Fish & Shellfish Immunology 41 (2014) 45–51

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Control of CSF-1 induced inflammation in teleost fish by a soluble form of the CSF-1 receptor

Aja M. Rieger [a], Patrick C. Hanington [b], Miodrag Belosevic [a,b], Daniel R. Barreda [a,c,*]

[a] Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada
[b] School of Public Health, University of Alberta, Edmonton, Alberta, Canada
[c] Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada

ARTICLE INFO

Article history:
Received 1 February 2014
Received in revised form
20 March 2014
Accepted 30 March 2014
Available online 12 April 2014

Keywords:
CSF-1
Evolution
Inflammation
Macrophage
Teleost fish

ABSTRACT

The colony-stimulating factor-1 (CSF-1) is the principal regulator of the survival, proliferation, differentiation, and function of macrophages and their precursors, and has been shown to play a role in the etiology of inflammation. We recently identified a novel mechanism for the control of CSF-1 activity in teleost fish, through the production of an inhibitory soluble form of the CSF-1 receptor (sCSF-1R). Primary goldfish kidney macrophages selectively expressed sCSF-1R during the senescence phase, which corresponds to a defined stage of *in vitro* culture development where inhibition of macrophage proliferation and apoptotic cell death are prominent. In contrast, primary macrophage cultures undergoing active proliferation displayed low levels of sCSF-1R expression. Addition of purified recombinant sCSF-1R to developing primary macrophage cultures leads to a dose-dependent decrease in macrophage proliferation and inhibits macrophage antimicrobial functions including chemotaxis, phagocytosis, and production of reactive oxygen intermediates. Using a goldfish *in vivo* model of self-resolving peritonitis, we found that sCSF-1R plays a role in the inhibition of inflammation, following an initial acute phase of antimicrobial responses within an inflammatory site. Soluble CSF-1R inhibits pro-inflammatory cytokine production, inhibits leukocyte recruitment to the inflammatory site and decreases ROS production in a dose-dependent manner. This sCSF-1R-dependent regulation of inflammation appears to be an elegant mechanism for the control of macrophage numbers at inflammatory sites of lower vertebrates. Overall, our results provide new insights into the evolutionary origins of the CSF-1 immune regulatory axis.

1. Introduction

The colony-stimulating factor-1 (CSF-1) or macrophage colony-stimulating factor (M-CSF) is the principal regulator of the survival, proliferation, and differentiation of macrophages and their precursors [1–5]. Indeed, cells of this lineage represent the main target population for this homodimeric sialoglycoprotein [6–9]. Outside of the female reproductive tract where CSF-1 expression is governed through a separate promoter, the high affinity tyrosine kinase CSF-1 receptor (CSF-1R) is exclusively found in cells of the macrophage lineage. This facilitates the identification of macrophages and their hematopoietic progenitors [1,10]. The level of CSF-1R progressively increases from primitive hematopoietic precursors to monocytes and further increases upon terminal maturation to macrophages [8]. Engagement of the CSF-1R by CSF-1 promotes proliferation of macrophage progenitor populations and increases expression of several other macrophage differentiation antigens [11–13]. CSF-1 is also a central regulator of macrophage function. It promotes monocyte and macrophage defense mechanisms through contributions to chemotactic, phagocytic, and killing activities [14–20]. Further, it increases production of plasminogen activator [21,22], prostaglandin E [23], reactive oxygen [20,24,25] and reactive nitrogen intermediates [26,27], in addition to several cytokines including G-CSF, GM-CSF, interleukin-1 (IL-1), IL-6, IL-8, IL-18, TNFα, and interferon [28–35]. Thus, based on its effects on this myeloid lineage, it is easy to recognize the importance of CSF-1 for the maintenance of tissue homeostasis, early defenses against invading pathogens, the regulation of downstream immunological responses, and tissue repair.

