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Role of Colony-Stimulating Factor-1 in Bone Metabolism

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Abstract Colony-stimulating factor-1 (CSF-1) is a cytokine required for proliferation, differentiation, activity, and survival of cells of the mononuclear phagocytic system. The growth factor is synthesized as a soluble, matrix, or membrane associated molecule. The specific functions of these forms are not clear. However, some data suggest a dependence of the development of various populations of tissue macrophages on the locally expressed and presented cytokine. Deficiency in CSF-1, as is the case in the murine mutant strain *op/op*, results in low numbers of macrophages and monocytes and, most striking, leads to osteopetrosis due to a virtual absence of osteoclasts. Using the *op/op* mutation as a model, CSF-1 was established as one of the growth factors for osteoclasts. The expression of CSF-1 receptors, encoded by the proto-oncogene *c-fms,* by osteoclast precursors and osteoclasts, suggested an effect of this cytokine not only during osteoclast formation but also on the mature cells. In fact, CSF-1 was shown to inhibit the resorbing activity, to stimulate migration, and to support survival of isolated osteoclasts in vitro. By these actions on cells of the osteoclast lineage, CSF-1 induces recruitment of new osteoclasts, leading to a net increase of bone resorption, and might govern the spatial distribution of resorption sites within the bone. During these processes, locally expressed and presented forms of the growth factor may play a crucial role, as will be discussed in this article. © 1994 Wiley-Liss, Inc.

Key words: osteoclast formation, resorption, CSF-1, bone, cytokine

Colony-stimulating factor-1 (CSF-1, also called macrophage colony-stimulating factor or M-CSF) is a lineage-specific cytokine required for proliferation, differentiation, survival, and activity of the cells of the mononuclear phagocytic system (MNPS). The growth factor is synthesized by mesenchymal cells such as fibroblasts, osteoblasts, or endothelial cells, but also by activated macrophages. Southern blot analysis revealed a single copy of the gene to be present both in the human and murine haploid genome. The gene, in human assigned previously to chromosome 5 and recently to chromosome 1 and in mouse to chromosome 3, contains 10 exons, of which exons 1–8 encode translated sequences. Sequence homology between human and murine CSF-1 is almost 70%. Investigations on species cross-reactivity revealed that human CSF-1 acts on murine, rat, and rabbit cells.

Alternative splicing yields several cytoplasmic mRNAs, derived from a common nuclear transcript (Fig. 1). The most abundant mRNA, both in human and murine cells, has a size of about 4 kb. In human, further transcripts of 2.4, 2.0, and 1.6 kb are found, in mouse such of 2.3 and 1.6 kb. All mRNA species except the 1.6 kb transcript contain the complete exon 6 of the M-CSF gene. The exon 6 encoded sequences are important in directing post-translational processing, as will be described later. The large transcripts, 4 kb and 2 kb, encode identical, rapidly secreted proteins, while the translation product of the 1.6 kb mRNA is integrated into the membrane and only slowly released [for reviews see Stanley, 1990; Sherr and Stanley, 1990].

All transcripts encode an aminoterminal signal peptide of 32 amino acids and a hydrophobic region of 23 amino acids, followed by a charged "stop transfer" sequence. This structure at the carboxyterminus is characteristic for many membrane-spanning proteins. During synthesis, the CSF-1 molecule is co-translationally glycosylated and remains integrated in the membrane, with the aminoterminal end in the lumen of the endoplasmic reticulum. Two peptide chains form a dimer, linked by a disulfide bridge. In the Golgi, they are O-glycosylated and the N-linked carbo-

