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Co-Inhibitory Regulation of Drug-Specific T-Cell Responses

Drug-specific T-cells have been isolated from patients with different forms of hypersensitivity; however, the factors governing inter-individual susceptibility remain ill-defined. In recent years the association of these reactions with specific HLA alleles has attracted much attention; however the majority of individuals who carry HLA risk alleles do not develop immunological reactions and so predisposition often relies on other immunological parameters. One such parameter may be the manipulation of T-cell activation thresholds, controlled in part by signalling through a multitude of T-cell co-inhibitory pathways. Two pathways in particular are thought to represent major immune inhibitory checkpoints; Programmed death -1 (PD-1), Cytotoxic T-lymphocyte associated prtotein-4 (CTLA4). A third T-cell inhibitory receptor, T-cell immunoglobulin domain and mucin domain-3 (TIM3), is of particular interest alongside these major checkpoints as it has been recently found to be upregulated in conjunction with PD-1 in both chronic viral and tumour-specific T-cell models.

It is now possible to prime human naïve T-cells in vitro to characterise primary T-cell responses to drugs. Utilising the model immunogen nitroso sulfamethoxazole (SMX-NO), we sought to investigate the comparative roles of PD-1, CTLA4, and TIM3 during drug-specific naïve and memory T-cell responses, and to compare the expression profiles of these receptors during the priming of naïve T-cells, and on drug-specific T-cell clones.

Naïve or memory T-cells were co-cultured with monocyte-derived dendritic cells in the presence of drug for a period of 8 days (±PDL1, CTLA4, TIM3 block), to expand the number of drug-responsive T-cells. The T-cells were then incubated with fresh dendritic cells and drug, and their antigen responsiveness analysed using readouts for proliferation, cytokine secretion, and cell phenotype. Cell phenotype was characterised by flow cytometry. T-cell clones were generated from these cultures to further characterize the inhibitory capacity of each pathway and assess the inter-cell variability of receptor expression.

Different T-cell regulatory pathways displayed varied abilities to manipulate T-cell activation. While blockade of PD-1 and CTLA4 enhanced T-cell activation subsequent to naïve T-cell priming, both were found to have less effect on memory T-cell responses. Blockade of TIM3 failed to enhance responses to drug from either naïve or memory T-cell compartments. Naïve T-cells expressed low levels of all three receptors, however CFSE analysis revealed a transient increase during priming of PD-1 and TIM3 in particular, with just a small increase detected for CTLA4 subsequent to re-stimulation with drug. PD-1, CLTA4 and TIM3 were stably expressed at different levels on drug-specific clones; however the level of expression did not correlate with the strength of the antigen-specific T-cell response from each clone.

Our work highlights an important level of regulation imposed on drug-specific T-cell responses. While these pathways individually do not have a great effect on T-cells previously exposed to antigen, drug-induced stimulation of naïve T-cells could be significantly affected by either PD-1 or CTLA4 blockade. Thus, inter-individual control of these T-cell co-inhibitory pathways may determine whether drug exposure leads to an aberrant T-cell response that may result in tissue injury in susceptible patients.