

Immunological Mechanisms of Specific Allergen Immunotherapy

Carsten B. Schmidt-Weber* and Kurt Blaser

Swiss Institute of Allergy and Asthma Research (SIAF), Obere Str. 22, CH-7270 Davos, Switzerland

Abstract: Allergy is an immunological disorder, which is driven by uncontrolled allergen-activated T cell subsets, leading to immediate type hypersensitivity against otherwise harmless environmental allergens. These allergens are tolerated by healthy individuals as well as by patients, who successfully underwent allergen-specific immunotherapy (SIT). The successful SIT is characterized by the induction of T cell unresponsiveness against the given allergen. Regulatory T cells (Tregs), which are installed or enhanced by SIT and govern the activity of potentially pro-allergic effector T cells, mediate this unresponsiveness. The current article reviews the mechanisms underlying the balance of these cell populations along with suppressive mechanisms of SIT, which may serve as future drug targets.

INTRODUCTION

Allergy is an immunological disorder, which manifests in upper airways, lung, skin and the gut. A key event in the pathogenesis of allergy is the activation of T cell subsets [1], which secrete inflammatory factors including IL-4 and IL-5 and/or IL-13 [2-4]. Both IL-4 and IL-13 are prerequisite for generation of allergen-specific IgE, mediating immediate hypersensitivity against allergens, that themselves are normally non-pathogenic and tolerated by healthy individuals. Allergen-specific T cells are found in both allergic and healthy individuals sensitized to the allergen [5]. In healthy individuals, however, their activity appears to be controlled by regulatory T cells (Treg) [6], which secrete suppressive cytokines such as IL-10 and/or TGF- β . Thus, Treg cells represent a dominant T cell population in healthy subjects, but not in the allergic counterparts [7]. The current understanding of allergy pathogenesis is that an imbalance between Th2 and Treg cells exists, which results in insufficient tolerance to the allergen [8-11].

In allergic disease, an individual's relevant allergen can usually be determined, which allows to restore specific tolerance by repetitive injections of high doses of allergen over a long period of time. This allergen-specific immunotherapy (SIT) specifically restores normal immunity against the allergen [8, 10, 12]. Successful SIT is characterized by the induction of a persistent T cell tolerance against the allergen [13-15], whereas other immunosuppressive treatments including glucocorticoids and other suppressive drugs only temporarily inhibit T cell activity and transiently improve allergy symptoms. Although SIT is most successfully applied in insect venom allergy, it is also effective in patients suffering from allergic rhinitis [16] and allergic asthma [12, 17-20]. Therefore, knowledge of cellular and molecular mechanisms underlying the SIT helps to identify molecular targets for treatment of allergic disease.

CELLULAR REGULATION OF ALLERGEN-SPECIFIC RESPONSES

The pathogenesis of allergy is characterized by excessive production of allergen-specific IgE antibodies, discriminating allergy from non-allergic inflammations. Clinical symptoms are mostly defined by immediate hypersensitivity reaction, which is initiated by crosslinking IgE bound to specific receptors on mast cells and basophils by the allergen. This leads to the release of various inflammatory mediators such as histamine and multiple proteases [21] and *de novo* production of prostaglandins, leukotrienes, platelet activating factor and proinflammatory cytokines [22, 23]. To generate allergen-specific IgE, naïve B cells have to recognize the allergen along with the coincidental help by IL-4 producing T cells [24]. The crucial role of T cells is reflected by the fact that the degree of T cell activation correlates with the number of low-affinity IgE receptor (CD23) bearing B cells [3], eosinophil cationic protein (ECP) and other serum factors [4], demonstrating that allergy is a T cell dependent disease [4]. Only Th2 cells produce IL-4 necessary to switch the B cells towards IgE production, suggesting that Th2 cells display a proallergic phenotype. In contrast, Th1 cells produce IFN- γ and induce IgE antibodies. Nevertheless, allergen-specific T cells of healthy donors also express IL-4 and other Th2 cytokines. It is assumed that the function and pathogenicity of allergen-specific Th2 cells is controlled by allergen-specific Treg cells, which suppress their Th2 effector-counterparts by secretion of suppressive cytokines and contact dependent mechanism [25]. The term effector T cells (Teff) is used to oppose Th1 and Th2 cells mediating inflammatory effects by regulating other immune cells to Treg cells that are defined by their regulatory function. It appears that Teffs and Tregs exist in a regulatory balance, which prevents excessive action of Teff and in turn generates a loss of peripheral tolerance against environmental antigens. The balance between Teffs and Tregs is influenced by antigen-presenting cells and environmental conditions [26]. The origin of the peripheral Tregs is still unclear, but it is assumed that a process similar to the differentiation of Th1 and Th2 cells may also generate a regulatory T cell population [27]. The process of Th1/Th2 differentiation depends on the uptake of

*Address correspondence to this author at the Swiss Institute of Allergy and Asthma Research (SIAF), Obere Str. 22, CH-7270 Davos, Switzerland; Tel: ++41814100853; Fax: ++41814100840, E-mail: csweber@siaf.unizh.ch

antigen by antigen-presenting cells (APC), which provide them to the T cells in an MHC-dependent fashion. The antigen-presentation requires the migration of the antigen-loaded APC to the lymph node, since naïve T cells are located in the draining LN and are normally not present in peripheral tissues. In addition to the antigen, T cell differentiation requires “decisive” signals such as IL-12 for Th1 or IL-4 for Th2 commitments, which are either provided by APC or by other lymphoid cells [28-31]. The intracellular key factor for Th1 differentiation is T-bet [32, 33], whereas GATA3, induced by IL-4 is indispensable for Th2 differentiation [34]. IL-10 may fulfill a similar role as it is observed for IL-4 and IL-12 for Th1 or Th2 differentiation [35], and may promote the expression of the transcription factor FOXP3, which has been shown to promote Treg phenotypes [36, 37]. The differentiation of naïve T cells using immature (CD83⁺) DCs generates a T cells population, which has a Treg phenotype characterized by IL-10 production [38, 39]. Since immature DC are characterized by reduced expression of costimulatory receptors [40], induction of Treg and anergy may be identical and characterized by the absence of “decisive” signals. Thus, it represents a pathway that is neither dominated by Th1 nor by Th2 driving conditions (Fig. 1). CD25⁺ Tregs are also released from the thymus [41], but can also develop from peripheral T cells in a thymus independent fashion [42].