In addition to its primary role in the maintenance of macrophage populations and their functions, CSF-1 activity is also relevant to other cells and tissues, as well documented in studies of

* Corresponding author. Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2P5, Canada. Tel.: +1 780 492 0375; fax: +1 780 492 4265.
E-mail address: dan.barreda@ualberta.ca (D.R. Barreda).

http://dx.doi.org/10.1016/j.fsi.2014.03.035
1050-4648/

CSF-1R knockout (KO) and CSf1^{op}/CSf1^{op} mice. The latter lacks active CSF-1 production due to a null mutation in the CSF-1 gene-coding region, which results in the generation of a biologically inactive truncated form of the growth factor [36,37]. As expected, CSf1^{op}/CSf1^{op} mice have a severe deficiency of tissue macrophages and bone-resorbing osteoclasts, absence of teeth, abnormal bone remodeling and osteopetrosis. However, they also exhibited abnormal breast development, decreased fertility, low body weight, and shortened life-span, that was reversed through by injections of recombinant CSF-1 early during development. Importantly, unlike CSF-1R KO models, the CSf1^{op}/CSf1^{op} mouse does not suffer from the confounding effects based on inhibition of IL-34, a ligand that has been recently shown to bind similar regions on the CSF-1R as CSF-1 with similar binding affinity [7]. However, despite the advantages of the CSf1^{op}/CSf1^{op} mouse, redundancy is expected to mask some additional contributions of CSF-1 to host physiology. Together, these studies identify CSF-1 contributions to immunity and inflammation, bone metabolism, atherogenesis, lipoprotein clearance, development and reproduction [4,7,8,38–41].

Observations on CSF-1 activity over the last 40 years have served as the basis for several clinical trials aimed at therapeutic interventions against microbes, autoimmune disorders, cancer and inflammatory diseases [42]. For example, recombinant human CSF-1 (rh-CSF-1) treatment alone or in combination with other cytokines effectively increased the numbers and activation of monocytes and macrophages, enhanced antibody-dependent cellular cytotoxicity (ADCC), antibody-independent tumor cell cytolysis, promoted macrophage antimicrobial responses, and lowered platelet numbers and cholesterol levels [27,38,42]. Administration of rh-CSF-1 also proved effective for the control of invasive fungal infections in patients undergoing bone marrow transplantation, resulting in a significant reduction in mortality rates when given in combination with standard antifungal treatments [43]. Acute myeloid leukemia (AML) patients also benefited from CSF-1 treatment, as shown by reduced incidence and shortened duration of febrile neutropenia and thrombopenia following chemotherapy, as well as shortening the period required to finish three courses of intensive consolidation therapy [27]. However, it is important to note that these and more recent trials also highlight the complexity of CSF-1 activity. For example, therapeutic benefits have been identified in models of Alzheimer's disease, kidney repair, chronic graft-versus-host disease (GVHD) after bone marrow transfer, and healing of gastric ulcers [7]. On the other hand, disease exacerbation was identified following CSF-1 administration in models of amyotrophic lateral sclerosis and lupus nephritis. Additional complexity is derived from differential effects on target cell populations and pathways that are affected. In immunity and inflammation, for example, CSF-1 has been associated with differential modulation of toll-like receptor (TLR) expression (e.g. promoting LPS but suppressing CpG DNA responses) and differential induction of cytokine production during innate antimicrobial responses (e.g. increases in expression of TNF-α, IL-6, IL-8 and IL-18 but decreases in IL-12 production) [3,28,44]. Further, among circulating monocyte subsets, CSF-1 appears to play a prominent pro-inflammatory role through selective effects on inflammatory CD14^{+}CD16^{+} monocytes in humans and their CX3CR1^{hi}CCR2^{-}GR1^{-} murine counterparts. Elevated levels of these monocytes have been identified in sepsis [45,46], chronic liver inflammatory disease [47], coronary heart disease [48], rheumatoid arthritis [49], chronic renal failure [50] and other severe inflammatory diseases. Thus, much remains to be learned with regards to CSF-1 activity and selectivity in order to understand the range of benefits and side effects when targeted as part of developing therapeutic interventions.

