

Cloning and expression of the first invertebrate tumor necrosis factor- α (TNF- α) gene in the Pacific oyster *Crassostrea gigas*

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Introduction

Tumor necrosis factor- α (TNF- α) is an inflammatory cytokine produced by monocytes/macrophages during acute inflammation and is responsible for a diverse range of signaling events within cells. Besides its well-defined role as an important mediator in resistance against parasitic, bacterial and viral infections (Czarniecki 1993; Goldfeld and Tsai 1996; Steinshamn *et al.* 1996). There is currently increased interest in resolving questions about the molecular mechanisms of defense in invertebrates, including mollusks, which represent the second largest group of the invertebrate phylum after insects. The mollusk's internal defense mechanisms involve cell-mediated and humoral reactions that interplay to recognize and eliminate pathogens. In this study, we describe the cloning and tissue-specific expression of a oyster gene with homology to the mammalian TNF- α gene.

Materials & Methods

The full cDNA sequence of the oyster TNF- α was obtained by using a cDNA fragment encoding a partial sequence of oyster TNF- α , based on EST analysis, as a probe to screen a previously constructed cDNA library from oyster hepatopancreas. cDNA clones were determined using the ABI 3100 automatic DNA sequencer (PE Applied Biosystems, CA, USA) and the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems). Oyster *Crassostrea gigas* were challenged by filling the shell and injecting into the adductor muscle either 100 μ l of saline peptone water (SPW: peptone 15 g/l, NaCl 15 g/l) and a mixture of four pathogenic *Vibrio* strains (*V. anguillarum*, *V. metshnikovii*, *V. tubiashii* and *Vibrio alginolyticus*). TNF- α gene expression was

detected by reverse transcription-polymerase chain reaction (RT-PCR).

Results & Discussion

A Pacific oyster, (*Crassostrea gigas*) gene for tumor necrosis factor- α (TNF- α) has been cloned and sequenced. The cDNA contains an open reading frame of 348 nucleotides that translate into a 115 amino-acid putative peptide, with a 5' untranslated region (UTR) of 110bp and a 3' UTR of 432bp. The identities between Oyster TNF- α and members of the mammalian TNF family were 40~45%. The positions of cysteine residues that are important for disulfide bonds were conserved with respect to those in mammalian TNF- α . Expression studies using RT-PCR have shown that the oyster TNF- α gene is constitutively expressed in the several tissue of unstimulated oyster. But, we also observed that oyster TNF- α mRNA accumulated during bacterial challenge. This pattern of expression suggests that oyster TNF- α may be the key components of defense mechanisms in mollusks. These findings serve to highlight a potentially important regulatory pathway to the study of oyster immunology, hence allowing comparison of the immune system in vertebrates and invertebrates, an important key issue to understand its evolution.

References

- Czarniecki CW, 1993, The role of tumor necrosis factor in viral disease. *Antiviral. Res.* 22:223-258.
- Goldfeld AE, Tsai EY, 1996, TNF- α and genetic susceptibility to parasitic disease. *Exp. Parasitol.* 84:300-303.
- Steinshamn S, Bemelmans MH, van Tits LJ, Bergh K, Buurman WA, Waage A, 1996, TNF receptors in murine *Candida albicans* infection: evidence for an important role of TNF receptor p55 in antifungal defense. *J Immunol* 157:2155-2159.
- Ikuo Hirono, Bo-Hye Nam, Tomofumi Kurobe and Takashi Aoki, 2000, Molecular Cloning, Characterization, and Expression of TNF cDNA and Gene from Japanese Flounder *Paralichthys olivaceus*, *J. Immunol*, 165: 4423-4427.
- C. Montagnani, C. Kappler, J. M. Reichhart and J. M. Escoubas, 2004, Cg-Rel, the first Rel/NF-B homolog characterized in a mollusk, the Pacific oyster *Crassostrea gigas*, *FEBS letters*, 561, 75-82.