

Regulation of IgE synthesis by T cells and cytokines

Régulation de la synthèse d'IgE par les cellules T et les cytokines

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RÉSUMÉ : L'atopie se caractérise par une surproduction d'IgE, invitant à considérer qu'il existe une dysrégulation de la synthèse de ces immunoglobulines et même en amont des anomalies du switching physiologique d'isotypes, au niveau du DNA. De nombreuses expérimentations *in vitro* ont montré que, à la suite de l'activation de lymphocytes T helper (CD4⁺), ces cellules sécrètent l'interleukine 4 qui induit la synthèse d'IgE à partir de lymphocytes B. Sur l'ensemble des lymphocytes B, la moitié ou le tiers se révèle capable de produire des IgE et des IgG, après avoir subi un switching transformant les B IgM⁺ en B IgG4⁺ puis ceux-ci en B IgE⁺. Si IL-4 est la seule interleukine capable d'induction, d'autres jouent un rôle modulateur : TNF alpha IL-5 et IL-6 amplifient la réponse IgE. Inversement, les interférons alpha et gamma, le TGF bêta et l'IL-10 l'inhibent. IL-10 inhibe à un stade précoce, en empêchant l'activation des CD4⁺. Les interférons inhibent la réponse à un stade ultérieur, interférant avec les stimuli activateurs émis par les CD4⁺. Le TGF bêta bloque l'induction de l'ARN messenger qui commande la synthèse Σ . Le TNF alpha stimule au contraire la transcription de l'ARN messenger pour Σ . IL-5 et IL-6 favorisent la synthèse d'IgE en agissant tardivement sur la prolifération des B Σ . L'interleukine 4 interagit avec la sécrétion de ces cytokines. Elle inhibe la production d'IL-6 TNF alpha et IL-10, et d'interféron gamma. La synthèse d'IgE induite par IL-4 est donc le résultat d'interactions cellulaires complexes. *In vivo*, on a pu montrer, chez les sujets atopiques, un taux élevé du récepteur soluble pour IL-4, CD23. A partir de cellules T prélevées chez eux, on obtient des taux élevés d'IL-4 et bas d'INF gamma, après activation, profil contraire à celui observé chez les sujets normaux. Les mêmes constatations ont été faites à partir de clones CD4⁺ spécifiques d'allergènes : taux élevés d'IL-4 et IL-5, taux bas d'IL-2 et INF gamma. Ces clones se révèlent capables d'induire la synthèse d'IgE à partir de B co-cultivés. Il a pu être prouvé que ceci s'exerçait par l'intermédiaire d'IL-4. En rendant ces CD4⁺ anergiques par un peptide de synthèse, correspondant à un épitope de la molécule de *Der p 1*, on n'observe ni synthèse d'IL-4, ni synthèse d'IgE. Cet état anergique peut être supprimé par l'interleukine 2. Des essais thérapeutiques par INF alpha et gamma chez des patients ayant un syndrome d'hypergammaglobulinémie E et un eczéma grave ont montré la disparition temporaire de l'eczéma et une diminution importante des taux d'IgE. La possibilité de bloquer la synthèse d'IgE en empêchant l'activation des CD4⁺ spécifiques d'un allergène, par des peptides de synthèse, est à envisager.

INTRODUCTION

The effector functions of antibodies are determined by the constant region of the immunoglobulin heavy chain. Antibodies retain their specificity, while their effector functions are changed by isotype switching at the DNA level [reviewed in 23]. IgE antibodies have the property to bind to high affinity IgE receptors on mast cells and basophils, and can cause these cells to degranulate when they are crosslinked by allergen. Therefore, human IgE responses to intravascular immunogens can lead to immediate type hypersensitivity reactions but, on the other hand, they may protect against extravascular parasites [1, 12, 14].

In early studies it has been demonstrated that the induction of most immunoglobulin isotypes is regulated by T cells [13, 17, 24]. Only recently, it has become clear that cytokines produced during

the immune response detect isotype switching [reviewed in 3].

