Mitochondrial ROS in the pro-hypertensive immune response

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Abstract

In the past decade, it has become clear that reactive oxygen species (ROS) and inflammation play an important role in the development of hypertension. Scavenging of mitochondrial superoxide and blocking either IL-17 or TNFα attenuates hypertension. T-cells, critical for development of hypertension, once activated intensively produce cytokines, proliferate and differentiate. Thus T-cell activation leads to expanded energy demand. In order to fulfill these needs, T-cells through tightly regulated mechanisms, supported by mtROS, alter their metabolic phenotype. In this review we summarize data and show evidence supporting new concept that mitochondrial ROS (mtROS) directly contributes to pro-hypertensive response of immune cells.
Hypertension is a multifactorial pathological condition which includes genetic, environmental, neural, endocrine, and humoral causes. In the past decade it has become clear that reactive oxygen species (ROS) and the immune system contribute to all of these factors (4). Phagocytic NADPH oxidase (Nox2) and mitochondria are two key ROS sources in immune cells. Nox2 plays an important role in immune cell regulation and blocking its expression or its activity reduces cytokine production and cell proliferation. Mitochondria contribute to Nox2 activation (2), regulation of cellular glycolysis and antigen-specific expansion of T-cells (9). The underlining precise mechanisms however, are not clear.

T-cells are critical for hypertension (5), however, the exact mechanism of T-cell activation in hypertension has not been fully understand. Once activated, T-cells intensively proliferate, produce cytokines that further stimulate immune system, alter vascular function and stimulate ROS production. MtROS regulates cellular signaling, cell functions, and metabolism. It controls major mitochondrial and cytoplasmic metabolic pathways, such as, glycolysis and the Krebs cycle. Rapidly proliferating cells, including immune cells, may rely on glycolysis to fulfill growing energy needs. To trigger changes in metabolism, cells increase ROS and through tightly regulated mechanisms alter metabolic phenotype. Cellular sources of ROS are also required for immune cell activation and their responses. Knocking down in T-cells p47 phox, a NOX2 subunit, prevents TNFα production, a cytokine that is critical for the development of hypertension (5). Inhibition of TNFα mutes hypertension; thus, scavenging ROS in immune cells may attenuate their pro-hypertensive responses. Targeting ROS-sensitive signaling pathways could be a new strategy to treat hypertension. In fact, overexpression of mitochondrial SOD in mice prevents development of hypertension or treatment mice with low doses of mitochondria-targeted superoxide dismutase mimetic reverses fully developed hypertension (3). We and others
have found that angiotensin II-induced hypertension leads to endothelial dysfunction dependent on mtROS and NADPH oxidases (3, 12). These pathological conditions can be reversed by preventing mitochondrial superoxide production. However, it remains unclear which tissue or organ is specifically targeted with systemic administration of mitochondria-targeted antioxidants. There are several potential sites of action for this antioxidant. It is conceivable that mitochondria-targeted antioxidants interfere with signal transduction in vascular cells; resulting in protection against the development of hypertension. Mitochondrial superoxide contributes to signaling events that are responsible for endothelial dysfunction (3) and smooth muscle cell proliferation. It is also feasible that mitochondria-targeted antioxidants act on other targets, such as immune cells, crucial for development of hypertension.

The fact that hypertension is dependent on ROS suggest that antioxidant administration should attenuate hypertension. Surprisingly, clinical trials showed limited or no effect of antioxidants in treatment of hypertension. This may indicate that antioxidants, such as vitamin E and vitamin C, are not targeted to sites of ROS generation that are crucial in this disease condition (3). Moreover, antioxidants used to treat hypertension have limited efficacy and inability to accumulate at high local concentration. Therefore, using more specific and organelle-targeted approaches may bring better results. Mitochondria are one of the locations where superoxide is produced at high local concentrations. Mitochondrial superoxide is dismutated to hydrogen peroxide, a product that is stable and able to transverse cellular membranes, and reach cytoplasmic signaling targets to initiate prolonged cellular responses. It is important to note that increased ROS is not equivalent to oxidative stress that results in massive damage to cells. Elevated ROS may result in the specific redox-dependent cell signaling but may not reach the threshold sufficient to induce oxidative damage leading to cell death.
There is limited data on the role of mtROS in immune cells mediated development of hypertension. However, there is evidence that mtROS can play a regulatory role in immune cell pro-hypertensive responses. In other disease conditions, lymphocytes, key players in development of hypertension, relay on mitochondrial superoxide to maintain their functions (9). Overexpression of mitochondrial SOD prevents T-cell activation and cytokine production (6). Naïve immune cells retained in the organism, while maintaining low metabolic profile, are designed to wait for a signal initiating their immediate response. Once activated, they need aggressively proliferate and produce cytokines. This dramatic change in their phenotype requires changes in their metabolism (10). This mtROS-dependent metabolic shift has been shown to be a target for anticancer therapy (8). Similarly, activated immune cells are a potential target for treatment in hypertension.

