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Forum Editorial

Redox Control of Red Blood Cell Biology: The Red Blood Cell as a Target and Source of Prooxidant Species

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ABSTRACT

Red blood cells may exert both an antioxidant and a prooxidant activity. The first is exerted in physiologic conditions, whereas the second can be detected in several human pathologies. These opposite characteristics can depend on the environmental milieu as well as on intrinsic alterations. Both these aspects are summarized in this brief review that takes into account the possible implications of redox-associated alterations of red blood cells in determining their function and fate. *Antioxid. Redox Signal.* 8, 1165–1169.

THE ERYTHROCYTE: A JANUS-FACED CELL

Because of its high iron concentration (~20 m*M*), the red blood cell (RBC) can be considered an "iron mine" but, paradoxically, is also one of the major components of blood antioxidant capacity and one of the cells more resistant to oxidative stress. Its very efficient intracellular reducing machinery coupled with its high cell density makes the erythrocyte an effective "sink" of reactive species. Probably not only the blood *per se* but, more important, the whole organism can benefit from RBC scavenging ability. However, this cell, during its 120-day life span, undergoes a tremendous chemical and mechanical stress, and thus it is not unexpected that a fine mechanism has been developed to "sense" and remove damaged cells.

Among all the possible causes of RBC chemical stress, the exposure to reactive oxygen/nitrogen species probably plays a dominant role. RBCs not only can generate free radicals during their life span but, in addition, are exposed to xenobiotics, pathogens, hyperglycemic conditions, and to radical-generating cells from the immune system (*e.g*., inflammatory cells). Moreover, sometimes tissues may represent a source of RBC oxidative alterations (*e.g.,* when subjected to ischemia–reperfusion).

Hemoglobin itself is at the same time a source and a "sink" of free radicals. In the partially oxygenated state, hemoglobin can undergo autoxidation, producing the superoxide anion radical (27, 33). Conversely, deoxygenated hemoglobin can reduce intracellular nitrite to nitric oxide ($NO^{\cdot}$) through its recently characterized nitrite reductase activity [as described by Rifkind *et al*. (30) in this Forum Issue]. Conversely, hemoglobin, being highly concentrated into the erythrocyte, is also a major target of reactive oxygen and nitrogen species. Not only is the porphyrin iron center a preferred target for several radicals and reactive oxidants, but also the iron center can "repair" oxidative insults occurring in the globin chain (5). Once the iron of hemoglobin is oxidized, the cell can reduce methemoglobin back to oxyhemoglobin through methemoglobin reductase/NADH/glycolysis and, under conditions of intense oxidative stress, through the pentose phosphate shunt/NADPH/glutathione and glutathione reductase system [for a more detailed picture of RBC reducing machinery, see Nickel *et al*. (23) and Tzantes *et al.* (34) in this Forum Issue]. All these reactions make hemoglobin within the RBC a highly efficient antioxidant system not yet fully appreciated. Moreover, to serve the important role of a circulating scavenger, the RBC is well equipped with nonenzymatic (*e.g.,* glutathione, thioredoxin, ascorbic acid, vitamin E) and enzymatic antioxidants. Compared with other cell types, RBCs exhibit high activities of the most important antioxidant enzymes, including superoxide dismutase [see also the review of Martin *et al*. (19) in this Forum Issue], thioredoxin system [thioredoxin peroxidase/peroxiredoxin; see for details the article of Nickel *et al*.

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(23) in this Forum Issue], catalase, glutathione peroxidase, glutathione reductase, and, last but not least, the plasma membrane oxidoreductases that RBCs use to reduce extracellular oxidants [see Kennett *et al*. (10) in this Forum Issue].

The antioxidant role of RBCs is schematically depicted in Fig. 1, in which it is shown that, crossing inflamed areas, the erythrocyte can contribute to detoxify reactive oxygen and nitrogen species and thus to rescue or partially "protect" cells under intense oxidative stress (*e.g*., endothelial cells).

