

Atopic allergy and other hypersensitivities

Editorial overview:

Technological advances and new insights into pathogenesis prelude novel therapeutic strategies

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Introduction

Atopic allergy is a genetically determined disorder characterized by the increased ability of B lymphocytes to produce IgE antibodies directed at groups of ubiquitous antigens that activate the immune system after inhalation or ingestion, and perhaps after penetration through the skin (allergens). IgE antibodies are able to bind to high-affinity Fcε receptors (FcεRI) present on the surface of mast cells/basophils, and allergen-induced FcεRI cross-linking triggers the release of vasoactive mediators, chemotactic factors and cytokines that are responsible for the allergic cascade. In addition to B cells that produce IgE and mast cells/basophils that bind IgE, eosinophils also appear to be involved in the pathogenesis of allergic reactions, as these cells usually accumulate at the sites of allergic inflammation and the toxic products they release significantly contribute to the induction of tissue damage.

Pathogenic role of allergen-specific Th2-like cells

The mechanisms accounting for the joint involvement of IgE-producing B cells, mast cells/basophils and eosinophils in the pathogenesis of allergic reactions have become clear with the demonstration of polarized forms of the specific immune response, as revealed on the basis of their profile of cytokine secretion [1,2]. Type 1 helper (Th1) CD4⁺ T cells produce interferon (IFN)-γ and are the principal effector of phagocyte-mediated host defense, which is highly protective against infections sustained by intracellular parasites. On the other hand, Th2 CD4⁺ T cells produce IL-4, which stimulates the production of IgE and IgG₁ antibodies, IL-5 (an eosinophil-activating factor), IL-10 and IL-13, which, together with IL-4, inhibit several macrophage functions. Therefore, the Th2 cell is mainly responsible for phagocyte-independent host defense (e.g. against certain nematodes [3]) as well as an excellent candidate to explain why the mast cell/eosinophil/IgE-producing

B cell triad is involved in the pathogenesis of allergy. Strong evidence has been accumulated to support this concept: allergens preferentially expand Th cells with a Th2-like profile [4,5]; Th2-like cells accumulate in the target organs of allergic patients [6-9]; challenge with allergen results in the local activation and recruitment of allergen-specific Th2-like cells [10]; and successful specific immunotherapy is associated with changes in the cytokine profile of allergen-reactive Th cells [11-13]. More recently, it was found that allergen-reactive Th2 cells expressing membrane CD30, an activation marker preferentially associated with the production of Th2-type cytokines, are present in the circulation of allergic patients during seasonal allergen exposure [14]. Moreover, CD30⁺ T cells are present in the lesional skin of patients with atopic dermatitis (M Caproni *et al.*, unpublished data), and high levels of soluble CD30 are detectable in the serum of patients with atopic dermatitis (L Giannarini, F Vinante, G Pizzolo, S Romagnani, E Maggi, unpublished data).

Mechanisms involved in the differentiation of Th2 cells

The mechanisms responsible for the preferential development of allergen-reactive Th2 cells in atopic subjects have not yet been clarified. In both murine and human *in vivo* and *in vitro* systems, IL-4 appears to be the most dominant factor in determining the likelihood for Th2 polarization [15-17]. Accordingly, mice that have had their IL-4 gene targeted fail to generate mature Th2 cells *in vivo* and to produce IgE antibodies [18], suggesting that early IL-4 production by a cell type distinct from the Th2 cell must be involved. Possible candidates include mast cells/basophils and eosinophils, which have been shown to release stored IL-4 in response to FcεR triggering [19-21], the LECAM-1^{dull} subset of CD4⁺ T cells [22], the CD4⁺NK1.1⁺ T-cell subset [23], or the naive Th cells themselves [24-26]. With regard to the role of mast cells/basophils, it is unlikely that parasites or allergens would be able to

Abbreviations

APC—antigen-presenting cell; IL—interleukin; IFN—interferon; Th—T helper.

cross-link their receptors prior to a specific immune response that had produced parasite-specific IgG and IgE antibodies. On the other hand, mice deficient in mast cells develop normal Th2 responses [27]. Finally, IL-4-deficient mice produce antigen-specific IgE only when reconstituted with IL-4-producing T cells (but not with IL-4-producing non-T cells) [28]. Thus, IL-4 production by mast cells/basophils or eosinophils triggered by antigen-IgE antibody immune complexes, might play a role in amplifying secondary responses to parasites, but cannot account for the Th2 development in primary immune responses. A way out of this dilemma may be a pathway of IL-4 secretion independent of FcεR cross-linking. It has been suggested that, because of their proteolytic activity, both helminth products and some allergens may induce FcεR1⁺ cells to release IL-4 [29]; however, obvious mechanisms of FcεR-independent IL-4 production by these cells have not yet been identified.