CELLULAR MECHANISMS UNDERLYING ALLERGEN-SPECIFIC IMMUNOTHERAPY

SIT against insect venom allergy induces T cell unresponsiveness, which is detected as anergy (viable T cell unresponsiveness) *in vitro* and can be reversed by the addition of IL-2 or IL-15 [13, 15]. The T cell anergy could be prevented by the addition of IL-10 neutralizing antibodies to the cell culture. An early and transient increase in allergen-specific IL-10 mRNA expression in PBMC could be shown to correlate with a successful therapeutic outcome after 1 year of SIT [43]. IL-10 production of T cells increased during the course of SIT, and could be observed later also on monocytes and B cells [14]. Interestingly, reduced IL-10 expression was found in children suffering from asthma or atopic dermatitis, compared to their healthy counterparts [44], suggesting that SIT restores IL-10 to normal levels. Also TGF- β plays an important role in suppressing mucosal T cell responses and maintaining peripheral tolerance. This was demonstrated in animals, which developed lethal autoimmune diseases, when the TGF- β RII was genetically eliminated [45]. In experimental asthma models it was shown that T cells engineered to produce TGF- β efficiently control allergy-inducing T cells in an antigen-specific manner [46]. Intranasal application of allergen induces tolerance by a TGF- β dependent mechanism [47]. In the absence of TGF- β 1, animals die of cardiac, pulmonary and gastric inflammation [48]. These TGF- β deficient mice illustrate that TGF- β is of particular importance in mucosal responses. This holds also true for SIT, where mucosal allergens, but not insect venoms trigger TGF- β following therapy. For example, in SIT against house dust mite allergy, IL-10 and TGF- β are induced, which results in decreased allergen-specific proliferation and cytokine secretion [49-51]. Important in mucosal immunity

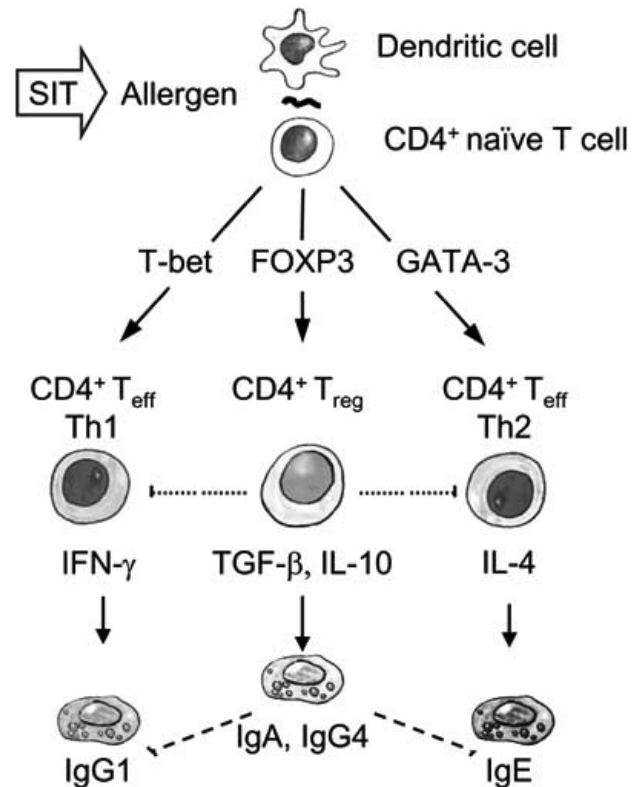


Fig. (1). Upon Allergen uptake dendritic cells can stimulate allergen-specific T cells to differentiate into effector T cells (Th1, Th2) or regulatory T cells. This process is dependent on T-bet for the Th1, GATA-3 for the Th2 and most probably FOXP3 for the Treg pathway. These T cell populations are balanced against each other and represent a key mechanism of peripheral tolerance. Characteristic cytokines represent IFN- γ for Th1 cells, TGF- β , IL-10 for Tregs and IL-4 for Th2 cells. The phenotype of dominating T cells is reflected by the B cell response (IgG1 for Th1, IgA and IgG4 for Tregs and IgE for Th2 cells).

is the generation of IgA, which is induced by TGF- β [52]. IL-10 promotes IgG4 expression [53]. Both cytokines directly suppress IgE production (Fig. 1). Accordingly, the allergen-specific isotype profile shifts during SIT towards normal IgG4/IgA levels and the ratio of specific IgE to IgG4 or IgA changes about 100 – 1000 fold. The IgG4/IgA response is a consequence of Treg-function and affects the immune response by blocking potential IgE epitopes and/or alliterating Ig-facilitated antigen uptake by antigen presenting cells. Taken together, current literature shows that SIT increases the suppression of the allergen-specific Th2 response, while a regulatory and protective response is installed or enhanced.

It is still unclear how repetitive allergen injections promote Treg cells. However, the persistent presence of antigens in the absence of inflammation was also observed to be immunologically tolerated or ignored in other models of peripheral tolerance, such as tolerance to transplants, where the absence of inflammation determines the transplant longevity [54-56]. The proposed “danger” model beyond these observations suggests that the immune system does not

