

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Role of Tyrosine Kinase A Receptor (TrkA) on Pathogenicity of *Clostridium perfringens* Alpha-Toxin

Masataka Oda, Masahiro Nagahama, Keiko Kobayashi and Jun Sakurai

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/48515>

1. Introduction

Clostridium perfringens (*C. perfringens*) is a toxin-producing anaerobic Gram-positive bacterium, which is well known for its role in human tissue infections and food poisoning. It is readily isolated from soil and a component of normal human intestinal and vaginal flora in many individuals. Apart from the classic clostridial myonecrosis of gas gangrene, *C. perfringens* can be responsible for a range of other clinical scenarios including sepsis, aspiration pneumonia, brain abscess, and enteritis necroticans. The potent exotoxins produced by various strains of *C. perfringens* are central to their effectiveness as pathogens, and include four major toxins used in strain classification: a phospholipase C (alpha-toxin, PLC), two pore-forming toxins (beta and epsilon toxins); and an ADP-ribosylation toxin (iota toxin). *C. perfringens* gas gangrene is one of the most fulminant necrotizing infections affecting humans. The infection can become well established in traumatized tissues in as little as 6-8 h and the destruction of adjacent healthy muscle can progress several inches per hour despite appropriate antibiotic coverage. Shock and organ failure occur in 50% of patients, and 40% of these individuals die. Even with modern medical advances and intensive care regimens, the centuries-old practice of radical amputation on an emergent basis remains the single best treatment. Histologically, this infection is characterized by widespread destruction of muscle and the absence of polymorphonuclear leukocytes at the site of infection. Instead, leukocytes accumulate within adjacent vessels.

C. perfringens alpha-toxin is the major virulence factor in gas gangrene with inflammatory myopathies (Williamson and Titball 1993, Awad et al. 1995). The toxin, which exhibits phospholipase C (PLC) and sphingomyelinase activities, causes hemolysis, necrosis, and death, and the activation of neutrophils and release of cytokines (Sakurai, Nagahama and Oda 2004). Bryant reported that the intramuscular injection of alpha-toxin caused a rapid

and irreversible decline in skeletal muscle blood flow due to toxin-induced intravascular aggregates of plates, leukocytes and fibrin (Bryant et al. 2000a, Bryant et al. 2000b). Neutrophils in these aggregates often bordered the endothelium but all remained intravascular (Bryant et al. 2000a). These findings suggested that the large heterotypic aggregates of platelets and leukocytes generated by alpha-toxin also contributed to impairment of the tissue inflammatory response. We have reported that alpha-toxin-induced activation of endogenous PLC and sphingomyelinase via a pertussis toxin (PT)-sensitive GTP-binding protein (Gi) plays an important role in the hemolysis of rabbit and sheep erythrocytes, respectively (Ochi et al. 1996, Ochi et al. 2004, Oda et al. 2008).

Recently, we revealed that the tyrosine kinase A (TrkA) receptor plays an important role in the release of superoxides and cytokines (Oda et al. 2006, Oda et al. 2008). This review will present findings about the signal transduction via TrkA receptor induced by alpha-toxin and summarize information about its likely role in inflammatory disease, especially septic shock.

2. Role of TrkA on a inflammation induced by alpha-toxin

2.1. Signal transduction via TrkA receptor

The TrkA receptor is a 140-kDa transmembrane protein encoded by a proto-oncogene located on chromosome 1 (Martin-Zanca, Hughes and Barbacid 1986). The family of Trk receptor tyrosine kinases consists of TrkA, TrkB and TrkC. While these family members have highly conserved sequences, they are activated by different neurotrophins: TrkA by nerve growth factor (NGF), TrkB by Brain-derived neurotrophic factor (BDNF) or neurotrophin 4 (NT4), and TrkC by NT3. TrkA regulates proliferation and is important for development and maturation of the nervous system (Pierotti and Greco 2006). This receptor comprises a tyrosine-kinase domain in its intra-cytoplasmic region and five extracellular domains, including two immunoglobulin-like domains involved in NGF binding and responsible for the specific selectivity to bind NGF (Wiesmann et al. 1999). In humans, the TrkA receptor is expressed on cells throughout the nervous system (Muragaki et al. 1995) as well as on structural cells and other non-neuronal cells in the immune and neuroendocrine systems (Levi-Montalcini et al. 1995, Aloe et al. 1997, Bonini et al. 2002, Levi-Montalcini 1987). When NGF binds to the TrkA receptor, it induces receptor homodimerization, which initiates kinase activation and transphosphorylation (Kaplan et al. 1991). This kinase activation involves small G proteins (Ras, Rac, Rap-1), PLC γ , protein kinase C (PKC) and phosphatidylinositol-3 kinase (PI3K) in neural cells (Obermeier et al. 1993b, Obermeier et al. 1993a, Melamed et al. 1999, York et al. 2000, Wu, Lai and Mobley 2001). Phosphorylation at Tyr490 is required for association with Shc and activation of the Ras-MAP kinase cascade. Residues Tyr674/675 lie within the catalytic domain, and phosphorylation at this site reflects TrkA kinase activity (Segal and Greenberg 1996, Stephens et al. 1994, Obermeier et al. 1993a, Obermeier et al. 1993b, Yao and Cooper 1995). Point mutations, deletions and chromosomal rearrangements (chimeras) cause ligand-independent receptor dimerization and activation of TrkA.

The mitogen-activated protein kinase (MAPK) pathways are activated next: extracellular-regulated protein kinase (ERK) by the small G proteins; ERK, p38 and JUN-N-terminal kinase (JNK) MAPK by PKC; and p38 and JNK by PI3K (Kaplan and Miller 1997). PI3K in turn induces activation of protein kinase B (PKB or Akt) and PKC ξ (York et al. 2000)(Fig. 1).

