

EDITORIAL

Clinical and Experimental Allergy

Sublingual immunotherapy: the quest for innovative adjuvants

This editorial discusses the findings of the paper in this issue by Lombardi et al. [7].

M. Goldman

Institute for Medical Immunology, Université Libre de Bruxelles 8, Rue Adrienne Bolland, B-6041 Charleroi, Belgium

The delivery of antigens through mucosal routes has been established in several experimental models as an efficient means to induce immunological tolerance. Our better knowledge of the unique characteristics of the mucosal immune system has recently shed light on the mechanistic basis of mucosally induced tolerance. Indeed, the dendritic cells (DC), which handle antigens in mucosal sites, appear to promote the emergence of regulatory T lymphocytes with potent suppressive properties. So far, sublingual immunotherapy (SLIT) for allergic rhinitis is the only therapeutic strategy based on this concept, which has reached clinical practice. There is a large consensus – primarily based on meta-analysis studies – that SLIT is an effective treatment in adults [1–3]. As a whole, about 35–40% of adult patients undergoing SLIT experienced a reduction in clinical symptoms with a decreased need for anti-allergic drugs. However, the optimal doses and schedules of administration to be used have not been defined so that the cost/benefit of SLIT is not demonstrated [1]. Furthermore, its efficiency is not as firmly established in children as in adults [4]. SLIT has not yet been approved by the U.S. Food and Drug Administration. Several factors limit the widespread acceptance of SLIT as standard of care for allergic rhinitis, among which the mode of preparation of the allergens is an important one, because aqueous allergen extracts are difficult to standardize [5]. Therefore, increased attention is paid to allergens obtained through recombinant technology. Recombinant allergens are less potent stimulators of the immune systems than allergen extracts, so that recombinant SLIT

products will have to include adjuvant-like molecules favouring differentiation of antigen-specific regulatory T lymphocytes. Cholera toxin B is an example of such adjuvants as it was shown to induce FOXP3⁺CD4⁺ regulatory T cells when administered with antigen via the sublingual route [6]. The study of Lombardi et al. [7] published in this issue indicates that agonists of Toll-like receptor (TLR) 2 should also be considered as potential SLIT enhancers.

Ligands of the TLR are currently considered as the most attractive adjuvant candidates for novel vaccines, because they stimulate DC to mature and migrate in draining lymph nodes where they induce immune responses through their interactions with T lymphocytes. In humans, 10 functional TLRs were identified so far. They differ in their pattern of expression and the signalling pathways they are associated with. Expressed on the membrane of myeloid DC, TLR2 and TLR4 are coupled to the adaptor protein named myeloid differentiation primary-response protein 88 (MyD88) which initiates activation of mitogen-activated protein (MAP) kinases and nuclear translocation of the transcription factor NF- κ B, resulting in transcription of genes controlling synthesis of key inflammatory cytokines such as interleukin-6 (IL-6) but also IL-10, a master anti-inflammatory mediator. TLR4 is also coupled to TIR domain – containing adaptor protein-inducing interferon (IFN)- β (TRIF). The TRIF pathway downstream of TLR3 and TLR4 is critically involved in the activation of genes controlled by IFN regulatory factors (IRF) 3 and 7, which include the genes encoding type I interferons and the p35chain of IL-12 in humans [8]. There is a complex cross-regulation between MyD88 and TRIF-dependent signalling pathways so that the patterns of cytokines produced by DC in response to TLR2 and TLR4 are distinct. A hallmark of DC responses to TLR2 agonists is the release of high levels IL-10 [9], a finding which was confirmed by Lombardi et al. [7] as far as Pam3CSK4 is concerned. Adjuvants inducing high levels of IL-10 by DC are likely to favour the emergence of regulatory T cells of the Tr1 type

Conflict of interest disclosure: The Institute for Medical Immunology is sponsored by GSKBiologicals (Rixensart, Belgium) and Michel Goldman serves as consultant for GSKBiologicals and OM-Pharma (Meyrin, Switzerland).

Correspondence:

Michel Goldman, Institute for Medical Immunology, Université Libre de Bruxelles 8, Rue Adrienne Bolland, B-6041 Charleroi, Belgium. E-mail: mgoldman@ulb.ac.be

which secrete IL-10 themselves [10]. Using an *in vitro* model, Lombardi et al. indeed observed that DC exposed to Pam3CSK4 promote the differentiation of CD4⁺ T cells secreting IL-10 but also IFN- γ [10]. The latter observation is of special interest in view of the previous report of the group of Umetsu et al. describing a population of regulatory T cells secreting both IL-10 and IFN- γ which protect against airway hyper-reactivity in a mouse model [11].

Lombardi et al. made the interesting observation that all TLR2 ligands are not equal in their ability to enhance SLIT. This might be related to the observation that certain TLR2 ligands might be endowed with the ability to induce a tolerogenic programme in DC characterized by high production of IL-10 and low production of IL-6 as shown previously for zymosan and schistosomal phosphatidylserine [12, 13]. In the case of zymosan, the induction of this programme depends on the simultaneous engagement of dectin-1 with subsequent activation of ERK/MAP kinase and also involves induction of TGF- β production by macrophages [12]. The example of schistosomal phosphatidylserine is especially relevant, because it has been shown to promote the emergence of adaptive regulatory T cells with potent suppressive activities on both Th1 and Th2 responses, which represents a mechanistic basis for the inhibition of allergic responses during schistosomiasis [14].

