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Understanding the Immunological Basis of Asthma; Immunotherapy and Regulatory T cells

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Introduction

Among Professor Ehrlich's many great ideas and achievements was his quest for a complete cure of bacterial infection. He said, "It will be easy to effect a cure, if substances have a specific affinity for these bacteria, and act on these alone, while they possess no affinity for the normal constituents of the body. Such substances would then be **magic bullets**".

Allergen Immunotherapy

The magic bullets for allergy might be allergen immunotherapy. Allergen immunotherapy is the only currently available therapy for allergy that alters the underlying immune system, and that can affect a cure. But the specific mechanisms by which immunotherapy works have been controversial for some time. Initially, it was thought that allergen immunotherapy induced blocking antibodies. Shortly after the introduction of the Th1 and Th2 paradigm in 1986, many people believed that allergen immunotherapy worked by inducing Th1 cells, and that allergen-specific Th1 cells could protect against allergy.

However, antigen-specific Th1 cells, if placed into the respiratory tract, induce very intense inflammation in the airways [1]. In addition, if both Th1 and Th2 cells are placed into the respiratory tract, the Th1 cells do not counterbalance the Th2 cells, but instead exacerbate airway inflammation. This suggests that immunotherapy may induce other forms of immunity, other than pure Th1 responses that can protect against allergy and asthma. For example, it is possible that antigen specific regulatory T (T_{Reg}) cells might be induced by immunotherapy, and these cells might be very effective in inhibiting allergic inflammation and protecting against allergy and asthma.

A mouse model of allergen immunotherapy

To study immunotherapy, we examined immunization using heat-killed *Listeria monocytogenes* (HKL) as an adjuvant for allergen immunotherapy. We showed that treatment of ovalbumin (OVA) sensitized mice with just one dose of OVA + HKL, could prevent airway hyperreactivity (AHR), a cardinal feature of asthma, and reduce airway inflammation [2]. Moreover, in a dog model of food allergy, immunization with peanut + HKL reversed established peanut allergy [3]. Thus, after one dose of peanut + HKL, the peanut allergic dogs could tolerate a much larger dose of peanuts, most without developing symptoms, whereas before treatment the dogs developed significant vomiting and diarrhea after ingestion of only small amounts of peanut.

We examined the mechanisms by which HKL induces protective immunity, by studying dendritic cells (DCs) isolated from mice immunized with OVA + HKL. The DCs were purified and then adoptively transferred into mice that were being sensitized with OVA in alum. The mice were subsequently challenged with OVA to induce AHR. We found that when CD8 α^+ DCs from the HKL immunized mice were transferred, there was a significant reduction in AHR. Surprisingly, only the CD8 α^+ DCs, the DCs that are known to induce Th1 responses, transferred this protection, while the CD8 α^- DCs, did not transfer protection [4].

The protective effect required that the DCs produce IL-10, since, adoptive transfer of DCs from IL-10 KO mice immunized with OVA + HKL failed to inhibit AHR. The protective effect also required that the DCs produce IL-12, since adoptive transfer of DCs from IL-12 KO mice, immunized with OVA + HKL also failed to inhibit AHR. These studies thus indicated that CD8 α^+ DCs from OVA + HKL immunized mice transferred protection, but only if these DCs produced both IL-10 and IL-12.

Allergen-specific regulatory T cells

We next determined if the transfer of DCs from HKL immunized mice induced the development of allergen-specific T_{Reg} cells in the recipients. In these experiments, we used DO11.10 mice crossed to RAG^{-/-} mice, since these mice do not contain any CD25⁺ T_{Reg} cells. OVA specific T cells purified from these mice were adoptively transferred to the mice that had received DCs from mice immunized with OVA + HKL. 7 days after transfer, the DO11.10 T cells were retrieved, and examined for cytokine production, and for regulatory cell activity. At 7 days after transfer, the DO11.10 T cells from these mice produced both IL-10 and IFN- γ . In contrast, transfer of DCs exposed to OVA without HKL, resulted in T cells producing IL-4, some IFN- γ , but not IL-10. This cytokine profile was very stable, since 14 days after transfer the T cells induced with the HKL DCs continued to produce both IL-10 and IFN- γ .