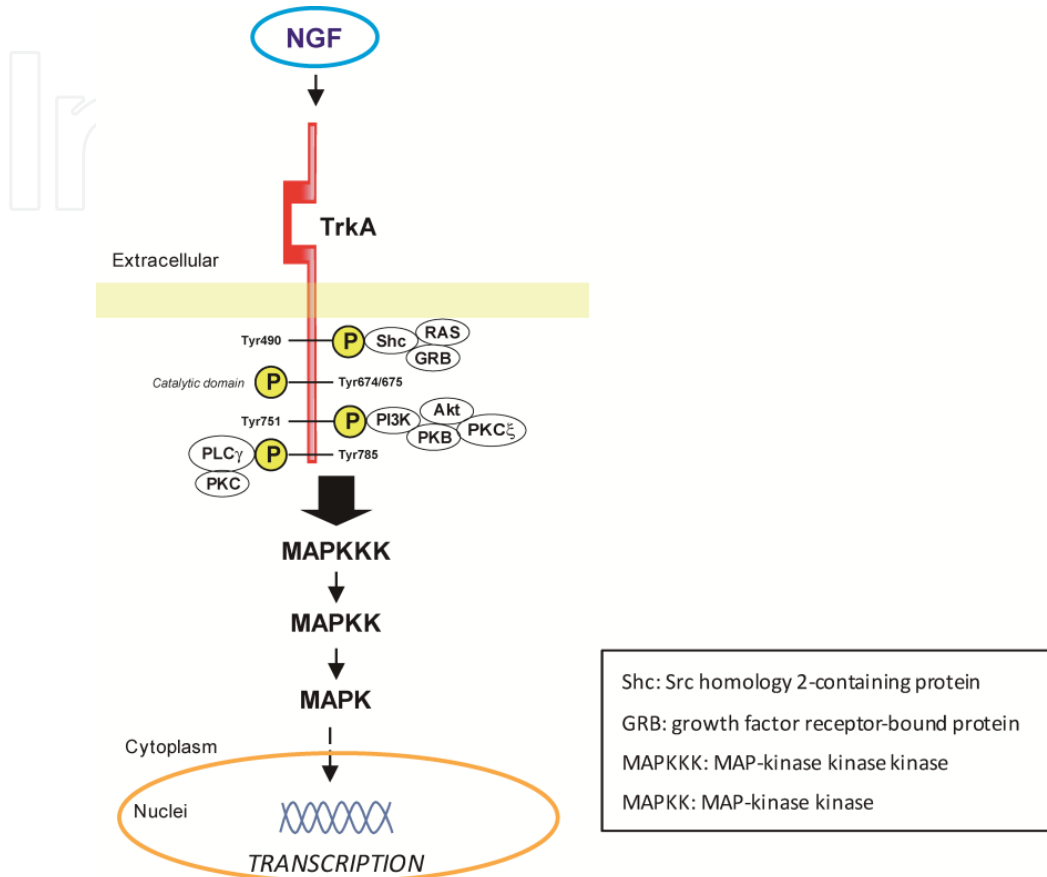


Figure 1. Signal transduction pathways of the TrkA receptor

2.2. Mechanism for the superoxide generation induced by alpha-toxin

The generation of superoxide in neutrophils has been reported to be stimulated by zymosan, 12-O-tetradecanoylphorbol 13-acetate (TPA), Ca²⁺ ionophores, and bacterial chemotactic peptides (Babior 1999). The signal transduction process leading to the stimulation has been studied extensively using *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) (Kusunoki et al. 1992), platelet-activating factor (Yasaka, Boxer and Baehner 1982), and TPA (Nick et al. 1997, Pongracz and Lord 1998). It has been reported that these stimuli activated MAPK or PI3K in neutrophils (Shenoy, Gleich and Thomas 2003, Yamamori et al. 2004). Furthermore, these studies have demonstrated that the interaction of the ligands with receptors on neutrophils activates endogenous PLC with the formation of diacylglycerol (DG), which activates PKC, and inositol 1, 4, 5-trisphosphate (IP₃), inducing the release of Ca²⁺ from the endoreticulum, and that these products act synergistically to generate superoxide. Several studies also reported that phosphorylation of tyrosine kinases and activation of phospholipase D (PLD) were closely related to the generation of superoxide in neutrophils stimulated with agonists

(Garland 1992, Mitsuyama, Takeshige and Minakami 1993) and that activation of PLD resulted in the formation of PA, which was linked to the activation of NADPH oxidase (Bellavite et al. 1988, Olson, Tyagi and Lambeth 1990). We revealed that alpha-toxin-induced generation of superoxide is closely related to the activation of endogenous PKC θ via a combination of two events: production of DG on activation of PLC through a PT-sensitive GTP-binding protein and activation of phosphatidylinositol kinase 1 (PDK1) through the TrkA receptor (Oda et al. 2006).

There are three classes of PKC isotypes: classical PKC isotypes (PKC α , β , and γ) which have a C1 and C2 domain, bind DG, 1-oleoyl-2-acetyl-3-phosphoglycerol (OAG) and TPA, and are regulated by DG and Ca²⁺; novel PKC isotypes (PKC δ , ϵ , η , and θ), which have a C1 domain and novel C2 domain and are regulated by DG but not Ca²⁺; and atypical isotypes (ζ/λ), which do not bind DG and are not regulated by these classical ligands (Le Good et al. 1998). Alpha-toxin induced phosphorylation of PKC θ and PKC ζ/λ , and the generation of superoxide induced by the toxin was inhibited by rottlerin and calphostin C, an inhibitor of PKC θ . We reported that the formation of DG induced by alpha-toxin in rabbit neutrophils plays an important role in the generation of superoxide (Ochi et al. 2002). It therefore appears that the toxin-induced generation of superoxide is dependent on the activation of PKC θ , through binding of PKC θ phosphorylated by PDK1 to DG (Parekh, Ziegler and Parker 2000, Toker and Newton 2000). PKC θ has been reported to play an important role in activation of the protein 1 and NF- κ B signaling pathway in T cells, production of interleukin-2, and apoptosis (Altman, Isakov and Baier 2000, Fan et al. 2004, Villalba et al. 1999, Villunger et al. 1999). Our data may provide clues to the role of PKC θ in neutrophils.

We reported that the alpha-toxin-stimulated generation of superoxide was related to the formation of DG through activation of endogenous PLC by a PT-sensitive GTP-binding protein in rabbit neutrophils (Ochi et al. 2002). U73122, an inhibitor of endogenous PLC, blocked the toxin-induced generation of superoxide and formation of DG in the cells, supporting that the toxin-induced increase in superoxide is dependent on the formation of DG by endogenous PLC. However, when the level of OAG incorporated into the cells was the same as the level of DG in the cells treated with 25 nM of the toxin, the level of OAG did not induce superoxide generation in the absence of the toxin but did in the presence of a near threshold dose (2.5 nM) of the toxin which did not induce production of DG. The result shows that the toxin-induced production of superoxide requires not only the formation of DG, but also the activation of other events.

