hyporesponsiveness upon engagement of the TCR/CD3 complex despite phenotypic evidence of former activation. We have previously shown that the central and crucial adaptor protein LAT (linker for activation of T cells), which plays a central and crucial role in the T cell receptor (TCR)-mediated signalling pathways, exhibits deficient phosphorylation due to displacement of the integral membrane protein from the plasma membrane in SF T lymphocytes. SF T lymphocytes exhibit several features of chronic oxidative stress, e.g. severely decreased intracellular levels of glutathione (GSH), and our previous studies have indicated that the subcellular localization of LAT is sensitive to changes in the intracellular GSH levels. The cysteine-to-serine substitutions of several cysteine residues (C26/29 or C117) within LAT creates LAT mutants that are resistant to reduced intracellular GSH levels and remain membrane-anchored in GSH-depleted cells.

Objectives

Methods

Results In this study, we have used the redox-insensitive LAT mutants to study the effect of redox balance alterations, like in SF T lymphocytes, on TCR signalling pathways downstream from LAT and on CD28 signalling pathways. In co-transfection experiments, we show that the presence of the redox-insensitive LAT mutants allows for the partial restoration of the TCR-mediated signalling pathways, but not the signalling pathways induced through the CD28 receptor. The data are indicative that the Raf1-ERK and the calcium-calcineurin pathways leading to transcriptional activation of AP-1 and NFAT, respectively, are very sensitive to reduced intracellular GSH levels, while the activation of the p38/Mpk2 pathway leading to AP-1-mediated transcription is mostly unaffected by chronic oxidative stress. A very proximal event in the CD28-mediated signalling pathways seems to be extremely sensitive to GSH depletion since costimulation did not affect the transcriptional activity of either AP-1 or NF- κ B.

Conclusion We conclude that the signalling pathways in SF T lymphocytes from RA patients are affected at several levels by chronic oxidative stress, all contributing to the observed hypo-responsiveness of these cells.

OP0002 DEOXYSPERGUALIN AND SPERMINE INDUCE APOPTOSIS IN QUIESCENT AND ACTIVATED HUMAN LYMPHOCYTES

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Background Deoxyspergualin (DSG) is a novel immunosuppressive drug currently undergoing clinical trials for treatment of transplant rejection or autoimmune diseases. The polyamine analogue DSG is a synthetic derivative of spergualin containing a spermidine moiety. Polyamines are known to induce apoptosis in several mesenchymal cell lines in presence of fetal calf serum (FCS).

Objectives The aims of the present study were

- to test whether DSG alone or in combination with other immunomodulating drugs induces apoptosis or necrosis in immunocompetent cells (fresh isolated cells, PHA generated lymphoblasts) and whether this effect depends on the presence of FCS;
- to analyse which cellular mechanisms are involved in the execution of the cell death program induced by DSG.

Methods Freshly isolated quiescent or activated human PBMCs, PHA generated lymphoblasts and several tumour cell lines (Jurkat, SKW-3, U937, RS4.11., MV4.11.) were incubated with different concentrations of DSG in presence of FCS or human AB serum (hABS). The rate of apoptotic cells was analysed after Propidium-Iodide/AnnexinV (PI/AxV) and PI/Triton staining by flow cytometric analysis.

Mitochondrial-membrane-potential ($\Delta\psi$ M) was measured by flow cytometric analysis after staining with Dihexyloxocarbocyanine (DiOC6).

Results By means of PI/Triton and PI/AxV staining we found an induction of apoptosis by DSG in presence of FCS in quiescent PBMCs, PHA generated lymphoblasts and the tumour cell lines Jurkat, SKW-3, U937. In presence of hABS no induction of apoptosis was found. Necrosis was excluded in any of the experiments.

DSG was also capable to induce apoptosis in the tumour cell lines RS4.11., MV4.11. which are refractory to conventional chemotherapeutic agents operating through the induction of apoptosis. In lymphoblasts coactivation with IL-4, IL-7 or IL-15 did not prevent DSG-induced apoptosis, whereas IL-2 had various effects.

A decrease of $\Delta\psi$ M was seen after treatment of cells with DSG in presence of FCS. DNA degradation measured by PI Triton staining was abolished by adding the caspase inhibitor zVAD FMK.

Combination of DSG with Cycloheximid (CHX), a protein synthesis inhibitor which is described to interact with the regulation of cellular polyamine synthesis, lead to a remarkable increase in the induction of apoptosis by DSG, while CHX alone showed no effects.

Similar effects as described above for DSG were achieved if spermine, a physiological polyamine, was added to the culture.

Conclusion These results show, that DSG in presence of FCS is able to induce apoptosis in several cell systems. Interestingly induction of apoptosis was seen in resting cells and two cell lines, that are resistant to conventional chemotherapeutic agents. Our findings point out that induction of apoptosis by DSG in presence of FCS requires activation of caspases and is associated with a decrease in $\Delta\psi$ M. The structural conjunction of DSG with polyamines and the additive effects in combination with CHX suggest, that alterations of the cellular polyamine contents might be involved in the induction of apoptosis by DSG.

OP0003 STAT1 AND STAT3 ACTIVATION DURING THE PRIMARY AND CHRONIC PHASES OF EXPERIMENTAL ARTHRITIS IN WILDTYPE AND IL-6 DEFICIENT MICE

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Background Experimental arthritis in Interleukin-6 deficient (IL-6^{-/-}) mice has shown the importance of this cytokine for chronicity of the synovitis. We^{1,2} and others have found previously that in IL-6 deficient mice the primary inflammation does not develop into a chronic synovitis. The JAK/STAT pathway is important for signaltransduction by IL-6 in the cell. Upon activation of the IL-6 receptor complex, the Signal Transducer and Activator of Transcription molecules STAT1 and STAT3 become phosphorylated and migrate to the nucleus to activate gene transcription.