

EDITORIAL

Clinical and Experimental Allergy

T cells and allergens relationships: are they that specific?

K. Botturi, D. Vervloet and A. Magnan

UPRES 3287, IPHM IFR125, Pathologie Respiratoire Liée à l'Environnement, Faculté de Médecine, Université de la Méditerranée, Marseille, France

Allergic diseases are T cell mediated conditions considered to be orchestrated by T-helper type 2 (Th2) cells, producing IL-4, IL-13 and IL-5. The two former cytokines are responsible for IgE synthesis while the latter attracts and activates eosinophils. This Th2 paradigm in allergy was imposed during the last 20 years, as a result of both experimental studies in animal models and human surveys examining allergic blood and tissues [1]. A primary Th2 deviation was described in atopic patients independently of any allergic disease, and demonstrated in allergic rhinitis, eczema and asthma. This predisposition was first attributed to an impaired capacity of T cells to develop a proper Th1 activation able to counter Th2. Because epidemiologic studies demonstrated at the same time a relationship between a reduced exposure to microbial agents in early life and occurrence of allergy, according to the so-called hygiene hypothesis, the deficient Th1 activation was widely accepted; indeed, microbial exposure schematically stimulates the Th1 system. However, it appeared that not only allergic but also autoimmune diseases, which are considered to be Th1 mediated, increased in parallel with the decreased microbial exposure [2]. It was therefore proposed that rather than a deficient Th1 activation, a lack of efficient tolerance could be responsible for the increased incidence of allergy and autoimmunity.

Efficient immune tolerance is a *sine qua non* condition for a harmonious development of living beings in an environment providing continuous exposure not only to microbial agents but also to a large variety of non-infectious antigens, including allergens. Indeed, it is crucial for the human organism to be able to prevent itself from any deleterious inflammation against non-aggressive antigenic stimulation. The best example is

probably the efficient tolerance developed towards the early exposure of the bowel to various bacteria species during neonatal colonization. It is clear that a very efficient tolerance machinery is necessary to avoid continuous bowel inflammation induced by these bacteria and to prevent them from becoming pathogens. Accordingly, the disruption in mice of the IL-10 gene has long been demonstrated to induce an inflammatory bowel disease reminiscent of human Crohn's disease or ulcerative colitis [3]. Indeed, IL-10 is one of the rare immunosuppressive cytokines identified to date and appears to be a major mediator of natural tolerance.

IL-10 is produced notably by T regulatory (Treg) cells, a small subset of T cells that, because its recent identification and characterization, has amassed most interest from researchers involved in the science of chronic inflammation. This subset itself recovers various T cell populations differing in their tissue localization, surface antigen expression, cytokine production and conditions of activation. Indeed, it is likely that some innate Treg cells are sentinel cells capable of inducing directly a tolerant response, although others are induced secondarily in response to cognate antigens. Forkhead box transcription factor (FoxP3) is a transcription factor initially regarded as a marker of certain subsets of Treg cells, but was recently proved to be directly related to the regulatory function of Treg cells, making it potentially a hallmark of all the Treg cells [4]. Because it circulates in blood and because it is easily identified by flow cytometry upon its expression of CD4 and of CD25 at a high level, the CD4⁺CD25^{high} T cell population is the most-studied Treg cell subset. Other markers such as CD127, which is expressed at a low level on these cells, FoxP3, which can now be detected at the protein level intracellularly, CD62L, and the absence of CD69 were used in various reports. It is now therefore recommended to identify the so-called population of CD4⁺CD25⁺ Treg cells as CD4⁺CD25^{high}CD127^{low}FoxP3⁺ cells [5].

After convincing data obtained in animal models, several reports recently indicated in humans a role for

Correspondence:

A. Magnan, UPRES 3287, IPHM IFR125, Pathologie Respiratoire Liée à l'Environnement, Faculté de Médecine, Université de la Méditerranée, 27, Bd Jean Moulin, 13005 Marseille, France.

