THU0016 SIALIC ACID, INTERCELLULAR ADHESION MOLECULE-1 AND RHEUMATOID ARTHRITIS: A STUDY ON THE ERYTHROCYTE MEMBRANE

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Background

Objectives We aimed to measure serum levels of soluble intercellular adhesion molecule-1 (sICAM-1) and erythrocyte membrane sialic acid (SA) activity and the correlation of these parameters in patients with rheumatoid arthritis (RA), and to investigate the correlation of these parameters with the disease activity.

Methods Serum sICAM-1 levels were determined with sandwich enzyme-linked immunosorbent assay (ELISA) and SA concentration was determined according to the method of Shamberger in sera from 42 patients with RA and in 30 healthy controls. Erythrocyte sedimentation rate (ESR) was determined according to the Westergren method and C-reactive protein (CRP) by nephelometric method. Disease activity was assessed by disease activity criterias.

Results Significantly lower erythrocyte membrane SA and higher serum levels of sICAM-1 were found in patients with RA than in healthy controls ($p < 0.001$ for both). Significant negative correlation between sICAM-1 level and erythrocyte membrane SA concentration ($r = -0.49$, $p < 0.001$), and positive correlation between sICAM-1 level with Ritchie articular index (RAI) score and CRP ($r = 0.32$, $p < 0.05$; $r = 0.44$, $p < 0.01$, respectively), were observed. No significant correlation was found between sICAM-1 level with ESR, age and disease duration. There was not any correlation between values of CRP, RAI score and ESR with erythrocyte membrane SA concentration.

Conclusion From these data, it is concluded that decreases in erythrocyte membrane SA concentration and increases in sICAM-1, ESR and CRP levels are present in RA, and that the decrease in erythrocyte membrane SA concentration in RA might be due to increased sICAM-1, and increased levels of sICAM-1 and correlations with other parameters may be a significant and novel marker for evaluating the disease status and the activity of RA.

THU0017 CHLOROQUINE RESISTANCE IN HUMAN CEM (T) CELLS IS MEDIATED BY MULTIDRUG RESISTANCE PROTEIN 1

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Background Drug resistance may play a role in the lack of efficacy of Disease Modifying Anti Rheumatic Drugs (DMARDs).

Objectives (1) Delineate mechanism of drug resistance to chloroquine (CHQ) and (2) does CHQ resistance confer resistance to other DMARDs.

Methods Human CEM (T) cells were made resistant to CHQ following a 4 months *in vitro* exposure to stepwise increasing extracellular CHQ concentrations.

Results CHQ resistant CEM cells (CEM/CHQ) displayed 3.3-fold resistance to CHQ compared to CEM cells (IC_{50} : 129 μ M vs 39 μ M, respectively, following 72 h drug exposure). The resistant phenotype was stable for at least one month when CEM/CHQ cells were grown in the absence of CHQ. CEM/CHQ cells exhibited full sensitivity to methotrexate (MTX) and

were even collaterally sensitive (1.7-fold) to sulfasalazine. Interestingly, CEM/CHQ cells displayed cross-resistance to doxorubicin and daunorubicin, suggesting that multidrug resistance proteins play a role in the resistant phenotype. In fact, immunological detection (Western blots) revealed a markedly increased expression of multidrug resistance protein 1 (MRP1) in CEM/CHQ cells compared to CEM cells. Involvement of MRP1 in CHQ resistance was further illustrated by observations that inhibitors of MRP1, probenecid and MK571, were able to reverse CHQ resistance in CEM/CHQ cells. Beyond MRP1, we noted that also cells transfected with MRP2 and MRP3 displayed resistance to CHQ (2.1- and 1.8-fold, respectively).

Conclusion Overexpression of multidrug resistance proteins (MRP1, MRP2 and MRP3) can confer *in vitro* resistance to CHQ and should be considered as a mechanism of lack of drug efficacy in a clinical setting.