Overproduction of IgE has been associated with various diseases, including atopy, which is a familial allergic disorder that results from hypersensitivity to allergens. During the last five years, significant progress has been made in understanding the cellular and molecular mechanisms underlying regulation of human IgE synthesis. Defining the regulatory pathways resulting in IgE switching and IgE synthesis in normal B cells are of importance for determining aberrant regulatory processes associated with overproduction of IgE in atopy. In addition, better understanding of these regulatory processes will allow us to define novel ways to intervene efficiently in hyper IgE production and IgE mediated allergic responses. Here, we describe current knowledge about the role of T cells and their secretory products in the regulation of the human IgE response.

T CELL HELP IN THE INDUCTION OF IgE PRODUCTION

The helper T cell function of CD4⁺ T cells is determined by a contact-mediated process in which the T cells induce activation and differentiation of B cells. This process can be separated into an induction- and an effector phase. In the induction phase, resting T cells interact with antigen primed B cells. This interaction which involves the T cell receptor/antigen/MHC class II complex results in bi-directional T-B cell activation. In the effector phase, B cell proliferation and differentiation is induced by both cytokines secreted by the activated T cells and by contact-mediated stimuli [7]. The induction phase of T cell help is antigen-dependent, however, the effector phase is antigen-independent and does not require MHC restriction. This implies that, once CD4⁺ have been activated, they can interact with any B cell, including primed B cells. Bystander B cell activation and differentiation may account for increased levels of polyclonal immunoglobulin production observed during an immune response.

INTERLEUKIN-4 INDUCES IgE SYNTHESIS

Interleukin-4 (IL-4) which is a cytokine predominantly produced by CD4⁺ T cells, induces the synthesis of IgE in cultures of mononuclear cells (MNC) derived from peripheral blood, tonsils or spleen *in vitro* [20]. IL-4 also induced IgE synthesis in cultures of MNC from cord blood, indicating that cord blood B cells are mature in their capacity to switch to IgE production [19]. In contrast to adult T cells, however, cord blood derived T cells failed to produce measurable quantities of IL-4 following activation. This lack or suppression of IL-4 production may account for the absence of detectable amounts of IgE in cord- or neonatal blood [19].

The induction of IgE synthesis by IL-4 reflects isotype switching and not a selective outgrowth of a few IgE committed B cells, since single IgM⁺ B cells could be induced to proliferate and to produce IgG4 and IgE with high frequencies: one out of every two to three B cells could be induced to produce IgG4 and IgE [8]. In addition, IL-4 induced B cells to switch to IgG4 producing cells with similar frequencies. Interestingly, in many cultures initiated from single B cells, not only IgE and IgG4, but also IgM, could be detected, suggesting that switching to IgE production succeeds in two steps, namely from IgM to IgG4 and from IgG4 to IgE [8]. This latter notion was confirmed by kinetics studies which indicated that the production of IgG4 preceded the synthesis of IgE [9]. This is in line with recent murine studies in which analysis of the excised switch circular DNA lead to the conclusion that immunoglobulin class swit-

ching in murine B cells occurred in successive steps from IgM to IgG1 and from IgG1 to IgE [16].

MODULATION OF IL-4 INDUCED IgE SYNTHESIS

The capacity to induce IgE synthesis *in vitro* is a specific property of IL-4 [21], since all other known cytokines tested to date were ineffective. However, many cytokines can modulate the IL-4 induced IgE synthesis. Both IFN- γ and IFN- α have strong inhibitory effects [20-22], whereas more recently it has been shown that the induction of IgE synthesis could also be blocked by TGF- β , and IL-10 [4, 5]. On the other hand, TNF- α , IL-5 and IL-6 [10, 26] have enhancing effects.

Inhibition of IgE synthesis by IFN- γ , IFN- α , TGF- β and IL-10 occurs at early stages of the cultures [2, 4, 5] but presently, little is known about the mechanism of their antagonistic effects.