One of the key molecules that regulates metabolic shift facilitating aggressive cell proliferation and intensified activity is hypoxia-inducible factor-1alpha, HIF1-α, a transcription factor induced by hypoxic conditions, rapid energy demand, and increased ROS (1). HIF1-α regulates expression and activity of glycolytic proteins, including glucose transporters and enzymes (hexokinases, phosphofructokinase, lactate dehydrogenase). HIF-1α modulates mitochondrial function by inactivating the pyruvate dehydrogenase complex and alteration of Krebs cycle substrates supply. As a result of these changes, activated T-cells in order to fulfill the bioenergetic demand, reprogram their metabolic pathways from aerobic glucose and fatty acid oxidation to the glycolytic, pentose-phosphate, and glutaminolytic pathways (11). HIF1-α activity can be potentially orchestrated by other signaling mechanisms regulating mitochondrial metabolism and function. TNF receptor associated protein, TRAP1, regulates metabolic switch between oxidative phosphorylation and aerobic glycolysis in
immortalized mouse fibroblasts and tumor cells. TRAP1-deficiency promotes an increase in mitochondrial respiration, fatty acid oxidation, and ROS. At the same time, glucose metabolism is suppressed. TRAP1-deficient cells also display enhanced invasiveness (13). This could indicate a tight correlation between metabolic phenotype of immune cells and ROS. It has been shown that mitochondrial superoxide is crucial for cytokine production in T-cells. SOD2 overexpression results in decreased IL-2, a cytokine required for T-cell survival and proliferation (6). Mitochondrial superoxide was also shown to regulate mitochondrial glycerol-3-phosphate dehydrogenase in T-cells. This metabolic shift results in increased glycolysis and increased reduction of mitochondrial respiratory chain (7). Described metabolic changes facilitate T-cell proliferation and cytokine production in models of other disease conditions; however, specific role of mitochondrial superoxide in the immune cells-mediated hypertension is not defined.

To address this question, first we investigated whether T-cells from hypertensive mice generate more mitochondrial superoxide than sham operated animals. We infused mice with angiotensin II (0.7 mg/ml), isolated T-cells from spleens and measured superoxide using HPLC fluorescence assay. We found that indeed there was increased superoxide production in cells isolated from hypertensive mice comparing to sham operated animals (not shown). In the next step, we measured TNF-α secretion from T-cells and found that ex vivo treatment with the mitochondria-targeted SOD2 mimetic, mitoTempo, significantly suppressed TNF-α production in T-cells isolated from hypertensive mice (Fig. 1A). We also found that those cells synthesized less ATP (Fig. 1B). These data demonstrate reversible metabolic changes mediated by mtROS. Evidence from previous studies and our data suggest that immune cells may utilize mtROS to induce metabolic reprogramming required for their activation, differentiation and proliferation. Indeed, ex vivo mitoTempo treatment prevented proliferation of T-cells from hypertensive
animals cultured on CD3-coated plates (Fig. 1C). This data shows that mtROS is required for T-cell proliferation. T-cell stimulation leads to increased ROS that triggers mtROS and further stimulates NADPH oxidases.

Taken together, several studies suggest that immune cell activation leads to increased cellular ROS production that triggers mtROS. The phenomenon known as ROS-induced ROS-release may contribute to further increase of cytoplasmic ROS by stimulation of NADPH oxidases that in turn has been shown to be required for immune cell activation and proliferation. To facilitate increased energy demand related to proliferation and cytokine production, cells switch their metabolism from mitochondrial ATP synthesis to much faster glycolysis by activation of ROS-sensitive expression of HIF1-α. These metabolic changes are supported by mitochondrial and cytosolic ROS (Fig.1D). Therefore, specific inhibition of mitochondrial ROS may represent a new pharmacological approach to prevent pro-hypertensive immune cells responses.

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Figure legend

Fig. 1 Role of mtROS in T-cells metabolism and activation. (A) TNFα production by T-cells isolated from Ang II-infused or sham operated mice. Cells cultured for 48h on CD3-coated plates. (B) ATP in cells isolated from spleens of Ang II-infused or sham operated mice. (C) Number of T-cells isolated Ang II-infused or sham operated mice after 72h culture on CD3-coated plates. (D) T-cells stimulation leads to increased ROS that triggers mtROS and further stimulates NADPH oxidases. Elevated ROS cause metabolic changes to support growing energy demand of proliferating cells. Mitochondrial ATP synthesis is inhibited and glycolysis becomes major pathway for ATP synthesis of stimulated cells. These changes facilitate development of T-cell mediated hypertension. Data are mean ± S.E.M. at least n = 3.

References


**Metabolic changes in immune cells**

**Immune cells activation**

**Cellular ROS**

**Mitochondrial ROS**

**Metabolic changes in immune cells**

**Cell proliferation/cytokine production**

**HYPERTENSION**