only distinguishes self from non-self, but it also recognizes the context in which antigens/allergens are presented [57,58]. In the setting of inflammation or tissue injury, "danger" signals induce the expression of critical costimulatory molecules on APC, thus permitting T cells to fully respond to antigens presented in this context. Conversely, it is proposed that under homeostatic conditions, in the absence of inflammation, antigen presentation occurs without costimulation, leading to specific T cell anergy and ultimately to tolerance. Potential danger for the organism has evolved pathogen-associated molecular patterns (PAMPs) that are recognized by various receptors on APCs and is assumed to be mediated by pattern recognition receptors [59]. Accordingly, SIT lacks signals, which may promote the activation of these pathways and allow the generation of regulatory responses. Interestingly, exposure of CD4⁺ T cells with high doses of specific peptides in the absence of APC also induces T cell anergy and CD25 expression [60]. In this experimental system, T cells themselves may act as APC, lacking costimulatory signals, allowing sufficient stimulation of effector responses, as suggested by the two-signal paradigm of Jenkins and Schwartz [61]. Further research is necessary to understand the role played by APC in SIT. However, the level of costimulation provided by APC, namely DC, differs in different tissues. Interestingly, sublingual application of allergens in SIT was successfully applied and it underlines that oral mucosa DCs facilitate the induction of Treg cells [62]. This capability was attributed to DCs expressing FcεRI, whose oral environment favors the expression of IL-10. This capability was not observed for DCs of the skin (Langerhans cells), which in established disease promote inflammation by driving T cells into a Th1 phenotype [63]. Thus, besides the PAMPs, tissue privileges play an important role in the regulation of DCs. A key question remains, how these tissue-specific signals are mediated to regulate the specific immune system. In this context, an interesting concept is the regulation of DC by CD8⁺ T cells. In contrast to the CD4⁺ T cells, which require the MHC-II antigen-presentation by professional APC, CD8⁺ T cells recognize antigens in context of the MHC-I, which is present on any cell in the tissue. Among the CD8⁺ T cells exists a regulatory population similar to CD25⁺CD4⁺ cells, which is characterized by the absence of CD28 [64]. Recently, it could be shown that these CD8⁺CD28⁻ Tregs cells upregulate immunoglobulin-like transcript 3 (ILT-3) and ILT-4 on DC. These receptors belong to a family of inhibitory receptors expressed by human monocytes and DCs. Induction of ILT-3 and ILT-4 on monocytes and DCs, renders the APCs into an tolerogenic phenotype, which is characterized by reduced expression of costimulatory molecules, and in turn induces antigen-specific unresponsiveness in CD4⁺ T helper cells [65]. This suggests that CD8⁺ T cells provide a link to antigens being present in tissues and presented by these under "danger" or homeostatic conditions. This model requires that tissue cells are able to modulate MHC-I mediated antigen presentation in response to "danger" signals. Although this concept is not yet fully confirmed, it provides a comprehensive model of how privileges could modulate the specific immune system in an antigen-specific manner. Of note, allergen-specific CD8⁺ T cells can regularly be detected in the periphery of healthy donors (C.B. Schmidt-Weber, unpublished) and the number

of CD8⁺ T cell infiltrates correlates with the number of mast cells and are increased in superficial submucosa of rhinitis patients [66]. In this model, a crosstalk of DC, CD8⁺ and CD4⁺ cells, altogether provides a comprehensive model of peripheral tolerance against antigens.

MOLECULAR MECHANISMS OF ALLERGEN-SPECIFIC IMMUNOTHERAPY

A key event in allergen-specific peripheral tolerance represents the induction of T cell unresponsiveness (anergy) mediated by Treg. However, the mechanism of suppression is still unclear. However, successful SIT goes along with the generation of IL-10/TGF-β producing Treg population, which is contained in the CD25⁺ T cell subset [51]. Other studies describe the CD25⁺ subset of T cells and show suppressive capacity *in vitro* and *in vivo* [67-71]. However, it is currently not fully established, whether CD25⁺ T cells are identical to IL-10 and/or TGF-β producing Tregs [70], since the suppression appears to be contact dependent [69]. However, suppressive cytokines may be secreted into immunological synapse [72] or can act in a surface bound form on the target cell [73]. Both IL-10 and TGF-β are currently the best described effector molecules for the induction of T cell unresponsiveness.

INTERLEUKIN-10

IL-10 suppresses the association of phosphatidylinositol-3-phosphatase (PI3K) with CD28 [74, 75] as well as other costimulatory molecules, which possess a -FYVE-phosphorylation site for K13K, by directly preventing costimulatory signals in T cells. The PI3K phosphorylates phosphatidyl inositols 2-phosphates (PIP2) into phosphatidyl 3-phosphates (PIP3). Interestingly, the immune inhibitory receptor of B cells or NK cells as also address PIP3-lipids by activating PIP-phosphatases (e.g. SHIP; [76]), suggesting that prevention of PIP3-Ω signaling-1 (SOCS) and SOCS-3 [79], which are known to inhibit the activity of the janus-kinase-1 (JAK1). JAK1 is necessary for cytokine mediated signaling and also inhibits IL-10 signal transduction, thus representing a negative feed-back loop. However, SOCS-3 maybe in balance with other transcription factors [80] and may even promote pro-allergic responses when artificially overexpressed [81].

Furthermore, IL-10 is known to reduce the expression of costimulatory molecules of antigen-presenting cells [82, 83], which, like the induction of SOCS-genes, operate via transcriptional regulation. In contrast to the interference with the PI3K pathway, the effect of this action is delayed.

TRANSFORMING GROWTH FACTOR-β

TGF-β mobilizes the SMAD2/3-4 complex which interacts with other transcription factors to bind DNA elements that will down regulate expression of the particular gene [84, 85]. Alternative ways of TGF-β suppression include the Transforming growth factor activated kinase-1 (TAK1) or the activation of phosphatases [86], which could immediately block or reduce signal transduction of TCR mediated signals [87]. TGF-β is a potent inhibitor of T cell

proliferation and cytokine production. Interestingly, the inhibition of proliferation versus IL-2 production is mediated by distinct, TGF- β induced signals. Disruption of Smad3 does not affect TGF- β -mediated inhibition of IL-2-induced proliferation, demonstrating that TGF- β signals through both Smad3-dependent and -independent pathways [88]. The inability of TGF- β to inhibit TCR-induced proliferation of Smad3(-/-) T cells suggests that SMAD independent mechanisms do also play an important role for TGF- β mediated suppression. Like IL-10, TGF- β has the ability to modulate the humoral immune response. Whereas IL-10 supports IgG4 production [53], TGF- β plays a key role in generation of IgA [52]. It represents both a switch factor for IgA and promotes IgA antibody production. On the other hand, like IL-10, TGF- β suppresses Ig production [89]. A major difference between TGF- β and IL-10 is the source of expression. Whereas IL-10 expression is restricted to cells of the immune system, TGF- β is expressed more diversely and can be detected in most tissues. Furthermore, TGF- β plays an important role in cellular repair functions by inducing extra cellular matrix production. In fact, platelets contain TGF- β , which is released upon activation [90] and supports tissue recovery after injury. However, if the expression pattern of TGF- β is compared with the pattern of the activated form of the downstream signaling target (phosphorylated SMAD2/3), it becomes obvious, that many cells have lost their TGF- β -responsiveness, that is increased in inflammation [91]. Differential TGF- β responsiveness was also observed in T cells and may be as important for successful suppression as the TGF- β production itself. Recent studies show that IFN- γ [92], TNF- α and IL-1 [93] can reduce the TGF- β sensitivity by upregulation of SMAD7 expression. SMAD7 is an inhibitory SMAD and blocks phosphorylation of SMAD2/3 [94]. It may allow binding of SMURF-1 and to subject the complex to ubiquitin mediated degradation [95]. The Smad anchor for receptor activation (SARA) and hepatic growth factor-regulated tyrosine kinase substrate (Hgs) are involved in TGF- β 1 signaling and inhibit TGF- β mediated signal transduction by attenuating SMAD2/3 [96]. However, in contrast to SMAD7, SARA and Hgs are induced as late as 12h by TGF- β (SARA) or T cell activation (Hgs), whereas SMAD7 is induced within a few hours [96]. Thus, T cells, which are involved in inflammatory reactions or have already been exposed to TGF- β , would not be susceptible to TGF- β mediated suppression. Studies revealed that T cells also express the TGF- β RIII, endoglin (CD105; [97]). Endoglin was found on activated cells, or cells which were previously stimulated. In fact, endoglin can be specifically upregulated by CD3 engagement, demonstrating that TGF- β responsiveness is regulated in an activation dependant manner. Interference with endoglin resolves TGF- β mediated suppression of T cells (C.B. Schmidt-Weber, unpublished).