A second possibility is that the endogenous source of IL-4 required for Th2 development is the LECAM-1⁺ subset of CD4⁺ T cells, which contains the majority of the memory/activated CD4⁺ cells and has been shown to secrete significant amounts of IL-4 upon primary stimulation *in vitro* [22,30], and/or the CD4⁺NK1.1⁺ T-cell subset [23]. The latter cells appear to represent a specialized population of CD4⁺ T cells that are particularly effective at producing cytokines and that are positively selected by the recognition of CD1, a nonpolymorphic MHC class I like molecule [31]. Interestingly, mice that are deficient in β₂-microglobulin (which are also deficient in CD1 expression) produce neither IL-4 nor IgE; moreover, SJL mice that are unable to produce IgE do not possess CD4⁺NK1.1⁺ T cells or express membrane IL-4 following *in vivo* injection of anti-CD3 antibody [32,33]. Therefore, it could be possible that immunogens having associated superantigens can interact not only with the naive CD4⁺ cells, but also with a sufficiently large fraction of CD4⁺NK1.1⁺ cells, thus making a pulse of IL-4 available for naive CD4⁺ T cells that are responding at the same time to the antigenic peptide. It is unlikely, however, that all antigens that are able to promote the differentiation of naive Th cells into the Th2 pathway can necessarily activate CD4⁺NK1.1⁺, CD1-restricted, T cells. A more likely possibility is that the source of IL-4 in the primary response is the naive CD4⁺ T cells themselves. This possibility has recently been supported by several findings. First, low intensity signalling of the TCR, such as that mediated by low peptide doses or by mutant peptides, led to secretion of low levels of IL-4 by murine naive T cells [24]. Second, human CD45RA⁺ (naive) adult peripheral blood T cells, as well as human neonatal T cells, were found to develop into IL-4-producing cells in the absence of any pre-existing source of IL-4, even in the presence of anti-IL-4 antibodies [25,26]. Finally, high proportions of T-cell clones showing a clear-cut Th2 profile of cytokine production can be generated from single CD4⁺ T cells isolated from thymus of small children (MC Mingari *et*

al., unpublished data). Thus, evidence is accumulating to suggest that the maturation of naive T cells into the Th2 pathway depends mainly on the level of, and the kinetics of, IL-4 production by naive T cells themselves at priming. These are likely to be determined by the genetic background of the individual and the nature and the intensity of TCR signalling by the peptide ligand. Obviously, when CD1-restricted antigens are expressed on APCs, CD4⁺NK1.1⁺ T cells, which rapidly release high amounts of IL-4, may also contribute to the development of the Th2 pathway.

Mechanisms responsible for development of Th2-like responses in atopic people

The demonstration that the production of IgE antibodies is strictly dependent upon the production of IL-4 (and/or IL-13) suggests that the mechanism(s) underlying heightened IgE responsiveness seen in atopic diseases (i.e. non-cognate regulation of IgE responsiveness) could primarily involve the development and/or the functional capability of Th cells. Such a possibility was strongly supported by experiments performed in the past few years in my laboratory.

First, it was found that CD4⁺ T-cell clones from atopic individuals were able to produce noticeable amounts of IL-4 and IL-5 in response to bacterial antigens, such as PPD and streptokinase, that usually evoke responses with a restricted Th1-like cytokine profile in nonatopic individuals [34]. Moreover, T-cell clones generated from cord blood lymphocytes of newborns with atopic parents produced higher concentrations of IL-4 than neonatal lymphocytes of newborns with nonatopic parents (M-P Piccinni *et al.*, unpublished data). More recently, large panels of *Parietaria officinalis* group 1 (Par O 1)-specific T-cell clones were generated from donors with low or high serum IgE levels and assessed for their profile of cytokine production and reactivity to two immunodominant Par O 1 peptides (p92 and p96). Interestingly, both p92-specific and p96-specific T-cell clones generated from 'high IgE' donors produced remarkable amounts of IL-4 and low levels of IFN-γ. In contrast, T-cell clones generated from 'low IgE' donors showed a different profile of cytokine production: the majority of those specific for p96 produced high amounts of both IL-4 and IFN-γ, whereas most p92-specific T-cell clones showed a Th1-like profile (high IFN-γ and low IL-4) (P Parronchi, S Sampognaro, L Giannarini, S Romagnani, E Maggi, unpublished data). Taken together, these data strongly suggest that allergen peptide ligand can influence the cytokine profile of Th cells; however, mechanisms underlying non-cognate regulation of IgE responsiveness are overwhelming.

