ANTIOXIDANTS & REDOX SIGNALING
Volume 11, Number 10, 2009

DOI: 10.1089/ars.2009.2377

FORUM REVIEW ARTICLE

Stress as an Intercellular Signal: The Emergence of Stress-Associated Molecular Patterns (SAMP)

Anna Rubartelli[1] and Roberto Sitia[2]

Abstract

Cells are continuously exposed to stressful situations that generally entail generation of reactive oxygen species and other redox alterations. Low levels of stress are physiological and can transmit essential survival or adaptive signals. At higher levels, however, the responses become maladaptive and cause damage. Frequently, stressful events occurring in a few cells propagate, resulting in tissue or even systemic response. Here we review recent evidence suggesting that stressed cells signal their state by expressing on their surface and secreting suitable molecular clues, which we propose to term Stress-Associated Molecular Patterns (SAMP). A unifying mechanism seems to involve the release of oxidoreductases and redox modifiers into the intercellular space, with structural and functional alterations in key signaling molecules. These observations open the way to novel therapeutic strategies. *Antioxid. Redox Signal.* 11, 2621–2629.

Stress: Physiologic, Pathologic, Intracellular, and Extracellular

Stress can be generated by far too many events along one's life. Working too hard, being the target of various types of psychological or physical hits, simply standing mutely the aggressiveness of close people, feeling deprived, or staying in a danger situation can be causes of severe stress. Likewise, tissues and single cells in our body are continuously exposed to stressful events. And as a person reacts to stress by starting a number of escape or defense mechanisms, living cells are equipped with devices that physiologically allow them to rapidly respond, adapt, or in extreme cases die. Cells can be subjected to internal or external stresses. The former includes metabolic processes such as, for instance, the exuberant synthesis and release of proteins by professional secretory cells that require large amounts of metabolites and energy (a hard work). Examples of external stress range from starvation and physical (*i.e.*, shearing, temperature changes, irradiation) or chemical (*e.g.*, exposure to drugs or poisons) insults, to contact with or invasion by pathogens. Particularly in the latter cases, the events occurring in a few cells elicit responses of the surrounding tissue, recruitment of specialized cell types, and stressful situations become systemic.

The organismic response to stress requires the participation of numerous cell types, cells of the innate and adaptive immune systems in charge of combating pathogens. How are these recruited in the areas where they are most needed? It has long been recognized that pathogens express a large array of pathogen-associated molecular patterns (PAMP). These are recognized by specific sensors located in diverse cellular districts (pattern recognition receptors, PRR), which activate signaling cascades leading to an inflammatory response. In sterile inflammatory conditions, a similar role is played by DAMP (damage-associated molecular patters). DAMP are cytosolic or nuclear molecules released upon cell necrosis whose presence in the extracellular space defines a potentially dangerous, nonphysiological situation (59). These molecules are also called Alarmins, for their property of eliciting danger signals and hence activating immunity (52). A third type of scenario is that of physiological stress situations. For instance, evidence is mounting that the differentiation of antibody secreting cells entails a multilevel chronic stress response that ultimately leads to cell death (16). In many aspects, the exuberant synthesis and transport of antibodies can resemble conditions of virus infection, the protein translation machinery being overwhelmed and skewed. It is reasonable to surmise that also the physiological overwork of some differentiating cells would attract the attention of immune cells, somehow like secret services have a look at areas of hyperactivity.

Here we review some recent evidence suggesting that cells suffering stress release signals, which we define SAMP for stress-associated molecular patterns, that inform vicinal cells of a changing situation, and can activate or sustain multicellular adaptive responses. As profound redox unbalances are common in stressed cells, SAMP are often generated or modulated by redox-based pathways. PAMP, DAMP, and

[1]Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy.
[2]Università Vita–Salute–San Raffaele Scientific Institute, Division of Genetics and Cell Biology, DIBIT, Milano, Italy.

SAMP may include macromolecules as well as small compounds, such as ATP, reactive oxygen species (ROS), reactive nitrogen species (RNS) and nonprotein thiols (NPSH, including reduced cysteine and glutathione), the proper integration of which seems to dictate the specificity and the duration of the danger signal.

Redox Unbalance Is a Hallmark of Stress

Most forms of stress have a common hallmark that is a profound unbalance of redox homeostasis due to generation of ROS or NRS. Respiration, hypoxia, signaling, the synthesis of certain proteins, metabolic imbalances are no exception. Of course, stress conditions are not always detrimental for cells, tissues, and organisms. Starvation, for instance, represents a stress; however, mild starvation can be beneficial, in that energy restriction may extend life span. A dose-dependent duality of the stress responses is encountered in many cases: thus, low doses of ROS are essential for cell survival, as they are necessary for many signaling pathways, whilst an excess of them generates damage and eventually cell death (56). To prevent the occurrence of maladaptive responses, cells are equipped with a vast array of defense mechanisms against free radical-induced oxidative stress (72). Among these, a major role is played by antioxidants. These include many enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), peroxiredoxins (PrX), heme oxygenase (HO), and glutathione synthases. The latter increase the production of glutathione (GSH), the prototypic and most abundant nonprotein thiol in eukaryotes. Other cellular nonenzymatic antioxidant systems include cysteine, vitamins C and E, carotenoids, and flavonoids. Under normal conditions, these systems balance the constitutive generation of ROS. Cells try to adapt to changing needs modulating the expression of antioxidants, enzymes, and NPSH.

Oxidative stress leads to the synthesis of heat shock proteins (Hsp) (24). This is not surprising, as thiol oxidation may lead to protein denaturation, as does thermal stress. However, these two stresses (oxidative and thermal) denature different proteins. The common feature of heat or oxidation-denatured proteins is the formation of a 'molten globule' that appears to trigger the synthesis of Hsp (24). Thiol oxidation is implicated as a key event leading to this conformational change, although other protein modifications probably also contribute. Protein thiol oxidation may, therefore, be a factor also in determining the heat shock response. In turn, Hsp, induced in cells by thiol specific reagents such as arsenite and diamide, are in part responsible for the consequent protection against the toxic effects of these compounds. Interestingly, in *Escherichia coli* the chaperone activity of Hsp33 is unleashed by exposure to oxidants. Hsp33 undergoes a conformational change and forms oxidized dimers, which bind to many substrates and prevent their aggregation. Thus, Hsp33 shifts between active (substrate-binding) and inactive conformations, according to its redox state, which in turn depends on the environment (27). In metazoans, a similar mechanism characterizes peroxiredoxins that form decamers endowed with chaperone activity upon exposure to H_2O_2 or heat shock (29).

Redox Differs Inside and Outside Cells

Although the environments inside and outside cells are in close communication, and an oxidative stress generated extracellularly can be promptly sensed intracellularly, the redox state inside and outside the plasma membrane is very different. Under normal conditions, glutathione is present in the cytosol in concentrations ranging between 1 and 10 m*M*, mostly in its reduced form (GSH). Conversely, micromolar concentrations are typically found in the plasma, where the oxidized form (GSSG) is predominant (53). Similarly, the amino acid cysteine, present in micromolar amounts, is predominantly reduced inside the cell. In contrast, oxidized cystine is the predominant species in the external space (42, 61).

