

REVIEW

Osteoclasts, mononuclear phagocytes, and c-Fos: new insight into osteoimmunology

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Abstract. Osteoimmunology is the emerging concept that certain molecules link the skeletal and immune systems. The transcription factor c-Fos, a component of activator protein-1 (AP-1), is essential for osteoclast differentiation. Mice lacking c-Fos are osteopetrotic owing to impaired osteoclast development. Recent studies suggest that in contrast to this positive role in osteoclastogenesis, c-Fos expression inhibits differentiation and activation of mononuclear phagocytes. Here, we focus on the contrasting roles of c-Fos in the bone and immune lineages. Both osteoclasts and mononuclear phagocytes are derived from common myeloid precursors. Osteoclasts resorb bone, whereas macrophages and myeloid dendritic cells phagocytose microbial pathogens, initiating innate and adaptive immunity. Differentiation of the common precursors into either bone or immune lineage is determined by ligand binding to cell-surface receptors, particularly receptor activator of NF- κ B (RANK) for osteoclasts, or Toll-like receptors (TLRs) for mononuclear phagocytes. Both RANK and TLRs activate the dimeric transcription factors NF- κ B and AP-1. Yet, c-Fos/AP-1 plays a positive role in osteoclasts but a negative role in macrophages and dendritic cells. Further study is necessary to clarify this dual role of c-Fos. (Keio J Med 53 (2): 78–84, June 2004)

Key words: AP-1, c-Fos, osteoclast, macrophage, dendritic cell

Introduction

Rheumatoid arthritis and other inflammatory bone diseases illustrate the close link between bone biology and immunology. Synovial inflammation, for example, results in joint destruction mediated by pathological interaction between T cells and osteoclasts.^{1,2} Patients with inflammatory bowel disease such as Crohn's disease are at high risk for osteoporotic vertebral fractures.³ Only recently, however, has it come to light that certain molecules actually regulate both the skeletal and immune systems. These findings rationalize the emerging concept of osteoimmunology.⁴ In this review, we describe the contrasting roles of the transcription factor c-Fos in osteoclasts and mononuclear phagocytes.

c-Fos was originally identified as the cellular homolog of the retroviral gene, v-Fos, which induces osteosarcomas in mice. Both in humans and in mice, three additional Fos proteins, Fra1, Fra2 and FosB have been identified (Fig. 1). Fos family members each carry the

highly conserved "basic leucine zipper" (bZip) structure. The basic region is responsible for DNA-binding, and the leucine-zipper is for heterodimerization, typically with the Jun proteins, c-Jun, JunB and JunD. The bZip structure is located in the middle of Fos proteins, but at the C-terminus of Jun proteins (Fig. 1). The resultant dimeric transcription factor complexes are collectively termed AP-1, and the consensus sequence of their binding sites is 5'-TGA(G/C)TCA-3'. c-Fos is transiently induced in response to various stimuli, and expressed in cell types ranging from neurons to keratinocytes. Thus it was surprising that mice lacking this transcription factor showed an overt phenotype only in bone, namely osteopetrosis.⁵⁻⁷ But in fact, recent findings indicate that c-Fos is likely a key regulator in the immune system as well.

Osteoclast and Macrophage-Dendritic Cell Lineages

Differentiation into osteoclasts or mononuclear

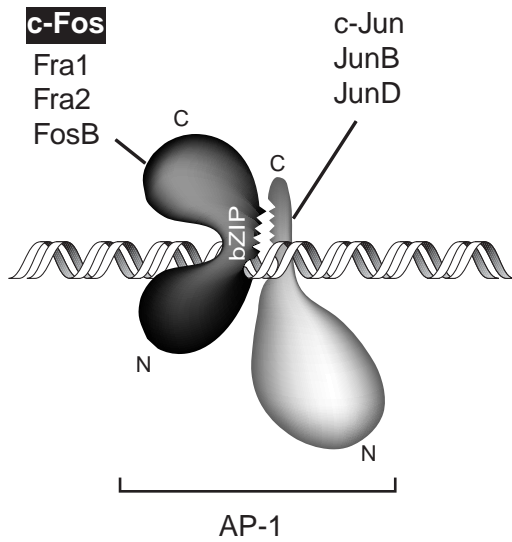


Fig. 1 c-Fos and other AP-1 family members.

