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Determinants and Mechanisms of Human Immune Responses to Bee Venom Phospholipase A_2

Abstract

The elicitation of an immune response to protein antigens depends on the specific recognition of antigenic determinants (epitopes) by T and B lymphocytes. Bee venom phospholipase A_2 (PLA) represents the major antigen/allergen of honey bee venom. It displays three dominant immunogenic peptide and one glycopeptide T cell recognition sites. These epitopes are equally recognized by both allergic and nonallergic individuals. A mixture of the three epitope containing peptides was successfully used in specific immunotherapy of bee venom-allergic patients. Both peptide and whole bee venom immunotherapy induced a state of specific anergy in T cells. The production of specific IgE and IgG4 antibodies directly correlated with the secreted interleukin-4:γ-interferon (IL-4:IFNγ) ratio, which itself depended on the concentration of available antigen and the strength of the T cell-activating signal. This signal comprises accumulated molecular interactions delivered by engagement of the antigenic peptide/MHC class II complex with the T cell receptor (TcR). Indeed the thermodynamic laws of chemical equilibrium reactions reveal that the antigen concentration, together with the equilibration constant K_i and the related Gibbs standard free energy $\Delta G°$ of the MHC-II/Ag/TcR complex reaction, may govern the secreted IL-4:IFNγ ratio, and in consequence, differential IgE and IgG4 antibody formation. K_i includes epitope and MHC-II haplotype variability and therefore represents a measure of immunological individuality. A major B cell epitope was determined by using point-mutated PLA. Specific antigen recognition by B cells can trigger distinct cytokine profiles in T cells and contribute to the differential regulation of specific IgE and IgG4 antibodies. Our results indicate that distinct cytokine profiles inducing allergic and nonallergic responses can be attributed to thresholds of T cell activation generated by the specific binding properties of individual MHC-II molecules to immunogenic T cell epitopes and their presentation to TcR.

Key Words
Anergy
Antigen concentration
Cytokine shift
Epitopes
IgE, specific
IgG4, specific
Thermodynamics

Introduction

Antibody responses to allergens represent appropriate models to elucidate the regulatory mechanisms of different antibody classes or isotypes. Excessive IgE antibody production against antigen generally leads to immediate-type allergic reactions, whereas specific IgG antibody production, in particular of IgG4 class, reflects normal, protective immunity to the same agent. Specific antibodies to allergens are elicited after differentiation and clonal expansion of B lymphocytes. These steps are under the control of specific T lymphocytes and depend on soluble factors that are released upon T cell activation [1]. The qualitative and quantitative cytokine composition, typically characterized

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by interleukin-4 (IL-4) and IL-13 or γ-interferon (IFNγ), respectively, regulates the specific IgE and IgG4 antibody synthesis by B cells [2]. Cytokine secretion results from activation of specific T cells by antigenic peptides which are processed by antigen-presenting cells (APC) and presented in the context of MHC class II molecules to specific T cell receptors (TcR) [3]. Therefore, the nature of the single components in the ternary MHC-II/peptide/TcR complex is absolutely crucial for the development of IgE and/or IgG4 antibody responses and IgE-mediated allergic diseases or IgG4-related immunity.

A specially suited model to study specific IgE and IgG regulation in humans is provided by the immune response to bee venom (BV). In most cases BV allergy results from dysregulated IgE/IgG antibody responses alone and is not complicated by the features of a true atopic condition. The BV phospholipase A_2 (PLA) represents the major antigen in both normal immunity and bee sting allergy [4]. Of BV-allergic patients 99% react against native PLA (nPLA) and 96% against recombinant PLA (rPLA) produced in *Escherichia coli* [5]. Hyaluronidase and melittin represent minor allergenic components of the venom. PLA is a glycoprotein of 14–16 kD consisting of 134-amino acid residues with a single carbohydrate side chain at position Asn^{13} [6]. The three-dimensional structure and the biochemical function of PLA are well defined [7]. Normally, most individuals initially elicit low affinity IgG1 anti-PLA antibodies and develop high affinity IgG4 antibodies to PLA after repeated exposure [8]. In contrast, some individuals generate PLA-specific IgE antibodies and develop bee sting allergy [4, 5].