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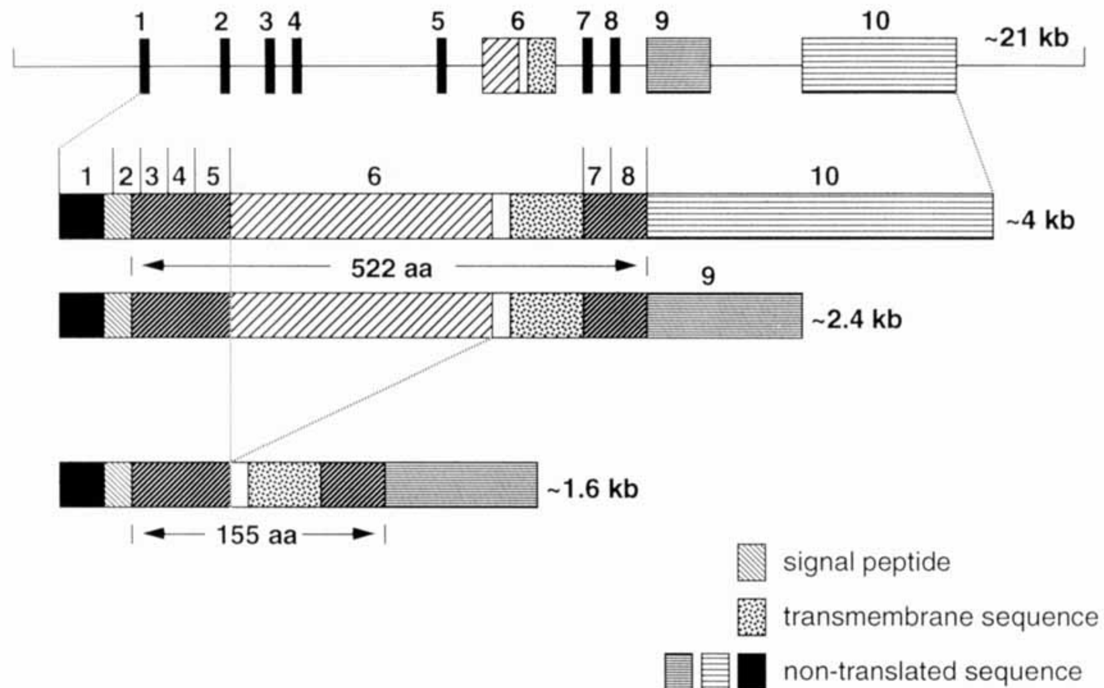

Fig. 1. Genomic organization and transcripts for human CSF-1. The three transcripts are derived from a common RNA precursor by alternative splicing. While the 4 kb and the 2.4 kb mRNAs encode an identical, rapidly secreted protein, the form of CSF-1 encoded by the 1.6 kb mRNA is integrated in the cell membrane and only slowly released. Reproduced from Stanley [1990].

hydrate moieties are further processed. CSF-1 can be further modified by the attachment of a glycosaminoglycan side chain to an exon 6 encoded amino acid [Price et al., 1992; Suzu et al., 1992]. This form of the growth factor binds to collagen type V. Further, it has been suggested that the proteoglycan adheres to the extracellular matrix, and indeed, this molecular form of the growth factor binds to pepsin extracted bone collagen and was found in bone [Ohtsuki et al., 1993].

Posttranslational processing of CSF-1 depends on the presence of exon 6 sequences within the transcripts. Besides a transmembrane domain, exon 6 also encodes a stretch of amino acids containing a proteolytic cleavage site, which is hydrolysed to release the peptide from the transmembrane domain. This processing occurs within the secretory vesicles of the cells, and the mature peptides are rapidly secreted. If exon 6 sequences are lacking in the transcript, as is the case for CSF-1 encoded by the 1.6 kb mRNA, the proteolytic cleavage site is missing in the translation product and therefore no hydrolysis occurs. Consequently, the peptide remains integrated in the cell membrane and is only slowly released from the cell surface [Rettenmier et al., 1987] (Fig. 2).

The effects of CSF-1 on its target cells are mediated by interaction with a single class of high affinity receptors which are expressed primarily on cells of the MNPS. CSF-1 receptors have been detected also on placental trophoblasts and on smooth muscle cells. Furthermore, the bone resorbing cells, the osteoclasts, contain CSF-1 receptor transcripts and express binding sites, as presented in this article. The receptor belongs to a family of growth factor receptors with an intrinsic tyrosine kinase activity and is encoded by the proto-oncogene *c-fms*. Binding of CSF-1 induces autophosphorylation within the intracellular domain of the receptor. The ligand-receptor complex is then internalized and subsequently degraded, and cell surface binding sites are rapidly down-modulated.

