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Methotrexate (MTX) inhibition of cytokine production: relationship with clinical outcome and genetic polymorphisms

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Introduction: Downregulation of TNF-α levels in synovial fluid of rheumatoid arthritis (RA) has been described following treatment with methotrexate (MTX). Although the mechanism of action of MTX is unknown, inhibition of methylenetetrahydrofolate reductase (MTHFR) could be implicated. Polymorphisms in MHTFR have been described (A1298T and C677T) that may condition clinical response.
Aim: To determine whether MHTFR genotype is related to the '*in vitro*' cytokine inhibition and '*in vivo*' clinical response to MTX.
Materials and methods: Twenty-three patients with early RA were studies along with 23 healthy donors. Blood (1/10 diluted in LPS-free Iscove's) was cultured in the presence of 1 μg/ml anti-CD3 and 1 μg/ml anti-CD28, with or without MTX (0.152 to 625 ng/ml; L. Aarden, personal communication). The effect of MTX was reverted with folinic acid (3 μg/ml). TNF-α, IFN-γ and IL-6 concentrations were measured by ELISA in sera and culture supernatants after 72 hours. MTHFR polymorphisms were determined by PCR-RFLP. DAS was used to evaluate the clinical effect of MTX at 6 months.
Results: 1) Basal and stimulated cytokine production was similar in controls and patients. 2) 40 ng/ml of MTX significantly inhibited IFN-γ, TNF-α and IL-6 production of T cells stimulated by anti-$CD3^+$anti-CD28 ($P < 0.001$). 3) A statistically significant correlation ($P < 0.05$) was found between ID-50 for TNF-α and clinical improvement as assessed by DAS (% decrease of DAS score). ID-50 for TNF-α was not related to MHTFR polymorphisms ($P = 0.076$). Median ID-50 was 27.13 for TNF-α (25th percentile = 18.5; 75th percentile = 34.16) and 19.74 for IFN-γ (25th percentile = 13.08; 75th percentile = 24.00). 4) ID-50 for IFN-γ was lower in homozygous individuals A1298A ($P < 05$), but was not associated with clinical outcome. No statistical differences were associated to the C677T mutation.
Conclusions: MTX inhibits stimulated T cell cytokine production. Individual susceptibility for MTX inhibition of cytokine production could help predict clinical response to the drug. Mutations in the MHTFR gene were associated with a lower response to MTX '*in vitro*'.

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Combination of cellular imaging and molecular analysis for evaluation of cellular gene therapy of RA

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Objective: Gene therapy has been developed as a promising tool for the treatment of autoimmune diseases such as rheumatoid arthritis (RA). In order to achieve the goal of establishing a targeted delivery of potentially immune-modulating gene products to inflamed joints in RA, the concept of adoptive cellular gene therapy was developed in an animal model of RA, collagen-induced arthritis (CIA). Adoptive cellular gene therapy utilizes immune cells with specific homing capacity as "vehicle cells" to deliver therapeutic gene products locally after *ex vivo* retroviral transduction. For the evaluation of adoptive cellular gene therapy two goals have to be achieved. One is the monitoring of cellular trafficking and homing to areas of inflammation. The other is the analysis of molecular effects in the synovium of inflamed target joints. In order to approach these goals, we have tested two analysis powerful techniques on animal and human specimens. For *in vivo*, real-time visualization of cellular trafficking, bioluminescence imaging has been developed in animal models. For the analysis of compartment-specific analysis of gene expression in human RA synovium, we established the combination of laser-mediated microdissection and differential display to analyze distinct gene expression profiles of histologically defined areas in RA synovium.
Methods: In the CIA model, antigen-specific T-cell hybridomas and dendritic cells (DC) were adoptively transferred into recipient animals after *ex-vivo* retroviral transduction to express luciferase. Repeated injection with the substrate luciferin and bioluminescence imaging on consecutive days allowed *in vivo* tracking of the adoptively transferred cells. For development of compartment-specific molecular analysis, cryosections derived from RA synovial tissues were used to obtain cells samples from synovial lining and sublining using a microbeam laser microscope. RNA was isolated and analyzed using nested RAP-PCR for differential display fingerprinting. Differentially expressed bands were cut out, PCR products were eluted, cloned and sequenced. Differential expression of identified sequences was confirmed by *in situ* hybridization and immunohistochemistry.
Results: It could be demonstrated that adoptively transferred T-cell hybridomas and DC homed to and accumulated in inflamed joints of CIA mice. In addition, microdissected RA synovial tissue sections containing about 600 cells were shown to yield sufficient RNA for a stable, reproducible RNA fingerprint. This method allowed us to identify several known and unknown genes as being expressed differentially between the synovial lining and sublining layers, including thrombospondin in the linig, Ciz/cip-1 in the sublining and fibronectin in both layers of RA synovium. All three molecules could be confirmed on the mRNA and protein level.
Conclusion: We tested two novel analysis techniques on animal and human specimens. Bioluminescence imaging allowed *in vivo* monitoring of the migration pattern of therapeutic "vehicle cells" in adoptive cellular gene therapy of CIA, an animal model of RA. Laser microdissection and subsequent RAP-PCR reliably enabled us to obtain novel insights into the area-dependent differential regulation of gene expression in human RA synovium and distinction between different cell types. In combination, these methods present a powerful tool for the evaluation of cellular gene therapy of RA.