NADPH Oxidase in Vascular Injury
A New Insight About Its Regulation and Role in T Cells

Jun-ichi Abe, Chang-Hoon Woo

Vascular proliferative disorders and their subsequent vascular stenosis resulting from neointimal formation and vascular remodeling have a very relevant clinical effect, especially following percutaneous coronary interventions. In this editorial, we would like to briefly summarize the regulatory mechanism of NADPH oxidase and its role in T cells during the process of neointimal formation.

Regulation of NADPH Oxidase Activity (Not Expression)

Many reports support the critical role of reactive oxygen species (ROS) in vascular injury. The signaling properties of ROS are largely attributable to the reversible oxidation of redox-sensitive target proteins, especially protein tyrosine phosphatases (PTPs). The PTP activity is dependent on the reactive cysteine residues (Cys-SH) that are readily susceptible to oxidation.1 Oxidative inhibition of PTPs by molecules including PTP1B and SHP2 can increase phosphorylation and activation of many receptor tyrosine kinase.2 Therefore, ROS production can be a very important mediator of signal transduction, because activation of receptor tyrosine kinase can initiate many signal transduction pathways. The balance between oxidases and antioxidant enzymes regulates ROS production. One of the prominent sources of vascular reactive oxygen species is NADPH oxidase and its cell-specific expression and localization may have a critical role in regulation of specific and unique ROS-mediated signal transduction pathways by “compartmentalization” of ROS production.3 Therefore, the regulatory mechanism of NADPH oxidase “activation” is of great interest. NADPH oxidases consist of membrane-associated cytochrome b558 comprising of the catalytic gp91phox and regulatory p22phox subunits and cytosolic components including p47phox, p67phox, p40phox, and the small GTPase Rac in phagocytic cells. In nonphagocytic cells, several homologs of gp91phox(Nox2) including Nox1 and Nox3–5, as well as the Dual oxidase (Duox), have been identified.4,5 Two major mechanisms have been reported to regulate NADPH oxidase activity (Figure): (1) Rac GTPase; and (2) p47phox phosphorylation.

Rac Activation

Rac, a member of the Rho-family small GTPases, plays an essential role in gp91phox/Nox2 activation. Rho GTPase can serve as a molecular switch to cycle between the GTP-bound active form that stimulates downstream effectors and the GDP-inactive form. Rac binds directly to p67phox (but not p47phox) and activates NADPH oxidase activity only in its GTP-bound active form. The importance of Rac GEFs (GDP/GTP exchange factors) such as Vav1 and Tiam1 on NADPH oxidase activation has been reported. Not only Rac/p67phox association but also Rac-mediated PAK (p21-activated kinase) are also involved in NADPH oxidase activation by direct phosphorylation of p47phox.4,5 Phosphatidylinositol 3-kinase activation can regulate Rac and p67phox complex formation,6 but the involvement of Akt on NADPH oxidase activity, which is downstream of phosphatidylinositol 3-kinase, is controversial.7

p47phox Phosphorylation

Protein kinase (PK)C and mitogen-activated protein kinase phosphorylation of p47phox (at different sites) can stimulate NADPH oxidase activity. Touyz et al have reported that c-Src activation increased p47phox phosphorylation and increased ROS production.8 Src can directly phosphorylate p47phox, but its tyrosine phosphorylation sites were not identified.9 Of note, the hierarchy of NADPH oxidase regulatory machinery remains unclear, because Src and PKC activation can be upstream of mitogen-activated protein kinase, and Src and PKCs may also regulate each others kinase activation.10 In addition, the involvement of Src on Rac activation via Vav tyrosine phosphorylation has been reported.11 The clarification of this hierarchy could be important to understand compartmentalization of localized ROS production and its subsequent pathological meaning.