The genetic alterations that are possibly responsible for immune disorders of atopic individuals are still unclear. Regarding a potential genetic link between atopy and specific IgE response, several studies have underlined the potential role of both MHC class II haplotype

[35], but the data remain controversial. With regard to TCR repertoire, a gene (or genes) in the α/δ complex has been described to influence the development of a specific IgE response in allergic subjects [36]. Restricted usage of V α 13 by T-cell clones specific for *Lolium perenne* group 1 (Lol p 1) was also reported [37]. More recently, we found an intra- and interindividually restricted usage of TCR variable regions in both Lol p 1-specific and *Poa pratensis* group 9 (Poa p 9)-specific T-cell lines (P Parronchi *et al.*, unpublished data; A Daser, P Parronchi, U Whan, S Romagnani, H Renz, unpublished data). On the other hand, the evidence for linkage of the IgE response to markers in chromosome 5q31.1, especially to the IL-4 gene [38], suggests that one or more polymorphisms exist in a coding region or, more probably, a regulatory region of the IL-4 gene.

Several studies have identified potential mechanisms governing the IL-4 gene expression in human and murine T cells. Transcription of IL-4 gene is stringently regulated by multiple promoter elements acting together [39,40]. Thus, the more likely possibility is that atopic subjects have an altered regulation at level of the IL-4 gene. Numerous genes map within 5q31.1, however, including IL-13, which might influence IgE production. Other possible candidates are IRF1, whose gene product upregulates IFN- α production, which in turn can downregulate IgE production and inhibit Th2-cell development [41] and IL-12B, which encodes the β chain of IL-12, a known inducer of IFN- γ production by CD4 $^{+}$ T cells, CD8 $^{+}$ T cells and by natural killer cells [42], as well as a powerful inducer of Th1 responses [43]. In this respect, it is noteworthy that depletion with a sublethal dose of ricin of a subpopulation of CD8 $^{+}$ T cells that produce IFN- γ results in a massive increase in the level of serum IgE [44]. More importantly, IFN- γ released by MHC class I-restricted CD8 $^{+}$ $\gamma\delta^{+}$ T cells prevents the development of Th2-like cells in response to nonreplicating antigens presented at mucosal surfaces [45,46].

Recent experiments performed in my laboratory also suggest a possible role for CD8 $^{+}$ T cells in controlling the allergen-specific Th2 response in humans. First, challenge with the Par O 1 peptide 92 (see above) resulted in the expansion of higher numbers of CD8 $^{+}$ T-cell clones that were 'low', rather than in those that were 'high', IgE producers. In addition, lactalbumin resulted in the expansion of higher numbers of CD8 $^{+}$ T-cell clones in nonatopic, rather than in atopic milk-sensitive, donors (P Parronchi, S Sampognaro, L Giannarini, E Maggi, S Romagnani, unpublished data), suggesting that allergen-specific CD8 $^{+}$ T cells may play an important role (via IFN- γ production?) in shifting the response of CD4 $^{+}$ Th cells to the same allergen from Th2 to Th1.

Thus, either alterations of molecular mechanisms directly involved in the regulation of IL-4 gene expression, or deficient regulatory activity of cytokines responsible for the inhibition of Th2-cell development (such as IFN- α/γ and IL-12), or both, may account for the

preferential Th2-type response towards environmental allergens in atopic people. An overexpression of other cytokine agents (IL-3, IL-5, granulocyte-macrophage colony-stimulating factor [GM-CSF] and IL-9) located together with IL-4 and IL-13 within the same cluster of chromosome 5 may also be present. The overexpression of the above genes can account for the preferential development of Th2 cells in response to allergens, as well as for the production by Th2 cells, and even by other cell types, of the cytokines involved in the allergic inflammation and can therefore explain the persistent histological, pathophysiological and clinical aspects of allergic disorders.

Novel therapeutic strategies now possible

The new insights into the pathogenesis of allergic disorders, as well as dramatic biotechnological advances, have provided novel opportunities for intervening more efficiently in the allergic disease process. Important technical progress was achieved when several groups started to isolate and analyze cDNAs coding for allergens. Current forms of *in vitro* and *in vivo* diagnosis of allergic diseases might be improved as soon as a complete panel of recombinant allergens covering plant, mite, animal and fungal allergy will be available for routine testing of allergic patients. Moreover, due to extensive cross-reactivities at level of allergen T-cell epitopes, it may be possible to use a few recombinant model allergens that bind a high percentage of specific IgE for the classic immunotherapy regimen. This field has been discussed in this issue by Valenta and Kraft (pp 751–756).