phagocytes can be considered a lineage bifurcation, similar to differentiation into either T versus B cells, or CD4⁺ versus CD8⁺ T cells. The common myeloid precursors commit to either the osteoclast or mononuclear phagocyte lineage, depending upon stimuli received from the external environment (Fig. 2).⁸⁻¹⁰ Generation

of myeloid precursors is dependent on the presence of macrophage-colony-stimulating factor (M-CSF) and the transcription factor PU.1, and mice devoid of either factor lack both osteoclasts and macrophages. Myeloid dendritic cells, the most potent antigen presenting cells (APCs) are central in eliciting T cell response. These dendritic cells also differentiate from the same myeloid precursors in the presence of granulocyte-macrophage-colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4).^{11,12} Myeloid precursors can be isolated not only from bone marrow, but also from other sites, including the spleen and peritoneal cavity.¹³

Osteoclast differentiation is associated with activation of genes encoding tartrate-resistant acid phosphatase (TRAP), calcitonin receptor, cathepsin K, β_3 -integrin, and ATP-dependent proton pump subunits. Once committed, mononuclear osteoclasts fuse with each other, forming multinucleated osteoclasts. They then polarize and adhere to bone matrix, induce actin ring formation, acidify bone surface and release osteolytic enzymes (Fig. 2).¹⁰ In contrast, macrophage differentiation is indicated by induction of various genes encoding cell-surface molecules, including F4/80, Mac-1 (CD11b), MOMA-2, and MHC class II molecules, and enzymes such as inducible nitric oxide synthase (iNOS). Dendritic cell differentiation is indicated by expression of MHC class II molecules as well as CD11c (Fig. 2). Signals from cell surface receptors determine which set of genes the myeloid precursors will eventually express.

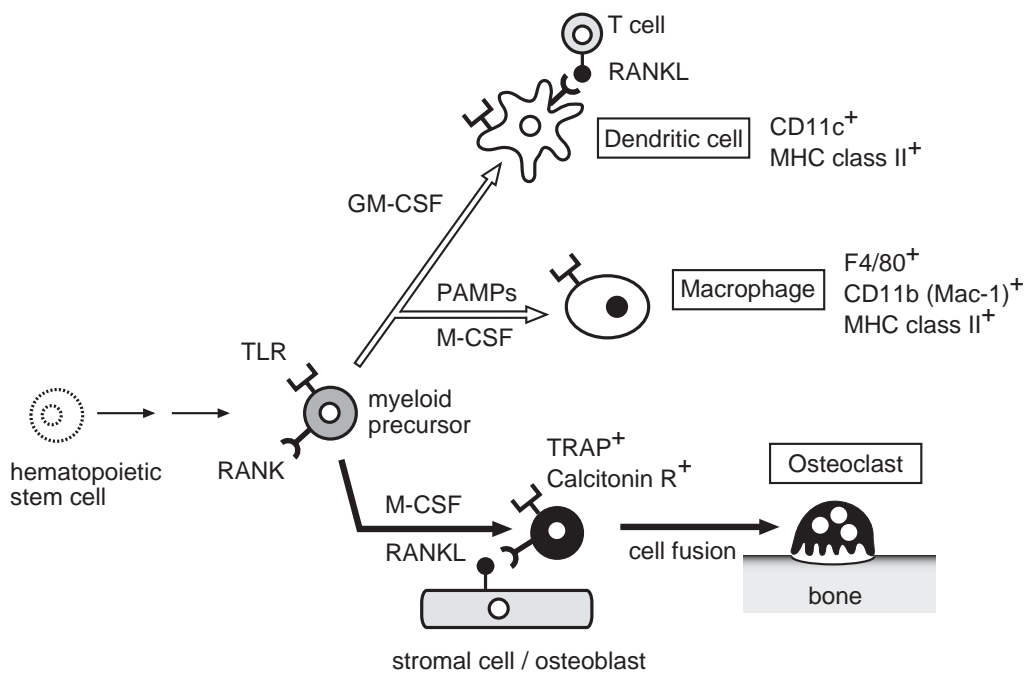


Fig. 2 Differentiation into osteoclasts or mononuclear phagocytes. PAMPs: pathogen associated molecular patterns, Calcitonin R: calcitonin receptor.