Development of IgE antibodies by B cells requires T cell contact and IL-4 for isotype switch [9], but also the isotype switch to IgG4 depends on IL-4 [10]. In human systems designated to study allergen-specific IgE and IgG4 antibody regulation in vitro [11, 12] it was demonstrated that increases in IL-4 correlated positively with IgE and negatively with IgG4 antibody production by memory B cells. Furthermore, the IL-4:IFN-γ ratios correlated with the specific IgE:IgG4 ratio. The involvement of enzymatic activity in the immunological response to PLA could be excluded by using enzymatically inactive rPLA that was mutated at His^{34}, the active center of the phospholipase, by Asn or Asp [13].

Fig. 1. T cell epitopes in BV PLA (dark) as found by overlapping synthetic peptides in specific T cell clones and PBMC of allergic and nonallergic subjects sensitized to bee sting. A T cell epitope contains the single carbohydrate side chain at position Asn^{13}. Lys^{25} which is a central part of a major B cell epitope in PLA is marked in black.

Allergic and Nonallergic Individuals Recognize the Same T Cell Epitopes in PLA

Upon specific stimulation with PLA differences in cytokine secretion between T cells from IgE-producing allergic and IgG4-producing nonallergic subjects were mostly quantitative. Therefore, other features may be of more importance for inducing distinct cytokine profiles in T cells than recognition of particular antigenic sites. By elucidating the epitope specificity of T cell clones and PBMC of sensitized individuals with 62 overlapping dodeca- and 19-octadeca-peptides, three immunodominant sites in the PLA molecule could be mapped [14–16]. These are represented by the peptides PLA40-63, PLA74-92 and PLA107-131, as indicated in figure 1.

Primary in vitro proliferative responses of unfractioned PBMC from allergic or normal hyperimmune individuals to the PLA peptides were restricted to the same immunogenic sites as found in T cell clones and no other of the overlapping PLA peptides was active in sensitized subjects (fig. 2). Same patterns of epitope reactivity were detected among all three groups of individuals. Thus, three dominant epitopes are involved in T cell recognition of PLA, independently of whether an individual is allergic or not and of its specific IgE and IgG4 antibody levels. Among BV allergic patients

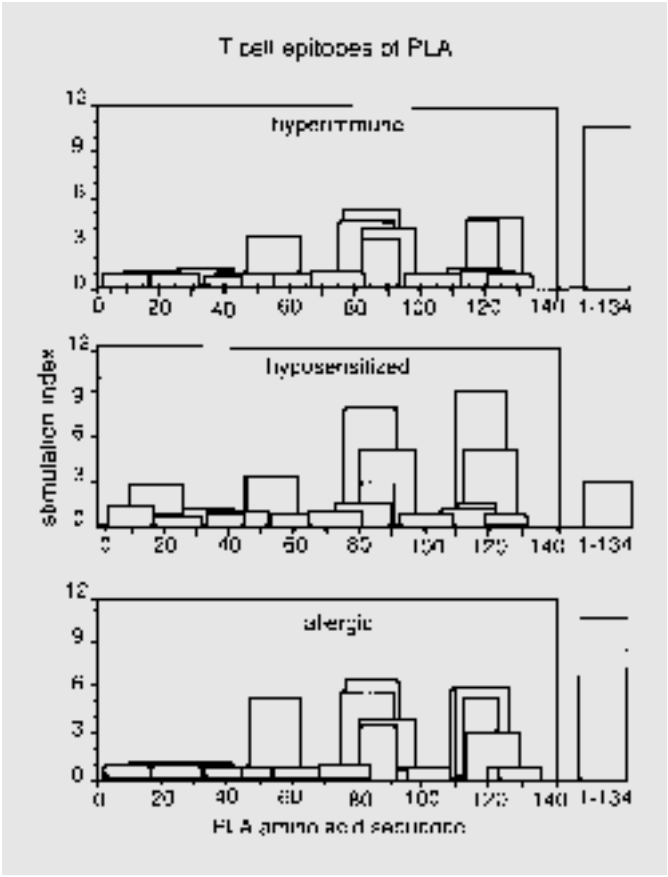

Fig. 2. T cell proliferation of unfractioned PBMC from bee sting-sensitized donors. PBMC from allergic (n = 20), hyposensitized (n = 8), and hyperimmune (n = 12) subjects were stimulated with PLA and PLA peptides. Bars show geometrical mean of the stimulation index (SI) induced at optimal peptide or PLA concentrations.