In this issue of Circulation Research, Lee et al investigated the role of Ref-1 as a Rac inhibitor on platelet-derived growth factor (PDGF) receptor signaling pathway and subsequent neointimal formation. The authors concluded that Ref-1 inhibits PDGF-mediated migration signal via the inhibition of ROS-mediated Syk activity in rat aortic smooth muscle cells. This study nicely shows the importance of Ref-1 in PDGF-mediated ROS production and subsequent cell migration. In addition, the inhibitory role of Ref-1 on neointimal formation after vascular injury was also shown in vivo. However, the investigation of downstream events of Ref-1 was not completely clarified, based on the nature of “chicken or egg” aspect of this proposed signaling pathway. The authors concluded that Ref-1 inhibited ROS production and subsequent ROS-sensitive Syk kinase activation. However, in figure 4A, the authors showed that Syk kinase inhibitor
completely inhibited PDGF-mediated ROS production, suggesting the possible role of Syk as an upstream mediator of NADPH oxidase activation. In addition, the authors showed that Ref-1 small interfering RNA enhanced PDGF-β receptor/Syk association also supports the possibility that Syk activation may be a direct downstream target of PDGF-β receptor and may be involved in the activation of NADPH oxidase activation like Src kinase family as an upstream mediator. In fact, the involvement of Syk activation of Vav1 Tyr174 phosphorylation, which is required for Vav1 GEF activity, has been reported.14,15 This event can contribute to Rac-GTP formation and subsequent NADPH oxidase activation. It may be critical to clarify the hierarchy of this signal transduction pathway, because most of the kinases involved in NADPH oxidase activation, as explained above, are also ROS-sensitive.

T Cells in Vascular Injury

CD8+ T cells belong to a subgroup of T lymphocytes that are capable of inducing the death of infected or damaged cells. CD4+ T cells recognize antigens on class II major histocompatibility complex molecules presented by antigen-presenting cells. CD4+ cells differentiate into the Th1 or Th2 lineage. Th1 cells stimulate macrophages, and Th2 cells produce antibodies by stimulating B cells. In addition, Th1 cells enhance the killing efficacy of macrophages and the proliferation of cytotoxic CD8+ T cells by secreting interferon-γ and tumor necrosis factor-α. In contrast, Th2 cells secrete interleukin-4, -5, -10, and -13. A significant contribution of Th1 cells in vascular inflammation and atherosclerosis by secreting tumor necrosis factor-α has been proposed. However, the hypothesis of a yin and yang between Th1 and Th2 adaptive responses controlling atherosclerosis and vascular injury has become oversimplified, and it has been suggested that both Th1 and Th2 responses may differently contribute to certain stages of vascular disease development, but the exact mechanism has not been clarified.16,17

CXCR chemokine receptor (CXCR)3 is a marker of Th1 and common receptor for CXC ligand (CXCL)10 (IP10), CXCL9 (MIG), and CXCL11.18 In this issue of Circulation Research, Schwarz et al19 report that CXCR3-dependent activation of mTORC is critical for activation of Th1 immune system and subsequent neointimal formation. Because CXCR3 is not expressed in vascular smooth muscle cells (VSMCs), these data suggest the critical role of CXCR3+ T cells in neointimal formation. The role of T cells in neointimal formation is somewhat discrepant with previous reports (Hansson et al20 and Remskar et al21). These studies showed that depletion of T cells resulted in significant augmentation of intimal thickening in response to injury. The involvement of the antiproliferative and antidifferentiating effects on VSMCs of IFN-γ secreted from T cells was suggested.

In this report, Schwarz et al19 have shown that CXCR3 activation increases the ROS production via mTORC1 activation in T cells. The authors proposed that CXCR3-mediated ROS production in T cells may be involved in the recruitment of CD3+ T cells, CD45+ leukocytes, and c-kit+ cells, as well as subsequent neointimal formation. Several recent studies have shown the involvement of T cell-mediated ROS production in vascular injury. Guzik et al22 have reported the critical role of T cell-mediated ROS production via NADPH oxidase on vascular dysfunction and hypertension using mice lacking T and B cells (RAG-1−/− mice) with transferring T cells from wild-type or p47phox knockout mice. The discrepancy regarding the role of T cells in vascular dysfunction and injury between the previous studies (Hansson et al20 and Remskar et al21) and recent reports (Schwarz et al and Guzik et al) remains unclear. However, the present report provides a new angle for investigating not only vascular injury from cytokines or chemokines but also ROS production from T cells.

The role of ROS production in vascular injury has been discussed extensively, but the accurate mechanism has not been identified. From the current issue of Circulation Research, the regulation of NADPH oxidase activation and subsequent ROS production, especially from T cells, will provide a new paradigm of this aspect.

Sources of Funding

This work is supported by grants from the American Heart Association to Dr. Woo (Scientist Development Grant 0930360N), and from the National Institute of Health to J.A. (GM-071485, HL-
Disclosures
None.

References

Key WORDS: NADPH oxidase T cells Rac p47phox phosphorylation neointimal formation
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Circ Res. 2009;104:147-149
doi: 10.1161/CIRCRESAHA.108.192518

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/104/2/147

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