Objectives In the present study we followed activation of STAT1 and 3 during zymosan-induced arthritis (ZIA) in wildtype (wt) and IL-6^{-/-} mice. We also studied expression of the STAT inhibitors Suppressor Of Cytokine Signalling (SOCS) 1 and 3 during the arthritis.

Methods Zymosan induced Arthritis was elicited by intra-articular injection of zymosan as described in (2). Protein lysates of inflamed and control synovia were subjected to Western blotting for both total and phosphorylated STAT1 and STAT3. RT-PCR was performed for STAT3, SOCS1 and SOCS3.

Results The primary inflammation lasted for a week in both strains. In wt mice this inflammation became chronic until the end of the experiments at week 4, while it decreased rapidly in IL-6^{-/-} mice. In wt mice STAT3 became activated from day 1 until the end of the experiments. Also the level of STAT3 mRNA and protein increased. STAT1 in contrast only became activated during the chronic phase after day 7. During the arthritis there was an increase of SOCS1 and SOCS3 mRNA expression. In IL-6^{-/-} mice STAT3 only became activated at day 1. No activation of STAT1 occurred in IL-6^{-/-} mice.

Conclusion STAT3 activation occurred during both the primary and chronic inflammation in wildtype mice. During the first week IL-6^{-/-} mice also developed a primary inflammation. IL-6 is not the only cytokine that activates STAT3, explaining STAT3 activation at day 1 in IL-6^{-/-} mice. Lack of further STAT3 activation during the primary inflammation suggests a major role for IL-6 in STAT3 activation. STAT1 only became activated in the chronic phase. Arthritis increased expression of SOCS1 and 3, but this seemed not enough to inhibit STAT activation. Future research will have to address the importance of activated STAT1 and 3 for chronic synovitis and the possibility of inhibiting these signalling molecules in the inflamed synovium.

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OP0004 PIVOTAL ROLE FOR THE SMALL GTPASE RAP1 IN THE REGULATION OF INTRACELLULAR GENERATED ROS AFTER T CELL STIMULATION

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Background Oxidative stress plays a critical role in various diseases including cancer, neuronal degeneration, atherosclerosis and rheumatoid arthritis. Identifying the target proteins for redox modification is crucial in understanding how oxidants mediate the pathological processes. As previously demonstrated activation of T cells with anti CD3 antibody or with phorbol ester results in a rapid production of intracellular reactive oxygen species (ROS). In this paper we further dissect the signalling pathways leading to ROS generation and we propose a possible critical role for the small GTPase Rap1 in antagonising the intracellular generated ROS.

Objectives

Methods In our experiments we made use of the free radical dye 6-carboxy-2',7'-dichlorofluoresceine DCF to measure radical generation at the single cell using flow cytometry. Transfected

Jurkat cells were incubated with the dye 20 min 37° before addition of stimuli. Readings were taken every 5-min over a 20 min period. The positive transfected Jurkat cell population was identified by detection of a co-transfected CD20 receptor.

Results T cells as well as Jurkat cells rapidly produce intracellular ROS in a time dependent manner, after stimulation with either CD3 or PMA+ionomycin. The intracellular ROS generated after T cell stimulation seem to be induced through a signalling pathway downstream of the small GTPase Ras, since Jurkat cells transfected with a constitutively active mutant of p21Ras, RasV12, produced large amounts of intracellular ROS, that could not be augmented by an additional stimulus. The observed ROS production after addition of a stimulus was suppressed by the expression of dominant negative mutant of Ras, RasN17. Jurkat cells transfected with the constitutive active mutant of another small GTPase, Rap1, failed to generate ROS after any stimulus tested. Moreover co-transfection of RapV12 and RasV12 showed that RapV12 abolishes the intracellular ROS generated by RasV12. The inhibition of ROS production appears to involve on a PI3K-dependent signalling pathway as is shown by inhibition of ROS production after Jurkat transfection with RapV12C40 (a Rap1 mutation that, analogue with RasV12C40 could affect specific binding to PI3K). Accordingly, pre-incubation with LY 294002, an inhibitor of the lipid kinase activity of PI3K, blocked the observed inhibition of ROS production by Rap1 in RasV12+RapV12 co-transfection experiments. In addition we report that Rap1 activation in T cells after activation with CD3 or PMA proved to be redox sensitive as shown by pretreatment of T cells with N-acetylcysteine and BSO. Furthermore hydrogen peroxide was capable of Rap1 activation in T cells, and pre-incubation with diphenylene iodonium (a flavoprotein inhibitor) completely blocked Rap1 activation.

Conclusion We define a role for ROS in T cell signalling pathways downstream from Ras and we propose a possible pivotal role for the small GTPase Rap1. Rap1 can be activated by ROS and antagonises the intracellular generated ROS in signalling pathways.

OP0005 LEFLUNOMIDE REDUCES TRANSENDOTHELIAL MIGRATION

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Background The disease modifying drug leflunomide is widely used in the therapy of rheumatoid arthritis (RA). Its active metabolite, A771726, leads to inhibition of dihydroorotate dehydrogenase, an enzyme necessary for pyrimidine de-novo synthesis. Since activated lymphocytes expand their pyrimidine pool, A771726 decreases their proliferation. A771726 also suppresses TNF mediated nuclear factor kappaB activation. We were therefore interested if A771726 also would be capable to influence transendothelial migration (TEM) of peripheral mononuclear cells (PBMC).

Objectives

Methods We investigated the TEM of PBMC, which migrated through endothelial cell monolayers in an in-vitro model. Human umbilical vein endothelial cells (EC) were cultured to confluence on collagen gels and then incubated with human PBMC of healthy blood donors. PBMC were recollected in three groups: 1) cells that did not adhere to the endothelium, 2) cells