>90% responded to the peptide corresponding to PLA107-131, 70% of patients recognized PLA74-92 and 34% PLA40-63. Moreover, 70% reacted against the two epitopes PLA74-92 and PLA107-131. Only 1 out of 40 tested donors, a hyposensitized patient, did not respond significantly in the primary in vitro response to at least one of the overlapping peptides spanning the entire PLA molecule [14, 17]. Some proliferative response to PLA9-27 was observed in 1 out of 48 BV-sensitized individuals (fig. 2) and T cell reactivity was recently demonstrated against the entire C-terminal fragment of PLA spanning from amino acid 90 to 134 [16]. Probably the real reactive site may be shorter or contain more than one epitope, since we found a T cell clone reacting against PLA94-111 [Joss et al., unpubl. data]. These findings may support the existence of minor or individual determinants within a larger cluster of antigenic sites in the PLA molecule. Generally, the lower proliferative responses were found among successfully hyposensitized patients, indicating that at least partial tolerance was induced during immunotherapy. None of the immunogenic peptides bound IgE antibodies or elicited skin reactions indicating that they did not contain B cell epitopes.

Some of the T cell clones generated from allergic subjects could not be mapped to any of the linear peptide sequences in PLA, although they expressed clear reactivity to the native allergen. A few of them reacted to melittin which was bound in traces to nPLA. Using nonglycosylated nPLA and rPLA as well as a number of different glycosylated and nonglycosylated PLA analogues, a glycosylation-dependent T cell epitope was found in PLA [18]. This carbohydrate-containing epitope was recognized by PBMC and T cell clones of some allergic individuals. The recognition of this epitope was HLA-DR-restricted and not inhibitable by the carbohydrate or peptide alone. Moreover, specific proliferation of T cell clones was abrogated after stepwise enzymatic deglycosylation of nPLA [18]. Based on the experimental design, superantigenic reactivity could be excluded. So far, such glycan-depending T cell epitopes were only found in experimental animal models using synthetic antigens. Whether such epitopes play a role in the development of allergy and elicitation of IgE antibodies remains to be established.

The Generation of Distinct Cytokine Profiles by Specific Th Cells Depends on Allergen Concentration and Affinity of Antigenic Peptide to MHC Class II and TcR

A panel of CD4+ T cell clones specific to PLA was established from PBMC of human subjects allergic, hyposensitized or hyperimmune to bee sting [19]. Depending on the T cell clone, optimal stimulation required 1–100 μg/ml of PLA [19]. Upon high dose antigen stimulation, all clones produced IL-4, IFNγ and glanulocyte-macrophage-colony-stimulating factor, beside other cytokines (fig. 3). However, both absolute and relative amounts of secreted cytokines depended on the antigen concentration in the T cell culture [19, 20], and typically 10–50 times lower threshold amounts were required for IL-4 induction than for IFNγ.

The PLA-specific T cell clones could be classified according to their increasing or decreasing ratio of IL-4:IFNγ production in response to the allergen doses [20, 21]. As demonstrated in figure 3, a typical Th0 clone clearly showed a Th2 cytokine pattern at low antigen concentration [21]. Increasing antigen concentrations, however, favored

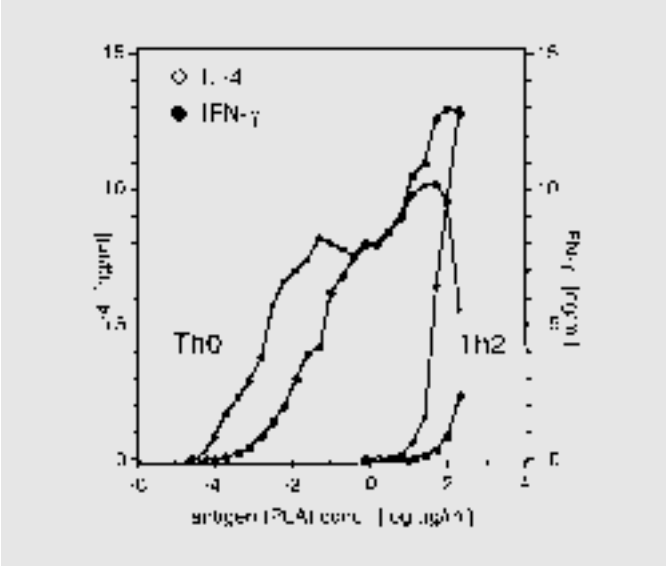

Fig. 3. Antigen concentration-dependent production of distinct cytokine profiles in a PLA-specific Th0 (BuT 1.A2) and Th2 (MeT 5.3A7) cell clone. Th0 cells require less antigen for stimulation than Th2 cells and IL-4 induction requires lower threshold amounts of the antigen than IFNγ.

