PERSPECTIVES

Interleukin-34: An Enigmatic Cytokine

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Abstract

Macrophage colony-stimulating factor (M-CSF) is required for the differentiation, proliferation and survival of cells of the monocyte lineage. M-CSF deficiency in mice (op/op mice) leads to a specific phenotype including skeletal deformities, a lack of teeth, and a marked osteopetrosis explained by an absence of osteoclasts. Recently, a novel cytokine, interleukin-34 (IL-34), has been discovered and shares a common receptor with M-CSF, resulting in functional overlaps in bone (similar effect on osteoclastogenesis) and in inflammatory systems (monocyte proliferation, and upmodulation of chemokine production). This Perspective focuses on the biology of IL-34 as suggested by recent published data and discusses IL-34's potential role in bone pathophysiology.

Introduction

Bone remodeling is tightly regulated by numerous parameters including mechanical constraints, soluble and membranous factors such as hormones, growth factors and cytokines, and extracellular matrix components such as collagen fragments. These factors contribute to the control of bone cell differentiation as well as to the functional coupling between osteoblasts and osteoclasts (1). Macrophage colony-stimulating factor (M-CSF, also named CSF-1) is a basic cytokine for the mononuclear phagocyte lineage and more particularly for monocytes (2;3). M-CSF is required for the physiological proliferation, differentiation and survival of macrophage-lineage cells (3). Mice bearing mutations in the M-CSF gene display a deficiency of circulating and resident monocytes (4). Interestingly, these mice exhibit numerous skeletal deformities including a toothless phenotype and congenital osteopetrosis (4) that are rescued by the administration of recombinant M-CSF (5). The osteopetrotic phenotype has been explained by a deficiency of osteoclasts, revealing that M-CSF is required for normal osteoclastogenesis (6;7). In May of 2008, Lin et al., published in Science the discovery of a novel cytokine designated interleukin-34 (IL-34) (8). They initially expressed the corresponding cDNA by transfecting human HEK 293T cells and have screened their biological activities (around 3,400 human secreted proteins) in an automated high-throughput system based on a wide spectrum of 30 assays such as metabolic, cardiovascular, immune response, cell survival, and differentiation assays (8). After analysis of 201,000 data points generated from these assays, they discovered IL-34, which supported the survival of human peripheral blood monocytes. Immediately these authors looked for the receptor of this new cytokine and demonstrated that IL-34 transduced signaling pathways through the receptor for M-CSF (MCSF-R) and exerted functional activities closely related to M-CSF (8). Two years later, this molecule is still mysterious as evidenced by the only 8 publications (5 original papers, 2 reviews and 1 editorial) available on this topic but stigmatises the discussion, especially
between bone specialists. Indeed, IL-34 is a real enigma protein unrelated to M-CSF, with a different tissue distribution, that may act through their common receptor (MCSF-R) after differential binding with no other clue to MCSF-R-independent activity. IL-34 and M-CSF open a new, unexplored path in bone and cytokine biology: is IL-34 another piece of the puzzle of the op/op mouse phenotype? How many other cytokines have such an obscure functional twin? In this specific context, this Perspective summarizes the main molecular characteristics of IL-34 and discusses the biological evidence for a role that this novel cytokine may have in bone biology.

**Interleukin-34 Is Highly Conserved Across Evolution**

Human IL-34 is a 27.5 KDa secreted dimeric glycoprotein consisting of 242 amino acids that is widely expressed in human and murine tissues including the brain, heart, liver, kidney, spleen, mammary glands and prostate (Table 1) (8;9). In humans, IL-34 is abundant mostly in the spleen where it is expressed by the sinusoidal endothelium in the red pulp. This expression pattern is consistent with its regulation of myeloid cell growth and differentiation (8). Two different human isoforms generated by alternative mRNA splicing have been described and differ by an additional glutamine between residues 80 and 81. The alignment of human, chimpanzee, rat and mouse IL-34 amino acid sequences clearly shows the highly conserved sequence of this cytokine (8). Similar to M-CSF, IL-34 possesses a signal peptide between amino acid residues 1 to 20 (32 amino acids for M-CSF). Structural analysis of the IL-34 protein is consistent with a four-helix bundle structure containing cysteine residues conserved with M-CSF and associated with intrachain disulfide bonds, but it differs from M-CSF for 7 other cysteine residues missing from IL-34 (10). These data also predict the presence of one interchain disulfide bond resulting in a secreted dimeric protein. Although it has been demonstrated that the extracellular forms of M-CSF result from proteolytic cleavage of the membrane form (11), soluble dimeric IL-34 might also be the result of a similar enzymatic reaction, thus controlling its bioavailability (10). IL-34 exerts more restricted cross-species reactivity than M-CSF (9). Although M-CSF exhibits cross-species specificity (human M-CSF activates similarly all M-CSF receptors for all species assessed, while murine M-CSF activity is restricted to nonprimate species), human IL-34 is a less potent MCSF-R activator than its murine homolog. Garceau et al. performed genomic comparative analysis between avian and zebrafish IL-34, M-CSF and MCSF-R and provided evidence that in these species, IL-34 and M-CSF bind similarly to MCSF-R and that these two cytokines are functionally conserved across all vertebrates, probably due to their partial redundancy (10).

