

Up regulation of c-FLIPs in a subgroup of patients with ALPS type III

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Rationale: Autoimmune lymphoproliferative syndrome (ALPS) is currently classified accordingly to the subjacent genetic defect as type Ia (mutation in *TNFRSF6*), Ib (*TNFSF6*), II (*CASP10*) and type III. Under the type III category are the patients where no defect in the *TNFRSF6*, *TNFSF6*, *CASP8* or *CASP10* are identified. We undertook a systematic analysis of critical intracellular molecular steps that proceed upon cross-linking of Fas as a means to characterize the molecular defect in type III ALPS patients.

Methods: EBV-transformed B cell lines from patients with ALPS and normal controls were used in all assays. Apoptosis assays were performed by adding the monoclonal antibody Apo1.3 followed by cross-linking with Staph aureus protein A. Cell death was determined by flow cytometry after staining with Dioc6 (•ψ mitochondrial) and propidium iodide. Surface expression of Fas was measured by flow cytometry. Recruitment of molecules to form the death inducing signaling complex (DISC) was evaluated by immunoprecipitation followed by western blotting. BID and c-FLIPs levels as well as cleavage of caspase-8 were evaluated using western blotting. The mRNA level of c-FLIPs was measured by real-time PCR using the TaqMan platform. siRNA transfection was performed by electroporation using the BTX system.

Results: A total of 6 ALPS type III EBV B cell lines were evaluated and 3 demonstrated a profound in vitro kill defect upon Fas cross-linking. All three had impaired processing of procaspase-8 to its active form, despite normal recruitment of this molecule to the Fas receptor (normal DISC) and normal cDNA sequencing for caspase-8. As a consequence, there was also no BID cleavage. FLIP is a caspase-8 homologue that inhibits procaspase-8 processing. In two out of the 3 B cell lines with procaspase-8 processing defects, we found significantly elevated amounts of c-FLIPs protein, when compared to cell lines from normal controls and patients with ALPS type 1a. Significant increase of the c-FLIPs mRNA was also documented by real-time PCR in the same cell lines, when compared to normal and type 1a controls. In preliminary experiments, knockdown of c-FLIPs by siRNA restored partially the sensitivity to Fas killing in the 2 cell lines tested. Additional work to confirm this finding is underway.

Conclusion: In some patients with ALPS type III the apoptotic defect appears to be related to abnormally high levels of c-FLIPs, a natural inhibitor of procaspase-8 processing and activation. As these findings were not universal to all patients tested, type III ALPS patients seem to have heterogeneous defects and further studies are underway in the uncharacterized patients to identify additional mechanisms underlying Fas mediated apoptotic defects.