

# Distinct regulation of IgE, IgG4 and IgA by T regulatory cells and toll-like receptors

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## INTRODUCTION/BACKGROUND

Allergic diseases result from the activation of the immune system and formation of IgE antibodies against normally innocuous environmental antigens. The T helper 2 (Th2) cells and their associated cytokines have been demonstrated to play a key role in the induction of IgE and in several other aspects of allergic inflammation. Both IL-10-secreting T regulatory type 1 (Tr1) cells and CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> T regulatory (Treg) cells suppress the allergic inflammatory or autoimmunity-related immune responses in several experimental models and their influence on antibody regulation remains to be elucidated. The present study investigates the influence of allergen-specific Tr1 cells and Treg cells on immunoglobulin (Ig) isotype production in peripheral blood mononuclear cell (PBMC) cultures.

## MATERIAL & METHODS

IL-10-secreting cells were purified by magnetic-activated cell sorting (MACS) and purity of the cytokine-secreting cells was checked by fluorescence-activated cell sorting (FACS). CD4<sup>+</sup>CD25<sup>+</sup> Tregs were purified by MACS in a two-step procedure. First, non-CD4<sup>+</sup> cells were labeled with a cocktail of biotin-conjugated antibodies and anti-biotin microbeads, after which labeled cells were depleted. In the second step, CD4<sup>+</sup>CD25<sup>+</sup> T cells were labeled with anti-CD25 microbeads and isolated by positive selection. IgE- and IgG4-mRNA levels were measured by qRT-PCR. Immunoglobulin concentrations in supernatants of PBMC cultures from healthy donors were measured by ELISA. The frequency of IgE- and IgG4-producing B cells was analysed by ELISPOT.

## RESULTS

Antibody synthesis and secretion in response to protein antigens are stimulated by CD40-mediated signals and B cell-activating cytokines expressed by T cells. Therefore, it was hypothesized that allergen-specific, IL-10-secreting Tr1 cells, which represent the dominant subset specific for common environmental allergens in healthy individuals, may also have an effect on Ig regulation. In the following experiments, we used IL-4 and sCD40L to induce IgE and IgG4, because IL-10-secreting Tr1 cells and IL-10 alone induced only very little IgG4 and no IgE. Der p 1-specific, IL-10-secreting, Tr1 cells are isolated from PBMC of healthy individuals and their role on IgE and IgG4 production is investigated. As shown in Fig. 1A, Tr1 cells suppressed germline  $\epsilon$  transcript expression and simultaneously, increased productive  $\gamma 4$  mRNA expression in IL-4- and sCD40L-stimulated PBMC. Consistently, quantification of the secreted immunoglobulins also verified our

findings. Tr1 cells decreased IgE- and enhanced IgG4-secretion (Fig. 1B). Similar results are found considering the frequency of IgE- and IgG4-secreting plasma cells. Tr1 cells decreased the number of IgE-secreting plasma cells, but enhanced the number of IgG4-secreting plasma cells (data not shown).