The notion that the cytosol is reducing, while the extracellular space is generally more oxidizing, has important consequences on protein structure and regulation. In membrane and secretory proteins, most cysteines are engaged in disulfide bonds that are often critical for their stability. Their reduction entails profound structural changes, which may serve a regulatory role in physiologic and pathologic conditions (see below). Cytosolic and nuclear proteins, instead, generally expose reduced cysteines on their surfaces. As a result, they are easily inactivated (or sometimes activated) by oxidation (37). This is counteracted primarily by GSH and thioredoxin (Trx), which are in charge of maintaining suitable redox conditions in the cytosol and nucleus during the basal metabolic activity of the cell and under stress. Not all the cytoplasm is equally reducing, however (Fig. 1). Islands with more oxidizing conditions are the lumen of the endoplasmic reticulum (ER) and other downstream compartments of the secretory pathway. In terms of redox and ionic compositions, these are similar to the extracellular space. This feature is crucial to favor the folding and transport of secretory proteins that are synthesized in the ER. Indeed, this organelle provides an efficient factory and test bench for secretory proteins, designed to operate in the high Ca^{2+} and largely oxidative conditions of the extracellular world (1, 20, 65). In the early secretory compartment, oxidative folding is facilitated by a vast array of thiol-disulfide oxidoreductases (protein disulfide isomerase (PDI), ERp57, ERp5, etc) that catalyze the oxidation, isomerization, and reduction of disulfides (21). In eukaryotes, conserved flavoproteins of the Ero1 family generate oxidative equivalents probably at the expenses of oxygen. At least, this reaction generates peroxide in stoichiometric amounts to the disulfides inserted in nascent proteins (28, 63, 71). The production of H2O2 during oxidative protein folding (28, 69) could serve signaling purposes, reporting on the protidosynthetic activity within the ER (43).

Mitochondria are reducing as well, but certain proteins need be oxidized for exerting their functions. The Erv1–Mia40 pathway, essential for mitochondrial protein import, transfers disulfide bonds into selected substrates (45), underscoring the versatility of redox-based mechanisms in regulating biological systems.

Little is known about the redox conditions found in other organelles. The development of suitable reporters for dynamically analyzing redox status in living cells is clearly needed.

Cell Responses to Stress Can Modify the Extracellular Redox

Intracellular redox modulation has long been recognized essential for many vital metabolic and signaling pathways (38, 56). Conversely, less attention has been paid so far to the extracellular redox as a modulator of cell activities. One rea-

son is that, until recently, oxidoreductases were considered to be strictly restricted inside cells. However, antioxidant enzymes such as superoxide dismutase (EC-SOD) are also present in the extracellular space, and intracellular oxidoreductases, including PDI, ERp5, and thioredoxin (Trx), undergo a regulated externalization by certain cell types (Fig. 2). In many cases, their extracellular activities are aimed at counteracting different kinds of stress. EC-SOD contains a unique heparin-binding domain that determines localization in the extracellular matrix, where the enzyme scavenges superoxide anions. The EC-SOD heparin-binding domain can be removed by proteolytic cleavage, releasing active enzymes into the extracellular fluids. In addition to protecting against extracellular oxidative damage, EC-SOD, by scavenging superoxide, preserves nitric oxide bioactivity and facilitates hypoxia-induced gene expression (51). Trx is secreted by several cell types through a "leaderless" pathway (58), and stimulates the growth of lymphocytes, fibroblasts, and a variety of leukemic and solid tumor cell lines (2, 38, 54, 67). Clearly, these effects rely on the Trx redox activity: a mutant in which the two cysteines in the active redox center (32 and 35) are replaced by serines fails to stimulate cell growth, even at 50-fold higher concentrations. The mechanisms underlying the proliferative effect are unclear, but they appear related to increased production of cytokines (*e.g.*, IL-1, IL-2, and TNF) and/or activation of basic fibroblast growth factor (bFGF) or other growth factors (reviewed in ref. 7).

Protein disulfide isomerase (PDI) has been implicated in the regulation of HIV entry into cells and in the activation of L-selectin shedding from the cell surface of leukocytes. Moreover, PDI is the epithelial cell receptor for *Chlamydia trachomatis Servoar E*, a bacterial agent responsible for sexually transmitted diseases (reviewed in ref. 60). As discussed further below, both PDI and another thiol isomerase, ERp5, normally present mainly on platelet intracellular membranes, are rapidly recruited to the platelet surface in response to many agonists, promoting aggregation (32).

In spite of these recognized functions of extracellular redox enzymes, few cell surface or soluble targets have been identified so far. One reason for this slow progress is the transient nature of thiol–disulfide exchange reactions, making the detection of intermediates and substrates rather difficult. The recent development of a mechanism-based kinetic trapping to identify individual cell surface target proteins that engage in disulfide exchange with thiol-dependent oxidoreductases will hopefully help to advance more rapidly (62). Among the few targets identified so far are the integrin IIb3, converted from a low to high affinity state by ERp5; the CD4 receptor, converted by Trx into a redox isoform permissive for HIV entry, and the HIV-1 envelope glycoprotein gp120, which also relies on the redox activity of PDI and Trx to promote virus–cell fusion. Recently, PDI has been recognized to control the redox state of an allosteric disulfide bond in tissue factor (TF), thereby regulating its activity (see below). In addition, secreted Trx has been found to activate the ion channel TRPC5 by breaking an intrachain disulfide bridge (75). Together, these examples indicate that redox enzymes in the extracellular space are capable of modulating intercellular signals or delivering novel ones, and reveal the existence of transduction mechanisms that can directly couple the activity of these enzymes to cell function.

Not only redox enzymes, but also nonprotein thiols (NPSH), by virtue of their ability to be reversibly oxidized, can influence the extracellular redox balance and hence many signaling pathways (47). Moreover, NPSH-mediated redox reactions are involved in the regulation of growth factor bioactivity, as in the case of transforming growth factor that is inactivated by NPSH in a nonenzymatic way (8).