As outlined before, the reverse side of RBC antioxidant power is its capability of being a source of reactive species. The superoxide radical generated within the RBC by deoxygenated or partially oxygenated hemoglobin (27, 33), usually found at low levels and likely under physiologic conditions, does not represent a big hazard for the cell. Similarly, the ability of RBCs to scavenge or generate nanomolar concentrations of $NO^{\bullet}$ can be easily handled by the methemoglobin reductase/NADH/glycolysis system. Completely different may be the situation when the erythrocyte becomes a target of xenobiotics (24), parasites [Nickel *et al*. (23) in this Forum Issue], or crosses a tissue where an intense production of reactive oxygen/nitrogen species occurs. Under these conditions, the cell may accumulate oxidative damage that reflects the oxidative stress of other tissues and organs. For this reason, cellular biomarkers of oxidatively modified RBCs are potential candidates for monitoring not only RBC-linked pathologies (*e.g*., thalassemia, sickle cell anemia) but also other pathologic conditions associated with oxidative stress and, more generally, to monitor the overall oxidative-stress status. The situation in which the RBC accumulates oxidative damage is illustrated in the lower part of Fig. 1.

UNDER THE FRIENDLY FIRE

On the basis of the scenario depicted, three different aspects must be taken into account: (a) the ability of the RBC to act as free radical scavenger through its antioxidant machinery; (b) the possibility of RBC being a target of unwanted oxidative stress (for example, after the interaction with xenobiotics), which in turn leads to RBC functional changes; or (c) the ability of the RBC to act as a sort of "prooxidant bullet" (see later). The RBC is under a sort of "friendly fire" due to the attack derived, for example, from inflamed tissues. This leads, among other effects, to RBC redox alterations. In turn, these redox alterations result in the disturbance of RBC integrity and function and to the inhibition of RBC antioxidant characteristics. Moreover, it is conceivable that oxidatively modified RBCs, as "dangerous friends," can act at the periphery as "prooxidant bullets" capable of modifying the behavior and fate of other vascular tissues (*e.g*., of endothelial cells). When the redox homeostasis of RBCs is deeply altered, the heme iron reveals its true face: an active-redox-metal and a source of dangerous radicals, an event that changes RBC characteristics from those of a scavenger cell to that of a dangerous prooxidant bullet.

However, this complex scenario, although accepted in principle, is far from being elucidated and still must be analyzed in detail in the *in vitro* as well as *in vivo* experimental studies. RBC redox manipulation can experimentally be induced *in vitro*, partially mimicking the *in vivo* alterations occurring in circulating erythrocytes (20). Some of these different aspects have been considered in this Forum issue. However, the previously mentioned alteration of the RBC considered as a target of oxidative stress or as a source of stress has been extensively analyzed in a number of experimental as well as clinical studies. Hence, findings reported in this Forum Issue represent only a small part of the scenario.

Redox alterations in RBCs

To depict this complex scenario, at least partially, in Table 1, we recapitulate some pathophysiologic implications of RBC alterations associated with oxidative imbalance. The first column indicates the RBC target that, if modified by an

