Modeling tumor-bone interactions in ATL with HTLV-1-infected peripheral blood cell lines

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Background:
Adult T-cell leukemia/lymphoma (ATL), caused by infection of human CD4+ T cells with HTLV-1, is associated with osteolytic lesions and hypercalcemia. HTLV-1 infected cells produce exosomes, previously shown to facilitate infection. We hypothesized that these exosomes also mediate bone loss.

Methods:
HTLV-1 infected T cell lines (HTLV/T) were generated by co-culturing lethally irradiated HTLV-1 producer cell lines with human peripheral blood mononuclear cells (hPBMCs). Exosomes were isolated from HTLV/T supernatants using a commercial kit (Total Exosome Isolation Reagent, Invitrogen), and fluorescently labelled with PKH-26. Mouse bone marrow macrophages (mBMMs) and hPBMCs were cultured in osteoclastogenic conditions with supernatant or exosomes from HTLV/T, then stained for TRAP. RNA was also isolated from HTLV/T and osteoclast (OC) cultures. HTLV/T lines were injected into the tibias of immunodeficient NCG mice, and bone mass was measured by viva CT. At sacrifice, human $CD4^+$ cell populations were analyzed in bone marrow and spleen.

Results:
Supernatant from HTLV/T cells were variable in their ability to stimulate OC differentiation, but showed similar effects on murine and human cultures. Expression of RANKL and OPG by these HTLV/T was also variable, but we found no correlation between OC numbers and RANKL/OPG. Isolated exosomes were taken up by mBMMs, and carry the OC stimulatory activity of supernatants. Proteomics of exosomes from high and low osteoclastogenic HTLV/T cell lines showed distinct compositions. In a pilot *in vivo* experiment, intratibial injection of HTLV/T clones with high *in vitro* OC activity led to systemic bone loss, and systemic spread of human CD4+ T cells.

Conclusions:
- HTLV/T cell lines variably affect OC differentiation.

- Osteoclastogenic effect of HTLV/T lines is mediated by exosomes.
- Proteomics of exosomes with high and low osteoclastogenic activity suggested possible candidates.
- Intra-tibially injected HTLV/T cells spread systemically and lead to bone loss in mice.

Disclosure of Interest Statement:
The authors declare no conflict of interest.