2. Evolutionary conservation of CSF-1 and CSF-1R

The CSF-1/CSF-1R axis is conserved across evolution. Among placental mammals, homologs for CSF-1 have been described in humans (*Homo sapiens*), chimpanzees (*Pan troglodytes*), rhesus monkeys (*Macaca mulatta*), cattle (*Bos taurus*), dogs (***Canis familiaris***), mice (*Mus musculus*) and rats (*Rattus norvegicus*). Alignment of corresponding protein sequences shows identity levels above 79% when compared to the human CSF-1 (97% chimpanzee XP_513655.3, 97% rhesus monkey XP_001090841.2, 84% cattle NP_776451.1, 82% dog XP_854600.1, 81% mouse NP_001107002.1 and 79% rat NP_076471.3 compared to human reference sequence for CSF-1 NP_000748.3). The CSF-1R is also well conserved among higher vertebrates. Homologs for CSF-1R have been described in humans (*H. sapiens*), chimpanzees (*P. troglodytes*), rhesus monkeys (*M. mulatta*), cattle (*B. taurus*), dogs (*C. familiaris*), mice (*M. musculus*), and rats (*R. norvegicus*). Alignment of corresponding protein sequences shows identity levels above 80% for placental mammals when compared to the human CSF-1R (98% chimpanzee XP_003310972.1, 97% rhesus monkey XP_001107711.2, 86% cattle NP_001068871.2, 86% dog XP_546306.2, 80% mouse NP_001032948.2 and 80% rat NP_001025072.1 compared to human reference sequence for CSF-1R NP_005202.2). Molecular support for the conservation of CSF-1 activity in lower vertebrates comes from identification of CSF-1 transcripts in birds [51], amphibians [52] and fish [53,54] as well as the identification of the CSF-1R in birds [51] and a number of fish species including zebrafish [55], goldfish [56], rainbow trout [57], Atlantic salmon [58], pufferfish [59] and the gilthead sea bream [19]. Sequence homology of CSF-1 transcripts decrease significantly compared to those found among placental mammals. For example, chicken, goldfish, zebrafish and trout CSF-1 predicted protein sequences display 31%, 21%, 21/15% (zebrafish MCSF-1/MCSF-2) and 22/16% (trout MCSF-1/MCSF-2) identity compared to that of humans, respectively [51,53,60]. A similar scenario is observed with the CSF-1R. The chicken predicted protein sequence for CSF-1R is 51% identical to that of human CSF-1R, whereas zebrafish, goldfish, trout and fugu share 45%, 44%, 45%, and 45% identity, respectively [55,57,59,61]. Although sequence homology for CSF-1 and CSF-1R is limited between lower vertebrates and their human counterpart, functional characterization of CSF-1 activity is largely consistent with roles previously defined in mammalian systems [2,53,62,63]. In chicken and Xenopus, CSF-1 promoted growth and survival of primary bone marrow-derived cultures [51,52]. In goldfish, CSF-1 macrophage expression was positively modulated by rgTNFα-2, rgIFNγ but not rgTGFβ [62]. *In vitro* macrophage treatment with CSF-1, in turn, increased the expression of IL-8, CCL-1, TNFα-1, TNFα-2, IL-1β1, IL-1β2, IL-12-p35, IL-12-p40, IFN, IL-10, and iNOS A and B. It also led to expression of TGF-β at later time points [62]. Functional responses for goldfish CSF-1 included enhancement of macrophage proliferation, differentiation from hematopoietic precursors, chemotaxis, phagocytosis and production of antimicrobial reactive oxygen and nitrogen intermediates [53,62,63]. *In vivo*, goldfish CSF-1 administration increased the number of circulating monocytes in the bloodstream [63].

Despite the functional similarities observed when comparing goldfish CSF-1 with mammalian CSF-1 [62], goldfish CSF-1 varies from its mammalian counterpart in a number of ways. Goldfish CSF-1 is composed of 199 amino acids making it significantly smaller than the secreted glycoprotein or the secreted/matrix bound proteoglycan form of mammalian CSF-1, and most similar to the membrane bound glycosylated form of the molecule [64]. Interestingly, all mammalian CSF-1 isoforms were shown to be functional as long as they possessed the first 150 amino acids found in the N-terminal of the CSF-1 protein. This region was shown to be