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

Rohan Singh Panwar

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Submitted in fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Science, University of Technology, Sydney
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.

Rohan Singh Panwar, Bsc (Hons).
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### Abbreviations

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<th>Description</th>
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<tbody>
<tr>
<td>AID</td>
<td>activation-induced cytidine deaminase</td>
</tr>
<tr>
<td>bp</td>
<td>base pairs</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>cDNA</td>
<td>complementary DNA</td>
</tr>
<tr>
<td>CDR</td>
<td>complementarity determining region</td>
</tr>
<tr>
<td>CDR</td>
<td>complementarity determining regions</td>
</tr>
<tr>
<td>CO₂</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>Cₜ</td>
<td>cycle threshold</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DTT</td>
<td>dithiothreitol</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme linked immunoabsorbant assay</td>
</tr>
<tr>
<td>EtBr</td>
<td>ethidium bromide</td>
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<tr>
<td>FAE</td>
<td>follicle associated epithelium</td>
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<tr>
<td>FCS</td>
<td>foetal calf serum</td>
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<tr>
<td>FITC</td>
<td>fluorescein isothiocyanate</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
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<tr>
<td>GALT</td>
<td>gut associated lymphoid tissue</td>
</tr>
<tr>
<td>GAPDH</td>
<td>glyceraldehyde-3-phosphate dehydrogenase</td>
</tr>
<tr>
<td>GC</td>
<td>gene conversion</td>
</tr>
<tr>
<td>HEPES</td>
<td>4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid</td>
</tr>
<tr>
<td>IEL</td>
<td>intraepithelial lymphocytes</td>
</tr>
<tr>
<td>Ig</td>
<td>immunoglobulin</td>
</tr>
<tr>
<td>IgNAR</td>
<td>immunoglobulin isotype new antigen receptor</td>
</tr>
<tr>
<td>ip</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>ip</td>
<td>peranal</td>
</tr>
<tr>
<td>IPTG</td>
<td>isopropyl-beta-D-thiogalactopyranoside</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------------------------------</td>
</tr>
<tr>
<td>KLH</td>
<td>keyhole limpet hemocyanin</td>
</tr>
<tr>
<td>L</td>
<td>litre/s</td>
</tr>
<tr>
<td>LB</td>
<td>luria broth</td>
</tr>
<tr>
<td>LPL</td>
<td>lamina propria lymphocytes</td>
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<tr>
<td>m</td>
<td>metre</td>
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<tr>
<td>M</td>
<td>molar</td>
</tr>
<tr>
<td>mAb</td>
<td>monoclonal antibody</td>
</tr>
<tr>
<td>Mamp</td>
<td>milliamps</td>
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<tr>
<td>MALT</td>
<td>mucosal associated lymphoid tissues</td>
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<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
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<tr>
<td>MMC</td>
<td>melanomacrophage centers</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
</tr>
<tr>
<td>NK</td>
<td>natural killer</td>
</tr>
<tr>
<td>°C</td>
<td>degrees celcius</td>
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<tr>
<td>OD</td>
<td>optical density</td>
</tr>
<tr>
<td>ORF</td>
<td>open reading frame</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>pH</td>
<td>potenz hydrogen</td>
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<tr>
<td>PIT</td>
<td>passive implantable transponders</td>
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<tr>
<td>PP</td>
<td>peyers patches</td>
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<tr>
<td>qRT-PCR</td>
<td>quantitative real time PCR</td>
</tr>
<tr>
<td>RACE</td>
<td>random amplification of cDNA ends</td>
</tr>
<tr>
<td>RAG</td>
<td>recombination activation genes</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>RPMI</td>
<td>Roswell park memorial institute</td>
</tr>
<tr>
<td>SHM</td>
<td>somatic hypermutation</td>
</tr>
<tr>
<td>slg⁺</td>
<td>surface Ig positive</td>
</tr>
<tr>
<td>TcR</td>
<td>T cell receptor homolog</td>
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<tr>
<td>TD</td>
<td>T-dependant</td>
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xiv
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>TdT</td>
<td>terminal deoxynucleotidyl transferase</td>
</tr>
<tr>
<td>TNP-LPS</td>
<td>trinitrophenyl conjugated to lipopolysaccharide</td>
</tr>
<tr>
<td>U</td>
<td>units</td>
</tr>
<tr>
<td>UPR</td>
<td>unfolded protein response</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>x g</td>
<td>gravitational force</td>
</tr>
<tr>
<td>X-gal</td>
<td>5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside</td>
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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-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.