CSF-1 as a regulator of macrophage activation and immune responses

Citation for published version:

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Archivum immunologiae et therapiae experimentalis

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
CSF-1 as a Regulator of Macrophage Activation and Immune Responses

MATTHEW J. SWEET* and DAVID A. HUME

CRC for Chronic Inflammatory Diseases, Institute for Molecular Bioscience and Departments of Microbiology/Parasitology and Biochemistry, University of Queensland, Qld 4072, Australia

Abstract. Macrophage activation is a key determinant of susceptibility and pathology in a variety of inflammatory diseases. The extent of macrophage activation is tightly regulated by a number of pro-inflammatory cytokines (e.g. IFN-γ, IL-2, GM-CSF, IL-3) and anti-inflammatory cytokines (e.g. IL-4, IL-10, TGF-β). Macrophage colony-stimulating factor (CSF-1/M-CSF) is a key differentiation, growth and survival factor for monocytes/macrophages and osteoclasts. The role of this factor in regulating macrophage activation is often overlooked. This review will summarize our current understanding of the effects of CSF-1 on the activation state of mature macrophages and its role in regulating immune responses.

Key words: CSF-1; macrophage; lipopolysaccharide; Toll-like receptors; inflammation.

Introduction

The production of circulating monocytes and tissue macrophages from the bone marrow is dependent on colony-stimulating factor 1 (CSF-1). This is highlighted by the gross deficiencies in macrophage development and numbers that occur in the op/op mouse, which has a natural inactivating mutation in the CSF-1 gene (for review see44), and by the demonstration that administration of CSF-1 to mice caused a 10-fold increase in blood monocyte numbers and increased macrophage numbers in the liver, spleen and peritoneal cavity41.

Via alternative splicing, post-translational modifications and proteolytic processing, CSF-1 is produced in multiple forms; a secreted homodimeric glycoprotein, a secreted proteoglycan and a membrane-bound glycoprotein73, 84. The CSF-1 receptor (CSF-1R), encoded by the c-fms protooncogene, is a type III receptor tyrosine kinase with structural similarity to c-kit and the platelet-derived growth factor receptor and the fms-like receptors flk, and flt 1, 2 and 3. Mice with a targeted disruption of the c-fms gene are essentially a phenocopy of the op/op mouse, indicating that all of the actions of CSF-1 are mediated by the CSF-1R18. Ligand binding to the CSF-1R initiates receptor dimerization, auto-


* Correspondence to: Dr. Matthew Sweet, Institute for Molecular Bioscience University of Queensland, Qld 4072, Australia, tel.: +61 7 3346 2072, fax: +61 7 3346 2101, e-mail: m.sweet@imb.uq.edu.au
phosphorylation, and activation of multiple signalling cascades including the mitogen-activated protein kinase and P-I-3 kinase/Akt pathways21, 35.

Although relatively few studies have addressed the biological roles of the different CSF-1 isoforms, it is likely that they have distinct functions in vivo. For example, the proteoglycan form is localized to specific types of extracellular matrix which might enable specialized functions such as regulation of bone homeostasis68. Daily administration of soluble recombinant CSF-1 to the op/op mouse from day 3 of life was able to correct some but not all of the deficiencies in macrophage numbers in different tissues13. This supports the argument that the full biological activity of CSF-1 requires all isoforms and not just soluble circulating CSF-1, although it is also possible that pre-natal absence of CSF-1 has effects that cannot be subsequently restored by exogenous CSF-1. The gene regulatory elements that are sufficient to reconstitute normal CSF-1 expression in vivo have been defined77. Transgenic expression on the op/op background of cDNAs encoding the secreted, proteoglycan or membrane-bound isoforms under the control of these elements should therefore provide clear evidence for any isoform-specific functions.

Regardless of the functions of the different isoforms, it is clear that CSF-1 is abundantly present in vivo. The major source of circulating CSF-1 is thought to be endothelial cells that line blood vessels76, but a range of other cell types, including fibroblasts, osteoblasts, monocytes, B cells, T cells and bone marrow stromal cells also produce CSF-1. In mice, CSF-1 levels are in the order of tens of nanograms per millilitre in the circulation and tens of picograms per milligram in a variety of tissues including liver, lung, spleen, kidney, intestine and heart77. CSF-1 levels are dramatically increased upon challenge with lipopolysaccharide (LPS)73 or with infectious agents such as Listeria monocytogenes32, Candida albicans14. In humans, a similar situation is apparent; CSF-1 levels were enhanced in patients with sepsis29, and LPS administration to cancer patients increased CSF-1 levels23. These observations would suggest that CSF-1 is likely to regulate immune responses to infection in both mice and humans.