Fig. 4. Substitution of amino acid residue Phe^{82} by alanine changes binding properties of epitope PLA$^{74–92}$ to HLA-DP and T cell-secreted IL-4:IFNγ cytokine ratio in favor in IFNγ.

IFNγ production, while IL-4 decreased at high antigen contents. In principle the same feature could be observed in a Th2 cell clone, however, cytokine production required higher antigen concentrations. Also T cell activation with anti-CD3 [21] induced significant IL-4 synthesis by allergen-specific Th cells, whereas IFNγ production by the same cells required stronger stimulatory signals [19–21]. These results demonstrate that particular cytokine patterns can be modulated by the dose of antigen and do not represent stable phenotypes. Thus, the individual HLA class II expression, determining the binding of antigenic peptide, influences the intensity of TcR engagement and the associated cytokine pattern [11, 21–24]. Accordingly, low antigen concentration and suboptimal antigenic peptide binding capacity of MHC class II will generate weak T cell activation, predominantly a Th2 cytokine pattern and therefore IgE antibodies [11]. Thus, at the same antigen concentration individuals with high affinity to immunogenic peptides display higher MHC-II/antigen complex density on the APC surface and induce stronger T cell activation inducing sufficient IFNγ to suppress IgE. The cytokine-regulating forces might be driven by individual HLA class II type expression and strength of epitope binding in an APC/peptide/T cell interaction [21–24]. The modulation of a T cell cytokine pattern by the dose of antigen may be a driving force for differential IgE and IgG antibody formation resulting in either allergy or immunoprotection.

Recently, it was demonstrated that altered peptide ligands (APL) can modify specific T cell activation and cytokine production by their diversity in binding the TcR or MHC class II-presenting molecules. By alanine substitution of the immunodominant epitope PLA74-92 a substantial decrease in proliferation of specific T cells and a proportional cytokine release were obtained [22]. Only the Phe^{82} substituted APL, altered in a HLA-DP binding residue, inhibited IL-4 but had no effect on IFNγ production. The location of the cytokine changing substitution is shown in figure 4. This effect of a particular amino acid substitution on cytokine secretion again appeared to result from changes in affinity to the MHC class II of the peptide ligand. Exposure of the specific T cells to APL induced anergy with abrogated proliferation and cytokine synthesis on antigen rechallenge. The defective T cell activation was associated with alterations in transmembrane signaling, specifically with a lack of tyrosine phosphorylation of the ZAP70 tyrosine kinase [22].

According to the thermodynamic laws of chemical equilibrium reactions [25], the number of MHC-II/antigenic peptide/TcR complexes between APC and T cell can be increased by higher concentrations of the given constituents (fig. 5). Under the assumption that in activated cells, at the

