

Immunotherapy of allergy: anergy, deletion, and immune deviation

Jennifer Rolland* and Robyn O'Hehir†

Decreased allergen-specific T cell proliferation and dysregulated cytokine synthesis accompany allergen immunotherapy, consistent with mechanisms of anergy and immune deviation. Recent studies emphasise the pivotal role of decreased T cell IL-4:IFN- γ ratios. A landmark clinical trial of T cell epitope peptides for venom-immunotherapy shows efficacy and safety; murine models suggest intramolecular epitope-suppression inhibits responses to the whole allergen.

Addresses

*Department of Pathology and Immunology, Monash University Medical School, Commercial Road, Prahran, Victoria 3181, Australia; e-mail: Jennifer.Rolland@med.monash.edu.au

†Department of Allergy and Clinical Immunology, Alfred Hospital and Monash University, Commercial Road, Prahran, Victoria 3181, Australia; e-mail: Robyn.O'Hehir@med.monash.edu.au

Current Opinion in Immunology 1998, 10:640–645

<http://biomednet.com/elecref/0952791501000640>

© Current Biology Ltd ISSN 0952-7915

Abbreviations

APC	antigen-presenting cell
HDM	house dust mite
IFN-γ	interferon γ
PLA₂	phospholipase A ₂
SIT	specific immunotherapy

Introduction

Conventional specific immunotherapy (SIT) with allergens involves the subcutaneous injection of incremental doses of native allergen-extract into sensitised subjects in order to achieve a state of clinical tolerance to subsequent natural allergen exposure. Although introduced in 1911, SIT still has variable efficacy — depending on the allergen — and carries significant risk of side effects. Current national guidelines (e.g. [1]) are based empirically on accumulated clinical evidence and risk/benefit analysis without consideration of the underlying immunological mechanisms. With identification of the pivotal role of T cells in the control of the allergic immune response, mechanistic questions are being re-examined.

Activated CD4⁺ T cells that drive an allergic response are polarised towards a Th2 phenotype; key cytokines made by such cells are IL-4, a major IgE switch factor, and IL-5 which regulates differentiation and activation of eosinophils [2]. Importantly, the T cell repertoires of both allergic and nonallergic subjects include cells that recognize allergen (e.g. see reference [3]) but it is the IL-4:IFN- γ ratio that determines the functional clinical phenotype (IFN- γ being a Th1-type cytokine) [4]. Increasing data suggest that repolarisation of the allergic response towards a Th1-type response accompanies successful SIT. Are allergen-specific Th2 cells being rendered anergic by SIT, exhibiting failure to proliferate and dysregulated cytokine production on specific immunogenic

rechallenge? Are they deleted, or are the observed changes all explained by immune deviation?

This review addresses these questions by examining both analyses of T cell function during current immunotherapy regimens and human or animal studies that have used defined T-cell-epitope peptides to modulate allergic immune responses.

Changes in allergen-specific T cell function with current immunotherapy

Despite different experimental designs, evaluation of successful SIT consistently demonstrates altered cytokine production by allergen-stimulated T cells, with a decreased IL-4:IFN- γ ratio [5–10,11*,12–14] (Table 1). Antigen specificity is indicated by the lack of similar changes following stimulation with control antigens such as tetanus toxoid. Venom-SIT consistently leads to decreased IL-4 levels, as protein or as mRNA, regardless of whether polyclonal or oligoclonal populations are analysed. Changes are detectable by seven days during 'rush' immunotherapy [5] and persist at three years with conventional SIT [9]. In contrast, allergen-induced production of IFN- γ is generally enhanced. Aeroallergen-SIT similarly decreases allergen-induced T cell IL-4 synthesis but IFN- γ production is more variable. Possible reasons for this different IFN- γ result compared with studies of venom-SIT may include the complexity both of allergen sensitivities (venom-allergic patients are often nonatopic) and of the allergens themselves.

Decreased IL-4 and, where studied, IL-5 production seen early during SIT could explain both the rapid downregulation of mast cell and basophil reactivity demonstrated by allergen challenge after rush immunotherapy [5] and the decreased inflammatory cell infiltrate [15]. Changes in circulating levels of specific IgE and IgG antibody occur later, consistent with progressive stimulation of naive B cells in an altered T-cell-cytokine milieu rather than modulation of the function of either memory B cells or fully differentiated IgE-producing B cells. Further interruption of allergen-specific B cell activation may be mediated by IFN- γ -induced failure of the seasonal upregulation of B cell CD23, CD40 and HLA-DR expression in SIT patients [16].