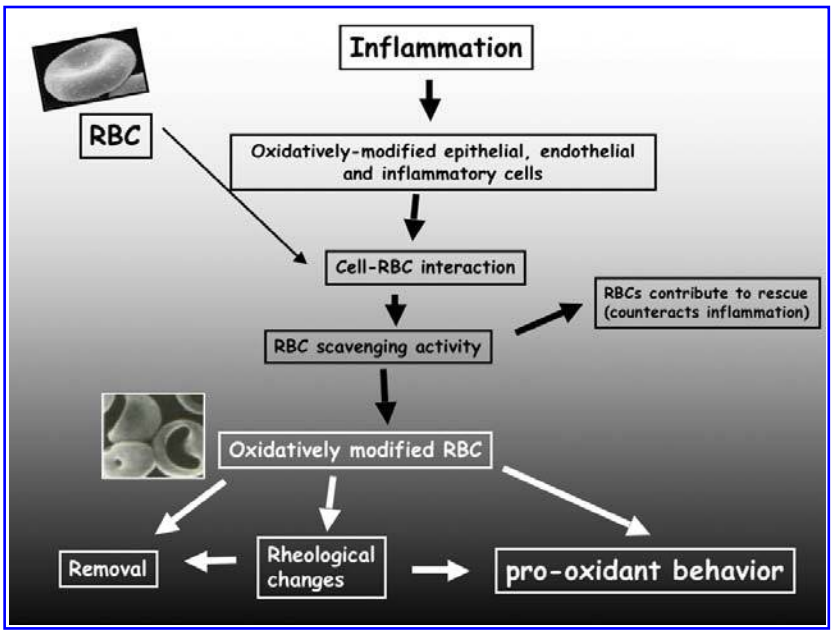

FIG. 1. Erythrocytes can exert both an antioxidant activity (white background) as well as a pro-oxidant activity once oxidized (grey background). Scanning electron microscopy micrographs show a normal discoid erythrocyte and an oxidatively modified erythrocyte (a so-called cup form).

Table 1. Cellular and Biochemical Targets of Erythrocyte Oxidative Damage

RBC Target	*Associated subcellular alterations*	*Functional alteration*	*Main cell fate*	*Systemic effects*	*Reference*
Band 3	Changes of membrane-cytoskeleton interaction, cell shrinkage, surface blebbing	Ionic imbalance	Senescence	Loss of RBC function, withdrawal from circulation	9, 29
Glycophorins	Cell shrinkage, changes of membrane-cytoskeleton interaction	Membrane remodeling, changes of rheological properties	Senescence	Withdrawal from circulation, blood group antigens, increased adhesion-aggregation	1, 7, 28
Phosphatydyl serine	Loss of membrane asymmetry	Increased RBC adhesion, RBC aggregation	Apoptosis	Contributes to thrombus formation, changes of rheological properties and loss of membrane stability	3, 18
Cytoskeletal proteins	Shape changes, membrane fragility	Loss of cell plasticity	Senescence	Changes of rheological properties, loss of membrane stability	21
Hemoglobin	Methemoglobin formation, glycolysis activation	Hemicromes, Heinz bodies	Senescence	Altered oxygen and NO delivery to tissues	24, 26, 27, 33
GSH	Metabolic imbalance	Loss of redox control	Senescence	Imbalance of protein thiols	8, 25, 35
Ca^{2+} ATPase	Calcium entry	Ionic imbalance	Apoptosis	Loss of membrane stability	18
Insulin receptor	Downregulation	Ionic Imbalance	Senescence	Hypertension	4, 14, 32
Thioredoxin system	Heinz bodies	Loss of protein redox control	Senescence	Hemolytic anemia	6
Cysteinyl and aspartyl proteases	Activation of proteolysis	PS externalization, Band 3 proteolysis	Apoptosis	Withdrawal from circulation, loss of membrane stability	2, 15, 16, 17, 20

oxidant, may produce the effects outlined in the following columns. The possible effects have been demonstrated experimentally or extrapolated arbitrarily from the known function of the target. It is worth noting that main subcellular oxidative changes occurring in RBCs have been referred to a singular target and may lead to specific pathologic conditions. Oxidative damage of RBCs can result in structural and functional alterations in terms of modifications of (a) their rheologic parameters, (b) their adhesion features, and (c) their delivery functions. For instance, once RBC plasticity is drastically reduced, its ability to flow also is impaired: the cell dimension (7 μm) does not allow its normal passage through the smallest blood capillaries (5 μm). Fittingly, RBCs modified by oxidants often shrink, and their normal plasticity is lost. Hence, RBC function is strictly associated with its deformability, in turn associated with the maintenance of redox homeostasis.

As a final event, RBCs also undergo senescence and apoptosis (also termed eryptosis; see the article of Lang *et al*. (11) in this Forum Issue). The first, senescence, is characterized by a series a specific cell alterations (see Table 1). The second, apoptosis, although unconventional, was described as a sort of a death of a mummy (2) [*i.e.,* of a cell that although devoid of those organelles that play a key role in apoptosis (mitochondria, nucleus) can still display protease activation somewhat similar to that of nucleated cells].