**IL-34 Exerts Its Biological Activity Through the MCSF-R but with an Alternative Binding Mode Compared to M-CSF**

Functional studies demonstrated that IL-34 and M-CSF similarly upregulate monocyte proliferation and viability. They also stimulate macrophage differentiation from human and animal bone marrow cells (8-10). Lin et al. demonstrated that IL-34 and M-CSF share a single tyrosine kinase receptor encoded by the proto-oncogene c-fms (12), MCSF-R (8), initially known to mediate M-CSF activities. The binding of MCSF to its receptor chain induces and stabilizes the homo-dimerization of its receptors resulting in their tyrosine phosphorylation and the phosphorylation of other cytoplasmic proteins (13). The binding affinity of human IL-34 to the immobilized human MCSF-R has been determined by surface plasmon resonance and exhibits a dissociation constant of 1 pM. M-CSF binds its receptor with a Kd of 34 pM (8), explaining the results of competitive experiments between M-CSF and IL-34 performed by Chihara et al. (14). Indeed, these authors demonstrated that the inhibition of M-CSF binding to MCSF-R by exogenous IL-34 was more efficient than the inhibition of IL-34 binding to MCSF-R by M-CSF. However, the activity of these cytokines is not directly related to their affinity for the receptor, and, for instance,
Table 1. The main characteristics of IL-34, M-CSF and MCSF-R.

<table>
<thead>
<tr>
<th></th>
<th>Human</th>
<th>Mouse</th>
</tr>
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<tbody>
<tr>
<td><strong>IL-34 mRNA expression</strong></td>
<td>2 isoforms</td>
<td>3 isoforms</td>
</tr>
<tr>
<td></td>
<td>Heart, brain, lung, liver, skeletal muscle, kidney, spleen, thymus, testes, ovary, prostate, small intestine, colon (8)</td>
<td>Uterus, placenta, brain (cerebral cortex, olfactory bulb, striatum, hippocampus, cerebellum), ear, pancreas, mammary gland, adipose tissue, salivary gland, heart, pituitary gland, kidney, spleen, skeletal muscle, osteoblast (9)</td>
</tr>
<tr>
<td><strong>Gene location</strong></td>
<td>Chromosome 16, locus: q22.1</td>
<td>Chromosome 8, locus: 8E1</td>
</tr>
<tr>
<td><strong>Protein size</strong></td>
<td>242 aa, 27.5 KDa</td>
<td>235 aa, 26.8 kDa</td>
</tr>
<tr>
<td><strong>M-CSF (CSF-1) expression</strong></td>
<td>3 isoforms</td>
<td>2 isoforms</td>
</tr>
<tr>
<td><strong>Gene location</strong></td>
<td>Chromosome 1</td>
<td>Chromosome 3</td>
</tr>
<tr>
<td><strong>Protein size</strong></td>
<td>554 aa, 60 kDa</td>
<td>552 aa, 60 kDa</td>
</tr>
<tr>
<td><strong>MCSF-R (CSF-1R, c-fms, CD115)</strong></td>
<td>Chromosome 5</td>
<td>Chromosome 18</td>
</tr>
<tr>
<td><strong>Gene location</strong></td>
<td>Chromosome 5</td>
<td>Chromosome 18</td>
</tr>
<tr>
<td><strong>Protein size</strong></td>
<td>972 aa, 168 kDa</td>
<td>977 aa, 190 kDa</td>
</tr>
</tbody>
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Note: aa = amino acids.

