Bone and Inflammation

George E. Fragoulis, MD

Metsovo 2012
Outline

- Physiology of bone turn-over
  - RANK-RANKL-OPG System
  - Wnt System
- Pathophysiology of Bone in Inflammation
  - Inflammation and bone destruction
  - Role of specific cytokines / Th17
- Disease Paradigms
  - RA
  - Spondyloarthropathies
  - Psoriatic Arthritis
  - Systemic Lupus Erythematosus
Osteoclasts (OCL) are multinucleated cells formed by fusion of mononuclear precursors in the monocyte/macrophage lineage.

Osteoclastogenesis is directed by Osteoblasts (OBL) & bone stromal cells.
RANK-RANKL-OPG System

- Major role in the bone turnover
  - Mice with a disruption in either RANK or RANKL
    - complete lack of osteoclasts
    - severe osteopetrosis
    - defective tooth eruption
  - mice lacking OPG
    - ↑ numbers and activities of osteoclasts
    - osteoporosis

- Regulator between T & DCs
  - RANKL is upregulated on T cells upon activation
    - Can drive a RANKL osteoclastogenesis & bone loss
RANK-RANKL-OPG System

Pathway

- Binding of RANKL to RANK
  - Signaling cascades that control lineage commitment and activation of osteoclasts
RANK-RANKL-OPG System
Pathway

Negative & Positive Regulators
Wnt Pathway
Bone formation

- **Mechanism**
  - OBL differentiation & activity
  - ↑ growth rate of OBL & inhibiting their apoptosis
  - Inhibiting osteoclastogenesis

- **Wnt proteins**
  - Secreted growth factors proteins (regulate key procedures)
  - 4 signaling pathways
    - Wnt/b-catenin pathway is the most important
  - Inhibitors
    - DKK-1: binds LRP5/6 + Kremen (LRP’s internalized)
    - SOST, produced by osteocytes: binds LRP5
Wnt Pathway

- **DKK-1 experimental models (mice)**
  - ↑: osteopenia, limb deformities, hairlessness, ↓ No of OBL
  - ↓: ↑ No of OBL, ↑ bone formation rate

- **LRP5**
  - LRP5 -/- mice
    - ↓ bone mass
  - Mice with gain of function mutation (G171V)
    - High bone mass phenotype

- **SFRPs (Secreted frizzled-related protein)**
  - able to bind Wnt proteins and prevents them from binding the Fz receptors
  - SFRP1 -/- mice
    - High trabecular bone density
  - In co-cultures of murine osteoblasts with spleen cells
    - Anti-sFRP-1: ↑ osteoclast formation
    - Recombinant sFRP-1: inhibited osteoclast formation
    - Bind and inactivate RANKL
      - thus SFRPs not only act blocking WNT but also via RANKL!
Possible interplay between
RANK/RANKL/OPG & Wnt Systems

- B-catenin regulate OPG expression in OBL
- Cocultures of mouse mononuclear spleen cells & OBL
  - Treated with
    - Wnt3a (activation) or Lithium (indirect activation)
      - Inhibition of formation of OCL
      - \( \downarrow \) RANKL (mRNA, protein)
Inflammation and bone destruction

IL-4
IL-12
IL-18
IFN-β
TGF-b

Promote bone resorption

Inhibit osteoclastogenesis
↑ OPG/RANKL
Inflammation and bone destruction

TNF-a

- Inhibits differentiation of mature OBL
- Stimulates Dkk-1
- Induces apoptosis of OBL, OBL precursors, chondrocytes

- ↑ expression of RANKL
  - Stromal cells
  - T lymphocytes
  - B lymphocytes
  - endothelial cells
- ↑ RANK
- ↑ OCL precursor population
  - mobilize CD11bhigh osteoclast from the bone marrow into the circulation
- ↑ OCL differentiation and activation
  - Along with IL-1 and RANKL
- ↑ M-CSF production
  - stromal cells

Mice deficient in TNFα: Some of them develop bone erosions (indicating that there are TNF independent pathways leading to erosions)
Inflammation and bone destruction

other cytokines

- **IL-1**
  - Direct actions
    - OCL precursors $\rightarrow$ OCL
    - Survival & function of OCL
  - act synergistically with TNFa
    - Mice lacking IL-1R1 treated with TNF
      - $\downarrow$ osteoclastogenesis

- **IL-6**
  - Mechanism
    - IL-6 $+$ IL-6sR bind OBL $\rightarrow$ $\uparrow$PGE2 $\rightarrow$ $\uparrow$ RANKL & $\downarrow$ OPG
    - Mice deficient IL-6: protected from CIA & AIA joint inflammation and tissue destruction but not from STA model

- **OSM**
  - $\uparrow$Osteoclastogenesis
    - $\uparrow$RANKL
    - Intrarticular injections of Ad-OSM: arthritis (pannus, destruction)
    - In CIA & AIA: neutralizing ab $\rightarrow$ $\downarrow$ inflammation & bone loss
Inflammation and bone destruction
other cytokines

- **IL-7**
  - Stimulating differentiation of OCL precursors → OCL
  - ↑ RANKL in T cells

- **PTHrP**
  - Induced by TNFα and IL-1
  - Acts synergistically with TNFα and IL-1
  - ↑ RANKL in OBL
  - ↓ OPG
  - Induces secretion of IL-6
  - CIA: ↑ in joint
  - SCW model: block
    - ↓ No of OCL
    - ↓ bone erosions
    - Inflammation: unaltered
Inflammation and bone destruction

