CHALLENGES IN PRODUCTION OF POLYCLONAL AND MONOCLONAL ANTIBODIES RECOGNIZING CYTOKINES OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

Tomáš Korytář[1]*, Fumio Takizawa[1]*, Zhen Xu[1,2], J. Oriol Sunyer[1§]

[1]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, United States of America
[2]Department of Aquatic Animal Medicine, College of Fisheries, Huazhong Agricultural University, Wuhan, Hubei, 430070, China

ABSTRACT

Thus far cytokine expression levels in teleost fish are being studied at the transcript level. This is in part due to the difficulties in generating cytokine-specific antibodies, as well as in developing the immunoassays and intracellular staining methods that would enable their accurate detection in biological samples and immune cells respectively. In the last few years, we have aimed to expand the fish immunologist toolkit by producing polyclonal and monoclonal antibodies against selected pro- and anti-inflammatory cytokines of rainbow trout. Herein, we provide examples that reflect some of the most common challenges we have encountered both in the production of antibodies as well as in their practical applications and offer possible solutions. Overall, we have found that except for the case of IL-10, none of the polyclonal antibodies produced against five other cytokines (including IL-1β, IL-6, IL-8, IFNγ and TNFα) appear to recognize the native proteins (i.e., cytokines produced by transfected trout cell lines). Interestingly, in several cases, the antibody recognized its target when expressed in mammalian but not trout cell lines. Moreover, most polyclonal antibodies (produced in rabbits) exhibited a high degree of non-specific binding when performing intracellular staining analysis, thereby precluding their usefulness for such application. Optimal reactivity of our anti-IL-10 pAbs with a STE-137 trout cell line transfected with an expression vector encoding trout IL-10 was only achieved after using the antibody Fab fragments. Moreover our newly generated anti-trout IL-10 mAbs provided also very good reactivity when using the same transfected cells while decreasing the non-specific binding to negligible levels. Identification of IL-10-producing leukocytes with these antibodies is now under investigation. In conclusion, our data strongly suggests the use of monoclonal antibody production for the efficient generation of antibodies to fish cytokines. Importantly, the use of transiently transfected teleost cell lines (rather than mammalian cell lines) is recommended for performing studies on the specificity of these antibodies. Finally, and at least in the case of trout IL-10, it would appear that this cytokine is expressed at levels that are undetectable with our current pAbs and mAbs, although further work is required to definitely draw this conclusion.

KEYWORDS

Cytokines, IL-10, monoclonal antibodies, polyclonal antibodies

*These authors have contributed equally to this work

[§]Corresponding author. TEL.: 215-573-8592; Fax number: 215-898-7887
E-mail: sunyer@vet.upenn.edu