Immune mechanisms of oral immunotherapy

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Oral immunotherapy (OIT) has demonstrated reproducibly successful desensitization in patients with food allergy completing clinical trials and, in some studies, sustained unresponsiveness. These clinical outcomes have been associated with characteristic modifications in the allergen-specific immune response, but a detailed synthesis of OIT's mechanisms of action is lacking. In this rostrum we review the current evidence regarding the human immune response to OIT, explore possible mechanisms, and identify knowledge gaps for future research. (J Allergy Clin Immunol 2018;141:491-8.)

Key words: *Oral immunotherapy, immune mechanisms, desensitization, sustained unresponsiveness, basophils, T cells, B cells, mast cells, antibodies*

Abbreviations used
Foxp3: Forkhead box protein 3
OIT: Oral immunotherapy
sIgE: Allergen-specific IgE
SPT: Skin prick test
SU: Sustained unresponsiveness
T_H2A: Proallergic T_H2
Treg: Regulatory T

The mechanisms of action of oral immunotherapy (OIT) remain poorly understood, with the literature comprised of primarily descriptive peripheral blood studies in human patients. Although stromal and immune cells in the gastrointestinal tract mucosa, its associated secondary lymphoid structures, the gastrointestinal microbiome, and so on are likely to critically influence human food allergy, the role of these structures in the mechanisms of OIT remains obscure given the inability to routinely sample these structures in human subjects. Although much can be learned from animal model systems, the knowledge gained is inherently limited by experimental conditions that do not resemble human food allergy. Despite these obstacles, the application of new technologies is enhancing our current understanding of the abundance and diversity of OIT's effect on immune cell subsets.

Our aim in this rostrum is to review what is known about clinically relevant OIT mechanisms, and we have chosen to focus primarily on human studies, supplementing them with data from animals, where appropriate. We have organized our approach sequentially in an attempt to outline the temporal changes from baseline during the OIT treatment protocol.

The primary clinical objective of most OIT programs for food allergy is to induce a desensitized state in the patient, which is defined here as a temporary increase in threshold reactivity to the allergen such that clinical protection from accidental ingestion can be achieved. This occurs through continuous stimulation of the immune system with subthreshold daily doses of allergen and then gradually escalating the dose level over time to reach a target maintenance dose. The oral route of administration might take advantage of the unique set of immune cells and pathways involved in induction of oral tolerance. Protocols vary in their approaches to the initial dose escalation phase, but they consistently begin OIT with low doses (eg, ≤5 mg of allergenic protein) and generally increase the doses by 25% to 100% at a periodic interval until the target maintenance dose is reached or dose-limiting toxicity occurs. Holding, reducing, or terminating dosing is occasionally required during this period of treatment because of allergic symptoms caused by the daily dose as participants transition from allergen avoidance before OIT to steadily progressive exposures. It is this period of transition that we will refer to in this article as the "initiation phase" to describe the mechanistic changes occurring during initial exposures.

Clinical studies have shown repeatedly that the majority of patients undergoing OIT in clinical trials will have adverse events related to dosing, usually mild to moderate in severity, and that they are more common during initiation, lessening in frequency over time.[1-3] In approximately 15% to 20% of subjects, more severe symptoms and/or dose-limiting toxicity can occur, and although clinical cofactors have been identified for systemic reactions, the biological basis (ie, the endotype) that explains this phenotype has not been elucidated. The repeated engagement of

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Disclosure of potential conflict of interest: S. U. Patil has received grants from the National Institutes of Health (NIH); Sanofi; the American Academy of Allergy, Asthma & Immunology Foundation; and Food Allergy Research & Education (FARE). E. Wambre has received grants from the National Institute of Allergy and Infectious Diseases (NIAID), Astellas, Aimmune, Anaptys Bio, and the Immune Tolerance Network and has a patent (US 9733247B2 Detection of TH2A Immune response) pending. B. P. Vickery has received personal fees and stock options from Aimmune Therapeutics and has received grants from the NIH/NIAID, FARE, and the American College of Allergy, Asthma & Immunology. M. D. Kulis declares that he has no relevant conflicts of interest.

Received for publication August 7, 2017; revised November 30, 2017; accepted for publication December 6, 2017.

Available online December 26, 2017.