Uncoupling inflammation from bone resorption

- OCL is the major player
  - Inflammatory arthritis can be present in mouse models lacking OCLs (i.e. cfos -/- / hTNFtg, RANKL KO) despite the complete loss of bone erosions
    - No altering in cellular populations
    - MMPs
    - No influence on TNF
Inflammation and bone destruction
Uncoupling inflammation from bone resorption

- Tx with OPG Fc in TNFtg mice
  - ↓ OCL No
  - ↓ focal erosion
  - Ongoing inflammation

- tgTNF mice treated with anti-Dkk-1: inhibition of bone erosions without altering inflammation attributed to ↓ OCL

Thus: TNF and other inflammatory cytokines enhance bone erosion but can’t lead alone to bone erosions
IL-17: a novel regulator

- A combination of TGF-b, IL-6, IL-1 and IL-23 is required for Th17 differentiation and IL-17 production
- Th17 cells
  - Express ↑RANKL
- Cartilage breakdown
  - Synergistic action with TNFa
- Osteoclastogenesis
  - Cocultures of OCL precursors with either RANKL, MCSF or OBL
    - Osteoclastogenesis was ↑ in the presence of IL-17
    - Probably via ↑RANKL in OBL
- AIA/CIA: In vivo blockade
  - ↓ joint destruction
  - ↓ IL-6
  - ↓ bone erosions
  - ↑ VEGF
Disease Paradigms

- **Inflammatory conditions**
  - Bone loss (e.g. Rheumatoid arthritis)
  - Bone loss & bone formation (e.g. Spondylarthritis)

- **Type of bone loss**
  - Generalized (osteopenia)
  - Focal (bone erosion)
  - Local (periarticular osteopenia)

- **Type of bone formation**
  - Sclerosis
  - Osteophyte formation
  - Calcification of soft tissues
Rheumatoid Arthritis

- Proinflammatory mediators in RA joints → production of OCL precursors → in bone/pannus → differentiating to OCL (under RANKL)

- Total hip BMD correlated with erosion scores
  - Stronger among patients with early RA
  - After adjustment disappeared
  - Relationship between focal erosions and generalized osteoporosis is complicated and modified by many factors
Rheumatoid Arthritis

Synovial tissue

- IL-17
  - ↑ spnt of synovial tissue
  - ↑ IL-6, IL-1, TNFa
    - By synoviocytes
  - Collagen destruction
  - Inhibition of collagen synthesis
- ↑ MMP-1
- ↑ RANKL
  - Synoviocytes
  - OBL
- ↓ OPG
  - OBL

↑ RANKL
- OBL
- T cells
- Fibroblasts

↓ OPG

IL-7
- MF
- Fibroblasts
- Endothelial cells

PTHrP
- Synovial lining cells
- Fibroblast like cells
Rheumatoid Arthritis

- **Synovial fluid**
  - ↑ IL-17
  - ↓ OPG
  - ↑ IL-6 & sIL-6R
    - Correlation with radiographic damage
  - ↑ OSM
  - ↑ IL-7
  - ↑ PTHrP

- **Serum**
  - ↑ Dkk-1
    - Association with radiographic progression
    - After anti-TNF Tx: ↓
  - ↑ IL-6 & sIL-6R
Ankylosing Spondylarthritis

- Osteopenia
  - Especially in the spine
  - Effective Tx with anti-TNFα

- New bone formation
  - Not effective Tx with anti-TNFα
  - Possibly due to overactive Wnt system
Ankylosing Spondylarthritis

- Osteopenia
  - Associated with
    - ↓ OPG
    - ↑ bone absorption markers (indicating role for RANKL)
Ankylosing Spondylarthritis
New bone formation

- Dkk-1 serum levels
  - ↑ in AS vs. Healthy, RA, PsA
    - However: ↓ Dkk-1 binding to LRP6
    - No association with inflammation markers or disease activity
  - ↑ in AS after anti-TNF Tx
    - Trying to balance the new bone formation resulting from resolution of inflammation ("TNF brake hypothesis")
Ankylosing Spondylarthritis

- Synovial tissue
  - ↑ TNFα
  - ↑ Bone Morphogenetic Protein (BMP) -2 and -6
    - Thought responsible for bone formation
    - Also found in RA
Psoriatic Arthritis

- Synovial tissue
  - \( \uparrow \) RANKL/OPG
    - Synovial fibroblasts the main source of RANKL
- PBMCs
  - \( \uparrow \) circulating OCL precursors
    - Higher in PsA pts with erosive arthritis
    - Inhibited by OPG
SLE

- Only 4-6% display erosive changes in Rx
  - Histological
    - Mild synovial hyperplasia
    - Microvascular changes
    - Perivascular inflammation
  - One possible explanation
    - ↑ IFNa in SLE skews myelomonocyte precursors to mDC instead of OCL
      - IFNαr1^-/- mice: ↑ OCL precursors & osteopenia
      - Does not explain the osteopenia in SLE
Take home messages

- Bone remodelling is a complex procedure
  - RANK/RANKL/OPG – Osteoclastogenesis
    - Inflammatory cytokines
    - RA
  - Wnt system – Bone formation
    - Dkk-1 is the major player
    - AS