

**P-093**

**OPTIMIZATION OF DRUG SPECIFIC MEMORY T-LYMPHOCYTES DETECTION  
IN A CASE OF SEVERE DRESS SYNDROME**

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Drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome is a severe multiorgan hypersensitivity reaction caused by some drugs and mediated by T-lymphocytes. When a DRESS is diagnosed, it is necessary to confirm that the suspicious drug is the cause of the reaction in order either to avoid a new exposition to this drug or to prevent from an unnecessary avoidance of a drug. However, in vitro techniques currently used have quite limitations. The aim of this work is to study the case of a severe DRESS syndrome probably caused by minocycline that triggered in a fulminant hepatitis and the requirement of liver transplantation in a fifteen years old patient . We also tried to improve the available techniques to detect these specific antigen responses. Three different techniques to test the activation and expansion of the drug-specific memory T-lymphocytes were performed: Cell Trace Violet Proliferation test, Ki67 staining, both analyzed through flow cytometry, and interferon-gamma IFN- $\gamma$ -based Enzyme-Linked Immunospot (ELISpot) assay. Different variables as time of co-incubation of PBMCs and the drug, use of different cytokines and different concentrations of the drug were tested in order to improve the sensibility of these techniques. The results obtained showed that both Cell Trace Violet Proliferation and the Ki67 detection did not show enough sensitivity for drug specific T-lymphocytes detection in this patient. ELISPOT assays allowed the detection of this response and a great improvement in sensitivity was achieved when PBMCs were cultured in presence of 0.1-2  $\mu\text{g/ml}$  of minocycline for 6 days prior to ELISPOT. The costimulation with IL2 or IL7+IL15 in these cultures potentiated the response although it also generated a nonspecific response in negative controls. All together, our results suggest that an optimized ELISPOT technique could be the best option for the detection of specific drug memory T-lymphocytes in DRESS syndrome.