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0091-6749/$36.00

https://doi.org/10.1016/j.jaci.2017.12.979

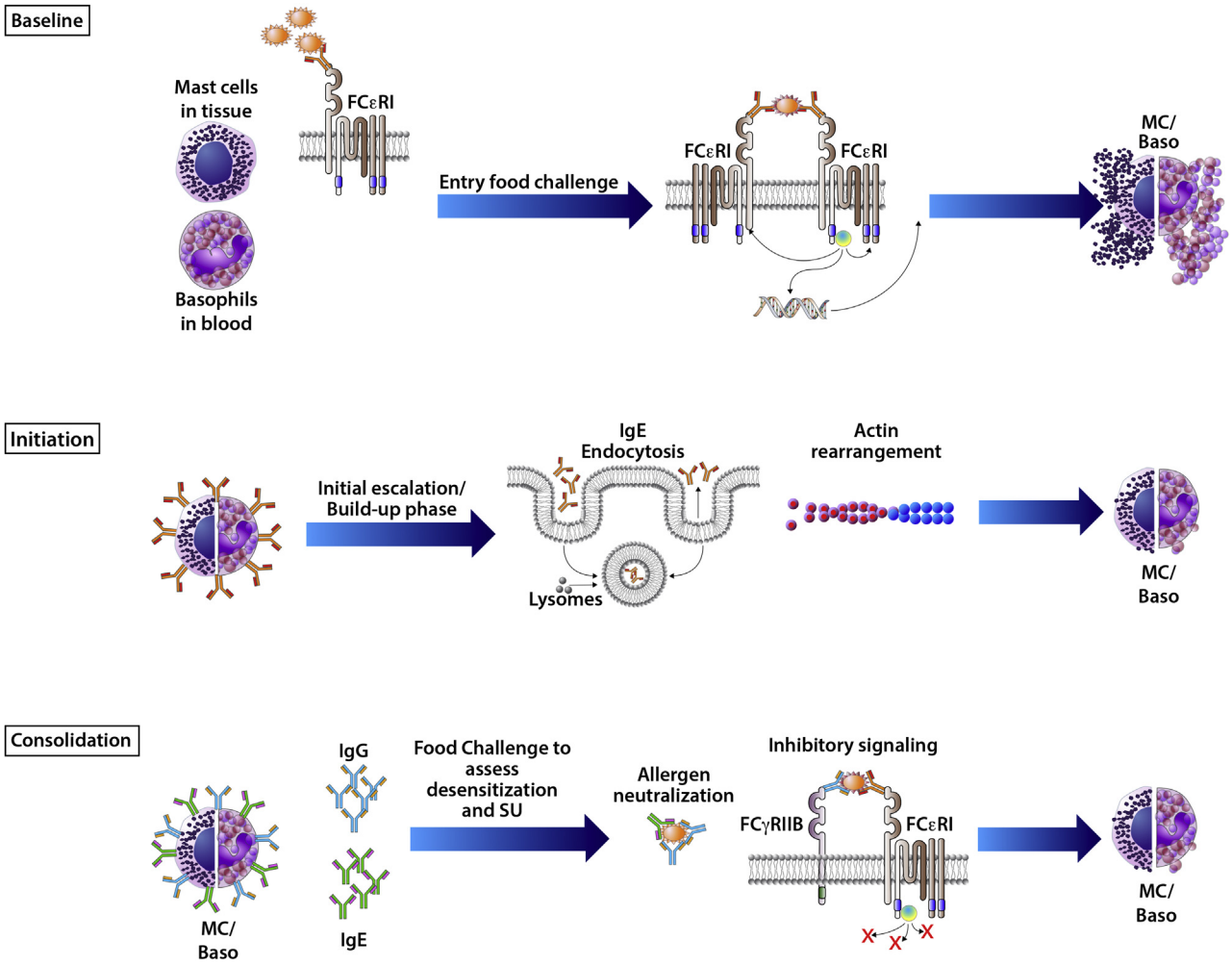

FIG 1. Modulation of mast cells and basophils during OIT. At baseline, allergic patients' mast cells and basophils are decorated with sIgE bound to cell-surface FcεRI receptors. On antigen exposure (eg, entry food challenge or accidental exposure), IgE molecules are cross-linked, leading to degranulation and subsequent manifestation of allergic symptoms. During the initiation phase of OIT, repeated exposures to low-dose antigen leads to direct effects on mast cells and basophils, including IgE endocytosis and actin rearrangement, rendering these effector cells hyporesponsive to allergen. As OIT continues and higher doses of antigen are administered, the production of allergen-specific IgG in the consolidation phase plays an important role and can lead to further, potentially long-lived inhibitory mechanisms seen clinically as SU. In particular, circulating allergen-specific IgG can neutralize allergen, such that IgEs are not cross-linked on effector cells, whereas IgG bound to cell-surface FcγRIIb can induce inhibitory signaling with IgE and IgG cross-linking, thus preventing degranulation. *MC/Baso*, Mast cell/basophil.

allergen-specific IgE (sIgE) on mast cells and basophils, which in many participants can lead to elicitation of some symptoms, can also contribute to OIT's mode of action, which later engages regulatory pathways that aim to control allergic inflammation through effector cell suppression and antibody production (eg, the modified T_H2 response), but the optimal relationship of excitation and inhibition is not well understood.

