

CHARACTERIZATION OF EVOLUTIONARILY CONSERVED CD4⁺ MONOCYTES/MACROPHAGES IN RAINBOW TROUT

Fumio Takizawa¹, David Parra², Zhen Xu^{1,3}, Tomáš Korytář¹,
Susana Magadan⁴, Pierre Boudinot⁴, J. Oriol Sunyer^{1*}

¹*Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, 19104*

²*Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, Barcelona, Spain*

³*Department of Aquatic Animal Medicine, College of Fisheries, Huazhong Agricultural University, Wuhan, Hubei, 430070, China*

⁴*Virologie et Immunologie Moléculaires, Institut National de la Recherche Agronomique, Jouy-en-Josas, France*

ABSTRACT

Mammalian CD4 is expressed on a variety of cell types in addition to helper T cells. The expression pattern of CD4 molecules shows species disparity but often defines distinct subsets in a certain myeloid cell population. Teleost fish possess two types of CD4 molecules, CD4-1 and CD4-2, however their expression on myeloid cells remain to be well characterized. The goal of this study was to identify the CD4⁺ subset within the myeloid leukocyte population in rainbow trout. Staining of head kidney cells with anti-CD4-1 and anti-CD4-2 mAbs revealed the presence of a significant population of myeloid cells with only CD4-1 surface expression. Gene expression analysis of myeloid cell markers revealed that CD4-1⁺ myeloid cells expressed high transcript levels of monocyte/macrophage markers, including *mcsfra/b*, *mpeg1*, and *lyz*. In contrast, the same cells expressed negligible amounts of *mpo*, a neutrophil marker. Conversely, CD4-1⁻ myeloid cells expressed high levels of *mpo* while expressing very low to negligible transcript levels of the aforementioned monocyte/macrophage markers. We then used cytochemical staining to characterize further the identity of CD4-1⁺ myeloid cells. Most of these cells were positive for β -glucuronidase (BG, ~97%) and naphthol AS-D chloroacetate esterase (NCAE, ~95%) staining while negative for myeloperoxidase (MPO) and Sudan Black B (SBB) stains. In contrast, CD4-1⁻ myeloid cells included mostly polymorphonuclear cells that stained positively with MPO and SBB (~80%). These results are consistent with reports that salmonid monocytes/macrophages are positive for BG and NCAE stains while neutrophils are positively stained with MPO and SBB stains. When combined, the above described gene expression and cytochemical analyses strongly suggest that CD4-1⁺ myeloid cells are monocytes/macrophages while CD4-1⁻ myeloid cells mostly comprise neutrophils. Furthermore, we assessed the phagocytic capacity of both CD4-1⁺ and CD4-1⁻ myeloid cells and found that CD4-1⁺ monocytes/macrophages represent the myeloid population with the highest phagocytic activity and capacity while CD4⁺ lymphocytes had a negligible phagocytic capacity. Our data represent the first description of monocytes/macrophages with surface CD4 expression in a non-mammalian species, thus suggesting that CD4 expression in these cells is the result of an ancient evolutionary event preceding the emergence of tetrapods. Importantly, the identification of these CD4-1⁺ monocytes/macrophages provides with an opportunity to study the role of trout monocytes/macrophages in immunity.

KEYWORDS

CD4, monocytes, macrophages, phagocytosis, rainbow trout

*Corresponding author. TEL.: 215-573-8592; Fax number: 215-898-7887

E-mail: sunyer@vet.upenn.edu