Review

Structure and role of neutrophil cytosol factor 1 (NCF1) gene in various diseases

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The neutrophil cytosol factor 1 (NCF1) gene consists of 11 exons and is found in two forms; one is wild type gene and the other is pseudogene. It has more than 98% homology. Both genes occupy the same chromosome region. The mutation in this gene leads to various types of diseases such as chronic granulomatous disease, multiple sclerosis, arthritis and parasitic infection. The common mutation of this gene in most diseases is GT deletion at the start of exon 2. The NCF1 gene interact with other subunits of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) and play an important role in innate immunity and produce reactive oxygen species and reduce the severity and duration of parasitic infection and autoimmune disease. NCF1 also has a role in T cell activation.

Key words: Neutrophil cytosol factor 1 (NCF1) gene, exons, T cell activation.

INTRODUCTION

An immune system is a system of biological structures and processes within an organism that protects against disease by identifying and killing pathogens. Detection is complicated as pathogens can evolve rapidly, producing adaptations that avoid the immune system and allow the pathogens to successfully infect their hosts. The mechanisms include antimicrobial peptides called defensins, phagocytosis, and the complement system (Beck and Gail, 1996). The innate leukocytes include the phagocytes (macrophages, neutrophils, and dendritic cells), mast cells, eosinophils, basophils, and natural killer cells. These cells identify and eliminate pathogens, either by attacking larger pathogens through contact or by engulfing and then killing microorganisms (Janeway et al., 2005). Immunity against microbial pathogens primarily depends on the recognition of pathogen components by innate receptors expressed on immune and non-immune cells. Innate receptors are evolutionary conserved germ-line-encoded proteins and include toll-like receptors (TLRs), retinoic acid-inducible gene-I-like receptors (RLRs [RIG-I]) and nod-like receptors (NLRs) (Kumar et al., 2009).

ROLE OF REACTIVE OXYGEN SPECIES IN KILLING PATHOGENS

The production of reactive oxygen species (ROS), via consumption of oxygen which is called oxidative burst, is one of the earliest cellular responses next to successful pathogen recognition. The generation of superoxide (O$_2^-$), or its dismutation product, hydrogen peroxide (H$_2$O$_2$), had been documented following recognition of a variety of pathogens (Doke, 1983; Auh and Murphy, 1995; Grant et al., 2000). The neutrophils are the main source of ROS and their primary functions in the innate immune response are to kill invading microbial pathogens (Doke, 1983). Neutrophils consist of potent antimicrobial components that includes oxidants, proteases, and antimicrobial peptides. Neutrophils also produce remarkable quantities of ROS and reactive nitrogen species (RNS) such as O$_2^-$.
and NO through the activity of oxidant-generating systems such as the phagocyte nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase (Ah and Murphy, 1995) and nitric oxide synthase (NOS), respectively (Grant et al., 2000; Kleinert et al., 2004). During ingestion (phagocytosis) of invading pathogens, antimicrobial compounds contained in granules and ROS generated at the phagosome membrane are released directly into the phagosome. This process compartmentalizes both the pathogen and the cytotoxic products and facilitates intracellular killing. Neutrophil cytosol factor 1 (NCF1) gene is involved in the generation of ROS (Moraes et al., 2006). The production of ROS and functions in response to pathogens is shown in Figure 1.

**Figure 1.** ROS production and functions in response to pathogens.

**NCF1 GENE AND PRODUCTION OF REACTIVE OXYGEN SPECIES**

The identification of mutations in the NCF1 gene related with low ROS production mediate the severity of disease (Olofsson et al., 2003; Hultqvist et al., 2005). The P47-phox protein is an essential component of the NADPH oxidase complex that catalyzes the transfer of a single electron from NADPH to oxygen, generating ROS. The release of ROS and its downstream products from phagocytosing cells is known as respiratory burst and is regarded as part of the protection against invading pathogens (Babior, 2000).

The functional version of NCF1 in macrophages protects the mice from arthritis (Gelderman et al., 2007). Lack of ROS was shown to break T cell tolerance to endogenously expressed collagen type II in mice, suggesting an effect on the regulation of T cell tolerance; however, it remains to be determined whether this occurs centrally in the thymus or in the periphery (Hultqvist et al., 2007).

**PRODUCTION OF REACTIVE OXYGEN SPECIES AND NADPH OXIDASE**

The NADPH oxidase complex is responsible for the reduction of oxygen, yielding superoxide anion (O2-) that is subsequently transformed into other ROS, including hydrogen peroxide (H2O2) and the hydroxyl radical (OH·) (Halliwell, 1987; Babior, 1999).

\[
\text{NADPH} + 2\text{O}_2 \rightarrow \text{NADP}^+ + 2\text{O}_2^- + \text{H}^+
\]

The NADPH complex consisted of five subunits, two of which (p22phox and gp91phox) are localized in cellular membranes, constituting the flavocytochrome b558. Upon activation, the three cytosolic subunits (p47phox, p67phox and p40phox) co-localize and migrate to the membrane where they, along with the membrane subunits and the small GTPase (Rac1 in monocytes and Rac2 in neutrophils), form the active NADPH complex...
Figure 2. Various components of NADPH oxidase and protective role of ROS in autoimmune disease.