human IL-34 induced a lower induction (around two-fold) of osteoclastogenesis than human M-CSF at the same concentration (15). In contrast, murine IL-34 has around two-fold lower affinity for the murine MCSF-R than murine M-CSF (9) and these affinities were in agreement with their biological activities. Indeed, Wei et al. demonstrated that murine MCSF-R was activated to a lesser degree by murine IL-34 compared to murine M-CSF, as shown by the level of phosphorylation (9). In all species assessed, IL-34 and M-CSF induced ERK1/2 and Akt phosphorylation in human/murine monocytes/macrophages (8;9;14;15) that are inhibited by GW2580, a specific MCSF-R inhibitor (8;15). However, human IL-34 effects on monocytes were blocked by anti-IL-34 antibodies but not by anti-M-CSF antibodies; in contrast, human M-CSF effects were abolished by anti-M-CSF antibodies but not by anti-IL-34 antibodies, demonstrating that the activities of both cytokines are independent (8). Interestingly, an anti-M-CSF-R monoclonal antibody blocked the interactions between both cytokines and MCSF-R but another antibody interacted only with M-CSF, suggesting that IL-34 and M-CSF could bind MCSF-R through two distinct domains (14). Furthermore, differences between IL-34 and M-CSF activities have been observed in monocytes (14). When both cytokines are used in combination to stimulate
macrophage differentiation, morphological differences can be observed such as lower fibroblastic-like elongated macrophages in the presence of IL-34. Furthermore, the cytokine profile produced by IL-34- and M-CSF-activated macrophages was distinct (e.g., a higher amount of eotaxin-2 and a lower amount of MCP-1 was secreted in the presence of IL-34) (14). Similarly, the phenotypes of activated macrophages were also distinct, highlighting the differential qualitative activation of these cells induced by IL-34 and M-CSF. Structural analysis has helped to explain these functional differences, and predicted that IL-34 and M-CSF interact with MCSF-R by two distinct contact points, thus revealing an alternative binding mode of IL-34 to MCSF-R (10). Consequently, the level of tyrosine phosphorylation induced by IL-34 and M-CSF is different. For instance, IL-34 phosphorylates Tyr546 or Tyr699 on MCSF-R more strongly than M-CSF (14). In short, the binding of both ligands to distinct binding sites on the single receptor may result in differences in the signaling pathways they elicit, explaining in part the difference in their biological activities (Fig. 1).

![Fig. 1. IL-34 and M-CSF bind MCSF-R by two distinct anchorage points and differentially induce the autophosphorylation of specific tyrosine residues [tyrosine phosphorylations induced by M-CSF (blue circles) or by IL-34 (yellow circles)]. MCSF-R belongs to the tyrosine kinase receptor family. The binding of the dimeric cytokines IL-34 or M-CSF to MCSF-R induces the dimerization and stabilization of the receptor chains and results in differential qualitative responses of the cells harboring the receptors (9;14).](image)

**IL-34 is Produced by Osteoblasts and Controls Osteoclastogenesis: A Role in the Bone Microenvironment**

The canonical pathway controlling osteoclastogenesis comprises M-CSF and receptor activator of NF-κB ligand (RANKL). While RANKL activity in osteoclastogenesis can be mimicked *in vitro* by different cytokines such as TNFα, the differentiation of osteoclasts *in vivo* is totally dependent on RANKL (16-18). Indeed, the phenotype exhibited by RANKL knockout mice is characterized by a complete lack of immature and mature multinucleated osteoclasts (19). M-CSF also contributes to
the control of osteoclastogenesis by stimulating both the adhesion and the proliferation of osteoclast precursors (20) but not their survival (21). The role of M-CSF in bone remodeling has been clearly demonstrated in osteopetrotic (op/op) mutant mice that suffer from congenital osteopetrosis due to a deficiency of osteoclasts associated with the absence of M-CSF (4). However, while RANKL action cannot be substituted for by other cytokines in vivo, M-CSF activity can be partly replaced by other soluble factors such as Flt3 ligand, vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF), eliciting the non-canonical pathway of osteoclastogenesis (22). MCSF-R knockout mice exhibit a similar but more pronounced phenotype than op/op mice characterized by a more severe osteopetrosis and reduction of tissue macrophages (23). This “exacerbated” phenotype confirmed the key function of M-CSF as well as its signaling through a single receptor during macrophage proliferation and differentiation. This phenotype has also suggested the existence of another potential ligand for its receptor such as IL-34. To assess this hypothesis, Wei et al. studied the potential compensatory effect of IL-34 in op/op mice and demonstrated that IL-34 expression is able to rescue the main defects of op/op mice: osteopetrosis and alterations in growth rate, tooth eruption and macrophage numbers in bone, the liver, and the kidneys (9). Thus, IL-34 and M-CSF may represent for the cells an extremely accurate method of adjustment of the biological activities transduced through the MCSF-R by inducing a distinct pattern of phosphorylation and signal transduction, according to the timing and the biological situation. Among the cell populations associated with the hematopoietic niche, osteoblasts produce numerous soluble factors sustaining the survival, proliferation and differentiation of hematopoietic cells. In contrast to M-CSF, which is highly expressed by osteoblasts (24), only a small amount of IL-34 is produced by murine osteoblasts (9). However, both cytokines are similarly upregulated by LPS and IFNγ (9).

