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Meeting Report

Redox regulation in acute and chronic inflammation

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Good things usually fly away faster than you realize, and in fact it is now >20 years ago that the first Italian–German Villa Vigoni conference on 'Redox reactions in cellular regulations' was held (1988, organized by Dr. Giorgio Bellomo and Dr. Volker Ullrich). This year, the role of redox regulation in acute and chronic inflammation has been the focus of the 10th conference, held at Loveno di Menaggio, Italy, in a fascinating venue that faces the breath-taking Lago di Como in northern Italy. Villa Vigoni was again an excellent environment to merge history and science, and a number of aspects of redox regulation were covered by 22 invited speakers (Figure 1).

Mitochondria

The first session was introduced by an overview delivered by one of the founders of the series of Villa Vigoni conferences on redox regulation, Dr. V. Ullrich, who depicted the manifold roles of nitric oxide (NO) and its peroxynitrite ($ONOO^-$) derivative on endothelial activation and arachidonate cascade. Accumulated evidence suggests that indeed peroxynitrite executes redox regulation of cellular reactions, bringing up the central role of mitochondria in its release. The existence of a mitochondrial nitric oxide synthase (NOS) has been hypothesized that generates NO within the organelle, in which also superoxide (O_2^-) is released, thus allowing *in situ* generation of peroxynitrite. In addition, the effect of NO on mitochondrial DNA (mtDNA) integrity was discussed as a key point for redox regulation of cellular functions and human pathologies like diabetes mellitus, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, hepatotoxicity, hypertension and, more in general, aging. Then, a mitochondrial enzyme was shown to impact redox regulation: telomerase. The mitochondrial isoform of telomerase is critical for protecting mtDNA against damage, and is more effective than its nuclear counterpart in protecting cells against apoptosis, by curbing mitochondrial reactive oxygen species (ROS) formation. In addition, an 'old friend' like thioredoxin was presented with a 'new face,' and in fact it was shown to be fully responsible for the anti-apoptotic effect of low doses of hydrogen peroxide (H_2O_2). The latter substance induces nuclear import of thioredoxin by the karyopherin system, leading to the regulation of a number of transcription factors of anti-apoptotic genes.

An interesting topic was covered on the role of infrared A radiation (IRA) as inducer of ROS formation and cause of skin disease. IRA was shown to act through mitochondria, engaging a retrograde signaling pathway that includes calcium flux. Then, the role of redox balance on myoblast differentiation and muscular dystrophies was on the floor. Endogenous NO was shown to be required to initiate myogenesis, and to control myoblast fusion and thus myotubes and fiber formation, in synergy with cyclic GMP (cGMP). In addition, NO was found to inhibit fission of mitochondria, through a DRP-1 (dynamin-related protein 1)-dependent pathway, and this strongly contributes to the myogenetic process. Then, a novel mitochondrial chaperone was described as an anti-apoptotic factor against stress-induced osteosarcoma and colorectal carcinoma cell death: TRAP-1 (tumor necrosis factor α (TNFα) receptor associated protein 1). This protein has a number of ligands, which help the cell to resist to chemicals, and seems to play a role in Parkinson's disease through a TRAP-1 phosphorylation-dependent mechanism. TRAP-1 knockdown enhances the opening of the PTP (permeability transition pore) of mitochondria, and contributes to its desensitization. PTP has manifold implications in prompting human diseases, and interestingly it was recently shown that hexokinase II detachment from mitochondria triggers apoptosis through the PTP, independently of voltage-regulated anion channels.

The remaining part of the session was devoted to the 'other side of the medal' of redox regulation, which is the role of mitochondrial antioxidants. The first talk addressed this issue in the context of hepatotoxicity. Manganese SOD-2 (superoxide dismutase 2) was shown to control isoprostane formation in hepatocytes, and its loss of function increased nitrotyrosine production. In addition, apoptosis and inflammation were associated to loss of SOD-2, which in addition initiated aberrant glutamine synthase expression and tumor