Table 1. Components of NADPH oxidase.

<table>
<thead>
<tr>
<th>Component</th>
<th>Gene</th>
<th>Location on chromosome</th>
<th>Location in cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>gp91phox</td>
<td>CYBB</td>
<td>Xp21</td>
<td>Cell membrane</td>
</tr>
<tr>
<td>P22phox</td>
<td>CYBA</td>
<td>16q24</td>
<td>Cell membrane</td>
</tr>
<tr>
<td>P47phox</td>
<td>NCF1</td>
<td>7q11</td>
<td>Cytosol</td>
</tr>
<tr>
<td>P67phox</td>
<td>NCF2</td>
<td>1q25</td>
<td>Cytosol</td>
</tr>
<tr>
<td>P40phox</td>
<td>NCF4</td>
<td>10q21.1</td>
<td>Cytosol</td>
</tr>
</tbody>
</table>

All cells are phagocyte dendritic cells.

(Figure 2 and Table 1). During activation, p47phox is heavily phosphorylated and its autoinhibitory confirmation is released, enabling it to co-localize with the flavocytochrome at the membrane (Ago et al., 2003). The p47phox protein is believed to be responsible for the transport of the cytosolic subunits to the membrane and is thus considered to be the organizing protein (Dusi et al., 1996).

STRUCTURE OF NCF1 GENE

The NCF1 gene expresses an important component called P47 phagocyte oxidase (P47phox), which has a molecular weight of about 47 Kd. This P47phox is activated by phosphorylation and produces super oxides and hypochlorous acid (Wientjes et al., 2001). Sequence analysis revealed that the NCF1 wild type gene is 15,236 bp long, having 11 exon, and its intron/exon structure is identical to highly homologous pseudogene (Gorlach et al., 1997). The NCF1 gene comprised of high portion of repetitive elements, GC contents, short and long interspersed elements (SINEs and LINEs). There are 21 Alu elements and 3 mammalian interspersed repetitive (MIR) elements throughout the NCF1 gene and is depicted in Figure 3 (Stephen et al., 2000). The pseudogene which is highly homologous to wild type located on same chromosome (7q11.23 of chromosome) (Francke et al., 1990). Comparative sequence analysis between the wild type gene and pseudogene demonstrates greater than 98% homology but the pseudogene has GT deletion (ΔGT) at the start of exon 2 (Chanock et al., 2000). The cause of GT deletion is recombination events which may occur between highly homologous sequence of pseudogene and the wild type gene sequence (Roesler et al., 2000).

COMPARISON OF HUMAN NCF1 GENE WITH MOUSE NCF1 GENE

The major features of mouse and human NCF1 genes are compared in Table 2 and shown in Figure 4. The number and size of exons is similar to the two orthologs that is of human and mice. However, the mouse gene is more compact as compared to human gene. The introns 1, 2, 4, 5, and 8 in the human gene are larger than the...
Figure 3. Structure of NCF1 gene and distribution of various repetitive sequences.

Table 2. Comparison of NCF1 gene of human and mouse.

<table>
<thead>
<tr>
<th>Exon</th>
<th>Exon length bp</th>
<th>Intron length bp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exon Human bp</td>
<td>Exon Mouse bp</td>
</tr>
<tr>
<td>1</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>2</td>
<td>81</td>
<td>81</td>
</tr>
<tr>
<td>3</td>
<td>76</td>
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<tr>
<td>4</td>
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<tr>
<td>5</td>
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<td>10</td>
<td>145</td>
<td>146</td>
</tr>
<tr>
<td>11</td>
<td>351</td>
<td>351</td>
</tr>
</tbody>
</table>

Figure 4. Structure of NCF1 gene of mouse.


Table 3. Contribution of various components of NADPH oxidase system in CGD.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Component</th>
<th>% Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGD</td>
<td>gp91phox</td>
<td>70</td>
</tr>
<tr>
<td>CGD</td>
<td>p47phox</td>
<td>25</td>
</tr>
<tr>
<td>CGD</td>
<td>p67phox</td>
<td>&lt;5</td>
</tr>
<tr>
<td>CGD</td>
<td>p22phox</td>
<td>&lt;5</td>
</tr>
<tr>
<td>CGD</td>
<td>Rac2</td>
<td>1 case</td>
</tr>
</tbody>
</table>

corresponding introns in the mouse gene except intron 7. The human introns contain more repetitive sequences while intron 1 of mouse is devoid of a repetitive sequence (Udaya et al., 2000).