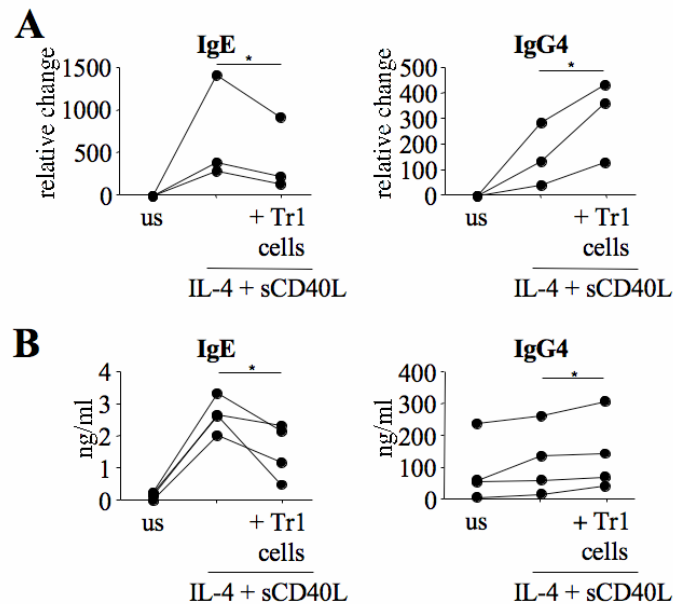


Fig. 1: Allergen-specific, IL-10-secreting Tr1 cells suppress IgE, but induce IgG4. Der p 1-specific, IL-10-secreting Tr1 cells are isolated from PBMC from healthy donors and incubated together with PBMC. (A) Quantitative RT-PCR determined at day 5 (n=3). (B) Supernatants analyzed for IgE and IgG4 at day 12 (n=4). Conditions in the same experiment are linked with a line. \*  $p < 0.05$ .

Similar to Tr1 cells, human peripheral blood  $CD4^+CD25^+$  Treg cells also secrete IL-10. Incubation of IL-4- and sCD40L-stimulated PBMC with  $CD4^+CD25^+$  Treg cells from healthy individuals inhibited germline  $\epsilon$  transcript expression, but they tend to induce productive  $\gamma 4$  transcript expression compared to  $CD4^+CD25^-$  T cells (Fig. 2A). The same effects of  $CD4^+CD25^+$  Treg cells were observed on the protein level. As shown in Fig. 2B,  $CD4^+CD25^+$  Treg cells suppressed IgE secretion, whereas IgG4 production was enhanced by IL-4- and sCD40L-stimulated PBMC. Not surprisingly, also the frequency of IgE- and IgG4-secreting plasma cells was decreased or enhanced, respectively, when  $CD4^+CD25^+$  Treg cells were added to the IL-4- and sCD40L-stimulated PBMC cultures (data not shown).  $CD4^+CD25^-$  T cells did not influence IL-4 and sCD40L-stimulated IgE or IgG4 production.

Because neither Tr1 cells nor  $CD4^+CD25^+$  Treg cells nor  $CD4^+CD25^-$  T cells nor IL-10 showed any influence on IgA production in PBMC as well as in pure B cell cultures (data not shown), we investigated an array of TLR agonists for their capacity to induce IgA. PBMC were cultured in the presence of different TLR agonists. While stimulation of TLR2, 3, 4, 5, or 8 was not able to influence the IgA production (data not shown), triggering of TLR7 or 9 highly enhanced both the mRNA level and the secretion of IgA compared to IgE and IgG4 (Fig. 3A and B). Interestingly, the addition of IL-4 and sCD40L to the cultures suppressed TLR7 or 9 induced IgA production, but upregulated IgE and IgG4 (data not shown). We conclude that the IgA production is not influenced by Tr1 and  $CD4^+CD25^+$  regulatory T cells, but rather induced by the innate immune system via activation of TLR7 or 9 stimulation.