**Macrophage-Priming by CSF-1**

The requirement for the CSF-1/CSF-1R system in macrophage development makes interpretation of in vivo studies aimed at addressing the role of CSF-1 in immunological responses difficult, since modulation of CSF-1 action affects both monocyte/macrophage production and survival, as well as mature macrophage functions. Additionally, CSF-1 is required for the maintenance of protein synthesis. Jessup et al.43 showed that CSF-1 greatly increased acetylated low-density lipoprotein uptake and foam cell formation, but this was entirely attributable to an increase in cell size; no effect was apparent when these parameters were assessed on a per microgram protein basis. This absolute requirement on CSF-1 for normal macrophage function raises an important issue with regard to the many in vitro studies that are performed on this cell type. Since it is normally present in vivo and because its absence has gross effects on normal cellular functions, CSF-1 should be present in culture medium during in vitro studies on macrophage function. This is often not the case, particularly in inflammatory studies.

A role for CSF-1 in regulating macrophage activation and inflammation is supported by in vitro studies in which CSF-1 enhanced macrophage phagocytic activity against Listeria monocytogenes32, microbicidal action against Leishmania mexicana67, Candida albicans47 and Aspergillus fumigatus42 and primed tumoricidal activity upon triggering with activating agents66. 79, 80. In other studies, CSF-1 did not enhance the ability of macrophages to kill intracellular organisms, including Leishmania major6, Mycobacterium tuberculosis19 and Listeria monocytogenes20. The basis for this inconsistency is unclear.

In contrast to in vitro studies, in vivo studies strongly support a role for CSF-1 in regulating primary immune responses to infection. For example, mice challenged with Listeria monocytogenes had an increased bacterial load following administration of an anti-CSF-1 antibody at the time of challenge52 and CSF-1 transgenic mice were more resistant to Listeria monocytogenes than wild-type controls5. CSF-1 administration also had protective effects for the host against challenge with Listeria monocytogenes99 and Candida albicans14.

As well as enhancing the ability of macrophages to destroy invading pathogens directly, CSF-1 is also a potent regulator of monokine production that regulates the magnitude of the inflammatory response. CSF-1 alone triggered production of mRNAs for interleukin (IL)-6, granulocyte macrophage (GM)-CSF, IL-1α and IL-1β in murine resident peritoneal macrophages25, 45. In other studies with different macrophage populations, CSF-1 alone did not trigger pro-inflammatory cytokine production, but instead acted as a potent priming signal for a subsequent activation stimulus. Pre-treatment of thioglycollate-elicited peritoneal macrophages or bone
zymosan are encountered. When less hazardous products such as CpG DNA and macrophage respiratory burst when zymosan, a TLR2/6 recognition; as a positive regulator of macrophage activation when extreme danger signals such as LPS are detected and to prevent excessive macrophage activation, which is required for responses to CpG DNA, was down-regulated by CSF-1. Levels of TLR2 and 6 mRNA, encoding receptors for Gram-positive components, were also moderately suppressed by CSF-1 (unpublished data and 86).

The biological significance of the potent inhibitory effect of CSF-1 on responses to different activating signals is, in part, mediated by selective effects of CSF-1 on expression of Toll-like receptors (TLRs) 86, which are required for signaling in response to microbial products 89. Whereas CSF-1 did not regulate expression of a variety of TLR members, including TLR3, 4, 5 and 7, expression of TLR9, which is required for responses to CpG DNA, was down-regulated by CSF-1. Levels of TLR2 and 6 mRNA, encoding receptors for Gram-positive components, were also moderately suppressed by CSF-1 (unpublished data and 86).