time of interaction, the numbers of surface-expressed MHC class II and TcR molecules are constant, and the reaction reaches an endpoint, the only accessible variable in the chemical equilibrium reaction remains the antigen concentration. Accessory molecules may also not change during interaction between an APC and the T cell. Thus, the chemical equilibrium constant K_i of the APC-T cell interaction comprises the immunological and genetic individuality of antigen recognition. Through the Gibbs law of thermodynamics [25] K_i is related to the Gibbs standard free reaction energy $\Delta G°$ of an individual's MHC-II/peptide/TcR complex forming reaction [17, 21, 23]. This individual constant K_i for T cell activation is the product of the equilibrium constants K_1 of the antigenic peptide binding to MHC-II and K_2 of the final MHC-II/peptide/TcR complex formation (fig. 5). Therefore, K_i may represent a measure for immunological individuality of antigen recognition. K_1 was determined at 10^{-6} to 10^{-8} *M* [26] and K_2 at 10^{-4} to 10^{-6} *M* [27]. Moreover, according to the Gibbs law [25] an equilibrium constant K is related to the change in free reaction energy ΔG_i of MHC-II/peptide/TcR complex formation by the equation $\Delta G° = -2.303RT \log K_i$ (R = 1.986 cal/mol; T = 315 °K at 36.8 °C). From this physicochemical relationship it follows that at suboptimal antigen doses a lower K_i (10^{-14}) is related to lower $\Delta G°$ (14.4 kcal/m^2), which is associated with the generation of IL-4-dominated cytokine patterns and IgE synthesis. In contrast, higher K_i (10^{-10}) and $\Delta G°$ (20.2 kcal/m^2) may lead to high IFNγ production and normal IgG4-related immunity. Therefore, it appears that thermodynamic reaction constants of MHC-II/peptide/TcR complex formation between APC and T cell fundamentally describe the generation of distinct states of T cell activation, cytokine patterns and finally development of either disease or normal immunity. Such allergen concentration-dependent counterregulatory effects on the secreted IL-4:IFNγ ratio and isotype production by mature B cells may reflect the physiological situation in allergen-specific immunotherapy in which repeatedly high allergen doses are injected into allergic patients over a long period of time [17, 23].

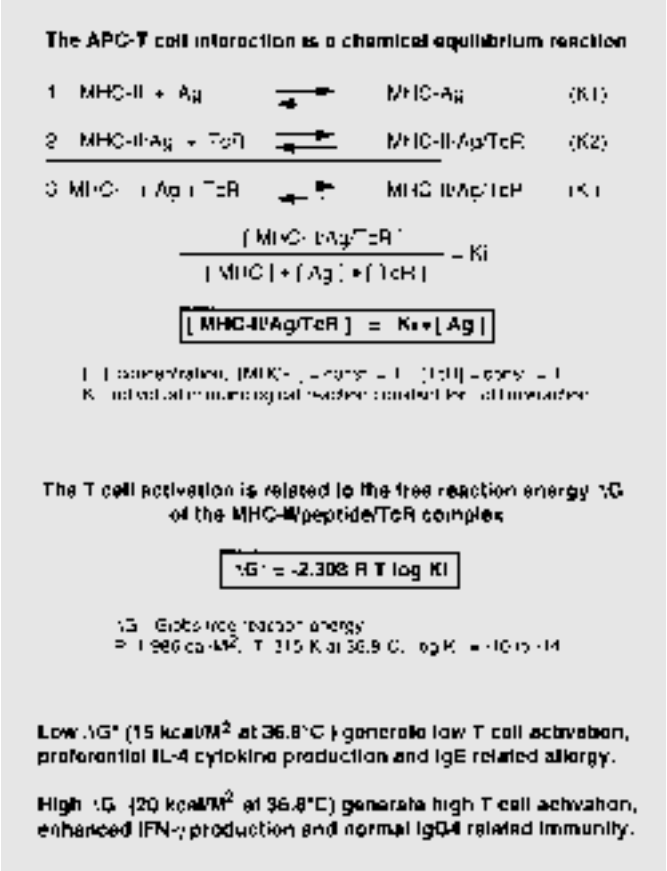

Fig. 5. The thermodynamic laws of chemical equilibrium reactions can be applied to the activation process of antigen-specific T cells and development of distinct cytokine profiles. The establishment of MHC class II/peptide/TcR complexes after APC-T cell interaction induces T cell proliferation and cytokine production. Only the antigen [Ag] concentration can be changed in this system. The constant K_i of the equilibrium reaction for the MHC class II/peptide/TcR complex is a measure for specfic antigen presentation by MHC class II and represents the immunological individuality in this reaction. According to the Gibbs law of thermodynamics, the constant K_i, consisting of the product of K1 and K2 of each single reaction step, is physicochemically related to the change in free reaction energy $\Delta G°$ of the MHC class II/peptide/TcR complex. Therefore, IL-4-dominated cytokine patterns and development of an allergic condition may be generated at lower antigen concentration and lower $\Delta G°$, whereas IFNγ production and normal immunity is favored by high allergen doses and higher $\Delta G°$ in specific molecular interaction between APC and T cells.