Ligand activation of the receptor kinase serves as the proximal signal that eventually elicits the mitogenic response. CSF-1 is required through the entire G1 phase of the cell cycle in order for mononuclear cells to enter S phase, but the growth factor is not necessary through S, G2, and M phases. Complete removal of the growth

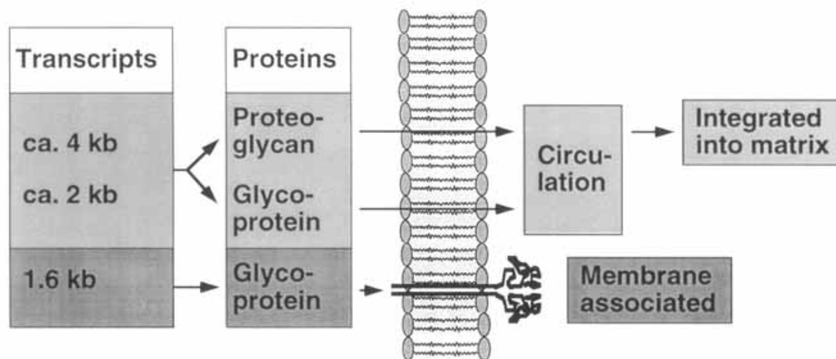

Fig. 2. Molecular forms of CSF-1. Three molecular forms of CSF-1 have been demonstrated. The growth factor is secreted rapidly as a glycoprotein or a proteoglycan. The proteoglycan form can be bound to the extracellular matrix. The translation product of the 1.6 kd mRNA is integrated into the cell membrane, from where it is slowly released by proteolytic cleavage.

factor, also in presence of serum, induces cells to enter a quiescent state and ultimately to die within several days.

CSF-1 induces both immediate and temporally delayed responses during G1 phase. Among the most rapid are alterations in membrane structure and increased pinocytosis. These events are accompanied by a rise in intracellular pH through an effect on the amiloride-sensitive Na^+/H^+-antiporter with compensatory stimulation of the Na^+/K^+-ATPase activity. The transcription of "immediate early" genes, such as *c-fos* and *c-myc,* is increased within minutes up to a few hours after CSF-1 stimulation. Furthermore, glucose uptake and protein synthesis are stimulated.

The mechanism of signal transduction upon CSF-1 binding is not clear as of yet. The growth factor initiates tyrosine phosphorylation of the receptor and of many not yet identified intracellular proteins. An association of phosphatidylinositol 3-kinase and of the *src* family kinases with the intracellular domain of the receptor was demonstrated [Courtneidge et al., 1993]. Whereas the *src* kinases seem to play a part in the signal transduction pathway leading to mitogenesis, phosphatidylinositol 3-kinase is not required for ligand stimulated cell growth. CSF-1 stimulation of diglyceride production via hydrolysis of phosphatidylcholine by phospholipase C has been found as a component of the signal transduction pathway leading to transcription of *c-fos* and *c-junB* [Xu et al., 1993]. The growth factor also increases hydrolysis of phosphatidylcholine by activating the cytoplasmic phospholipase A2 [Nakamura et al., 1992]. The signal events responsible for the stimulation of this enzyme are not known. Furthermore, a rapid phosphorylation of protein tyrosine phosphatase-1C on tyrosine in response to CSF-1 has been described [Yeung et al., 1992]. This observation could, however, not be confirmed by another group [Yi and Ihle, 1993]. There are many questions left open about CSF-1 signal transduction, either in macrophages or in fibroblasts transfected with *c-fms.* For more information on CSF-1 effects on these cells see the reviews by Sherr and Stanley [1990] and Vairo and Hamilton [1991].

THE *op/op* MOUSE: A NATURAL KNOCKOUT MODEL

Animals defective in the production of growth factors represent ideal models to study the effects of these proteins during development and function of organs and cells. However, animals with inborn deficiencies in the production of growth factors are scarce. This is thought to be due to their essential role during prenatal development, so that their absence will lead to abortion of the embryo. To our knowledge, three murine strains carrying mutations in a growth factors or a growth factor receptor have been described. The *SI* mutant is defective in the production of stem cell factor, also called mast cell growth factor, Steel factor, or Kit ligand; the *W* (dominant white spotting locus) mutant synthesizes a defective *c-kit* proto-oncogene product, the receptor for the Kit ligand [Broxmeyer et al., 1991]; the *op/op* mutant displays an impairment in the production of CSF-1 [Wiktor-Jedrzejczak et al., 1990; Felix et al., 1990a].

The murine mutant strain *op/op* is characterized by an osteopetrotic phenotype due to im-

paired bone resorption. At the cellular level, the numbers of monocytes and macrophages are reduced and osteoclasts are virtually absent. The mice lack biologically active CSF-1, due to a point mutation within the coding region of the CSF-1 gene [Yoshida et al., 1990]. This mutation is not lethal. However, since the teeth do not erupt, the animals have to be fed a soft diet to survive. As will be described in this article, studies on the *op/op* mutant demonstrated that macrophages of various tissues differ in their dependence on circulating CSF-1. Furthermore, using this mouse strain as a model, CSF-1 was proven to be a growth factor for osteoclasts.

