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Differential spontaneous killing of human and murine tumour cell lines by leucocyte subpopulations from carp peripheral blood leucocytes

Chihaya Nakayasu[a,*], Tomonori Somamoto[a], Satoshi Hasegawa[b], Tomoyasu Yoshitomi[c], Nobuaki Okamoto[b]

[a]*Inland Station, National Research Institute of Aquaculture, Tamaki, Mie 519-0423, Japan*
[b]*Department of Marine Biosciences, Tokyo University of Marine Science and Technology, Konan 4, Minato, Tokyo 108-8477, Japan*
[c]*Field Studies Institute for Environmental Education, Tokyo Gakugei University, 4-1-1 Nukuikitamachi, Koganei, Tokyo 184-8501, Japan*

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Abstract

Cell populations from carp (*Cyprinus carpio* L.) peripheral blood leucocytes (PBLs) were examined for nonspecific cytotoxicities. By using monoclonal antibodies (MAbs) against carp thrombocytes (TCL-HB8) and both neutrophils and monocytes (TCL-BE8), PBLs with a density of 1.08 g ml^{-1} were separated into three fractions: thrombocytes, a mixture of neutrophils and monocytes, and other cells (mainly lymphocytes), and the separated cells were tested for cytotoxic activities against mammalian tumour cell lines (K562, HeLa, P815 and Yac-1 cell). Consequently, the mixture of neutrophils and monocytes exhibited cytolysis against these target cells, whereas the lymphocyte-rich and thrombocyte fractions did not show any cytolysis. To isolate only neutrophils, which do not contain monocytes, the MAb (TCL-BE8) positive cells from PBLs with a density of 1.08–1.09 g ml^{-1} were separated. Pure isolated neutrophils showed cytotoxic activities against K562 cells, but not P815 cells. Furthermore, analysis of the cytolytic mechanisms indicated that killing of these cells depended on H_2O_2 or HOCl. These results suggest that both neutrophils and monocytes are effectors for nonspecific cytotoxicity in carp PBLs, and neutrophils may be distinct from monocytes in their reactivity in cytolysis, including target cell selectivity and/or target cell sensitivity, and the cytolytic pathway. In

* Corresponding author. Tel.: +81 596 58 6411; fax: +81 596 58 6413.
E-mail address: cnakayas@fra.affrc.go.jp (C. Nakayasu).

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carp, cytotoxicity of target cells can be mediated by several populations of their leucocytes which have cytotoxic capacities with various recognition and cytolytic mechanisms.

Keywords: Carp; Nonspecific cytotoxicity; Cell separation; Neutrophil; Monocyte; Cytolytic mechanisms; Monoclonal antibodies

1. Introduction

Cell-mediated cytotoxicities have been recognised to be important in the immune system. This function is effected by cells of lymphoid and myeloid lineages, such as granulocytes, monocytes and probably natural killer (NK) cells and has been found in many species from invertebrates to vertebrates. In crayfish (*Astacus astacus*), granular and semigranular cells have a cytotoxic capacity [1]. In birds, granulocytes are the main effector cells for natural cytotoxicity [2], and in mammals, a number of effector cells including NK cells [3,4], granulocytes [5–7], neutrophils [8,9], basophils [10], monocytes and macrophages [11,12] have been reported to be cytotoxic. Blood platelet-mediated cytotoxicity has also been reported [13]. In fish, several types of effectors for spontaneous cytotoxicity are known: small agranular lymphocytes from channel catfish (*Ictalurus punctatus*) [14–16], neutrophils from carp (*Cyprinus carpio* L.) [17] and rainbow trout (*Oncorhynchus mykiss*) [18], and eosinophilic granular cells from the peritoneal cavity of sea bass (*Dicentrarchus labrax*) [19].

In mammals, different types of effector cells show unique reactivities in cytotoxicity. On the other hand, in invertebrates, there are no reports about these. It is interesting whether different types of effector cells, which have unique cytolytic mechanisms and target cell selectivity in cytotoxicity exist in lower vertebrates. It was previously reported that nonspecific cytotoxic cells (NCCs) from catfish head kidney (HK) killed mammalian tumour targets [14–16] and lymphocytes from catfish PBLs, which were distinct from NCC, spontaneously killed allogeneic or virus-infected targets [20–22]. These studies indicate that catfish have several types of nonspecific cytotoxic effector cells. Kurata et al. reported that neutrophils from carp HK exhibited a spontaneous cytotoxicity against K562 cells, and their killing depended on H_2O_2 [17]. However, although carp neutrophils have been identified as nonspecific cytotoxic effector cells, it is currently unclear whether lymphocytes or other leucocytes possess a spontaneous cytotoxicity.

Previously MAbs reacting with thrombocytes, or both neutrophils and monocytes in carp were developed, and cell populations from the PBLs using a combination of an immuno-magnetic separation system and a density gradient centrifugation method were separated [23,24]. In this study, using the separated cell populations, the cells that possessed nonspecific cytotoxicity against several mammalian tumour cells were identified, and the cytolytic mechanisms in cytotoxicity were examined.

2. Materials and methods

2.1. Fish

Carp (*Cyprinus carpio* L.) weighing approximately 500 g were obtained from Yoshida Research and Training Station, Tokyo University of Marine Science and Technology. The fish were maintained in a 400-l tank with running water at a temperature of 25 ± 1 °C at Tokyo University of Fisheries and fed commercial pellets.