Increasing evidence indicates that in case of stress, the adaptive antioxidant response triggered by cells, with overexpression of oxidoreductases and NPSH, goes beyond the cell border and alters the extracellular redox. The idea that disulfide bonds can act as dynamic redox switches, specifically operated by secreted redox catalysts, represents an

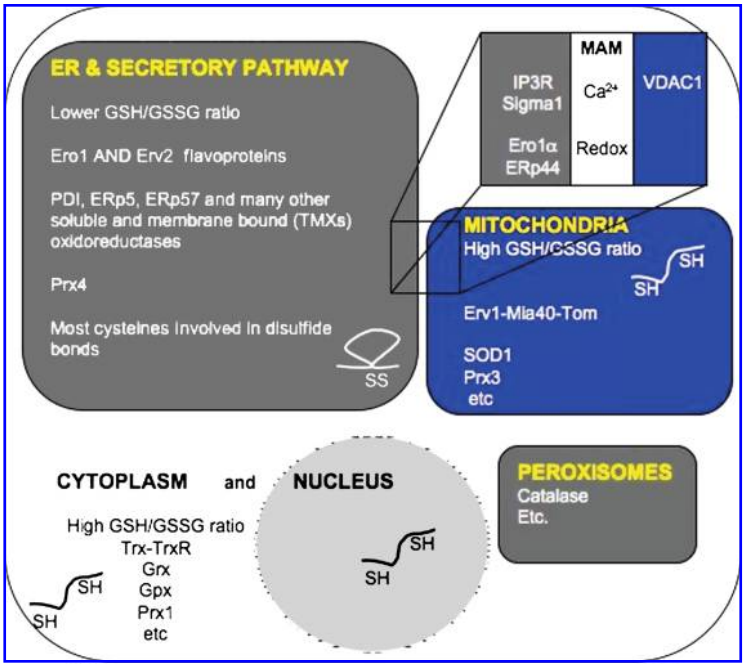

FIG. 1. Compartmentalized redox control in mammalian cells. The figure summarizes the main regulators of the redox state in the different subcellular compartments. The endoplasmic reticulum (ER) and downstream stations of the exocytic pathways are generally oxidizing. Ero1 and Erv2 are flavoproteins that oxidize PDI and other resident oxidoreductases (70). The GSSG/GSH ratio is significantly higher than in the cytoplasm and mitochondria (43). In the latter compartment, Erv1 oxidizes Mia40, thus allowing import of proteins from the cytosol (45). Redox active proteins seem to preferentially accumulate at mitochondria–ER interface. MAM, mitochondrial associated membranes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

emerging concept in signal transduction (31). This could be particularly relevant in close intercellular contacts, particularly in neural or immunological synapses, where the release of a small quantity of redox-active molecules could have dramatic consequences on receptors or channels, as proposed (75). A few well-defined examples are reported below.

1. Tuning immune responses by redox and metabolic stress

Many lines of evidence demonstrate that GSH and Trx regulate immune responses (34, 50). The intracellular GSH levels in macrophages influence the Th1/Th2 cytokine response patterns (48). Glutathione depletion of donor organs inhibits host T-cell responses and prolongs allograft survival *in vivo* (44). Owing to their antiviral and immuno-modulatory properties, the use of novel pro-glutathione compounds (*e.g.*, S-acetylglutathione, an acetylated GSH derivative permeable to the plasma membrane, see (23)), have been proposed in many diseases such as viral infections, immune dysfunction, and cancer.

The notion that the extracellular environment is oxidizing seems at first in contrast with the long-standing evidence that resting B and T lymphocytes require a reducing milieu for activation and proliferation (6, 74). One underlying reason is that unlike most cell types, resting T and B cells do not express the Xc- transporter that allows internalization of oxidized cystine (14, 25). They hence rely on exogenous cysteine, which is in shortage not only in tissue culture media, but also in most body extracellular fluids. Intercellular contacts with macrophages (64) or dendritic cells (2) become hence crucial, and contribute in regulating the strength of immune responses. In fact, these cell types can uptake cystine, reduce it intracellularly via Trx-dependent pathways, and eventually release the cysteine necessary for lymphocyte activation. Interestingly, upon antigen-dependent contacts, dendritic cells can also release Trx, which might generate further cysteine (2). It remains to be seen whether and to what extent also Trx-reductase and NADPH are released so as to produce an extracellular source of reducing power in the immunological synapse.

In vivo, lymphoid organs are characterized by low oxygen tension (0.5–4.5%) (12), which favors a reduced intracellular environment, with high GSH/GSSG ratios (3). As a result, lymphoid organs are generally more reducing than average tissues. Furthermore, the production of NPSH increases dramatically during the immune response (14). Not only dendritic cells, but also activated B-lymphocytes in germinal centers can contribute to the generation of the reducing microenvironment of the immune response. Antibody secreting cells release abundant thiols, perhaps as byproducts of their intense metabolic activity (14; M. Fabbri and R. Sitia, unpublished observations). It is tempting to speculate that plasmacytic differentiation contributes in generating an environment that is suited for further lymphocyte activation, should antigen not be neutralized by the antibodies produced. When a long-lived, resting B cell is activated by mitogens or antigens, a structural and functional metamorphosis ensues: the ER and other secretory organelles expand so as to sustain massive Ig production (73). Elements of the UPR, a multidimensional signaling pathway elicited by ER stress, are activated and play an essential role in the physiological differentiation pathways (9, 41, 73). At the end of the process, a single mature antibody secreting cell can release up to 10^3 IgM per second, each containing 10^2 disulfides. It follows that 10^5 disulfides must be formed each second solely to sustain IgM production. Ero1α and β, the rate limiting factors in disulfide bond formation (46), increase significantly during terminal B cell differentiation, likely providing the required oxidative power. If oxygen is used as an electron acceptor, as described *in vitro* for yeast Ero1p (28, 71), huge amounts of peroxide would be generated as by-products, which could serve signaling purposes. Differentiating B cells activate Nrf2-dependent pathways (49; S. Cozza, S. Masciarelli, and R. Sitia, unpublished observations), suggesting that they indeed experience oxidative stress. Initially, peroxides could reinforce BCR- and NF-kB-dependent differentiation signals. Later on, the buffering power could be saturated by the increasing flux of Igs, thus conveying apoptotic signals (see ref. 43, and references therein). Antibody secreting cells undergo yet another kind of stress (16): surprisingly, in fact, proteasome capacity decreases in the late phases of their differentiation, when Ig production becomes maximal. The unfavorable proteasome load/capacity ratio correlates with increased sensitivity to proteasome inhibitors and spontaneous apoptosis both *in vitro* (16) and *in vivo* (13). These observations have profound implications for the therapy of multiple myeloma, a pathologic condition in which proteasome inhibitors display remarkable efficacy (57). Interestingly, proteasome inhibitors displayed promising synergies with inhibitors of glutathione biosynthesis (49) and ER stressors (G. Bianchi, S. Cenci, and R. Sitia, unpublished observations) suggesting that indeed different kinds of stress (redox, proteasomal, ER-dependent) conjure in inducing plasma cell death, thus limiting antibody responses (16, 43).

Collectively, these findings support that extracellular redox tunes intercellular communication during adaptive immune responses. The notion that the generation of an efficient immune response requires a local reduced microenvironment (27, 45) may pose the basis for therapeutic interventions.

