ANTIOXIDANTS & REDOX SIGNALING
Volume 4, Number 1, 2002

Forum Editorial

Redox Regulation of Neutrophil Function

MARK B. HAMPTON and CHRISTINE C. WINTERBOURN

THE NEUTROPHIL IS MORE THAN A KILLER OF MICROBES

ONE could be forgiven for thinking of neutrophils as simple phagocytic cells, useful remnants of a primitive immune system, with their sole function restricted to waste disposal at inflammatory sites. When needed, they are attracted from the circulation by a scent of chemotactic factors generated by cells under assault, and upon arrival they engulf and destroy anything labeled as foreign. Their means of destroying an ingested pathogen includes a blast of reactive oxidants so toxic that it leads to the kamikaze-like death of the neutrophil itself. However, most neutrophils never achieve this goal. They rarely live beyond a day, only to be replaced in circulation by cells mass-produced in the bone marrow.

As terminally differentiated cells, neutrophils give the impression of prepackaged killers. The cytoplasm is filled with granules containing antimicrobial and digestive compounds, ready for translocation and emptying into the phagosome. The NADPH oxidase, the primary source of oxidants, is already synthesized and awaits a signal for assembly and activation. This preparedness allows a response within seconds—an inducible response would be inadequate as a first line of defense.

As such, it is not surprising that transcription factors, gene expression, and protein synthesis have received little attention in neutrophils. However, there is a collection of studies that warn against underestimating the complexity of these cells. The ability of neutrophils to synthesize and release cytokines and chemokines is well documented (13), as is the fundamental role of these products in coordinating the immune response. There is also a recent report that neutrophils are able to act as antigen-presenting cells (11). Nuclear factor-κB activation has been reported in neutrophils treated with different stimuli *in vitro* (8), and we have observed nuclear translocation of this transcription factor in neutrophils present in inflammatory exudates (Cheah *et al.*, unpublished observations). Also of considerable interest is the detailed investigation showing changes in the expression of several hundred neutrophil genes during bacterial phagocytosis (14). This is indeed remarkable for a cell under extreme oxidative stress and destined to die within an hour or two. Some gene products may be important for the neutrophil that generates them, perhaps to facilitate the apoptosis and clearance that is a key feature of successful resolution of inflammation. However, it is worth considering that these processes may also be important in bystander neutrophils, which are attracted to the site but are yet to be engaged in phagocytosis. These cells

Free Radical Research Group, Department of Pathology, Christchurch School of Medicine and Health Sciences, Christchurch, New Zealand.

are exposed to a range of inflammatory mediators, some of which are capable of delaying the rate of spontaneous apoptosis and thereby extending the functional lifetime of the neutrophil (7). Others are able to prime the bystander neutrophil, such that it is prepared for a rapid and extensive oxidative burst upon subsequent activation (15). Altered gene expression and cytokine production by these cells may play an important role in the regulation of the inflammatory response.

NEUTROPHIL OXIDANTS AS SIGNALING MOLECULES

As evidenced by the recent establishment of this journal, there is growing interest in the concept of redox signaling. Reactive oxidants are major products of stimulated neutrophils, and the question arises as to whether these oxidants act as signals, either for the neutrophils themselves or for other cells at an inflammatory site. This is the focus of this special forum. We have taken a broad approach to the topic with articles considering how oxidant production is regulated, as well as potential regulatory roles of the oxidants themselves.

The pathways and processes of oxidant production are well defined in the neutrophil. On stimulation, the NADPH oxidase is rapidly assembled in the plasma membrane, leading to the production of O_2^- on its external surface (1). Successful ingestion of the targeted microorganism results in O_2^- release into the phagosomal space. This rapidly dismutates to H_2O_2, which can then be converted to HOCl by the granule enzyme myeloperoxidase. Other secondary oxidants of the oxidative burst are chloramines, formed upon the reaction of HOCl with amine groups. These oxidant products make a significant contribution to the microbicidal arsenal of the neutrophil (4).

There are various threads of evidence that suggest neutrophil oxidants also perform other functions. It is known that oxidants are capable of activating or modulating signaling pathways in cells. H_2O_2 is the best characterized; it activates pathways leading to kinase activation, cell proliferation, growth arrest, and apoptosis. Chlorinated oxidants have been shown to cause some of these changes (9, 16).

Potential targets include any cells in the immediate vicinity of the activated neutrophil, particularly those to which it is adhered, as well as the activated neutrophil itself. Soluble stimuli can cause neutrophils to release oxidants into the surroundings, as does incomplete phagosome closure. Also, H_2O_2, HOCl, and some chloramines are small uncharged molecules that can cross membranes and diffuse from the site of generation. They should not, therefore, be restrained in the phagosomal space. The volume of a neutrophil is 1,000 times that of a typical bacterium, so the oxidants need not travel far for the neutrophil to become the major reactive sink. Indeed, recent work from our laboratory indicates that the majority of tyrosine chlorination by HOCl generated during phagocytosis is on neutrophil rather than bacterial protein (Chapman *et al., J Biol Chem*, in press). There is also evidence that oxidant production is not restricted to the phagosome. Work from Dahlgren and colleagues suggests that NADPH oxidase activation occurs in granule membranes, increasing the likelihood of significant extraphagosomal oxidation (5).