POTENTIAL DRUG TARGETS SUPPORTING ALLERGEN-SPECIFIC IMMUNOTHERAPY AND TOLERANCE

There is no doubt that induction of lymphocyte tolerance against allergen will be a hallmark of allergy cure, as it is currently realized by the application of SIT. It is questionable though, whether it is possible to design drugs

which promote tolerance independent of SIT and without administration of allergen. This would be necessary for patients who suffer from allergy against multiple allergens. It appears to be difficult to induce a specific unresponsiveness to an allergen, while potential dangerous pathogens remain to be recognized and are eliminated. However, the history of tolerance research shows that such treatments are successful and are surprisingly well tolerated, as demonstrated by anti-CD4 or anti-CD3 treatments in transplantation [98-100]. Interestingly, superagonistic anti-CD28 mab were shown to induce tolerance *in vivo*, promote Tregs selectively and were well tolerated in several experimental disease models [101-104]. It may be possible to facilitate tolerance by adjuvant treatment, so that thresholds necessary for tolerance induction can be reached, particularly when the allergen appears in a seasonally restricted time frame. Several examples like usage of Vitamin D3, Tacrolimus, Rapamycin or glucocorticoids illustrate reduction of costimulatory molecules or cytokine expression by DC, rendering them pro-tolerogenic (reviewed in [26]). Glucocorticoids enhance FOXP3 expression [105] and promote the differentiation of IL-10 expressing cells [106]. Histamine enhances TGF- β mediated signal transduction and suppression of T cell proliferation and IL-4 expression by a histamine receptor-II dependent mechanism [107]. These examples show that such drugs are already available and clinically used. A combination of treatments may allow more efficient induction of allergen-specific tolerance, such as an anti-histamine receptor-I treatment prior to immunotherapy [108], which appears to improve tolerance induction.

For the design of new drugs, it is important to select molecules that are specific for lymphocytes, and which reduce the risk of unwanted side effects. A promising candidate is the transcription factor FOXP3, which is highly expressed in Treg cells and whose ectotropic expression is sufficient to induce T cells with a regulatory phenotype [36, 109]. However, the regulation and function of FOXP3 is currently not fully known and requires further investigations. The IL-10 and TGF- β pathway may provide further interesting targets, however, for both genes exist family members, which partially use the same receptors or have overlapping functions. These are for IL-10: IL-19, IL-20, IL-22, IL-24 and IL-26 and for TGF- β : activins, inhibins and growth and differentiation factors (GDFs). The TGF- β -family members are particularly interesting for the design of anti-allergy drugs, since they also play a role in tissue remodeling and cannot be suppressed by glucocorticoids [110]. The direct neutralization of TGF- β has been shown to prevent disease in experimental animal models of glomerulo nephritis [111-113] and attenuate diabetes [114]. Beside the use of anti-TGF- β antibodies or TGF- β R- Fc chimeras, also matrix components (decorin) binding TGF- β have been successfully used for *in vivo* neutralization of TGF- β [115]. Recently ursolic acid, which is a naturally occurring triterpenoid isolated as active compound from plants used in Chinese folk medicine against nephritis, acts as an antagonist for TGF- β [116]. Consistently, ursolic acid was reported to enhance the immune system [117], which however remains to be attributed to TGF- β . Moreover, synthetic derivatives were generated and proved as SMAD-targeting molecules, which enhance Smad signaling [118, 119]. These anti-TGF- β strategies are focused on TGF- β neutralization to overcome

disease induced fibrosis, however, their effect on the immune system remains to be clarified. An alternative to TGF- β neutralization is the strategy to target the TGF- β receptors as it has already been shown for ALK-4, -5 and -7 [120]. Small molecules targeting TGF- β RI were recently described [121-123]. Particularly interesting would be receptors, which are expressed on a restricted number of cells, specifically endoglin, which is restricted to immune cells and endothelial cells. The downstream signaling targets of TGF- β are also promising drug targets. In this context, it is important to consider that the SMAD-factors usually act in concert with other factors [124-127], and may represent promising targets to modulate the TGF- β response. Furthermore, distinct phosphate residues within the SMAD2 and SMAD3 molecules appear to have different functions [128], which may allow selective targeting of pro-immunosuppressive or anti-fibrotic responses, if the relevant kinase source is identified [128]. Taken together, it appears that existing drugs need to be re-evaluated in tolerizing treatment strategies and new drugs need to be characterized for their effect on the immune system as well as for anti-fibrosis effect.

CONCLUSION

Specific immunotherapy is currently the only curative treatment of allergy and therefore of major interest for the development of new anti-allergy and -asthma drugs. The key mechanism in SIT is the induction of T cell unresponsiveness against allergens. The specific unresponsiveness is mediated by complex cellular and molecular mechanisms involving the effector T cells, Tregs, DCs and possibly CD8⁺ T cells. The differentiation of T cells into Tregs as well as the effectiveness of Treg mediated suppression by TGF- β and IL-10 is a prime target for facilitated induction of T cell unresponsiveness. The development of new drugs as well as the use of existing protolerogenic and anti-fibrosis drugs may improve treatment strategies of allergy and asthma.

