

TNF- α Is not a Miscreant: A Hero for Basal Nrf2-Antioxidant Signaling

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ABSTRACT | About three decades of intensive research suggest that tumor necrosis factor- α (TNF- α) is a “miscreant”. Although it is obvious that supra-physiological TNF- α levels are deleterious to cellular activities leading to a variety of pathological conditions, it is unlikely that complete removal of TNF- α is cytoprotective. Are we rejecting the basal physiological role of TNF- α as a reactive oxygen species (ROS) producer that is key and essential for numerous basal cell signaling processes? We believe that there are important protective roles for TNF- α under basal/physiological conditions. We propose that one such role is that of signaling through nuclear erythroid 2 p45 related factor-2/antioxidant response element (Nrf2/ARE). Confirming our hypothesis that TNF- α is necessary and sufficient for the basal activation of Nrf2/ARE transcriptional pathways, will change the existing paradigms on the function of TNF- α . This article briefly reviews the canonical role of TNF- α as miscreant and introduces a new role as a hero in the context of Nrf2-antioxidant signaling.

KEYWORDS | Antioxidant signaling; Myocardium; Nrf2; TNF- α

ABBREVIATIONS | ARE, antioxidant response element; Keap1, Kelch like ECH associated protein; Nrf2, nuclear erythroid 2 p45 related factor-2; ROS, reactive oxygen species; TNF- α , tumor necrosis factor- α ; TNFR, TNF- α receptor

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1. TNF- α AS AN INFLAMMATORY MARKER AND A SIGNALING MOLECULE

Tumor necrosis factor-alpha (TNF- α), a potential cytokine (an inflammatory marker and a signaling molecule), released upon acute inflammation by the monocytes and macrophages [1–3]. TNF- α is pivotal for intracellular signaling and generates reactive oxygen species (ROS) by activating NADPH oxidases, which are major sources of ROS production in cells. The ROS are critical for regulating various physiological processes in cells and provide basal activation of key signaling pathways [1, 4, 5]. A trimeric TNF- α binds to one of its major receptors on the cell membrane (TNFR1 of 55 kDa or TNFR2 of 75 kDa) and exerts its pleiotropic effects in cell signaling cascades [6, 7]. In this review, we hypothesize a novel role for TNF- α involving the nuclear erythroid 2 p45 related factor-2 (Nrf2)/Kelch like ECH associated protein (Keap1) signaling pathway, which is a master regulator of hundreds of antioxidant/cytoprotective genes.

We recently described that increased intracellular reducing power (reduced form of glutathione [GSH], NAD(P)H, cysteine) termed “reductive stress” is causally linked to protein aggregation and cardiac hypertrophy/heart failure in mice harboring a human mutant transgene for a small heat shock protein, α B-crystallin (hR120GCryAB) [8]. Of interest, we found an increased and sustained nuclear translocation of Nrf2 in the hR120GCryAB hearts [9]; but basal mechanisms for Nrf2 function remain elusive. Our recent report showed that exogenous TNF- α supplementation to the HL-1 cardiac myocytes significantly increased Nrf2 activity and its nuclear translocation, whereas knock-down of TNF- α prevented Nrf2 nuclear translocation [10]. These results indicate a vital role for TNF- α in Nrf2/antioxidant response element (ARE) signaling. Evidence from the last three decades of research using TNF- α over expression models [11] revealed pivotal roles for TNF- α in several oxidative stress diseases including diabetes, cancer, cardiac hypertrophy, and cardiomyopathy [12–14]. Consistent with this evidence, abolishing TNF- α using TNFR1/R2 knockout mouse models showed increased protection against oxidative stress diseases [15]. In contrast, other studies have shown that chronic TNF- α blockade in the elderly resulted in impaired immune function, increased risk of cancer, and cardiovascular complications [16, 17], suggest-

ing that basal signaling mechanisms through ROS generation may be important for redox homeostasis. Thus, a critical link for such TNF- α deficiency-mediated disorder could be related to a decrease in Nrf2/ARE dependent cytoprotection, which has yet to be elucidated in a great detail. Considering the fact that either supra-physiological or sub-physiological (abrogation) levels of TNF- α are detrimental to cellular life, we believe that its physiological level is essential for regulating Nrf2-Keap1 signaling under basal conditions.