THU0018 DIFFERENTIAL CHLOROQUINE SENSITIVITY OF HUMAN CEM (T) CELLS WITH VARIOUS MECHANISMS OF RESISTANCE TO METHOTREXATE

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Background Methotrexate (MTX) is the anchor drug in rheumatoid arthritis treatment, either as monotherapy or in combination with other antirheumatics, e.g. chloroquine (CHQ).

Objectives Does resistance to MTX influence sensitivity to CHQ, and if so, by what mechanism?

Methods *In vitro* CHQ sensitivity was evaluated in human CEM (T) cell lines, wild type CEM (CEM^{wt}) and 3 CEM sublines resistant to MTX due to reduced cellular uptake (CEM/MTX-transport^{low}), elevated levels of dihydrofolate reductase (CEM/MTX-DHFR^{up}) or impaired polyglutamylation (CEM/MTX-FPGS^{low}).

Results CEM/MTX-transport^{low} cells were 1.5-fold more sensitive to CHQ than CEM^{wt} cells (IC_{50} : 25 μ M vs 39 μ M, respectively, after 72 h drug exposure). However, CEM/MTX-DHFR^{up} and CEM/MTX-FPGS^{low} cells were 2.3-fold (IC_{50} : 89 μ M) and 1.9-fold (IC_{50} : 74 μ M) resistant to CHQ, respectively, compared to CEM^{wt} cells. The mechanism of CHQ resistance in CEM/MTX-DHFR^{up} and CEM/MTX-FPGS^{low} cells is likely due to overexpression of multidrug resistance protein 1 (MRP1). This was suggested by a low level (1.5–2-fold) cross-resistance to doxorubicin and daunorubicin, and observations that an inhibitor of MRP1, MK571, was able to reverse CHQ resistance. The same phenotypic changes were noted in CEM/MTX-transport^{low} cells with acquired resistance to CHQ (4-fold) after *in vitro* exposure to stepwise increasing extracellular CHQ concentrations.

Conclusion Development of MTX resistance can be associated with up regulated expression of MRP1, which, concomitantly, can confer cross-resistance to CHQ. Analysis of MRP1 expression in inflammatory cells should therefore be considered as an important parameter determining the efficacy of anti-rheumatic (combination) therapy.

THU0019 INTRACELLULAR EXPRESSION OF CXCR3 ON RHEUMATOID ARTHRITIS SYNOVIAL TISSUE CELLS

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Background Inflammatory cell infiltration and synovial activation are important processes in rheumatoid arthritis. Chemotactic gradients of various chemokines are responsible for cell attraction and possibly for their activation. We have previously detected strong expression of chemokine receptor CXCR3 in the rheumatoid joint by immunostaining.

Objectives Characterisation of the cells expressing CXCR3 in RA synovial membrane.

Methods Synovial tissue samples were obtained from RA patients undergoing either synovectomy or a total joint replacement. Cells were released by digestion with collagenase, DNase and briefly with hyaluronidase. A three colour fluorescence analysis was performed with FITC conjugated anti-CXCR3 mouse MAb (R&D) and with a panel of phycoerythrin (PE) conjugated MAb (anti-CD3, CD4, CD8, CD19, CD55, CD31, CD68, CD14 and CD45). Live cells were identified by propidium iodide. PBCs were stained using the same protocol.

Results As expected, a proportion of CD3⁺ and CD4⁺ blood and synovial cells was CXCR3 positive. In addition, CXCR3 was also seen in synovial cells positive for CD55, CD14, CD8 and to a less extent CD31. However, in contrast to the surface staining of cells from peripheral blood, CXCR3 staining was identified only intracellularly in synovial cells. No CXCR3 staining could be detected on the surface of any type of viable synovial cell, including CD3 positive lymphocytes.