Results of analyses of mucosal tissue parallel changes observed in peripheral blood T cells. After effective SIT — with inhibition of early- and late-phase responses to allergen challenge — analyses of target tissues indicate Th1-cell dominance [15].

Thus current SIT is associated with decreased Th2-type T cell reactivity and a shift in the net cytokine balance towards Th1 predominance. But is the observed repolar-

Table 1**Changes in allergen-specific T cell function following immunotherapy.**

Allergen	Immunotherapy regimen	Cultured cells	Cytokine change			Proliferation response	Reference
			Assay	IL-4	IFN- γ		
Bee venom	Rush	PBMCs	ELISA	↓	↑	↓	[5]
Wasp and bee venom	Rush	PBMCs	ELISA	↓	↑	↓	[6]
Bee venom	Rush	Enriched T cells	ISH	↓	↑	↓	[7]
Bee venom	Rush	T cell lines	ELISA	↓	↓	↓	[8]
Bee venom	Standard	T cell lines	ELISA	NS ↓	↑	↓	[9]
Bee venom	Standard	PBMCs	ELISA	↓	↑	↓	[10]
Timothy grass	Standard	T cell clones	ELISA	↓	No change	↓	[11•]
Grass	Standard	PBMCs	ELISA	↓	NS ↓	↓	[12]
HDM and grass	Standard	T cell lines	ELISA	↓	No change	↓	[13]
HDM	Standard	PBMCs	RT-PCR	↓	↓	Not tested	[14]

ELISA, enzyme-linked immunosorbent assay; ISH, *in situ* hybridisation; NS, nonsignificant; PBMCs, peripheral blood mononuclear cells; RT-PCR, reverse-transcriptase polymerase chain reaction.

isation due to deletion or anergy of allergen-specific Th2 cells, or to immune deviation?

Deletion or anergy?

Studies consistently demonstrate a decrease in allergen-stimulated lymphoproliferative responses following SIT, regardless of whether or not Th1 cytokine production is enhanced (Table 1). Deletion of allergen-specific T cells and/or induction of anergy by allergen treatment may be the mechanism. In experimental models of mucosal tolerance that do not use allergens, high-dose antigen treatment induces clonal deletion via apoptosis [17] but to date T cell apoptosis due to allergen SIT has not been reported. The precursor frequency of allergen-reactive T cells is comparable between atopic and nonatopic donors [4] but any decreases following SIT — which would support the proposal regarding deletion — have not been described.

Anergy as the mechanism of SIT is supported by the observed diminution of antigen-specific proliferative response and decreased cloning efficiency [11•], together with modified cytokine production. These changes mirror anergy induction by high-dose antigen under defined *in vitro* conditions [18–20]; moreover, anergy reversal by IL-2 [19] has been demonstrated in one clinical study of allergen-SIT [8]. High-dose IL-2 restored oligoclonal T cell proliferation to specific allergen (bee-venom phospholipase A₂ [PLA₂]) and production of IL-2 and IFN- γ (but not IL-4) to pre-SIT levels; however, due to the non-monoclonal nature of these cultures, distinction between re-activation of anergised T cells and activation of naive T cells is difficult. The increased production of IL-10 following SIT [6] would favour the induction of T cell anergy [21•]. Further elucidation of regulatory factors and of SIT-induced differences in biochemical activation-pathways of allergen-stimulated T cells is required to define the cellular defects.

Immune deviation?

