ABSTRACT: Background: Our previous results showed that intrasynovial Rifamycin SV caused the lysis of synoviocites and freed the autoantigens which in turn stimulated the immunoregulatory rather than autoreactive T cell response in rheumatoid patients. Here, we hypothesize that disruption in vitro of peripheral blood mononuclear cells, by freeze/thawing or by lytic action of Rifamycin SV, would induce the release of endosomal pathogenic autoantigens from APCs present in the circulation, which could then be isolated from degrading enzymes by ultrafiltration. Methods: The preparation of the ultrafiltrates are based on the rupture of PBMCs (5 × 10(6) cells/mL) by the addition of Rifamycin SV in culture (250 μg/mL), which causes the lysis of 90 % of the cells in 3 h, or by three cycles of freeze/thawing of the PBMC, from -80 °C to room temperature. The lysate and the fragmented cells were then centrifuged and ultrafiltered by passage through a filtration device with a cut-off of 10 kDa. Also the synovial fluid was subjected to ultrafiltration. Results and conclusions: At clinical monitoring of the 30th day, 22/58 (38 %) patients subcutaneously treated with the autologous ultrafiltrate prepared by the freeze/thawing of PBMCs reached an ACR20. Comparable results were obtained with the other two ultrafiltrates. Cell cultures The addition of ultrafiltrates to rheumatoid PBMCs cultures led to the upregulation of a marker for T-regulatory cells, and downregulation of a cell proliferation marker; changes that together have the meaning of a global immunomodulatory response and that only a specific antigen (ultrafiltrate UF-f/t) might induce in the rheumatoid patient, probably by activating pre-existing protective network. Experimental arthritis All the ultrafiltrates except that prepared by Rifamycin SV were able to modulate the adjuvant arthritis in rats. In particular, longlasting synovial fluid induced a significant reduction of the severity of subsequent arthritis ($p < 0.01$) while SF from recent RA effusion (5-10 days after a previous complete extraction) and knee osteoarthrosis were ineffective. It is reasonable to assume there are at least two unknown endosomal immunoactive epitopes; one developing its immunotherapeutic property in RA, and the other, related to the molecule of HSP60, reduces the severity of oncoming arthritis. Both epitopes are present in humans, have a molecular weight of ≤10 kDa and do not appear to be bystander antigens. Please see Additional file 1 for the abstract in Italian.

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