ASSOCIATION OF NCF1 GENE WITH OTHER GENES

The NCF1 gene is a crucial component of the NADPH oxidase complex and this complex is involved in production of ROS which reduce the duration of infection such as malaria (Rosen et al., 1995). Molecular and biochemical analysis highlighted the importance of at least four components of the NADPH oxidase: gp91-phox, p67-phox, p47-phox, and p22-phox (Clark and Hunt, 1991; Roos et al., 1995; Roos et al., 1996). Interestingly, p47-phox deficiency is roughly five times more common than p22-phox or p67-phox deficiency. The mutations were reported in patients with gp91-phox as well as p22-phox and p67-phox deficient; the majority of p47-phox deficient patients have only the GT deletion at the start of exon 2 (Volpp and Lin, 1993; Roesler et al., 2000). The resultant frame shift generates a pre terminal stop codon; the predicted truncated protein of approximately 50 amino acids has not been detected in patient samples. Several groups suggested that this region represented a mutational hot spot, and it was postulated that either DNA strand slippage or hairpin loops could account for the striking over representation of the dinucleotide deletion (Casimir, 1991; Volpp and Lin, 1993).

ASSOCIATION OF NCF1 GENE WITH MALARIA

The release of ROS plays an important part in innate immune responses against pathogens (Rosen et al., 1995). The majority of ROS including hydrogen peroxide ($H_2O_2$) or superoxide ions ($O_2^-$) are produced via two pathways, involving phagocyte NADPH oxidase or hypoxanthine metabolism (Beauchamp and Fridovich, 1970; Iyer et al., 1961). It had been reported that pseudogene has no effect on clearance and time course of parasite (Brad et al., 2004). During malaria, ROS production can contribute to both rapid parasite clearances in mild malaria (Greve et al., 1999) but in severe malaria, a high capacity to produce ROS was associated with anaemia. It means that ROS has a possible role for both parasite clearance and anemia during Plasmodium falciparum infection (Greve et al., 2000). Genetic variation in components of the phagocyte NADPH oxidase may, therefore, influence disease susceptibility and disease course of parasitic infection and autoimmune disease (Uhlemann et al., 2004). The ratio of AGT/GTGT in NCF1 gene had also been correlated with clinical parameters and ROS production during P. falciparum malaria infection (Greve et al., 2008).

ASSOCIATION OF NCF1 GENE WITH CHRONIC GRANULOMATOUS DISEASE

Chronic granulomatous disease (CGD) was first described in 1959 and was known as fatal granulomatous disease of childhood (Prando et al., 2004). The mutational defect in the NADPH oxidase complex leads to CGD. The phagocytes fail to produce high amount of ROS (Bridges et al., 1959; Baehner and Nathan, 1967). These mutational changes range from X-linked recessive defect in CYBB (Xp21) encoding gp91phox component of NADPH oxidase to autosomal recessive defects in CYBA (16q24), NCF1 (7q11), NCF2 (1q25), and RAC2 (22q12) genes encoding p22phox, p47phox, p67phox, and Rac2 components, respectively (Table 3) (Winkelstein et al., 2000).

The mutation may be due to deletions, nonsense mutations, insertions, and missense mutations (Williams et al., 2000). The evidence to hyper inflammatory, non infectious complications in CGD, suggest a role for ROS in lowering inflammatory responses. The CGD patients do not only suffer from increased frequency of infectious diseases but are also more prone to develop other inflammatory diseases in case of mutation (De Ravin et al., 2008).

AUTOIMMUNE DISEASES AND NCF1 GENE

Autoimmunity is the failure of an organism to recognize its own constituent parts as self, which allows an immune response to act against its own cells and tissues. The NCF1 in humans is quite complex because it also contains a variable number of genetic duplications (Gorlach et al., 1997; Hockenhull et al., 1999). The association of NCF1 gene with autoimmune disease is difficult due to its complexity; however, genetic variants in other genes of the NADPH complex could result in similar effects (Moreno et al., 2007). Association studies of all the genes encoding the components of the NADPH oxidase complex have been implicated to have a link with autoimmune diseases (RA) (Figure 5) (Olsson et al., 2007).
NCF1 GENE AND AUTOREACTIVE T CELLS

The NCF1 gene is involved in the production of ROS through various components of NADPH complex. The complex formed in the adenomatous polyposis coli (APC) endosomal membrane is translocated within lipid rafts to the cellular membrane where contact with the T cell occurs (Babior, 1999). The ROS produced are directed towards endosomal compartments, where the peptides are processed and major histocompatibility complex (MHC) complex is formed which facilitate T cells interaction with the APC (Figure 6). The ROS directed into endosomal compartments raise the pH and modify antigen processing in dendritic cells (DCs) but not in macrophages (Savina et al., 2006; Mantegazza et al., 2008). Macrophages might use the oxidative burst in such a way that may down regulate interacting T cells by oxidizing their cell membranes (Shao et al., 2003).

