.P., Lefer, D.J., Walsh, K., 2003. Endothelial cell overexpression of fas ligand attenuates ischemia-reperfusion injury in the heart. J. Biol. Chem. 278, 15185–15191.

Yoshida, M., Westlin, W.F., Wang, N., Ingber, D.E., Rosenzweig, A., Resnick, N., Gimbrone, M.A. Jr, 1996. Leukocyte adhesion to vascular endothelium induces E-selectin linkage to the actin cytoskeleton. J. Cell Biol. 133, 445–455.

Zadeh, M.S., Kolb, J.P., Geromin, D., D'Anna, R., Boulmerka, A., Marconi, A., Dugas, B., Marsac, C., D'Alessio, P., 2000. Regulation of ICAM-1/CD54 expression on human endothelial cells by hydrogen peroxide involves inducible NO synthase. J. Leukoc. Biol. 67, 327–334.