TGF- β blocks the induction of germline ϵ mRNA [4] which precedes, and is a prerequisite for, ϵ -switching [11]. IFN- α and IFN- γ efficiently block IL-4 induced IgE synthesis when CD4⁺ T cells are present, but these cytokines fail to block IgE synthesis in culture systems in which the T cells have been replaced by anti-CD40 mAb [9]. Taken together with the observation that IFN- γ and IFN- α failed to inhibit the induction of germline ϵ transcripts, these results suggest that these cytokines interfere with the costimulatory signals of the CD4⁺ T helper cells.

IL-10 blocked IL-4 induced IgE synthesis in cultures of MNC, but was ineffective when B cells were co-cultured with activated T cells and IL-4 [5]. The failure of IL-10 to block IgE synthesis once the CD4⁺ T cells were activated, indicated that IL-10 prevents activation of these cells. This prevention of T helper cell activation by IL-10 was indirect and due to inhibition of accessory cell function of monocytes [6]. Both TGF- β and IL-10 failed to block constitutive IgE production by B cells of atopic individuals. IL-5 and IL-6 both act at relatively late stages of B cell growth and differentiation and promote IgE production without isotype preference, which probably explains their enhancing effects of IL-4 dependent IgE production. This is in agreement with the observation that IL-5 and IL-6 did not modulate IL-4 induced germline ϵ transcripts, which is an early event in the IgE switch process.

In contrast, TNF- α enhances germline ϵ transcripts which probably accounts for its enhancing effects on IL-4 dependent IgE synthesis [10]. It is of importance to note that IL-4 induces interaction between MNC and modulates cytokine production by these cells, including the secretion of cytokines that modulate IL-4 dependent IgE synthesis. IL-4 inhibits the production of IL-6, TNF- α and IL-10

by activated monocytes. In addition, IL-4 blocks the IFN- γ production in cultures of activated MNC which reflects the IFN- γ production of NK cells in these cultures. Hence, IgE synthesis induced by IL-4 is the result of complicated cellular interactions in which many cytokines are involved.

REGULATION OF HUMAN IgE SYNTHESIS *IN VIVO*

The finding that IL-4 is responsible for induction of IgE synthesis *in vitro* prompted us to investigate whether IL-4 was operational *in vivo* and was associated with elevated serum IgE levels in atopic patients and patients with the hyper IgE syndrome. Although no circulatory IL-4 could be detected, indirect evidence was obtained indicating that levels of IL-4 in these patients were enhanced. Firstly, MNC of approximately 20 % of these patients produced IL-4 spontaneously *in vitro*, a phenomenon we never observed with MNC from healthy individuals. Secondly, levels of circulating soluble CD23, which is specifically induced by IL-4, were strongly enhanced in these patients, as compared to control donors. Thirdly, activated MNC of patients with elevated serum IgE levels produced significantly higher levels of IL-4 and lower levels of IFN- γ , than did MNC of healthy control donors [22].

Based on these observations, we hypothesized whether allergen-specific T cell could be responsible for enhanced levels of IgE produced in atopic patients. Indeed, CD4⁺ T cell clones, specific for the major house dust allergen *Der p* I, obtained from allergic patients had the intrinsic property to produce extremely high levels of IL-4 and IL-5, whereas they produced levels of IFN- γ and IL-2 that were in the low or non-detectable range [27]. The *Der p* I-specific T cell clones induced IgE upon cocultivation with purified B cells, which was mediated by endogenously produced IL-4. These results indicate that an aberrant IL-4/IFN- γ production ratio is already sufficient for inducing IgE synthesis *in vitro*. Based on these observations, it is tempting to speculate that high levels of IL-4 production by allergen-specific T cells could be responsible for enhancing allergen-specific IgE synthesis *in vivo*.