2. Stress, REDOX, and coagulation

To promptly and selectively accomplish its multiple functions, blood coagulation is functionally inactive in the intact vasculature but rapidly triggered when the barrier function of the endothelium is perturbed. Following vascular wall damage, platelets aggregation and fibrin formation at the site of injury ensure sealing of the ruptured vessel and prevention of blood loss. Apart from its immediate actions necessary to arrest bleeding, fibrin also regulates the subsequent steps of wound healing. Interestingly, coagulation seems to be strictly redox-regulated (17). Indeed, platelet activation results in a marked increase in the number of cell surface protein thiols (10), in particular on integrins (22). This event is paralleled by an increase in cell surface exposure for both PDI (10) and ERp5 (32). Inhibition of surface PDI blocks platelet aggregation, suggesting that PDI-dependent thiol/disulfide interchange reactions account for alteration of integrin conformation (35). In addition to integrins, a number of pro- or anti-thrombotic proteins contain one or more disulfide bonds that undergo modifications during the coagulation process. The best example is tissue factor (TF), a membrane protein predominantly expressed on endothelial and blood cells, which

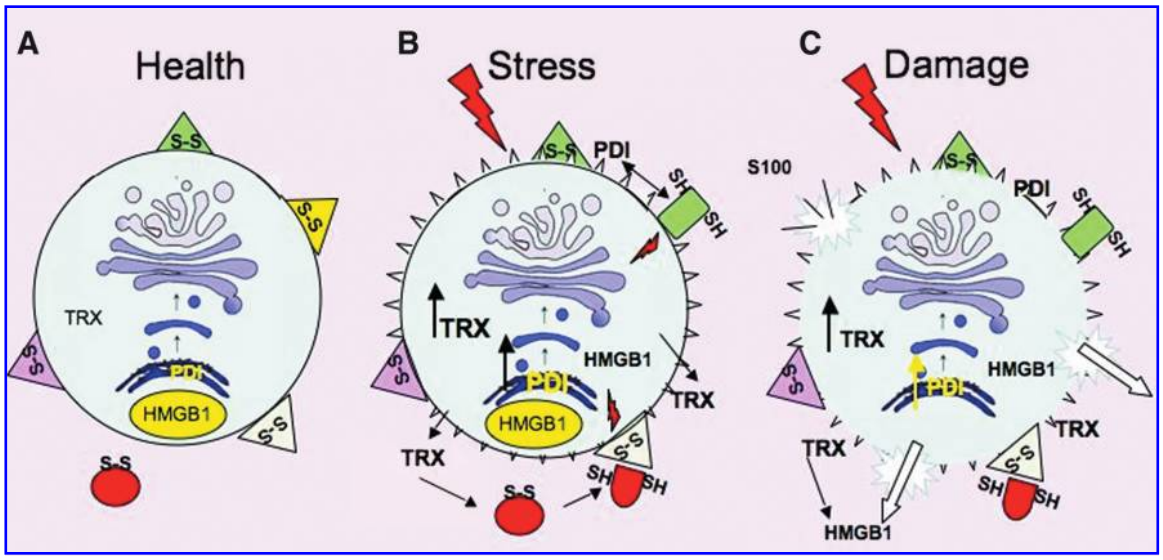

FIG. 2. SAMPs and DAMPs. (**A**) In healthy cells, cytosolic proteins are mostly reduced, whereas secretory and membrane-bound proteins contains many disulfides (S–S). (**B**) When cells undergo stress, there is an increased production of intracellular stress proteins, and certain oxido-reductases normally retained intracellularly (*e.g.* Trx, PDI, RERp5) are externalized. These released oxido-reductases may reduce disulfide bonds on soluble or membrane-bound proteins, resulting in conformational and functional alterations of key signaling molecules. (**C**) Injured cells undergo membrane damage and release intracellular proteins (*i.e.*, HMGB1) as DAMP. In principle, DAMP function only briefly and locally, before they become inactivated by the oxidizing extracellular milieu. This restricts their range of action both temporally and spatially. This control may be lost by the concomitant reduction of the microenvironment due to the externalizations of SAMP such as oxido-reductases and NPSH that may maintain the functional folding of DAMP or revert their oxidation, thereby supporting prolonged activity of the DAMP (39, 59). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

promotes fibrin formation by increasing the proteolytic activity of the coagulation factor VIIa by several orders of magnitude. An allosteric disulfide bond in the membrane-proximal fibronectin type III domain controls TF activity. TF exists in three forms on the cell surface: a cryptic form that is inert, a coagulant form that rapidly binds factor VIIa to initiate coagulation, and a signaling form that binds factor VIIa and cleaves protease-activated receptor 2, which promotes inflammation, tumor progression, and angiogenesis. Reduction and oxidation of the Cys186–Cys209 disulfide bond is central to the transition between the three forms of TF. The redox state of the bond is controlled also in this case by PDI (and by NO). PDI acts as a SAMP since it is not detected on the surface of the unperturbed vessels and its extracellular exposure is injury specific. PDI accumulates rapidly in the thrombus that evolves at the site of wall damage, preceding platelet accumulation, and remains associated with the thrombus, indicating that it is externalized both by stressed endothelial cells and by aggregating platelets (18, 55).

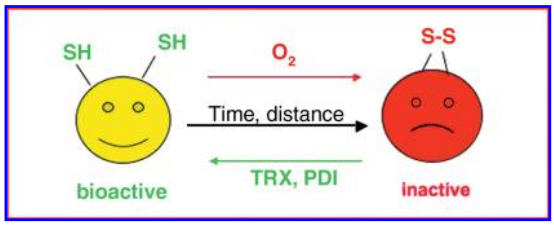

FIG. 3. Redox-dependent timing of DAMP's activity. Cytosolic and nuclear protein usually require free thiols for their activity, either because they bear the active enzymatic redox site C-X-X-C (such as enzymes of the thioredoxin family) or because intrachain or interchain disulfide bond formation cause conformational changes that are detrimental for their function (FGF1, galectins, and HMGB1). When these proteins are delivered outside, the oxidizing milieu of the extracellular space will cause oxidation and therefore inactivation as a function of time and distance from the producing cell (39, 59). The presence of oxido-reductase systems may delay or revert inactivation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

3. Stress, REDOX and cancer

Generation of ROS in the site of chronic inflammation and ROS-mediated tissue injury are recognized as deeply involved in cancer development (72). The cancer promoting effects of ROS and RNS are exerted at many levels, including promotion of angiogenesis, increase in DNA damage and mutagenesis, and immunosuppression (5). In support of these pro-tumor effects, higher levels of NO and H_2O_2 are detected in tumors, and correlation to tumor grade was often observed (reviewed in ref. 5). Cancer cells undergoing oxidative stress react by mounting a protective antioxidant response, resulting in overexpression of reducing enzymes. Unexpectedly, also the overexpression of reducing enzymes such as Trx (66, 68) has been associated with poor prognosis. Intracellular Trx may result advantageous for cancer by increasing cell proliferation and resistance to apoptotic cell death (7). In addition, we have recently observed that overexpression of Trx is accompanied by production and secretion of NPSH