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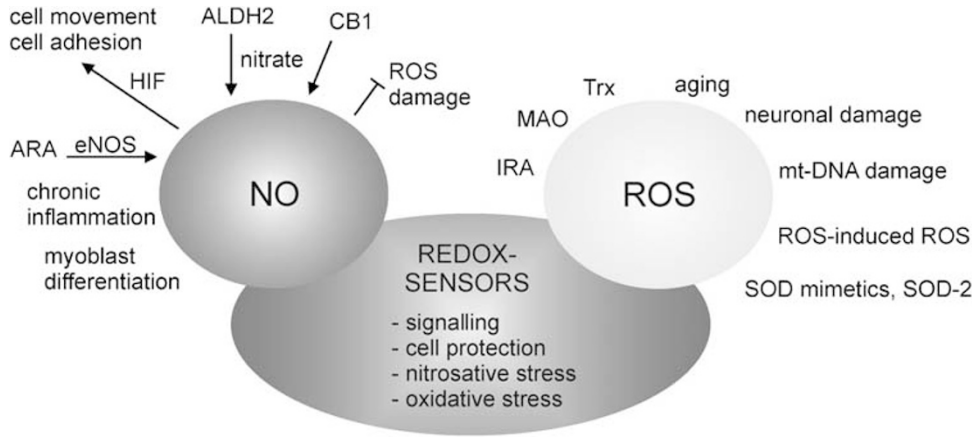

Figure 1 Redox signals in acute and chronic inflammation. The figure summarizes aspects of redox signalling discussed during the meeting, covering the sphere of NO *versus* ROS formation/action in the context of redox-senor signalling. For details and abbreviations see the text

development, overall causing liver failure. Then, the association of antioxidant enzymes with mtDNA was proposed as a defensive strategy. The proximity of mtDNA to the respiratory chain makes it more vulnerable to oxidative damage. Therefore, mtDNA is organized in protein–DNA macrocomplexes called 'nucleoids.' Evidence was presented to show that SOD-2 and GPx-1 (glutathione peroxidase 1) associate with mtDNA within nucleoids, suggesting that an antioxidant system is an integral constituent of nucleoids, aimed at preserving DNA integrity. Finally, nicotine adenine dinucleotide phosphate oxidases (Nox) were discussed as part of the mitochondrial arsenal against oxidative stress, in addition to SOD-2, GPx-1, and ALDH-2 (aldehyde dehydrogenase 2). Nox activation was shown to play a role in diabetes, hypertension, and aging, along a novel signaling pathway whereby mitochondrial ROS, through Nox activation, induce the formation of ROS in the cell cytosol.

Inflammation and redox signalling

The second session moved from mitochondria to inflammation and redox signaling. First, HIF-1α (hypoxia-inducible factor 1α) was shown to regulate metastasis and invasion, as well as chemoresistance, of human gastric or breast cancer cells. Integrin α5 was found to be essential for the induction of HIF-1α, and hence for its biological activity, and in turn it was shown to be sensitive to O_2^- levels. Then, it was shown that ubiquitination of HIF-1α is blocked by NO through the inhibition of prolyl hydroxylase activity at the oxygen-dependent degradation domain for ubiquitination and attenuation of proline hydroxylation of HIF-1α. Additionally, HIF-1α works synergistically with NO to induce migration of macrophages and angiogenesis by controlling gene regulation. Then, a bridge was made with the first session by presenting evidence that hypoxia increases the activity of Ca^{2+}-activated K^+ channel (BK-channel) in the inner mitochondrial membrane, reducing the activity of PTP. A further link between the two sessions was presented next, when bioactive lipids widely expressed in our body (the 'endocannabinoids') were shown to regulate mitochondrial function and atherogenic inflammation. Adipocytes express the machinery to metabolize and bind the two major endocannabinoids (*N*-arachidonoylethanolamine [anandamide] and 2-arachidonoylglycerol), and their differentiation is stimulated by activation of type-1 cannabinoid receptors (CB1) by these compounds. Instead, blockade of CB1 up-regulates endothelial NOS and mitochondrial biogenesis, thus promoting energy expenditure, whereas CB1 overactivity impairs adipocyte mitochondrial function in mice with diet-induced obesity. Furthermore, CB1 antagonism up-regulates adiponectin and down-regulates TNFα in obese Zucker rats, thus curbing atherogenic inflammation and reducing atherosclerosis. Control of chronic inflammation by NO-releasing drugs was the focus of the following presentation, where redox regulation was discussed in the context of EAE (experimental autoimmune encephalomyelitis), an animal model of multiple sclerosis. It was shown that NO-releasing flurbiprofen improves the clinical score of EAE mice and even protects these animals from the gastric toxicity of flurbiprofen alone. The therapeutic efficacy of flurbiprofen–NO was due to a reduction of lymphocyte proliferation, leading to a reduced release of pro-inflammatory cytokines, and a reduced infiltration of T lymphocytes in the central nervous system. Then, the same EAE model was used to discuss the central effects of neuroinflammation. Oxidative damage of mtDNA and mitochondrial dysfunction have been recognized as crucial events along the mechanism behind multiple sclerosis, forming a basis for glutamate exitotoxicity that is responsible for neuronal damage. In this context, Na^+/Ca^{2+} exchanger contributes to increased glutamate release in EAE, and additionally infiltrating CD3+ lymphocytes are able to alter glutamate transmission by activating microglia and promoting TNFα release. A case for redox regulation by the chloroplast, another energy-producing organelle in plants, was presented next. Here, 2-Cys peroxiredoxin was discussed as a novel 'redox sensor.' This protein belongs to the thiol-disulfide redox network, where multiple players were described and grouped as redox input elements, redox transmitters, and redox sensors. Remarkably peroxiredoxins, which have peroxidase activity, are expressed also in yeasts and humans, expanding