It has been reported that the PI3K signaling pathway has an important role in several effector functions including the generation of superoxide (Yamamori et al. 2004). PI3K is known to generate phosphatidylinositol 3, 4, 5-trisphosphate (PIP₃), which is recognized by a pleckstrin homology domain identified as a specialized lipid-binding module (Le Good et al. 1998). Several papers have reported that PDK1 requires PIP₃ as its activator for effective catalytic activity (Le Good et al. 1998). Le Good et al. reported that there is a cascade involving PI3K, PDK1, and various members of the PKC superfamily in signal transduction (Le Good et al. 1998). Furthermore, the function of PKC family members is reported to

depend on the phosphorylation of an activation loop by PDK1 (Le Good et al. 1998). LY294002 and wortmannin, both PI3K inhibitors, inhibited alpha-toxin-induced generation of superoxide and phosphorylation of PDK1 but did not affect the toxin-induced formation of DG. The result shows that the toxin-induced activation of PI3K occurs upstream of the phosphorylation of PDK1, which is an important step in the toxin-induced generation of superoxide. It is likely that the toxin-induced phosphorylation of PDK1 is a process independent of the toxin-induced formation of DG.

Tyrosine phosphorylation is thought to be crucial to the regulation of effector functions in neutrophils (Rollet et al. 1994). It is known that stimuli that induce tyrosine kinase activity in cells evoke the generation of PIP₁, PIP₂, and PIP₃. This tyrosine kinase activity is linked to the NGF receptors with intrinsic tyrosine kinase activity. Kannan et al. reported that NGF enhances the generation of superoxide induced by TPA in murine neutrophils (Kannan et al. 1991). Ehrhard et al. reported that human monocytes express the *trk* proto-oncogene, encoding the signal-transducing receptor unit for NGF, and that the interaction of NGF with monocytes triggers respiratory burst activity (Ehrhard et al. 1993). NGF, which did not induce the generation of superoxide in rabbit neutrophils, potentiated the events triggered by the toxin and caused superoxide to form in the presence of OAG, suggesting that a combination of the production of DG and stimulation of the NGF receptor induces severe activity in the generation of superoxide. The TrkA receptor was detected in rabbit neutrophils and found to be phosphorylated when the cells were treated with the toxin. Furthermore, immunoprecipitation using the anti-TrkA receptor antibody revealed direct binding of the toxin to the TrkA receptor. In addition, the antibody inhibited the toxin-induced generation of superoxide. These observations indicate that the interaction of alpha-toxin with TrkA receptors is important to the production of superoxide. In rabbit neutrophils, K252a, a TrkA inhibitor, and LY294002 inhibited the toxin-induced generation of superoxide and phosphorylation of PDK1 within specific concentration ranges, but PP2, a Src inhibitor, and AG1478, an epidermal growth factor receptor inhibitor, did not, supporting the finding that the TrkA receptor is involved in the toxin-induced increase in superoxide. The results obtained with the anti-TrkA antibody, LY294002, and K252a show that the activation of PI3K through direct binding of the toxin to the TrkA receptor results in production of PIP₃, which activates PDK1. In addition, PT inhibited the alpha-toxin-induced generation of superoxide and formation of DG, but not phosphorylation of PDK1, suggesting that a PT-sensitive GTP-binding protein plays a crucial role in the coupling to endogenous PLC, but not phosphorylation of PDK1. These observations indicate that the toxin independently induces activation of both endogenous PLC via a PT-sensitive GTP-binding protein and PDK1 via the TrkA receptor.

NGF, which binds to the TrkA receptor, is reported to be required for the differentiation and survival of sympathetic and some sensory and cholinergic neuronal populations (Howe et al. 2001). Furthermore, it has been reported that NGF is involved in inflammatory responses, an increase in mast cells in neonatal rats (Woelf et al. 1996), the degranulation of rat peritoneal mast cells (Woelf et al. 1996), and the differentiation of specific granulocytes (Kannan et al. 1991). The injection of *C. perfringens* cells or alpha-toxin into tissues is known

to cause inflammation. Therefore, it is possible that the activation of the TrkA receptor by alpha-toxin is related to inflammation caused by *C. perfringens* in humans and animals.

H148G induced phosphorylation of PKC θ , but not production of DG, suggesting that the enzymatic activity of the toxin is essential for activation of endogenous PLC, but not activation of the TrkA receptor. It has been reported that binding of the C-domain, which does not contain the enzymatic site, to erythrocytes is important for the hemolysis induced by the toxin (Nagahama et al. 2002). It therefore is possible that the C-domain, the binding domain of alpha-toxin, plays a role in the binding of the toxin to the TrkA receptor and in the activation of signal transduction via the TrkA receptor.

Several studies have reported that the activation of PKC by various stimuli results in the generation of superoxide via the activation of MAPK systems (Coxon et al. 2003, Dewas et al. 2000, McLeish et al. 1998, Zu et al. 1998). K252a and U73122 inhibited the toxin-induced phosphorylation of PKC θ and ERK1/2 and generation of superoxide, suggesting that the toxin-induced production of superoxide is linked to the stimulation of the MAPK system via the activation of PKC θ . The toxin causes phosphorylation of ERK1/2, but not p38 and SAPK/JNK, implying that the process is dependent on a MAPK system containing MEK1/2 and MAPK/ERK1/2, but not systems containing p38 and SAPK/JNK.

It has been reported that PA directly or indirectly activated NADPH oxidase in a cell-free system of neutrophils (Erickson et al. 1999) and that PKC δ regulates phosphorylation of p67^{phox} in human monocytes (Zhao et al. 2005). PKC also has been reported to activate directly NADPH oxidase (Johnson et al. 1998). However, PD98059 almost completely inhibited the toxin-induced production of superoxide near the inhibitory threshold dose of the inhibitor. Thus, it is unlikely that PA and PKC directly activate NADPH oxidase under the conditions used here.

We have shown that alpha-toxin induces formation of DG through the activation of endogenous PLC by a PT-sensitive GTP-binding protein and phosphorylation of PDK1 via stimulation of the TrkA receptor, so that DG and PDK1 synergistically activate PKC θ , and that the activation of PKC θ stimulates generation of superoxide through MAPK-associated signaling events in rabbit neutrophils (Fig. 2).