THE EFFECT OF CSF-1 ON THE GROWTH OF MACROPHAGES

CSF-1 was originally characterized as the growth-, differentiation-, activation-, and survival factor for cells of the MNPS [Stanley et al., 1983]. The cytokine may be part of the tissue specific microenvironment, since it is assumed that the differentiation of resident macrophages is governed by tissue specific matrix components and/or locally produced growth factors. Therefore, the CSF-1 deficient *op/op* mouse was an appropriate model to elucidate the dependence of tissue macrophages on this cytokine [Naito et al., 1991].

To study the influence of CSF-1 administered to *op/op* mice, macrophages bearing the marker antigen F4/80 were investigated in phenotypically normal +/*?* and compared to osteopetrotic *op/op* littermates up to an age of 3 months. In phenotypically normal animals, the density of F4/80$^+$ cells either decreased, transiently increased, or gradually increased with age, depending on the tissue, whereby the highest levels generally correlated with the period of maximum organogenesis and/or cell turnover. In *op/op* mice, tissues of macrophage populations could be classified as either absent, reduced, initially normal and then subsequently reduced, or normal [Cecchini et al., in press].

To assess the dependence of macrophage development on soluble CSF-1, *op/op* animals, in which the concentration of circulating CSF-1 were corrected by daily injections above normal levels, were analyzed. The results demonstrated differences in the dependence on CSF-1 among the different populations of tissue macrophages and in the presentation of the growth factor to the target cells [Cecchini et al., in press].

A first group of cells, among them the resident bone marrow macrophages and the macrophages of the connective tissues, was absent in newborn *op/op* mice. These cell populations differ in their response to treatment with CSF-1. While the bone marrow macrophages were partially restored by circulating CSF-1, the macrophages of the connective tissues were not corrected at all. This suggests that the cells are fully dependent on CSF-1 during prenatal development and that the inborn CSF-1 deficiency can not be overcome by circulating maternal cytokine. The failure to fully restore these macrophage populations by CSF-1 administration might be due to the limited access of the growth factor to the tissue sites where macrophages develop. Alternatively, the growth of these cell populations might be regulated mainly by membrane and/or matrix bound CSF-1. The macrophage populations of a second group, among them the Kupffer cells of the liver, were normal at birth, decreased during postnatal development, and recovered completely after CSF-1 treatment, suggesting that these cells grow prenatally in response to circulating maternal CSF-1 passing through the placenta. The last group of macrophages, such as Langerhans cells, bone marrow monocytes, macrophages of the thymus, and lymph nodes, were not decreased in newborn *op/op* mice and were not affected by the administered circulating CSF-1. These cell populations are involved in inflammatory and immune responses and appear to require other growth factors, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) [Wiktor-Jedrzejczak et al., 1992]. These findings suggest that in contrast to the function of GM-CSF in immunological defense, CSF-1 would be involved in organogenesis and tissue turnover by supporting the growth of tissue macrophages. The osteoclasts participating in bone turnover can also be included in this group.

THE EFFECT OF CSF-1 ON OSTEOCLASTOGENESIS

Several years ago, various laboratories demonstrated osteoblasts in vitro to synthesize colony stimulating factors, including CSF-1. The involvement of these factors in osteoclast development however was not clear. For CSF-1, contradictory observations were reported, some supporting, others negating a role in osteoclastogenesis. Only the studies on the murine osteopetrotic mutant strain *op/op* demonstrated that

CSF-1 is one of the growth factors for osteoclasts.

The Role of CSF-1 in Osteoclast Formation In Vivo

For a long time it has been known that transplantation of normal hemopoietic cells into *op/op* recipients did not restore formation of phagocytic mononuclear cells. This indicated that the defect in the *op/op* mouse does not reside within the hemopoietic compartment. Wiktor-Jedrzejczak and coworkers further observed that marrow and spleen cells from these animals gave rise in vitro to almost homogeneous colonies of fibroblastoid cells, containing only very low numbers of macrophages. They suggested that *op/op* mice are deficient in a colony stimulating activity that supports differentiation of cells of the monocyte-macrophage lineage [Wiktor-Jedrzejczak et al., 1982]. For a long time this message was not appreciated by other laboratories. In 1988 we reported that whole calvaria, calvaria cells, and fibroblasts from *op/op* mice do not produce CSF-1 activity in vitro, in contrast to tissue and cells from phenotypically normal littermates [Felix et al., 1988; Felix et al., 1990a]. This finding was then confirmed by other laboratories [Yoshida et al., 1990; Wiktor-Jedrzejczak et al., 1990].