Mechanisms of CSF-1-Mediated Priming of Cytokine Production

In human monocytes/macrophages, the mechanisms by which CSF-1 primes activation have not been extensively studied. CSF-1 marginally increased expression of CD14 on human monocytes and this may partially account for increased sensitivity to LPS 3. Whether CSF-1 regulates LPS recognition in murine macrophages has not been adequately addressed, but CSF-1 did not regulate expression of the receptor for LPS, TLR4 or early signaling events in response to maximal LPS doses 86. Instead, CSF-1 appears to prime murine macrophage responses to LPS through a number of distinct mechanisms. The synergism between CSF-1 and either LPS or TNF-α for IL-6 production, from resident peritoneal macrophages was largely-mediated by CSF-1-stimulated autocrine GM-CSF production, since synergism was greatly reduced, although not abolished, in GM-CSF-deficient mice 26. The mechanism by which CSF-1 augments IL-12 production is not known, but may also involve autocrine GM-CSF, since this factor synergises with LPS for IL-12 production (unpublished data and 98). Another possibility is that synergy occurs at the transcriptional level, since Ets-2, which binds to and activates the IL-12 p40 promoter 56, 57, is activated in a sustained fashion in response to CSF-1 128. The priming effect for TNF-α production is downstream of mRNA regulation since CSF-1 enhanced LPS-induced TNF-α secretion but not LPS-induced TNF-α mRNA levels in BMM 86. Since CSF-1 is known to increase production of matrix metalloproteinase (MMP) 92, one possibility is that CSF-1 also induces production of the MMP-related protein TNF-α converting enzyme that is required for cleavage of membrane-bound TNF-α 60, 61.

CSF-1 as a Regulator of Leukocyte Recruitment

Apart from affecting macrophage activation, CSF-1 acts at multiple levels to regulate cellular recruitment, a hallmark of inflammatory responses. Firstly, CSF-1 has pronounced effects on macrophage motility. Treatment of the CSF-1-dependent murine macrophage cell line BAC1.2F5 with CSF-1 rapidly triggered actin reorganization, membrane ruffling and cell spreading 2, 11. Further, CSF-1 had chemokinetic and chemotactic activity on human monocytes 86. BAC1.2F5 cells 99 and myeloid progenitor cells-transfected with a c-fms expression plasmid 3. Thus, the enhanced production of CSF-1 during responses to infection may serve to re-
cruit monocytes rapidly to the inflammatory site. Secondly, CSF-1 regulates expression of many genes that encode mediators of adhesion and migration. These include the integrins, α4β1 and α5β1, urokinase plasminogen activator, plasminogen activator inhibitors, and MMPs. Finally, CSF-1 may enhance chemokine production from monocytes/macrophages, thereby allowing for recruitment of other effector cells; CSF-1 increased IL-8 production from human monocytes. The importance of CSF-1 for the recruitment of monocytes during inflammatory responses has also been documented in vivo. Blockade of CSF-1 action suppressed monocyte recruitment during Listeria monocytogenes infection and renal inflammation.

CSF-1 Involvement in Viral Infections

Many viruses, including respiratory syncytial virus, measles virus, dengue virus and HIV-1, replicate within macrophages as a means of escaping immune detection, and in many cases CSF-1 regulates macrophage function during viral infection. This is particularly true for HIV-1, where CSF-1 is an important factor in the HIV-1 replicative strategy. Firstly, HIV-1 replication has been associated with increased CSF-1 production in vivo and replication of HIV-1 within monocyte-derived macrophages triggered CSF-1 production. Secondly, CSF-1 enhanced HIV-1 replication in macrophages, possibly by regulating expression of the HIV-1 co-receptor CCR5. Thus, HIV-1 appears specifically to induce CSF-1 production from macrophages and this, in turn, enhances its ability to replicate within this cell type. CSF-1/CSF-1 receptor antagonists may therefore have therapeutic potential as agents to block HIV-1 replication within macrophages.

Whilst CSF-1 is a positive regulator of HIV-1 replication, it may be protective to the host for other viral pathogens. Intranasal administration of CSF-1 offered almost complete, protection to BALB/c mice upon challenge with otherwise lethal doses of Sendai virus, and CSF-1 treatment of macrophages in vitro induced resistance to vesicular stomatitis virus. Interestingly, Epstein-Barr virus (EBV) encodes a protein, BARF1, that bound to and neutralized CSF-1. Although the biological consequences of this have not been addressed, one might predict that blockade of CSF-1 signaling via BARF1 would provide a selective advantage to EBV over the host. Indeed, there are reports of CSF-1R expression on B cells, which may be relevant to EBV infection. Whilst some of the anti-viral actions of CSF-1 may be attributed to production of type 1 and type 2 interferons, one report demonstrated that a CSF-1 expression construct, when administered with a DNA vaccine, enhanced cytotoxic T lymphocyte responses. Therefore, CSF-1 may also enhance the development of antigen-specific anti-viral mechanisms.

Effects of CSF-1 on the Acquired Response

Whilst most of the studies described above indicate that CSF-1 enhances macrophage activation and the innate response, CSF-1 is actually immunosuppressive for antigen-specific responses. T cell responses against allogeneic cells were suppressed in the presence of CSF-1-stimulated macrophages from tumor-bearing hosts, and CSF-1 suppressed MHC class II expression in the placenta. Other studies have substantiated this immunosuppressive effect of CSF-1 in vitro and CSF-1 administration in vivo suppressed proliferation of purified splenocytes to T cell mitogens.