E-mail: antoine.magnan@ap-hm.fr

CD4⁺CD25⁺ cells in preventing allergic diseases. In grass pollen-allergic patients, Ling et al. [6] co-cultured sorted CD4⁺CD25⁺ and CD4⁺CD25⁻ cells in non-atopic controls, sensitized subjects sampled outside the pollen season and allergic patients naturally exposed to grass pollen during the season. In response to allergen extract *ex vivo*, CD4⁺CD25⁻ cells proliferate and produce IL-5 whatever the patient group. However, CD4⁺CD25⁺ cells from non-atopics but not from symptomatic allergics suppressed both CD4⁺CD25⁻ proliferation and IL-5 production. An intermediate pattern was found in non-symptomatic sensitized subjects. Akdis et al. [7] showed concordant results in birch-, house dust mite- and food-allergic patients. In addition, these authors demonstrated that IL-10 secretion was responsible for the suppressive effect of CD4⁺CD25⁺ cells. Karlsson et al. [8] have studied a cohort of children allergic to milk proteins and have compared the CD4⁺CD25⁺ cell activation in patients recovering spontaneously from their allergy vs. patients with persisting allergy after at least 2 months of avoidance. They elegantly demonstrated a deficient CD4⁺CD25⁺ cell activation in children with persisting allergy compared with spontaneously cured children. This paper clearly demonstrates a coincidence of CD4⁺CD25⁺ cell maturation and tolerance induction. Lastly, several reports in pollen allergy and hymenoptera venom allergy demonstrated the induction of CD4⁺CD25⁺ Treg cells during specific immunotherapy [9, 10]. Interestingly, we demonstrated recently in wasp venom immunotherapy that Treg induction was delayed in the more severe patients [10]. According to a protective role of CD4⁺CD25⁺ cells in allergy, we also recently demonstrated that subjects occupationally exposed to allergens but not sensitized or displaying allergic symptoms showed an increase in their blood CD4⁺CD25⁺ cells' proportion after realistic challenge with the allergen, although asthmatic patients showed an increase in their Th2 and Th1 subsets concomitant to a decrease of lung function in response to such challenge [11].

It therefore appears to be established that Treg cell activation is deficient in allergy, both in sensitized and symptomatic patients, and both in rhinitis and asthma [12]. This is especially important considering that it is not unrealistic to envisage for severe patients curative strategies aiming to stimulate or use Treg cells. However, several issues still have to be addressed and clarified before envisaging such an approach. One of these is the specificity of Treg cells in the context of allergy.

Indeed, although the first reports demonstrating a role for Treg cells in preventing allergy were based on *ex vivo* specific stimulations with allergen extracts, it is likely that *in vivo*, the Treg cell populations involved are specific of a large panel of antigens rather than of the targeted allergen only. Accordingly, it is shown in the initial Akdis's paper that the frequency of single allergen-

specific CD4⁺CD25⁺IL-10⁺ cells ranges between 1 in 1000 and 1 in 20 000 of the whole CD4⁺CD25⁺ cell population, which itself represents 10–15% of CD4⁺ T cells [7]. However, in papers studying the variation of CD4⁺CD25⁺ cells during immunotherapy or in response to allergen challenges *in vivo*, it is well demonstrated that the proportion of CD4⁺CD25^{high} cells can be doubled under *in vivo* stimulation [9–11]. This indicates that even under 'specific' stimulation, non-specific Treg cells proliferate. This phenomenon could result from an effect of cytokines produced by stimulated allergen-specific T cells on bystander non-specific Treg cells, inducing them to proliferate and produce cytokines, including IL-10. The bystander effect can also be attributed to the stimulation of the innate immune system during antigen stimulation. Indeed, such an *in vivo* stimulation is never due to the action of the antigen alone but of various compounds associated with allergic substances, including pathogen-associated molecular patterns (PAMPs). These PAMPs are ligands of toll-like receptors (TLRs) found at the surface of antigen-presenting cells (APC). Their engagement makes APC differentiate into pro-inflammatory cells activating Th1 or Th2 cells, or on the contrary Treg cells [13]. According to an effect on innate immune cells during specific immunotherapy, monocytes and dendritic cells are activated during this treatment [14]. The bystander effect of immunotherapy could account for its preventive action against further sensitizations in children [15].

The bystander non-allergen-specific effect of Treg cells can also be demonstrated in experiments comparing the CD4⁺CD25⁺ cell function in response to various antigens. In this issue of *Clinical and Experimental Allergy*, Thunberg et al. [16] very elegantly report the effect of CD4⁺CD25⁺ Treg cells in birch-allergic and non-allergic subjects, in response to birch allergens or to influenza antigens. As expected, they show that in response to birch pollen extract or recombinant Bet v 1, CD4⁺CD25⁺ cells inhibit the proliferation of CD4⁺CD25⁻ T cells in non-allergics but not in allergics. Less expectedly, they found that CD4⁺CD25⁺ cells exert the same effect on cytokine production in both groups of patients under allergen stimulation: inhibition of Th1 (IL-2, IFN- γ , TNF- α) production with no effect on Th2 cytokines IL-4 and IL-5, suggesting that allergen stimulation induces a non-suppressible Th2 activation whatever the allergic status of the patients. The importance of this result is tempered by the finding that birch pollen induces low levels of Th2 cytokines in non-allergic subjects as compared with patients. But the most unexpected result resides in that under stimulation by a recall viral antigen, influenza, CD4⁺CD25⁺ cells fail to suppress the Th2 cytokine production from allergics although both Th1 and Th2 cytokines are inhibited in non-allergics. This reflects a global deficiency of Treg cells towards Th2 activation in allergic patients, which is therefore not limited to allergens but

extends to viral antigens. Also, this result illustrates that in response to viral stimulation, T cells from allergic subjects react by secreting non-suppressible Th2 cytokines, able to amplify the allergic inflammation. It might be the illustration in humans of a global predisposition to develop a Th2 reaction, as suggested by the authors, or to the presence of a bystander action of allergen-specific Th2 cells, educating influenza-specific T cells to react to their cognate antigen by producing Th2 cytokines, as shown in animals more than 10 years ago [17].