Because participants undergoing OIT progress through dose escalation, the initial initiation phase of the desensitization process gives way to a consolidation phase. In this phase the clinical benefit of the regimen is preserved through maintenance dosing (ie, no further escalation), and effector cells remain stably suppressed. Lymphocytes and their products (cytokines and antibodies) are modulated further, culminating in some participants in a result known as sustained unresponsiveness (SU), a persistent state of increased allergen threshold in the absence of daily dosing. The mechanistic changes associated with SU will be discussed in this section, followed by some selected key knowledge gaps that serve as future research needs in this field.

INITIATION PHASE

Mast cells and basophils

At baseline, the mast cells and basophils of participants with OIT express the high-affinity IgE receptor FcεRI on their cell surfaces, are primed with sIgE, and are the major effector cells of IgE-mediated allergic reactions to foods because of their granule contents. These primed effector cells are activated rapidly by a signaling cascade through FcεRI signaling when untreated patients with food allergy accidentally and occasionally encounter allergen in suprathreshold amounts (Fig 1). However,

the steady subthreshold dosing used in OIT trials for peanut, egg, and milk allergies have consistently demonstrated significantly decreased skin prick test (SPT) wheal size and basophil activation (as measured by upregulation of CD63, CD203c, or both) in response to the antigen used for OIT,[4-11] and this effect likely accounts for the initial desensitization seen clinically.

Suppression of these effector cell responses occurs within the first few months of OIT and therefore might be linked to escalating antigen dose. It is important to note that this desensitization occurs in the absence of a decrease in sIgE levels and often during the period of time that sIgE levels are actually increasing from baseline.[5,11] This finding across several studies implies that desensitization of mast cells and basophils does not rely on decreased sIgE levels as its underlying mechanism. However, decreased IgE levels caused by omalizumab treatment before initiating OIT allow for much higher doses of antigen to be safely given in the initial escalation phase.[12,13] The effects of anti-IgE therapy on reducing circulating IgE levels and downregulating FcεRI levels on basophils might explain this finding. Therefore although low sIgE levels are not requisite for desensitization, removal of IgE by omalizumab allows for a rapid escalation in antigen dose.

More detailed mechanistic studies of effector cells have tended to focus on basophils, which are easier targets given their circulation in peripheral blood, compared with tissue-resident, long-lived mast cells. It should be noted that there are key differences between basophils and mast cells, and their functional equivalence should not be assumed. Interestingly, OIT appears to inhibit the entire IgE-signaling pathway in basophil activation assays as polyclonal anti-IgE and egg allergen responses on basophils are decreased with peanut OIT, pointing to a peanut-nonspecific mechanism.[14] Given the technical difficulty in studying cellular mechanisms and inaccessibility of mucosal tissues in human subjects undergoing OIT, findings from orally induced desensitization mouse models might provide important further mechanistic insights; however, there is a relative scarcity of literature from OIT models.

Mouse models of rapid desensitization, along with supporting cellular studies, indicate that short-term desensitization is induced by inhibiting calcium flux and remodeling of actin through repeated stimulation of sIgE on mast cells,[15] whereas another report demonstrates that endocytosis of surface-bound IgE is critical for mast cell desensitization (Fig 1).[16] A model of oral desensitization in mice with egg allergy demonstrated that allergic mice can be rendered nonreactive to oral egg challenge.[17] However, these mice reacted when given an intraperitoneal injection of egg antigen, indicating that effector cells could still respond vigorously to antigen in the bloodstream. This study hints at the role of local effects, presumably on mast cells in the gastrointestinal tract, that prevent allergic reactions on oral challenge and emphasize the temporal changes that occur during OIT. Within weeks of starting dosing, we hypothesize that the effector cell suppression is likely to be predominantly mediated by the intrinsic responses of those effector cells to repeated low-level allergen exposure, which is consistent with *in vitro* studies of desensitization.[15,16] Early antibody responses, which are just beginning to change at this time, might also contribute. Peripheral allergen-specific antibodies and B cells also emerge within weeks of beginning OIT,[18] likely interacting with T cells, and these concerted regulatory actions ultimately lead to further changes in antibody repertoire that interact with and can suppress basophil responses through multiple pathways[19,20] late in the initiation phase and into consolidation, which is discussed in greater detail below.

T cells

In allergic patients T-cell activation drives the main effector phases of allergy, including eosinophil activation and B-cell production of allergen-specific IgE. This takes place primarily through a T_H2-biased response pathway initiated by epithelially derived soluble mediators, such as thymic stromal lymphopoietin, IL-25, and IL-33.[21] Conversely, regulatory T (Treg) cells, $CD4^+$ T cells, or both able to produce the anti-inflammatory IL-10 are generally considered to be significant contributors to the induction and maintenance of peripheral tolerance to allergen; regulatory B cells might also contribute IL-10.[22,23] With antigen-specific T_H2 cells at the core of the allergic process in atopic patients, changes in the magnitude and polarization of allergen-specific $CD4^+$ T cells are likely to be a key component to the effectiveness of OIT driven by the duration and dose of antigen exposure.