We next asked if the IL-10/IFN- γ producing T cells had regulatory activity, by adoptively transferring these T cells into mice that had been sensitized with OVA 7

days earlier. The mice were then challenged with OVA i.n. to induce AHR. We found that adoptive transfer of the IL-10/IFN- γ producing cells greatly inhibited AHR. In contrast, OVA sensitized and challenged mice developed severe AHR and transfer of control DO11.10 T cells did not inhibit AHR. The inhibitory effect of the IL-10/IFN- γ secreting T cells on AHR was due to the production of IL-10, since treatment of these mice with an anti-IL-10 mAb reversed the inhibitory effects of the T_{Reg} cells. The effect was not due to IFN- γ , since treatment with an anti-IFN- γ mAb failed to reverse this inhibitory effect. Transfer of the T_{Reg} cells also greatly reduced airway inflammation and reduced mucus production in the airways. In contrast, transfer of naive DO11.10 cells or of control OVA-activated DO11.10 T cells resulted in severe inflammation, with increased mucus production.

For the aficionados of AHR, we examined AHR not only by examination of Penh in unrestrained mice, but also by directly examining airway resistance and dynamic compliance in anesthetized, tracheostomized, intubated, and mechanically ventilated mice. We found that the mice that received the IL-10/IFN- γ producing cells, had no increase in airway resistance on challenge with methacholine, nor did they have a drop in dynamic compliance with methacholine, indicating the absence of AHR in the mice that received the T_{Reg} cells.

Natural and adaptive T_{Reg} cells

The T_{Reg} cells that were induced with OVA + HKL were OVA specific, and developed on immunization with OVA in the periphery (adaptive T_{Reg} cells), whereas CD25⁺ (natural) T_{Reg} cells, which are the T_{Reg} cells involved in protection against autoimmune diseases develop in the thymus, and are auto antigen specific. Neonatal thymectomy eliminates the CD25⁺ T cells, and results in a wasting disease, associated with autoimmune disease [5]. The transcription factor Foxp3 is a very important gene for CD25⁺ cells, since deletion of Foxp3 results in the absence of CD25⁺ cells and in a similar wasting syndrome associated with autoimmune disease. In addition, Foxp3 is important in humans since mutations in the Foxp3 gene result in a syndrome associated with severe autoimmune diseases, called IPEX.

Because humans with IPEX develop not only autoimmune disease, but also food allergy and eczema, we thought that Foxp3 might also be important for antigen-specific T_{Reg} cells. We therefore examined our antigen-specific T_{Reg} cells for expression of Foxp3 by quantitative RT-PCR. We found that the IL-10/IFN- γ secreting T_{Reg} cells induced with HKL expressed high levels of Foxp3. Moreover, the antigen-specific T_{Reg} induced during respiratory tolerance that we had studied previously also expressed high levels of Foxp3. In contrast, Foxp3 was not expressed by the control OVA-specific T cells, nor by naive DO11.10 T cells.

Subsets of antigen-specific T_{Reg} cells: expression of T-bet and GATA-3

We next examined the HKL induced T_{Reg} cells for expression of T-bet, the master Th1 transcription factor, using flow cytometry. We found that the antigen-specific

T_{Reg} cells induced with *Listeria* were similar to the Th1 cells in expressing high levels of T-bet. In contrast, the T_{Reg} cells induced with respiratory tolerance expressed much lower levels of T-bet [6,7].

We next examined these T_{Reg} cells for expression of GATA-3, the master Th2 transcription factor, by RT-PCR. We found that Th2 cells expressed GATA-3 as expected, whereas Th1 cells did not. The OVA-specific T_{Reg} cells induced by respiratory tolerance also expressed GATA-3, whereas the antigen-specific *Listeria* induced T_{Reg} cells did not. These studies indicate that the OVA-specific T_{Reg} cells induced with *Listeria* had features of both CD25⁺ cells and Th1 cells. On the other hand, the T_{Reg} cells induced by respiratory tolerance had features of both CD25⁺ T_{Reg} cells and Th2 cells. Both OVA-specific T_{Reg} cell types had the capacity to inhibit AHR and airway inflammation, suggesting that a family of adaptive, antigen-specific T_{Reg} cells can inhibit the development of asthma. Moreover, these adaptive T_{Reg} cells are related to natural T_{Reg} cells, since they all express Foxp3.