INDUCTION OF ANERGY IN ALLERGEN-SPECIFIC T CELL CLONES

Because of the central role that allergen-specific CD4⁺ T cells play in the induction of the human IgE response, prevention of activation of these cells and hence of their capacity to produce IL-4 may block the induction of the synthesis of IgE. One of the approaches we have followed is the use of *Der p* I derived synthetic peptides to render *Der p* I-specific T cells non-responsive [18]. Preliminary

data have indicated that incubation of T cell clones with high concentrations of a synthetic peptide, representing a T cell activation inducing epitope present on the *Der p* I molecule, induced a state of non-responsiveness in these cells. The T cell clones did not proliferate in response to stimulation with *Der p* I and failed to produce IL-4. Importantly, the cells rendered non-responsive in this way also failed to provide B cell help for IgE synthesis (YSSEL *et al.*, manuscript in preparation). The state of non-responsiveness could be reversed: culturing of the cells in IL-2 resulted in proliferation, whereas activation via a pathway not involving triggering of the T cell receptor CD3 complex, lead to the production of cytokines, indicating that cells were only unable to respond when activated specifically via the T cell receptor/CD3 complex. We are currently characterizing this state of non-responsiveness further and are exploring its usefulness in future therapeutic approaches.

IFN- α THERAPY REDUCES SERUM IgE LEVELS IN A PATIENT WITH HYPER IgE SYNDROME

The notion that IL-4 is responsible for enhanced serum levels in atopic patients and patients with the hyper IgE syndrome, combined with our finding that IFN- α inhibits both IL-4 induced [20] and ongoing IgE production by MNC of patients with elevated serum IgE levels *in vitro* [22] lead us to treat one patient with the hyper IgE syndrome and severe eczema. Administration of IFN- α twice per week for 4 weeks resulted in a 50 % reduction in serum IgE levels, without affecting the serum IgM, IgG and IgA levels in this patient [25]. In addition, the atopic dermatitis disappeared completely after two weeks of treatment. This effect was transient because the skin lesions reappeared two weeks after the treatment was stopped, but in much milder form.

Recently, KING *et al.* [15] demonstrated that administration of IFN- γ to patients with the hyper IgE syndrome also resulted in transient reduction of serum IgE levels. These data indicate that IFN- α and IFN- γ are also operational *in vivo* in reducing IgE production.

CONCLUSION

Figure 1 shows a simplified schematic representation of the role of various cytokines in the regulation of the IgE response, as well as potential targets for interference. It is clear that novel approaches to block IgE synthesis in an early stage should be aimed at inhibition or neutralization of IL-4 production *in vivo* by using IL-4 antagonists such as soluble IL-4 receptors, inhibitors of IL-4 synthesis, or compounds that interact with productive IL-4/IL-4 receptor interactions. It

