3 CD3ζ-CHAIN EXPRESSION IS REGULATED BY TUMOR NECROSIS FACTOR VIA SRC-LIKE ADAPTOR PROTEIN DEPENDENT PROTEOSOMAL DEGRADATION IN HUMAN T LYMPHOCYTES

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Background and objectives decreased expression of the T cell receptor (TCR) ζ-chain has been reported in several autoimmune, inflammatory and malignant diseases, suggesting that

ζ-chain downregulation is common at sites of chronic inflammation. While ζ-chain is critically important in T lymphocyte activation, the mechanism of the decreased ζ-chain expression is not known. Src-like adaptor protein (SLAP) is a master regulator of T cell activation; previous data indicated that SLAP regulates immunoreceptor signaling. The authors have examined the mechanism and the functional consequences of CD3 ζ-chain downregulation.

Materials and methods CD3ζ and SLAP protein levels of Jurkat cells and primary human T lymphocytes were measured by Western blot. Jurkat cells were transiently transfected with siRNAs to silence SLAP, knockdown efficiency of the siRNAs was measured by real-time RT-PCR and by Western blot. For confocal microscopy experiments cells were transfected with eGFP-SLAP expression-ready, full-length cDNA vector or control eGFP vector. The colocalisation between CD3ζ and SLAP were determined by laser confocal microscopy. CD3ζ mRNA was measured by quantitative real-time RT-PCR, IL-2 level was measured by ELISA method.

Results TNF treatment of human T lymphocytes (15–40 ng/ml) selectively downregulates CD3 ζ-chain expression in a dose dependent manner ($p<0.001$), and decreases the activation induced IL-2 synthesis ($p<0.01$). Although blocking of the lysosomal compartment fails to restore the TNF-induced CD3 ζ-chain downregulation, the inhibition of the proteasome prevented the effect of TNF. Both SLAP expression and the colocalisation of SLAP with CD3 ζ-chain was enhanced by TNF treatment ($p<0.05$; $p<0.009$ respectively), while TNF induced ζ-chain downregulation was inhibited by silencing SLAP with siRNA ($p<0.01$).

Conclusions Our present data suggest that TNF modulates T cell activation during inflammatory processes, by regulating the amount of CD3 ζ-chain expression via SLAP dependent mechanism. These data indicate that SLAP dependent regulation of CD3 ζ-chain provides fine control of TCR signaling.