2.3. Mechanism for the cytokine release induced by alpha-toxin

Cytokines are immunoregulatory peptides with a potent inflammatory action, mediating the immune/metabolic response to an external noxious stimulus and fueling the transition from sepsis to septic shock, multiple organ dysfunction syndromes, and/or multiple organ failure (Tracey et al. 1987, Dinarello 2004, Riedemann, Guo and Ward 2003). It is thought that synergistic interactions between cytokines can cause or attenuate tissue injury (Calandra, Bochud and Heumann 2002). TNF- α , which is released early from neutrophils and macrophages, is one of the important cytokines involved in the pathophysiology of sepsis (Tracey et al. 1987, Lum et al. 1999). TNF- α -induced tissue injury is largely mediated through neutrophils, that respond by producing elastase, superoxide ion, hydrogen

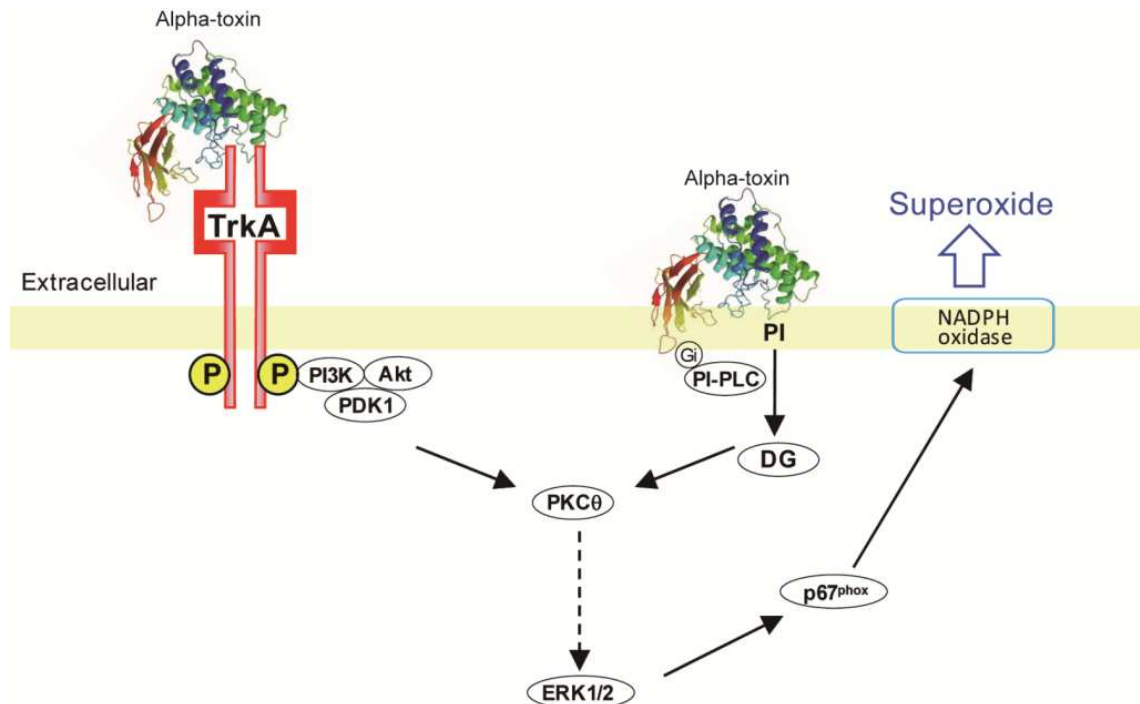


Figure 2. Signaling events involved in alpha-toxin-activated generation of superoxide

peroxide, sPLA2, PAF, leukotriene B1, and thromboxane A2 (Aldridge 2002). IL-1 stimulates the synthesis and release of prostaglandins, elastases, and collagenases and transendothelial microvascular cells, which respond by releasing the powerful neutrophil-stimulating agents, PAF and IL-8 (Leirisalo-Repo 1994). IL-1 and TNF- α are synergistic and share many biological effects in sepsis (Herbertson et al. 1995).

Anti-TNF- α antibody inhibited the death of mice induced by alpha-toxin. Furthermore, TNF- α -deficient mice were resistant to alpha-toxin. These observations suggest that the lethal effect of alpha-toxin is closely related to the release of TNF- α into the bloodstream. Stevens et al. and Bunting et al. suggested that alpha-toxin contributes indirectly to shock by stimulating production of endogenous mediators such as TNF- α and platelet-activating factor (Bunting et al. 1997, Stevens and Bryant 1997). It therefore appears that TNF- α released by alpha-toxin is important in enhancing the toxic actions of alpha-toxin in vivo. Consequently, inhibitors for release and expression of TNF- α may be worth pursuing as a novel therapeutic approach to the treatment of gas gangrene and sepsis caused by *C. perfringens*.

Cytokines such as the pro-inflammatory TNF- α , interleukin-1 β (IL-1 β) or transforming growth factor- β (TGF- β), increase the synthesis of NGF in airway structural cells. This stimulation has been evidenced in vitro in human pulmonary fibroblasts (Olgart and Frossard 2001, Micera et al. 2001), A549 epithelial cells (Pons et al. 2001) and bronchial smooth muscle cells (Freund et al. 2002). Studies also show that pro-inflammatory cytokines can act in concert to stimulate additional NGF secretion: TNF- α , for example, increases the secretion of NGF induced by IL-1 β and interferon γ (IFN- γ) in fibroblasts (Hattori et al. 1994) and by interleukin-4 (IL-4) in astrocytes (Brodie et al. 1998). NGF synthesis in

inflammatory conditions has also been demonstrated *in vivo*: elevated NGF concentrations are observed in cutaneous inflammation (Safieh-Garabedian et al. 1995) and in asthmatic airways (Olgart and Frossard 2001, Kassel, da Silva and Frossard 2001, Virchow et al. 1998). Taken together, these results suggest that pro-inflammatory cytokines, which are present at high levels in the airways of patients with asthma (Tillie-Leblond et al. 1999), might contribute to the elevated levels of NGF synthesis.