An alternative explanation for the observed T cell changes is immune deviation — a shift from predominantly Th2-like

to a more Th1-like functional phenotype. Carballido *et al.* [22•] demonstrated that PLA₂-specific T cell clones shift from IL-4 to IFN- γ production with increasing antigen dose; furthermore receptivity to B7 co-stimulatory signals depends on the strength of the TCR signal, with naive CD4⁺ T cells being receptive to CD28-dependent IL-4 production only in response to weak TCR signals [23]. These observations suggest that the higher allergen concentrations achieved with SIT, compared with natural exposure, could induce a shift from IL-4 to IFN- γ production by allergen-reactive T cells. Additionally, the route of allergen administration may affect cytokine profiles by influencing the predominant antigen-presenting cell (APC) type. Using allergen-specific T cell lines from donors who are sensitive to grass pollen and house dust mite (HDM), IL-4 production was maximal at low doses of antigen, especially with B cells as APCs, but minimal with high concentrations of allergen presented by monocytes [24]. Differential expression of co-stimulatory molecules on different APCs may mediate these effects. Engagement of CD28 on T cells with B7.1/CD80 on APCs has been shown to favour Th1-cell differentiation while interaction with B7.2/CD86 favoured Th2-cell differentiation [25]; however, while B7.2/CD86 is constitutively expressed on monocytes and dendritic cells, B cells show early rapid induction of B7.2/CD86 expression following activation but later show expression of B7.1/CD80 [26]. Thus the kinetics of expression of co-stimulatory molecules may confound these studies on APC regulation of Th-cell differentiation; moreover, the activation state of interacting T cells also influences outcome, since engagement with the alternative T cell ligand CTLA-4 (cytotoxic T lymphocyte antigen 4; expressed on activated T cells) delivers a tolerogenic signal [27].

More convincing is the role of the cytokine milieu in influencing Th-cell differentiation. The balance between local IL-4 and IFN- γ is a key determinant of CD4⁺ T cell response to antigen. Importantly, IL-12 is the major immunomodulatory cytokine associated with Th1-polarised immune responses and indeed increased expression of

IL-12 mRNA in macrophages has been detected at sites of allergen challenge post-SIT [28]. The observed local increase in IFN- γ may be driving the enhanced IL-12 production (reviewed in [29]) or alternatively the IL-12 may be induced by direct interaction between the T cell and the APC [30]. Naive Th0 cell populations can readily be induced to differentiate along Th1 or Th2 pathways, depending on the relative mix of IL-4 and IL-12/IFN- γ [31]. The greater efficacy of bee-venom-SIT compared with aeroallergen SIT and the more pronounced effects on T cell cytokine responses (Table 1) may reflect the more Th0-like profile of the circulating allergen-specific T cells. This may be due to the small, infrequent doses of venom naturally encountered by patients compared with chronic high-dose exposure to aeroallergens. Manipulation of cytokine profiles of committed Th2-cell subsets is more difficult; however, a combination of IL-12 and IFN- γ has switched Th2 cells to IFN- γ production [32]. Thus T cells with enhanced IFN- γ production after SIT might mediate immune deviation by acting as regulatory cells for other allergen-reactive T cells and for subsequent naive T cells encountering allergen.

The possible role of CD8⁺ antigen-specific suppressor cells in effective SIT was suggested by Rocklin *et al.* [33]. Certainly CD8⁺ T cells can produce large amounts of IFN- γ and thus both potentially inhibit CD4⁺ Th2-cell expansion and antagonise the effects of IL-4 on B cell switching to IgE production; however, increased CD8⁺ T cell numbers and/or activity following SIT have been difficult to substantiate. An observed increase in the proportion of IFN- γ ⁺ peripheral blood CD3⁺ T cells measured by flow cytometry after bee-venom-SIT lay with the CD4⁺ T cell population [34[•]]. Thus current evidence favours the induction of an IFN- γ ⁺ regulatory CD4⁺ T cell population by SIT.

Epitope mapping of T cells further explores the possible SIT-induced expansion of new populations of regulatory T cells with different antigen specificities; however, studies of conventional grass-pollen-SIT found no evidence of determinant spreading and so the observed switching of the predominant Th2/Th0 cytokine profile to Th1/Th0 instead suggested modulation of existing T cell populations [11[•]]. Interestingly, the allergen's T cell epitope specificities are similar for allergic and nonallergic subjects [35].

It therefore remains unclear whether SIT effects are due predominantly to induction of T cell anergy or to immune deviation. Both possibilities seem likely, since the decreased ratio of IL-4:IFN- γ produced by anergised T cells would create the milieu favourable for differentiation of allergen-stimulated naive T cells into Th1 cells. Likewise, the particular circumstances for stimulation of T cells by allergens during SIT — including high antigen dose, different route of antigen administration and different APC types — may directly switch T cell responses from Th2- to Th1-type.