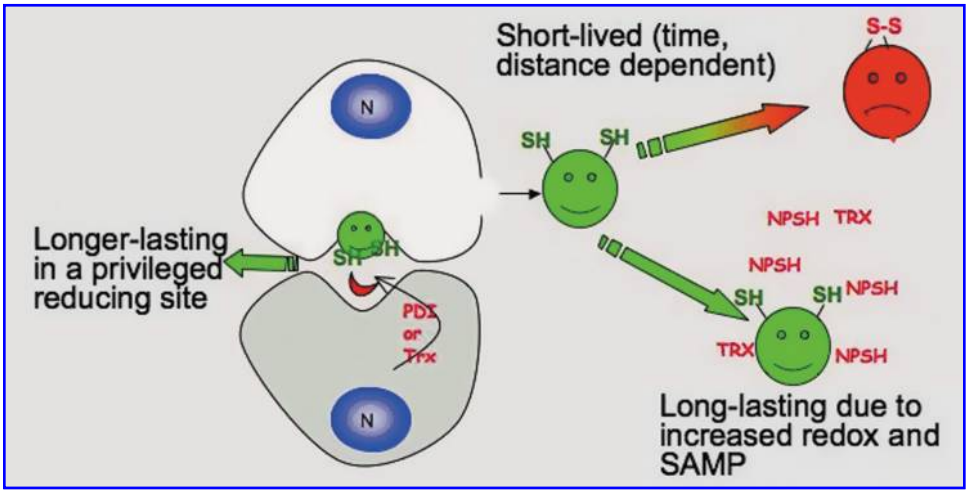

FIG. 4. SAMPs and DAMPs as signals in intercellular space and synapses. A cytosolic or nuclear protein released either through leaderless secretion or upon cell death is rapidly inactivated unless it localizes in a privileged site, such as the synaptic pocket between two interacting cells, where a reduced microenvironment may be generated. Another condition that may prolong extracellular stability and bioactivity of a protein bearing free cysteines is the concomitant modification of the extracellular milieu toward a reducing state, such as it may occur in site of chronic inflammation or cancer (15). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

(15). Also, Trx may be externalized by either active secretion or passive release following cell necrosis. Externalization of oxidoreductases and NPSH may change the extracellular redox dramatically, switching from an oxidizing to a reducing state. Necrosis occurs frequently within a neoplastic tissue, due to the presence of large areas of hypoxia or scarce nutrients, and results in the externalization of intracellular components (DAMP) that induce production of cytokines, chemokines, and angiogenic factors maintaining and worsening the chronic inflammatory state. DAMP are originally located inside cells in an active reduced state. When externalized in an oxidizing extracellular environment, DAMP are subjected to inactivation by oxidation (39, 59) (Fig. 3). The reducing extracellular milieu generated in certain cancer contexts by abundant NPSH and Trx might delay oxidation and support a prolonged activity of DAMP that further promotes cancer growth.

Trx (and its partner Trx-reductase) is not the only extracellular oxidoreductase correlating with poor prognosis. PDI expression has been found related to the invasive properties of malignant glioma (26). Moreover, a recent report indicates that the surface expression of ERp5 by tumor cells favors the shedding of MIC-A, a MHC class I-like molecule that interacts with cognate receptors on NK cells (NKGD2). Released MIC-A can bind to and hence inhibit NKGD2-dependent recognition of cancer cells, thereby inhibiting immune surveillance by NK cells and promoting tumor immune evasion (33). Interestingly, it has been recently shown that the progression of benign proliferation of plasma cell clones (MGUS) into life-threatening multiple myeloma correlates with ERp5-mediated MIC-A shedding. ERp5 acts hence as a tumor progression factor (30). It remains to be seen whether extracellular NPSH or other factors are able to reduce ERp5, allowing it to continue its enzymatic function.

Exogenous Antioxidants: A Double-Edge Sword?

The ambiguity regarding the roles of pro- and antioxidants in tumor progression is reflected by the difficulties encountered in the design of therapeutic approaches aimed at manipulating the redox status. Antioxidants, that can neutralize free radicals and prevent cell and tissue damage, have been proposed as novel and effective anti-tumor drugs, providing promising results (reviewed in ref. 11). However, several anticancer drugs cause an increase of ROS, which contributes to their anti-tumor activity (4, 36). Moreover, targeting the Trx/Trx reductase system by the pro-oxidant arsenic trioxide [As_2O_3, (40)] has beneficial results in experimental cancer therapy. We have recently observed that the same dose of As_2O_3 is toxic for tumor cell lines displaying high levels of Trx, but induces release of Trx and NPSH and cell proliferation on clones derived from the same cell line that express low Trx (15). This suggests that As_2O_3 kills neoplastic cells in which the antioxidative response has reached the maximum extent and are hence under a "reductive stress" (19), but not tumors that can still mount effective antioxidant responses. The implication of these findings is that pro-oxidantbased therapeutic interventions may be effective on neoplastic tissues in which no further responses to oxidative hit are possible, but may actually stimulate tumor progression through the enhanced production of Trx and NPSH in neoplastic cells still able to adapt to an oxidative stress. Clearly, further studies on the biology of tumor-oxidative stress relationships are needed.

Concluding Remarks

Intercellular redox control emerges as a signal generator and rheostat. Cell stress, caused by pathogens, damage, or simply metabolic overwork, often entails profound redox unbalances. These rapidly lead to the appearance of stress-associated molecular patterns (SAMP), including the selective release of certain intracellular oxidoreductases and possibly NPSH that alter the conformation and activity of diverse signaling and regulatory molecules. As a result, intercellular communication pathways are altered. Due to the rapid diffusion of small molecules in the interstitial space, and to the

oxidizing state of the extracellular environment, this mechanism is conceivably active in the "short range," suggesting that redox-mediated signaling can be particularly relevant in privileged sites as immune or neural synapses (Fig. 4).

Acknowledgments

We thank Drs. Michael Lotze, Bruno Goud, Graça Raposo, Simone Cenci, Margherita Cortini, Silvia Masciarelli, and Francesca Tosetti for helpful discussion, and AIRC, Cariplo, MIUR-CoFin, Ministero della Salute, ISS, Foundation Pierre-Gilles de Gennes pour le Recherche, and Telethon for financial support. We apologize to the many colleagues whose seminal work we could not cite solely for space limitations.

References

1. Anelli T and Sitia R. Protein quality control in the early secretory pathway. *EMBO J* 27: 315–327, 2008.
2. Angelini G, Gardella S, Ardy M, Ciriolo MR, Filomeni G, Di Trapani G, Clarke F, Sitia R, and Rubartelli A. Antigen-presenting dendritic cells provide the reducing extracellular microenvironment required for T lymphocyte activation. *Proc Natl Acad Sci USA* 99: 1491–1496, 2002.
3. Atkuri KR, Herzenberg LA, Niemi AK, Cowan T, and Herzenberg LA. Importance of culturing primary lymphocytes at physiological oxygen levels. *Proc Natl Acad Sci USA* 104: 4547–4552, 2007.
4. Baker AF, Landowski T, Dorr R, Tate WR, Gard JM, Tavenner BE, Dragovich T, Coon A, and Powis G. The antitumor agent imexon activates antioxidant gene expression: Evidence for an oxidative stress response. *Clin Cancer Res* 13: 3388–3394, 2007.
5. Ben–Baruch A. Inflammation-associated immune suppression in cancer: The roles played by cytokines, chemokines and additional mediators. *Semin Cancer Biol* 16: 38–52, 2006.
6. Bevan MJ, Epstein R, and Cohn M. The effect of 2-mercaptoethanol on murine mixed lymphocyte cultures. *J Exp Med* 139: 1025–1030, 1974.
7. Biaglow JE and Miller RA. The thioredoxin reductase/thioredoxin system: novel redox targets for cancer therapy. *Cancer Biol Ther* 4: 6–13, 2005.

