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Therapeutic Tissue Regeneration by a Macrophage Colony-Stimulating Factor Fc Conjugate

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Macrophage colony-stimulating factor 1 (CSF1) is a hematopoietic growth factor involved in the proliferation, differentiation, and survival of monocytes, macrophages, and bone marrow progenitor cells. Current clinical interest in CSF1 lies in relatively short-term treatments using recombinant protein for applications such as tissue regeneration. However, because the small size of the CSF1 protein ensures rapid renal clearance, a continuous intravenous application is needed to maintain an effective dose. In this issue of Molecular Therapy, Göw et al. describe how they addressed this issue by developing an Fc conjugate of CSF1. They then go on to dissect its biological effects and capacity to induce tissue regeneration, showing that the conjugate augments the circulating half-life of the protein, which in turn stimulates the capacity of the mononuclear phagocyte system (MPS) to induce tissue regeneration in the liver. Although this provides us with an exceptional new therapeutic tool in situations in which liver homeostasis is compromised, caution is required before moving into clinical application, because of the possible effects on other organs and tissues.

The MPS is a part of the immune system that is composed primarily of monocytes and macrophages that accumulate in lymph nodes and the spleen, and it includes the Kupffer cells of the liver. In many tissues, macrophages are derived from progenitor cells of fetal origin or, possibly, circulating monocytes, and these tissue-resident macrophages may self-renew by proliferation. During infection or inflammation there is generally an influx of monocytes that will also differentiate to become macrophages. CSF1 is essential to macrophage survival and function, and there are profound macrophage deficiencies in op/op mice carrying an inactive CSF1 gene.6 Besides playing a vital role in innate immunity, the MPS is involved in other essential processes, such as the regulation of inflammation, scavenging of tissue debris, angiogenesis, and facilitating tissue repair. An initial human studies have indicated that recombinant CSF1 exhibits a broad action profile by activating the MPS and influencing a variety of other processes, such as enhancing antibody-dependent cytotoxicity, augmenting macrophage-mediated microbial killing, and lowering of blood cholesterol and circulating platelets.7 As illustrated by Hume and MacDonald,8 a number of studies have since established the pleiotropic effects of CSF1.

These findings have sparked interest in CSF1 as a therapeutic agent. During wound healing, tissue-resident macrophages expand in response to CSF1. Together with recently recruited macrophages, they engulf apoptotic cells and clear cell debris, forming a first line of immune defense and fostering tissue repair. The significance of their role in tissue repair has been established by depletion studies showing failed regeneration in the absence of macrophages.11 Gow et al. observed similar regenerative effects in the livers of CSF1-Fc-treated animals. The engineered Fc-bound form gave rise to a 10- to 100-fold greater concentration of CSF1 in vivo that was maintained for up to 72 hours. As depicted in Figure 1a, the Fc-fusion protein was able to achieve this by avoiding renal clearance and via protection from endosomal degradation in the MPS through binding to the Brambell receptor (neonatal Fc receptor) upon internalization, resulting in recycling into the extracellular milieu. As illustrated in Figure 1b, liver-resident Kupffer cells were activated by CSF1-Fc to support matrix remodeling and hepatocyte proliferation, probably by expression of the urokinase plasminogen activator (Plau) and production of tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6). This was accompanied by increased spleen size, which was due mainly to extramedullary hematopoiesis and not directly related to the tissue-regeneration response.
It is both surprising and highly clinically relevant that CSF1-Fc is able to increase the size of a healthy liver by induction of a local tissue-repair response. CSF1-Fc may be applicable in several clinical situations. Certain chemotherapeutics, for example, rely on active liver metabolism for clearance or activation and can induce liver damage as a side effect. Peritherapeutic overdosing or toxin-induced liver injury.

The study by Gow et al. suggests that CSF1 might also be beneficial in the resolution phase of heart diseases, spinal cord injury, fertility issues, or bone repair.

