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

Susana Magadan[1,2,§], Luc Jouneau[1], Wahiba Chara[3,4], Aurélie Lunazzi[1], Alexandra Walzcak[5], Thierry Mora[6], Edwige Quillet[7], Øystein Ovensen[8], Adrien Six[3,4], Oriol Sunyer[9], Pierre Boudinot[1,§].

[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