7a. Bianchi G, Olive L, Cascio P, Pengo N, Fontana F, Cerruti F, Orsi A, Pasqualetto E, Mezghrani A, Colbi V, Palladini G, Giuliani N, Anderson KC, Sitia R, Cenci S. The proteasome load vs. capacity balance determines apoptotic sensitivity of multiple myelome cells to proteasome inhibition. *Blood* 2009 (in press).

8. Blakytny R, Erkell LJ, and Brunner G. Inactivation of active and latent transforming growth factor beta by free thiols: Potential redox regulation of biological action. *Int J Biochem Cell Biol* 38: 1363–1373, 2006.
9. Brewer JW and Hendershot LM. Building an antibody factory: A job for the unfolded protein response. *Nat Immunol* 6: 23–29, 2005.
10. Burgess JK, Hotchkiss KA, Suter C, Dudman NP, Szöllösi J, Chesterman CN, Chong BH, and Hogg PJ. Physical proximity and functional association of glycoprotein 1 b alpha and protein-disulfide isomerase on the platelet plasma membrane. *J Biol Chem* 275: 9758–9766, 2000.
11. Cabello CM, Bair WB, and Wondrak GT. Experimental therapeutics: Targeting the redox Achilles heel of cancer. *Current Opinion in Investigational Drugs (London, England, 2000)* 6: 1022–1037, 2007.
12. Caldwell CC, Kojima H, Lukashev D, Armstrong J, Farber M, Apasov SG, and Sitkovsky MV. Differential effects of physiologically relevant hypoxic conditions on T lymphocyte development and effector functions. *J Immunol* 167: 6140–6149, 2001.
13. Cascio P, Oliva L, Cerruti F, Mariani E, Pasqualetto E, Cenci S, and Sitia R. Dampening Ab responses using proteasome inhibitors following *in vivo* B cell activation. *Eur J Immunol* 2008, 38: 658–667, 2008.
14. Castellani P AG, Delfino L, Matucci A, and Rubartelli A. The thiol redox state of lymphoid organs is modified by immunization: Role of different immune cell populations. *Eur J Immunol* 38: 2419–2425, 2008.
15. Ceccarelli J DL, Zappia E, Castellani P, Borghi M, Ferrini S, Tosetti F, and Rubartelli A. The redox state of the lung cancer microenvironment depends on the levels of thioredoxin expressed by tumor cells and affects tumor progression and response to prooxidants. *Int J Cancer* 123: 1770–1778, 2008.
16. Cenci S and Sitia R. Managing and exploiting stress in the antibody factory. *FEBS Lett* 581: 3652–3657, 2007.
17. Chen VM and Hogg PJ. Allosteric disulfide bonds in thrombosis and thrombolysis. *J Thomb Haemost* 4: 2533–2541, 2006.
18. Cho J, Furie BC, Coughlin SR, and Furie B. A critical role for extracellular protein disulfide isomerase during thrombus formation in mice. *J Clin Invest* 118:1123–1131, 2008.
19. Dimmeler S and Zeiher AM. A "reductionist" view of cardiomyopathy. *Cell* 130: 401–402, 2007.
20. Ellgaard L and Helenius A. Quality control in the endoplasmic reticulum. *Nat Rev Mol Cell Biol* 4: 181–191, 2003.
21. Ellgaard L and Ruddock LW. The human protein disulphide isomerase family: Substrate interactions and functional properties. *EMBO Rep* 6: 28–32, 2005.
22. Essex DW and Li M. Redox control of platelet aggregation. *Biochemistry* 42: 129–136, 2003.
23. Fraternale A, Paoletti MF, Casabianca A, Oiry J, Clayette P, Vogel JU, Cinatl J, Palamara AT, Sgarbanti R, Garaci E, Millo E, Benatti U, and Magnani M. Antiviral and immunomodulatory properties of new pro-glutathione (GSH) molecules. *Curr Med Chem* 13: 1749–1755, 2006.
24. Freeman ML, Borrelli MJ, Meredith MJ, and Lepock JR. On the path to the heat shock response: Destabilization and formation of partially folded protein intermediates, a consequence of protein thiol modification. *Free Radic Biol Med* 26: 737–745, 1999.
25. Gmünder H, Eck HP, and Dröge W. Low membrane transport activity for cystine in resting and mitogenically stimulated human lymphocyte preparations and human T cell clones. *Eur J Biochem* 211: 113–117, 1991.
26. Goplen D, Wang J, Enger PØ, Tysnes BB, Terzis AJ, Laerum OD, and Bjerkvig R. Protein disulfide isomerase expression is related to the invasive properties of malignant glioma. *Cancer Res* 66: 9895–9902, 2006.
27. Graf PC, Martinez–Yamout M, Van Haerents S, Lilie H, Dyson HJ, and Jakob U. Activation of the redox-regulated chaperone Hsp33 by domain unfolding. *J Biol Chem* 279: 20529–20538, 2004.
28. Gross E, Sevier CS, Heldman N, Vitu E, Bentzur M, Kaiser CA, Thorpe C, and Fass D. Generating disulfides enzymatically: reaction products and electron acceptors of the endoplasmic reticulum thiol oxidase Ero1 p. *Proc Natl Acad Sci USA* 103: 299–304, 2006.
29. Jang HH, Lee KO, Chi YH, Jung BG, Park SK, Park JH, Lee JR, Lee SS, Moon JC, Yun JW, Choi YO, Kim WY, Kang JS,

