

ALLERGY IS AN IMMUNE DISORDER RELATED TO A LACK OF REGULATION: THE GLUING ROLE OF IL-2

by

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Running Title: Interleukin-2: adjuvant therapy for allergies

Introduction

Allergy is the immune system's overreaction to certain antigens called allergens, making a specific class of immune response that produces skin, mucosal, or systemic inflammatory pathologies (1). Allergy has a pandemic nature in the modern world, especially in countries with a high Human Development Index (HDI) (1, 2). In this essay, I shall discuss the current mechanistic view, comparing it to an alternative and testable theoretical framework that may lead to more efficacious treatment.

The actual mechanistic view

Allergens are proteins that elicit a Th2, IgE-producing immune response (3). The Th2 response depends on the differentiation of T cells that produce IL-4, IL-5, and IL-13, among other cytokines (3). IL-4 is not produced by undifferentiated T cells (Th0) but is necessary for polarization to Th2 or, as recently shown, Th0 might differentiate in Th2 cells in the presence of IL-33 (3). Interleukin-33 is an alarmin released upon damage to epithelial cells (4, 5). ST2, the receptor for IL-33, is expressed in many cells, including eosinophils, basophils, NK, NKT, Th1, Th2, and Treg cells (4). IL-33 is also essential for the maturation of type 2 innate lymphoid cells (ILC2) in the lungs and may act directly on dendritic cells to induce Th2 polarization and Treg generation (6-8). Many allergens are proteases and may trigger IL-33 release directly from epithelial cells (9-11). For instance, the honeybee venom phospholipase A2 elicits a Th2 immune response by disrupting the epithelial cell membrane and releasing IL-33, promoting a Th2 immune response (5). Also, four (out of more than 20) aeroallergen groups from the dust mite *Dermatophagoides pteronyssinus* are proteases (9-11). They might elicit a Th2 immune response by acting as described above for the bee venom phospholipase A2 or by directly

inducing the release of another Th2 immune response-promoting cytokine, the IL-4, from mast cells suggested by an *in vitro* study (12).

As pointed out above, unprimed T cells need an external source of IL-4 to develop the Th2 functional pattern (13). Candidates such as ILC-2, basophils, mast cells, and NK T lymphocytes may be a primary source of IL-4 (13-15). Still, the neonate T cells or dendritic cells may also produce the initial IL-4 to polarize Th0 unprimed CD4 T cells (15-17). The Th2 polarized response may induce the production of IgG1 and IgE by B cells (13, 18). However, the amount of IL-4 necessary to cause IgE production is higher than the quantity required for IgG1 secretion (18). Therefore, allergens must induce a robust Th2 response to make IgE⁺ plasmablasts. The strong, uncontrolled, Th2 response could occur in allergic patients because they might not develop Tregs to allergens while having normal frequencies of Tregs to other antigens and, therefore, cannot control the allergen-induced immune response (19). These findings open up at least three possibilities: 1- Allergy is due to Treg repertoire holes; 2- Treg proliferation or function is inhibited in the priming microenvironment; 3- Allergy would be accompanied by a lack of expansion of a particular set of Treg reactivities implicated in the immune regulation of a given group of non-self molecules, contributing to make them, allergens.

Central and peripheral regulatory T cells

It is widely accepted that a specialized regulatory T cell subpopulation is responsible for controlling several aspects of the immune system activity (20). Treg cells are positively selected in the thymus by relatively high-affinity interactions with self-peptides in the context of MHC class II molecules. They mature as CD4⁺ Foxp-3⁺ T cells, expressing the high-affinity receptor for IL-2 (CD25) (20). They remain activated after migration to peripheral lymphoid organs, supposedly by recognizing peripheral self-antigens (20). The repertoire of Treg cells may suffer modifications by addition/deletion or expansion/contraction of different clones within the population (21). These modifications on the Treg cell pool may involve the continuous positive thymic selection, with the presentation of a different set of self or non-self peptides in the thymus (22), the activation of a precursor pool of thymic-derived CD4⁺ Foxp-3⁺ CD25⁺ peripheral T cells (23, 24) or the conversion of recently thymic emigrants CD4⁺ Foxp-3⁺ T cells into the regulatory pathway (21, 25). Thymic and peripheral Treg generation complement each other to maintain tolerance (21). Many cytokines are involved in Treg generation, differentiation, proliferation, and survival (26). TGF-beta is essential for producing thymic and peripheral Tregs (26), whereas IL-2 is crucial in their expansion, maintenance, and functional activity (26). Evidence suggests that IL-33 is also vital in generating an IL-33 receptor⁺ (ST2⁺) Treg subpopulation in conjunction with IL-2 (8). Tregs do not produce IL-2 but instead use the IL-2 produced by dendritic or effector T cells to grow (27). Therefore, some degree of

