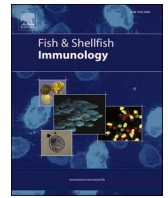




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CD4-1 and CD4-2 single positive are two major CD4 lymphocyte subpopulations in ginbuna crucian carp *Carassius auratus langsdorffii*

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ABSTRACT

In this study, we established a murine cell line that expresses ginbuna crucian carp (ginbuna) CD4-2 and used it to develop an anti-CD4-2 monoclonal antibody (mAb). An established mAb, named D5, showed good reactivities to BALB/c 3T3 cells expressing CD4-2 and a lymphocyte population in the ginbuna leukocytes. Gene expression analysis showed that D5⁺ cells express CD4-2 and TCR β genes but not CD4-1 and IgM genes, meanwhile May Grunwald-Giemsa staining of sorted D5⁺ cells had the typical morphology of lymphocytes. Two-color immunofluorescence analysis with anti-CD4-1 mAb (6D1) and anti-CD4-2 mAb (D5) by flow cytometry revealed that the percentages of CD4-1 single positive (SP) and CD4-2 SP lymphocytes were comparatively higher than CD4-1/CD4-2 double positive (CD4 DP) lymphocytes in all tissues examined in ginbuna. The highest percentage of CD4-2 SP cells (~40%) was found in the thymus, while the head-kidney exhibited the highest percentages of CD4-1 SP (~30%) and CD4 DP (~5%) cells. These findings indicated that ginbuna CD4⁺ lymphocyte population consists of two major subpopulations (CD4-1 SP and CD4-2 SP) and a minor subset (CD4 DP).

1. Introduction

Cell-mediated immunity (CMI) and humoral immunity (HMI) are two main mechanisms of immunity within the adaptive immune system. T lymphocytes are an essential leukocyte population that plays a critical role in the adaptive immune system. Cluster of differentiation (CD) molecules such as CD4 and CD8 are important surface markers of helper T (Th) lymphocytes and cytotoxic T lymphocytes, respectively. In mammals, CD4⁺ Th cells play a crucial role in coordinating the entire adaptive immune response by acting either as effector cells or as memory cells. When stimulated by an antigen, CD4⁺ Th cells can further differentiate into a variety of effector T cell subsets such as Th1, Th2, Th17, or Treg that secrete various cytokines to regulate immune responses to diverse infections [1,2]. Based on the immunoglobulin domain structures, teleost fish differ from mammals by having two distinct CD4 molecules: CD4-1 resembles the mammalian CD4, whereas CD4-2 is a second CD4 homolog in teleost fish [3,4]. To date, teleost fish CD4-1 has been discovered in Japanese pufferfish *Takifugu rubripes* [5,6], spotted green pufferfish *Tetraodon nigroviridis* [7], rainbow trout *Oncorhynchus mykiss* [8,9], common carp *Cyprinus carpio* [10], channel catfish *Ictalurus punctatus* [11], European seabass *Dicentrarchus labrax* [12], ginbuna crucian carp (ginbuna) *Carassius auratus langsdorffii* [13],

Atlantic halibut *Hippoglossus hippoglossus* [14], Atlantic salmon *Salmo salar* [15], Japanese flounder *Paralichthys olivaceus* [16], zebrafish *Danio rerio* [17], grass carp *Ctenopharyngodon idella* [18], and orange-spotted grouper *Epinephelus coioides* [19] that contain four immunoglobulin domains. In contrast, teleost fish CD4-2 has been identified in rainbow trout [9,20], channel catfish [11], ginbuna [13], Atlantic salmon [15], spotted green pufferfish [7], Japanese flounder [16], zebrafish [17], and grass carp [18] with fewer (two or three) immunoglobulin domains.

Several studies in rainbow trout [9], Japanese flounder [16], and grass carp [18] showed that the presence of three distinct CD4⁺ lymphocyte subpopulations: CD4-1/CD4-2 double positive (DP), CD4-1 single positive (SP) and CD4-2 SP population in teleost fish. Kato et al. [16] demonstrated that in Japanese flounder, CD4-2 mRNA level increased significantly following infection with intracellular parasitic pathogens like *Edwardsiella tarda* and viral hemorrhagic septicemia virus, but did not increase following infection with extracellular pathogen *Streptococcus iniae*. Furthermore, Yamaguchi et al. [21] revealed that CD4-1 SP cells only exhibited the Th2 phenotype, whereas a tiny population of CD4-2 SP cells displayed both Th1 and Th2 phenotypes in common carp. Somamoto et al. [22] showed that CD4-1⁺ T cells had a helper function in secondary HMI but not secondary CMI against crucian carp hematopoietic necrosis virus in ginbuna. Wen et al. [7] suggested

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