Analysis of the B cell repertoire in the systemic and mucosal tissues of rainbow trout (*Oncorhynchus mykiss*)

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Declaration

I declare that this thesis has not been already submitted for any degree and is not being submitted as part of candidature for any degree.

I also declare that the thesis has been written by me and that any help I received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

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Abbreviations

AID	activation-induced cytidine deaminase
bp	base pairs
BSA	bovine serum albumin
cDNA	complementary DNA
CDR	complementarity determining region
CDR	complementarity determining regions
CO_2	carbon dioxide
CRP	C-reactive protein
C_t	cycle threshold
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic
ELISA	enzyme linked immunoabsorbant assay
EtBr	ethidium bromide
FAE	follicle associated epithelium
FCS	foetal calf serum
FITC	fluorescein isothiocyanate
g	gram
GALT	gut associated lymphoid tissue
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GC	gene conversion
HEPES	4-(2-hydroxyethyl)-1- piperazineethanesulfonic acid
IEL	intraepithelial lymphocytes
Ig	immunoglubulin
IgNAR	immunoglobulin isotype new antigen receptor
ip	intraperitoneal
ip	peranal
IPTG	isopropyl-beta-D-thiogalactopyranoside

KLH	keyhole limpet hemocyanin
L	litre/s
LB	luria broth
LPL	lamina propria lymphocytes
m	metre
M	molar
mAb	monoclonal antibody
Mamp	milliamps
MALT	mucosal associated lymphoid tissues
MHC	major histocompatability complex
MMC	melanomacrophage centers
mRNA	messenger ribonucleic acid
NK	natural killer
^{o}C	degrees celcius
OD	optical density
ORF	open reading frame
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pH	potenz hydrogen
PIT	passive implantable transponders
PP	peyers patches
qRT-PCR	quantitative real time PCR
RACE	random amplification of cDNA ends
RAG	recombination activation genes
RNA	ribonucleic acid
rpm	revolutions per minute
RPMI	Roswell park memorial institute
SHM	somatic hypermutation
sIg^{+}	surface Ig positive
TcR	T cell receptor homolog
TD	T-dependant

TdT	terminal deoxynucleotidyl transferase
TNP-LPS	trinitrophenyl conjugated to lipopolysaccharide
U	units
UPR	unfolded protein response
UV	ultraviolet
x g	gravitational force
X-gal	5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside

Abstract

Acquired immune responses against hapten-carrier systems have been used in mammals and teleosts *in vivo* as an effective means of identifying and characterizing T and B cell activity. Fish immunized by intraperitoneal (ip) injection of the hapten-carrier system FITC-KLH develop strong serum antibody responses to both the hapten (FITC) and carrier protein (KLH; Jones *et al.*, 1999). In contrast, fish immunized with FITC-KLH by peranal (pa) intubation results in a failure to develop a detectable anti-KLH response in the serum, yet they still develop a significant anti-FITC response. To investigate the nature of the antibody secreting cell (ASC) response in the systemic (head kidney and spleen) and mucosal tissues (hindgut and gills) of trout, ELISA-based serum and cellular assays were utilized to detect serum and tissue-specific antibody responses to FITC and KLH. Given the distinction in serum responsiveness to FITC-KLH through the ip and pa routes, an identification of where the B cell tissue-specific responses were occurring would identify if there is a restricted B cell population in the mucosal tissues of rainbow trout. In the primary response to immunization with FITC-KLH, systemically challenged fish presented ASC activity in systemic tissues, while mucosally challenged fish presented ASC activity in a mixture of both systemic and mucosal tissues. In the secondary response to FITC-KLH, in fish immunized via a combination of systemic and mucosal routes there was a utilization of both systemic and mucosal tissues in the response. It is possible that immunization with FITC-KLH through the mucosal or systemic routes may cause presentation of FITC-KLH in the systemic tissues, resulting in trafficking of ASC or antigen presenting cells (APC) between the mucosal and systemic tissues. To examine the relationships within and between the B cell populations of the lymphoid tissues, the rearranged V_H gene repertoire was examined in the tissues with ASC activity. Identification of preferential or restricted use of the different V_H genes may provide further insight into the restricted serum response to KLH in mucosally challenged fish, and whether restricted populations of B cells exist within the mucosal tissues. A qRT-PCR assay was developed and optimized using non-immunized trout to analyze the use of V_H gene families V_H-I to V_H-XI in rearranged Ig genes in the lymphoid tissues of trout. All V_H gene families were amplified across the tissues tested. In the primary response to immunization with FITC-KLH, families with the highest fold changes in gene expression for both systemic and mucosal tissues were V_H-VI, V_H-VIII, V_H-IX, V_H-X and V_H-

XI. In the secondary response, families V_H-I, V_H-II, V_H-V, V_H-IX, V_H-X and V_H-XI had the highest fold changes in gene expression. Gaps in the repertoire were also apparent within the primary ASC response to FITC-KLH, and were mainly associated with families V_H-I, V_H-II, V_H-III, V_H-IV, V_H-V and V_H-VII. In the secondary ASC response study to FITC-KLH, systemic tissue contained few repertoire gaps, however in mucosal lymphoid tissues there was evidence of repertoire gaps for families V_H-I, V_H-II, V_H-III, V_H-IV, V_H-V, V_H-VI, V_H-VII and V_H-VIII. A similar pattern of expression for the V_H gene families could suggest B cells migrate to the different tissues from a common source, perhaps from the head kidney given its role as a primary lymphoid tissue. Alternatively, families present in high abundance may have a larger number of germline members, or are highly expressed due to an unknown antigenic challenge. This study is one of the first to identify the extent of the usage of the V_H gene families in these tissues.