autoreactivity or response to non-self antigens might exist to stimulate the production of IL-2 by effector T cells (28). For non-self antigens, other sources of IL-2 are being proposed to feed up their Treg correspondent compartment, including effector T cells or even dendritic cells (27). The increased IL-2 production would promote the expansion and proper function of antigen-best fitting linked Treg cells, adjusting the quality and quantity of recruited clones along time and setting up different plateaus of regulatory circuits defined by antigen reactivity/quantity/persistence (28, 29).

The need for an alternative view

We have hypothesized three possible scenarios to explain the Th2 overresponse, as pointed out above. Although possible, holes in repertoires derived from a lack of Tregs' positive or excessive negative selection were not demonstrated. Furthermore, the potential recruitment of other reactivities from peripheral CD4⁺ T cells decreases the potential of having repertoire holes (23, 25). Additionally, allergies would have to be linked to MHC/HLA alleles since negative or deficient positive thymic selection would determine the lack of antigen-reactive T cells (22). Despite the relative importance of MHC/HLA alleles, no strong link was reported for allergies, contrary to autoimmune conditions.

Furthermore, allergic patients have specific effector Th2 responses to the allergens, showing that allergen-reactive T cells can be positively selected (30). The presence of effectors CD4⁺ T cells does not rule out the possibility of MHC/HLA playing a role since the selection rules for Treg cells and thymic emigrants CD4⁺ T cells seem different (31). Yet, I would prefer to call the readers' attention to the fact that environmental antigens are not abundant in the thymus during the selection process, which operates mostly based on self-peptides/MHC/HLA screening (31). Therefore, the immune responses to environmental antigens must work based on degeneracy and crossreactivity of effector and regulatory T cells. In that case, one must hypothesize that a time gap between the generation of effector and expansion of antigen-related regulatory T cells in peripheral lymphoid organs must exist to allow immune responses and their subsequent control. Again, one should not expect a significant time gap for self-antigens as the players should be already balanced from the start by the continuous presence of self. Hence, the T cell repertoire has a high degree of degeneracy and crossreactivity, as first proposed by Don Mason (32). Therefore, the hypothesis considering physical holes in repertoire does not fit the evidence.

Recently, a study supporting the functional inactivation of Treg cells was published (14). It shows that IL-33-expanded ILC-2, through the production of IL-4, could enhance mast cell activity, inhibiting the expansion and function of peripheral Treg precursors in food allergy (14). However, IL-4 has been strongly linked to augmenting the functional activities of Treg cells in many models, including the intestinal microenvironment, upon helminth infection (33-

35). Furthermore, IL-33 and IL-4 increase Treg cell proliferation and aid in producing many regulatory cytokines, arguing against this possibility (34, 36).

Although the above theoretical framework explains the amplification of Th2 responses by lacking control of their expansion, it cannot be responsible for both: Th2 polarization and the absence of Treg cells at the same time since IL-33 and IL-4 may help regulatory T cells.

Therefore, allergy would be better explained by a theoretical framework that could accommodate both lack of Treg cell activity/expansion and polarization of Th0 to Th2 as one single mechanism.

The gluing role of IL-2: an alternative view

As discussed previously, the amplification of Treg frequencies depends on effector T cells that secrete IL-2, and in some anatomical places, the IL-2 secretion may be dendritic cell-dependent (27).