Consistent with increased production of related sIgE commonly observed during the initiation phase of OIT,[5,7,10,11,24] the first low-dose exposures to allergen might not only reinforce the pathogenic T_H2 cell effector responses but also create an inhibitory milieu that hampers establishment of Treg cells (Fig 2).

Data from various models inform these concepts. For instance, IL-4 production has been shown to cause T_H2 functional activities to become resistant to Treg cell–mediated suppression and to antagonize the postthymic development of forkhead box protein 3 $(Foxp3)^+$ Treg cells.[25-27] Subsequent increasing doses of allergen exposure during escalation are associated with a decrease in T_H2 cell activity and clonal expansion[28] and an increased frequency of IL-10–producing $CD4^+$ T cells.[29] This in turn leads to production of allergen-specific IgG_4 antibody that could attenuate IgE-mediated allergic symptoms[30] and might create a milieu that suppresses *de novo* generation of pathogenic T_H2 cells. However, at this stage, a high frequency of allergen-specific $CD4^+$ T cells is still present (Fig 2). Therefore this could explain why the clinical benefit of OIT can be lost or significantly decreased when dosing is interrupted or discontinued at this point.

One possible mechanism to explain and integrate all these results into a cohesive schema is that chronic stimulation of allergen-specific T_H2 cells during the initial initiation phase of OIT might culminate in a counterregulatory immune response, which consists of pathogenic T_H2 cells driven to an anergic, regulatory-like phenotype transiently preventing allergic symptoms through production of suppressive factors, such as IL-10. However, solid evidence for induction of allergen-specific Treg cells in human subjects mediating T-cell tolerance through IL-10 or other means during current OIT protocols remains elusive.

Although the suppression of T_H2 cytokine production has been observed in subjects undergoing OIT, multiple groups have examined $Foxp3^+$ Treg cells, with inconsistent results. During this phase, if treatment is not continued long enough, the initial pathogenic properties of allergen-specific T_H2 cells can gradually recover, which is consistent with transient clinical benefits. This idea is supported by work demonstrating that during chronic inflammation, IL-10–producing T_H2 cells (which fulfill