Human and murine IL-34 support RANKL-induced osteoclastogenesis in the absence of M-CSF in several in vitro models: human CD14+ cells, murine CD11b+ cells, and myelomonocytic RAW264.7 cells (15). Multinucleated cells generated in the presence of IL-34 expressed osteoclastic markers such as TRAP and cathepsin K in agreement with their ability to resorb calcified matrix and NFATc1, a transcription factor found in several immune cells and upregulated in response to RANKL. Similarly to M-CSF, IL-34 promoted the adhesion and the proliferation of osteoclast progenitors but did not modulate the survival of osteoclasts. Moreover, the positive expression (transcriptional and protein levels) of multinucleated cells composing giant cell tumors of bone strengthens the pathophysiological role of IL-34 in osteoclastogenesis (Fig. 2).

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**Fig 2.** Immunolocalization of IL-34 and MCSF-R in human giant cell tumors of bone. Bone-resorbing osteoclasts are immunoreactive to IL-34 (left panel) probably due its internalization following its binding to MCSF-R. Osteoclasts also strongly express MCSF-R (right panel). Materials and methods: see in (15). Original magnification: X 200.
IL-34 thus defines another non-canonical pathway of osteoclastogenesis (15). The recent observation by Wei et al. provides a better understanding of the functional specificity of IL-34 compared to M-CSF (9). These authors studied the pattern of IL-34 and M-CSF expression during mouse development and demonstrated that these expression patterns differ substantially in a spatiotemporal manner. Thus, IL-34 and M-CSF exert specific activities and some overlapping functionalities that could constitute a spatiotemporal compensatory mechanism implicating a single receptor. In this case, IL-34 and M-CSF could be considered more as local molecular effectors according to the spatial and temporal situation (e.g., communication between osteoblasts, macrophages and osteoclasts during bone formation or bone remodeling) than as systemic factors. A comparative analysis of IL-34 and M-CSF levels in the serum of mice and patients and in the local microenvironment can address this hypothesis. Furthermore, we must keep in mind that the activity of recombinant IL-34 and M-CSF proteins may differ to some degree from the native proteins, particularly when membrane-bound forms exist as shown for M-CSF [see Table 1, (11)].

Conclusion: What Is the Future of IL-34?

IL-34 is a novel cytokine, functionally related to M-CSF and with which it shares a single receptor. IL-34 and M-CSF differentially bind their receptor chains through two distinct binding sites, explaining their partial functional overlap but also their specificities. This mode of action is original but remains enigmatic. Consequently, the major role played by these two cytokines in macrophage proliferation/differentiation could have a strong influence on bone remodeling and in osteoimmunology through an effect on osteoclastogenesis. In this case, IL-34 can completely substitute for M-CSF during osteoclastogenesis and defines a novel non-canonical pathway of osteoclastogenesis. IL-34 might also be involved in the pathological development of giant cell tumors of bone and in osteolysis associated with tumor development such as occurs with bone metastases as shown for M-CSF (26). The exacerbated osteopetrosis exhibited by MCSF-R knockout mice, compared to op/op mice, might be explained by the recently discovered role of IL-34. Interestingly, IL-34 and M-CSF expression patterns differ in a spatiotemporal manner during development, suggesting that IL-34 might be specifically coordinating the cell communication network at the local microenvironment level, rather than at the systemic level. Thus, IL-34 might have a key function at basic multicellular units by regulating cell interactions of the canopy (25). The other domain in which we will very soon learn of novelties of IL-34 will probably be responses to infections. Indeed, the replication of the HIV-1 virus seemed to be higher in macrophages treated with IL-34 than with M-CSF (14) and the S2 protein of the equine infectious anemia virus (a virus closely related to HIV) specifically enhances the expression of IL-34 by macrophages in vitro (27). In this case, the role of IL-34 will probably be critical in inflammation biology as shown by its ability to increase IL-6 and chemokine levels in human whole blood (IP-10/CXCL10, IL-8/CXCL8, and MCP-1/CCL2) (28). Future functional studies will improve our understanding of IL-34 in bone biology and in human pathophysiology as well.

Conflict of Interest: None reported.

Peer Review: This article has been peer-reviewed.

References