The considerable progress in our understanding of the mechanisms underlying the pathogenesis of atopic disorders has also stimulated the development of alternative immunotherapy regimens. Immunotherapy can be addressed to target allergen-specific T cells (allergen-specific immunotherapy) or their effector molecules (non allergen-specific immunotherapy). Novel approaches to allergen-specific immunotherapy consist of: induction of T cell anergy with allergen-derived peptides; induction of T-cell class switch (from Th2 to Th0/Th1). Non allergen-specific immunotherapy is based on antagonizing Th2 cytokines and anti-IgE therapies (Fig. 1).

Allergen-specific immunotherapies

The *in vitro* and *in vivo* experimental models of peptide modulation of allergen-specific responses are extensively discussed in this issue by Hoyne *et al.* (pp 757–761). Preliminary results of clinical studies using the first peptide employed in therapy have recently been reported [46]. Treatment with this T-cell reactive (but not B-cell reactive) peptide, obtained from the major cat dander allergen (Fel d 1), appeared to be free of toxicity and, after a short cycle of four weekly injections, resulted in a significant improvement of symptoms, as compared with the placebo-treated group. More importantly, neither significant side effects, nor induction of anti-peptide IgE

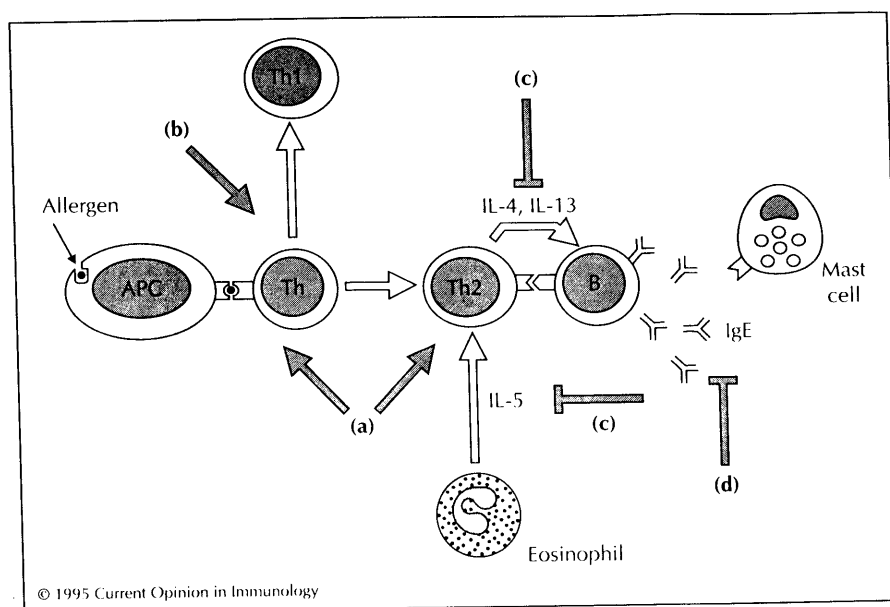


Fig. 1. Novel allergen-specific (a,b) and non-allergen-specific (c,d) immunotherapeutic strategies for allergic disorders. (a) induction of T-cell anergy or Th2-cell tolerance; (b) induction of T cell class switch from Th2 to Th1 (allergen-specific); (c) antagonizing Th2 cytokines (IL-4, IL-5 and IL-13); (d) targeting B-cell membrane and soluble IgE (allergen-non-specific).

antibodies, were observed in any of the peptide-treated patients [47].

At present, the induction of a T cell class switch, which is discussed in this issue by Daser *et al.* (pp 762–770), is one of the hottest topics, not only for the treatment of allergy, but of other immunopathological conditions, such as chronic infectious diseases and autoimmune disorders. As Th2-type responses against common environmental allergens do represent the initial triggering event in the allergic cascade, the possibility to switch the T-cell response toward a less dangerous pathway appears of primary importance in the development of a new efficient form of specific immunotherapy. A very important issue is how to achieve such a switch. One possibility may be to downregulate established allergen-specific Th2 responses (i.e. by acting at level of memory T cells). Downregulation of Th2 cells might result from either induction of tolerance, as well as by selective manipulation of cytokine secretion patterns (reduction of IL-4 production and increase in IFN- γ production). Indeed, although several studies in the animal models have previously demonstrated a failure to tolerize Th2 clones [48,49], more recent studies have demonstrated tolerance induction in both the Th1-like and Th2-like subpopulations [50,51]. More importantly, such a possibility has been supported by recent studies performed in allergic patients treated with the traditional immunotherapy regimen [12–14]. Tolerization of antigen-specific Th2 cells might be achieved by induction of high dose anergy by peptides [52] or by their partial activation with altered peptide ligands [53].