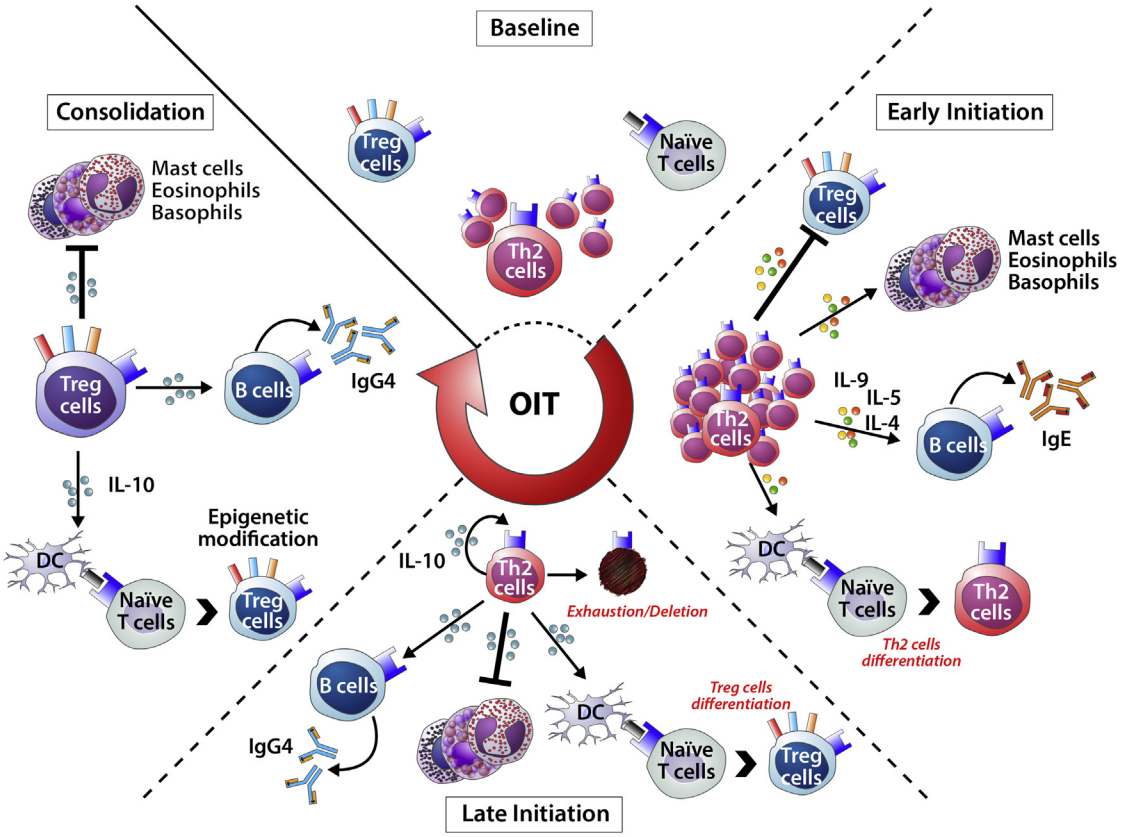

FIG 2. Sequential immune mechanisms of OIT. At baseline, T_H2A cells are at the core of the allergic process in patients with food allergy. During the early initiation phase of OIT, the first low-dose exposures to food allergen reinforce the pathogenic effector responses, increasing proinflammatory cell and B-cell pathogenic activities while creating an inhibitory milieu that hampers early establishment of Treg cells. Subsequent chronic stimulation of allergen-specific T_H2 cells with increasing doses of OIT culminate rapidly in a counterregulatory immune response to prevent excessive effector responses. These in turn drive a desensitization state through a decrease in T_H2A cell activity and IL-10 production and change in IgE/IgG_4 ratio. At this point, the clinical benefit of OIT might be significantly decreased when dosing is interrupted or discontinued. The consolidation phase of OIT arises once a specific threshold of activation is achieved and triggers selective T-cell exhaustion/deletion-skewing effector responses away from the proallergic T_H2 response. Prolonged continuous antigenic stimulation during maintenance OIT can also have other direct consequences associated with SU, enhancing epigenetic modifications at the *Foxp3* locus during Treg cell differentiation mechanisms.

the criteria of inducible Treg cells) can arise directly from nonsuppressive T_H2 cells once a specific threshold of activation is achieved.[31] In support of the presence of anergic T cells, a recent study showed that allergen-specific $CD4^+$ T cells expand during OIT and shift toward an anergic T_H2 cell phenotype.[28]

Antigen-specific B cells and their antibodies: Bridging the initiation and consolidation phases

High-affinity specific antibody is a hallmark of an adaptive immune response and is a characteristic of IgE-mediated hypersensitivity. IgE-mediated food allergy is driven by sIgE antibodies, and the association between food challenge outcomes and circulating levels of sIgE, as well as specific-to-total IgE ratios, is well known.[32] Qualitative aspects of sIgE, such as clonality, epitope specificities, and posttranslational modifications, might play a decisive role in the allergic immune response. Linear epitope analysis of sIgE in allergic patients' sera has revealed not only variability in the number of bound epitopes but also a positive association with reaction severity during food challenge outcomes.[33,34] Our understanding of how the sIgE level, sIgE/total IgE ratio, and sIgE clonality and affinity can affect effector cell degranulation[35] has been further expanded by data from *in vitro* model systems using basophils sensitized with recombinant sIgE antibodies.

The study of antigen-specific B cells has provided new insight into how the clonal contribution of these cells might be important in the humoral response to peanut OIT. An early and transient population of rare, circulating, antigen-specific memory and plasmablast B cells can be identified early in the initiation phase of peanut OIT by using an Ara h 2 fluorescent multimer.[18] New techniques to isolate and clone recombinant allergen-specific antibodies induced during peanut OIT have proved highly informative. The majority of allergen-specific antibodies from patients with OIT bind to conformational epitopes[36]; this observation is also supported by phage display analysis of sera.[37] Interestingly, even though antibody repertoires are