Current conventional SIT involves administration of intact native allergen extracts or, at best, purified whole allergens; these are recognised by different T cell populations with particular specificities and affinities. Further insight into mechanisms of SIT can be obtained from animal and human studies using peptides, identified as dominant T cell epitopes of allergens, to target particular T cell populations.

Peptide immunotherapy

Modulation of human T cell effector function by peptides that are epitopes for T cells was first reported for influenza-virus-specific cloned human CD4⁺ T cells [18] and later applied to modulation of allergen-specific T cell reactivity. Dominant T cell epitope peptides are the most potent tolerogens [36]. Treatment of cloned HDM-specific CD4⁺ T cells with supraoptimal concentrations of their specific ligand resulted in decreased proliferation to subsequent immunogenic challenge, decreased IL-4 and IL-5 synthesis but persistent IFN- γ production [20]. Characteristic surface phenotypic changes of anergy were seen, including blunting of the upregulation of CD28 expression normally observed after immunogenic challenge [37]. In addition, altered signalling pathways are utilised by anergic T cells [38]. Studies of peptide-anergised, PLA₂-specific, cloned CD4⁺ T cells identified abrogation of the activity of p56^{lck} and ZAP-70 tyrosine kinases [39[•]].

Murine experimental models

Inhalation of a dominant peptide of Der p 1, a major HDM allergen, decreased T cell responses in both naive and sensitised mice [36]. Initial cytokine upregulation was followed by abrogation of both antigen-specific proliferation and cytokine synthesis [40]. These findings suggest transient activation prior to anergy induction and may explain apparent differences in cytokine profiles following SIT (Table 1). There is evidence that these effects are mediated by regulatory cells. Firstly, mixing lymph-node cells from mice tolerised to the Der p 1 peptide with immune spleen cells completely inhibited specific antibody production. Secondly, and importantly, treatment with the immunodominant peptide abrogated T cell response to the whole, multideterminant Der p 1 molecule; however, CD8⁺ T cells did not play a role in this tolerance model. Evidence against deletion of epitope-specific T cells was provided since the tolerance could be 'uncoupled' by subsequent immunisation with a minor epitope and adjuvant. The downregulation was most efficient for epitopes on the same molecule, suggesting the mechanism of intramolecular suppression rather than bystander suppression [41^{••}]. Together these data support a regulatory role for the anergised CD4⁺ T cells. This mechanism appears to be favoured by higher antigen doses [42]. Similar inhibition of responses to whole allergens by dominant peptides was reported for other models (e.g. see references [43,44]), stimulating recent human trials of epitope peptide vaccines.

Human clinical trials

Five bee-venom-allergic patients were effectively desensitised by subcutaneous administration of three peptide epitopes of PLA₂ [45••]. Analysis of T cell responses revealed both reduced PLA₂-induced proliferation and decreased IL-2, IL-4, IL-5, IL-13 and IFN- γ production. Intramolecular suppression is again suggested, as vaccination with these three dominant epitopes induced tolerance to the whole PLA₂ molecule.

Clinical trials of SIT using peptides of a cat allergen (Fel d 1) have shown variable efficacy, but large protein determinants were administered rather than minimal epitopes. Immunological analysis showed decreased IL-4 production but no significant change in IFN- γ production or proliferative response to Fel d 1 [46]. Possible explanations for the disparity between the studies with PLA₂ and Fel d 1 peptides would include the different total peptide doses and the use of smaller epitope peptides for PLA₂ (i.e. 11, 12 and 18 amino acids) compared with Fel d 1 (i.e. 27 amino acids). Again, bee-venom sensitivity may be easier to downregulate.

An alternative strategy for enhancing peptide efficacy, that is suggested from *in vitro* studies, is to use peptide analogues in which secondary TCR contact residues are substituted. Such altered peptide ligands may act as partial agonists or antagonists, but importantly they can cause increased IFN- γ production or decreased IL-4 production by allergen-specific human T cell clones [30,47,48*].

Conclusions

Clinical efficacy of SIT is associated with altered allergen-specific T cell function. T cells show decreased proliferative response to subsequent allergen challenge, together with dysregulated cytokine production. T cell anergy and immune deviation as potential underlying mechanisms of successful SIT are not mutually exclusive. With ongoing SIT, the redirection to Th1 cytokine production by allergen-stimulated T cells would be perpetuated since naive T cells will now be maturing in an environment with a decreased IL-4: IFN- γ ratio. To date, T cell deletion accompanying SIT for allergy has not been examined but remains a theoretical possibility.

