

Regulation of IgE and IgG₄ responses by allergen specific T-cell clones to bee venom phospholipase A₂ in vitro

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Abstract

An in vitro antibody response to bee venom phospholipase A₂ (PLA) from peripheral blood mononuclear cells of bee sting-sensitized individuals was achieved after stimulation with PLA and pokeweed mitogen. This stimulation resulted in a secretion of T_{H1}-associated cytokines and induced PLA-specific and nonspecific IgG₄ antibody production but not IgE production. The addition of interleukin-4 (IL-4) to this system decreased the secretion of IgG antibodies, whereas secretion of polyspecific IgE was induced. The mitogen was not required if peripheral blood mononuclear cells were enriched with autologous, PLA-specific, resting T-cell clones in the presence of the antigen. In these experiments the cytokine profile of the particular clone determined the antibody class generated. Low ratios of IL-4 to interferon- γ , induced by the antigen alone or obtained by neutralizing anti-IL-4 antibodies, enhanced IgG₄ antibody formation, whereas IgE levels increased at high ratios of IL-4 to interferon- γ . These results suggest a complementary regulation of the main isotypes, IgE and IgG₄, implicated in allergic and protective hyperimmune responses. (J ALLERGY CLIN IMMUNOL 1994;93:758-67.)

IFN-γ	Interferon- γ
IL	Interleukin
mAb	Monoclonal antibody
MHC	Major histocompatibility complex
PBMCs	Peripheral blood mononuclear cells

PLA	Bee venom phospholipase A ₂
PWM	Pokeweed mitogen

Key words: [T_H cells](#), [interleukin-4](#), [IgG₄](#), [IgE](#), [bee sting allergy](#)

Physical contact of B lymphocytes with activated T helper (T_H) lymphocytes renders B cells responsive to subsequent cytokine-mediated differentiation signals.¹ These contact-dependent signals are not restricted by major histocompatibility complex (MHC) or species.^{2,3} However, under physiologic conditions, most of the cellular interactions are antigen-specific.⁴ Cytokines have been shown to play a crucial role in the regulation of isotype production in both mouse and human systems.⁵ Particularly, murine interleukin (IL)-4 induces IgG₁ and IgE antibody formation,⁶ whereas interferon- γ (IFN- γ) inhibits this activity and enhances the production of IgG_{2a} subclass.⁷ In human beings the antagonistic effects of IL-4 as inducer and IFN- γ as suppressor of the IgE production are well established.^{8,9} IgG₄ could be induced by IL-4 and phorbol 12-myristate 13-acetate stimulation of purified human B cells cocultured with the mouse thymoma line EL-4.¹⁰ Also, peripheral blood mononuclear cells (PBMCs) from atopic and normal donors have been shown to produce both IgG₄ and IgE on stimulation with IL-4.¹¹⁻¹⁴ Furthermore, addition of anti-CD40 monoclonal antibody (mAb) or stimulated CD4 + T-cell clones to purified human B cells in the presence of IL-4 induced switching to the IgG₄ class.¹⁴ On the other hand, some drugs such as disodium cromoglycate can inhibit the in vitro production of human IgE and simultaneously enhance the formation of the IgG₄ subclass.¹⁵ To date, involvement of IFN- γ in the induction of human IgG antibody secretion has not been clearly demonstrated.

Bee venom phospholipase A₂ (PLA) is a chemically defined glycoprotein of 14 to 16 kd, which consists of 134 amino acids that are responsible for both allergy and immunity to bee sting.¹⁶⁻¹⁹ After initial responses to bee sting, low-affinity anti-PLA IgG₁ antibodies are generally elicited.^{20,21} With repeated exposure, as in the case of bee keepers, most subjects mount protective immune responses involving the generation of high-affinity IgG₄ antibodies to PLA.^{21,22} In contrast, in some individuals allergy to bee venom develops on generation of PLA-specific IgE antibodies.^{19,22} Successful immunotherapy of these allergic patients generally results in a strong decline in the PLA-specific IgE to IgG₄ ratio.^{20,23-25} Thus immunoprotection in response to bee sting commonly correlates with the preferential expression of IgG₄ antibodies, whereas type I hypersensitivity is caused by excessive IgE formation.²⁶

Recently, we have demonstrated that PLA-specific T-cell clones from individuals who are hyperimmune, hyposensitized, or allergic to bee sting change their cytokine pattern with the dose of antigen used during stimulation.^{27,28} The present study was designed to elucidate the role of IL-4 and IFN- γ in the formation of human IgE and IgG₄ isotypes in bee sting-compromised individuals in vitro.