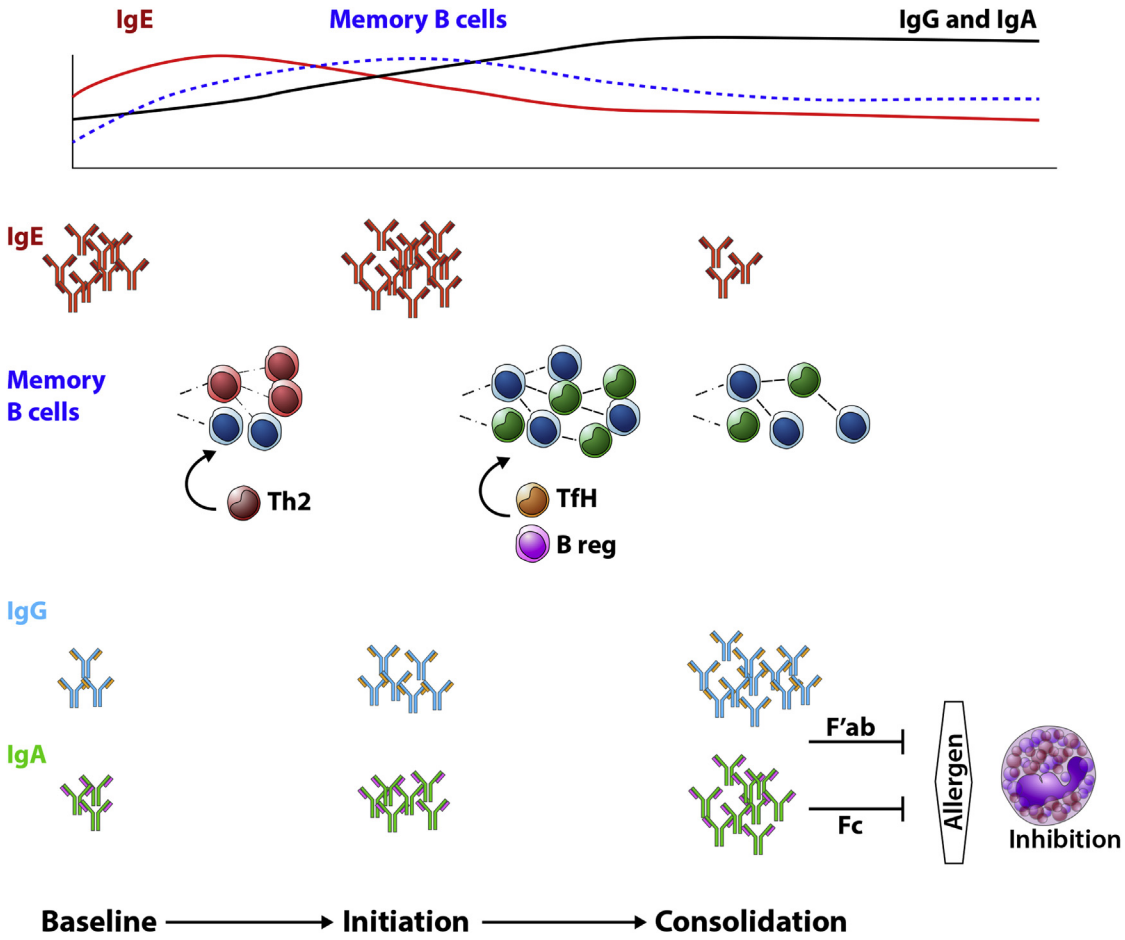

FIG 3. Humoral mechanisms of OIT. The diverse pool of sIgE antibodies are a marker of food allergies in affected patients at baseline. On antigenic re-exposure in the form of OIT, allergen-specific memory B cells are reactivated to undergo somatic hypermutation and affinity maturation. During the induction phase, these memory cell responses contribute to plasma cells that will promote the increase in functional allergen-specific IgG and IgA responses. On the other hand, pathogenic T_H2 cells, on reactivation by these low allergen exposures, might in part drive allergen-specific IgG memory B cells to IgE-producing cells, hence transiently increasing sIgE levels. During the consolidation phase, follicular T_H *(TfH)* and regulatory B *(B reg)* cell compartments can drive memory B-cell responses. In turn, the continued increases in titers of diversified, affinity-matured, allergen-specific IgG and IgA result in persistent suppression of allergic effector cells and the lasting efficacy of OIT.

considered to be highly individual, selection of homologous Ara h 2–specific antibody clones in the repertoires of multiple patients has been observed during OIT. It remains to be proved whether these homologous clones recognize the same epitope, as would be expected, or have unique functional significance such as in suppressing IgE-dependent reactivity.[18]

The increase in the frequency of memory B cells and plasmablasts during the initiation phase of peanut OIT coincides with the increase in Ara h 2–specific IgG_4 antibody levels, as well as total Ara h 2–specific IgG and IgA levels, suggesting that these cells might have a clonal contribution to the functionally suppressive antibodies after peanut OIT (Fig 3). This suggestion is supported further by induction of new Ara h 2–specific IgG_4-recognizing linear epitopes after peanut OIT,[38] as well as the observation of increased somatic hypermutation in a clonal lineage of IgG_4.[36] These changes in the context of effector cell suppression by allergen-specific IgG_4[19] suggest that reactivation of the memory response and development of new allergen-specific antibodies can contribute to post-OIT SU. However, the relevance of the newly emergent clones and even their isotype to clinical outcomes in peanut OIT remains the subject of investigation.

CONSOLIDATION PHASE

Changes in antibody and effector cell responses during the consolidation phase of OIT are likely associated with significant and stable changes at the T-cell level. This might be due in part to selective exhaustion/deletion of allergen-specific T_H2 cells induced by persistent higher-dose allergen exposure, allowing concurrent regulatory immune responses to emerge slowly during the consolidation/maintenance phase of OIT (Fig 2). Recently, allergen-specific T_H2 cells have been shown to represent a phenotypically distinct T_H2 subpopulation confined to atopic subjects, and they display greater adverse activity relative to conventional T_H2 cells.[39] This proallergic T_H2 subset, denoted as the proallergic T_H2 (T_H2A) cell subset, is characterized by stable coexpression of chemoattractant receptor-homologous molecule expressed on T_H2 cells, CD161, and IL-33 receptor and low

expression of CD45RB, CD27, and Bcl-2, which is consistent with cells that are highly sensitive to activation-induced cell death.[40] Furthermore, *ex vivo* analysis of the peanut-specific T_H2A responses in peripheral blood of patients during the course of OIT demonstrated that elimination of allergen-reactive T_H2 cells from the periphery was associated with clinical benefit. This is consistent with the notion that skewing allergen-specific effector T cells away from the proallergic T_H2 response could facilitate other protective changes and might be a causative mechanism for clinical benefits seen during OIT.