The questions arose whether CSF-1 was an obligate requirement for osteoclastogenesis and whether treatment of *op/op* mice with CSF-1 would reverse the osteopetrotic phenotype. This was shown to be the case, since daily injections of CSF-1 induced osteoclast formation in these animals [Felix et al., 1990b; Kodama et al., 1991]. As a consequence, the osteopetrotic phenotype was reversed. This observation demonstrated that CSF-1 is an essential growth factor for cells of the osteoclast lineage.

Subsequently, a single injection of CSF-1 was shown to be sufficient to induce osteoclastogenesis [Kodama et al., 1993]. Osteoclasts appeared within 2 days after CSF-1 administration and were most numerous between 4 and 9 days. After 25 days, the number of osteoclasts was decreased to 20% of the maximum. It came as a surprise that osteoclast formation proceeded after a single CSF-1 injection, although the factor is cleared very efficiently from circulation. Furthermore, in the absence of the growth factor, osteoclasts could survive for a minimum of 2 weeks.

The Role of CSF-1 in Osteoclast Formation In Vitro

The dependence of osteoclast formation on CSF-1 was further confirmed in two in vitro systems. In one of these systems, murine embryonic metatarsals/metacarpals were cultivated in the presence or absence of CSF-1. In the other system, osteoblasts from *op/op* or +/? mice were cultured together with hemopoietic precursors, also derived from both types of mice, as a source of osteoclast progenitor cells.

In metatarsals from 16-day-old murine embryos, locally proliferating, tartrate resistant acid phosphatase (TRAP)-negative osteoclast precursors are present on the outer diaphyseal surface. One day later, in 17-day-old embryos, the osteoclastic precursors proceeded to a postmitotic, TRAP-positive, stage. At day 18, the mineralized cartilage core within the rudiment is resorbed by invading, multinuclear, mature osteoclasts and replaced by bone [Scheven et al., 1986].

When metatarsals/metacarpals from normal 17-day-old embryos were cultured in the presence of 5–10 ng/ml of CSF-1 [Antonioli-Corboz et al., 1992], both osteoclast formation and mineral resorption were increased. This effect was abolished, when the bones were irradiated before the culture period, suggesting CSF-1 action on proliferating osteoclastic precursor cells. In this experimental system, osteoclast formation was stimulated at concentrations that are required to support growth of macrophages in vitro. The growth factor also was able to induce osteoclastogenesis in metatarsals from *op/op* mice, however, only at much higher concentrations [Morohashi et al., 1994].

The function of CSF-1 in osteoclastogenesis was further investigated in a coculture system, in which hemopoietic precursor cells from the spleen proliferate and differentiate to TRAP-positive osteoclasts in the presence of osteoblasts [Takahashi et al., 1991a]. Cocultures of precursors and stromal osteoblastic cells from *op/op* and +/? littermates gave rise to osteoclasts only, when the mesenchymal cells as the source for CSF-1 were derived from phenotypically normal +/? animals. The origin of the hemopoietic precursors was of no importance in these experiments. When the osteoblasts were derived from *op/op* mice, osteoclast formation was observed only upon addition of very high

concentrations (100 ng/ml) of exogenous CSF-1. These results demonstrated that osteoblast-derived CSF-1 is essential for osteoclast formation.

The coculture system also helped to determine during which period of the development of osteoclastic precursors to mature osteoclasts CSF-1 was required. As mentioned above, the CSF-1 induced increase of osteoclast recruitment in embryonal metatarsals was blocked by irradiation, demonstrating an effect of the factor on proliferative precursors. In cocultures, antibodies against CSF-1 or its receptor could inhibit osteoclast formation when added either during the period of proliferation or of differentiation, suggesting that the cytokine stimulates growth as well as differentiation of precursor cells [Tanaka et al., 1993]. Neutralising antibodies against GM-CSF had no effect on osteoclast formation. Thus this cytokine seems not to be involved in the processes of osteclastogenesis. However, precursor cells grown in the presence of GM-CSF or interleukin 3 are capable to form osteoclasts in coculture with osteoblasts [Takahashi et al., 1991b]. These cytokines may therefore support proliferation of very early precursor cells, but are unable to replace CSF-1 at later developmental stages.