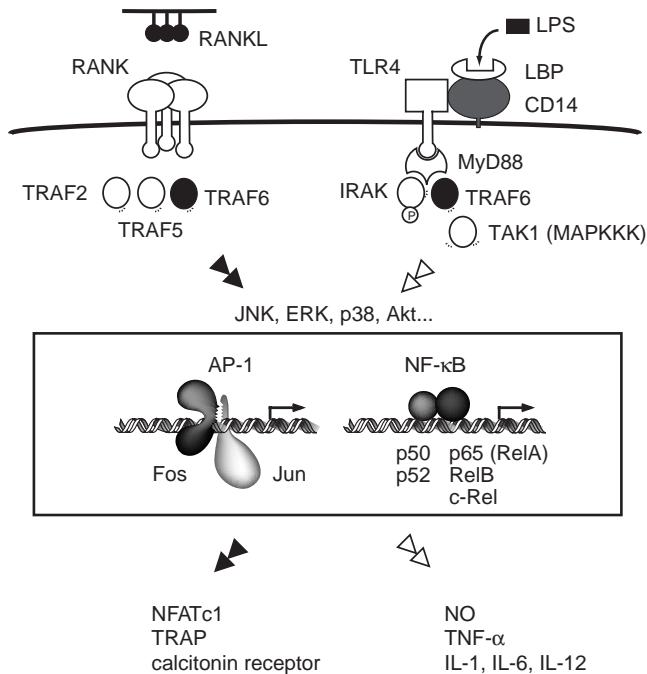


Fig. 3 RANK and TLR signals both activate NF- κ B and AP-1, but with different target gene expression. IRAK: IL-1 receptor-associated kinase, Akt: protein kinase B.

RANK and TLRs

RANKL¹⁴⁻¹⁷ (encoded by *Tnfrsf11*) is expressed as a trimer on osteoblasts. The RANKL receptor, RANK¹⁴ (encoded by *Tnfrsf11a*), which is mainly expressed in the myeloid lineages, belongs to the tumor necrosis factor (TNF) receptor superfamily. RANKL-RANK interaction initiates a signal cascade involving recruitment of intracellular tumor necrosis factor receptor-associated factors (TRAFs) such as TRAF2, TRAF5, and TRAF6¹⁸ (Fig. 3). Genetic experiments in mice have revealed that RANKL, RANK, TRAF6 and the transcription factors c-Fos and NF- κ B (double knock-out for p50 and p52) are essential for precursors to commit into the osteoclast lineage (Table 1). Mice lacking any of these molecules are osteopetrotic due to lack of bone resorption. It is worth noting that RANK is necessary for lymph node formation as well (Table 1). In the presence of M-CSF, the RANK pathway can activate all three groups of mitogen-activated protein (MAP) kinases: c-Jun-N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and p38. TRAF6 associates with transforming growth factor- β -activated kinase 1 (TAK1), which activates JNK and AP-1, as well as inducing nuclear translocation of NF- κ B through I κ B degradation (Fig. 3).¹⁹ The only component of AP-1 known to be necessary for osteoclast

Table 1 Immunological Phenotypes of Mice with Osteopetrosis Due to Impaired Osteoclast Differentiation