In my opinion, however, a potentially more successful approach would be the upregulation of allergen-specific Th1 responses, which is directed to prime naive T cells in a manner that selects for a prevalent Th1 phenotype. Predominant secretion of Th1-type

cytokines in response to allergen will not only result in the development and recruitment of different effector responses (IgG production and macrophage activation instead of IgE production and eosinophil activation), but it will also probably inhibit the function of established Th2 responses as a result of cross-regulatory circuits. Indeed, although *in vitro* generated Th2 cells could not be converted into IFN- γ producers, in as much as they lose their sensitivity to IL-12 [54], compelling evidence that established Th2 responses can be shifted *in vivo* to Th1-like responses has been provided in both mice [55] and humans [14]. Upregulation of allergen-specific Th1 responses has already been achieved both *in vitro* and *in vivo* by using cytokines, such as IFN- γ or IFN- α [41,56] and IL-12 [43]. More recently, we have found that, at least *in vitro*, IFN- α not only modulates the cytokine profile, but also affects the T-cell repertoire of allergen-specific T cells (P Parronchi *et al.*, unpublished data). Alternative ways to upregulate Th1-type responses to allergens may be the use of recombinant *Mycobacteria* or other adjuvants, APCs that select for the Th1 phenotype, or plasmid DNA (allergen epitope plus cytokine) — gene therapy.

Non-allergen-specific immunotherapies

Other potentially efficient therapeutic strategies involve targeting the effector molecules produced as a consequence of activation of Th2 cells (non-allergen-specific immunotherapy). This approach has become conceptually available after evidence was accumulated to suggest that Th2 responses are probably not critical for survival and protection. First, IL-4-deficient mice, which do not develop Th2 responses, are better protected than wild-type animals against the great majority of infections [18]. Th2 cells are indeed more protective than Th1 cells only against gastrointestinal nematodes; Th2 cells do not kill nematodes, but make their life habitat unpleasant via

the effects exerted by IL-4 on gastrointestinal function [57]. At present, however, gastrointestinal nematodes do not represent a health problem, at least in developed countries. Subjects homozygous for deletions involving the ϵ gene, however, appear perfectly normal [58], suggesting that at least one of the main IL-4-dependent effector mechanisms (i.e. production of IgE antibody) is not critical for human life, or its quality. IL-4 activity may be antagonized by soluble IL-4 receptor [59] or more effectively by a human IL-4 mutant protein, described in this issue by de Vries *et al.* (pp 771–778). Interestingly, this protein also antagonizes the biological activity of IL-13, as well as the IL-4-driven differentiation of Th2 cells *in vitro*. IL-5 activity has now been antagonized by humanized antibodies to IL-5, which appeared to be capable of inhibiting eosinophil infiltration and normalizing airway hyperactivity in monkeys challenged with *Ascaris suis* [60,61]. IL-5 activity has also been antagonized by a IL-5-specific gene transcription inhibitor [62]. Finally, 'intelligent' humanized anti-human IgE antibodies have recently been prepared. These antibodies, which are described by Paula Jardim in this issue (pp 779–782), bind to IgE residues critical for receptor binding, thus reacting with both membrane IgE from B cells and soluble IgE, but not with IgE bound to mast cells or basophils. Human preclinical safety of anti-IgE therapy appears to be satisfying and clinical studies look very promising.