ACKNOWLEDGEMENTS

This work was supported by the Swiss National Foundation Grants Nr: 31-65436.01 and 32-100266 and 3100A0-100164, the Ehmann Foundation, the Saurer Foundation, the Ernst Göhner Stiftung, the OPO-Foundation, Zürich, the Rentenanstalt/Swiss Life Jubiläumsstiftung and Bonizzi-Theler Foundation.

REFERENCES

- Carballido, J.M.; Carballido-Perrig, N.; Terres, G.; Heusser, C.H.; Blaser, K. *Eur. J. Immunol.*, **1992**, 22, 1357.
- Walker, C.; Virchow, J.C. Jr.; Bruijnzeel, P.L.; Blaser, K. *J. Immunol.*, **1991**, 146, 1829.
- Walker, C.; Bode, E.; Boer, L.; Hansel, T.T.; Blaser, K.; Virchow, J.C., Jr. *Am. Rev. Respir. Dis.*, **1992**, 146, 109.
- Walker, C.; Kagi, M.K.; Ingold, P.; Braun, P.; Blaser, K.; Bruijnzeel-Koomen, C.A.; Wuthrich, B. *Clin. Exp. Allergy*, **1993**, 23, 145.
- Halvorsen, R.; Bosnes, V.; Thorsby, E. *Int. Arch. Allergy Appl. Immunol.*, **1986**, 80, 62.
- Kay, A.B. *Allergy*, **1999**, 54, 29.
- Akdis, M.; Verhagen, J.; Taylor, A.; Karamloo, F.; Karagiannidis, C.; Cramer, R.; Thunberg, S.; Günnur, D.; Valenta, R.; Fiebig, H.; Kegel, C.; Disch, R.; Schmidt-Weber, C.B.; Akdis, C.A. *J. Exp. Med.*, **in press**.
- Ewan, P.W. *Curr. Opin. Allergy Clin. Immunol.*, **2001**, 1, 367.
- Sheffer, A.L. *Ann. Allergy*, **1993**, 71, 327.
- Schmidt-Weber, C.B.; Blaser, K. *Springer Semin. Immunopathol.*, **2004**, 25, 377.
- Curotto de Lafaille, M.A.; Lafaille, J.J. *Springer Semin. Immunopathol.*, **2004**, 25, 295.
- Bousquet, J.; Demoly, P.; Michel, F.B. *Ann. Allergy. Asthma Immunol.*, **2001**, 87, 38.
- Bellinghausen, I.; Metz, G.; Enk, A.H.; Christmann, S.; Knop, J.; Saloga, J. *Eur. J. Immunol.*, **1997**, 27, 1131.
- Akdis, C.A.; Blaser, K. *FASEB J.*, **1999**, 13, 603.
- Akdis, C.A.; Blesken, T.; Akdis, M.; Wuthrich, B.; Blaser, K. *J. Clin. Invest.*, **1998**, 102, 98.
- Durham, S.R.; Walker, S.M.; Varga, E.M.; Jacobson, M.R.; O'Brien, F.; Noble, W.; Till, S.J.; Hamid, Q.A.; Nouri-Aria, K.T. *N. Engl. J. Med.*, **1999**, 341, 468.
- Rak, S.; Heinrich, C.; Jacobsen, L.; Scheynius, A.; Venge, P. *J. Allergy Clin. Immunol.*, **2001**, 108, 921.
- Gruber, W.; Eber, E.; Mileder, P.; Modl, M.; Weinhandl, E.; Zach, M.S. *Clin. Exp. Allergy*, **1999**, 29, 176.
- Cantani, A.; Arcese, G.; Lucenti, P.; Gagliesi, D.; Bartolucci, M. *J. Investig. Allergol. Clin. Immunol.*, **1997**, 7, 90.
- Arvidsson, M.B.; Lowhagen, O.; Rak, S. *Allergy*, **2004**, 59, 74.
- Lutzel Schwab, C.; Pejler, G.; Aveskogh, M.; Hellman, L. *J. Exp. Med.*, **1997**, 185, 13.
- Wang, J.; Qi, J.C.; Konecny, P.; Krilis, S.A. *Curr. Drug Targets Inflamm. Allergy*, **2003**, 2, 293.
- Hart, P.H. *Immunol. Cell Biol.*, **2001**, 79, 149.
- Shimoda, K.; van Deursen, J.; Sangster, M.Y.; Sarawar, S.R.; Carson, R.T.; Tripp, R.A.; Chu, C.; Quelle, F.W.; Nosaka, T.; Vignali, D.A.; Doherty, P.C.; Grosveld, G.; Paul, W.E.; Ihle, J.N. *Nature*, **1996**, 380, 630.
- Schmidt-Weber, C.B.; Blaser, K. *Allergy*, **2002**, 57, 762.
- Lagaraine, C.; Lebranchu, Y. *Transplantation*, **2003**, 75, 375.
- Shevach, E.M. *Nat. Rev. Immunol.*, **2002**, 2, 389.
- KleinJan, A.; Dijkstra, M.D.; Boks, S.S.; Severijnen, L.A.; Mulder, P.G.; Fokkens, W.J. *J. Allergy Clin. Immunol.*, **1999**, 103, 441.
- Till, S.; Durham, S.; Dickason, R.; Huston, D.; Bungre, J.; Walker, S.; Robinson, D.; Kay, A.B.; Corrigan, C. *Immunology*, **1997**, 91, 53.
- Huang, S.K.; Xiao, H.Q.; Kleine-Tebbe, J.; Paciotti, G.; Marsh, D.G.; Lichtenstein, L.M.; Liu, M.C. *J. Immunol.*, **1995**, 155, 2688.
- Bodey, K.J.; Semper, A.E.; Redington, A.E.; Madden, J.; Teran, L.M.; Holgate, S.T.; Frew, A.J. *Allergy*, **1999**, 54, 1083.
- Szabo, S.J.; Kim, S.T.; Costa, G.L.; Zhang, X.; Fathman, C.G.; Glimcher, L.H. *Cell*, **2000**, 100, 655.
- Smits, H.H.; van Rietschoten, J.G.; Hilken, C.M.; Sayilir, R.; Stiekema, F.; Kapsenberg, M.L.; Wierenga, E.A. *Eur. J. Immunol.*, **2001**, 31, 1055.
- Zheng, W.; Flavell, R.A. *Cell*, **1997**, 89, 587.
- Groux, H.; O'Garra, A.; Bigler, M.; Rouleau, M.; Antonenko, S.; Devries, J.E.; Roncarolo, M.G. *Nature*, **1997**, 389, 737.
- Hori, S.; Nomura, T.; Sakaguchi, S. *Science*, **2003**, 299, 1057.
- Fantini, M.C.; Becker, C.; Monteleone, G.; Pallone, F.; Galle, P.R.; Neurath, M.F. *J. Immunol.*, **2004**, 172, 5149.
- Jonuleit, H.; Schmitt, E.; Schuler, G.; Knop, J.; Enk, A.H. *J. Exp. Med.*, **2000**, 192, 1213.
- Dhodapkar, M.V.; Steinman, R.M.; Krasovsky, J.; Munz, C.; Bhardwaj, N. *J. Exp. Med.*, **2001**, 193, 233.
- Jin, Y.; Fuller, L.; Ciancio, G.; Burke, G.W., 3rd; Tzakis, A.G.; Ricordi, C.; Miller, J.; Esquenazi, V. *Hum. Immunol.*, **2004**, 65, 93.
- Ng, W.F.; Duggan, P.J.; Ponchel, F.; Matarese, G.; Lombardi, G.; Edwards, A.D.; Isaacs, J.D.; Lechler, R.I. *Blood*, **2001**, 98, 2736.
- Karim, M.; Kingsley, C.I.; Bushell, A.R.; Sawitzki, B.S.; Wood, K.J. *J. Immunol.*, **2004**, 172, 923.
- Savolainen, J.; Laaksonen, K.; Rantio-Lehtimäki, A.; Terho, E.O. *Clin. Exp. Allergy*, **2004**, 34, 413.
- Koning, H.; Neijens, H.J.; Baert, M.R.; Oranje, A.P.; Savelkoul, H.F. *Cytokine*, **1997**, 9, 427.
- Gorelik, L.; Flavell, R.A. *Immunity*, **2000**, 12, 171.

