INTRODUCTION

Peanut allergy is one of the most serious hypersensitivity reactions to foods in terms of persistence and severity. Currently, there is no approved treatment to address the growing prevalence of severe peanut allergies, leaving these patients at risk of life-threatening anaphylaxis upon accidental exposure to peanuts. To address this need, food oral immunotherapy (OIT) has been investigated as a treatment and potentially disease modifying approach. OIT is the process of gradual increases in antigen ingestion until relevant quantities of foods that correspond to accidental exposure can be consumed without life threatening symptoms. While peanut oral immunotherapy has been shown to be encouraging, safety and efficacy as a desensitization therapy for peanut allergy, little is known about corresponding immune responses. It is generally accepted that enumeration and characterization of antigen-specific T cells provide essential information about the potency of the immune response and can serve as useful biomarker. The clinical application of food immunotherapy would greatly benefit from the development of reliable immune-monitoring assays that could address the complexity and functional heterogeneity of food allergy. The aim of this study is to evaluate the frequency and phenotypic heterogeneity of peanut specific effector and regulatory T cells as baseline in a subset of peanut allergic patients undergoing characterized oral desensitization immunotherapies (CODIT) with AR101, an experimental orally administered biological drug containing the antigenic profile found in peanuts. This dual assessment of peanut-specific effector (Teff) and regulatory T cells (Treg) as a potential to provide essential information determining which patients are most likely to tolerate up-dosing and which patients are at risk for developing GI symptoms associated with early discontinuation from AR101 therapy thereby helping to reveal the best course of treatment for the individual patient.

METHODS

Blinded samples to the operator are provided during a randomized 3:1, double blind, placebo controlled Phase 3 ongoing trial (ARC003) of the efficacy and safety of AR101 in a characterized desensitization (CODIT™) approach in patients with peanut allergy (Figure 1). Eligible subjects, 4-50 years of age, reacted to ≥ 100 mg peanut protein during a screening double blind placebo controlled food challenge (DBPCFC). To measure basophil allergen threshold sensitivity, basophils are stimulated with peanut extract dilution and cytoplasmic IgE assessed. A threshold frequency of basophils reacting to a specific dose was reached based on detection of the activation marker CRTH2. The magnitude and quality of peanut specific allergen-reactive T helper type 2 (Th2) was determined using a CD137- and CRTH2+ upregulation assay respectively, following short restimulation of PBMCs with a pool of peanut peptide library derived from Ara h 1, h 2, h 3, h 6 and Ara h 8. Following bead enrichment, the CD4+ and CD8+ enriched fraction are separately stained with various combinations of surface and intracellular antibodies for phenotyping using 16 color flow cytometry platform.

RESULTS

Here we have used methods for parallel ex vivo assessment of peanut-specific effector and regulatory CD4+ T cells to provide meticulous immune-monitoring of patients receiving patients receiving AR101 or placebo through the 24 months duration of the trial. At any given time-point, positive allergic patients (positive to peanut extract compared with those patients who did not react to a 100 mg DBPCFC (Figure 2b). The absence of a clinical response in DBPCFC non-reactors patient was accompanied by a significantly lower number of peanut-reactive effector CD4+ T cells (Figure 2c). These data extends a previously CD27+ CD69- memory phenotype consistent with protective immune responses observed in non-allergic individuals (Figure 2d). Surprisingly, subsets who did not react to the enter DBPCFC also exhibit significant increase in the oral challenge (Figure 3). In all peanut allergic patient enrolled in ARC003 trial, the DBPCFC protocol at baseline led to significant increased expression of the cell-surface activation marker CD38 within peanut reactive T cells (Figure 2e), confirming their role in food sensitivity. At this dose, the data suggests a model wherein a pathogenic subpopulation of peanut-specific CD4+ T cells possessing a distinct effector feature in effector function are generated in vivo in atopic individuals and are crucial for allergic pathogenesis, regardless of the balance of T helper cell subsets. Our data at baseline of the ARC003 trial also emphasizes the heterogeneity of allergen-reactive T cell responses in peanut allergic subjects, with two mutually exclusive immunotypes associated with food allergy (Figure 4). This raises important questions regarding the pathophysiological role of each peanut specific CD4+ T cell subset in food allergy and suggested that phenotyping may indicate or predict clinical treatment outcomes following CODIT in subjects with peanut allergy. These immunotypes are likely the result of different immunologic mechanisms and therefore may require different immunotherapeutic approaches to bring about resolution. These biomarkers are currently being examined prospectively during the Immune Phase III (ARC003) trial to determine if it can be used to help predict which patients are most likely to benefit from this investigational therapy, and which immune mechanisms are involved in the response and screen out those candidates in whom OIT may lead to unnecessary risks.

CONCLUSIONS

The methodology presented here, testing a large set of CD4+ effector and regulatory T cell phenotypes in patients with peanut allergy, may provide novel information about the immune responses to peanut allergy. This approach allows for the assessment of the immune responses to peanut extract in patients with peanut allergy and may help to identify those patients who are most likely to benefit from immunotherapy. Moreover, the methodology presented here may help to identify the mechanisms involved in the protective immune responses against peanut allergy and to screen out those patients who may not benefit from OIT. The data also highlights the importance of understanding the heterogeneity of peanut-specific T cell responses in peanut allergic subjects, which may be crucial for developing effective immunotherapies for peanut allergy.

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Dual assessment of peanut-specific-efector and regulatory T cells in patients undergoing oral immunotherapy: A preliminary review of ARC003

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