Regarding antibodies, it is now well established that levels of allergen-specific IgG, particularly IgG_4, are increased within a few months after starting OIT and often increased more than 10-fold from baseline values and that these remain increased, even after many years of OIT.[4-6,9,11] Induction of peanut-specific IgG during OIT has been linked to suppression of allergic effector cells by using 2 mechanisms, suggesting a gradual temporal convergence in suppressive mechanisms involving humoral and effector cell responses (Fig 1).

The first postulated mechanism is that allergen-specific IgG can block allergen-IgE interactions, thus sequestering the allergen.[41] Functional blocking antibodies correlate with clinical outcomes in subcutaneous immunotherapy.[41] Not only do peanut-specific IgG_4 antibody levels increase during the course of peanut OIT, but IgG_4 from post–peanut OIT sera can suppress peanut-stimulated basophil and mast cell activation.[19]

The second hypothesis highlights the Fc portion of IgG. Human basophil suppression by post-OIT IgG has been shown to be mediated by interactions through the inhibitory receptor FcγRIIb, as shown in murine models of food allergy.[20,42] Blocking FcγRIIb with an mAb prevented inhibition of basophil degranulation, indicating that specific IgG binds this inhibitory receptor and prevents antigen-driven activation by inhibitory signaling. The interactions of antibodies with inhibitory Fc receptors can be influenced by antibody Fc subtypes (eg, levels of $sIgG_1$, $sIgG_2$, and $sIgG_3$ are all increased during peanut OIT[20]), as well as by posttranslational modifications, such as glycosylation. The increase in allergen-specific IgG_4 levels might be related to IL-10 production from Treg cells or regulatory cells B, as has been shown in other forms of immunotherapy.[30,43]

More recently, serum Ara h 2–specific IgG and IgA levels have also been shown to increase during peanut OIT.[18] These antibodies might play a blocking role[44] or might have a deeper role in disease pathogenesis and treatment. Alternatively, the increase in specific IgA levels during OIT might point to a mucosal origin of allergen-specific B cells, which could ultimately shape the allergen-specific B-cell repertoire.[45] For example, significant increases in IgA and IgA_2 levels were found in patients undergoing egg OIT, and these can contribute to effector cell suppression.[46]

SU

SU is a relatively new and loosely defined term referring to the durability of the clinical effect after cessation of the dosing protocol. The term SU was coined to differentiate this post-immunotherapy outcome from true immunologic tolerance, which is regarded as the default state in healthy subjects and can be naturally re-established when food allergies spontaneously resolve (eg, "outgrowing" egg or milk allergy). Nonetheless, although SU probably differs from true tolerance, it is a significant clinical achievement, allowing more flexible consumption of the previously allergenic food in its natural forms. There are 2 main explanations for SU: simple desensitization occurring after extended maintenance treatment, such that the elimination kinetics of the effect are prolonged, or an intermediate-phase change that is neither simple desensitization nor full tolerance.

Our understanding of SU in patients undergoing OIT and its association with the humoral response has been significantly strengthened recently through development of novel tools and methodologies. There might be a pre-existing bias within the adaptive response of those without SU for the propagation of sIgE. For example, SU has been associated with lower quantities of pretreatment peanut and milk sIgE,[9,10,47] whereas the importance of diversity and clonality in patients with persistent and severe food allergy[33,34] suggests that qualitative differences can also exist. Whether this is due to IgE-switched memory B cells or another compartment, such as IgG-switched memory B cells, T_H2 cells, or follicular T_H cells, is still unknown. The induction of antibodies directed against new linear epitopes and oligoclonal allergen-specific memory B cells with somatically hypermutated antibodies suggests that OIT modulates the B-cell repertoire (Fig 3). Although we can speculate that these post-OIT allergen-specific antibodies in patients with SU might effectively suppress allergen effector cells through antigen sequestration[19] or engagement of inhibitory Fc receptors,[20] SU can be more related to the longevity of the induced B-cell memory response or novel immunomodulatory functions of allergen-specific antibodies.

As previously discussed, this effector cell suppression occurs rapidly with continuous administration of antigen, but SPT responses rarely become negative, and some activation is seen in basophil assays, even after many months or years of therapy. This implies that once OIT is stopped, cells can become increasingly responsive to antigen. Indeed, clinical desensitization resulting from OIT can be short-lived, with a large percentage of subjects regaining allergic reactivity within 2 weeks after stopping OIT[4,7,9,24] and in some cases as soon as 1 week after stopping OIT.[6] In these cases it appears that the suppressive effects on mast cells and basophils are transient and that these effector cells will become reactive once antigen administration is stopped.