Enhancement of the shift to Th1 cytokines to increase efficacy, especially for more complex allergens, may be achieved by recombinant allergen approaches. These include either mutated recombinant proteins — that retain dominant T cell epitopes but have abrogated IgE reactivity — for use as hypoallergenic vaccines [49] or recombinant major allergens coupled to bacterial crystalline surface proteins [50]. The expression of a Der p 1 epitope in mycobacterial vaccine strains induced peptide-specific IFN- γ responses in mice [51*]. An alternative approach utilises expression vectors containing allergen genes linked to cytokine genes [52]. DNA vaccines may themselves promote Th1 cytokine

responses [53]. Each of these strategies favours Th1 cytokine synthesis in the local environment of allergen-responsive T cells.

Clarification of the precise underlying mechanisms of clinically effective SIT will permit the rational design of new immunotherapeutics for allergic disease.

Acknowledgements

We wish to acknowledge the support of the National Health and Medical Research Council of Australia, the Australian Allergy Foundation and the Alfred Hospital, Melbourne.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Anonymous: **Specific allergen immunotherapy for asthma. A position paper of the Thoracic Society of Australia and New Zealand and the Australasian Society of Clinical Immunology and Allergy.** *Med J Australia* 1997, **167**:540-544.
 2. O'Hehir RE, Garman RD, Greenstein JL, Lamb JR: **The specificity and regulation of T-cell responsiveness to allergens.** *Annu Rev Immunol* 1991, **9**:67-95.
 3. Blaher B, McCluskey J, Puy R, Czarny D, Rolland JM: **T cell response to grass pollen allergens: correlation with skin test reactivity and serum IgE levels.** *Immun Cell Biol* 1995, **73**:17-22.
 4. Li Y, Simons ER, Jay FT, Hay-Glass KT: **Allergen-driven limiting dilution analysis of human IL-4 and IFN- γ production in allergic rhinitis and clinically tolerant individuals.** *Int Immunol* 1996, **8**:897-904.
 5. Jutel M, Pichler WJ, Skrbic D, Urwyler A, Dahinden C, Muller UR: **Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN- γ secretion in specific allergen-stimulated T cell cultures.** *J Immunol* 1995, **154**:4187-4194.
 6. Bellinghausen I, Metz G, Enk AH, Christmann S, Knop J, Saloga J: **Insect venom immunotherapy induces interleukin-10 production and a Th2-to-Th1 shift, and changes surface marker expression in venom-allergic subjects.** *Eur J Immunol* 1997, **27**:1131-1139.
 7. Akoum H, Tsicopoulos A, Vorng H, Wallaert B, Dessaint JP, Joseph M, Hamid Q, Tonnel AB: **Venom immunotherapy modulates interleukin-4 and interferon- γ messenger RNA expression of peripheral T lymphocytes.** *Immunology* 1996, **87**:593-598.
 8. Akdis CA, Akdis M, Blesken T, Wymann D, Alkan SS, Muller U, Blaser K: **Epitope-specific T cell tolerance to phospholipase A₂ in bee venom immunotherapy and recovery by IL-2 and IL-15 in vitro.** *J Clin Invest* 1996, **98**:1676-1683.
 9. Kammerer R, Chvatchko Y, Kettner A, Dufour N, Corradin G, Spertini F: **Modulation of T-cell response to phospholipase A₂ and phospholipase A₂-derived peptides by conventional bee venom immunotherapy.** *J Allerg Clin Immunol* 1997, **100**:96-103.
 10. McHugh SM, Deighton J, Stewart AG, Lachmann PJ, Ewan PW: **Bee venom immunotherapy induces a shift in cytokine responses from a TH-2 to a TH-1 dominant pattern: comparison of rush and conventional immunotherapy.** *Clin Experiment Allergy* 1995, **25**:828-838.
 11. Ebner C, Siemann U, Bohle B, Willheim M, Wiedermann U, Schenk S, Klotz F, Ebner H, Kraft D, Scheiner O: **Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from TH₂ to TH₁ in T-cell clones specific for Phl p 1, a major grass pollen allergen.** *Clin Experiment Allergy* 1997, **27**:1007-1015.
- This reports a longitudinal study of standard SIT in eight grass-pollen-sensitive patients. Clonal analysis revealed a shift from Th2 to Th1 cytokines. Together with decreased cloning efficiency, these results support anergy as the mechanism for SIT. No evidence was found for induction of CD8⁺ regulatory cells or for changes in T cell repertoire.
12. Giannarini L, Maggi E: **Decrease of allergen-specific T-cell response induced by local nasal immunotherapy.** *Clin Experiment Allergy* 1997, **28**:404-412.

