Investigating T cell responses to a major catechin found in Green Tea (Epigallocathechin-3-O-gallate – EGCG) in healthy donors expressing HLA-B*35:01.

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Introduction: Green Tea, derived from the Camellia sinensis plant, is a common constituent of many herbal supplements and is used globally. Green Tea extracts have been linked with immune-mediated idiosyncratic liver injury and associated with the expression of HLA-B*35:01. Epigallocathecin-3-O-gallate or 'EGCG' is the most abundant catechin found in green tea extracts and has been implicated in the liver injury observed. This study utilises *in vitro* peripheral blood mononuclear cells (PBMC) and T-cell assays to investigate the immunogenicity of EGCG in healthy donors positive and negative for the HLA-B*35:01 risk allele.

Methods: PBMC from HLA-B*35:01 positive and negative healthy donors were cultured with EGCG for 21 days. PBMCs were restimulated weekly with EGCG, IL-2 and irradiated autologous PBMCs before re-challenge and proliferative responses measured by [^{3}H] thymidine incorporation. Naïve T cells and dendritic cells from random healthy donors were also cultured with EGCG for 12 days, re-challenged with EGCG in the presence of immune-checkpoint blockade in day 12 and a proliferative readout was taken on day 14. EGCG-responsive T cell clones (TCC) were generated from HLA-B*35:01 positive donors via serial dilution and repetitive mitogen stimulation. TCC were examined for specificity, phenotype, cytokine secretion and pathways of T cell activation.

Results: Long term PBMC cultures were interrogated for EGCG-specific priming. Significant proliferative responses were identified 2/4 donors from both allele positive and negative groups. EGCG-specific priming of naïve T cells was observed in random healthy donors in the presence of immune checkpoint inhibition, a marked increase in the number of wells demonstrating medium to severe proliferative responses was observed in the presence of PD-1 and PD-L1, but not CTLA-4 blockade. A number of EGCG-specific TCCs were identified from healthy donors expressing HLA-B*35:01. TCCs proliferated in a dose-dependent manner when stimulated with EGCG and secreted IFNγ, IL-5, IL-13 and Granzyme B. TCC had a CD4+ phenotype and demonstrated a chemokine profile (CCR9, CXCL3) which confers a Th2 subset. Activation of TCC was restricted to MHC Class II, more specifically HLA-DR.

Conclusion: These data suggest EGCG has an intrinsic ability to be immunogenic and can effectively prime naïve T cells from both HLA-B*35:01 positive and negative healthy donors. Co-inhibitory signalling is likely to down regulate an immune-response and blockade of this pathways potentiates the priming seen. EGCG is able to activate CD4+ TCC, stimulating the release of pro-inflammatory and cytolytic cytokines, driven through MHC Class II HLA-DR interactions.