Studies have demonstrated a return in SPT and basophil responses in subjects with failed oral food challenges several weeks after stopping OIT. However, the opposite was seen in subjects achieving SU, in whom SPT responses and basophil activation remained suppressed.[9,48] Importantly, a study of egg OIT demonstrated that longer treatment regimens led to a higher proportion of subjects with SU,[49] possibly because of a further reduction in sIgE levels or increasing $sIgG_4$ or sIgA levels or more permanent changes in mast cell signaling pathways.

Prolonged continuous antigenic stimulation during maintenance treatment can also have other direct consequences on $CD4^+$ T cells, enhancing epigenetic mechanisms that have been associated with SU.[8] The disease induction model[50] proposes that the presence of a pathogenic $CD4^+$ T-cell subset with distinct phenotypic and functional properties might be sufficient for the pathogenesis of an immune-mediated disease regardless of the balance of other T_H subsets. Similarly, it is possible that current OIT protocols can target allergen-specific T_H2A cells in a stepwise way, including T-cell exhaustion followed by T-cell deletion, to restore a hyporesponsive state to allergen. This is consistent with previous studies suggesting that allergen-specific T cells might represent a suitable therapeutic target during OIT.

CONCLUSIONS/FUTURE DIRECTIONS

There has been significant progress in understanding how OIT suppresses mast cell and basophil reactivity, whereas newer methodologic approaches are beginning to uncover the roles of T and B cells in OIT-induced immunomodulation. However, several key knowledge gaps remain. We need to understand specifically how effector cells are desensitized at the molecular level because this could lead to targeted therapies for food allergies. We need to know whether the basophil activation assay can be used as a biomarker to reliably determine a state of allergy before treatment and then to monitor desensitization, SU, or both as outcomes of treatment. Recently, it was demonstrated that basophil activation assays can predict allergy versus sensitization and eliminate the need for oral food challenges in some patients.[51]

In addition, it is of paramount importance to have a better understanding of the cellular changes associated with different clinical outcomes during or after OIT; for example, it is not known why some subjects achieve partial or full desensitization and others achieve SU. For example, is there a change in the signaling pathways through FcεRI that re-emerges on cessation or does sIgE and/or FcεRI density increase, leading some subjects to become reactive again?

SU likely requires concerted coordination of the adaptive response to delete pathogenic T_H2A cells and induce protective and functionally suppressive allergen-specific clonal memory B-cell responses to suppress effector cell responses for long-lasting clinical efficacy of OIT, but the relative importance of these mechanisms and their kinetics need further study. It will require the endophenotyping of larger numbers of subjects to do this. Ultimately, this work should lead to development of a reliable biomarker assay or group of assays for diagnosis, treatment response monitoring, or both, which will facilitate widespread OIT implementation.

For many years, mechanistic studies investigating the effect of OIT on B cells and $CD4^+$ T cells have been hampered by the absence of adequately sensitive approaches that directly assess immunologic changes within these rare allergen-specific cell populations. Therefore there is a need for a comprehensive understanding of the targeted $CD4^+$ T-cell population, which is critical to designing more effective immunotherapy. However, new technologies, such as polychromatic flow cytometry, mass cytometry, and transcriptional profiling, have been applied to the study of patients with food allergy and are now making it possible to characterize these and other cells with unprecedented resolution.[52-54] As with other routes of allergen-specific immunotherapy, OIT can alter T-cell responses through multiple parallel or overlapping mechanisms, including exhaustion/deletion of proallergic T-cell responses (immune disease induction model), the switch in T-cell effector immune responses (immune deviation model), or the induction of concurrent immune-regulating T cells (immune regulation model). More research is needed to develop a more unified understanding of which of these T-cell mechanisms, or others yet undiscovered, are operative in the short- and long-term outcomes during OIT. Data from such studies should inform rational strategies to enhance OIT by combining it with immune-modulating strategies (eg, mAbs) that can either induce a counterregulatory immune response or block *de novo* generation of proallergic T_H2 cells, leading to improved safety and durable clinical benefit. On the B-cell side, emerging data suggest that we might be approaching a new era of antibody-directed enhancement for OIT through modulating the antibodies produced during therapy to induce long-lasting clinical tolerance. The next generation of antibody-directed vaccine efforts might involve careful shaping of the antibody repertoire, either using antigen-specific B-cell modulation[55] or sequential vaccination strategies, such as used in HIV vaccine trials, to drive the generation of protective antibodies.[56]

Finally, local immune mechanisms in the gut associated with OIT remain to be investigated further, most particularly with respect to the factors involved in antigen uptake and response at the site of administration, such as epithelial cells, innate lymphoid cells, and local microbial factors. Emerging evidence suggests that dendritic cells can play a role in OIT outcomes,[8,57] and this is a key area requiring more investigation. We look forward to future studies that will fill in these and other knowledge gaps and lead us to a better unified understanding of the complex interplay of the molecular, cellular, and humoral changes that occur during and after OIT.

We thank Wesley Burks, William Kwok, and Wayne Shreffler for their careful review of the manuscript and helpful suggestions.

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