Corticosteroids are well known for their anti-inflammatory properties, particularly in asthmatic airways. Numerous studies report that the glucocorticoids dexamethasone and budesonide affect NGF expression. They cause a significant reduction in the increased NGF expression induced by pro-inflammatory cytokines; in one study, this action was shown to result from the repression of NGF gene transcription in endoneural fibroblasts from the rat sciatic nerve (Lindholm et al. 1990). Olgart and Frossard have reported that glucocorticoid treatment decreases the NGF secretion that the pro-inflammatory cytokines IL-1 β and TNF- α stimulate in cultures of human pulmonary fibroblasts (Olgart and Frossard 2001) and in A549 epithelial cells (Pons et al. 2001).

These results suggested that the initial release of pro-inflammatory cytokines induced by alpha-toxin *in vivo* leads to the production of NGF, and the NGF released synergistically causes systemic inflammation such as sepsis and shock via activation of the TrkA receptor (Fig. 3).

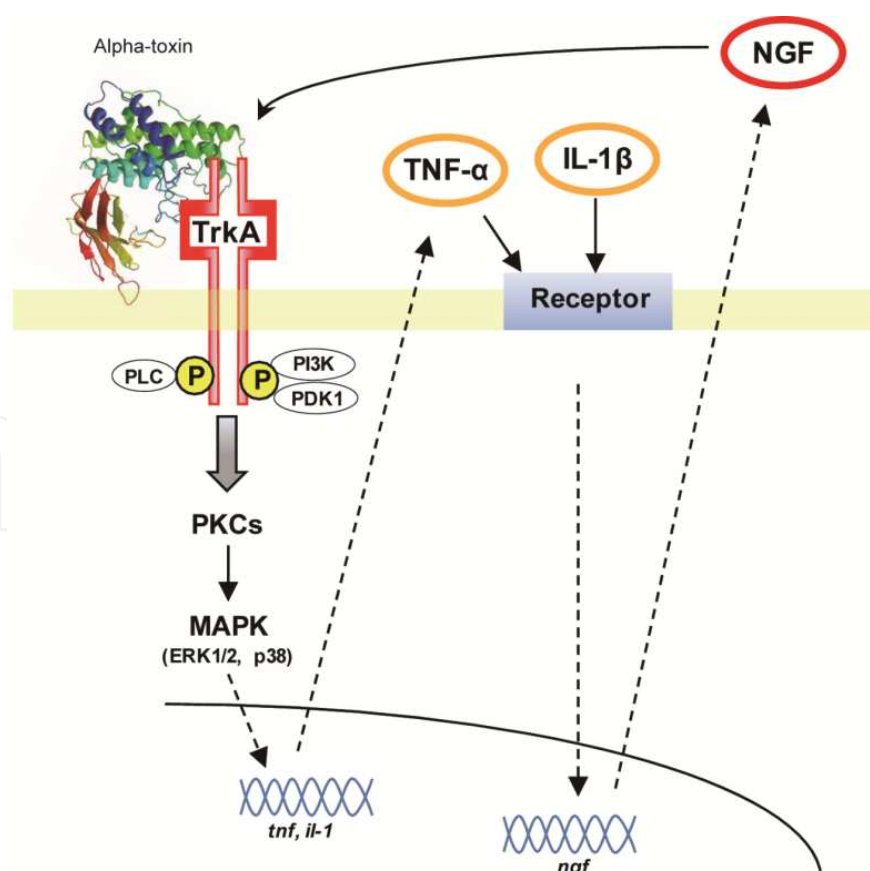


Figure 3. Alpha-toxin-induced release of pro-inflammatory cytokines and NGF

3. Conclusion

C. perfringens alpha-toxin, the main agent involved in the development of gas gangrene and septicemia, induces death, hemolysis, and the activation of macrophages and neutrophils. The toxin activated the MAPK-associated signal transduction from phospholipid metabolism and phosphorylation of TrkA. Penicillin is known to be highly effective in preventing the growth of microorganisms. In conclusion, treatment with TrkA inhibitors (tyrosine kinase inhibitors) and high doses of penicillin would be effective against diseases caused by *C. perfringens*.

Author details

Masataka Oda, Masahiro Nagahama, Keiko Kobayashi and Jun Sakurai
Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Japan

4. References

- Aldridge, A. J. (2002) Role of the neutrophil in septic shock and the adult respiratory distress syndrome. *Eur J Surg*, 168, 204-14.
- Aloe, L., L. Bracci-Laudiero, S. Bonini & L. Manni (1997) The expanding role of nerve growth factor: from neurotrophic activity to immunologic diseases. *Allergy*, 52, 883-94.
- Altman, A., N. Isakov & G. Baier (2000) Protein kinase C θ : a new essential superstar on the T-cell stage. *Immunol Today*, 21, 567-73.
- Awad, M. M., A. E. Bryant, D. L. Stevens & J. I. Rood (1995) Virulence studies on chromosomal alpha-toxin and theta-toxin mutants constructed by allelic exchange provide genetic evidence for the essential role of alpha-toxin in *Clostridium perfringens*-mediated gas gangrene. *Mol Microbiol*, 15, 191-202.
- Babior, B. M. (1999) NADPH oxidase: an update. *Blood*, 93, 1464-76.
- Bellavite, P., F. Corso, S. Dusi, M. Grzeskowiak, V. Della-Bianca & F. Rossi (1988) Activation of NADPH-dependent superoxide production in plasma membrane extracts of pig neutrophils by phosphatidic acid. *J Biol Chem*, 263, 8210-4.
- Bonini, S., A. Lambiase, G. Lapucci, F. Properzi, M. Bresciani, M. L. Bracci Laudiero, M. J. Mancini, A. Procoli, A. Micera, G. Sacerdoti, F. Levi-Schaffer, G. Rasi & L. Aloe (2002) Nerve growth factor and asthma. *Allergy*, 57 Suppl 72, 13-5.
- Brodie, C., N. Goldreich, T. Haiman & G. Kazimirsky (1998) Functional IL-4 receptors on mouse astrocytes: IL-4 inhibits astrocyte activation and induces NGF secretion. *J Neuroimmunol*, 81, 20-30.
- Bryant, A. E., R. Y. Chen, Y. Nagata, Y. Wang, C. H. Lee, S. Finegold, P. H. Guth & D. L. Stevens (2000a) Clostridial gas gangrene. I. Cellular and molecular mechanisms of microvascular dysfunction induced by exotoxins of *Clostridium perfringens*. *J Infect Dis*, 182, 799-807.
- Bryant, A. E., R. Y. Chen, Y. Nagata, Y. Wang, C. H. Lee, S. Finegold, P. H. Guth & D. L. Stevens (2000b) Clostridial gas gangrene. II. Phospholipase C-induced activation of