Methods

Reagents

PLA (Sigma Chemical Co., St. Louis, Mo.) was reduced and carboxymethylated, as described elsewhere,²⁹ to remove enzymatic activity and associated cell toxic effects. The treated PLA showed the same antibody-binding capacity in ELISA as in the native form. The following mouse mAbs were used in the experiments: neutralizing anti-IL-4 mAb 8F12,³⁰ anti-IFN- γ clone 69 (kindly provided by Dr. H. Gallati, Hoffman-La Roche & Co. AG, Basel, Switzerland), anti-HLA-DP B7/21 (Becton Dickinson, Basel, Switzerland), and MOPC-21 (Organon Teknica Corp., Durham, N.C.). All of these mAbs are of the γ 1 isotype.

Cells and culture conditions

Cloning and maintenance of human PLA-specific T-cell clones have been previously described in detail.²⁷ The clone BuT 1,2A2 was isolated from a nonallergic hyperimmune bee keeper. It is HLA-DP-restricted. On stimulation with 1 ng/ml PLA for 20 hours, 10⁶ cells characteristically produce 1.5 ng/ml IL-4 and more than 6

ng/ml IFN- γ . This reflects a cytokine profile produced by the same number of PBMCs after pokeweed mitogen (PWM) stimulation. BuT 6.21 is a PLA-nonspecific T-cell clone from the same individual.²⁷ The PLA-specific clone ReT83 originates from a patient with bee sting allergy, and 10^6 cells produce more than 5 ng/ml IL-4 and no measurable IFN- γ .^{27, 28} on stimulation with an allergen concentration of 50 μ g/ml PLA. This is a cytokine pattern characteristic of a T_{H2} clone. Peripheral blood was obtained by venipuncture from the nonallergic bee sting hyperimmune bee keepers BU and CH and from the allergic patient RE after informed consent was obtained. PBMCs were isolated by Ficoll-Paque (Pharmacia, Uppsala, Sweden) density gradient centrifugation, washed three times with Earle's balanced salt solution, counted, and resuspended in fresh medium. In all cases RPMI 1640 culture medium containing 1 mmol/L sodium pyruvate, 1% MEM nonessential amino acids, 1% MEM vitamins, 2 mmol/L L-glutamine, 100 μ g/ml kanamycin, 100 U/ml penicillin, 100 μ g/ml streptomycin, and 0.05 mmol/L 2-mercaptoethanol (all from Gibco, Basel, Switzerland) supplemented with 10% fetal calf serum (Sera-Lab, Crawley Down, Sussex, England) was used.

PLA-specific antibody production

Samples of 6×10^6 PBMCs were stimulated with PLA in 3 ml of RPMI in the presence of PWM (Gibco) at a final dilution of 1:200 or in the presence of 3×10^5 autologous PLA-specific, resting T-cell clones. Cloned T cells, routinely stimulated with phytohemagglutinin (Wellcome Diagnostics Ltd., Dartford, England) and feeder cells and expanded in IL-2, were used on day 12 after the last restimulation. The PBMC cultures were set up in 6-well Costar tissue culture plates (Costar Corp., Cambridge, Mass.). They were incubated for 3 days at 37° C in a humidified atmosphere of 5% CO₂. Supernatants were harvested and assayed for cytokine production while the cells were washed and reincubated for an additional 7 days in fresh medium at 2×10^6 cells/ml in 24-well Costar plates. In some experiments neutralizing antibodies to IL-4 or IFN- γ were included during the first 3 days of stimulation. All conditions were assayed in triplicate. Antibody formation in culture supernatants was determined by ELISA on day 10.

