Transcriptome changes during peanut oral immunotherapy and omalizumab treatment

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To the Editor,

Peanut allergy is a common food allergy and the main cause of anaphylaxis among children[1]. In recent years, oral immunotherapy has emerged as a promising treatment for children with different IgE-mediated food allergies, although safety issues must be considered[2]. The main aim of immunotherapy is to induce tolerance or desensitization to an allergen which otherwise causes an allergic reaction. For oral immunotherapy this means ingesting the allergen in a controlled manner with gradually increasing dosages. Specifically, peanut oral immunotherapy (pOIT) is able to induce tolerance/desensitization[3]. While the pathogenesis of food allergy in general is relatively well-studied[4], mechanisms of OIT-induced tolerance are not well understood. Omalizumab (anti-IgE) used as treatment for severe allergic asthma and other IgE-driven allergies, can facilitate OIT initiation[5], however, little is known about the involved mechanisms, including possible changes at the transcriptional level. We therefore investigated transcriptional changes in whole blood using RNA-sequencing profiles during omalizumab treatment and pOIT in participants from the FASTX (Food Allergen Suppression Therapy with Xolair ®) study previously described in detail elsewhere[5].

In brief, peanut-allergic adolescents (n=23 of whom 17 completed the study, age 12-18 years) were started on omalizumab (baseline) and treated for at least 8 weeks before starting pOIT (pOIT start) while on omalizumab. The peanut-dose was gradually increased during the 8 weeks until reaching a maintenance dose. Guided by a basophil activation test (BAT/CD-sens)[6] after 8 weeks on the maintenance dose, participants decreased the omalizumab dose by 50% (maintenance) and continued to decrease the omalizumab dose if pOIT was tolerated. Eleven patients were able to tolerate pOIT without omalizumab protection for >8 weeks and then passed an open peanut food challenge (final); 6 patients could not discontinue omalizumab, but blood samples were obtained for analysis after 2-3 years of omalizumab treatment (final); 6 patients dropped out of the study. RNA-sequencing was performed on whole blood at baseline, pOIT start, maintenance and final time-points using the NovaSeq 6000 platform. DESeq2 was used for differential expression analysis of the omalizumab effect and a linear mixed-effect model for analyses during pOIT in combination with

omalizumab (pOIT+O) after adjustment for treatment outcome and cell type. A complete description of the treatment protocol and method is given in Appendix S1.

General characteristics of the study participants at baseline can be found in Table S1 . To elucidate if omalizumab treatment alone induced alterations in peripheral blood gene expression, we investigated the two first timepoints, baseline and pOIT start, however no significant differences were observed (Figure S1). In the longitudinal analysis (pOIT start to final), 680 genes associated with pOIT+O at nominal p <0.005 (Table S2). The Gene Ontology (GO) biological process of the up- and down-regulation of these 680 genes are presented in Figure 1A,B . Upregulation of 337 genes were linked to GO terms "protein regulation and modification", while "neutrophil degranulation, immune response, phagocytosis, and metabolic process" were among the top terms for the downregulated 343 genes. Out of the 680 genes, 16 were differentially expressed at false discovery rate (FDR) adjusted p<0.05 (Table 1, Figure S2). The three genes with the largest negative and positive coefficients, respectively, are displayed in Figure 1C,D ; downregulation of *ASGR2* ,*GPBAR1* and *HM13,* and upregulation of *USP44* ,*ICOS* and *CDKN2AIP* . Finally, we evaluated the enrichment of 680 pOIT+O-associated genes, relative to peripheral blood gene expression associated with acute peanut allergic reactions in a recently published clinical study by Watson et al using the same p-value cut-off (p<0.005)[7]. Out of our 680 significant genes, 108 genes overlapped with the differentially expressed genes in Watson et al[7], mostly with opposite direction, $P_{enrichment} = 0.0095$ (Figure 2).

Our results demonstrate that omalizumab treatment alone does not induce alterations in whole blood gene expression in patients with severe food allergy. This is not surprising given that these patients were unexposed to peanut allergen at the time of blood sampling, and any concomitant asthma, rhinitis or eczema were well controlled. However, the longitudinal analysis during pOIT+O identified up- and downregulation of several immune-related genes. CD278/ICOS (Inducible T-cell costimulatory) is expressed on activated T-cells and appears to play a role in directing effector T-cell differentiation and responses during inflammatory conditions[8]. ICOS-expression on T regulatory cells and T follicular helper cells may be involved in the allergic disease mechanism[9]. In the pathway analyses, we observed significant enrichment for several GO biological process terms related to T-cell function and immune responses. Notably, we have previously described alterations in T-cell polyclonal *in vitro*activation during pOIT +O in the FASTX study[10]. Comparing our findings with data described by Watson *et al*[7] , suggests that pOIT+O may alter the expression level of many genes that were found activated during an acute peanut allergy reaction.

The main limitations of this study are lack of any control subjects and small sample size. Moreover, further studies are needed to evaluate the long-term biological effect of pOIT+O.

In conclusion, omalizumab treatment alone does not alter the transcriptional signature in peripheral blood of peanut allergic patients, but during pOIT+O, several immune-related signatures were observed. These results may provide insights into mechanisms of allergen tolerance.

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CONLFICT OF INTEREST

None.

ETHICAL APPROVAL

Approved by the ethics committee in Stockholm: 2013/827-31/3, 2014/1980-32, 2016/1390-32, 2020-00807 and the Swedish Drug Agency: 5.1-2013-46183; The trial is registered at EudraCT: 2012-005625-78, ClinicalTrails.gov; NCT02402231. Patients and caregivers provided written informed consent.

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FIGURE LEGENDS

Figure 1. *(A, B)* Gene ontology (GO) biological process analysis of upregulated *(A)* and downregulated *(B)*genes. The x-axis shows the gene ratio of the overlapping genes of our gene list with the pathway gene set. The colour bar represents the adjusted p-value and the circle size is the count of overlapping genes.*(C, D)* Boxplots display log2 expression values for six pOIT genes (C : upregulated, D : downregulated) throughout the treatment protocol at FDR p<0.05. Red box: pOIT start, green box: Maintenance, blue box: Final. ORA = Over-representation analysis.

Figure 2. Overlap of 108 genes from the FASTX pOIT study with the peanut-related genes found in *Watson et al* . at nominal p<0.005. The y-axis represents changes in gene expression between the means at baseline and the four-hour time point of the peanut challenge in *Watson et al* , and the x-axis is effect size estimates from the mixed-effect model in the FASTX pOIT study. The green colour shows opposite direction, and the pink same direction.

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