References

- Mosmann TR, Coffman RL: **TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties.** *Annu Rev Immunol* 1989, 7:145–173.
- Romagnani S: **Lymphokine production by human T cells in disease states.** *Annu Rev Immunol* 1994, 12:227–257.
- Romagnani S: **Biology of human Th1 and Th2 cells.** *J Clin Immunol* 1995, 15:121–126.
- Wierenga EA, Snoek M, De Groot C, Chretien I, Bos JD, Jansen HM, Kapsenberg ML: **Evidence for compartmentalization of functional subsets of CD4⁺ T lymphocytes in atopic patients.** *J Immunol* 1990, 144:4651–4656.
- Parronchi P, Macchia D, Piccinni M-P, Biswas P, Simonelli C, Maggi E, Ricci M, Ansari AA, Romagnani S: **Allergen- and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production.** *Proc Natl Acad Sci USA* 1991, 88:4538–4542.
- Maggi E, Biswas P, Del Prete GF, Parronchi P, Macchia D, Simonelli C, Emmi L, De Carli M, Tiri A, Ricci M, Romagnani S: **Accumulation of Th2-like helper T cells in the conjunctiva of patients with vernal conjunctivitis.** *J Immunol* 1991, 146:1169–1174.
- Van der Heijden FL, Wierenga EA, Bos JD, Kapsenberg ML: **High frequency of IL-4-producing CD4⁺ allergen-specific T lymphocytes in atopic dermatitis lesional skin.** *J Invest Dermatol* 1991, 97:389–394.
- Hamid Q, Azzawi M, Ying S, Moqbel R, Wardlaw AJ, Corrigan CJ, Bradley B, Durham SR, Collins JV, Jeffery PK *et al.*: **Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma.** *J Clin Invest* 1991, 87:1541–1546.
- Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, Corrigan CJ, Durham SR, Kay AB: **Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma.** *N Engl J Med* 1992, 326:295–304.
- Del Prete GF, De Carli M, D'Elis MM, Maestrelli P, Ricci M, Fabbri L, Romagnani S: **Allergen exposure induces the activation of allergen-specific Th2 cells in the airway mucosa of patients with allergic respiratory disorders.** *Eur J Immunol* 1993, 23:1445–1449.
- Varney VA, Hamid Q, Gaga M, Ying S, Jacobson M, Frew AJ, Kay AB, Durham SR: **Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses.** *J Clin Invest* 1993, 92:644–651.
- 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.
- 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.
- Del Prete GF, De Carli M, Almerigogna F, Daniel CK, D'Elis MM, Zancuoghi G, Pizzolo G, Romagnani S: **Preferential expression of CD30 by human CD4⁺ T cells producing Th2-type cytokines.** *FASEB J* 1995, 9:81–86.
- Swain SL: **IL-4 dictates T-cell differentiation.** *Res Immunol* 1993, 144:616–620.
- Seder RA, Paul WE, Davis MM, Fazekas de St Groth B: **The presence of interleukin 4 during *in vitro* priming determines the lymphocyte-producing potential of CD4⁺ T cells from T cell receptor transgenic mice.** *J Exp Med* 1992, 176:1091–1098.
- Maggi E, Parronchi P, Manetti R, Simonelli C, Piccinni M-P, Santoni-Rugiu F, De Carli M, Ricci M, Romagnani S: **Reciprocal regulatory role of IFN- γ and IL-4 on the *in vitro* development of human Th1 and Th2 clones.** *J Immunol* 1992, 148:2142–2147.
- Kopf M, Le Gros G, Bachmann M, Lamers MC, Bluthmann H, Kohler G: **Disruption of the murine IL-4 gene blocks Th2 cytokine responses.** *Nature* 1993, 362:245–248.
- Bradding P, Feather IH, Howarth PH, Mueller R, Roberts JA, Britten K, Bews JPA, Hunt TC, Okayama Y, Heusser CH *et al.*: **Interleukin 4 is localized and released by human mast cells.** *J Exp Med* 1992, 176:1381–1386.
- Brunner T, Heusser CH, Dahinden CA: **Human peripheral blood basophils primed by interleukin 3 (IL-3) produce IL-4 in response to immunoglobulin E receptor stimulation.** *J Exp Med* 1993, 177:605–611.
- Moqbel R, Ying S, Barkans J, Newman TM, Kimmitt P, Vakeelin M, Taborda-Barata L, Meng Q, Corrigan CJ, Durham SR, Kay AB: **Identification of mRNA for interleukin-4 in human eosinophils with granule localization and release of the translated product.** *J Immunol* 1995, in press.
- Gollob KJ, Coffman RL: **A minority subpopulation of CD4⁺ T cells directs the development of naive CD4⁺ T cells into IL-4-secreting cells.** *J Immunol* 1994, 152:5180–5188.
- Yashimoto T, Paul WE: **CD4^{pos}, NK1.1^{pos} T cells promptly produce interleukin 4 in response to *in vivo* challenge with anti-CD3.** *J Exp Med* 1994, 179:1285–1295.
- Pfeiffer C, Stein UJ, Southwood S, Ketelaar H, Sette A, Bottomly K: **Altered peptide ligands can control CD4 T lymphocyte differentiation *in vivo*.** *J Exp Med* 1994, 181:1569–1574.
- Kalinski P, Hilkens CMU, Wierenga EA, Van der Pouw-Kraan TCTM, Van Lier RAW, Bos JD, Kapsenberg ML, Sijndewint FGM: **Functional maturation of human naive T helper cells in the absence of accessory cells. Generation of IL-4-producing T helper cells does not require exogenous IL-4.** *J Immunol* 1995, 154:3753–3760.
- Demeure CE, Yang LP, Byun DG, Ishihara H, Vezzio N, Delespessis G: **Human naive CD4 T cells produce interleukin-4 at priming and acquire a Th2 phenotype upon repetitive stimulations in neutral conditions.** *Eur J Immunol* 1995, 25:in press.
- Wershil BK, Theodos CM, Galli SJ, Titus RG: **Mast cells augment lesion size and persistence during experimental *Leishmania major* infection in the mouse.** *J Immunol* 1994, 152:4563–4571.
- Schmitz J, Thiel A, Kuhn R, Rajewsky K, Muller W, Assenmacher M, Radbruch A: **Induction of interleukin 4 (IL-4)**