Expression of CSF-1 and CSF-1 Receptors in Bone

After the demonstration of CSF-1 being an obligate requirement for osteoclastogenesis and consequently bone resorption, the question arose, whether CSF-1 is produced in bone, and which cells would express the receptor for this growth factor. To investigate time and site of the expression of growth factor and receptor, in situ hybridization was performed on embryonic murine metatarsals representing different stages of bone formation and osteoclast development.

In metatarsals from embryos younger than 15 days, no CSF-1 transcripts were detected. On day 16, low expression was observed in a portion of the cells lining the rudiment (Table I). The expression increased during the following 24 h, but was still limited to the bone surface. Only at day 18 and later, a signal for CSF-1 transcripts could be detected within the metatarsal rudiment. The intensity of the CSF-1 signal was further increased in the bones of 1-day-old animals. In more mature bones, CSF-1 expressing cells were lining all bone surfaces, be it cortical or trabecular bone [Hofstetter et al., 1991].

Transcripts for the CSF-1 receptor, *c-fms,* were not detected within cells in close relation to the surface of or within the metatarsal rudiments from 16-day-old embryos (Table I). One day later, few cells on or close to the bone collar were positive for *c-fms* transcripts. At the same time, TRAP-positive cells were observed at the same location. At day 18, *c-fms* transcripts were evident in individual cells within the metatarsals at sites, where TRAP-positive, multinuclear osteoclasts resorb the mineralized cartilage. Furthermore, isolated osteoclasts were shown to contain transcripts for *c-fms* [Hofstetter et al., 1992]. In the same tissues and cells, the expression of binding sites was confirmed by binding studies with [^{125}I]CSF-1 [Hofstetter et al., 1993].

These experiments demonstrated that: (1) transcription of the gene for CSF-1 is locally and temporally related to osteoclastogenesis, and (2) CSF-1 receptors are not only expressed by TRAP-positive, mononuclear osteoclast precursors, but also by mature osteoclasts. Therefore it is suggested that local sources for CSF-1 govern the recruitment and activation of osteoclasts during bone development via a direct action on osteoclastic precursors and the mature cells.

TABLE I. Differentiation of Osteoclasts and Detection of CSF-1 and *c-fms* Transcripts in Developing Murine Metatarsals*

	Days after conception			
	16	17	18	19
Osteoclast differentiation stage	Mitotic mononuclear TRAP-negative	Post-mitotic mononuclear TRAP-positive	Multinuclear TRAP-positive	Multinuclear TRAP-positive
CSF-1	(+)	+	+	+
c-fms	–	+	+	+

*Transcripts encoding CSF-1 and its receptor *c-fms* are locally and temporally related to osteoclastogenesis. + = transcripts could be detected; – = no transcripts could be detected.

The demonstration of CSF-1 receptors on mature osteoclasts indicates that osteoclasts are closely related to macrophages, since *c-fms* expression is considered to be a marker for the MNPS among the cells of the hemopoietic compartment [Guilbert and Stanley, 1980]. The macrophage populations from different tissues are heterogeneous. Thus osteoclasts may represent a population of bone-specific macrophages, and the stimulus required for the differentiation would be provided by bone cells and the extracellular matrix.

Action of CSF-1 on Mature Osteoclasts

Already several years ago, an inhibitory effect of CSF-1 on active osteoclasts in an in vitro resorption assay was reported [Hattersley et al., 1988]. These results were surprising, because the cytokine would exert opposite effects on bone resorption. On one side, the growth factor increases bone resorption by supporting osteoclast formation, on the other side CSF-1 would inhibit osteoclast activity. However, such an inhibitory action of CSF-1 was not observed in other resorption assays. No effect of the growth factor could be detected in cultured calvariae or radii, two organ culture systems in which osteoclast recruitment is negligible and resorption is completely dependent on already present, mature osteoclasts [Antonioli-Corboz et al., 1992]. Finally, CSF-1 does not induce contraction or alter cytoplasmic mobility in disaggregated osteoclasts, in contrast to other inhibitors of bone resorption acting directly on the mature osteoclasts, like calcitonin and prostaglandins. Consequently, the effect of CSF-1 on mature osteoclasts remained somewhat controversial.

