**Background:** In OA joint, synovial membrane contain immunocompetent cells (1-4), which produce predominantly pro-inflammatory cytokines (2,5), justifying the name "ostearthritis" and directing the development of disease towards mild systemic inflammatory condition (6). Granulysin (GNLY) is mediator of cellular immunity expressed in T and NK cells in 15 kDa precursor and 9 kDa cytotoxic forms (2). It is regulated by interleukin 15. We investigated GNLY expression in peripheral blood lymphocytes and GNLY mediated apoptosis in vitro, serum concentration of IL-15 and the correlation of GNLY expression with intensity of the pain in the knee of OA patients and with 6-minute walk distance.

**Objectives:** With knee OA (20), and healthy control (17) were medically examined, and their blood samples tested. All of them signed informed consent before medical sampling of peripheral blood (PB).

**Methods:** Visual analogue scale (VAS) of pain and results of 6-minute walk test were noticed in all participants. Peripheral blood mononuclear cells were isolated by gradient density centrifugation and intracellular staining were performed and evaluated by flow cytometry. NK cells' apoptotic activity against NK sensitive K-562 cells was measured in 18-hour PKH-26 (red) cytotoxicity assay with evaluation of propidium iodide-annexin V+ target cells by flow cytometry. In some samples anti-GNLY and/or anti-perforin antibodies were added. IL-15 concentration was measured by ELISA. Nonparametric Kruskal-Wallis and Mann-Whitney U-test, as well as Spearman correlation test were used for statistical evaluation.

**Results:** In lymphocytes of OA patients GNLY expression and NK cell-mediated apoptosis of K-562 cells did not differ significantly from the healthy control. In OA patients only, RC8 antibody against cytotoxic granulysin (GNLY) molecule significantly decreased apoptosis of K-562 cells. RF10 anti-15 kDa GNLY did not show such effect. Anti-perforin antibody completely abolished apoptosis in both groups tested and the effects of additionally added RC8 or RF10 anti-GNLY antibodies were not observed. Serum IL-15 concentration in healthy controls and OA patients was low and did not show statistically significant difference. GNLY expression in lymphocytes, and particularly in NK subset, positively correlated with VAS of pain and 6-minutes walking distance.

**Conclusion:** In OA patients, GNLY mediated apoptosis is involved in apoptosis of NK sensitive K-562 cells in vitro and might be involved in the killing of damaged joint cells in vivo after direct contact.

**REFERENCES**


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