- expression in T helper (Th) cells is not dependent on IL-4 from non-T cells. *J Exp Med* 1994, 179:1349-1353.
29. Finkelman FD, Urban JF: Cytokines: making the right choice. *Parasitol Today* 1992, 8:311-314.
 30. Macatonia SE, Hosken NA, Litton M, Vieira P, Hsieh C-S, Culpepper JA, Wysocka M, Trinchieri G, Murphy KM, O'Garry A: Dendritic cells produce IL-23 and direct the development of Th1 cells from naive CD4⁺ T cells. *J Immunol* 1995, 154:5071-5079.
 31. Bendelac A, Lantz O, Quimby ME, Yewdell JW, Bennink JR, Butkiewicz RR: CD1 recognition by mouse NK1.1⁺ T lymphocytes. *Science* 1995, 268:863-865.
 32. Yashimoto T, Bendelac A, Watson C, Hu-Li J, Paul WE: CD-1-specific, NK1.1^{pos} T cells play a key *in vivo* role in a Rh2 response and in IgE production. *Science* 1995, in press.
 33. Yashimoto T, Bendelac A, Hu-Li J, Paul WE: Defective IgE production by SJL mice is linked to the absence of a subset of T cells that promptly produce IL-4. *Proc Natl Acad Sci USA* 1995, in press.
 34. Parronchi P, De Carli M, Manetti R, Simonelli C, Piccinni M-P, Macchia D, Maggi E, Del Prete G-F, Ricci M, Romagnani S: Aberrant interleukin (IL)-4 and IL-5 production *in vitro* by CD4⁺ helper T cells from atopic subjects. *Eur J Immunol* 1992, 22:1615-1620.
 35. Marsh DG, Lockhart A, Holgate ST: *The Genetics of Asthma*. Oxford: Blackwell Scientific Publications; 1993.
 36. Moffatt MF, Hill MR, Cornelis F, Schou C, Faux JA, Young RP, James AL, Ryan G, Souef P, Musk AW *et al.*: Genetic linkage of T-cell receptor α/δ complex to specific IgE response. *Lancet* 1994, 343:1597-1600.
 37. Mohapatra SS, Mohapatra S, Yang M, Ansari AA, Parronchi P, Maggi E, Romagnani S: Molecular basis of cross-reactivity among allergen-specific human T cells: T cell receptor V gene usage and epitope structure. *Immunology* 1994, 81:15-20.
 38. Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich-Kautzky E, Schou C, Krishnaswamy G, Beaty TH: Linkage analysis of IL-4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 1994, 264:1152-1156.
 39. Murphy KM, Murphy TL, Gold JS, Szabo SJ: Current understanding of IL-4 gene regulation in T cells. *Res Immunol* 1993, 144:575-578.
 40. Matsuda I, Nato Y, Arai K, Arai N: The structure of IL-4 gene and regulation of its expression. *Res Immunol* 1993, 144:569-574.
 41. Parronchi P, De Carli M, Manetti R, Simonelli C, Sampognaro S, Piccinni M-P, Macchia D, Maggi E, Del Prete G-F, Romagnani S: IL-4 and IFN(s) (alpha and gamma) exert opposite regulatory effects on the development of cytolytic potential by Th1 or Th2 human T cell clones. *J Immunol* 1992, 149:2977-2982.
 42. Chehimi J, Trinchieri G: Interleukin-12: a bridge between innate resistance and adaptive immunity with a role in infection and acquired immunodeficiency. *J Clin Immunol* 1994, 14:149-161.
 43. Manetti R, Parronchi P, Giudizi M-G, Piccinni M-P, Maggi E, Trinchieri G, Romagnani S: Natural killer cell stimulatory factor (interleukin 12) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4 producing Th cells. *J Exp Med* 1993, 177:1199-1204.
 44. Kemeny M: The role of CD8⁺ T cells in the regulation of IgE. *Clin Exp Allergy* 1993, 23:466-470.
 45. McMenamin C, Holt PG: The natural immune response to inhaled soluble protein antigens involves major histocompatibility complex (MHC) class I-restricted CD8⁺ T cell-mediated but MHC class-II-restricted CD4⁺ T cell-dependent immune deviation resulting in selective suppression of immunoglobulin E production. *J Exp Med* 1993, 178:889-899.
