

EDITORIAL

Clinical and Experimental Allergy

Searching for regulatory T cells in allergy: are we nearing the 'Finish'?

This editorial discusses the findings of the paper in this issue by Skrindo et al. [10].

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Allergic diseases are characterized by cellular infiltration and production of mediators at the site of the affected organ and the presence of circulating specific IgE antibodies against either environmental or food allergens. In contrast to what was initially thought, there is growing evidence that clinical tolerance in healthy individuals is not the result of immunological unresponsiveness but the result of active homeostatic protective mechanisms.

Functionally diverse T cells play a role in both the induction and the manifestation of allergic diseases on the one hand and of clinical tolerance on the other. Local overproduction of T-helper type 2 (Th2) cytokines at the site of allergic inflammation as well as increased systemic Th2 cytokine production, and an intrinsic defect in the production of IFN- γ by Th1 cells in atopic individuals, have now been reported in several cohort studies. The cause of the allergen-specific Th2 predominance in atopics is not fully explored but could be related to a dysfunction of certain regulatory T (Treg) cells that modulate both Th1 and Th2 responses [1].

Among the CD4⁺ Treg, at least two subsets of Tregs can be distinguished by their distinct suppressive mechanisms. Type 1 T regulatory (Tr1) cells are contact independent and their suppressive activity is based mainly on the production of cytokines such as IL-10 and TGF- β [2]. Naturally occurring CD4⁺CD25⁺ T cells, on the other hand, exert their suppressive effects *in vitro* mainly *via* cell-cell contact; however, *in vivo*, these cells can also function through the induction of inhibitory cytokines such as IL-10 and TGF- β [1, 3].

Loss of functional suppression by CD4⁺CD25⁺ T cells in humans has been observed in several diseases, including

rheumatoid arthritis [4] and multiple sclerosis [5]. With respect to allergic diseases, Ling et al. [6] demonstrated that isolated CD4⁺CD25⁺ T cells from non-atopic donors suppressed proliferation and IL-5 production by their own allergen-stimulated CD4⁺CD25⁻ T cells, whereas the inhibition of proliferation by CD4⁺CD25⁺ T cells from atopic donors was significantly reduced and was even more diminished by CD4⁺CD25⁺ T cells isolated from pollen-allergic patients during the pollen season.

However, limitations to use CD25 in order to identify and purify Treg cells exist, as CD25-expression among CD4⁺ cells is not limited to naturally occurring Treg cells alone. Indeed, CD25 is also expressed on CD4⁺ T cells upon activation. As patients with autoimmune diseases and allergic diseases might possess circulating pre-activated T effector cells expressing CD25 (albeit less intensively than expressed by naturally occurring Treg cells), the use of CD25 to purify Treg cells might be even more problematic in patients than in healthy subjects. In order to study the *in vitro* suppressive capacity of naturally occurring Treg cells in different disease models, researches started an *Olympic sprint* to find other membrane or intracellular markers that could be used to identify and purify (the) unique and pure population(s) of naturally occurring Treg cells. The forkhead/winged helix transcription factor Scurfin (FOXP3) was shown to be specifically expressed in regulatory CD4⁺CD25⁺ T cells both from mice and from humans to control their development as well as their function [7]. However, there is growing evidence that FOXP3 can also be induced upon activation [8]. Moreover, as the transcription factor FOXP3 is located intracellularly, its use to study Treg cells functionally is limited.

Recently, Liu et al. [9] described that naturally occurring human CD4⁺CD25⁺ Treg cells were further characterized by down-regulation of the IL-7 receptor, CD127. Interestingly, in the current issue of *Clinical and Experimental Allergy*, Skrindo et al., depleted peripheral blood

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mononuclear cell (PBMC) from grass-pollen allergic and healthy individuals from CD25⁺ T cells as well as CD4⁺CD25⁺CD127^{lo} T cells (outside the grass-pollen season). Depleting PBMC from those fractions did not result in increased allergen-induced proliferation or cytokine production in allergic or healthy individuals [10]. On the other hand, purified CD4⁺CD25⁺CD127^{lo} T cells perfectly suppress an allogeneic mixed lymphocyte reaction. These data show that CD4⁺CD25⁺CD127^{lo} T cells are not involved in the *in vitro* suppression of allergen-specific T cell responses in healthy individuals nor in pollen-allergic patients outside the pollen season. Whether CD4⁺CD25⁺CD127^{lo} Treg cells are also irrelevant *in vivo* to maintain clinical tolerance against allergens or instead could play an important role during the pollen season is, however, not addressed. Recent work suggested that upon activation, T cells can also down-regulate CD127, complicating the use of CD127 for identification of Tregs in patients suffering from inflammatory and probably also from allergic diseases [11].

Moreover, two functionally different subsets of FOXP3⁺ naturally occurring Treg cells in the human thymus and periphery have been described recently [12]. Inducible costimulator (ICOS)⁺FOXP3⁺ Treg cells used IL-10 to suppress dendritic cell function and TGF- β to suppress T cell function, whereas the ICOS⁻FOXP3⁺ Treg cells used TGF- β only [12]. Studying the relevance of these different subsets in the maintenance of clinical tolerance in allergy would certainly be of interest.

Besides naturally occurring Treg cells, inducible populations of antigen-specific IL-10-secreting Treg cells also inhibit inappropriate immune responses in experimental models (reviewed in [13]). Healthy individuals indeed possess IL-10-producing allergen-specific T cells that are involved in suppressing allergen-induced T cell proliferation [14]. In a recent study, we could furthermore demonstrate that allergen-specific T cells from healthy control children not only produce IL-10 and IFN- γ but also express ICOS [15]. The relation of these cells with the naturally occurring ICOS⁺FOXP3⁺ Treg cells needs to be clarified. Successful immunotherapy also induces a population of IL-10-producing allergen-specific T cells, which could even be limited to immunotherapy patients only [16].

In conclusion, different Treg cell populations exist and their role in allergy is only partly unravelled. Ever since the race was started, the finish seemed to be reachable in an Olympic record time, but today we should adjust our

training schedules in order to overcome the obstacles that limit the use of markers in order to identify Treg cells in allergy.

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