CASCADE OF EVENTS IN IgE MEDIATED ALLERGIC REACTIONS

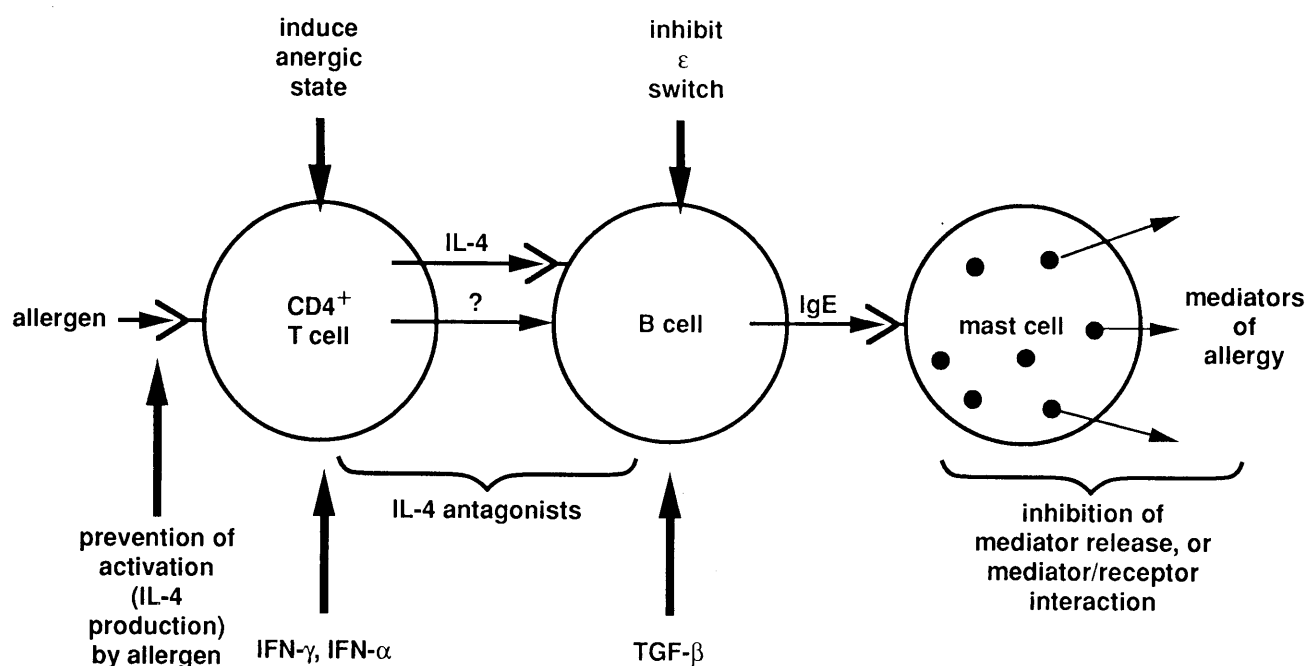


Fig. 1. — Schematic representation of the cytokines involved in the human IgE response.

is of interest to note that the first experiments carried out with « natural » IL-4 antagonists like IFN-γ and IFN-α seem to be promising in lowering serum IgE levels in patients with the hyper IgE syndrome. Their application in the treatment of severe allergy deserves further exploration.

The finding that allergen specific CD4⁺ T cell clones established from atopic patients produce unusual high levels of IL-4 following activation, indicates that treatment methods should be developed that are aimed at blocking or preventing of allergen specific activation of these T cell clones *in vivo*. One approach which we are currently exploring is to use *Der p* I derived synthetic peptides, that block T cell activation and IL-4 production, which may allow us to intervene in a precise and harmless way to prevent overproduction of IgE in allergic disease and IgE mediated hypersensitivity reactions.

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ABSTRACT : Human IgE synthesis is tightly regulated by cytokines. IgE production by normal B cells is specifically induced by IL-4, but requires additional, yet to be defined, signals that are provided by CD4⁺ T cells. Single surface IgM⁺ B cells can be induced to proliferate and switch to IgG4 and IgE producing cells, indicating that the induction of IgE synthesis by IL-4 and CD4⁺ T cells reflects direct isotype switching. Although IL-4 is the sole inducing cytokine of IgE synthesis known thus far, multiple cytokines modulate IL-4 induced IgE synthesis *in vitro*. IFN- α , IFN- γ , TGF- β and IL-10 are inhibitory, whereas IL-5, IL-6 and TNF- α act synergistically with IL-4. Results obtained with animal models, as well as from clinical studies in the human have indicated that IL-4, IFN- α and IFN- γ are operational *in vitro*. Cocultivation of B cells with allergen-specific CD4⁺ T cell clones producing high levels of IL-4 and IL-5, but normal to undetectable levels of IL-2 and IFN- γ , following activation resulted in the synthesis of IgE, in the absence of exogenously added IL-4. These results indicate that aberrant ratios of IL-4 and IFN- γ production are sufficient for induction of IgE synthesis *in vitro*. The notion that an imbalance of cytokine production may account for the high serum IgE levels in allergic patients was supported by the finding that IFN- α and IFN- γ administered to patients with the hyper IgE syndrome resulted in a selective reduction in serum IgE levels which was accompanied by an improvement of clinical symptoms.