2. TNF- α BLOCKERS AND POTENTIAL THERAPEUTIC PROBLEMS

Use of TNF- α blockers such as monoclonal antibodies against TNF- α or specific receptor blockers has been found to result in deleterious consequences in various cells and organ systems [17]. Recent evidence indicates that anti-TNF- α therapy using different agents is associated with adverse risk of cancer, demyelinating disorders, and cardiovascular complications [16, 17]. Several other reports indicate that prolonged TNF- α deficiency results in a wide range of immune system disorders starting from asymptomatic immunological problems to life-challenging autoimmune diseases. Although, at present, the associated mechanisms for TNF- α deficiency are not known, issues linked to basic cellular redox status could be of potential importance. Generation of ROS is a crucial requirement for any living cell in order to trigger the signaling cascades and perform physiological processes [4, 5, 18]. In this context, the role of TNF- α is important [19] and its chronic deficiency might lead to cell injury. Currently, it is uncertain to what extent TNF- α deficiency might be associated with dysregulation of transcriptional mechanisms that drive cytoprotective antioxidant gene expression.

3. DRAWBACKS OF TNF- α KNOCKDOWN ON NRF2-DEPENDENT TRANSCRIPTIONAL/REDOX SIGNALING

In spite of emerging evidence showing that TNF- α deficiency leads to cellular injury and disease, there is currently no in vivo or in vitro model that directly examines the mechanistic issues related to antioxidant/cytoprotective defense mechanisms under TNF-

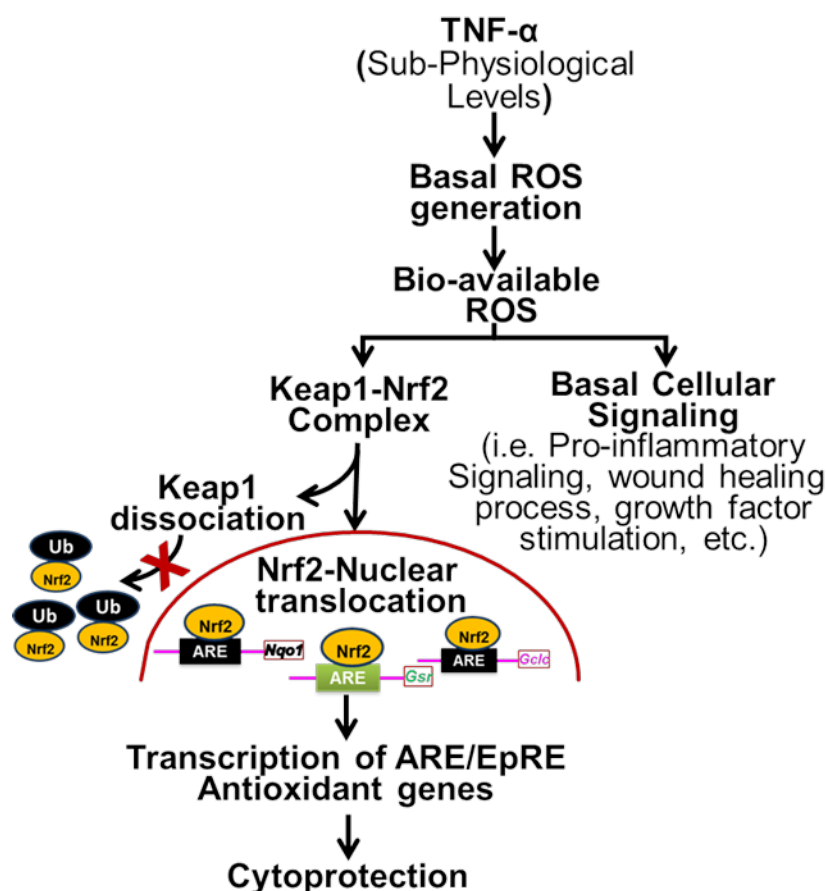


FIGURE 1. A heroic role for TNF α in regulating basal Nrf2-antioxidant signaling. See text for detailed description. Ub, ubiquitin; Nqo1, NAD(P)H:quinone oxidoreductase 1; Gst, glutathione S-transferase; Gclc, glutamate-cysteine ligase catalytic subunit; EpRE, electrophile response element.

α deficient conditions. Based on the preliminary studies performed in several cell culture models (H9c2 myoblasts, HL-1 cardiomyocytes, and olfactory epithelial cells—OP6/OP27), we believe that abolishing TNF- α will lead to chronic impairment of cellular redox defense mechanisms [11, 20]. A welter of information establishes that the transactivation mechanisms of ARE-containing defense genes, at basal conditions, are dependent on the stabilization and biological function of Nrf2 [21, 22]. Under basal physiological conditions, the bioavailability of ROS (generated through various metabolic and physiological processes including via TNF- α and NADPH oxidases) is necessary for the stabilization and activation of Nrf2 [10, 23]. However, the degree of

Nrf2 activation varies according to the level of stress. We believe that TNF- α contributes to physiological concentrations of ROS required for the basal ARE signaling mechanisms of the cell (Figure 1).

