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

NOR OMAIMA HARUN

UNIVERSITY OF ABERDEEN

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

Nor Omaima Harun

BSc (Hons Biology – University Putra Malaysia, Malaysia)

A thesis submitted to the School of Biological Sciences for the Degree of Master of Science at the University of Aberdeen, Scotland

2006

UNIVERSITY OF ABERDEEN

THESIS SUBMITTED FOR HIGHER DEGREE BY RESEARCH

Full Name: (block capitals) NOR OMAIMA HARUN

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

Under Library Regulations, any thesis, unless restricted, may be:

a) consulted for research purposes by any user within the University Library, whether that user is a registered staff member or student of the University or a member of the public
b) under certain conditions, borrowed for reading outside the Library, as i) an inter-library loan or ii) by registered Library members, including external users who have paid for Library borrowing rights.

A candidate may stipulate a period of time, up to a maximum of 5 years, during which the thesis, although still available for consultation, as above, can not be borrowed or sent on inter-library loan. See 2 and 3 below.

A candidate can restrict all access to a thesis i.e. no consultation, borrowing or inter-library loan, only if the Librarian is requested in writing to do so by the Academic Standards Committee (Postgraduate). A copy of the request must accompany the thesis. See 1 below.

1. This thesis contains confidential information which should not be divulged to any third party without written consent.
☑ Yes ☐ No

If Yes, there is no access to this thesis until _____ (date)
See written request from ASC (Postgraduate)

2. I give permission for a copy of my thesis to be supplied to any *bona fide* library or research worker, at the Librarians discretion, *immediately/after _____ years (not more than 5)

3. I give permission for a copy of my thesis to be sent on inter-library loan for consultation in any other library at the Librarian's discretion, *immediately/after_____ years (not more than 5)

Signature _____ Date 2006

*Delete as appropriate

NB Authors of theses are advised to consult a supervisor or other member of staff before signing. Authors should not that any user of their work will be required to sign a copyright declaration and conform to the provisions of current copyright law.

To be completed and returned to:

The Postgraduate Registry
University Office
University of Aberdeen
Regent Walk
Aberdeen
AB24 3FX

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

ACKNOWLEDGMENTS

First of all I would like to take this opportunity to dedicate this appreciation to my supervisor; Prof. C. J Secombes for his support, guidance, criticisms and invaluable time spent throughout my study.

My appreciation also goes to Dr. Jun Zou for helping me in preparing recombinant protein that has been used in Chapter 3 and providing vaccination used in Chapter 4, in addition to providing assistance with the gene expression studies carried out in Chapter 4. For his guidance in preparing the recombinant protein, I would like to acknowledge Dr. Yong-An Zhang and Dr. Allison Carrington for her help with the chemotaxis work in Chapter 3.

My special thanks also goes to all who work in the Scottish Fish Immunology Research Centre, University of Aberdeen and to Dr. Scott Peddie (New Zealand King Salmon Company Limited). For his help with TEM, I would like to acknowledge Mr. Kevin McKenzie. To those who have been involved either direct or indirectly in sharing their knowledge and assistance during this project, I would like to record my gratefulness.

Thanks also go to my current employer in Malaysia, College University of Science and Technology Malaysia (KUSTEM) and to the Malaysian Government; Public Service Department for sponsoring this study.

Finally, I would like to express my gratitude to my beloved family; mum Zaimah Ali and dad Harun Mohamad, brothers and sisters for their patience and concern throughout my period of study in Aberdeen. Not forgotten to my parent in Aberdeen, Frances and Bob Farquhar. Thank you for all your support, encouragement, belief and love that you have given me in making this ambition come true. Last word but not least from Confucious; '*a journey of a thousand mile starts with a single step*'.

TABLE OF CONTENTS

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-1β	: 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	: CDIIb
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 1μg ml^{-1} and 2mg ml^{-1} for stimulation of the respiratory burst and detection of superoxide onion 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.