Cytokine and antibody detection assays

The solid-phase ELISAs for IL-4 and IFN- γ , total IgG, anti-PLA IgG, anti-PLA IgG₄, and anti-PLA IgE are described in detail elsewhere.^{27, 29, 31, 32} In all cases specific mAbs were used. Total IgE and IgG₄ were measured by sandwich ELISA. Mouse anti-human IgE mAb TN-142 (a gift of Dr. G. Delespesse, University of Montreal, Quebec, Canada) and alkaline phosphatase-conjugated goat anti-human ϵ -chain-specific antibodies (Tago, Inc., Burlingame, Calif.) were used for IgE. This assay detected 50 pg/ml of a human IgE standard preparation (Behringwerke AG, Marburg, Germany). Mouse anti-human IgG₄ mAb RJ4, (Unipath Limited, Basingstoke, England) purified by mixed-bed ion-exchange high-performance liquid chromatography and horseradish peroxidase-conjugated mouse anti-human κ - and λ -chain-specific mAb (The Binding Site Ltd., Birmingham, England), both diluted at 1:500, were used to quantify IgG₄. This assay detected 1 ng/ml of human IgG₄ calibrated against the World Health Organization reference serum 67/97.

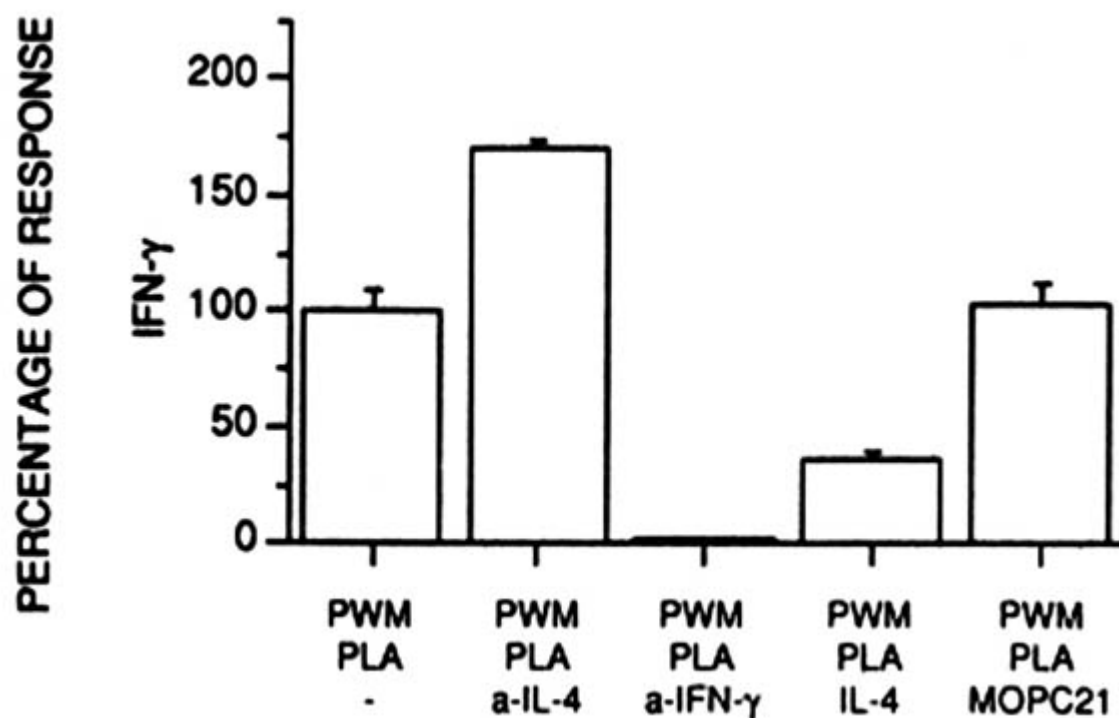
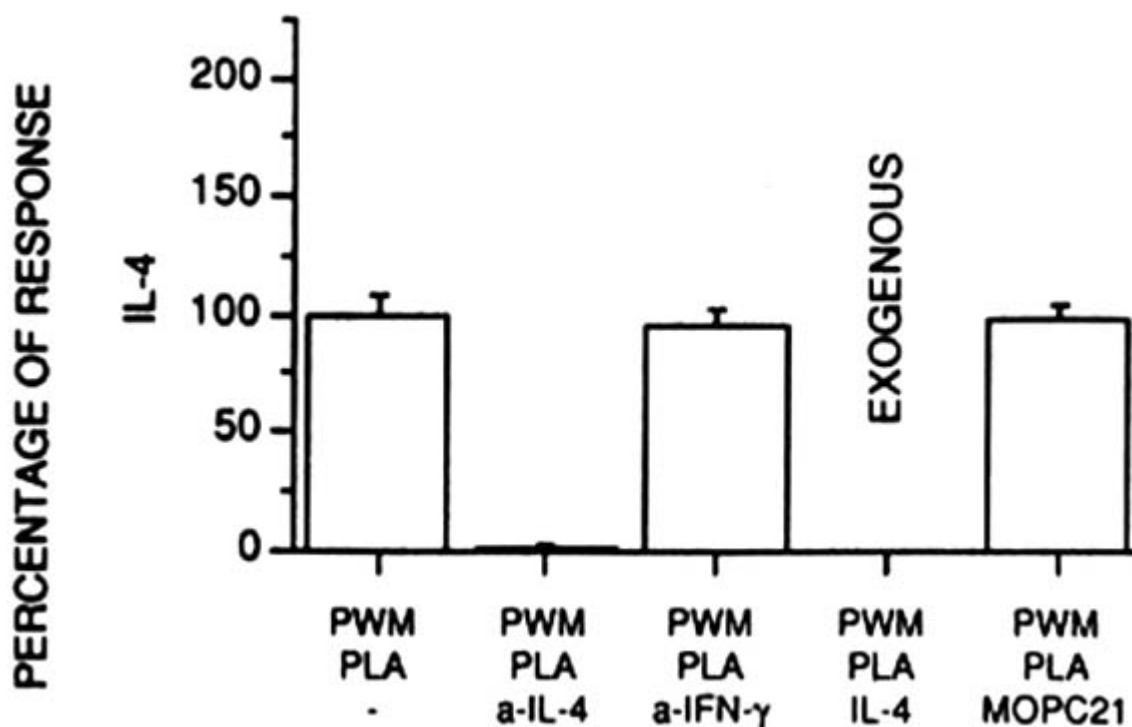
Results