 46. McMenamin C, McKersy M, Kuhnlein P, Hunig T, Holt PG: $\gamma\delta$ T cells down-regulate primary IgE responses in rats to inhaled soluble protein antigens. *J Immunol* 1995, 154:4390-4394.
 47. Norman PS, Ohman JL, Long AA: Clinical experience with T cell reactive peptides from cat allergen Fel d 1. *J Allergy Clin Immunol* 1994, 93:231.
 48. Gilbert KM, Hoang KD, Wiegler WO: Th1 and Th2 clones differ in their response to a tolerogenic signal. *J Immunol* 1990, 144:2063-2071.
 49. Williams ME, Shea CM, Lichtman AH, Abbas AK: Antigen receptor-mediated anergy in resting T lymphocytes and T cell clones: correlation with lymphokine secretion patterns. *J Immunol* 1992, 149:1921-1926.
 50. Romball CG, Wiegler WO: *In vivo* induction of tolerance in murine CD4⁺ subsets. *J Exp Med* 1993, 178:1637-1644.
 51. Garside P, Steel M, Worthey EA, Satoskar A, Alexander J, Bluethmann H, Liew FY, Mowat AM: T helper 2 cells are subject to high dose oral tolerance and are not essential for its induction. *J Immunol* 1995, 154:5649-5655.
 52. Yssel H, Fasler S, Lamb J, de Vries J: Induction of non-responsiveness in human allergen-specific type 2 T helper cells. *Curr Opin Immunol* 1994, 6:847-852.
 53. Sloan-Lancaster J, Evavold BD, Allen PM: Th2 cell clonal allergy as a consequence of partial activation. *J Exp Med* 1994, 180:1195-1205.
 54. Perez VL, Lederer JA, Lichtman AH, Abbas AK: Stability of Th1 and Th2 population. *Int Immunol* 1995, 7:869-874.
 55. Nabors GS, Alfonso LCC, Farrell JP, Scott P: Switch from a type 2 to a type 1 T helper cell response and cure of established *Leishmania major* infection in mice is induced by combined therapy with interleukin 12 and pentostam. *Proc Natl Acad Sci USA* 1995, 92:3142-3146.
 56. Lack G, Renz H, Saloga J, Bradley KL, Loader J, Leung DYM, Larsen G, Gelfand EW: Nebulized but not parenteral IFN- γ decreases IgE production and normalizes airways function in a murine model of allergen sensitization. *J Immunol* 1994, 152:2546-2554.
 57. Urban JF, Maliszewski CR, Madden KB, Katona IM, Finkelman FD: IL-4 treatment can cure established gastrointestinal nematode infections in immunocompetent and immunodeficient mice. *J Immunol* 1995, 154:4675-4684.
 58. Brusco A, Cariota U, Bottaro A, Boccazzi C, Plebani A, Ugazio AG, Galanella R, Guerra MG, Carbonara A: Variability of the immunoglobulin heavy chain constant region locus: a population study. *Hum Genet* 1995, 95:319-326.
 59. Renz H, Enssle K, Lauffer L, Kurrel R, Gelfand EW: Inhibition of allergen-induced IgE and IgG1 production by soluble IL-4 receptor (sIL-4R). *Int Arch Allergy Immunol* 1995, 106:46-54.
 60. Egan RW, Athwahl D, Chou C-C, Emtage S, Jehn C-H, Kung TT, Mauser PJ, Murgolo NJ, Bodmer MW: Inhibition of pulmonary eosinophilia and hyperreactivity by antibodies to interleukin-5. *Int Arch Allergy Immunol* 1995, 107:321-322.
 61. Kung TT, Stelts DM, Zurcher JA, Adams GK, Egan RW, Kreutner W, Watnick AS, Jones H, Chapman RW: Involvement of IL-5 in the murine model of allergic pulmonary inflammation: prophylactic and therapeutic effect of an anti-IL-5 antibody. *Am J Respir Cell Mol Biol* 1995, 13:360-365.
 62. Mori A, Suko M, Kaminuma P, Nishizaki Y, Nagahori T, Mikami T, Ohmura T, Hosino A, Hasakura Y, Okudaira H: Enhanced production and gene expression of IL-5 in bronchial asthma. Possible management of atopic diseases with IL-5 specific gene transcription inhibitor. In *Molecular Biology of Allergens and the Atopic Immune Response*. Edited by Sehon A, Kraaft D. New York: Plenum Press; 1995: in press.

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