Summary

We believe that immunotherapy with HKL as an adjuvant induces Th1-like T_{Reg} cells that can inhibit AHR and airway inflammation. These antigen-specific T_{Reg} cells are induced with CD8 α ⁺ DCs producing IL-12 and IL-10, produce IFN- γ and IL-10, and also express T-bet and Foxp3. These Th1 $_{Reg}$ cells are distinct from antigen-specific T_{Reg} cells induced with respiratory tolerance, which can also inhibit AHR and airway inflammation. These Th2-like T_{Reg} cells are induced with CD8 α ⁻ DCs producing IL-10, they express IL-10, GATA3 and Foxp3. So allergen immunotherapy is effective in large part because it induces regulatory T cells. With HKL you get Th1 $_{Reg}$ cells, and with other forms of immunotherapy you may get Th2 $_{Reg}$ cells. We believe that with further refinements, allergen immunotherapy that rapidly induces allergen specific T_{Reg} cells will indeed be the magic bullets for allergy and asthma.

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Discussion

Bufe: Thank you very much for these really fascinating studies. My question is, what happens to the antibodies in your system?

Umetsu: In the heat-killed *Listeria* system where we actually transfer the dendritic cells from mice that have been immunized with ovalbumin together with *Listeria*, the transfer is relatively short. And so we actually have not looked at serum from these mice. In previous studies, where we have looked at mice that have been treated with heat-killed *Listeria* plus OVA, you get lots of IgG2a. So you get a Th1-like response. Initially, we are actually looking at Th1 responses and you get a significant reduction in IgE. In the respiratory tolerance model, that I did not have much time to talk about, we also get some form of immune deviation where you get much more IgG1 and also a significant reduction in IgE. So there are effects, definitely, on the humoral immune compartment along with the development with these regulatory T cells. But that is to be expected because in order to induce antibody you have to have some T cell that is inducing it.

Valenta: I would like to know if you have studied whether the cell transfer would affect IgE-related parameters, such as skin sensitivity, basophil or mast cell degranulation, that are mediated by IgE and the allergen.

Umetsu: We have not looked at mast cell effects, it was not part of the set-up.

Valenta: What about parameters where you would see that the cell transfer would affect IgE-mediated inflammation, such as basophil or mast cell degranulation, which is the hallmark of allergic inflammation in most of the cases.

Umetsu: We know that IgE levels go down, but we have not specifically looked at mast cell or basophil effects in this system.

Moingeon: Those T_{Reg} cells with shared features with Th1 and Th2 cells are extremely interesting. The current view is that we can try to modulate T cell differentiation out of Th0 and committed or naive progenitors, but would you go as far as to suggest that we could reprogram committed Th2 cells, for example, to make them *bona fide* T_{Reg} cells?

Umetsu: I think that many people have looked at trying to reprogram very committed effector T cells. If you have effector Th2 cells, those cells tend to be very set in their ways. However, *in vivo* there are always lots of less committed cells that are coming up. And I believe the ones that are less committed can be reprogrammed.

Casanovas: What was the reason to use heat-killed *Listeria* and not other bacteria?

Umetsu: There has actually been a fairly long literature looking at *Listeria*. Unanue and many other people have looked at *Listeria* as a stimulus that induces very potent Th1 responses. And so, when we began these studies almost 10 years ago, we had

216

actually been thinking that Th1 cells were the ones that were very important in preventing allergic inflammation. So, initially we were looking for a signal that really induced a Th1 response. But along the way, we noticed that there were lots of IL-10 and TGF- β being produced and so we started looking at other parts of that immune response. We found that lots of regulatory T cells were part of that immune response.

Casanovas: In your presentation it seems that these bacteria act more as an adjuvant.

Umetsu: That is exactly right. It is an adjuvant, that is exactly how we look at it.