Antigenic T Cell Epitopes Are Also Inducers of Specific Anergy

It is an interesting observation that only a few distinct sites in the PLA antigen are antigenic. These immunogenic determinants are recognized by specific T of both allergic and nonallergic individuals and are able to generate IgE-inducing Th2 and IgG4-inducing Th0 cytokine patterns. Moreover, the same immunogenic peptides which activate T cell responses can induce a state of anergy in Th0 and Th2 clones [28, 29]. Accordingly, a mixture of immunogenic PLA peptides, each containing a single T cell epitope, was used for specific immunotherapy of patients with IgE-mediated systemic allergic reactions to BV [30]. Increasing doses of the peptide mixture were subcutaneously administered in weekly intervals, over a 2-month period. After therapy,

specific proliferative responses to PLA and the peptides were decreased in PBMC of successfully treated patients. Moreover, the production of IL-4, IL-5 and IL-13, but also of IFNγ and IL-2 was inhibited, indicating that a state of anergy was induced in T cells against the respective T cell epitopes of PLA. The three PLA peptides neither elicited immediate nor delayed skin reactions in allergic patients and did not bind IgE antibodies in vitro. Accordingly, no allergic side effects were caused by the peptide immunotherapy. The patients were challenged 1 week after therapy with PLA and later with a live bee sting. Two patients developed a mild systemic allergic reaction following the bee sting challenge, probably because of an inadequate treatment schedule or aberrant responses to BV constituents. B cells were not affected in their capacity to produce specific antibodies and serum levels of specific antibodies did not significantly change during peptide immunotherapy but increased in favor of IgG4 after PLA challenge. These results indicate that successful peptide immunotherapy is effective due to induction of epitope-specific T cell anergy to major allergen without risk of anaphylaxis.

Similar studies were undertaken in bee sting-allergic patients treated with whole BV for 2 months [31]. In this study PLA- and PLA epitope-specific T cell and cytokine responses in PBMC of bee sting-allergic patients were investigated before and after 2 months of rush immunotherapy with whole BV. After successful immunotherapy, PLA and T cell epitope peptide-specific T cell proliferation were suppressed, and the PLA- and PLA-peptide-induced secretion of type 2 (IL-4, IL-5 and IL-13), as well as type 1 (IL-2 and IFNγ) cytokines were abolished. The suppressive effect was specific as the tetanus toxoid- or PPD-induced cytokine production and proliferation remained unchanged. In spite of the allergen-specific anergy in T cells, in vitro produced anti-PLA IgE and IgG4 antibody and their corresponding serum levels slightly increased during immunotherapy, while the PLA-specific IgE:IgG4 ratio changed in favor of IgG4. These findings indicated that BV immunotherapy induces peripheral anergy in allergen-specific T cells, whereas B cells remained unaffected [31].

The molecular mechanism of anergy induction in allergen-specific T cells was further elucidated in PLA-specific T cells [28]. We have demonstrated that incubation of human CD4+ PLA-specific Th2 cells with antigenic peptide, in the absence of professional APC or a second signal, results in a state of nonresponsiveness. The anergic T cells failed to proliferate, and were unable to secrete IL-2 and IL-4 in response to optimal stimulation with PLA and autologous APC. Also the secretion of IL-5 and IL-13 was inhibited. Induction of anergy appeared to be related to alterations in signaling pathways mediated through the TcR. In contrast to untreated Th2 cells, anergized Th2 cells failed to respond to anti-CD3 mAb with either increased tyrosine kinase activity or increased levels of tyrosine phosphorylation of $p56^{Ick}$ or ZAP70. Strong and sustained intracellular calcium flux, observed in untreated Th2 cells in response to anti-CD3 mAb, was absent in anergic Th2 cells. The induction of anergy represents an active process, associated with increased levels of basal tyrosine kinase activity, cytokine production and CD25 upregulation in anergic Th2 cells [22, 28]. Together, these results indicate that anergy in Th2 cells in associated with defective transmembrane signaling through the TcR.