- [46] Thorbecke, G.J.; Umetsu, D.T.; deKruyff, R.H.; Hansen, G.; Chen, L.Z.; Hochwald, G.M. *Cytokine Growth Factor Rev.*, **2000**, *11*, 89.
- [47] Wiedermann, U.; Jahn-Schmid, B.; Bohle, B.; Repa, A.; Renz, H.; Kraft, D.; Ebner, C. *J. Allergy Clin. Immunol.*, **1999**, *103*, 1202.
- [48] Shull, M.M.; Ormsby, I.; Kier, A.B.; Pawlowski, S.; Diebold, R.J.; Yin, M.; Allen, R.; Sidman, C.; Proetzel, G.; Calvin, D. *et al. Nature*, **1992**, *359*, 693.
- [49] Jutel, M.; Akdis, M.; Budak, F.; Malolepsky, J.; Casaulta, C.; Wrzyszc, M.; Blaser, K.; Akdis, C.A. *J. Allergy Clin. Immunol.*, **2001**, *107*, S90.
- [50] Secrist, H.; Chelen, C.J.; Wen, Y.; Marshall, J.D.; Umetsu, D.T. *J. Exp. Med.*, **1993**, *178*, 2123.
- [51] Jutel, M.; Akdis, M.; Budak, F.; Aebischer-Casaulta, C.; Wrzyszc, M.; Blaser, K.; Akdis, C.A. *Eur. J. Immunol.*, **2003**, *33*, 1205.
- [52] van Vlasselaer, P.; Punnonen, J.; de Vries, J.E. *J. Immunol.*, **1992**, *148*, 2062.
- [53] Jeannin, P.; Lecoanet, S.; Delneste, Y.; Gauchat, J.F.; Bonnefoy, J.Y. *J. Immunol.*, **1998**, *160*, 3555.
- [54] Contreras, J.L.; Wang, P.X.; Eckhoff, D.E.; Lobashevsky, A.L.; Asiedu, C.; Frenette, L.; Robbin, M.L.; Hubbard, W.J.; Cartner, S.; Nadler, S.; Cook, W.J.; Sharff, J.; Shiloach, J.; Thomas, F.T.; Neville, D.M., Jr.; Thomas, J.M. *Transplantation*, **1998**, *65*, 1159.
- [55] Anderson, C.C.; Carroll, J.M.; Gallucci, S.; Ridge, J.P.; Cheever, A.W.; Matzinger, P. *J. Immunol.*, **2001**, *166*, 3663.
- [56] Waldmann, H.; Cobbold, S. *Annu. Rev. Immunol.*, **1998**, *16*, 619.
- [57] Matzinger, P. *Annu. Rev. Immunol.*, **1994**, *12*, 991.
- [58] Matzinger, P. *Science*, **2002**, *296*, 301.
- [59] Gordon, S. *Cell*, **2002**, *111*, 927.
- [60] Faith, A.; Akdis, C.A.; Akdis, M.; Simon, H.U.; Blaser, K. *J. Immunol.*, **1997**, *159*, 53.
- [61] Jenkins, M.K.; Chen, C.A.; Jung, G.; Mueller, D.L.; Schwartz, R.H. *J. Immunol.*, **1990**, *144*, 16.
- [62] Van Wilsem, E.J.; Van Hoogstraten, I.M.; Breve, J.; Scheper, R.J.; Kraal, G. *Immunology*, **1994**, *83*, 128.
- [63] Novak, N.; Allam, J.P.; Betten, H.; Haberstok, J.; Bieber, T. *Allergy*, **2004**, *59*, 5.
- [64] Cortesini, R.; LeMaout, J.; Ciubotariu, R.; Cortesini, N.S. *Immunol. Rev.*, **2001**, *182*, 201.
- [65] Suciu-Foca, N.; Manavalan, J.S.; Cortesini, R. *Transpl. Immunol.*, **2003**, *11*, 235.
- [66] Powe, D.G.; Huskisson, R.S.; Carney, A.S.; Jenkins, D.; McEuen, A.R.; Walls, A.F.; Jones, N.S. *Allergy*, **2004**, *59*, 204.
- [67] Suri-Payer, E.; Amar, A.Z.; Thornton, A.M.; Shevach, E.M. *J. Immunol.*, **1998**, *160*, 1212.
- [68] Cederbom, L.; Hall, H.; Ivars, F. *Eur. J. Immunol.*, **2000**, *30*, 1538.
- [69] Thornton, A.M.; Shevach, E.M. *J. Exp. Med.*, **1998**, *188*, 287.
- [70] Shevach, E.M.; McHugh, R.S.; Piccirillo, C.A.; Thornton, A.M. *Immunol. Rev.*, **2001**, *182*, 58.
- [71] Jonuleit, H.; Schmitt, E.; Kakirman, H.; Stassen, M.; Knop, J.; Enk, A.H. *J. Exp. Med.*, **2002**, *196*, 255.
- [72] Stinchcombe, J.C.; Griffiths, G.M. *Immunology*, **2001**, *103*, 10.
- [73] Nakamura, K.; Kitani, A.; Strober, W. *J. Exp. Med.*, **2001**, *194*, 629.
- [74] Joss, A.; Akdis, M.; Faith, A.; Blaser, K.; Akdis, C.A. *Eur. J. Immunol.*, **2000**, *30*, 1683.
- [75] Bhattacharyya, S.; Sen, P.; Wallet, M.; Long, B.; Baldwin, A.S., Jr.; Tisch, R. *Blood*, **2004**, *104*, 1100.
- [76] Ravetch, J.V.; Lanier, L.L. *Science*, **2000**, *290*, 84.
- [77] Marte, B.M.; Downward, J. *Trends Biochem. Sci.*, **1997**, *22*, 355.
- [78] Schmidt-Weber, C.B.; Wohlfahrt, J.G.; Akdis, C.; Blaser, K. *Eur. J. Immunol.*, **2002**, *32*, 1196.
- [79] Ding, Y.; Chen, D.; Tarcsfalvi, A.; Su, R.; Qin, L.; Bromberg, J.S. *J. Immunol.*, **2003**, *170*, 1383.
- [80] Arakawa, S.; Hatano, Y.; Katagiri, K. *Clin. Exp. Immunol.*, **2004**, *135*, 505.
- [81] Seki, Y.; Inoue, H.; Nagata, N.; Hayashi, K.; Fukuyama, S.; Matsumoto, K.; Komine, O.; Hamano, S.; Himeno, K.; Inagaki-Ohara, K.; Cacalano, N.; O'Garra, A.; Oshida, T.; Saito, H.; Johnston, J.A.; Yoshimura, A.; Kubo, M. *Nat. Med.*, **2003**, *9*, 1047.
- [82] Ding, L.; Shevach, E.M. *J. Immunol.*, **1992**, *148*, 3133.
- [83] Ding, L.; Linsley, P.S.; Huang, L.Y.; Germain, R.N.; Shevach, E.M. *J. Immunol.*, **1993**, *151*, 1224.
- [84] Dong, Y.; Tang, L.; Letterio, J.J.; Benveniste, E.N. *J. Immunol.*, **2001**, *167*, 311.