- platelet gpIIb/IIIa mediates vascular occlusion and myonecrosis in *Clostridium perfringens* gas gangrene. *J Infect Dis*, 182, 808-15.
- Bunting, M., D. E. Lorant, A. E. Bryant, G. A. Zimmerman, T. M. McIntyre, D. L. Stevens & S. M. Prescott (1997) Alpha toxin from *Clostridium perfringens* induces proinflammatory changes in endothelial cells. *J Clin Invest*, 100, 565-74.
- Calandra, T., P. Y. Bochud & D. Heumann (2002) Cytokines in septic shock. *Curr Clin Top Infect Dis*, 22, 1-23.
- Coxon, P. Y., M. J. Rane, S. Uriarte, D. W. Powell, S. Singh, W. Butt, Q. Chen & K. R. McLeish (2003) MAPK-activated protein kinase-2 participates in p38 MAPK-dependent and ERK-dependent functions in human neutrophils. *Cell Signal*, 15, 993-1001.
- Dewas, C., M. Fay, M. A. Gougerot-Pocidalo & J. El-Benna (2000) The mitogen-activated protein kinase extracellular signal-regulated kinase 1/2 pathway is involved in formyl-methionyl-leucyl-phenylalanine-induced p47phox phosphorylation in human neutrophils. *J Immunol*, 165, 5238-44.
- Dinarello, C. A. (2004) Therapeutic strategies to reduce IL-1 activity in treating local and systemic inflammation. *Curr Opin Pharmacol*, 4, 378-85.
- Ehrhard, P. B., U. Ganter, A. Stalder, J. Bauer & U. Otten (1993) Expression of functional trk protooncogene in human monocytes. *Proc Natl Acad Sci U S A*, 90, 5423-7.
- Erickson, R. W., P. Langel-Peveri, A. E. Traynor-Kaplan, P. G. Heyworth & J. T. Curnutte (1999) Activation of human neutrophil NADPH oxidase by phosphatidic acid or diacylglycerol in a cell-free system. Activity of diacylglycerol is dependent on its conversion to phosphatidic acid. *J Biol Chem*, 274, 22243-50.
- Fan, Y. Y., L. H. Ly, R. Barhoumi, D. N. McMurray & R. S. Chapkin (2004) Dietary docosahexaenoic acid suppresses T cell protein kinase C theta lipid raft recruitment and IL-2 production. *J Immunol*, 173, 6151-60.
- Freund, V., F. Pons, V. Joly, E. Mathieu, N. Martinet & N. Frossard (2002) Upregulation of nerve growth factor expression by human airway smooth muscle cells in inflammatory conditions. *Eur Respir J*, 20, 458-63.
- Garland, L. G. (1992) New pathways of phagocyte activation: the coupling of receptor-linked phospholipase D and the role of tyrosine kinase in primed neutrophils. *FEMS Microbiol Immunol*, 5, 229-37.
- Hattori, A., S. Iwasaki, K. Murase, M. Tsujimoto, M. Sato, K. Hayashi & M. Kohno (1994) Tumor necrosis factor is markedly synergistic with interleukin 1 and interferon-gamma in stimulating the production of nerve growth factor in fibroblasts. *FEBS Lett*, 340, 177-80.
- Herbertson, M. J., H. A. Werner, C. M. Goddard, J. A. Russell, A. Wheeler, R. Coxon & K. R. Walley (1995) Anti-tumor necrosis factor-alpha prevents decreased ventricular contractility in endotoxemic pigs. *Am J Respir Crit Care Med*, 152, 480-8.
- Howe, C. L., J. S. Valletta, A. S. Rusnak & W. C. Mobley (2001) NGF signaling from clathrin-coated vesicles: evidence that signaling endosomes serve as a platform for the Ras-MAPK pathway. *Neuron*, 32, 801-14.
- Johnson, J. L., J. W. Park, J. E. Benna, L. P. Faust, O. Inanami & B. M. Babior (1998) Activation of p47(PHOX), a cytosolic subunit of the leukocyte NADPH oxidase.

- Phosphorylation of ser-359 or ser-370 precedes phosphorylation at other sites and is required for activity. *J Biol Chem*, 273, 35147-52.
- Kannan, Y., H. Ushio, H. Koyama, M. Okada, M. Oikawa, T. Yoshihara, M. Kaneko & H. Matsuda (1991) 2.5S nerve growth factor enhances survival, phagocytosis, and superoxide production of murine neutrophils. *Blood*, 77, 1320-5.
- Kaplan, D. R., B. L. Hempstead, D. Martin-Zanca, M. V. Chao & L. F. Parada (1991) The trk proto-oncogene product: a signal transducing receptor for nerve growth factor. *Science*, 252, 554-8.
- Kaplan, D. R. & F. D. Miller (1997) Signal transduction by the neurotrophin receptors. *Curr Opin Cell Biol*, 9, 213-21.
- Kassel, O., C. da Silva & N. Frossard (2001) The stem cell factor, its properties and potential role in the airways. *Pulm Pharmacol Ther*, 14, 277-88.
- Kusunoki, T., H. Higashi, S. Hosoi, D. Hata, K. Sugie, M. Mayumi & H. Mikawa (1992) Tyrosine phosphorylation and its possible role in superoxide production by human neutrophils stimulated with FMLP and IgG. *Biochem Biophys Res Commun*, 183, 789-96.
- Le Good, J. A., W. H. Ziegler, D. B. Parekh, D. R. Alessi, P. Cohen & P. J. Parker (1998) Protein kinase C isotypes controlled by phosphoinositide 3-kinase through the protein kinase PDK1. *Science*, 281, 2042-5.
- Leirisalo-Repo, M. (1994) The present knowledge of the inflammatory process and the inflammatory mediators. *Pharmacol Toxicol*, 75 Suppl 2, 1-3.
- Levi-Montalcini, R. (1987) The nerve growth factor 35 years later. *Science*, 237, 1154-62.
- Levi-Montalcini, R., R. Dal Toso, F. della Valle, S. D. Skaper & A. Leon (1995) Update of the NGF saga. *J Neurol Sci*, 130, 119-27.
- Lindholm, D., B. Hengerer, R. Heumann, P. Carroll & H. Thoenen (1990) Glucocorticoid Hormones Negatively Regulate Nerve Growth Factor Expression In Vivo and in Cultured Rat Fibroblasts. *Eur J Neurosci*, 2, 795-801.
- Lum, L., B. R. Wong, R. Josien, J. D. Becherer, H. Erdjument-Bromage, J. Schlondorff, P. Tempst, Y. Choi & C. P. Blobel (1999) Evidence for a role of a tumor necrosis factor-alpha (TNF-alpha)-converting enzyme-like protease in shedding of TRANCE, a TNF family member involved in osteoclastogenesis and dendritic cell survival. *J Biol Chem*, 274, 13613-8.
- Martin-Zanca, D., S. H. Hughes & M. Barbacid (1986) A human oncogene formed by the fusion of truncated tropomyosin and protein tyrosine kinase sequences. *Nature*, 319, 743-8.
- McLeish, K. R., C. Knall, R. A. Ward, P. Gerwins, P. Y. Coxon, J. B. Klein & G. L. Johnson (1998) Activation of mitogen-activated protein kinase cascades during priming of human neutrophils by TNF-alpha and GM-CSF. *J Leukoc Biol*, 64, 537-45.
- Melamed, I., H. Patel, C. Brodie & E. W. Gelfand (1999) Activation of Vav and Ras through the nerve growth factor and B cell receptors by different kinases. *Cell Immunol*, 191, 83-9.
- Micera, A., E. Vigneti, D. Pickholtz, R. Reich, O. Pappo, S. Bonini, F. X. Maquart, L. Aloe & F. Levi-Schaffer (2001) Nerve growth factor displays stimulatory effects on human skin and lung fibroblasts, demonstrating a direct role for this factor in tissue repair. *Proc Natl Acad Sci U S A*, 98, 6162-7.