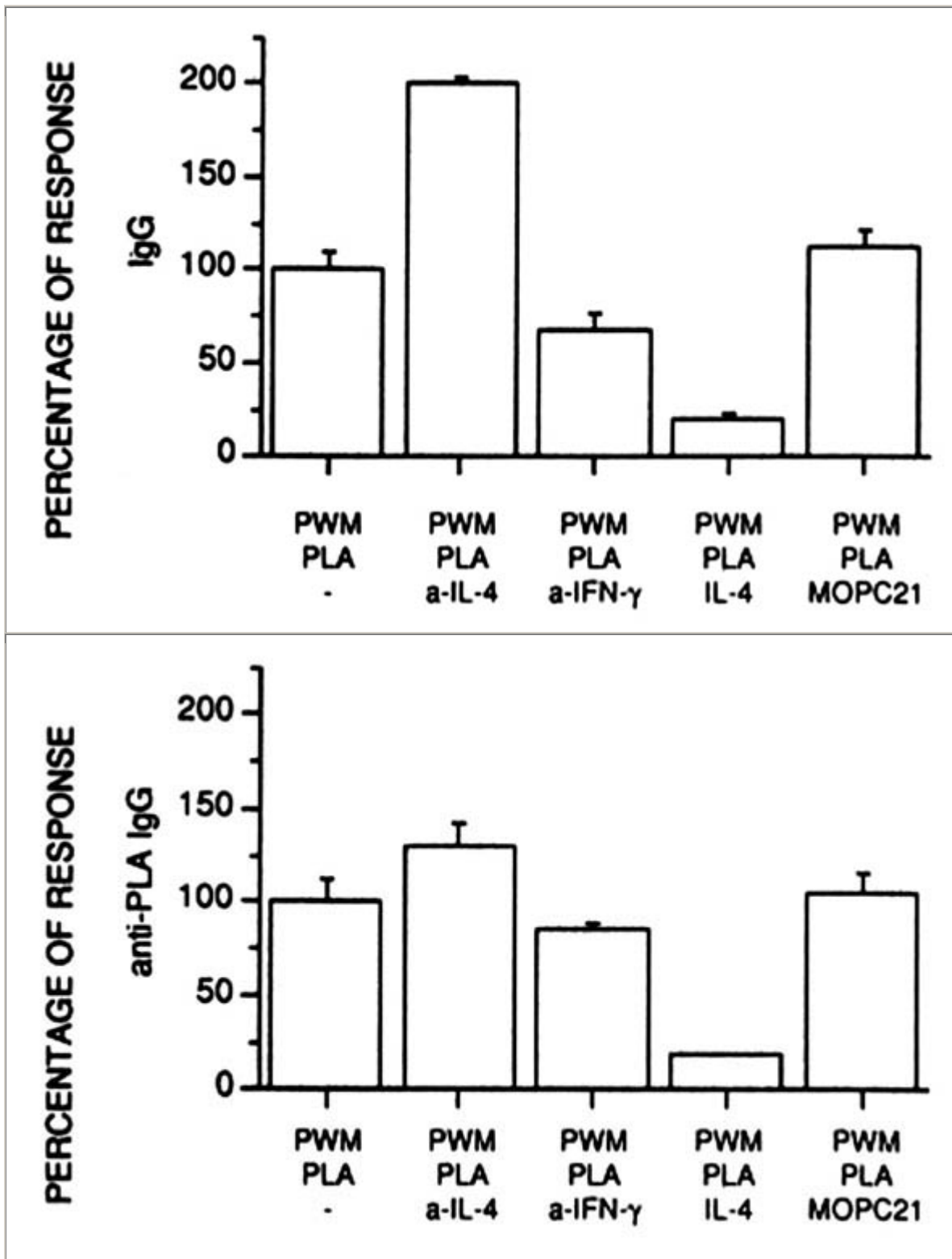
The ratio of IL-4 to IFN- γ determines the relative production of IgE and IgG₄ antibodies in PWM-driven in vitro systems

PBMCs from bee sting hyperimmune donors, stimulated with PLA and PWM, were shown to produce anti-PLA IgG₄ antibodies but not IgE.²⁹ The cytokine secretion pattern generated under these conditions was dominated by IL-2 and IFN- γ (4 to 12 ng/ml per 10^6 cells in 20 hours), whereas less than 0.3 ng/ml IL-4 was produced. As shown in Fig. 1, the addition of IL-4 or neutralizing anti-cytokine antibodies during the first 3 days of culture modified the proportion of total and PLA-specific antibody secretion. Neutralization of IL-4 enhanced the production of IFN- γ and the formation of total and PLA-specific IgG. Neutralizing anti-IFN- γ antibodies neither affected the IL-4 levels nor significantly influenced the antibody secretion. The production of both total and PLA-specific IgG was suppressed by the addition of IL-4 which also decreased IFN- γ formation. No significant effects were observed after addition of the control MOPC-21 IgG₁ myeloma protein.