- [85] Schmidt-Weber, C.B.; Kunzmann, S.; Blaser, K. *Curr. Allergy Asthma Rep.*, **2002**, *2*, 259.
- [86] Petritsch, C.; Beug, H.; Balmain, A.; Oft, M. *Genes Dev.*, **2000**, *14*, 3093.
- [87] Choudhry, M.A.; Sir, O.; Sayeed, M.M. *Shock*, **2001**, *15*, 193.
- [88] McKarns, S.C.; Schwartz, R.H.; Kaminski, N.E. *J. Immunol.*, **2004**, *172*, 4275.
- [89] Meade, R.; Askenase, P.W.; Geba, G.P.; Neddermann, K.; Jacoby, R.O.; Pasternak, R.D. *J. Immunol.*, **1992**, *149*, 521.
- [90] Hosgood, G. *Vet. Surg.*, **1993**, *22*, 490.
- [91] Rosendahl, A.; Checchin, D.; Fehniger, T.E.; ten Dijke, P.; Heldin, C.H.; Sideras, P. *Am. J. Respir. Cell Mol. Biol.*, **2001**, *25*, 60.
- [92] Ulloa, L.; Doody, J.; Massague, J. *Nature*, **1999**, *397*, 710.
- [93] Bitzer, M.; von Gersdorff, G.; Liang, D.; Dominguez-Rosales, A.; Beg, A.A.; Rojkind, M.; Bottinger, E.P. *Genes Dev.*, **2000**, *14*, 187.
- [94] Wotton, D.; Massague, J. *Curr. Top. Microbiol. Immunol.*, **2001**, *254*, 145.
- [95] Kavsak, P.; Rasmussen, R.K.; Causing, C.G.; Bonni, S.; Zhu, H.; Thomsen, G.H.; Wana, J.L. *Mol. Cell*, **2000**, *6*, 1365.
- [96] Kunzmann, S.; Wohlfahrt, J.G.; Itoh, S.; Asao, H.; Komada, M.; Akdis, C.A.; Blaser, K.; Schmidt-Weber, C.B. *FASEB J.*, **2003**, *17*, 194.
- [97] Schmidt-Weber, C.B.; Wohlfahrt, J.G.; Blaser, K. *Int. Arch. Allergy Immunol.*, **2001**, *126*, 1.
- [98] Alters, S.E.; Shizuru, J.A.; Ackerman, J.; Grossman, D.; Seydel, K.B.; Fathman, C.G. *J. Exp. Med.*, **1991**, *173*, 491.
- [99] Wood, K.J.; Bushell, A.R.; Darby, C.R.; Pearson, T.C.; West, L.; Morris, P.J. *Transplant. Proc.*, **1991**, *23*, 133.
- [100] Norman, D.J.; Bennett, W.M.; Cobanoglu, A.; Hershberger, R.; Hosenpud, J.D.; Meyer, M.M.; Misiti, J.; Ott, G.; Ratkovec, R.; Shihab, F. *et al. Transplant. Proc.*, **1993**, *25*, 802.
- [101] Schmidt, J.; Elflein, K.; Stienekemeier, M.; Rodriguez-Palmero, M.; Schneider, C.; Toyka, K.V.; Gold, R.; Hunig, T. *J. Neuroimmunol.*, **2003**, *140*, 143.
- [102] Elflein, K.; Rodriguez-Palmero, M.; Kerkau, T.; Hunig, T. *Blood*, **2003**, *102*, 1764.
- [103] Lin, C.H.; Hunig, T. *Eur. J. Immunol.*, **2003**, *33*, 626.
- [104] Luhder, F.; Huang, Y.; Dennehy, K.M.; Guntermann, C.; Muller, I.; Winkler, E.; Kerkau, T.; Ikemizu, S.; Davis, S.J.; Hanke, T.; Hunig, T. *J. Exp. Med.*, **2003**, *197*, 955.
- [105] Karagiannis, C.; Akdis, M.; Holopainen, P.; Woolley, N.J.; Hense, G.; Ruckert, B.; Mantel, P.Y.; Menz, G.; Akdis, C.A.; Blaser, K.; Schmid-Weber, C.B. *J. Allergy Clin. Immunol.*, **2004**, *114*, 1425-1433.
- [106] Barrat, F.J.; Cua, D.J.; Boonstra, A.; Richards, D.F.; Crain, C.; Savelkoul, H.F.; de Waal-Malefyt, R.; Coffman, R.L.; Hawrylowicz, C.M.; O'Garra, A. *J. Exp. Med.*, **2002**, *195*, 603.
- [107] Kunzmann, S.; Mantel, P.Y.; Wohlfahrt, J.G.; Akdis, M.; Blaser, K.; Schmidt-Weber, C.B. *FASEB J.*, **2003**, *17*, 1089.
- [108] Muller, U.; Hari, Y.; Berchtold, E. *J. Allergy Clin. Immunol.*, **2001**, *107*, 81.
- [109] Fontenot, J.D.; Gavin, M.A.; Rudensky, A.Y. *Nat. Immunol.*, **2003**, *4*, 330.
- [110] Chakir, J.; Shannon, J.; Molet, S.; Fukakusa, M.; Elias, J.; Laviolette, M.; Boulet, L.P.; Hamid, Q. *J. Allergy Clin. Immunol.*, **2003**, *111*, 1293.
- [111] Border, W.A.; Okuda, S.; Languino, L.R.; Sporn, M.B.; Ruoslahti, E. *Nature*, **1990**, *346*, 371.
- [112] Ziyadeh, F.N.; Hoffman, B.B.; Han, D.C.; Iglesias-De La Cruz, M.C.; Hong, S.W.; Isono, M.; Chen, S.; McGowan, T.A.; Sharma, K. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*, 8015.
- [113] Isaka, Y.; Akagi, Y.; Ando, Y.; Tsujie, M.; Sudo, T.; Ohno, N.; Border, W.A.; Noble, N.A.; Kaneda, Y.; Hori, M.; Imai, E. *Kidney Int.*, **1999**, *55*, 465.
- [114] Sharma, K.; Jin, Y.; Guo, J.; Ziyadeh, F.N. *Diabetes*, **1996**, *45*, 522.
- [115] Isaka, Y.; Brees, D.K.; Ikegaya, K.; Kaneda, Y.; Imai, E.; Noble, N.A.; Border, W.A. *Nat. Med.*, **1996**, *2*, 418.
- [116] Murakami, S.; Takashima, H.; Sato-Watanabe, M.; Chonan, S.; Yamamoto, K.; Saitoh, M.; Saito, S.; Yoshimura, H.; Sugawara, K.; Yang, J.; Gao, N.; Zhang, X. *FEBS Lett.*, **2004**, *566*, 55.
- [117] Raphael, T.J.; Kuttan, G. *Phytomedicine*, **2003**, *10*, 483.
- [118] Suh, N.; Roberts, A.B.; Birkey Reffey, S.; Miyazono, K.; Itoh, S.; ten Dijke, P.; Heiss, E.H.; Place, A.E.; Risingsong, R.; Williams,