- Mitsuyama, T., K. Takeshige & S. Minakami (1993) Tyrosine phosphorylation is involved in the respiratory burst of electropermeabilized human neutrophils at a step before diacylglycerol formation by phospholipase C. *FEBS Lett*, 322, 280-4.
- Muragaki, Y., N. Timothy, S. Leight, B. L. Hempstead, M. V. Chao, J. Q. Trojanowski & V. M. Lee (1995) Expression of trk receptors in the developing and adult human central and peripheral nervous system. *J Comp Neurol*, 356, 387-97.
- Nagahama, M., M. Mukai, S. Morimitsu, S. Ochi & J. Sakurai (2002) Role of the C-domain in the biological activities of *Clostridium perfringens* alpha-toxin. *Microbiol Immunol*, 46, 647-55.
- Nick, J. A., N. J. Avdi, S. K. Young, C. Knall, P. Gerwins, G. L. Johnson & G. S. Worthen (1997) Common and distinct intracellular signaling pathways in human neutrophils utilized by platelet activating factor and FMLP. *J Clin Invest*, 99, 975-86.
- Obermeier, A., H. Halfter, K. H. Wiesmuller, G. Jung, J. Schlessinger & A. Ullrich (1993a) Tyrosine 785 is a major determinant of Trk--substrate interaction. *EMBO J*, 12, 933-41.
- Obermeier, A., R. Lammers, K. H. Wiesmuller, G. Jung, J. Schlessinger & A. Ullrich (1993b) Identification of Trk binding sites for SHC and phosphatidylinositol 3'-kinase and formation of a multimeric signaling complex. *J Biol Chem*, 268, 22963-6.
- Ochi, S., K. Hashimoto, M. Nagahama & J. Sakurai (1996) Phospholipid metabolism induced by *Clostridium perfringens* alpha-toxin elicits a hot-cold type of hemolysis in rabbit erythrocytes. *Infect Immun*, 64, 3930-3.
- Ochi, S., T. Miyawaki, H. Matsuda, M. Oda, M. Nagahama & J. Sakurai (2002) *Clostridium perfringens* alpha-toxin induces rabbit neutrophil adhesion. *Microbiology*, 148, 237-45.
- Ochi, S., M. Oda, H. Matsuda, S. Ikari & J. Sakurai (2004) *Clostridium perfringens* alpha-toxin activates the sphingomyelin metabolism system in sheep erythrocytes. *J Biol Chem*, 279, 12181-9.
- Oda, M., S. Ikari, T. Matsuno, Y. Morimune, M. Nagahama & J. Sakurai (2006) Signal transduction mechanism involved in *Clostridium perfringens* alpha-toxin-induced superoxide anion generation in rabbit neutrophils. *Infect Immun*, 74, 2876-86.
- Oda, M., T. Matsuno, R. Shiihara, S. Ochi, R. Yamauchi, Y. Saito, H. Imagawa, M. Nagahama, M. Nishizawa & J. Sakurai (2008) The relationship between the metabolism of sphingomyelin species and the hemolysis of sheep erythrocytes induced by *Clostridium perfringens* alpha-toxin. *J Lipid Res*, 49, 1039-47.
- Olgart, C. & N. Frossard (2001) Human lung fibroblasts secrete nerve growth factor: effect of inflammatory cytokines and glucocorticoids. *Eur Respir J*, 18, 115-21.
- Olson, S. C., S. R. Tyagi & J. D. Lambeth (1990) Fluoride activates diradylglycerol and superoxide generation in human neutrophils via PLD/PA phosphohydrolase-dependent and -independent pathways. *FEBS Lett*, 272, 19-24.
- Parekh, D. B., W. Ziegler & P. J. Parker (2000) Multiple pathways control protein kinase C phosphorylation. *EMBO J*, 19, 496-503.
- Pierotti, M. A. & A. Greco (2006) Oncogenic rearrangements of the NTRK1/NGF receptor. *Cancer Lett*, 232, 90-8.

