Characterization of the specificity and functionality of drug-responsive T-cells isolated piperacillin hypersensitive patients with cystic fibrosis

Introduction: Piperacillin is a β-lactam antibiotic that is frequently used to combat bacterial infections in patients with cystic fibrosis. However, its use is associated with a high incidence of delayed-type hypersensitivity reactions. Our previous studies have identified lymphocyte proliferative responses and cytokine secretion from PBMCS isolated from approximately 75% of hypersensitive patients. In contrast, PBMCs from tolerant controls are not specifically activated. Moreover, these studies have shown that the drug forms a hapten and binds covalently to specific lysine residues on serum proteins such as human serum albumin (HSA). The aim of this study was to generate a synthetic piperacillin HSA conjugate and characterize hapten binding sites. Levels of modification were quantified using a synthetic piperacillin-peptide standard. The conjugate was then used to explore T-cell antigenic determinants in hypersensitive patients and the nature of the induced response focussing on TCR Vβ usage and cytokine release.

Methods: Piperacillin was cultured with HSA at different molar ratios (10:1–250:1 drug:protein) and mass spectrometry was used to characterize and quantify the adducts formed. PBMCs from hypersensitive patients were cultured in the presence of parent drug and the piperacillin-HSA conjugate and T cell clones were generated by serial dilution. TCR Vβ usage was analyzed by flow cytometry. Antigen-specific proliferative responses and cytokine release was assessed using [^{3}H]thymidine and ELISpot, respectively.

Results: The piperacillin hapten was detected on 10-20% of the lysine residues in HSA. The level of piperacillin-modified lysine 541 ranged from 3-24%. Sixty CD4+ clones displayed reactivity against piperacillin-modified HSA. Activation of the clones was dependent on: (1) protein processing by antigen presenting cells; and (2) the level of modification with the piperacillin hapten; low levels of modification failed to activate the clones. The T-cell response was antigen-specific as other β-lactam-HSA conjugates did not stimulate a response. TCR Vβ expression was restricted to expression of TCR Vβ 9. Importantly, many clones were not activated with unbound piperacillin. Thus, over 100 clones were generated from PBMC cultured with piperacillin itself. These clones were activated with piperacillin-pulsed antigen presenting cells (APCs) in a time-dependent manner and T-cell responses were blocked following inhibition of protein processing; however, the synthetic piperacillin-HSA conjugate did not stimulate proliferation. TCR Vβ usage was diverse on these clones. Clones secreted of IFNγ (85% of the clones), IL5 (65%), IL13 (35%), perforin (60%), granzyme B (25%) and FasL (65%) following antigen stimulation.

Conclusion: We have shown that drug-responsive CD4+ T-cells with divergent antigen specificities circulate in hypersensitive patients. These data have important implications for studies relying exclusively on the parent drug as it is highly likely that the full repertoire of T-cells is not being investigated.

18 ,Immunological mechanisms: T cells, cell activations, Treg, cytotoxicity, NK