Deficiency	Immunological phenotypes
c-Fos*	elevated IL-12 production in macrophages ³⁶
NF- κ B (p50, p52)*	impaired cytokine production; impaired B-cell, thymus, and spleen development ^{39,40}
RANK*	lack of lymph nodes but Peyer's patches present; impaired B-cell development ^{41,42}
RANKL	lack of lymph nodes but Peyer's patches present; defects in early differentiation of T and B cells ⁴³
<i>microphthalmia (mi)</i>	mast cell defects ⁴⁴
PU.1	lack of macrophages ⁴⁵ ; reduced lymphoid-myeloid progenitors ⁴⁶
<i>op/op</i> (M-CSF)	reduced macrophages ⁴⁷
TRAF6*	lack of IL-1, CD40 and LPS signaling; lack of lymph nodes; lack of dendritic cell maturation ⁴⁸⁻⁵⁰

* indicates cell-autonomous differentiation block.

formation is c-Fos. Interestingly, however, mice lacking another Fos family protein, Fra2 appear to be osteopetrotic (E.F. Wagner, personal communications).

Signaling by TLRs induces macrophage differentiation and activation. TLRs recognize pathogen-associated molecular patterns (PAMPs) from a wide variety of microorganisms. For example, lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria, is recognized by TLR4 with the help of LPS-binding protein (LBP) and CD14 (Fig. 3).²⁰ LPS stimulation leads to production and release of various inflammatory mediators including TNF- α , IL-1, IL-6, IL-12, as well as nitric oxide (NO). TLR3, TLR5 and TLR9 recognize double-stranded RNA, flagellin and unmethylated CpG DNA, respectively. TLR1, TLR2 and TLR6 can cooperate to recognize peptidoglycan and several other specific PAMPs. TLR1 to TLR9 are all expressed in myeloid precursors.²¹ Typically, TLR signaling is mediated by adaptor molecules such as MyD88 and TRAF6, which eventually activate NF- κ B and AP-1. This results in production of inflammatory cytokines (Fig. 3), and subsequent induction of innate and adaptive responses. RANK and TLR signaling appear surprisingly similar in that both activate NF- κ B and AP-1, including c-Fos. Yet, this similarity must only be superficial since RANK signaling promotes osteoclastogenesis, while TLR signaling is generally believed to inhibit osteoclast differentiation.^{13,21,22} Furthermore, overexpression of c-Fos has been shown to inhibit maturation of a murine myeloblastic leukemia cell line into macrophages.²³ How exactly is c-Fos involved in RANK and TLR signaling in myeloid cells?

Roles of c-Fos in Bone Biology

The necessity of c-Fos in osteoclast differentiation is revealed by the severe osteopetrotic phenotype of c-Fos deficient (*Fos*^{-/-}) mice.^{5,6} A similar cell-autonomous differentiation block in the osteoclast lineage is also demonstrated in other osteopetrotic mice (Table 1). The critical target genes of c-Fos in osteoclast differentiation have been extensively analyzed by rescue experiments. In these experiments, putative c-Fos target genes were expressed in *Fos*^{-/-} precursors via retroviral vectors or transgenes to determine whether they could overcome this block in differentiation.

Retroviral gene transfer of any Fos family genes, particularly *Fra1* (also known as *FosL1*, for Fos-like 1), results in osteoclast formation *in vitro*, demonstrated by expression of *Acp5* (encoding TRAP) and *Calc* (encoding calcitonin receptor), as well as bone resorption.²⁴ The rescue of osteoclastogenesis in *Fos*^{-/-} precursors by *Fra1* has also been shown *in vivo* by crossing *Fra1* transgenic mice with *Fos*^{-/-} mice.²⁴ This was further confirmed by using a knock-in technique, which allows *Fra1* to be expressed under the *Fos* promoter, at the *Fos* locus.²⁵ In *Fos*^{-/-} osteoclast precursors, *Fra1* expression is hardly detectible. Furthermore, *Fra1* carries functional AP-1 sites in intron 1,²⁶ suggesting that *Fra1* expression is dependent on c-Fos during osteoclast differentiation. Unlike c-Fos, *Fra1* has no detectible transactivation domains. The activation potential of *Fra1*-containing AP-1 may actually be determined by the heterodimerizing partner. It is not known at present whether Jun proteins are the partners of *Fra1* during osteoclast formation or not.

