AB0031

METABOLIC ADAPTATION OF HUMAN NEUTROPHILS TO GLUCOSE DEPRIVATION IS IMPAIRED WITH INCREASING AGE

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Background: Age-related impairment of classical neutrophil functions is well described (Fortin, McDonald et al. 2008). However, experimental evidence for age-related alterations of neutrophil metabolic adaptation towards nutrient deprivation - a feature of neutrophil battlefields - remains elusive. Moreover, age-related differences in neutrophil metabolic adaptation may contribute to age-related pathologies such as atherosclerosis, cancer, and autoimmune diseases including rheumatoid arthritis.

Objectives: Therefore, we hypothesized that metabolic adaptation of human neutrophils to glucose deprivation is impaired with increasing age.

Methods: We isolated human peripheral CD15⁺ neutrophils from four healthy young donors (mean age: 23.4 ± 2.7) and four healthy donors with a mean age of 58.7 ± 2.4. First, we analyzed the survival of neutrophils either stimulated with PMA or left untreated and subsequently incubated for 0h and 6h under varying glucose concentrations (0, 0.5, 1, 5, and 10 mM). To address this, we used 7-AAD staining and flow cytometry. Using Seahorse™ technology, we determined basal respiration, ATP-bound respiration, and maximal and spare capacity.

Results: We show that neutrophils (purity > 95%) survived for 6 hours *in vitro*, independent of treatment with PMA or concentrations of glucose in the culture medium. With negligible differences between the various concentrations of glucose used, the percentage of living cells after 6h was 95% ± 2.5 without PMA and 75% ± 4.7 with PMA stimulation. No differences were uncovered in this respect between the two age groups. However, Seahorse™ technology revealed significant differences in basal, maximal, and spare respiratory capacity. Briefly, OCR (pmol/min/cell count) with respect to basal, maximal and reserve respiratory capacity was lower in the elderly donors compared to the young donors. For instance, with a concentration of 5 mM glucose, the basal respiration (OCR) was 17 ± 0.7 in elderly donors compared to 22.5 ± 1 in young donors, while the maximal respiration was 25 ± 0.8 in elderly and 41 ± 0.6 in the young donor group. Interestingly, these differences were independent of glucose concentration in the medium.

Conclusion: Our data show that basal metabolic parameters differ between neutrophils from young and older donors. Further experiments are needed to understand in detail the mechanisms and effects of age-related differences in metabolism on neutrophil functions.

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AB0032

DIFFERENTIAL IMPACT OF BIOLOGICS AND GLUCOCORTICOIDS ON TNF SECRETION AND CD14⁺CD16⁺ MONOCYTES PERCENTAGE IN CULTURE DERIVED FROM SYNOVIAL FLUIDS OF PATIENTS WITH PSORIATIC ARTHRITIS-IN VITRO RESEARCH

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Background: Inflammatory joint diseases, such as psoriatic arthritis (PsA), are frequently treated by biologics. Assessment of treatment efficacy is based upon change in clinical activity scores and in tender and swollen joint counts. Although the response to these agents may be attributed to their central anti-inflammatory effects, synovial response may be operating in parallel. The potential beneficial role of intra-articular injection of tumor necrosis factor (TNF) blockers compared to glucocorticoids (GCs) in reducing synovitis was shown by means of clinical and instrumental validated measures (1). The inflamed synovial fluid is rich in mononuclear cells, however, the different mode of action of the *in vitro* response of these cells to drugs may contribute to the understanding of cellular response to therapeutic agents in central as opposed to peripheral compartments.

Objectives: To evaluate the effect of biologics used in the management of PsA on synovial fluid mononuclear cells (SFMCs) *in vitro*, and to compare their modes of action to GCs that are used to locally alleviate synovial inflammation.

Methods: SFMCs were obtained from PsA patients (n=11) during therapeutic knee arthrocentesis. The cells were cultured *in vitro* for 7 days in the presence of biologics (adalimumab, infliximab, secukinumab and ustekinumab, 10ug/ml) and

GCs (betamethasone and methylprednisolone, 1 ug/ml and 10ug/ml) or medium as control. Levels of the secreted TNF were measured by ELISA. Changes in %CD14⁺CD16⁺ monocytes were analyzed by flow cytometry.

Results: Both TNF inhibitors (adalimumab *p*<0.01, infliximab *p*=0.0003) and GCs (betamethasone and methylprednisolone at 1ug/ml *p*<0.01 and at 10ug/ml *p*<0.04) significantly reduced TNF levels in cultured media derived from SFMCs of PsA patients (n=8) compared to medium. None of the other biologics reduced TNF levels in culture (Fig. 1A). Additionally, %CD14⁺CD16⁺ SFMCs derived from PsA patients (n=11) were significantly reduced by TNF inhibitors (*p*=0.0003) compared to medium, however, other biologics and GCs did not display similar activity (Fig. 1B).

Figure 1. Both TNF inhibitors and GCs block TNF secretion but exhibit different activity on inflammatory CD14⁺CD16⁺ monocytes derived from SFMCs of PsA patients. SFMCs were co-cultured for 7 days in the presence of adalimumab, infliximab, secukinumab and ustekinumab at 10ug/ml or with betamethasone and methylprednisolone at 1ug/ml and 10ug/ml. Medium alone was used as a control. (A) Culture supernatants were analyzed for TNF levels by ELISA (n=8). (B) Cells were analyzed for %CD14⁺CD16⁺ monocytes by flow cytometry (n=11). All *p* values were calculated by the non-parametric one-way ANOVA Kruskal-Wallis test and Dunn's multiple comparison test, **p*<0.04, ***p*<0.01 and ****p*=0.0003.

Conclusion: Our data demonstrated marked activity mediated by TNF inhibitors in comparison with other biologics tested for their ability to suppress TNF secretion and inflammatory CD14⁺CD16⁺ monocytes. In contrast, GCs suppressed TNF secretion but did not significantly change the proportion of inflammatory CD14⁺CD16⁺ monocytes. These findings suggest an additional mechanism of action exerted directly by TNF inhibitors on synovial monocytes and which differs from that of GCs. These results warrant further studies of the therapeutic potential of local peripheral activity of TNF inhibitors for clinical application.

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AB0033

MACROPHAGE ACTIVATION IN PATIENTS WITH ACTIVE RHEUMATOID ARTHRITIS AND SYSTEMIC LUPUS ERYTHEMATOSUS (PRELIMINARY DATA)

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Background: The study of the ability of monocytes to activate associated with the clinical activity of immunological markers of inflammation in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) will provide important and fundamentally new information on the involvement of these cells in the development of autoimmune rheumatic diseases (ARDs).

Objectives: To study macrophage activation in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) patients (pts).

Methods: A total of 21 active ARDs pts (11 RA, 10 SLE) were enrolled in the study (median age was 55[44; 63] years; disease duration was 8 [2; 14] months). There are 11 pts with early RA (disease duration was ≤12 months), moderate to high activity (DAS28 was 6.1[4.9;6.7]; SDAI was 25[22;31]; ACCP was positive in 73% and RF in 87% cases) and 10 pts with active SLE (SLDAI-2K was 7 [6;8])