SURFACE EXPRESSION OF TROUT CD4-1 AND CD4-2 DEFINES NOVEL POPULATIONS OF FUNCTIONALLY DISTINCT CD4$^+$ T CELLS IN TELEOST FISH

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ABSTRACT

The largest subset of T cells in jawed vertebrates express a TCR bearing α and β chains that recognize antigens bound to MHC molecules. Such T cells use two main co-receptors, CD4 and CD8 which show mutually exclusive expression on naive helper T (Th) and cytotoxic T cells respectively. Tetrapods contain a single CD4 co-receptor with four immunoglobulin domains that likely arose from a primordial two-domain ancestor. Notably, teleost fish contain two CD4 genes. Like tetrapod CD4, CD4-1 of rainbow trout includes four-immunoglobulin domains while CD4-2 contains only two. Thus far the concurrent surface expression of CD4-1 and CD4-2 on teleost leukocytes has not been determined due to the lack of reagents capable of detecting both molecules in a single species. In the absence of those, transcript levels have been used to assess expression patterns of CD4-1 and CD4-2, but results in different teleost species have been inconclusive. Hence, very little is known regarding the existence of different teleost CD4$^+$ T cells subsets and their roles in immunity. Here we generated monoclonal antibodies against trout CD4-1 and CD4-2 that enabled the identification of two *bona fide* CD4$^+$ T-cell populations, a predominant lymphocyte population co-expressing surface CD4-1 and CD4-2 (CD4 DP) and a minor subset expressing only CD4-2 (CD4-2 SP). While both subsets produced equivalent levels of Th1, Th17, and Treg cytokines in response to *Yersinia ruckeri* infection, CD4-2 SP lymphocytes were less proliferative to PHA and alloantigen stimulations and displayed a more restricted TCRβ repertoire. These data suggest that CD4-2 SP cells represent a functionally distinct population and may embody a vestigial CD4$^+$ T cell subset, the roles of which reflect those of primeval CD4$^+$ T cells. In addition, significant disparities in their transcriptomes as well as in their abundance in systemic and mucosal organs indicate further functional differences between CD4 DP and CD4-2 SP subsets. This study fills in an important gap in the knowledge of teleost CD4-bearing lymphocytes thus revealing critical insights into their functional roles and evolutionary origins. Importantly, as our knowledge on CD4$^+$ T-cell responses in teleosts is very scarce, our findings will be critical for the design of more effective vaccines for fish that induce strong effector and memory CD4$^+$ T cell responses.

KEYWORDS
CD4-1, CD4-2, $CD4^+$ T cell, rainbow trout, cytokines

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