The NADPH complex is located in the lipid rafts and probably releases the ROS into the immunological synapse formed between interacting T cells and APCs. The $\text{O}_2^-$ radical has a short half-life but it forms stable hydrogen peroxide which enables diffusion to distant targets, such as interacting cell membranes (Gelderman...
et al., 2006; Kramarenko et al., 2006). The intracellular oxidation might lead to apoptosis and thereby reduce the number of responding T cells. However, the regulatory effect of the NCF1 polymorphism does not lead to a differential cell number or susceptibility to apoptosis (Gelderman et al., 2006). This argues for a more limited role of NCF1, operating by regulating ROS production from interacting macrophages and resulting in effects on T-cell membranes and membrane-associated proteins (Figure 6) (Gelderman et al., 2007). The T cells isolated from RA synovial joints are under oxidative stress, which affects linker for activation of T cells (LAT) signaling (Gringhuis et al., 2000). This indicates that ROS exposure also comprises a regulatory mechanism in human RA. Furthermore, ROS-mediated modification of the structure of the protein tyrosine kinase, Lck, had been reported in T cells from synovial fluid (Romagnoli et al., 2001), suggesting that ROS under certain conditions can modify cysteine residues. These residues are targets for S-acetylation and could result in delocalization of proteins from T cell membrane lipid rafts and inhibition of T cell activation (Jury et al., 2007). A more direct effect on T cell activation had been suggested because redox levels affect the fine tuning of T cell receptor (TCR) signaling (Hehner et al., 2000; Reyes et al., 2005). Furthermore, ROS not only affect the TCR signaling structures but also promote the assembly of the T cell-activation complex within the lipid rafts and enhances lipid raft formation (Lu et al., 2007) and the clustering of proteins (Nakashima et al., 2002).

ASSOCIATION OF NCF1 GENE WITH ARTHRITIS

The etiology of RA is not exactly known but there is possibility that environmental and genetic factors play an important role in its severity and disabling (Symmons et al., 1997; MacGregor et al., 2000). A linkage analysis and positional cloning in animal model identified that NCF1 gene regulate the severity of RA (Vingsbo et al., 1998; Olofsson et al., 2003). A dramatic increase was observed in the severity of RA due to low capacity of NADPH oxidase complex to produce ROS, so a decrease in membrane proteins and activation of T cell and arthritogenic T cells was observed mainly through low oxidative burst (Gelderman et al., 2006). Thus, NCF1 polymorphism and its effects on the decreased ROS production was concluded to be associated with the regulation of arthritogenic CD4 T cells in the immune priming phase (Olofsson et al., 2003; Hultqvist et al., 2005). It was found that T cells of rats and mice with impaired ROS production could change the oxidation state and reduce the proteins on their cell membrane surfaces, that is, they had more free sulfhydryl (–SH) groups (Gelderman et al., 2006). It showed that other cells, such as APCs, in the donor strains modify the T cell membrane redox levels before transfer. The functional expression of p47phox on macrophages protect from arthritis in mice (Gelderman et al., 2007). The low level of ROS was shown to break T cell tolerance to endogenously expressed collagen type II in mice, suggesting an effect on the regulation of T cell tolerance; however, it remains to be explored whether this occurs centrally in the thymus or in the periphery (Hultqvist et al., 2007).

THERAPEUTIC APPROACHES

Polycyclic aromatic hydrocarbons such as benzo (a) pyrene (BaP) are known to regulate the gene expression through aryl hydrocarbon receptor (AhR). The BaP markedly increased the expression of P47phox factor. It was confirmed by the NADPH oxidase specific inhibitor apocynin and the chemical AhR inhibitor alpha-naphthoflavone increase the expression of P47phox (Pinel et al., 2009).

There is an increasing number of findings suggesting that ROS produced by the NOX2 complex are anti-inflammatory and prevent autoimmune responses, thus challenging existing dogma (Malin et al., 2009).

The cytosolic activator and organizer proteins and GTP-Rac are used to regulate the activities of NOX, a family of NADPH oxidases. It is regulated through (a) Formation of Nox-p22phox heterodimeric complexes allowing plasma membrane translocation, and (b) phospholipids-binding specificities of PX domain-containing organizer proteins (p47phox or Nox organizer 1 (Noxo1 and p40phox) (Thomas et al., 2009).

Treatment of arthritis with ROS promoting substances such as phyto1 (3,7,11,15-tetramethyl-2-hexadecene-1-ol) targets a newly discovered pathway leading to autoimmune inflammatory disease and introduces a novel class of therapeutics for treatment of RA and possibly other chronic inflammatory diseases (Malin et al., 2006).

REFERENCES