FIG. 1. Effect of neutralizing anti-cytokine mAb and exogenous IL-4 on the production of IL-4 and IFN- γ (day 3) and the secretion of total and PLA-specific IgG (day 10) on PBMCs from hyperimmune individuals. PWM was used at 1:200 and PLA at 1 ng/ml. Anti-IL-4, anti-IFN- γ , and

MOPC21 IgG₁ were all used at 5 ng/ml, and IL-4 was added at 10 U/ml. Bars represent mean results $\pm$ SEM obtained from three different donors (BU, CB, and MR). In all cases the results are normalized with the data obtained with PWM and PLA alone as 100% response. Mean values, corresponding to stimulation with PWM and PLA, were 0.29 ng/ml for IL-4; 7.41 ng/ml for IFN- γ ; 35.72 ng/ml for IgG; and 30.02 ng/ml for anti-PLA IgG.

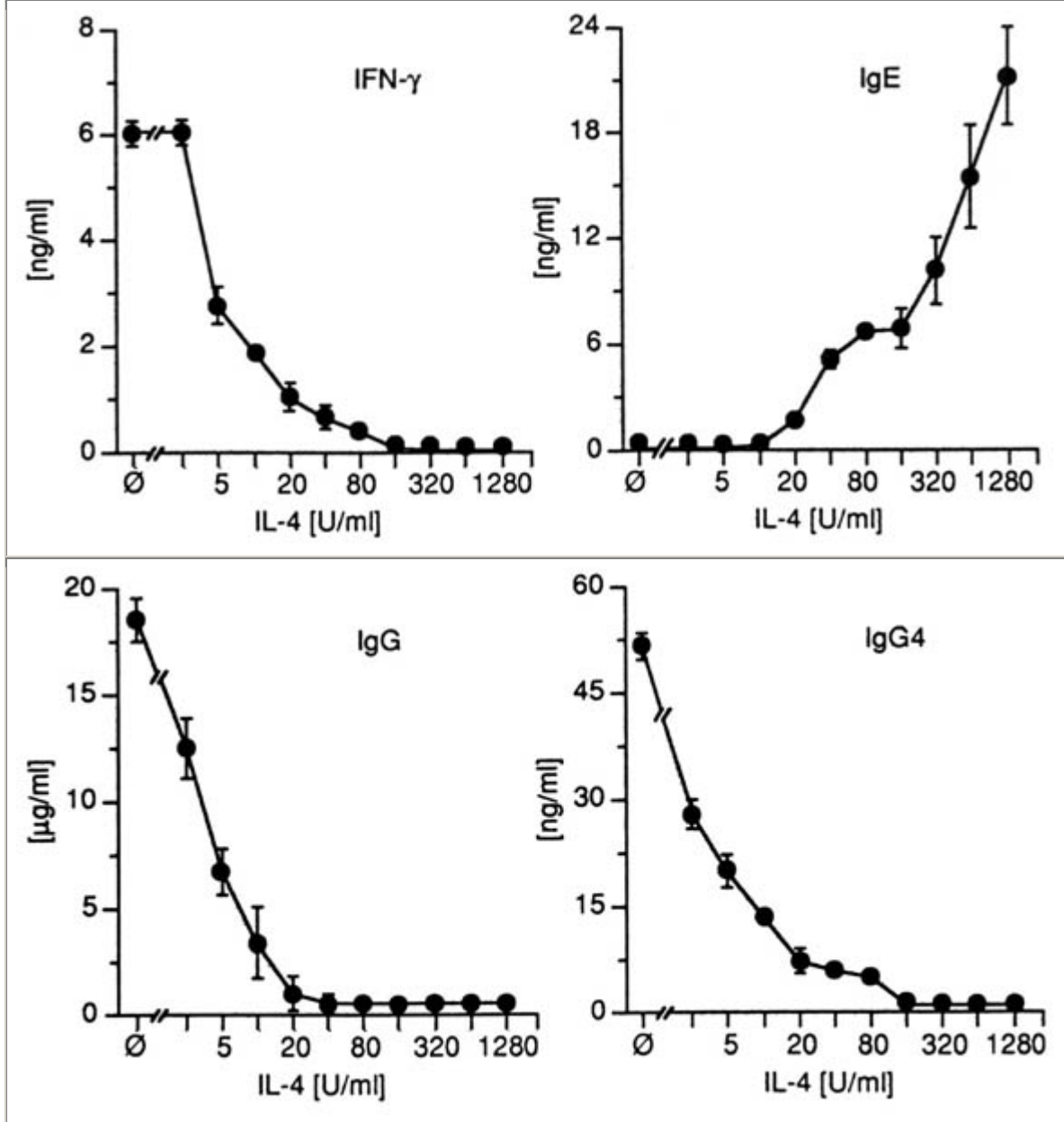


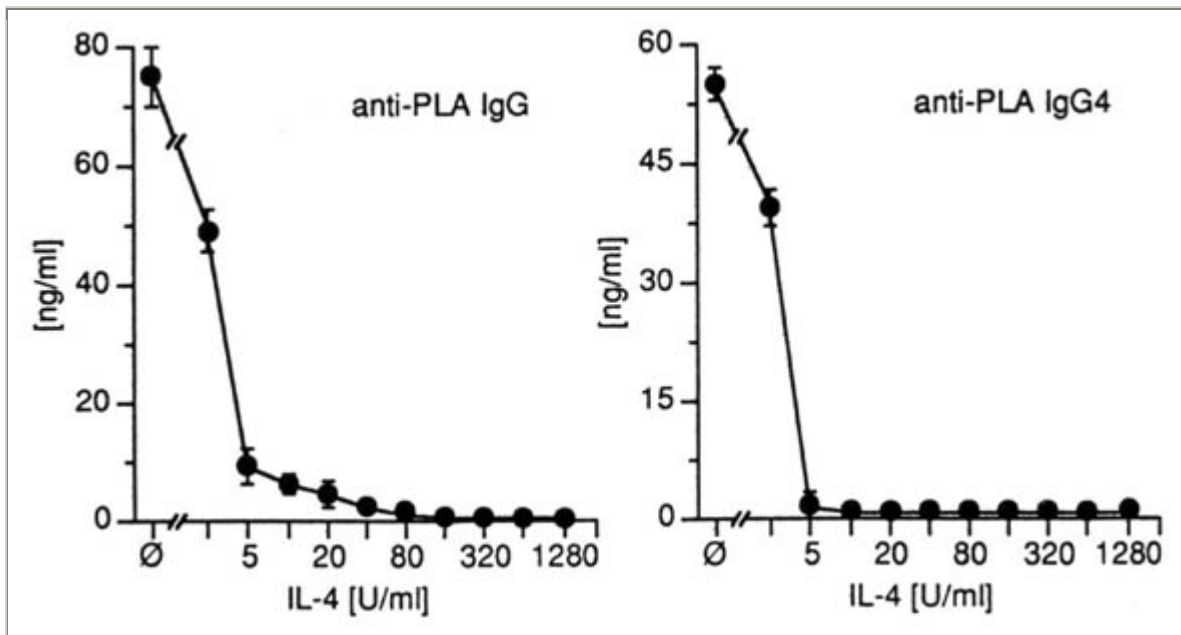


A change in the ratio of IL-4 to IFN- γ by the addition of IL-4 to the culture system substantially influenced the formation of IgE and IgG isotypes. As demonstrated in Fig. 2, the addition of IL-4 suppressed the production of IFN- γ in a dose-dependent manner and the formation of total and PLA-specific IgG and IgG₄. In contrast to IgG, increasing ratios of IL-4 to IFN- γ , resulting from the addition of IL-4 and suppression of IFN- γ , induced an allergen dose-dependent formation of IgE. PLA-specific IgE was not produced in measurable amounts.

FIG. 2. Total and PLA-specific antibody formation at different IL-4 to IFN- γ ratios. PBMCs from the hyperimmune donor CH were stimulated with PWM and PLA as indicated in Fig. 1. IFN- γ

production was measured on day 3 and antibody formation on day 10. Values represent means of triplicates $\pm$ SEM.