Cheong GW, Yun DJ, Rhee SG, Cho MJ, and Lee SY. Two enzymes in one; two yeast peroxiredoxins display oxidative stress-dependent switching from a peroxidase to a molecular chaperone function. *Cell* 117: 625–635, 2004.
30. Jinushi M, Vanneman M, Munshi NC, Tai YT, Prabhala RH, Ritz J, Neuberg D, Anderson KC, Carrasco DR, and Dranoff G. MHC class I chainrelated protein A antibodies and shedding are associated with the progression of multiple myeloma. *Proc Natl Acad Sci USA* 105: 1285–1290, 2008.
31. Jordan PA and Gibbins JM. Extracellular disulfide exchange and the regulation of cellular function. *Antioxid Redox Signal* 8: 312–324, 2006.
32. Jordan PA SJ, Hubbard GP, Barrett NE, Sage T, Authi KS, and Gibbins JM. A role for the thiol isomerase protein ERP5 in platelet function. *Blood* 105: 1500–1507, 2005.
33. Kaiser BK, Yim D, Chow IT, Gonzalez S, Dai Z, Mann HH, Strong RK, Groh V, and Spies T. Disulphide-isomerase-enabled shedding of tumour-associated NKG2D ligands. *Nature* 47: 482–486, 2007.
34. Kondo N, Ishii Y, Kwon YW, Tanito M, Horita H, Nishinaka Y, Nakamura H, and Yodoi J. Redox-sensing release of human thioredoxin from T lymphocytes with negative feedback loops. *J Immunol* 172: 442–448, 2004.
35. Lahav J, Jurk K, Hess O, Barnes MJ, Farndale RW, Luboshitz J, and Kehrel BE. Sustained integrin ligation involves extracellular free sulfhydryls and enzymatically catalyzed disulfide exchange. *Blood* 100: 2472–2478, 2002.
36. Lecane PS, Karaman MW, Sirisawad M, Naumovski L, Miller RA, Hacia JG, and Magda D. Motexafin gadolinium and zinc induce oxidative stress responses and apoptosis in B-cell lymphoma lines. *Cancer Res* 65: 11676–11688, 2005.
37. Leichert LI and Jakob U. Global methods to monitor the thiol-disulfide state of proteins *in vivo*. *Antioxid Redox Signal* 8: 763–772, 2006.
38. Lillig CH and Holmgren A. Thioredoxin and related molecules—from biology to health and disease. *Antioxid Redox Signal* 9: 25–47, 2007.
39. Lotze MT, Zeh HJ, Rubartelli A, Sparvero LJ, Amoscato AA, Washburn NR, Devera ME, Liang X, Tör M, and Billiar T. The grateful dead: Damageassociated molecular pattern molecules and reduction/oxidation regulate immunity. *Immunol Rev* 220: 60–81, 2007.
40. Lu J, Chew EH, and Holmgren A. Targeting thioredoxin reductase is a basis for cancer therapy by arsenic trioxide. *Proc Natl Acad Sci USA* 104: 12288–12293, 2007.
41. Ma Y and Hendershot LM. The stressful road to antibody secretion. *Nat Immunol* 4: 310–311, 2003.
42. Mansoor MA, Svardal AM, and Ueland PM. Determination of the in vivo redox status of cysteine, cysteinylglycine, homocysteine, and glutathione in human plasma. *Anal Biochem* 200: 218–229, 1992.
43. Masciarelli S and Sitia R. Building and operating an antibody factory: Redox control during B to plasma cell terminal differentiation. *Biochim Biophys Acta* 1783: 578–588, 2008.
44. McKenna GJ, Kim PT, Mui AL, Ong CJ, Rotstein OD, Warnock GL, and Chung SW. Glutathione depletion of stimulator cells inhibits responder T-cell immunogenicity *in vitro* and prolongs allograft survival *in vivo*. *Am J Surg* 191: 588–592, 2006.
45. Mesecke N, Terziyska N, Kozany C, Baumann F, Neupert W, Hell K, and Herrmann JM. A disulfide relay system in the intermembrane space of mitochondria that mediates protein import. *Cell* 121: 1059–1069, 2005.
46. Mezghrani A, Fassio A, Benham A, Simmen T, Braakman I, and Sitia R. Manipulation of oxidative protein folding and PDI redox state in mammalian cells. *EMBO J* 20: 6288–6296, 2001.
47. Moran LK, Gutteridge JM, and Quinlan GJ. Thiols in cellular redox signalling and control. *Curr Med Chem* 8: 763–772, 2001.
48. Murata Y, Shimamura T, Tagami T, Takatsuki F, and Hamuro J. The skewing to Th1 induced by lentinan is directed through the distinctive cytokine production by macrophages with elevated intracellular glutathione content. *Int Immunopharmacol* 2: 673–689, 2002.
49. Nerini–Molteni S, Ferrarini M, Cozza S, Caligaris–Cappio F, and Sitia R. Redox homeostasis modulates the sensitivity of myeloma cells to bortezomib. *Br J Haematol* 141:494–503, 2008.
50. Nishinaka Y, Nakamura H, and Yodoi J. Thioredoxin cytokine action. *Methods Enzymol* 347: 332–338, 2002.
51. Nozik–Grayck E, Suliman HB, and Piantadosi CA. Extracellular superoxide dismutase. *Int J Biochem Cell Biol* 37: 2466–2471, 2005.
52. Oppenheim JJ, Tewary P, de la Rosa G, and Yang D. Alarmins initiate host defense. *Adv Exp Med Biol* 601:185–194, 2007.
53. Pastore A, Federici G, Bertini E, and Piemonte F. Analysis of glutathione: implication in redox and detoxification. *Clin Chim Acta* 333: 19–39, 2003.
54. Powis G and Montfort WR. Properties and biological activities of thioredoxins. *Annu Rev Pharmacol Toxicol* 41: 261–295, 2001.
55. Reinhardt C, von Brühl ML, Manukyan D, Grahl L, Lorenz M, Altmann B, Dlugai S, Hess S, Konrad I, Orschiedt L, Mackman N, Ruddock L, Massberg S, and Engelmann B. Protein disulfide isomerase acts as an injury response signal that enhances fibrin generation via tissue factor activation. *J Clin Invest* 118: 1110–1122, 2008.
56. Rhee SG. Cell signaling. H2O2, a necessary evil for cell signaling. *Science* 312: 1882–1883, 2006.
57. Richardson PG, Mitsiades C, Hideshima T, and Anderson KC. Bortezomib: Proteasome inhibition as an effective anticancer therapy. *Annu Rev Med* 57: 33–47, 2006.
58. Rubartelli A, Bajetto A, Allavena G, Wollman E, and Sitia R. Secretion of thioredoxin by normal and neoplastic cells through a leaderless secretory pathway. *J Biol Chem* 267: 24161–24164, 1992.
59. Rubartelli A and Lotze MT. Inside, outside, upside down: Damage-associated molecular-pattern molecules (DAMPs) and redox. *Trends Immunol* 28: 429–436, 2007.
60. Sahaf B, Heydari K, Herzenberg LA, and Herzenberg LA. The extracellular microenvironment plays a key role in regulating the redox status of cell surface proteins in HIV-infected subjects. *Arch Biochem Biophys* 434: 26–32, 2005.
61. Sato H, Shiiya A, Kimata M, Maebara K, Tamba M, Sakakura Y, Makino N, Sugiyama F, Yagami K, Moriguchi T, Takahashi S, and Bannai S. Redox imbalance in cystine/glutamate transporter-deficient mice. *J Biol Chem*, 2005, 280: 37423–37429, 2005.
62. Schwertassek U, Balmer Y, Gutscher M, Weingarten L, Preuss M, Engelhard J, Winkler M, and Dick TP. Selective redox regulation of cytokine receptor signaling by extracellular thioredoxin-1. *EMBO J* 26: 3086–3097, 2007.
63. Sevier CS, Qu H, Heldman N, Gross E, Fass D, and Kaiser CA. Modulation of cellular disulfide-bond formation and the ER redox environment by feedback regulation of Ero1. *Cell* 129: 333–344, 2007.
64. Sido B, Braunstein J, Breitkreutz R, Herfarth C, and Meuer SC. Thiol-mediated redox regulation of intestinal lamina propria T lymphocytes. *J Exp Med* 192: 907–912, 2000.