4. WHY IS THIS DIRECTION/IDEA SIGNIFICANT?

We propose a novel signaling mechanism that may explain numerous observations regarding TNF- α deficiency associated with chronic TNF- α blockade, or diseases associated with age-related oxidative stress. Over three decades of intensive research on cytokines and inflammatory responses reveal potential

pathological consequences for TNF- α in various human diseases [24]. In contrast, the elderly suffer impairments to their immune system, evidenced by higher susceptibility to infections, cancer, and many diseases believed to be directly associated with TNF- α deficiency [25–27]. However, elucidating the TNF- α deficiency associated mechanisms for transcriptional regulation of antioxidants and cytoprotective genes in different cell types/organs will be vital. Since TNF- α induces ROS generation, it is important to determine whether it contributes to Nrf2/ARE signaling under physiological (aging) or pathological (e.g., cardiovascular diseases) conditions associated with oxidative stress. Our future research will investigate the molecular cross talk between TNF- α -induced ROS and Nrf2/ARE activation in musculoskeletal and cardiac systems. Further, transcriptional regulation of antioxidant/cytoprotective mechanisms involved in the pathogenesis of inflammation and/or unresolved-inflammation mediated cardiac hypertrophy/heart failure also will be investigated.

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REFERENCES

- Kim YS, Morgan MJ, Choksi S, Liu ZG. TNF-induced activation of the Nox1 NADPH oxidase and its role in the induction of necrotic cell death. *Mol Cell* 2007; 26(5):675–87. doi: 10.1016/j.molcel.2007.04.021.
- Keogh C, Fong Y, Marano MA, Seniuk S, He W, Barber A, et al. Identification of a novel tumor necrosis factor alpha/cachectin from the livers of burned and infected rats. *Arch Surg* 1990; 125(1):79–84; discussion 5.
- Kinouchi K, Brown G, Pasternak G, Donner DB. Identification and characterization of receptors for tumor necrosis factor-alpha in the brain. *Biochem Biophys Res Commun* 1991; 181(3):1532–8.
- D'Autreaux B, Toledano MB. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nat Rev Mol Cell Biol* 2007; 8(10):813–24. doi: 10.1038/nrm2256.
- Hensley K, Robinson KA, Gabbita SP, Salsman S, Floyd RA. Reactive oxygen species, cell signaling, and cell injury. *Free Radic Biol Med* 2000; 28(10):1456–62.
- Chen G, Goeddel DV. TNF-R1 signaling: a beautiful pathway. *Science* 2002; 296(5573):1634–5. doi: 10.1126/science.1071924.
- Wajant H, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling. *Cell Death Differ* 2003; 10(1):45–65. doi: 10.1038/sj.cdd.4401189.
- Rajasekaran NS, Connell P, Christians ES, Yan L-J, Taylor RP, Orosz A, et al. Human α B-crystallin mutation causes oxido-reductive stress and protein aggregation cardiomyopathy in mice. *Cell* 2007; 130(3):427–39. doi: 10.1016/j.cell.2007.06.044.
- Rajasekaran NS, Varadharaj S, Khanderao GD, Davidson CJ, Kannan S, Firpo MA, et al. Sustained activation of nuclear erythroid 2-related factor 2/antioxidant response element signaling promotes reductive stress in the human mutant protein aggregation cardiomyopathy in mice. *Antioxid Redox Signal* 2011; 14(6):957–71. doi: 10.1089/ars.2010.3587.
- Shanmugam G, Narasimhan M, Sakthivel R, Kumar R R, Davidson C, Palaniappan S, et al. A biphasic effect of TNF- α in regulation of the Keap1/Nrf2 pathway in cardiomyocytes. *Redox Biology* 2016; 9:77–89. doi: 10.1016/j.redox.2016.06.004.
- Sriram K, Matheson JM, Benkovic SA, Miller DB, Luster MI, O'Callaghan JP. Deficiency of TNF receptors suppresses microglial activation and alters the susceptibility of brain regions to MPTP-induced neurotoxicity: role of TNF- α . *FASEB J* 2006; 20(6):670–82. doi: 10.1096/fj.05-5106com.
- Mariappan N, Soorappan RN, Haque M, Sriramula S, Francis J. TNF- α -induced mitochondrial oxidative stress and cardiac dysfunction: restoration by superoxide dismutase mimetic Tempol. *Am J Physiol Heart Circ Physiol* 2007; 293(5):H2726–37. doi: 10.1152/ajpheart.00376.2007.
- Berthonneche C, Sulpice T, Boucher F, Gouraud

