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Title: Effect of enginereed GOLD nanoparticles targeted to mesenchymal cells on inflammatory response

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Body: In a previous work we proved that gold nanoparticles engineered with a specific anti-CD44 antibody (GNP-HCe) directed against mesenchymal cells (MCs)from BOS patients were able to specifically inhibit their proliferation and increase their apoptosis. Aim of this work is to prove that GNP-HCe do not stimulate inflammatory cells in vitro since inflammatory process plays a pivotal role in BOS onset and its activation would exacerbate the reactive milieu. Macrophages were isolated from BAL of three subjects by adhesion procedure. Lymphocytes and neutrophils were obtained from peripheral blood by Lympholyte gradient. IL-8 was assayed by ELISA test while IFN-g, IL-17 and IL-10 by Elispot. Elastase release was evaluated by enzymatic procedure. Apoptosis and proliferation rate were evaluated by flow cytometry (Annexin V and CFSE). In vitro experiments proved that 24 and 48 h of incubation with GNP-HCe did not increase IL-8 secretion by macrophages but instead a significant (P<0,001) reduction was observed. Similarly, IFN-g, IL-17 and IL-10 produced by lymphocytes significantly decreased in presence of functionalized nanoparticles. Neutrophils were not stimulated by incubation with GNP-HCe as evidenced by unchanged elastase release in the culture medium. Lymphocyte proliferation rate in presence of GNP-HCe was not significantly different from controls while a significant increase ($p<0.01$) of apoptosis was recorded at 24-48 h incubation. This work opens new scenarios on the possibility to target a drug to specific cells without rising a further inflammatory response that would contribute to perpetuate the vicious cycle epithelial damage/inflammatory response/fibroproliferative process.