A major issue to be addressed is the targeting of the agent. As noted above, CSF1 has a broad activity spectrum, and targeting specific tissues might help to focus these activities to address clinical needs. In addition to extending the compound’s half-life, conjugation to Fc appears to provide partial targeting to the liver, particularly to Kupffer cells, the primary blood-exposed phagocytes of the MPS. Interestingly, a previous study using recombinant CSF1 (ref. 12) indicated that the kidney, not the liver, was the primary target organ of CSF1 in mice, and it showed efficacy of recombinant CSF1 on kidney tissue repair after ischemic reperfusion injury. At first, this seems contradictory, but it actually shows that tissue targeting of CSF1 is possible and effective; in the study by Alikhan et al.,12 the recombinant CSF1 was secreted primarily by the kidneys, which therefore were exposed to the highest concentrations of CSF1. By contrast, in the study by Gow et al.,1 the Fc conjugate rescued CSF1 from endosomal degradation in Kupffer cells, ensuring accumulation of high local CSF1 concentrations in liver sinusoids. Renal excretion was excluded by the size of the conjugate, thereby rendering the liver the

Figure 1 Working mechanism of CSF1-Fc versus recombinant CSF1. (a) Left panel: recombinant colony-stimulating factor 1 (CSF1) is rapidly degraded by lysosomal degradation following pinocytosis and CSF1 receptor (CSF1R)-mediated uptake, mainly by liver-residing Kupffer cells. Additionally, owing to its small protein size, CSF1 is rapidly cleared by renal excretion. Collectively this results in a very short circulating half-life and low bioavailability of recombinant CSF1. Right panel: The CSF1-Fc conjugate protein has an augmented circulating half-life and therefore strongly enhanced bioavailability and efficacy. It is rescued from lysosomal degradation by binding of its Fc portion to the neonatal Fc receptor (FcRN), leading to recycling into the extracellular milieu. Moreover, because of its increased protein size, it is not directly excreted by the kidneys. (b) CSF1-Fc induces hepatocyte proliferation by, for example, inducing expression of Plau in Kupffer cells via the CSF1R, which induces the urokinase plasminogen activator (uPA). This mediates remodeling of the extracellular matrix as well as releasing and activating hepatocyte growth factor. Additionally, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) are produced in the livers of CSF1-Fc–treated mice. Unlike to be induced directly by CSF1 triggering, it might be that in vivo, through accumulation of CSF1-Fc in liver sinusoids, the Fc portion of the CSF1-Fc conjugate achieves sufficient triggering of the CD64 Fc receptor to induce a proinflammatory response in the liver Kupffer cells. This could induce production of cytokines such as TNF-α and IL-6, which prime hepatocytes for growth and proliferation in response to hepatocyte growth factor.
primary target organ. These results invite further investigation into antibody- and Fc-receptor-mediated targeting of CSF1 to different tissues, with the aim of resolving specific pathological situations.

A major limitation of prolonged CSF1 use is its effects on the immune system: CSF1 reduces the expression of Toll-like receptors 1, 2, 6, and 9 on induced monocytes or macrophages, thereby diminishing effective immune sensing of bacteria, fungi, and viruses. Additionally, the expression of the purinergic P2x7 receptor is augmented, which can exaggerate NLRP3 inflammasome-dependent inflammatory responses driven by extracellular adenosine triphosphate, such as those that occur during bacterial infections or contact hypersensitivity responses. Moreover, the differentiation and expansion of dendritic cells (DCs) is under CSF1 control. CSF1 differentiates and expands dendritic hypersensitivity responses. Moreover, the cur during bacterial infections or contact

Another concern is the other ligand of the CSF1 receptor (CSF1R): IL-34. Persistent occupation of the CSF1R by exogenous CSF1 or CSF1-Fc might outcompete IL-34 for CSF1R interaction. This could lead to overactivation of as-yet-unknown alternative IL-34 targets or attenuation of IL-34-driven immune activity through CSF1R. Finally, the use of a fusion protein, even if fully humanized, raises the possibility of generating a (neutralizing) immune response, which is important for applications involving prolonged or repetitive use. These issues notwithstanding, the study by Gow et al. establishes CSF1-Fc as a novel therapeutic agent for possible use in liver regeneration that also holds promise for novel therapeutic interventions through its functional impact on the MPS.

REFERENCES