Transcription factors of the nuclear factor of activated T cells (NFAT) family contain the Rel-homology domain, a conserved DNA-binding domain distantly related to that of NF- κ B.²⁷ NFATs were originally identified in T cells and are involved in regulation of genes encoding IL-2 and other cytokines. Mice lacking NFATc1 die *in utero* due to impaired heart development.^{28,29} The critical role of NFATc1 in osteoclastogenesis has recently been demonstrated.^{30,31} Importantly, we as well as others have observed that expression of NFATc1 is induced in *Fos*^{+/+}, but not *Fos*^{-/-} precursors during osteoclast differentiation.³² The lack of NFATc1 expression in the absence of c-Fos is the cause, not the consequence of the differentiation block. Exogenously expressed NFAT can rescue expression of *Acp5* and *Calc*, as well as bone-resorbing activity in cells from *Fos*^{-/-} precursors *in vitro*.³² These data establish the RANK – c-Fos – NFATc1 transcriptional cascade, which is critical for osteoclastogenesis. NFATc1 is usually localized in the cytosol in phosphorylated form. Dephosphorylation by calcineurin, a Ca²⁺-dependent phosphatase, is required for NFATc1

to translocate into the nucleus. It is worth noting that NFATc1 and AP-1 can interact at the protein level and bind cooperatively to DNA at certain promoters, including those of *Acp5* and *IL-2*. In summary, RANK activates osteoclastogenic target genes, through c-Fos and NFATc1, resulting in differentiation of myeloid precursors into osteoclasts.

Unlike *Fra1* or NFAT, c-Fos target genes in the osteoclast lineage are not always positive regulators of osteoclastogenesis. Microarray analyses comparing osteoclastogenic cultures of *Fos*^{+/+} and *Fos*^{-/-} precursors revealed that *Fos*^{-/-} precursors did not express interferon (IFN)-inducible genes. It turns out that transcription of *IFN- β* is downregulated in the absence of c-Fos. A functional AP-1 site was identified in the *IFN- β* promoter, suggesting that *IFN- β* is a c-Fos target gene in osteoclast precursors.³³ Surprisingly, signals from the IFN- β receptor cause reduction of c-Fos protein. Thus, there exists a c-Fos negative-feedback loop, which is mediated by IFN- β . Since IFN- β production in response to Newcastle disease virus is still intact in *Fos*^{-/-} myeloid precursors, *IFN- β* seems to be a c-Fos target gene specifically during osteoclast differentiation.³³ It was unexpected that IFN- β , an antiviral cytokine, would have an important role in bone physiology as well.

Downregulation of c-Fos by cytokines seems to be a general regulatory mechanism for suppressing osteoclast differentiation. GM-CSF and IL-3 are cytokines which both act directly on early osteoclast precursors, irreversibly blocking RANKL-induced osteoclast differentiation by downregulating c-Fos transcripts. As a result these cells become macrophages or dendritic cells.^{11,34}

Roles of c-Fos in the Immune System: Macrophages and Dendritic Cells

In contrast to its role in bone biology, the role of c-Fos in the immune system remains unclear. Osteopetrosis in mice is often accompanied by various immunological phenotypes (Table 1), and *Fos*^{-/-} mice are no exception to this phenomenon. Research examining the functions of c-Fos in mononuclear phagocytes suggests that c-Fos induction may be important in both innate and adaptive immune responses, as we shall see below.

c-Fos/AP-1 has been shown to negatively regulate transcription of *iNOS*, which is responsible for production of the microbiocidal compound, NO (Fig. 4A).³⁵ Stimulation by LPS, IFN- γ , or a combination of the two induces *iNOS* in macrophages. c-Fos is also upregulated in these macrophages post-LPS stimulation. By using an inducible c-Fos transgene (*Mx-c-fos*), Okada *et al.* showed that c-Fos overexpression suppressed *iNOS*, preventing NO production in macrophages after LPS