Recently, new data on the action of CSF-1 on mature osteoclasts were reported [Fuller et al., 1993]. The cytokine, acting as a chemotactic agent, stimulated spreading and migration of isolated osteoclasts in vitro and supported survival of the cells by preventing apoptosis. Within this experimental system, total osteoclastic resorptive activity was inhibited by reducing the proportion of resorbing cells. The authors put forward the hypothesis that CSF-1 in vivo acts on the mature osteoclasts on resorptive sites (Fig. 3). Cells of the osteoblastic lineage adjacent to the osteoclasts, would control the balance between migrating and resorbing cells by expressing the growth factor. Through its chemotactic activity, CSF-1 would regulate not only the quantity, but also the spatial pattern of resorption.

In conclusion, CSF-1 might be a factor regulating the remodelling of the bone by two mechanisms. It would exert a stimulating effect on resorption, supporting local recruitment of osteoclasts. Furthermore, the cytokine might regulate the spatial distribution of resorption sites within the bone by inducing a chemotactically directed migration of the mature osteoclasts.

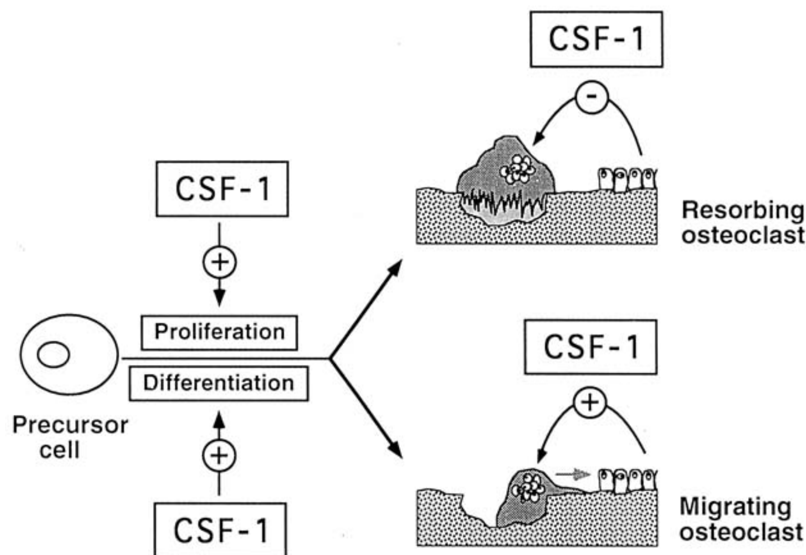

Fig. 3. Target cells for CSF-1 action. CSF-1 is required both for proliferation and differentiation of progenitor cells. Furthermore, CSF-1 acts chemotactically on mature osteoclasts, inhibiting resorption and inducing migration on these cells.

UNRESOLVED QUESTIONS

The Molecular Forms of CSF-1 Differ in Their Biological Effects

CSF-1 is synthesized either as a soluble, a matrix, or a membrane bound molecule [Price et al., 1992; Rettenmier et al., 1987; Suzu et al., 1992]. The different molecular forms of CSF-1 may mediate specific biological effects, and may fulfill different functions during osteoclast development. The soluble growth factor would act mainly on early precursor cells, whereas the membrane or matrix bound form could then attract late precursors to specific sites, favouring further differentiation.

This hypothesis may explain the finding that in culture systems in which cells or tissues were derived from *op/op* animals, high CSF-1 concentrations were required for osteoclast formation [Takahashi et al., 1991a; Morohashi et al., 1994], whereas in cultures of normal bones, the low CSF-1 concentrations sufficient for macrophage proliferation in vitro stimulate osteoclastogenesis [Antonioli-Corboz et al., 1992]. Thus, in normal tissues all molecular forms of the cytokine are synthesized. The exogenously added soluble CSF-1 may increase osteoclast formation by stimulating proliferation of precursors only and may not be involved in further differentiation, which would be under the control of the locally presented forms of the growth factor. If the tissues are derived from *op/op* animals, locally bound forms of CSF-1 are lacking and the exogenously added factor would have to act on each stage during proliferation and differentiation, although with limited efficiency on differentiation.

However, the dependence of osteoclast formation on CSF-1 seems to be different in vivo. Upon treatment of *op/op* mice with CSF-1, the growth factor acted immediately and efficiently [Felix et al., 1990b; Kodama et al., 1991]. Furthermore, a single administration was sufficient to initiate the complete osteoclastic differentiation cascade. This observation suggests that an initial action of CSF-1 on osteoclastic precursors is an absolute requirement, whereas for further development, the presence of the growth factor would no longer be necessary in vivo.

