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Title: Peripheral blood Th17 / Treg ratio increases in late asthmatic response

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Body: Background The late asthmatic response follows the early asthmatic response to allergen inhalation challenge (AIC) in half of atopic asthmatics (dual responder, DR). DRs develop airway hyperresponsiveness (AHR) and more prominent and sustained airway inflammation after AIC. While Th17 and regulatory T (Treg) cells have been studied in asthma, their roles have not been fully understood in isolated early responders (ERs) and DRs. A new method has been utilized to quantify immune cell subsets based on DNA methylation. Aims We aimed to measure immune cells and gene expression profiles in blood cells pre and post AIC, comparing ERs and DRs. Methods Eight ERs and 6 DRs underwent AIC. Blood cells were collected pre- and 2 hours post-challenge. DNA was used for epigenetic cell counting (Epiontis, Germany). Th17 and Treg cells were counted as percentage of demethylation of the cell-specific gene region; IL17A gene and FOXP3 Treg-specific demethylated regions, respectively, and the Th17/Treg ratio was compared between ERs and DRs using t-test. Gene expression was measured with Affymetrix Human Gene 1.0 ST array (Affymetrix, USA). After normalization, identified genes significantly correlated to each cell-type. GeneGo network analysis was performed for biological functions. Results The genes significantly correlated to Th17 and Treg counts were enriched in GeneGo analyses for Th17 functions and regulatory cellular functions, respectively. Th17/Treg was significantly higher at baseline in ERs compared to DRs (p 0.002). Th17/Treg significantly increased in DRs compared to ERs (p 0.023). Conclusion Th17/Treg imbalance may contribute to development of late phase bronchoconstriction and the associated AHR and inflammation.