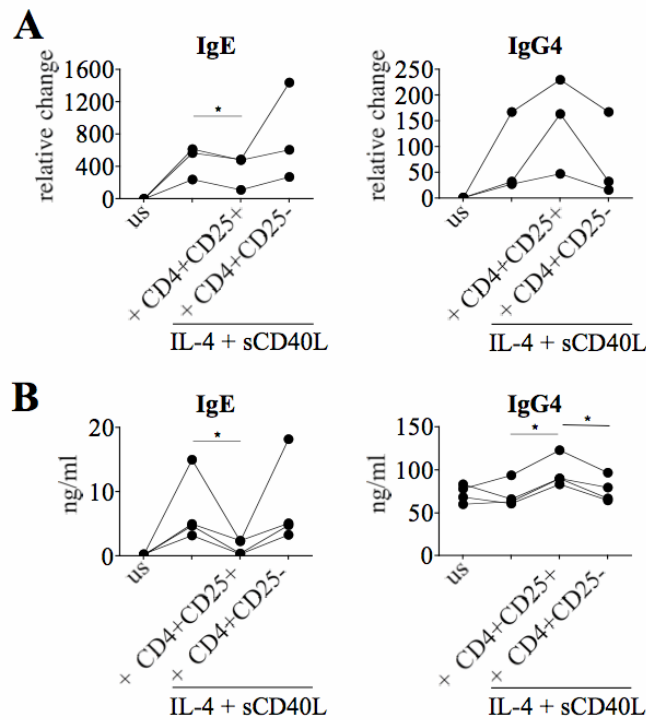


Fig. 2: Suppression of IgE and induction of IgG4 by CD4<sup>+</sup>CD25<sup>+</sup> Treg cells. CD4<sup>+</sup>CD25<sup>+</sup> and CD4<sup>+</sup>CD25<sup>-</sup> T cells are isolated from PBMC from healthy donors and cultured together with PBMC. (A) Quantitative mRNA for RT-PCR measured at day 5 (n=3). (B) IgE and IgG4 concentrations in the supernatants at day 12 (n=4). \* p < 0.05.

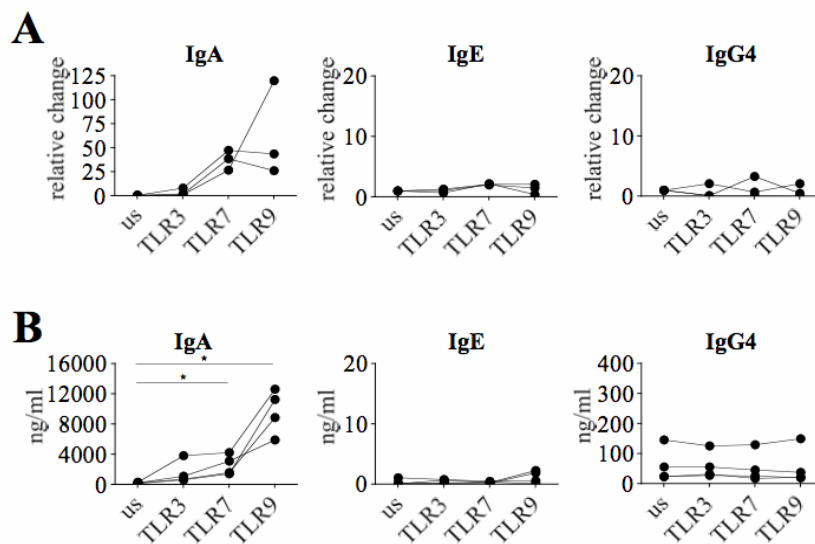


Fig. 3: Induction of IgA requires TLR7 and 9 triggering. PBMC from healthy individuals are stimulated with TLR3, 7 or 9 ligands. (A) Quantitative RT-PCR determined after 5 days of incubation (n=3). (B) IgA, IgE and IgG4 concentrations in the supernatants measured after 12 days (n=4).

## CONCLUSIONS & OUTLOOK

We conclude that peripheral tolerance utilizes multiple mechanisms to suppress allergic inflammation. Apparently, Treg cells contribute to the control of allergen-specific immune responses in several ways: Suppression of antigen-presenting cells that support the generation of effector Th2 and Th1 cells; suppression of Th2 and Th1 cells; suppression of mast cells, basophils and eosinophils; interaction with resident tissue cells and remodeling. In addition to the above mechanisms, the present study demonstrates suppression of IgE and induction of the non-inflammatory antibody isotype IgG4 by Tr1 and CD4<sup>+</sup>CD25<sup>+</sup> Treg cells.

The clinical relevance of Treg cells is of great interest. Generation of Treg cells or the increase of their suppressive capacity by drugs, cytokines or costimulatory molecules is an important target not only for the application in allergy and asthma, but also for transplantation and autoimmunity. However, it must be considered that Treg cells are not always responsible for a healthy immune response, since they can also induce chronicity of infections and tumor tolerance. Cellular therapy with antigen-specific Treg cells is another promising approach, since maintenance of long-term immune modulation and avoiding of general immunosuppression could be realized.

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