Investigating the survival of osteoclasts, further differences were observed between in vitro and in vivo conditions. In the absence of serum and CSF-1, isolated osteoclasts undergo apoptosis in vitro within hours [Fuller et al., 1993]. Contrary to this, osteoclasts survived in vivo for at least 14 days after a single administration of the growth factor to *op/op* mice [Kodama et al., 1993], suggesting that in vivo the microenvironment is suitable to support osteoclast survival for extended periods of time also in the absence of CSF-1.

Restoration of Osteoclastogenesis in *op/op* Mice and *tl/tl* Rats Is Not Complete

The osteopetrotic toothless (*tl/tl*) rat expresses some similar traits as the *op/op* mouse, namely an impaired bone resorption due to a decreased number of osteoclasts. Furthermore, femoral, peritoneal, and pleural macrophages are reduced. Like *op/op* mice, *tl/tl* rats are not cured by bone marrow transplantation. After treatment with endotoxin, the increase in CSF-1 activity in sera from *tl/tl* animals is substantially smaller than the increase in sera from phenotypically normal littermates. It is proposed that the osteopetrosis in *tl/tl* rats is due to CSF-1 defienciency [Marks et al., 1992]. This is supported by the finding that osteoclast formation and bone resorption are restored in *tl/tl* rats upon treatment with CSF-1.

Most interesting was the absence of osteoclasts in the subepiphyseal region of the long bones in CSF-1 treated *tl/tl* rats [Marks et al., 1993], as was the case also for long bones of treated *op/op* mice [Sundquist et al., 1993]. Two possible explanations for this finding are that either the injected soluble recombinant human CSF-1 does not reach the subepiphyseal region of the long bones, or the presence of a locally presented matrix or membrane associated form of the factor would be required.

CSF-1 in Other Types of Osteopetrosis

Osteopetrosis is a disease, enclosing a multitude of causes that lead to the common phenotype of impaired bone resorption. The CSF-1 deficient *op/op* strain is just one case. Another murine osteopetrotic strain is the microphthalmic *mi/mi* strain. The mouse is characterized by an increased number of osteoclasts as compared to normal littermates, and there is no evidence for CSF-1 deficiency. However, in *mi/mi* mice, osteoclasts are inactive. Upon treatment with a combination of CSF-1 and interferon-γ, bone resorption in *mi/mi* mice was stimulated, but this stimulation was not sufficient to form a normal marrow cavity. Furthermore, hemopoietic function and physical activity were im-

proved and the body weight was increased [Rodriguiz et al., 1993]. The contribution of CSF-1 in the reversal of *mi/mi* osteopetrosis is not yet clear.

The question for a human analogy to the murine *op/op* osteopetrosis has been raised too. However, there is no evidence as of yet that any of the human forms of osteopetrosis is due to CSF-1 deficiency [Orchard et al., 1992].

Spontaneous Recovery of *op/op* Mice

Of great interest is the fact that *op/op* mice undergo an age-dependent hemopoietic recovery and a resolution of osteopetrosis [Marks and Lane, 1976; Wink et al., 1991; Begg et al., 1993]. Since the gene for CSF-1 is mutated in *op/op* animals, the recovery cannot be due to age-dependent expression of this factor. Thus, an alternative salvage pathway to compensate for the lack of CSF-1 seems most probable, the mechanism of which however is not known.

CONCLUDING REMARKS

The murine osteopetrotic mutant strain *op/op* was instrumental in establishing CSF-1 as one of the growth factors for osteoclasts. However, while proving this point, several further questions concerning the role of the growth factor in the recruitment of osteoclasts were raised. CSF-1 is synthesized as a soluble, matrix, or membrane associated molecule. What is the function of these individual forms during the formation of osteoclasts? During aging, the osteopetrotic phenotype of *op/op* mice is reversed. What is the mechanism of this spontaneous recovery?

CSF-1 binding sites could be found on osteoclasts. This suggested a direct action of the growth factor also on the mature cell. Indeed, in vitro CSF-1 influences the proportion of migrating and resorbing cells. This observation suggests that cells of the osteoblastic lineage control the spatial resorption pattern in vivo by their expression of CSF-1. What regulates the local expression of CSF-1 by osteoblasts?

It is well established that CSF-1 plays an essential role in osteoclastogenesis and, therefore, in bone resorption. However, the function of the various molecular forms of the growth factor, as well as the regulation of their expression, within the processes of osteoclast formation and regulation of activity are not known. The elucidation of these mechanisms could provide important progress toward the understanding of bone resorption.

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