- L, de Leiris J, O'Connor SE, et al. New insights into the pathological role of TNF- α in early cardiac dysfunction and subsequent heart failure after infarction in rats. *Am J Physiol Heart Circ Physiol* 2004; 287(1):H340–50. doi: 10.1152/ajpheart.01210.2003.
14. Wender M, Michałowska-Wender G, Szczech J. Minimal changes of TNF and MCP-1 expression in blood serum of rats subjected to experimental cardiac arrest. *Folia Neuropathologica* 2005; 43(2):109–11.
 15. Liu JY, Brass DM, Hoyle GW, Brody AR. TNF- α receptor knockout mice are protected from the fibroproliferative effects of inhaled asbestos fibers. *Am J Pathol* 1998; 153(6):1839–47.
 16. Onel KB, Onel K. Anti-tumor necrosis factor therapy and cancer risk in patients with autoimmune disorders. *Arthritis Care Res* 2010; 62(7):1024–8. doi: 10.1002/acr.20228.
 17. Lis K, Kuzawińska O, Bałkowiec-Iskra E. Tumor necrosis factor inhibitors: state of knowledge. *Arch Med Sci* 2014; 10(6):1175–85. doi: 10.5114/aoms.2014.47827.
 18. Ha H, Lee HB. Reactive oxygen species as glucose signaling molecules in mesangial cells cultured under high glucose. *Kidney Int Suppl* 2000; 77:S19–25.
 19. Keller C, Keller P, Giralte M, Hidalgo J, Pedersen BK. Exercise normalises overexpression of TNF- α in knockout mice. *Biochem Biophys Res Commun* 2004; 321(1):179–82. doi: 10.1016/j.bbrc.2004.06.129.
 20. Morimoto Y, Gai Z, Tanishima H, Kawakatsu M, Itoh S, Hatamura I, et al. TNF- α deficiency accelerates renal tubular interstitial fibrosis in the late stage of ureteral obstruction. *Exp Mol Pathol* 2008; 85(3):207–13. doi: 10.1016/j.yexmp.2008.08.003.
 21. Sykietis GP, Bohmann D. Keap1/Nrf2 signaling regulates oxidative stress tolerance and lifespan in *Drosophila*. *Dev Cell* 2008; 14(1):76–85. doi: 10.1016/j.devcel.2007.12.002.
 22. Eggler AL, Liu G, Pezzuto JM, van Breemen RB, Mesecar AD. Modifying specific cysteines of the electrophile-sensing human Keap1 protein is insufficient to disrupt binding to the Nrf2 domain Neh2. *Proc Natl Acad Sci USA* 2005; 102(29):10070–5. doi: 10.1073/pnas.0502402102.
 23. Rushworth SA, Shah S, MacEwan DJ. TNF mediates the sustained activation of Nrf2 in human monocytes. *J Immunol* 2011; 187(2):702–7. doi: 10.4049/jimmunol.1004117.
 24. Mohammed FF, Smookler DS, Taylor SE, Fingleton B, Kassiri Z, Sanchez OH, et al. Abnormal TNF activity in Timp3^{-/-} mice leads to chronic hepatic inflammation and failure of liver regeneration. *Nat Genet* 2004; 36(9):969–77. doi: 10.1038/ng1413.
 25. Daynes RA, Enioutina EY, Jones DC. Role of redox imbalance in the molecular mechanisms responsible for immunosenescence. *Antioxid Redox Signal* 2003; 5(5):537–48. doi: 10.1089/152308603770310185.
 26. McAfoose J, Koerner H, Baune BT. The effects of TNF deficiency on age-related cognitive performance. *Psychoneuroendocrinology* 2009; 34(4):615–9. doi: 10.1016/j.psyneuen.2008.10.006.
 27. Aggarwal BB, Shishodia S, Takada Y, Jackson-Bernitsas D, Ahn KS, Sethi G, et al. TNF blockade: an inflammatory issue. *Ernst Schering Res Found Workshop* 2006; (56):161–86.