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PHYSIOPATHOLOGICAL MECHANISMS OF OSTEOIMMUNE ALTERATIONS IN GAUCHER DISEASE

Gaucher disease (GD) is a lysosomal storage disorder caused by mutations in the gene encoding β–glucocerebrosidase (GBA) (E.C. 3.2.1.45). Deficient GBA enzymatic activity leads to accumulation of the substrate glucocerebrosidase mainly in macrophages (*Grabowski, 2009*), resulting in lipid-laden activated macrophages referred as "Gaucher cells". Skeletal disorder of Gaucher disease is the major cause of morbility and is highly refractory to enzyme replacement therapy. The pathological mechanisms of bone alterations in Gaucher disease are still poorly understood.

In different experimental models of bone disease, cytokines and chemokines are usually released within the inflammatory milieu, impliying interplay between immune system and bone in the pathophisiology. Especially in Gaucher disease, various reports have shown significant elevations of cytokines, chemokines and bone disease markers. Skeletal disease results from a disruption of the fine balance between osteoblastic bone formation and osteoclastic bone resorption. Osteoclasts originate from the fusion of precursors belonging to the monocyte/macrophage lineage, originating multinucleated cells. M-CSF is a crucial cytokine for the proliferation and survival of osteoclastic precursor cells. Osteoblasts express RANKL at cell surface and binding to its receptor RANK results in the activation of signaling cascades controlling lineage commitment and activation of osteoclasts (*Leibbrandt, 2008*). This process is inhibited by the presence of osteoprotegerin (OPG). T-cell-derived cytokines influences osteoclastogenesis: IFN-γ, IL-4, and IL-10 in a negative way but IL-17 enhancing this process (*Okamoto, 2011*). Under pro-inflammatory conditions, RANKL and TNF-α cooperate to enhance osteoclastogenesis. Theories have been postulated to explain the disruption of homeostatic balance of bone in GD, implying dysfunction of osteoclasts, osteoblasts and mesenchimal cells. In our work, we hypothesized that cellular alteration in GD produces a proinflammatory milieu leading to bone destruction through enhancement of machrophages differentiation to osteoclasts and osteoclasts resorption activity, and/or deficiency of bone formation by osteoblasts.

Previous results from our group have shown mononuclear cells from an in vitro Gaucher disease model secrete factors that enhance osteoclastogenesis, and cytokines are playing a role in this process.

The aims of our project is to identify factors secreted by Gaucher cells that mediate osteoclastogenesis.

Activities

- Patients and samples:

This study is approved by IBYME (a CONICET institute) ethics committee. We will include in the study patients with confirmed Gaucher disease. Samples of peripheral blood will be taken by venopuncture. Mononuclear cells will be isolated by Ficoll sedimentation.

- Flow cytometry:

To identify osteoclast precursors, PBMC will be stained with the following antibodies specific for: CD51, CD14 and CD16. Moreover intracytoplasmic cytokine analyses will be carried out by staining with antibodies specific for TNF-α, IL-6, IL-1β.

- Osteoclastogenesis potential of PBMC

PBMC will be cultured in a-MEM, supplemented with 10% fetal bovine serum and maintained at 37ºC in a humidified atmosphere of 5% CO2. To obtain fully differentiated human OCs, PBMCs will be cultured in presence or absence of recombinant human M-CSF and RANKL (30 ng/ml), for 15 days.

In independent experiments, PBMCs will be cultured in the presence of anti-TNF-antibodies at 2 ng/ml to neutralize this cytokine or an isotype control. To identify osteoclasts, cells will be fixed in 4% paraformaldehyde and stained for tartrate-resistant acid phosphatase (TRAP).

- Gelatinase activity

Metalloproteinase (MMP) levels will be assayed by zymography according to the method of *Hibbs et al.*

-Expression of cytokines, chemokines and osteoclasts markers

Expression of osteoclasts markers catepsine K, RANK and creatine kinase B (CKB) and chemokine CCL-2 will be tested by ELISA.

- Assessment of possible alterations in osteoblast activity

Conditioned media from PBMC will be added to a human osteoblast cell line (SaOS-2, MG-63 or hFob). Production of proinflammatory cytokines, MMP, expression of KC and MCP-1, apoptosis (assessed by TUNEL) and bone mineralization (assessed by Alizarin red S staining) will be tested.