Some or several of these modifications have been encountered in clinical and *ex vivo* studies aimed at the analysis of the role of RBC changes in human pathology [see, for example, Lucantoni *et al*. (13) in this Forum Issue]. These studies encompass (a) those aimed at the comprehension of the pathogenetic mechanisms of human diseases, as well as (b) those aimed at the identification of the possible implications

of RBC changes as bioindicators or biomarkers in certain pathologic conditions (31), and, finally (c) those aimed at the evaluation of a possible therapeutic clinical intervention (*e.g.*, by the use of antioxidant compounds such as, for example, *N*-acetyl-cysteine or vitamin E). Altogether these works supported the idea that the RBC features, although intensively studied in the course of the last 50 years, still hide biologic information of great relevance to the comprehension of the pathogenetic mechanisms of human diseases as well as to the improvement of therapeutic strategies relevant to the maintenance of RBC homeostasis).

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ABBREVIATIONS

GSH, Reduced glutathione: NO, nitric oxide; PS, phosphatidyl serine; RBC, red blood cells.

REFERENCES

1. Corbett JD and Golan DE. Band 3 and glycophorin are progressively aggregated in density-fractionated sickle and normal red blood cells: Evidence from rotational and lateral mobility studies. *J Clin Invest* 91: 208–217, 1993.
2. Daugas E, Cande C, and Kroemer G. Erythrocytes: Death of a mummy. *Cell Death Differ* 8: 1131–1133, 2001.
3. de Jong K, Emerson RK, Butler J, Bastacky J, Mohandas N, and Kuypers FA. Short survival of phosphatidylserine-exposing red blood cells in murine sickle cell anemia. *Blood* 98: 1577–1584, 2001.
4. Ferreira A, Rivera A, and Romero JR. Na^+/Mg^{2+} exchange is functionally coupled to the insulin receptor. *J Cell Physiol* 199: 434–440, 2004.
5. Goldstein S and Samuni A. Intra- and intermolecular oxidation of oxymyoglobin and oxyhemoglobin induced by hydroxyl and carbonate radicals. *Free Radic Biol Med* 39: 511–519, 2005.
6. Grattagliano I, Russmann S, Calmieri VO, Portincasa P, Palasciano G, and Lauterburg BH Glutathione peroxidase, thioredoxin, and membrane protein changes in erythrocytes predict ribavirin-induced anemia. *Clin Pharmacol Ther* 78: 422–432, 2005.
7. Head DJ, Lee ZE, Poole J, and Avent ND. Expression of phosphatidylserine (PS) on wild-type and Gerbich variant erythrocytes following glycophorin-C (GPC) ligation. *Br J Haematol* 129: 130–137, 2005.
8. Imanishi H, Nakai T, Abe T, and Takino T. Glutathione metabolism in red cell aging. *Mech Ageing Dev* 32: 57–62, 1985.
9. Kay MM, Flowers N, Goodman J, and Bosman G. Alteration in membrane protein band 3 associated with accelerated erythrocyte aging. *Proc Natl Acad Sci U S A* 86: 5834–5838, 1989.
10. Kennett EC and Kuchel PW. Plasma membrane oxidoreductases: Effects on erythrocyte metabolism and redox homeostasis. *Antioxid Redox Signal,* this Issue.
11. Lang F, Karl S, Lang KS, Lang PA, Huber SM, and Wieder T. Mechanisms and significance of eryptosis. *Antioxid Redox Signal,* this Issue.
12. Lang KS, Lang PA, Bauer C, Duranton C, Wieder T, Huber SM, and Lang F. Mechanisms of suicidal erythrocyte death. *Cell Physiol Biochem* 15:195–202, 2005.
13. Lucantoni G, Pietraforte D, Matarrese P, Gambardella L, Metere A, Paone G, Li Bianchi E, and Straface E. The red blood cell as a bio-sensor for monitoring oxidative imbalance in chronic obstructive pulmonary disease (COPD): An *ex vivo* and *in vitro* study. *Antioxid Redox Signal,* this Issue.
14. Makris TA, Paizis I, Krespi PG, Stavroulakis GA, Papazachou OG, Papadopoulos DP, Hatzizacharias AN, and Votteas VV. Insulin receptor number is reduced in healthy offspring of patients with essential hypertension. *Am J Hypertens* 17: 911–914, 2004.
15. Mandal D, Baudin-Creuza V, Bhattacharyya A, Pathak S, Delaunay J, Kundu M, and Basu J. Caspase 3-mediated proteolysis of the N-terminal cytoplasmic domain of the human erythroid anion exchanger 1 (band 3). *J Biol Chem* 278: 52551–52558, 2003.
16. Mandal D, Mazumder A, Das P, Kundu M, and Basu J. Fas-, caspase 8-, and caspase 3-dependent signaling regulates the activity of the aminophospholipid translocase and phosphatidylserine externalization in human erythrocytes. *J Biol Chem* 280: 39460–39467, 2005.
17. Mandal D, Moitra PK, Saha S, and Basu J. Caspase 3 regulates phosphatidylserine externalization and phagocytosis of oxidatively stressed erythrocytes. *FEBS Lett* 513: 184–188, 2002.
18. Manno S, Takakuwa Y, and Mohandas N. Identification of a functional role for lipid asymmetry in biological membranes: Phosphatidylserine-skeletal protein interactions modulate membrane stability. *Proc Natl Acad Sci U S A* 99: 1943–1948, 2002.
19. Martin FA, Bydlon G, and Friedman JS. SOD2-deficiency, sideroblastic anemia and red blood cell oxidative stress. *Antioxid Redox Signal,* this Issue.
20. Matarrese P, Straface E, Pietraforte D, Gambardella L, Vona R, Maccaglia A, Minetti M, and Malorni W. Peroxynitrite induces senescence and apoptosis of red blood cells through the activation of aspartyl and cysteinyl proteases. *FASEB J* 19: 416–418, 2005.
21. Mohandas N, An XL, Lecomte MC, and Gratzer W. A dynamic spectrin-dimer self association regulates of red cell membrane mechanical stability. *Cell Mol Biol Lett* 6: 221, 2001.
22. Mulquiney PJ and Kuchel PW. Model of 2,3-bisphosphoglycerate metabolism in the human erythrocyte based on detailed enzyme kinetic equations: Computer simulation and metabolic control analysis. *Biochem J* 342: 597–604, 1999.
23. Nickel C, Rahlfs S, Deponte M, Koncarevic S, and Becker K. Thioredoxin networks in the malarial parasite *Plasmodium falciparum*. *Antioxid Redox Signal,* this Issue.

