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Cross-reactivity and epitope analysis of anti-ginbuna CD4-1 and CD8α monoclonal antibodies with lymphocytes of selected cyprinid species

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Statement of the problem: The two major subsets of T lymphocytes, helper and cytotoxic T cells, are defined by expression of CD4 or CD8 glycoproteins, respectively. Activation of CD4 T cells leads to their proliferation and differentiation into effector or regulatory cells that mediate or control the immunity; whereas, CD8 T cells destroy virus-infected cells as cytotoxic T cells. Both are essential for protecting the host from pathogens. Therefore, it is important to understand the function of CD4 and CD8 T cells. However, analysis of fish immune mechanisms has been hampered by the lack of suitable tools such as monoclonal antibodies (mAbs) against CD4^{+} and CD8^{+} T cells.

Methodology & Theoretical Orientation: We have generated mAbs against CD4-1 and CD8α in ginbuna crucian carp. In this study, we analyzed the cross-reactivity of these antibodies against the lymphocytes from eleven cyprinid species by flow cytometry. According to the reactivity of antibodies, we categorized them into high-, medium- and low-reactivity groups. Additionally, we cloned the ORFs of CD4 and CD8 and analyzed their protein sequences from each fish.

Findings: We identified those lymphocytes from four fish species cross-reacted with ginbuna CD4-1 mAb and lymphocytes from seven species cross-reacted with ginbuna CD8α mAb. High-reactivity group shared similar sequence characteristics with ginbuna CD4-1 and CD8α, especially in terms of the candidate epitopes of antibodies. By comparing the sequences of each groups, we identified the potential candidate epitopes, including a few epitopes for CD4-1 mAb, and one epitope for CD8α mAb.

Conclusion & Significance: The epitopes of our antibodies have been well-conserved in examined cyprinid species. Our antibodies will be available for analysis of the immune mechanisms in cyprinid fish. Furthermore, present strategy can be applied to predict the epitope recognized by antibodies in other fish species than cyprinid.

Biography

Ryuichiro Miyazawa, the second year student of the Doctor course, Graduate School of Veterinary Medicine, Nihon University. He has been involved in the studies on fish immune mechanisms using zebra fish and ginbuna as model fish. Recently, he found that monoclonal antibodies against ginbuna CD4-1 and CD8α cross react with lymphocytes of zebra fish and other cyprinid fishes. He is interested in T cell function in fish and investigates the autoimmune mechanisms from an evolutionary perspective at present. Monoclonal antibodies against T cell subsets are indeed quite helpful to analyze T cell function in zebra fish suffering from autoimmune disease. He has published four papers in international journals including Miyazawa *et al.*, Dev. Comp. Immunol, 2016. He presented six papers at international conferences and domestic meetings.

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