COMPARISON OF CLONAL COMPLEXITY OF PRIMARY AND SECONDARY TROUT IGM AND IGT RESPONSE USING DEEP SEQUENCING.

Susana Magadan1,2,§, Luc Jouneau1, Wahiba Chara3,4, Aurélie Lunazzi1, Alexandra Walczak5, Thierry Mora6, Edwige Quillet7, Øystein Ovensen8, Adrien Six3,4, Oriol Sunyer9, Pierre Boudinot1,§.

1 Virologie et Immunologie Moléculaires, Institut National de la Recherche Agronomique, Université Paris Saclay, Jouy-en-Josas, France.
2 Current address: Center for Evolutionary and Theoretical Immunology (CETI), Department of Biology, University of New Mexico, Albuquerque, NM, USA
3 UPMC University Paris 06, UMR 7211, Immunology-Immunopathology-Immunotherapy (I3), Paris, France.
4 CNRS, UMR 7211, Immunology-Immunopathology-Immunotherapy (I3), Paris, France.
5 Laboratoire de Physique Statistique, UMR8550, CNRS and Ecole Normale Supérieure, Paris, France.
6 Laboratoire de Physique Théorique, UMR8549, CNRS and Ecole Normale Supérieure, Paris, France.
7 Génétique Animale et Biologie Intégrative, Institut National de la Recherche Agronomique, Université Paris Saclay, Jouy-en-Josas, France.
8 Department of Basic Sciences and Aquatic Medicine, Norwegian University of Life Sciences, Faculty of Veterinary Medicine and Biosciences, Oslo, Norway.
9 Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA.

Fish infection or vaccination induces the production of antigen-specific antibodies by B lymphocytes. These B cells are recruited based on the specificity of their surface Ab, among the vast diversity of receptors produced through the random and imprecise genomic rearrangement of V, D and J genes during lymphocyte differentiation. In fish, the monitoring of B cell response to infections or vaccines has been mainly performed by serological and molecular techniques that provide a limited insight into the complexity of humoral adaptive immune response. We have developed a deep sequencing based approach to compare the clonal structure of the rainbow trout B cell primary and secondary response against the fish rhabdovirus VHSV. In this approach, unique barcode labels are incorporated on each starting cDNA molecule before amplification, allowing the correction of PCR/sequencing errors by generating consensus sequence and a safer quantification of sequence relative abundance. We characterized the clonal complexity of the IgM and IgT repertoire during the primary and secondary responses: we identified B cell clonal expansions generated in primary response to VHSV that are still detectable five months after immunization, and analyzed their frequency after a challenge with the same virus. Our data will be useful to model the development of the Ig landscape, and to understand the mechanisms of B-cell memory after infection by pathogens or vaccination in fish.

Key words: Immunoglobulin, repertoire, NGS, trout.

§ Corresponding authors:

P.B Tel: +33 1 34652585 E-mail address: pierre.boudinot@jouy.inra.fr
S.M Tel.: +1 5055508360 E-mail address: smagadan@unm.edu