Noteworthy in this paper, no difference was found between allergics and controls with regard to the CD4⁺CD25⁺ cells effect on CD4⁺CD25⁻ non-specific proliferation. The global defect seen in allergy therefore seems to be limited to Th2 cytokine production. This limitation could explain why atopy is a predisposition to allergy but not to autoimmune diseases.

Taken together, these considerations interestingly indicate that the specificity of T cell activation in allergy is not as clear as it is dogmatically considered. There is a global predisposition to allergy in atopic subjects attributed to deficient Treg cell machinery, allowing the Th2 cell activation to occur in response to all antigens. The Treg cell deficiency can be globally corrected spontaneously as seen in milk allergy, or using allergen stimulation during immunotherapy. These hallmarks of allergy result from the activation of specific T cells, of innate immune cells and of bystander Th2 and Treg cells.

A next step will be to determine what the CD4⁺CD25⁺ cells really do: IL-10, TGF- β and/or CTLA-4-mediated activation of dendritic cells, as questioned in the paper by Thunberg et al. [16]. Another one will be to definitely identify the other Treg cell populations, because if not all CD4⁺CD25⁺ cells produce IL-10, not all IL-10-producing T cells are CD4⁺CD25⁺. Probably one of the last steps will be to determine to what extent the innate system stimulation will help, together with stimulation of T cells with antigens, to reverse the Treg cell deficiency and the Th2 deviation in allergy [18].

Acknowledgement

Karine Botturi was awarded a UCB Pharma and ANRT grant through a CIFRE contract.

References

- 1 El Biaze M, Boniface S, Koscher V *et al.* T cell activation, from atopy to asthma: more a paradox than a paradigm. *Allergy* 2003; 58:844–53.
- 2 Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* 2002; 347:911–20.
- 3 Davidson NJ, Leach MW, Fort MM *et al.* T helper cell 1-type CD4⁺ T cells, but not B cells, mediate colitis in interleukin 10-deficient mice. *J Exp Med* 1996; 184:241–51.
- 4 Lin W, Haribhai D, Relland LM *et al.* Regulatory T cell development in the absence of functional Foxp3. *Nat Immunol* 2007; 8:359–68.
- 5 Banham AH. Cell-surface IL-7 receptor expression facilitates the purification of FOXP3 (+) regulatory T cells. *Trends Immunol* 2006; 27:541–4.
- 6 Ling EM, Smith T, Nguyen XD *et al.* Relation of CD4⁺CD25⁺ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. *Lancet* 2004; 363:608–15.
- 7 Akdis M, Verhagen J, Taylor A *et al.* Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 2004; 199:1567–75.
- 8 Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD4⁺CD25⁺ regulatory T cells in children who have outgrown cow's milk allergy. *J Exp Med* 2004; 199:1679–88.
- 9 Francis JN, Till SJ, Durham SR. Induction of IL-10⁺CD4⁺CD25⁺ T cells by grass pollen immunotherapy. *Allergy Clin Immunol* 2003; 111:1255–61.
- 10 Mamelessier E, Birnbaum J, Dupuy P, Vervloet D, Magnan A. Ultra-rush VIT induces differential T cell activation and regulatory patterns according to the severity of allergy. *Clin Exp Allergy* 2006; 36:704–13.
- 11 Mamelessier E, Milhe F, Guillot C *et al.* T cell activation in occupational asthma and rhinitis. *Allergy* 2007; 62:162–9.
- 12 Magnan A, Humbert M. Is deficient tolerance the true paradigm for atopic diseases? *Clin Exp Allergy* 2005; 35:1507–10.
- 13 Kanzler H, Barrat FJ, Hessel EM, Coffman RL. Therapeutic targeting of innate immunity with toll-like receptor agonists and antagonists. *Nat Med* 2007; 13:552–9.
- 14 Magnan A, Marin V, Mely L *et al.* Venom immunotherapy induces monocyte activation. *Clin Exp Allergy* 2001; 31:1303–9.
- 15 Des Roches A, Paradis L, Menardo JL, Bouges S, Daures JP, Bousquet J. Immunotherapy with a standardized dermatophagoides pteronyssinus extract. VI. Specific immunotherapy prevents the onset of new sensitizations in children. *J Allergy Clin Immunol* 1997; 99:450–3.
- 16 Thunberg S, Akdis M, Akdis C *et al.* Immune regulation by CD4⁺CD25⁺ T cells and IL-10 in birch-pollen patients and non-allergic controls. *Clin Exp Allergy* 2007; 37:1205–14.
- 17 Coyle AJ, Erard F, Bertrand C, Walti S, Pircher H, Le Gros G. Virus-specific CD8⁺ cells can switch to interleukin 5 production and induce airway eosinophilia. *J Exp Med* 1995; 181:1229–33.
- 18 Creticos PS, Schroeder JT, Hamilton RG *et al.* Immune tolerance network group. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. *N Engl J Med* 2006; 355:1445–55.