65. Sitia R and Braakman I. Quality control in the endoplasmic reticulum protein factory. *Nature* 426: 891–894, 2003.
66. Soini Y, Kahlos K, Näpänkangas U, Kaarteenaho–Wiik R, Säily M, Koistinen P, Pääakkö P, Holmgren A, and Kinnula VL. Widespread expression of thioredoxin and thioredoxin reductase in non-small cell lung carcinoma. *Clin Cancer Res* 7: 1750–1757, 2001.
67. Tagaya Y, Maeda Y, Mitsui A, Kondo N, Matsui H, Hamuro J, Brown N, Arai K, Yokota T, and Wakasugi H. ATL-derived factor (ADF), an IL-2 receptor/Tac inducer homologous to thioredoxin; possible involvement of dithiol-reduction in the IL-2 receptor induction. *EMBO J* 8: 757–764, 1989.
68. Tomiyasu M, Yoshino I, Suemitsu R, Okamoto T, and Sugimachi K. Quantification of macrophage migration inhibitory factor mRNA expression in non-small cell lung cancer tissues and its clinical significance. *Clin Cancer Res* 8: 3755–3760, 2002.
69. Tu BP, Ho–Schleyer SC, Travers KJ, and Weissman JS. Biochemical basis of oxidative protein folding in the endoplasmic reticulum. *Science* 290: 1571–1574, 2000.
70. Tu BP and Weissman JS. Oxidative protein folding in eukaryotes: Mechanisms and consequences. *J Cell Biol* 164: 341–346, 2004.
71. Tu BP and Weissman JS. The FAD- and O(2)-dependent reaction cycle of Ero1-mediated oxidative protein folding in the endoplasmic reticulum. *Mol Cell* 10: 983–994, 2002.
72. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, and Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 39: 44–84, 2007.
73. van Anken E, Romijn EP, Maggioni C, Mezghrani A, Sitia R, Braakman I, and Heck AJ. Sequential waves of functionally related proteins are expressed when B cells prepare for antibody secretion. *Immunity* 18: 243–253, 2003.
74. Watanabe T, Yoshizaki K, Yagura T, and Yamamura Y. *In vitro* antibody formation by human tonsil lymphocytes. *J Immunol* 113: 608–616, 1974.
75. Xu SZ, Sukumar P, Zeng F, Li J, Jairaman A, English A, Naylor J, Ciurtin C, Majeed Y, Milligan CJ, Bahnasi YM, Al-Shawaf E, Porter KE, Jiang LH, Emery P, Sivaprasadarao A, and Beech DJ. TRPC channel activation by extracellular thioredoxin. *Nature* 451: 69–72, 2008.

Address correspondence to:
Anna Rubartelli, M.D.
Largo Rosanna Benzi 10
16132 Genova, Italy

E-mail: anna.rubartelli@istge.it

Date of first submission to ARS Central, November 24, 2008; date of acceptance, December 29, 2008.

Abbreviations Used

DAMP = damage associated molecular patterns
ER = endoplasmic reticulum
Hsp = heat shock proteins
NPSH = nonprotein thiols
PAMP = pathogen-associated molecular patterns
PDI = protein disulfide isomerase
RNS = reactive nitrogen species
ROS = reactive oxygen species
SAMP = stress-associated molecular patterns
Trx = thioredoxin

This article has been cited by:

1. E. Venereau, M. Casalgrandi, M. Schiraldi, D. J. Antoine, A. Cattaneo, F. De Marchis, J. Liu, A. Antonelli, A. Preti, L. Raeli, S. S. Shams, H. Yang, L. Varani, U. Andersson, K. J. Tracey, A. Bachi, M. Uguccioni, M. E. Bianchi. 2012. Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release. *Journal of Experimental Medicine* **209**:9, 1519-1528. [CrossRef]
2. Milena Bertolotti , Roberto Sitia , Anna Rubartelli . 2012. On the Redox Control of B Lymphocyte Differentiation and Function. *Antioxidants & Redox Signaling* **16**:10, 1139-1149. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
3. Brian Henderson, A. Graham Pockley. 2012. Proteotoxic stress and circulating cell stress proteins in the cardiovascular diseases. *Cell Stress and Chaperones* . [CrossRef]
4. Michela Vezzoli , Patrizia Castellani , Gianfranca Corna , Alessandra Castiglioni , Lidia Bosurgi , Antonella Monno , Silvia Brunelli , Angelo A. Manfredi , Anna Rubartelli , Patrizia Rovere-Querini . 2011. High-Mobility Group Box 1 Release and Redox Regulation Accompany Regeneration and Remodeling of Skeletal Muscle. *Antioxidants & Redox Signaling* **15**:8, 2161-2174. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
5. C.E. Angulo-Valadez, M.C. Reyes-Becerril, G.M.J. Romero, R. Cepeda-Palacios, D.R. López-Aguilar, T. Zenteno, F. Ascencio. 2011. Antioxidant enzymes in erythrocytes from goats seropositive to the sheep nose bot fly (Oestrus ovis L., Diptera: Oestridae) infection. *Veterinary Parasitology* . [CrossRef]
6. Daolin Tang , Rui Kang , Herbert J. Zeh III , Michael T. Lotze . 2011. High-Mobility Group Box 1, Oxidative Stress, and Disease. *Antioxidants & Redox Signaling* **14**:7, 1315-1335. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
7. Alessandra Castiglioni, Valentina Canti, Patrizia Rovere-Querini, Angelo A. Manfredi. 2011. High-mobility group box 1 (HMGB1) as a master regulator of innate immunity. *Cell and Tissue Research* **343**:1, 189-199. [CrossRef]
8. Éva Margittai, Roberto Sitia. 2011. Oxidative Protein Folding in the Secretory Pathway and Redox Signaling Across Compartments and Cells. *Traffic* **12**:1, 1-8. [CrossRef]
9. Roberta Vené , Laura Delfino , Patrizia Castellani , Enrica Balza , Milena Bertolotti , Roberto Sitia , Anna Rubartelli . 2010. Redox Remodeling Allows and Controls B-Cell Activation and Differentiation. *Antioxidants & Redox Signaling* **13**:8, 1145-1155. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
10. Michela Vezzoli, Patrizia Castellani, Lara Campana, Gianfranca Corna, Lidia Bosurgi, Angelo A. Manfredi, Marco E. Bianchi, Anna Rubartelli, Patrizia Rovere-Querini. 2010. Redox remodeling: a candidate regulator of HMGB1 function in injured skeletal muscle. *Annals of the New York Academy of Sciences* **1209**:1, 83-90. [CrossRef]