In the natural development in mice and humans, neonate T cells are polarized to the Th2 pathway without an apparent source of non-T cell IL-4 or ongoing immune responses to pathogens or self-antigens (16, 17). Interestingly, in mice, splenic neonatal T cells do not produce IL-2 until a particular time after birth (37). Also, in the mouse model, Tregs appear a little later after birth (20). More intriguingly, IL-2 knockout mice have high levels of IgG1 and IgE besides signs of autoimmunity (38-41). Elevated serum IgG1 and IgE levels have diminished in the IL-2/IL-4 double knockout mice, demonstrating that IL-4 was responsible for this phenotype (41). These studies unequivocally show that Th2 cells can be developed in the relative or total absence of IL-2, even though IL-2 is essential to induce Th2 cells *in vitro* (42). Therefore, we propose that the Th2 pathway would be the default response when a Th0 cell is activated without IL-2 *in vivo*.

Consequently, limited amounts of IL-2 could link the polarization of Th0 to Th2 and low numbers/activity of Tregs in the early allergic response. Hence, no other cell lineages other than T cells would be necessary for the initial Th2 polarization (42). This hypothesis, although not formally demonstrated, is completely amenable to experimentation.

Following this theoretical framework, allergens would be molecules that would not elicit enough IL-2 during its recognition, potentially generating Th2 cells without expanding regulatory T cells. However, one study shows an increased frequency of total CD4⁺ CD25⁺ Foxp-3⁺ T cells in patients with allergic asthma, thus contradicting a supposedly Treg deficiency in this clinical condition (43). Yet, this study did not consider antigen-related Tregs. Therefore, their increased percentages might be due to the associated inflammatory process and not the regulation of allergen responsive Th2 effectors (44). It should be noted that the indexation between IL-2 producers and Treg expansion/activity mentioned above must fail somehow in immune-related diseases so that homeostasis is lost. For instance, an increase in CD4⁺ Foxp-

$3^{+/low}$ CD25⁻ T cells in human lupus correlates with a worsening condition, indicating that these cells may have a pathogenic role (45, 46). It would be interesting to know if low-dose IL-2 treatment (47) would recruit these cells through the stimulation of CD122 instead of CD25, thus recovering their primordial regulatory function and demonstrating that lack of IL-2-producer T cells, rather than diminished Treg levels, contribute to this disease. Hence, this is a crucial point to investigate because the IL-2 molecule has been engineered to target the high-affinity IL-2 receptor (CD25). Therefore, these constructs might reinforce pathological conditions as they do not target CD4⁺ CD25⁻ Foxp-3^{+/low} cells. In addition, another experimental study has used IL-2/anti-IL-2 complexes to drive the IL-2 activity to regulatory cells already expressing the high-affinity CD25 molecules in an experimental model of allergic asthma (48). Contrarily to the expectation, the authors observed an increased inflammation that aggravated the allergic condition in mice treated with engineered IL-2 (48). However, as the authors pointed out, the previous contact with the allergen abolished the harmful effect of engineered IL-2 on their model, promoting regulation. An obvious interpretation of these results is that exposure to the allergen induces the expression of CD25 by TCR activation on an idle allergen-reactive Treg population, making them susceptible to the modified IL-2. Thus, IL-2 might be preferable in pathological conditions over modified forms that increase binding to CD25 unless the antigenic stimulation suffices.

This theoretical framework would support treatments where low doses of IL-2 may be employed to facilitate the development of Treg cells in association with low-dose allergenic stimulation, as demonstrated for experimental food allergy in mice (49). A seminal study recently showed that IL-2 is produced by a subpopulation of allergen-specific effector Th2 cells in the intestinal mucosa of allergic patients upon oral allergen immunotherapy (50). In successfully treated patients, an IL-2-dependent allergen-reactive Treg expansion could be observed, demonstrating for the first time that human oral tolerance is dependent on Treg cells and is mediated by antigen-related CD4⁺ CD25⁺ Foxp-3⁺ T cells peripherally expanded by IL-2 (50).

Low-dose allergenic therapy usually results in a low clinical success rate, and its improvement is highly desired (51, 52). In addition, a previous study shows a potentially interesting IL-2 and IL-4 combination effect, further potentiating Treg proliferation and functional activities (53). Therefore, making allergies strong candidates for IL-2 adjuvant therapy.

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