T cell Nonresponsiveness in Specific Immunotherapy Is Associated with Increased IL-10 Production

By treatment with whole BV the secretion of Th2 type (IL-4, IL-5 and IL–13), as well as Th1 type (IFNγ) cytokines was significantly inhibited already after 7 days of immunotherapy [32]. Simultaneously, the IL-10 production increased continuously. Intracytoplasmic staining of peripheral blood lymphocytes combined with surface marker expression as analyzed by multicolor FACS analysis revealed that IL-10 was first produced by allergen-specific CD4+CD25+ T cells and appeared later in B cells and monocytes [32]. Neutralization of IL-10 in PBMC cultures reconstituted the abolished proliferative and cytokine responses of T cells. Moreover, successful immunotherapy led to a decrease in allergen-specific IgE:IgG4 ratio. In vitro, IL-10 inhibited both allergen-specific and total IgE synthesis and enhanced IgG4 antibody formation by directly affecting memory B cells. These results indicate that increased IL-10 production during immunotherapy is responsible for peripheral anergy in specific T cells and, therefore, plays a decisive role in skewing the antigen-specific IgE:IgG4 ratio in favor of IgG4.

IL-2 and IL-15 Recover T Cells from Epitope-Specific Anergy

It is known that anergized T cells can be reactivated by IL-2 [29, 33, 34]. By culturing PBMC from BV-treated patients in the presence of the antigen and IL-2 or IL-15 the specifically anergized T cell response could be restored with respect to specific proliferation and secretion of the type 1 cytokines, IL-2 and IFNγ [31]. In contrast, IL-4, IL-

5 and IL-13 remained suppressed. Treatment of anergized T cells with IL-4 only partially restored proliferation and induced formation of a type 2 cytokine pattern at low levels. Reactivation of anergic T cells and modulation of cytokine patterns suggest an important role of microenvironmental cytokines resulting in success or failure of specific immunotherapy. In particular IL-15, being released by professional APC and various tissue cells in normal immune defense, may be of importance for establishing normal immunity. Indeed, it was demonstrated in mice that T cell tolerance can be broken by infection [35]. In contrast, long-lasting success of allergen-specific immunotherapy may be difficult to achieve in an established atopic and polyallergic environment.

Specific Antigen Recognition by B Cells Influences Differential Expression of Distinct T Cell Cytokine Profiles and Regulation of Specific Isotypes

B cells recognizing antigenic determinants can specifically take up antigen and act as APC in immune responses. The type of APC which is involved in the response strongly influences the generation of cytokine pattern by T cells [36]. By using lysine-modified or point-mutated PLA analogues we have mapped recognition sites of human IgG4/λ anti-PLA monoclonal (huMab) and serum antibodies [37, 38]. Two huMabs from the heteromyelomas BVA1 and BVA2 [37], and one from the EBV-B cell line BUB1 [21] showed identical epitope specificity involving Lys^{25} of PLA. By using lysine-modified and point-mutated PLA it was demonstrated that this residue is an essential constituent of a major binding site for human anti-PLA antibodies [37–39]. In ELISA, binding of both BVA1 and BVA2 to PLA was inhibited by each other and about 90% of serum anti-PLA IgG4 antibody binding could be inhibited by the huMabs. Although the affinity of specific antibodies to the PLA mutants and analogues varied among different individuals, these antibodies represent a major segment of the polyclonal PLA-specific antibody spectrum of each sensitized individual. Antibodies to the glycan moiety of PLA, although they were found [40], might be of minor relevance. Interestingly, nonrefolded rPLA or reduced and alkylated nPLA were not recognized by any of the huMabs or the polyclonal antisera. These observations support other reports indicating a restricted clonotype spectrum among PLA-specific antibodies of hyperimmune individuals [17, 41] and suggest a conserved antigen recognition in hyperimmune individuals.

By using structural and conformational variants of PLA which have lost conformational antibody binding properties, we have studied specific activation of T cells and secretion of cytokines regulating the specific IgE and IgG4 antibody formation [42]. PLA preparations expressing the correctly folded tertiary structure, and which were recognized by specific antibodies and B cells from bee sting-allergic patients induced high IL-4, IL-5 and IL-13 levels in vitro. In contrast, nonrefolded rPLA or reduced and alkylated nPLA generated high levels of IFNγ and IL-2. The differences in proliferation and cytokine patterns between correctly folded and nonrefolded PLA resulted from conformation-dependent involvement of different types of APC. Whereas B cells, recognizing PLA only in its natural conformation, stimulated type 2 cytokines in T cells and induced IgE antibody synthesis, monocytes nonspecifically recognized and presented nonrefolded PLA and, by high IL-1 and IL-12 production, induced an IFNγ-dominated T cell cytokine profile and IgG4 antibodies by B cells [41]. A change from specific IgE to IgG4 could be induced by nonrefolded rPLA that carried the major T cell epitopes, but was not recognized by specific antibodies and B cells of sensitized patients [41]. These findings demonstrate the pivotal role of antigen recognition by different APC, depending on the structure of the antigen, in differential regulation of specific IgE and IgG4 antibodies and the potential usage of structurally altered allergens, in particular of recombinant origin, which are selectively presented by Th1 cytokine-inducing APC in immunotherapy.

