Development of novel tools based on immune molecules for antibody detection in fish

［課程博士・論文博士共通］

博士学位論文内容要旨
Abstract

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論文題目 Title	Development of novel tools based on immune molecules for antibody detection in fish（魚類抗体検出のための免疫学的な新規手法の開発）		

Antibody response is one of the key features of the vertebrate adaptive immunity. Detection of antibody has become a useful analytical tool in immunological studies and immunodiagnostic of numerous diseases. Moreover, antibody response is widely detected to evaluate the vaccine efficacy and to study the vertebrate immune system. To date, antibodies specific to immunoglobulin (Ig) molecules of various fish species have been developed and used in immunoassays. However, there are many economically important fish species in Japan. While their specific immune systems are broadly studied, the development of specific antibody for each fish species would be expensive and time-consuming. To reduce the cost and time for development of specific antibody, tools available for detection of antibody responses in various fish species are needed. In this study, the tools based on immune molecules for antibody detection in various fish species were developed.

Ig molecule consists of heavy (H) and light (L) chains, both of which are composed of variable and constant regions. Variable and constant regions are responsible for antigen recognition and mediation of signaling pathway, respectively. Here, the tools specific to fish IgM heavy chain were evaluated for fish antibody detection. Two synthetic peptides (IgH-1 and IgH-2) were designed based on the conserved sequence of fish IgH including Japanese flounder *Paralichthys olivaceus*, seabream *Pagrus major*, yellowtail *Seriola quinqueradiata*, carp *Cyprinus carpio* L., rainbow trout *Oncorhynchus mykiss*, hybrid sturgeon *Huso huso* x *Acipenser ruthenus* and banded houndshark *Triakis scyllium*. The synthetic peptides were used for immunization of rabbit to produce peptide polyclonal antisera (anti-IgH-1 and anti-IgH-2). The specificity of peptide antisera was determined by Western blot and enzyme-linked immunosorbent assay (ELISA). Anti-IgH-1 antiserum showed reactivity to IgM of Japanese flounder, seabream, yellowtail, carp, rainbow trout and hybrid sturgeon under reducing and non-reducing condition of Western blot. Anti-IgH-2 antiserum reacted to IgM of seabream, yellowtail and rainbow trout under reducing condition. However, under non-reducing condition, anti-IgH-2 antiserum solely reacted to IgM of rainbow trout. Attempts to use the antisera to measure fish antibody titer by ELISA were unsuccessful. These results indicate the application of anti-IgH-1 peptide antiserum for detection of IgM in various fish species by Western blot. Moreover, the antibody detection tools were further developed based on IgM light chain of Japanese flounder. Here, recombinant proteins of three isotypes of Japanese flounder IgL (L1, L2 and L3) were produced and used for immunization of rabbit to produce specific antiserum. The cross-reactivities of three antisera to IgLs of seven fish species (Japanese flounder, seabream, yellowtail, carp, rainbow trout, hybrid sturgeon and banded houndshark) were determined by Western blot (under reducing and non-reducing conditions) and ELISA. Anti-JF IgL1 and L2 antisera could react to Japanese flounder, seabream and yellowtail IgLs under reducing condition. However, fish IgLs were not clearly detected by anti-JF IgL3 antiserum under reducing condition. Unexpectedly, measurements of fish

antibody titer by ELISA using polyclonal antisera were unsuccessful. These results demonstrate the cross-reactivities of anti-JF IgL antisera to fish IgLs. The antisera are useful for detection of Japanese flounder, seabream and yellowtail IgLs by Western blot.

The tools were developed based on Ig-related immune molecules. Since Fc receptors (FcRs) are known as Ig receptor which play important roles in regulation of immune systems through the binding to the Fc portion of Ig molecules, they might be able to use as antibody detection tool. However, FcRs have not yet been well characterized in teleost. In this study, four Fc receptors-like (FcR1-like, FcR2-like, FcR3-like and FcR4-like) were identified in Japanese flounder and their binding activities to Japanese flounder serum proteins was analyzed. Recombinant FcR-like proteins showed binding activity to Japanese flounder serum proteins as determined by ELISA with different binding affinity. Interestingly, recombinant JF-FcR-like proteins also had binding properties to formalin-killed cells of *Edwardsiella tarda* and *Streptococcus iniae*. Therefore, attempts to use the recombinant protein as tool for antigen-specific antibody detection were unsuccessful. These results indicate that all FcR-like proteins are involved in regulation of immune response through binding not only to the Japanese flounder IgM molecules but also to pathogenic bacteria.

Finally, the tool for fish antibody detection was developed. Phage display assay is one of the most effective ways to study protein-ligand interactions and to produce large amounts of antibody. The cDNA library was generated from tuna IgM-immunized rabbit spleen. The polypeptides derived from cDNA library were expressed on the surface of lysogenic filamentous bacteriophages. Fish IgM-specific phages were enriched by two rounds of bio-panning with purified yellowtail IgM and further two rounds of bio-panning with purified red seabream IgM. The enriched phages demonstrated increase in binding specificity to the purified tuna, yellowtail, red seabream and rock bream IgMs compared with an unpanned library. The single clonal phage protein binding specificity to fish IgM will be characterized and applied for antibody detection in multiple fish species.