- Pongracz, J. & J. M. Lord (1998) Superoxide production in human neutrophils: evidence for signal redundancy and the involvement of more than one PKC isoenzyme class. *Biochem Biophys Res Commun*, 247, 624-9.
- Pons, F., V. Freund, H. Kuissu, E. Mathieu, C. Olgart & N. Frossard (2001) Nerve growth factor secretion by human lung epithelial A549 cells in pro- and anti-inflammatory conditions. *Eur J Pharmacol*, 428, 365-9.
- Riedemann, N. C., R. F. Guo & P. A. Ward (2003) Novel strategies for the treatment of sepsis. *Nat Med*, 9, 517-24.
- Rollet, E., A. C. Caon, C. J. Roberge, N. W. Liao, S. E. Malawista, S. R. McColl & P. H. Naccache (1994) Tyrosine phosphorylation in activated human neutrophils. Comparison of the effects of different classes of agonists and identification of the signaling pathways involved. *J Immunol*, 153, 353-63.
- Safieh-Garabedian, B., S. Poole, A. Allchorne, J. Winter & C. J. Woolf (1995) Contribution of interleukin-1 beta to the inflammation-induced increase in nerve growth factor levels and inflammatory hyperalgesia. *Br J Pharmacol*, 115, 1265-75.
- Sakurai, J., M. Nagahama & M. Oda (2004) *Clostridium perfringens* alpha-toxin: characterization and mode of action. *J Biochem*, 136, 569-74.
- Segal, R. A. & M. E. Greenberg (1996) Intracellular signaling pathways activated by neurotrophic factors. *Annu Rev Neurosci*, 19, 463-89.
- Shenoy, N. G., G. J. Gleich & L. L. Thomas (2003) Eosinophil major basic protein stimulates neutrophil superoxide production by a class IA phosphoinositide 3-kinase and protein kinase C-zeta-dependent pathway. *J Immunol*, 171, 3734-41.
- Stephens, R. M., D. M. Loeb, T. D. Copeland, T. Pawson, L. A. Greene & D. R. Kaplan (1994) Trk receptors use redundant signal transduction pathways involving SHC and PLC-gamma 1 to mediate NGF responses. *Neuron*, 12, 691-705.
- Stevens, D. L. & A. E. Bryant (1997) Pathogenesis of *Clostridium perfringens* infection: mechanisms and mediators of shock. *Clin Infect Dis*, 25 Suppl 2, S160-4.
- Tillie-Leblond, I., J. Pugin, C. H. Marquette, C. Lamblin, F. Saulnier, A. Brichet, B. Wallaert, A. B. Tonnel & P. Gosset (1999) Balance between proinflammatory cytokines and their inhibitors in bronchial lavage from patients with status asthmaticus. *Am J Respir Crit Care Med*, 159, 487-94.
- Toker, A. & A. C. Newton (2000) Cellular signaling: pivoting around PDK-1. *Cell*, 103, 185-8.
- Tracey, K. J., Y. Fong, D. G. Hesse, K. R. Manogue, A. T. Lee, G. C. Kuo, S. F. Lowry & A. Cerami (1987) Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. *Nature*, 330, 662-4.
- Villalba, M., S. Kasibhatla, L. Genestier, A. Mahboubi, D. R. Green & A. Altman (1999) Protein kinase ctheta cooperates with calcineurin to induce Fas ligand expression during activation-induced T cell death. *J Immunol*, 163, 5813-9.
- Villunger, A., N. Ghaffari-Tabrizi, I. Tinhofer, N. Krumbock, B. Bauer, T. Schneider, S. Kasibhatla, R. Greil, G. Baier-Bitterlich, F. Uberall, D. R. Green & G. Baier (1999) Synergistic action of protein kinase C theta and calcineurin is sufficient for Fas ligand expression and induction of a crmA-sensitive apoptosis pathway in Jurkat T cells. *Eur J Immunol*, 29, 3549-61.

- Virchow, J. C., P. Julius, M. Lommatzsch, W. Luttmann, H. Renz & A. Braun (1998) Neurotrophins are increased in bronchoalveolar lavage fluid after segmental allergen provocation. *Am J Respir Crit Care Med*, 158, 2002-5.
- Wiesmann, C., M. H. Ultsch, S. H. Bass & A. M. de Vos (1999) Crystal structure of nerve growth factor in complex with the ligand-binding domain of the TrkA receptor. *Nature*, 401, 184-8.
- Williamson, E. D. & R. W. Titball (1993) A genetically engineered vaccine against the alpha-toxin of *Clostridium perfringens* protects mice against experimental gas gangrene. *Vaccine*, 11, 1253-8.
- Woolf, C. J., Q. P. Ma, A. Allchorne & S. Poole (1996) Peripheral cell types contributing to the hyperalgesic action of nerve growth factor in inflammation. *J Neurosci*, 16, 2716-23.
- Wu, C., C. F. Lai & W. C. Mobley (2001) Nerve growth factor activates persistent Rap1 signaling in endosomes. *J Neurosci*, 21, 5406-16.
- Yamamori, T., O. Inanami, H. Nagahata & M. Kuwabara (2004) Phosphoinositide 3-kinase regulates the phosphorylation of NADPH oxidase component p47(phox) by controlling cPKC/PKCdelta but not Akt. *Biochem Biophys Res Commun*, 316, 720-30.
- Yao, R. & G. M. Cooper (1995) Requirement for phosphatidylinositol-3 kinase in the prevention of apoptosis by nerve growth factor. *Science*, 267, 2003-6.
- Yasaka, T., L. A. Boxer & R. L. Baehner (1982) Monocyte aggregation and superoxide anion release in response to formyl-methionyl-leucyl-phenylalanine (FMLP) and platelet-activating factor (PAF). *J Immunol*, 128, 1939-44.
- York, R. D., D. C. Molliver, S. S. Grewal, P. E. Stenberg, E. W. McCleskey & P. J. Stork (2000) Role of phosphoinositide 3-kinase and endocytosis in nerve growth factor-induced extracellular signal-regulated kinase activation via Ras and Rap1. *Mol Cell Biol*, 20, 8069-83.
- Zhao, X., B. Xu, A. Bhattacharjee, C. M. Oldfield, F. B. Wientjes, G. M. Feldman & M. K. Cathcart (2005) Protein kinase Cdelta regulates p67phox phosphorylation in human monocytes. *J Leukoc Biol*, 77, 414-20.
- Zu, Y. L., J. Qi, A. Gilchrist, G. A. Fernandez, D. Vazquez-Abad, D. L. Kreutzer, C. K. Huang & R. I. Sha'afi (1998) p38 mitogen-activated protein kinase activation is required for human neutrophil function triggered by TNF-alpha or FMLP stimulation. *J Immunol*, 160, 1982-9.