Conclusion Flow cytometry identifies synovial cells that display intracellular CXCR3 staining. These cells are comprised of T lymphocytes, macrophages, possibly synovial fibroblasts and endothelial cell populations. The intracellular presence of CXCR3 suggests a possible internalisation of this molecule, which may be a consequence of ligand binding. The significance of this phenomenon and of CXCR3 expression in cell types other than leukocytes remains to be determined.

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THU0020 ROLE OF FCGAMMARII AND FCGAMMARIII IN INFLAMMATION AND CARTILAGE DAMAGE DURING MURINE EXPERIMENTAL ANTIGEN-INDUCED ARTHRITIS

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Background IgG-containing immune complexes, which are found in most RA joints, communicate with haematopoietic cells using three classes of Fc receptors (FcγRI, II, III). In a previous study we found that if an experimental arthritis was elicited in knee joints of FcR γ-chain deficient mice which lack functional FcγRI and III, joint inflammation was lower and severe cartilage destruction was absent.

Objectives To examine the role of FcγRII and III in inflammation and functional cartilage damage in knee joints with chronic experimental antigen-induced arthritis (AIA) using FcγRIII and FcγRII deficient mice.

Methods FcγRII and FcγRIII ^{-/-} mice and their controls were immunised with methylated BSA in complete adjuvant followed by induction of arthritis by local injection of mBSA into the right knee joint. The course of inflammation was studied by ^{99m}Tc uptake. Chronic inflammation and cartilage damage (depletion of proteoglycans, chondrocyte death and matrix erosion) was studied histologically in total knee joints sections stained with haematoxylin or safranin-O. Aggrecan breakdown in cartilage caused by metalloproteinases (MMP) was studied by immunolocalisation (VDIPEN neopeptides) and image analysis.

Results Three weeks after immunisation both humoral (total IgG, IgG1, IgG2a and IgG2b levels) and cellular (T cell responses as measured by lymphocyte proliferation) immunity raised against mBSA was comparable in all groups examined. IgG2a levels were comparable in FcγRIII ^{-/-} but higher in FcγRII ^{-/-} if compared to controls. Joint swelling at day 1, 3 and 7 was similar in FcγRIII ^{-/-} mice and significantly (103%) higher in FcγRII ^{-/-} on day 1 but not on later time-points. Histologically, at day 7 after AIA induction, exudate and infiltrate in the knee joint was similar in FcγRIII ^{-/-} and significantly higher (100% and 388%) in FcγRII ^{-/-} mice if compared to controls. MMP-induced neopeptides determined in various cartilage layers (tibia and femur) were comparable in FcγRIII ^{-/-} and higher (respectively 155–360% and 67–75%) in FcγRII ^{-/-}. Initial depletion of proteoglycans was similar (40–70%) in all groups. In the chronic phase, chondrocyte and cartilage matrix erosion in tibia and femur stage was significantly elevated in FcγRII ^{-/-} but not in FcγRIII ^{-/-} mice.

Conclusion FcγRIII is redundant or not involved in the presence of FcγRI whereas FcγRII is a crucial inhibiting factor in acute and chronic inflammation and cartilage erosion during antigen-induced arthritis.

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THU0021 DEFECTIVE RAP1 ACTIVATION IN SYNOVIAL FLUID T LYMPHOCYTES FROM PATIENTS WITH RHEUMATOID ARTHRITIS

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Background Rap1 is a small G-protein, member of the Ras GTPase family. Rap1 has been linked to T cell anergy and it is suggested that Rap1 has the potential to inhibit Ras-mediated oncogenic or growth promoting activity. Synovial fluid (SF) T cells from patients with rheumatoid arthritis (RA) display a defective phosphorylation pattern of several pivotal signalling proteins and severe hyporesponsiveness upon antigenic stimulation. Our previous data showed that the hyporesponsive state of the SF T cells in RA correlates with markers of oxidative stress and replenishment of the intracellular level of the anti-oxidant (GSH) by treatment of cells with N-acetyl-cysteine (NAC) seems to partly restore the observed signalling defects.