13. Secrist H, Chelen CJ, Wen Y, Marshall JD, Umetsu DT: **Allergen immunotherapy decreases interleukin 4 production in CD4⁺ T cells from allergic individuals.** *J Exp Med* 1993, **178**:2123-2130.
 14. O'Brien RM, Byron KA, Varigos GA, Thomas WR: **House dust mite immunotherapy results in a decrease in Der p 2-specific IFN- γ and IL-4 expression by circulating T lymphocytes.** *Clin Exp Allergy* 1997, **27**:46-51.
 15. Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, Kay AB, Hamid QA: **Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4⁺ T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon- γ .** *J Allerg Clin Immunol* 1996, **97**:1356-1365.
 16. Hakansson L, Heinrich C, Rak S, Venge P: **Activation of B-lymphocytes during pollen season. Effect of immunotherapy.** *Clin Experiment Allergy* 1998, **28**:791-798.
 17. Chen Y, Inobe JJ, Marks R, Gonnella P, Kuchroo VK, Weiner HL: **Peripheral deletion of antigen-reactive T cells in oral tolerance.** *Nature* 1995, **376**:177-180.
 18. Lamb JR, Skidmore BJ, Green N, Chiller JM, Feldmann M: **Induction of tolerance in influenza virus-immune T lymphocyte clones with synthetic peptides of influenza haemagglutinin.** *J Exp Med* 1983, **157**:1434-1447.
 19. Fasler S, Aversa G, Terr A, Thestrup-Pedersen K, de Vries JE, Yssel H: **Peptide-induced anergy in allergen-specific human Th2 cells results in lack of cytokine production and B cell help for IgE synthesis.** *J Immunol* 1995, **155**:4199-4206.
 20. O'Hehir RE, Yssel H, Verma S, de Vries JE, Spits H, Lamb JR: **Clonal analysis of differential lymphokine production in peptide and superantigen induced T cell anergy.** *Int Immunol* 1991, **3**:819-826.
 21. Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K: **Role of interleukin 10 in specific immunotherapy.** *J Clin Invest* 1998, **102**:98-106.
- Here, a role for IL-10 in induction of T cell anergy by SIT is proposed. Decreased allergen-specific proliferative and cytokine responses following bee-venom-SIT could be reversed by addition of a neutralising anti-IL-10 monoclonal antibody to cultures. Increased IL-10 following SIT was shown, by intracytoplasmic staining, to be initially produced predominantly by activated CD4⁺ T cells but later by monocytes and B cells.
22. Carballido JM, Faith A, Carballido-Perrig N, Blaser K: **The intensity of T cell receptor engagement determines the cytokine pattern of human allergen-specific T helper cells.** *Eur J Immunol* 1997, **27**:515-521.
- The authors show that allergen-specific T cell clones can be induced to shift from IL-4 to IFN- γ production by increasing the concentration of antigen (PLA₂) in the culture.
23. Tao X, Constant S, Jorritsma P, Bottomly K: **Strength of TCR signal determines the costimulatory requirements for Th1 and Th2 CD4⁺ T cell differentiation.** *J Immunol* 1997, **159**:5956-5963.
 24. Secrist H, DeKruyff RH, Umetsu DT: **Interleukin 4 production by CD4⁺ T cells from allergic individuals is modulated by antigen concentration and antigen-presenting cell type.** *J Exp Med* 1995, **181**:1081-1089.
 25. Kuchroo VK, Das MP, Brown JA, Ranger AM, Zamvil SS, Sobel RA, Weiner HL, Nabavi N, Glimcher LH: **B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy.** *Cell* 1995, **80**:707-718.
 26. Hathcock KS, Pucilla LG, Linsley P, Hodes RJ: **Comparative analysis of B7-1 and B7-2 costimulatory ligands: expression and function.** *J Exp Med* 1994, **180**:631-640.
 27. Perez V, Van PL, Biuckians A, Zheng X, Strom T, Abbas A: **Induction of peripheral T cell tolerance *in vivo* requires CTLA-4 engagement.** *Immunity* 1997, **6**:411-417.
 28. Hamid QA, Schotman E, Jacobson MR, Walker SM, Durham SR: **Increases in IL-12 messenger RNA⁺ cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy.** *J Allerg Clin Immunol* 1997, **99**:254-260.
 29. Smith W, Sly PD: **Immunotherapy - anergy, deviation or suppression?** *Clin Experiment Allergy* 1998, **28**:911-916.
 30. Matsuoka T, Kohrogi H, Ando M, Nishimura Y, Matsushita S: **Altered TCR ligands affect antigen-presenting cell responses: up-regulation of IL-12 by an analogue peptide.** *J Immunol* 1996, **157**:4837-4843.
 31. Kelso A: **Cytokines: principles and prospects.** *Immun Cell Biol* 1998, **76**:300-317.