What processes in the neutrophil may be under redox control? At low levels, oxidants are known to modulate transcription factor activity, and therefore may affect cytokine production and release. They are also known to prime cells for subsequent activation and to affect cell-to-cell interactions. Both these phenomena are addressed in this issue (12, 15, 17). There is evidence that neutrophil apoptosis is under redox control, with NADPH oxidase-derived oxidants proposed to regulate spontaneous neutrophil apoptosis (6) and trigger phosphatidylserine exposure and clearance of activated neutrophils (2). These mechanisms are defective in chronic granulomatous disease, a genetic defect resulting in a nonfunctional NADPH oxidase, and can lead to impaired neutrophil uptake (Hampton *et al.*, *J Leukoc Biol,* in press). Others have observed an elevation in the number of inflammatory neutrophils in chronic granulomatous disease, independent of defective microbial killing, and have hypothesized

that a defective NADPH oxidase is associated with dysfunctional regulation of the inflammatory response (3, 10).

FUTURE DIRECTIONS

As discussed in this issue, there are several indications that neutrophil oxidants play a regulatory role. However, this field is in its infancy, and more experimentation is required to define which neutrophil processes are under redox control. Interesting questions arise as to whether any of the changes in gene expression following phagocytosis are redox-regulated, and how oxidant production leads to neutrophil apoptosis and recognition for clearance. Mitogen-activated protein kinases are involved in many of the phosphorylation events leading to activation of the NADPH oxidase and generation of superoxide. In other cell types, this pathway is redox-sensitive. The possibility that oxidants amplify this response in bystander neutrophils warrants consideration. NADPH oxidase-knockout mice are available and may provide the vehicle for elucidating the processes other than bacterial killing that are dependent on an oxidative burst.

REFERENCES

1. Babior BM. NADPH oxidase: an update. *Blood* 93: 1464–1476, 1999.
2. Fadeel B, Åhlin A, Henter J, Orrenius S, and Hampton MB. Involvement of caspases in neutrophil apoptosis: regulation by reactive oxygen species. *Blood* 92: 4808–4818, 1998.
3. Gallin JI and Buescher ES. Abnormal regulation of inflammatory skin responses in male patients with chronic granulomatous disease. *Inflammation* 7: 227–232, 1983.
4. Hampton MB, Kettle AJ, and Winterbourn CC. Inside the neutrophil phagosome: oxidants, myeloperoxidase, and bacterial killing. *Blood* 92: 3007–3017, 1998.
5. Karlsson A and Dahlgren C. Assembly and activation of the neutrophil NADPH oxidase in granule membranes. *Antioxid Redox Signal* 4: 49–60, 2002.
6. Kasahara Y, Iwai K, Yachie A, Ohta K, Konno A, Seki H, Miyawaki T, and Taniguichi N. Involvement of reactive oxygen intermediates in spontaneous and CD95(Fas/APO-1)-mediated apoptosis of neutrophils. *Blood* 89: 1748–1753, 1997.
7. Lee A, Whyte MKB, and Haslett C. Inhibition of apoptosis and prolongation of neutrophil longevity by inflammatory mediators. *J Leukoc Biol* 54: 283–288, 1993.
8. McDonald PP, Bald A and Cassatella MA. Activation of the NF-κB pathway by inflammatory stimuli in human neutrophils. *Blood* 89: 3421–3433, 1997.
9. Midwinter RG, Vissers MCM, and Winterbourn CC. Hypochlorous acid stimulation of the mitogen-activated protein kinase pathway enhances cell survival. *Arch Biochem Biophys* 394: 13–20, 2001.
10. Morgenstern DE, Gifford MAC, Li L, Doerschuk CM, and Dinauer MC. Absence of respiratory burst in X-linked chronic granulomatous disease mice leads to abnormalities in both host defence and inflammatory response to *Aspergillus fumigatus*. *J Exp Med* 185: 207–218, 1997.
11. Potter NS and Harding CV. Neutrophils process exogenous bacteria via an alternate class I MHC processing pathway for presentation of peptides to T lymphocytes. *J Immunol* 167: 2538–2546, 2001.
12. Pricop L and Salmon JE. Redox regulation of Fcγ receptor-mediated phagocytosis: implications for host defense and tissue injury. *Antioxid Redox Signal* 4: 85–95, 2002.
13. Scapini P, Lapinet-Vera JA, Gasperini S, Calzetti F, Bazzoni F, and Cassatella MA. The neutrophil as a cellular source of chemokines. *Immunol Rev* 177: 195–203, 2000.
14. Subrahmanyam YVBK, Yamaga S, Prashar Y, Lee HH, Hoe NP, Kluger Y, Gerstein M, Goguen JD, Newburger PE, and Weissman SM. RNA expression patterns change dramatically in human neutrophils exposed to bacteria. *Blood* 97: 2457–2468, 2001.
15. Swain SD, Rohn TT, and Quinn MT. Neutrophil priming in host defense: role of oxidants as priming agents. *Antioxid Redox Signal* 4: 69–83, 2002.
16. Vissers MCM, Pullar JM, and Hampton MB. Hypochlorous acid causes caspase activation and apoptosis or growth arrest in human endothelial cells. *Biochem J* 344: 443–449, 1999.
17. Wang Q and Doerschuk CM. The signaling pathways induced by neutrophil-endothelial cell adhesion. *Antioxid Redox Signal* 4: 39–47, 2002.

Address reprint requests to:
Dr. Mark Hampton
Free Radical Research Group
Department of Pathology
Christchurch School of Medicine and Health Sciences
P.O. Box 4345
Christchurch, New Zealand

E-mail: mark.hampton@chmeds.ac.nz

This article has been cited by:

1. Marisa Freitas, José L.F.C. Lima, Eduarda Fernandes. 2009. Optical probes for detection and quantification of neutrophils' oxidative burst. A review. *Analytica Chimica Acta* **649**:1, 8-23. [CrossRef]