24. Nohl H and Stolze K. The effects of xenobiotics on erythrocytes. *Gen Pharmacol* 31: 343–347, 1998.
25. Piccinini G, Minetti G, Balduini C, and Brovelli A. Oxidation state of glutathione and membrane proteins in human red cells of different age. *Mech Ageing Dev* 78: 15–26, 1995.
26. Powers HJ and Thurnham DI. Riboflavin deficiency in man: Effects on haemoglobin and reduced glutathione in erythrocytes of different ages. *Br J Nutr* 46: 257–266, 1981.
27. Reeder BJ, Svistunenko DA, Cooper CE, and Wilson MT. The radical and redox chemistry of myoglobin and hemoglobin: From in vitro studies to human pathology. *Antioxid Redox Signal* 6: 954–966, 2004.
28. Reid ME and Mohandas N. Red blood cell blood group antigens: Structure and function. *Semin Hematol* 41: 93–117, 2004.
29. Rettig MP, Low PS, Gimm JA, Mohandas N, Wang J, and Christian JA. Evaluation of biochemical changes during in vivo erythrocyte senescence in the dog. *Blood* 93:376–384, 1999.
30. Rifkind J M, Nagababu E, and Ramasamy S. Nitric oxide redox reactions and red cell biology. *Antioxid Redox Signal,* this Issue.
31. Rogowski O, Berliner S, Zeltser D, Serov J, Ben-Assayag E, Justo D, Rozenblat M, Kessler A, Deutsch V, Zakuth V, and Shapira I. The erythrosense as a real-time biomarker to reveal the presence of enhanced red blood cell aggregability in atherothrombosis. *Am J Ther* 12:286–292, 2005.
32. Suchankova G, Vlasakova Z, Zicha J, Vokurkova M, Dobesova Z, and Pelikanova T. Erythrocyte membrane ion transport in offspring of hypertensive parents: Effect of acute hyperinsulinemia and relation to insulin action. *Ann N Y Acad Sci* 967: 352–362, 2002.
33. Svistunenko DA, Davies NA, Wilson MT, Stidwill RP, Singer M, and Cooper CE. Free radical in blood: A measure of haemoglobin autoxidation in vivo? *J Chem Soc Perkin Trans* 2: 2539–2543, 1997.
34. Tsantes AE, Bonovas S, Travlou A, and Sitaras NM. Redox imbalance, macrocytosis and RBC homeostasis. *Antioxid Redox Signal,* this Issue.
35. Usberti M, Lima G, Arisi M, Bufano G, D'Avanzo L, and Gazzotti RM. Effect of exogenous reduced glutathione on the survival of red blood cells in hemodialyzed patients. *J Nephrol* 10: 261–265, 1997.