their potential role in redox regulation beyond the plant kingdom. The 2-Cys peroxiredoxin works as a decamer, which exists in a reduced or overoxidized form and *in vivo* can undergo to fine conformational changes in dependence of the redox state of the cell. The reduced decamer has peroxidase activity, whereas the overoxidized decamer attaches to the tylacoid membranes and seems to act as a chaperone. Furthermore, MAO-A (monoamine oxidase A) has been shown to be a relevant source of ROS, with a strong impact on ischemia/reperfusion injury and myocardial damage. For instance, MAO-A inhibition reduces arachidonate-induced H_2O_2 production in cardiomyocites, where it antagonizes the contractility impairment observed on ischemia, and prevents ROS formation occurring during post-ischemia. On the other hand, the P66 protein is a complex substance that on stress stimuli translocates to the mitochondria, where it generates H_2O_2. Deletion of P66 confers protection against contractility impairment and prevents lipid peroxidation, an observation confirmed by an *in vivo* model of coronary microembolosis. A further step toward a clinical perspective of redox regulation was taken in the next presentation, where nitrate-induced endothelial preconditioning was shown to improve the outcome of angina in a human study. The underlying mechanism might depend on the protective effect of NO against ROS damage of endothelial cells, and/or it might engage PTP functionality. In this context, also mitochondrial ALDH-2 might be critical, because it could stimulate cGMP formation, and thus NO release, PTP activation, and vasodilation. The next topic on the floor was chronic inflammation, a 'secret killer' that strongly depends on SOD-mediated redox regulation. SOD mimics are metal-based artificial enzymes (so-called 'synzymes') that have the advantage of being smaller than SOD and membrane permeable. These substances have been shown to have a positive effect as therapeutics by reducing prostaglandin production and increasing ICAM-1 (intercellular adhesion molecule 1) expression. The same compounds reduce colon injury and pancreatitis, as well as ischemia/reperfusion insult, arthritis, and spinal cord injury, by protecting cells against DNA/RNA damage, mitochondrial alterations, and apoptosis. Then, arachidonate signaling in neuroinflammation was discussed, with a focus on the regulation of astrocyte survival. These cells respond to agents that increase intracellular Ca^{2+} concentration with a linear release of arachidonic acid (ARA), due to activation of cytosolic phospholipase A_2. In addition, a reduction of NO release, due to ARA-dependent inhibition of neuronal NOS, is observed, which causes reduction of $ONOO^-$ tone and subsequent PTP-dependent apoptosis. In addition, inhibition of neuronal NOS by ARA initiates the inflammatory response, allowing NF-kB activation and subsequent expression of NF-kB-dependent genes. Finally, the last talk focused on the role of DAMPs (damage-associated molecular-pattern molecules) in inflammation. In fact, DAMPs oxidation impacts the outcome of acute inflammation, and outside the cell DAMPs might even adopt novel conformations or alter the redox state of the extracellular environment to mimic more closely the redox state inside the cell. It can be proposed that chronic inflammation associated with autoimmunity, chronic viral infection, and cancer are likely mediated by persistent release and function of DAMPs.

Concluding remarks

Responses to redox signals are determined by a complex array of proteins (so-called 'redox proteome'), which are able to transduce alterations initiated by superoxide (oxysome), NO (nitrosome), or hypoxia (hypoxysome), and that invariably engage mitochondria. The conference ended with a general consensus that future research should focus on the impact of the redox proteome on human pathology, both within the brain and in peripheral tissues, as well as on gene regulation brought about by redox-sensitive transcription factors. Additionally, the wish was made by all participants that the next Villa Vigoni conference might start a new series, where the Italian–German meeting is turned into a wider event, open to all 'redox enthusiasts' within the European Union.

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