- C.R.; Honda, T.; Gribble, G.W.; Sporn, M.B. *Cancer Res.*, **2003**, 63, 1371.
- [119] Mix, K.S.; Coon, C.I.; Rosen, E.D.; Suh, N.; Sporn, M.B.; Brinckerhoff, C.E. *Mol. Pharmacol.*, **2004**, 65, 309.
- [120] DaCosta Byfield, S.; Major, C.; Laping, N.J.; Roberts, A.B. *Mol. Pharmacol.*, **2004**, 65, 744.
- [121] Laping, N.J.; Grygielko, E.; Mathur, A.; Butter, S.; Bomberger, J.; Tweed, C.; Martin, W.; Fornwald, J.; Lehr, R.; Harling, J.; Gaster, L.; Callahan, J.F.; Olson, B.A. *Mol. Pharmacol.*, **2002**, 62, 58.
- [122] Li, H.Y.; Wang, Y.; Yan, L.; Campbell, R.M.; Anderson, B.D.; Wagner, J.R.; Yingling, J.M. *Bioorg. Med. Chem. Lett.*, **2004**, 14, 3585.
- [123] Scott Sawyer, J.; Beight, D.W.; Britt, K.S.; Anderson, B.D.; Campbell, R.M.; Goodson, T., Jr.; Herron, D.K.; Li, H.Y.; McMillen, W.T.; Mort, N.; Parsons, S.; Smith, E.C.; Wagner, J.R.; Yan, L.; Zhang, F.; Yingling, J.M. *Bioorg. Med. Chem. Lett.*, **2004**, 14, 3581.
- [124] Li, J.M.; Nichols, M.A.; Chandrasekharan, S.; Xiong, Y.; Wang, X.F. *J. Biol. Chem.*, **1995**, 270, 26750.
- [125] Pessah, M.; Marais, J.; Prunier, C.; Ferrand, N.; Lallemant, F.; Mauviel, A.; Atfi, A. *J. Biol. Chem.*, **2002**, 277, 29094.
- [126] Nishihara, A.; Hanai, J.I.; Okamoto, N.; Yanagisawa, J.; Kato, S.; Miyazono, K.; Kawabata, M. *Genes Cells*, **1998**, 3, 613.
- [127] Jinnin, M.; Ihn, H.; Asano, Y.; Yamane, K.; Trojanowska, M.; Tamaki, K. *Oncogene*, **2004**, 23, 1656.
- [128] Kondo, M.; Suzuki, H.; Takehara, K.; Miyazono, K.; Kato, M. *Cancer Sci.*, **2004**, 95, 12.