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1. Isaac Ginsburg, Ron Kohen, Erez Koren. 2011. Quantifying Oxidant-Scavenging Ability of Blood. *New England Journal of Medicine* **364**:9, 883-885. [CrossRef]
2. Yi-Ting Wu, Che-Yi Lin, Ming-Yuan Tsai, Yi-Hua Chen, Yu-Fen Lu, Chang-Jen Huang, Chao-Min Cheng, Sheng-Ping L Hwang. 2011. Beta-Lapachone induces heart morphogenetic and functional defects by promoting the death of erythrocytes and the endocardium in zebrafish embryos. *Journal of Biomedical Science* **18**:1, 70. [CrossRef]
3. Hee-Young Yang, Joseph Kwon, Hoon-In Choi, Seong Hwa Park, Ung Yang, Hyang-Rim Park, Lina Ren, Kyoung-Jin Chung, Youn U. Kim, Byung-Ju Park, Sang-Hun Jeong, Tae-Hoon Lee. 2011. In-depth analysis of cysteine oxidation by the RBC proteome: Advantage of peroxiredoxin II knockout mice. *PROTEOMICS* n/a-n/a. [CrossRef]
4. Jean-Daniel Tissot, Olivier Rubin, Giorgia Canellini. 2010. Analysis and clinical relevance of microparticles from red blood cells. *Current Opinion in Hematology* **17**:6, 571-577. [CrossRef]
5. Kellie M. Jaremko, Jing Chen-Roetling, Lifen Chen, Raymond F. Regan. 2010. Accelerated hemolysis and neurotoxicity in neuron-glia-blood clot co-cultures. *Journal of Neurochemistry* no-no. [CrossRef]
6. Michalis G. Nikolaidis, Athanasios Z. Jamurtas. 2009. Blood as a reactive species generator and redox status regulator during exercise. *Archives of Biochemistry and Biophysics* **490**:2, 77-84. [CrossRef]
7. Irene L. Lisovskaya, Irina M. Shcherbachenko, Rimma I. Volkova, Fazoil I. Ataullakhanov. 2009. Clotrimazole enhances lysis of human erythrocytes induced by t-BHP. *Chemico-Biological Interactions* **180**:3, 433-439. [CrossRef]
8. Maurizio Minetti , Thomas L. Leto , Walter Malorni . 2008. Radical Generation and Alterations of Erythrocyte Integrity as Bioindicators of Diagnostic or Prognostic Value in COPD?. *Antioxidants & Redox Signaling* **10**:4, 829-836. [Abstract] [Full Text PDF] [Full Text PDF with Links]