

PROTECTION OF TELEOST FISH

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1. Field of the Invention

This invention relates to the protection of teleost fish against microorganismic pathogens. Sodium orthovanadate, a protein phosphatase inhibitor, or a vanadate-mimetic protein phosphatase inhibitor is used to protect fish therapeutically or prophylactically from infection or disease caused by microorganismic pathogens.

2. Background of the Invention

Nonspecific cytotoxic cells (NCC) from teleost fish are believed to be comparable to mammalian natural killer (NK) cells. NCC lyse a wide variety of mammalian transformed tumor target cells and may participate as major effector cells in killing protozoan parasites. Compared to all known effector functions of specific and nonspecific immunity in fish, NCC may represent the most broadly functional defense against infectious agents as well as participating in immunoregulation.

NCC and NK cells share many characteristics, some of which are: lack of restriction for target cell recognition by MHC or H2 antigens, inability to produce secondary responses, and ability to lyse tumor and virus transformed target cells and various parasitic protozoans. The differences between NCC, mouse, and human NK cells provide perhaps the most significant information regarding the evolution of NK/NCC cells as well as providing clues regarding their roles in natural immune responses. For example, NCC are small agranular lymphocytes (SAL) [Evans et al. (1987) *Dev. Comp. Immunol.* 11:95], and chickens and pigs have SAL [Sieminski-Brodzina et al. (1991) *Dev. Comp. Immunol.* 15:181], and large granular lymphocytes (LGL) [Takamatsu et al. (1985) *Jpn. J. Vet. Sci.* 47:749], while mice, rats, and humans have LGL. Fresh NCC, unlike similarly prepared murine and human NK cells, have a wide range of target cell specificities [Graves et al. (1984) *Dev. Comp. Immunol.* 8:293; Evans et al. (1984) *Dev. Comp. Immunol.* 8:303] like mammalian LAK and ALAK [Jaso-Friedmann et al. (1993) *J. Natl Immun.* 12:316-325]. These comparisons provide some evidence for phylogenetic equivalencies.

Recently, a receptor-like molecule on NCC and NK cells was isolated by the inventors from many different species [Evans et al. (1988) *J. Immunol.* 141:324; Harris et al. (1991) *Proc. Natl. Acad. Sci.* 88:3006; Jaso-Friedmann et al. (1992) *Cell Immunol.* 141:293]; this work provides the best evidence yet for an evolutionary relationship. The expression of this protein on NK cells from diverse species closely links natural immune mechanisms of target cell recognition.

Monoclonal antibodies (mab) specific for the putative receptor (e.g., mab 5C6) inhibited cytotoxicity of NCC and NK cells [Evans et al. (1988) supra, Harris et al. (1991) supra, Jaso-Friedmann et al. (1992) supra]. The mab 5C6 binding to NCC and NK cells also triggered many different levels of activation responses [Evans et al. (1990a) *Dev. Comp. Immunol.* 14:223; Evans et al. (1990b) *Dev. Comp. Immunol.* 14:295; Evans et al. (1990c) *Nat. Immun. Cell Growth Regul.* 9:353-365; Evans et al. (1992) *Dev. Comp. Immunol.* 16:383]. NCC and NK cells appeared to have some equivalent second messenger responses, whereas in other important comparative areas of signaling, agonist binding appeared to initiate additional requirements for NCC activation. These included an increased dependence on intracellular calcium levels for activation and lack of synergistic requirements for costimuli effects to trigger lytic

responses. For example, calcium appeared to participate in more direct pathways of signaling in NCC compared to NK cells [Evans et al. (1990a) supra, Evans et al. (1990b) supra, Evans et al. (1992) supra]. The calcium ionophore A23187 in the absence of PMA or mab 5C6 activated NCC cytotoxicity and increased "receptor" expression.

There appears to be general agreement [Jaso-Friedmann et al. (1993) *Cell Immunol.* 148:208; Evans et al. (1990) *Dev. Comp. Immunol.* 14:223; Evans et al. (1990) *Dev. Comp. Immunol.* 14:295; Evans et al. (1992) supra; and Stahls et al. (1992) *Eur. J. Immunol.* 22:611] that both-kinases and phosphatases regulate some signaling pathways. The prototype protein phosphatase which provides the best evidence for the role of these enzymes in signaling responses in lymphocytes is the CD45 glycoprotein. CD45 is expressed on all (human) lymphocytes, and it belongs to the "receptor-type" phosphotyrosyl-protein phosphatase (PTPase) family of enzymes (including LAR-leukocyte antigen related and leukocyte common antigen-related phosphatase-LRP). Inclusion of these proteins in this family is based on amino acid sequence homology [Fischer et al. (1991) *Science* 253:401; Alexander et al. (1990) *The New Biologist* 2:1049] with a PTPase purified from human placental tissue (i.e., PTB1B). Within this group of membrane and cytosolic enzymes, all share significant conserved cytoplasmic domain sequences (707 amino acids). More than 90% of the cytoplasmic portion of CD45 of mouse, rat and humans is identical [Evans et al. (1988) supra]. The extracellular regions of protein phosphatase generally are composed of several immunoglobulin-like and fibronectin type-III-like domains [Alexander et al. (1990) supra; Krueger et al. (1990) *EMBO J.* 9:3241] and they are markedly heterogenic in sequence and size (i.e., vary from 27-1599 amino acids). Although a sequence motif is common to many of the extracellular portions of protein phosphatases, there is not enough sequence homology between any of the protein phosphatases in the extracellular region to suggest that they have common substrates to which they bind. The nonreceptor-type protein phosphatases lack extracellular and transmembrane domains and have a significantly different structure compared to the receptor-type protein phosphatases [Graves et al. (1984) supra; Schechter et al. (1980) *Nature* 284:556].

There is considerable information to suggest that CD45 may be involved in T-cell signaling responses. Crosslinking CD45 with CD3 or with CD2 affects T-cell activation [Tamura et al. (1984) *J. Biol. Chem.* 259:6650; Gil et al. (1988) *J. Biol. Chem.* 263:1868; Fantus et al. (1990) *Endocrin.* 127:2716; Earp et al. (1983) *FEBS Lett.* 161:180; Ledbetter et al. (1988) *Proc. Natl. Acad. Sci.* 85:8628]. Crosslinkage of CD45 with CD4 induces intracellular calcium release. The possible functional as well as physical association between these molecules suggested that the CD45 protein phosphatase plays a role in T-cell activation responses. Additional evidence that CD45 participates in T-cell signaling was shown in studies where CD45 deficient (mutant) cell lines failed to produce phosphatidylinositol metabolites following stimulation [Kiener et al. (1989) *J. Immunol.* 143:23; Shaw et al. (1991) *Curr. Opin. Cell Biol.* 3:862].

Although the substrates recognized by many of the protein phosphatases have not yet been detected, a likely candidate for the CD45 phosphatase is p56^{lck}. This cytoplasmic kinase is a member of the src oncogene family of kinases [Livanainen et al. (1990) *Eur. J. Immunol.* 20:2509] and in T-cells is biochemically and physically associated with the cytoplasmic domains of CD4 and CD8 [Odum et al.