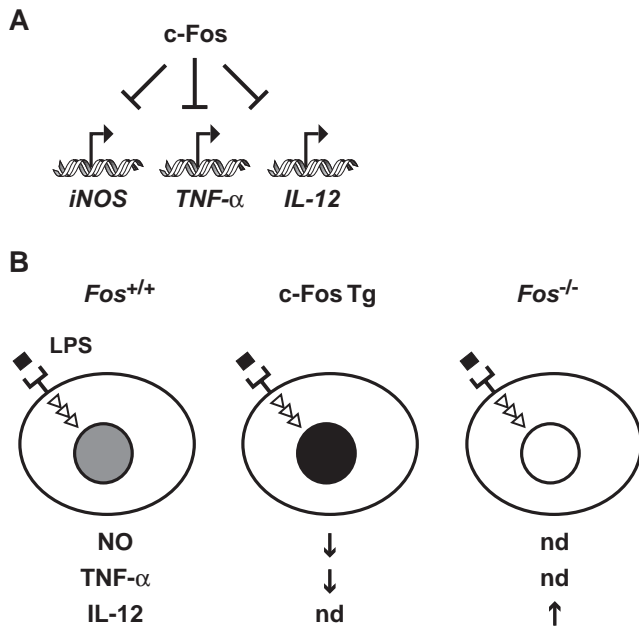


Fig. 4 c-Fos acts as a negative regulator in macrophages. (A) c-Fos can suppress genes encoding iNOS, TNF- α , and IL-12. (B) Lack of c-Fos results in IL-12 overproduction in LPS-stimulated macrophages, and overexpression of c-Fos causes decreased production of NO and TNF- α .

stimulation (Fig. 4B).³⁵ Interestingly, production of TNF- α was also significantly reduced in these transgenic macrophages overexpressing c-Fos. On the other hand, peritoneal macrophages from mice lacking c-Fos did not show an abnormal increase in NO production when stimulated with LPS and IFN- γ . In this context, it is possible that other Fos family members are substituting for c-Fos.³⁵

The negative regulatory function of c-Fos on the *iNOS* promoter may be explained by the interaction between c-Fos and nuclear factor-IL6 (NF-IL6), another basic leucine zipper family transcription factor.³⁵ NF-IL6 can induce *iNOS* by binding to its promoter region. Protein-protein interaction between c-Fos and NF-IL6 may interfere with the DNA-binding activity of NF-IL6, resulting in inhibition of *iNOS*. The *TNF- α* gene also possesses binding sites for NF-IL6, AP-1 and additionally NF- κ B. However, the mechanism of *TNF- α* downregulation due to c-Fos overexpression is not yet known. Overproduction of NO and other inflammatory mediators is implicated in inflammatory bowel disease, experimental arthritis, and septic shock. A possible physiological role of c-Fos may be to suppress unnecessary production of these inflammatory mediators.

IL-12, a heterodimeric cytokine composed of two subunits (IL-12 p40 and IL-12 p35), is released by macrophages and dendritic cells upon stimulation by LPS. c-Fos appears to inhibit IL-12 production in both

macrophages and dendritic cells.³⁶⁻³⁸ Using *Fos*^{-/-} murine peritoneal macrophages, Roy *et al.* demonstrated that absence of c-Fos results in overproduction of IL-12 in response to LPS stimulation.³⁶ This overproduction of IL-12 observed in *Fos*^{-/-} macrophages can be mimicked in *Fos*^{+/+} and *Fos*^{+/-} macrophages when they are treated with IFN- γ prior to LPS stimulation. IFN- γ priming in these cells enhances IL-12 production presumably through downregulation of c-Fos. This is consistent with the fact that production of IL-12 in *Fos*^{-/-} macrophages is not further increased by IFN- γ priming.³⁶

Similar to IFN- γ pre-treatment, IL-4 pre-treatment of *Fos*^{+/+} peritoneal macrophages results in increased production of IL-12 in response to LPS stimulation, also likely via decrease in c-Fos transcripts.³⁷ The already high IL-12 production by *Fos*^{-/-} macrophages in response to LPS is not further affected by IL-4 pre-treatment. This effect of IL-4 is abolished, however, if c-Fos is constitutively overexpressed in macrophages. Thus, both IFN- γ and IL-4 can prime macrophages similarly for IL-12 production by downregulating c-Fos. IL-12 production promotes Th1 response, and therefore suppression of c-Fos may be a mechanism by which the immune system modulates Th1 versus Th2 responses.