Apart from targeting MHC class II expression, the immunosuppressive effect of CSF-1 may be dependent on the enzyme indoleamine 2,3-dioxygenase (IDO), which was induced in co-cultures of CSF-1-derived macrophages and T cells. IDO rapidly degrades tryptophan, an amino acid that is essential for T cell proliferation. Several studies have reported inducible expression of membrane-bound CSF-1 on T cells upon activation, possibly by regulating expression of the HIV-1 co-receptor CCR5. Thus, HIV-1 appears specifically to induce CSF-1 production from macrophages and this, in turn, enhances its ability to replicate within this cell type. CSF-1/CSF-1 receptor antagonists may therefore have therapeutic potential as agents to block HIV-1 replication within macrophages.

Whilst CSF-1 is a positive regulator of HIV-1 replication, it may be protective to the host for other viral pathogens. Intranasal administration of CSF-1 offered almost complete, protection to BALB/c mice upon challenge with otherwise lethal doses of Sendai virus, and CSF-1 treatment of macrophages in vitro induced resistance to vesicular stomatitis virus. Interestingly, Epstein-Barr virus (EBV) encodes a protein, BARF1, that bound to and neutralized CSF-1. Although the biological consequences of this have not been addressed, one might predict that blockade of CSF-1 signaling via BARF1 would provide a selective advantage to EBV over the host. Indeed, there are reports of CSF-1R expression on B cells, which may be relevant to EBV infection. Whilst some of the anti-viral actions of CSF-1 may be attributed to production of type 1 and type 2 interferons, one report demonstrated that a CSF-1 expression construct, when administered with a DNA vaccine, enhanced cytotoxic T lymphocyte responses. Therefore, CSF-1 may also enhance the development of antigen-specific anti-viral mechanisms.

CSF-1 and Chronic Inflammatory Responses

The involvement of macrophages in chronic inflammatory diseases such as rheumatoid arthritis (RA) is well established and the effects of CSF-1 in such diseases have also been studied. In collagen-induced arthi-
ritis, a mouse model of RA, administration of CSF-1 exacerbated disease severity whilst an anti-CSF-1 antibody reduced the severity of established arthritis. CSF-1 has also been implicated as a contributor to disease severity in other arthritic models. Evidence exists for CSF-1 involvement in RA itself; CSF-1 levels were elevated in RA patient sera and synovial fluid, and synovial fibroblasts from RA patients produce CSF-1.

Apart from arthritic diseases, there is an extensive literature on the contribution of CSF-1 to kidney disease. Macrophage accumulation is a predictor of renal outcome in glomerulonephritis and correlates with kidney dysfunction in humans, and elevated levels of renal CSF-1 are apparent in glomerulonephritis patients. Other studies have also documented enhanced CSF-1 levels in sera of patients with chronic renal disease. In experimental disease models, there is clear evidence for the involvement of CSF-1 in directing excessive macrophage proliferation and tissue damage. The severity of lupus nephritis in MRL-lpr mice correlated with CSF-1 levels, treatment with anti-CSF-1R antibody reduced local macrophage proliferation during experimentally induced renal inflammation, and treatment of mice with CSF-1 enhanced LPS-induced glomerular macrophage accumulation and proteinuria.

**Conclusion**

Determination of the exact roles of CSF-1 during immune responses has been difficult because of its essential role in the differentiation, proliferation and normal cellular function of macrophages. Nonetheless, numerous studies have documented effects of CSF-1 on macrophage activation in vitro and inflammatory responses in vivo. The fact that CSF-1 levels are elevated during responses to infection in mice and humans strongly supports the concept that this factor has key immunoregulatory functions. As with other pro-inflammatory cytokines, CSF-1 exerts protective effects against many infectious agents (with some notable exceptions), but elevated levels of CSF-1 may contribute to pathology in both acute and chronic inflammatory diseases. Hence, specific antagonists of CSF-1 action may have therapeutic potential as a means of preventing pathology associated with excessive macrophage accumulation and activation.

**Acknowledgment.** This work was supported by the NHMRC of Australia through a C. J. Martin fellowship (Reg Key No. 987038).

**References**

14. CENC E., BARTOCCHI A., Puccetti P., MOCCI S., STANLEY E. R.


Received in January 2003
Accepted in February 2003