Conclusion

Relatively few linear epitopes or epitope clusters in the PLA molecule are recognized by T or B cells. The immunological features of the dominant T cell epitopes are summarized in table 1. They are able to elicit both allergic and nonallergic immune responses and can induce peripheral anergy in T cells, independent of whether an individual developed allergy or normal immunity. This argues against the existence of special allergenic epitopes and suggests that other factors may be of more relevance for Th2 cytokine and IgE antibody induction. Indeed, elicitation and regulation of IL-4 and IgE on one hand, and IFNγ and IgG4 on the other hand appear mainly to depend on an individual's ability to fully activate specific T cells to produce an entire panel of cytokines for IgG4 production. Differences between allergic and nonallergic individuals were observed in antigen concentration-dependent development of IL-4:IFNγ ratio and the threshold amounts required for T cell activation

Table 1. Immunological determinants of PLA

T cell epitopes
Contains four peptide and one glycopeptide epitopes
Epitopes are recognized by both allergic and nonallergic individuals
Lower antigen tresholds are required for IL-4 production than for IFNγ
Antigen dose-dependent decrease of IL-4:IFNγ ratio
Antigen dose-dependent decrease of IgE:IgG4 antibody ratio
Tyr^{82}-APL decreases IL-4:IFNγ ratio
Epitopes can induce peripheral T cell anergy in vivo and in vitro
Epitope peptides do not bind IgE
B cell epitopes
B cell epitopes are discontinuous
Lys^{25} is part of a major B cell epitope and antibody binding site
Reduction and alkylation of cystine prevents antibody binding to PLA
No antibody binding of unrefolded rPLA
Conformation-dependent selection of APC in PLA-specific responses
Nonconformational PLA activates phagocytes and preferentially induces Th1 cytokines and IgG4 antibodies
Conformational PLA is presented by B cells and preferentially induces Th2 cytokines and IgE

and induction of different cytokines. The generation of distinct cytokines appears to greatly depend on binding forces of antigenic peptide to MHC class II molecules and TcR. At low affinity of immunogenic peptide in the HLA-II/peptide complex only few contact sites between T cells and APC will be displayed, and weak T cell activation signals will be generated. This may solve the question of where IL-4, required for type 2 Th cell development, may initially come from. The number of MHC class II/peptide/TcR complexes linking APC and T cells together can be increased by higher antigen concentrations. This is applied in specific immunotherapy by administrating high allergen doses to allergic patients. The chemical binding properties of peptide ligands can be changed by chemical alterations, activating by this way similar mechanisms.

It is, therefore, an interesting conclusion that development of certain immunological disorders or normal protective immunity is based on thermodynamic laws of chemical equilibrium reactions and individual reactivity properties. In addition, uptake of antigen by different APCs regulates the formation of distinct cytokine patterns by T cells. Special engineering of the PLA molecule, as used for this purpose for other allergens [43], is not required because unfolded PLA does not bind any antibody and does not elicit allergic reactions. Nonrefolded rPLA can be produced in *E. coli*.

As immunogenic epitopes are also potent anergens or tolerogens, immunization of allergic patients with short immunodominant T cell epitope containing peptides can be used to induce specific anergy in T cells without risk of IgE-mediated anaphylactic reactions. The induction of specific anergy in peripheral T cells and recovery from this state by cytokines from the tissue environment represent key steps in immunotherapy by both immunogenic epitope peptides or entire allergen [30, 31].

Taken together, these results demonstrate that the activation of distinct cytokine profiles in specific T cells and generation of antigen-specific IgE and IgG4 antibodies depend on antigen concentration and physicochemical properties of individual antigen recognition. Accordingly, the same T cell epitopes possess the potential to act as an antigen, an allergen or an anergen, a fact that is applied routinely in specific immunotherapy.

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