Beside their direct effect on antigen-presenting cells, TLR2 ligands might also directly act on T cells including regulatory T cells. The consequences of TLR2 engagement on T cells are rather complex and could be either detrimental or beneficial in the context of allergy. Potentially favourable effects include inhibition of the production of Th2 cytokines by allergen-specific T and increased regulatory T cell activity after extinction of TLR2 signalling (reviewed in reference [15]). It is important to realize that TLR2 is also expressed and functional on other cell types involved in allergic responses, including mast cells and epithelial cells. Indeed, TLR2 agonists were found to activate mast cells and to enhance mucin production in airways [16, 17]. The latter findings are obviously relevant to the safety of TLR2 agonists, as well as their ability to elicit caspase-dependent cell apoptosis [18].

Although the experimental data reported by Lombardi et al. suggest that TLR2 ligands could be developed as enhancers of SLIT, it is essential to keep in mind the pleiotropic expression of TLR and their multiple actions that might impact their safety profile. As a matter of fact, subcutaneous immunotherapy using products based on a TLR4 agonist (monophosphoryl lipid A) is currently under scrutiny by the U.S. Food and Drug Administration. More than ever, SLIT should be guided by the Hippocrates' aphorism *primum non nocere*.

References

- 1 Cox LS, Linnemann DL, Nolte H, Weldon D, Finegold I, Nelson HS. Sublingual immunotherapy: a comprehensive review. *J Allergy Clin Immunol* 2006; **117**:1021–35.
- 2 Frew AJ. Sublingual immunotherapy. *N Engl J Med* 2008; **358**:2259–64.
- 3 Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy* 2005; **60**:4–12.
- 4 van Wijk RG. Sublingual immunotherapy in children. *Expert Opin Biol Ther* 2008; **8**:291–8.
- 5 O'Hehir RE, Sandrini A, Anderson GP, Rolland JM. Sublingual allergen immunotherapy: immunological mechanisms and prospects for refined vaccine preparation. *Curr Med Chem* 2007; **14**:2235–44.
- 6 Sun JB, Czerkinsky C, Holmgren J. Sublingual 'oral tolerance' induction with antigen conjugated to cholera toxin B subunit generates regulatory T cells that induce apoptosis and depletion of effector T cells. *Scand J Immunol* 2007; **66**:278–86.
- 7 Lombardi V, Van Overtvelt L, Horiot S et al. The TLR2 agonist Pam3CSK4 enhances the induction of antigen-specific tolerance via the sublingual route. *Clin Exp Allergy* 2008; **38**: (doi: 10.1111/j.1365-2222.2008.03056.x).
- 8 Gorieli S, Neurath MF, Goldman M. How microorganisms tip the balance between interleukin-12 family members. *Nat Rev Immunol* 2008; **8**:81–6.
- 9 Re F, Strominger JL. IL-10 released by concomitant TLR2 stimulation blocks the induction of a subset of Th1 cytokines that are specifically induced by TLR4 or TLR3 in human dendritic cells. *J Immunol* 2004; **173**:7548–55.
- 10 Battaglia M, Gregori S, Bacchetta R, Roncarolo MG. Tr1 cells: from discovery to their clinical application. *Semin Immunol* 2006; **18**:120–7.
- 11 Stock P, Akbari O, Berry G, Freeman GJ, Dekruyff RH, Umetsu DT. Induction of T helper type 1-like regulatory cells that express Foxp3 and protect against airway hyper-reactivity. *Nat Immunol* 2004; **5**:1149–56.
- 12 Dillon S, Agrawal S, Banerjee K et al. Yeast zymosan, a stimulus for TLR2 and dectin-1, induces regulatory antigen-presenting cells and immunological tolerance. *J Clin Invest* 2006; **116**:916–28.
- 13 van der Kleij D, Latz E, Brouwers JF et al. A novel host–parasite lipid cross-talk. Schistosomal lyso-phosphatidylserine activates Toll-like receptor 2 and affects immune polarization. *J Biol Chem* 2002; **277**:48122–9.
- 14 van Riet E, Hartgers FC, Yazdanbakhsh M. Chronic helminth infections induce immunomodulation: consequences and mechanisms. *Immunobiology* 2007; **212**:475–90.
- 15 Goldman M. Translational mini-review series on Toll-like receptors: Toll-like receptor ligands as novel pharmaceuticals for allergic disorders. *Clin Exp Immunol* 2007; **147**:208–16.
- 16 Chu HW, Jeyaseelan S, Rino JG et al. TLR2 signaling is critical for *Mycoplasma pneumoniae*-induced airway mucin expression. *J Immunol* 2005; **174**:5713–9.
- 17 Varadaradjalou S, Feger F, Thieblemont N et al. Toll-like receptor 2 (TLR2) and TLR4 differentially activate human mast cells. *Eur J Immunol* 2003; **33**:899–906.
- 18 Aliprantis AO, Yang RB, Weiss DS, Godowski P, Zychlinsky A. The apoptotic signaling pathway activated by Toll-like receptor-2. *EMBO J* 2000; **19**:3325–36.