

THE ROLE OF INTERLEUKIN-8 IN
INFLAMMATORY RESPONSES OF FISH; STUDIES
IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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The Role of Interleukin-8 in Inflammatory Responses of Fish; Studies in
Rainbow Trout (*Oncorhynchus mykiss*)

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DECLARATION

I declare that this thesis was composed by myself and that all research presented here was performed by myself between July 2002 and March 2004. This thesis has not been submitted in any previous application for a higher degree. All sources of information have been acknowledged in the text.

Omaima Harun

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LIST OF ABBREVIATIONS

C5a	: A split product of complement protein/anaphylatoxin
BCR	: B Cell Antigen Receptor
CD11a	: Integrin alpha chains/alpha-1
CD11b	: Leukocyte integrins
CD11c	: Leukocyte integrins
CD18	: Integrin alpha chains/beta-2
cDNA	: Competent DNA
COX-2	: Cyclooxygenase-2
dNTP	: Deoxynucleotide triphosphate
EB	: Elution Buffer
ELR	: Glutamic acid-leucine-arginine motif
ENA-78	: Neutrophil attractant-78
f-MLP	: N-formyl-methionyl-leucyl-phenylalanine
GCP-2	: Granulocyte chemotactic protein-2
GRO- α	: Growth regulated oncogenes- α
HBSS	: Hanks Balances Salt Solution
HOCL	: Hydrogen Peroxide
IgE	: Immunoglobulin E
IgM	: Immunoglobulin M
IL-1 β	: Interleukin-1 beta
iNOS	: Inducible Nitric Oxide Synthase
IL-8	: Interleukin-8
IP-10	: Interferon- γ inducible protein-10
LPS	: Lippolysaccharide
LTB4	: Leukotriene B4
Mac-1	: CD11b
MCP-1	: Human Macrophage Chemoattractant Protein-1
MDP	: Muramyl Dipeptide
MHC	: Major Histocompatibility Complex
Mig	: Monokine induced by interferon- γ
MIP	: Macrophage Inflammatory Protein

NADPH	: Nicotinamide Adenine Dinucleotide Phosphate
NAP-2	: Neutrophil Activating Protein-2
NK	: Natural Killer
NMR	: Nuclear Magnetic Resonance and Crystallography
PF-4	: Platelet Factor-4
PFA	: Platelet Activating Factor
PMA	: Phorbol-12-Myristate-Acetate
PMN	: Polymorphonuclear leukocytes
ROS	: Reactive Oxygen Species
RT-PCR	: Reverse Transcriptase-Polymerase Chain Reaction
SDF-1	: Stromal Cell-Derived Factor-1
SOD	: Sodium Oxidase Dismutase
TCR	: T cell Antigen Receptor
TNF- α	: Tumor Necrosis Factor-alpha

ABSTRACT

Interleukin-8, a novel chemotactic cytokine, has been shown to play an important role in activation and recruitment of neutrophils, and thus acts as a traffic controller. This study builds upon the breakthrough in cloning several fish cytokine genes including IL-8. The predicted rainbow trout *Oncorhynchus mykiss* mature interleukin-8 peptide has been produced as a recombinant protein in *E. coli*; interleukin-8 (rIL-8a) and a mutated form lacking a receptor binding motif (rIL-8b) were studied initially *in vitro* and *in vivo*. The bioactivity of this molecule has been studied using trout head kidney cell preparations, using a microchemotaxis chamber designed to study the migration of cell populations *in vitro*. Various concentration gradients were tested (0.1, 1.0 and 10.0ng/ml) to determine optimal assay conditions and suitable attractants for such an assay and the numbers of cells migrating counted. The recombinant trout IL-8 and the mutated recombinant IL-8 induced specific dose-dependent *in vitro* chemotaxis of leukocytes at doses as low as 1.0ng/ml.

To study respiratory burst kinetics; optimal final concentration of PMA and Ferricytochrome C were established at $1\mu\text{g ml}^{-1}$ and 2mg ml^{-1} for stimulation of the respiratory burst and detection of superoxide anion respectively. *In vitro* dose-response studies demonstrated an increased stimulation with an optimal concentration of the recombinant protein of 1.0ng ml^{-1} .

Following on from these preliminary studies, the recombinant proteins were injected into fish, and the numbers of leukocyte subpopulations elicited in exudates and blood, and their activation state determined. The aim of this experiment was to investigate the ability of trout IL-8 to modulate the expression of certain immune parameters and disease resistance *in vivo*. Immunostimulatory activity *in vivo* was elucidated with respect to induction of a localised immune response as a consequence of intraperitoneal peptide administration, where the responses characterised included leukocyte migration to the peritoneal cavity.

Lastly, oil-based and water-based adjuvanted vaccines for *Vibrio anguillarum* (strain M2017) were used to study the effect of these two vaccines on several non-specific immune defenses of rainbow trout. Thus, at various times after injection ranging from 7 to 28 days, whether IL-8 and other pro-inflammatory genes were expressed by the elicited cells was determined.