 32. Nakamura T, Lee RK, Nam SY, Podack ER, Bottomly K, Flavell RA: **Roles of IL-4 and IFN- γ in stabilizing the T helper cell type 1 and 2 phenotype.** *J Immunol* 1997, **158**:2648-2653.
 33. Rocklin RE, Sheffer AL, Greineder DK, Melmon KL: **Generation of antigen-specific suppressor cells during allergy desensitization.** *N Engl J Med* 1980, **302**:1213-1219.
 34. Lack G, Nelson HS, Amran D, Oshiba A, Jung T, Bradley KL, Giclas PC, Gelfand EW: **Rush immunotherapy results in allergen-specific alterations in lymphocyte function and interferon- γ production in CD4⁺ T cells.** *J Allerg Clin Immunol* 1997, **99**:530-538.
- This demonstrates that clinically effective mite-SIT was associated with both ablation of lymphoproliferative response to mite (but not to control [cat] extract) and increased serum IFN- γ . This was due to a selective increase in the frequency of IFN- γ ⁺ CD4⁺ cells rather than CD8⁺ cells.
35. Mark PG, Segal DB, Dallaire ML, Garman RD: **Human T and B cell immune responses to Fel d 1 in cat-allergic and non-cat-allergic subjects.** *Clin Experiment Allergy* 1996, **26**:1316-1328.
 36. Hoyne GF, O'Hehir RE, Wraith DC, Thomas WR, Lamb JR: **Inhibition of T cell and antibody responses to house dust mite allergen by inhalation of the dominant T cell epitope in naive and sensitized mice.** *J Exp Med* 1993, **178**:1783-1788.
 37. Lake RA, O'Hehir RE, Verhoef A, Lamb JR: **CD28 mRNA rapidly decays when activated T cells are functionally anergized with specific peptide.** *Int Immunol* 1993, **5**:461-466.
 38. Schwartz RH: **T cell clonal anergy.** *Curr Opin Immunol* 1997, **9**:351-357.
 39. Faith A, Akdis CA, Akdis M, Simon HU, Blaser K: **Defective TCR stimulation in anergized type 2 T helper cells correlates with abrogated p56^{lck} and ZAP-70 tyrosine kinase activities.** *J Immunol* 1997, **159**:53-60.
- This paper shows that allergen-induced anergy of Th2 cells is associated with defective transmembrane signaling through the TCR.
40. Hoyne GF, Askonas BA, Hetzel C, Thomas WR: **Regulation of house dust mite responses by intranasally administered peptide: transient activation of CD4⁺ T cells precedes the development of tolerance *in vivo*.** *Int Immunol* 1996, **8**:335-342.
 41. Hoyne GF, Jarnicki AG, Thomas WR, Lamb JR: **Characterization of the specificity and duration of T cell tolerance to intranasally administered peptides in mice: a role for intramolecular epitope suppression.** *Int Immunol* 1997, **9**:1165-1173.
- This paper demonstrates a role for regulatory CD4⁺ T cells in downregulating T cell responses to minor epitopes that are found on the same molecule as the immunodominant T cell anergen.
42. Lowrey JA, Savage NDL, Palliser D, Corsin-Jimenez M, Forsyth LMG, Hall G, Stewart GA, Tan KAL, Hoyne GF, Lamb JR: **Induction of tolerance via the respiratory mucosa.** *Int Arch Allergy Immunol* 1998, **116**:93-102.
 43. Bauer L, Bohle B, Sahn-Schmid B, Wiedermann U, Daser A, Renz H, Kraft D, Ebner C: **Modulation of the allergic immune response in BALB/c mice by subcutaneous injection of high doses of the dominant T cell epitope from the major birch pollen allergen Bet v 1.** *Clin Exp Immunol* 1997, **107**:536-541.
 44. Cao Y, Yang M, Luo Z, Mohapatra SS: **Vaccination with a multi-epitopic recombinant allergen induces specific immune deviation via T-cell anergy.** *Immunology* 1997, **90**:46-51.
 45. Muller U, Akdis A, Fricker M, Akdis M, Blesken T, Betens F, Blaser K: **Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom.** *J Allerg Clin Immunol* 1998, **101**:747-754.
- This is the first convincing demonstration of clinically effective allergen immunotherapy using T cell epitope peptides. No allergic side-effects to the peptide treatment were experienced. Anergy induction and cytokine dysregulation were demonstrated.
46. Marcotte GV, Braun CM, Norman PS, Nicodemus CF, Kagey-Sobotka A, Lichtenstein LM, Essayan DM: **Effects of peptide therapy on *ex vivo* T-cell responses.** *J Allerg Clin Immunol* 1998, **101**:506-513.
 47. Tsitoura DC, Verhoef A, Gelder GM, O'Hehir RE, Lamb JR: **Altered T cell ligands derived from a major house dust mite allergen enhance IFN- γ but not IL-4 production by human CD4⁺ T cells.** *J Immunol* 1996, **157**:2160-2165.

