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EDITORIAL

Th1/Th2 paradigm: not seeing the forest for the trees?

A.J.M. van Oosterhout and A.C. Motta

In this issue of the *European Respiratory Journal*, IRIFUNE *et al.* [1] describe the effects of adoptive transfer of T-helper cell type 1 (Th1) cells in a mouse asthma model. Since the 1980s, the Th1/Th2 dichotomy has been considered the cornerstone of immune responses [2]. Th1 cells (secreting interleukin (IL)-2 and interferon (IFN)-γ) lead to cell-mediated responses, whereas Th2 cells (secreting IL-4, IL-5 and IL-13) mediate for humoural immune responses [2]. Accordingly, allergy results from an imbalance in favour of a Th2 response, and is negatively regulated by Th1 cells. However, while numerous studies support this theory, concurrently, data are accumulating that do not fit with the model in its original form.

Data in the literature show that both the prevalence of Th1-mediated autoimmune and Th2-mediated allergic diseases have increased simultaneously in recent decades [3–5]. Furthermore, helminth infections, which induce strong Th2 responses, have paradoxically been shown to protect against allergy [6]. These observations are in contradiction to the view of mutual cross-regulation between the two arms of the Th1/Th2 balance. Finally, studies regarding this balance in mouse asthma models have failed to provide clear evidence of counter-regulation between the two subtypes. For these latter studies, at least two strategies have been explored: adoptive transfer of *in vitro* polarised T-cells and treatment with Th1-skewing factors *in vivo*.

Typically, Th1- and Th2-polarised cells are generated by culturing CD4+ T-cells derived from ovalbumin (OVA)-specific T-cell receptor (TCR) transgenic mice (DO11.10) in the presence of T-cell skewing (anti-)cytokines. This protocol allows generation of polarised T-cells, which maintain their characteristics after they have been injected in recipient mice. Surprisingly, despite down regulation of airway eosinophilia, adoptively transferred Th1 cells have been shown to potentiate cellular inflammation in the lungs, and not to reduce airway hyperresponsiveness (AHR) [7, 8]. In agreement with this, the Th1-type cytokine IFN-γ is implicated in antigen-induced AHR in, at least, some mouse models of asthma [9, 10]. RANDOLPH *et al.* [11] even showed that co-transfer with Th1 cells is required for antigen-induced eosinophilic inflammation in the lungs of mice after transfer of OVA-TCR transgenic Th2 cells.

The present data of IRIFUNE *et al.* [1] are in contrast with these studies. They sensitised mice with OVA and subsequently Th1 cells were injected in the tail vein prior to the inhalation challenge. In comparison with the positive control asthma group, mice from the asthma Th1-group showed marked decreases in both AHR and eosinophilia, concurrent with a noneosinophilic inflammation in the lungs. Studies of the BALF cytokine profile showed increased IFN-γ levels and decreased Th2 cytokines, IL-5 and IL-13, in the asthma-Th1 group, indicating that the observed effects on asthma manifestations were due to tipping of the Th1/Th2 balance. Although suggested by IRIFUNE *et al.* [1], differences in the level of polarisation of the injected T-cells appear unlikely to explain the differences with previous transfer studies. A novel approach was recently taken by ARONICA *et al.* [12], who transferred OVA-TCR transgenic Th1 and Th2 cells to naïve mice and allowed these cells to convert from an activated effector phenotype to a resting memory phenotype. Upon subsequent OVA inhalation, mice that received Th2 cells displayed airway eosinophilia and AHR but co-transfer of Th1 cells completely failed to counterbalance these asthma manifestations [12]. Besides these conflicting results, adoptive transfer studies remain rather artificial. The injection of 1–10 million polarised T-cells in the above-mentioned studies is in sharp contrast to the number of *in vivo* generated antigen-specific CD4+ T-cells required to induce eosinophilic lung inflammation, which is estimated to be <1,200 [13].

A common observation in most adoptive transfer studies is a Th1-induced noneosinophilic lung inflammation characterised by markedly increased numbers of macrophages and lymphocytes [1, 7, 8]. Interestingly, it is well known that alveolar macrophages suppress the induction of immunoglobulin (Ig)E responses in rats [14] and that the adoptive transfer of allergen-loaded macrophages inhibits airway eosinophilia and AHR in mouse models of asthma [15–17]. Therefore, transferred polarised Th1 cells may not directly suppress Th2 cells but may act through macrophages.

The second, more physiological, approach is to promote a Th1 polarised response *in vivo via* treatment with Th1-skewing factors, such as IL-12, IL-18, bacteria or bacterial fragments. Treatment with the Th1-skewing cytokines IL-12 and IL-18 or with bacteria-derived CpG-DNA inhibits antigen-induced AHR and eosinophilia in mouse models of allergic asthma [18–21]. Although data strongly support a role for Th1-derived IFN-γ, additional mechanisms are at play [22, 23]. Interestingly, it was shown recently that adoptive transfer of CpG-ODN-activated OVA-loaded macrophages potently suppressed allergic airway inflammation and AHR in an IL-10 dependent fashion [24]. This suggests that reduction of asthma manifestations by CpG-DNA treatment *in vivo* may, at least partially, be mediated by IL-10-producing antigen-presenting cells.

Lab. Allergology and Pulmonary Diseases, Dept Pathology and Lab. Medicine, University Medical Center Groningen (UMCG), Groningen, The Netherlands.

CORRESPONDENCE: A.J.M. van Oosterhout, Laboratory of Allergology and Pulmonary Diseases, Dept of Pathology and Laboratory Medicine, UMCG, P.O. Box 30.001, 9700 RB, Groningen, The Netherlands. Fax: 31 503121576. E-mail: a.j.m.van.oosterhout@path.umcg.nl

Th1 responses can also be promoted by *in vivo* administration of entire bacteria, like killed *Mycobacteria* or *Listeria monocytogenes*, either one of which markedly reduces AHR and eosinophilia [25, 26]. Recently however, suppression of these asthma manifestations in mice after administration of killed *M. vaccae* or *L. monocytogenes* has been attributed to OVA-specific T-regulatory cells that mediate suppression by IL-10 and transforming growth factor (TGF)-β [27, 28].

Twenty-five years after the initial T-helper cell type 1/type 2 concept, T-regulatory cells are taking the centre stage as crucial immunoregulatory cells that are capable of suppressing T-helper cell type 1- and T-helper cell type 2-mediated adaptive immune responses [29–31]. At present, little is known about the role of T-regulatory cells in allergic asthma. Interestingly, interleukin-10- and transforming growth factor-β-producing antigen-specific T-regulatory cells are increased in the blood and airway tissue after allergen-immunotherapy [32–34]. Moreover, in a mouse model of allergen-immunotherapy, blocking of the interleukin-10 receptor largely abrogated the reduction of airway eosinophil numbers and airway hyperresponsiveness after therapy [35]. It is feasible that T-regulatory cells offer an explanation for many of the observations that cannot be adequately explained by the T-helper cell type 1/type 2 balance. Blinded by the T-helper cell type 1/type 2 paradigm we could not see the forest for the trees.

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