Research examining immature human dendritic cells indicates that phosphorylation of c-Fos may be a mechanism by which these cells differentially initiate a Th2 instead of Th1 response, depending upon the nature of stimulation received (Fig. 5).³⁸ LPS and flagellin promote a Th1 response, illustrated by production of IFN- γ and IL-12 by the dendritic cells. Pam3cys and SEA, in contrast, promote a Th2 response as shown by IL-5 and IL-13 production by the dendritic cells. c-Fos acts as a negative regulator of IL-12 production in dendritic cells as it does in macrophages. Both Pam3cys and SEA stimulation appear to stabilize c-Fos through phosphorylation via the ERK1/2 pathway. This results in suppression of IL-12, favoring the Th2 response.³⁸ Thus, the role of c-Fos goes beyond that of a simple negative regulator to possibly being part of a mechanism for fine-tuning adaptive immunity.

Emerging data indicates that c-Fos is likely involved in differentiation and activation of immune cells. A better understanding of the roles of c-Fos in the immune system may lead to more effective therapies for suppressing an overactive immune response.

Conclusion

The research we have discussed demonstrates the breadth of functions that the transcription factor c-Fos has in both osteoclasts and mononuclear phagocytes. c-Fos is among several molecules which are important in osteoimmunology, others include IFN- β , IFN- γ ,

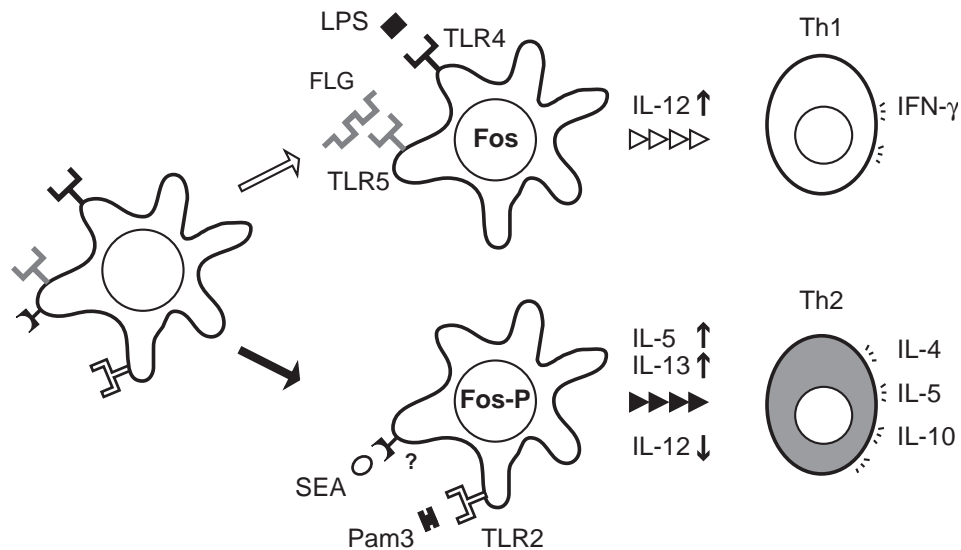


Fig. 5 c-Fos phosphorylation in dendritic cells may result in preferential induction of Th2 response. FLG: flagellin, SEA: schistosome egg antigens, Pam3: Pam3Cys-Ser-Lys4, Fos-P: phosphorylated c-Fos.

RANKL, TRAF6, NFAT, and NF- κ B. What makes c-Fos especially interesting is the contrasting roles it plays. Overall, it appears to be a positive regulator in the osteoclast lineage, but a negative regulator in macrophages and dendritic cells. A clearer understanding of this dual nature of c-Fos may shed more light on the complex interplay between the skeletal and immune systems.

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