48. Fasler S, Aversa G, de Vries JE, Yssel H: **Antagonistic peptides specifically inhibit proliferation, cytokine production, CD40L expression, and help for IgE synthesis by Der p 1-specific human T-cell clones.** *J Allerg Clin Immunol* 1998, **101**:521-530.
This study shows that altered peptide ligands offer an alternative strategy for modulating the function of allergen-specific T cells by inhibiting proliferation, dysregulating cytokine secretion and abrogating B cell help for IgE synthesis.
49. Smith AM, Chapman MD, Taketomi EA, Platts-Mills TA, Sung SS: **Recombinant allergens for immunotherapy: a Der p 2 variant with reduced IgE reactivity retains T-cell epitopes.** *J Allerg Clin Immunol* 1998, **101**:423-425.
50. Jahn-Schmid B, Siemann U, Zenker A, Bohle B, Messner P, Unger FM, Sleytr UB, Scheiner O, Kraft D, Ebner C: **Bet v 1, the major birch pollen allergen, conjugated to crystalline bacterial cell surface proteins, expands allergen-specific T cells of the T_H1/T_H0 phenotype *in vitro* by induction of IL-12.** *Int Immunol* 1997, **9**:1867-1874.

51. Hetzel C, Janssen R, Ely SJ, Kristensen NM, Bunting K, Cooper JB, Lamb JR, Young DB, Thole JER: **An epitope delivery system for use with recombinant mycobacteria.** *Infect Immun* 1998, **66**:3643-3648.
This manuscript supports the use of mycobacteria as Th1-inducing delivery systems for vaccination using allergen peptides. Immunized mice generated peptide-specific IFN- γ responses.
52. Kim TS, DrKruyff RH, Rupper R, Maecker HT, Levy S, Umetsu DT: **An ovalbumin-IL-12 fusion protein is more effective than ovalbumin plus free recombinant IL-12 in inducing a T helper cell type 1-dominated immune response and inhibiting antigen-specific IgE production.** *J Immunol* 1997, **158**:4137-4144.
53. Raz E, Tighe H, Sato Y, Corr M, Dudley JA, Roman M, Swain SL, Spiegelberg HL, Carson DA: **Preferential induction of a Th1 immune response and inhibition of specific IgE antibody formation by plasmid DNA immunization.** *Proc Natl Acad Sci USA* 1996, **93**:5141-5145.