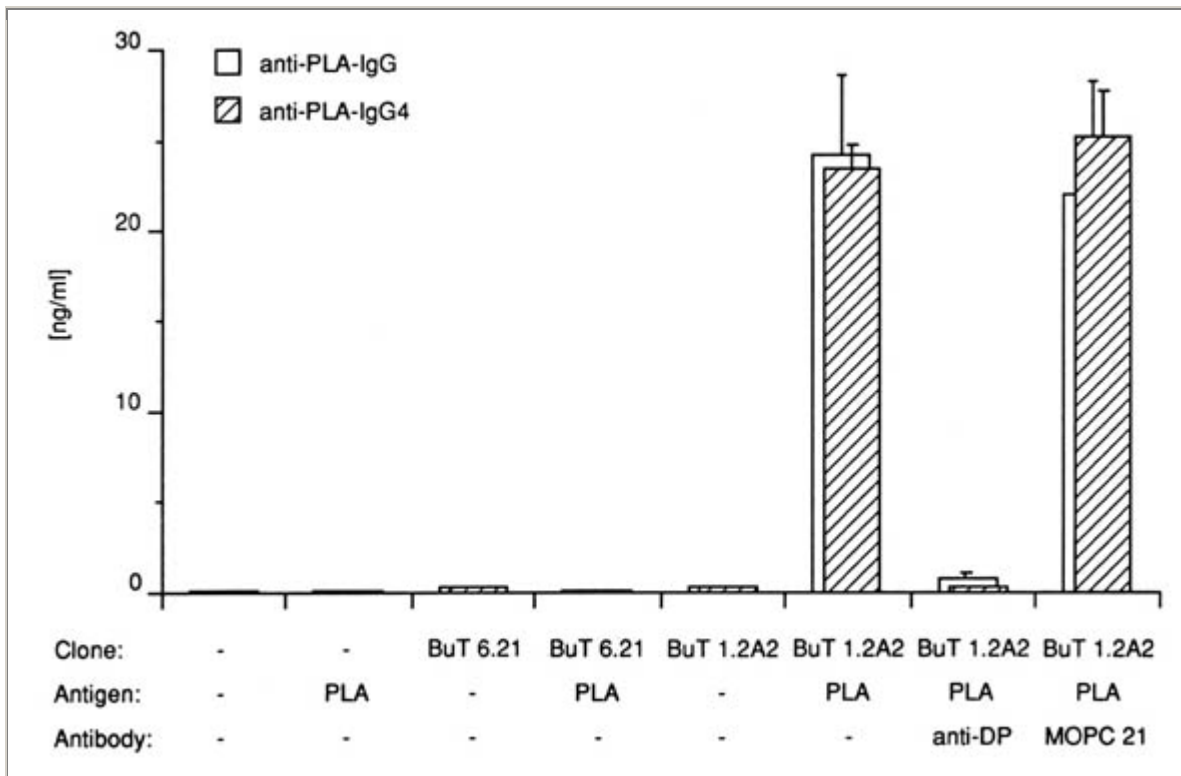




PLA-specific T-cell clones induce antibody production in PBMCs

In an improved in vitro system without mitogens, PWM was replaced by PLA-specific T-cell clones. ■Fig. 3 shows that antigen stimulation of PBMCs from the bee venom hyperimmune individual BU, enriched with the autologous, resting, PLA-specific T-cell clone BuT 1.2A2,²⁷ was necessary and sufficient to produce anti-PLA IgG antibodies. Almost all of the anti-PLA IgG was of the IgG₄ class, which reflects the serum proportions of BU (not shown). The resting, non-PLA-specific T-cell clone BuT 6.21, isolated from the same individual, failed to induce any response, demonstrating that the activation was not due to mitogenic effects of PLA. Furthermore, ■Fig. 3 shows that the T-cell–B-cell interaction was HLA-class II–restricted because antibodies to HLA-DP, the restriction element of clone BuT 1.2A2,³² completely blocked the immunoglobulin formation. Control MOPC-21 IgG₁ did not influence the antibody production. Also, other PLA-specific T-cell clones, from the same individual or from other hyperimmune or allergic donors, assisted corresponding autologous PBMCs in anti-PLA formation (not shown).

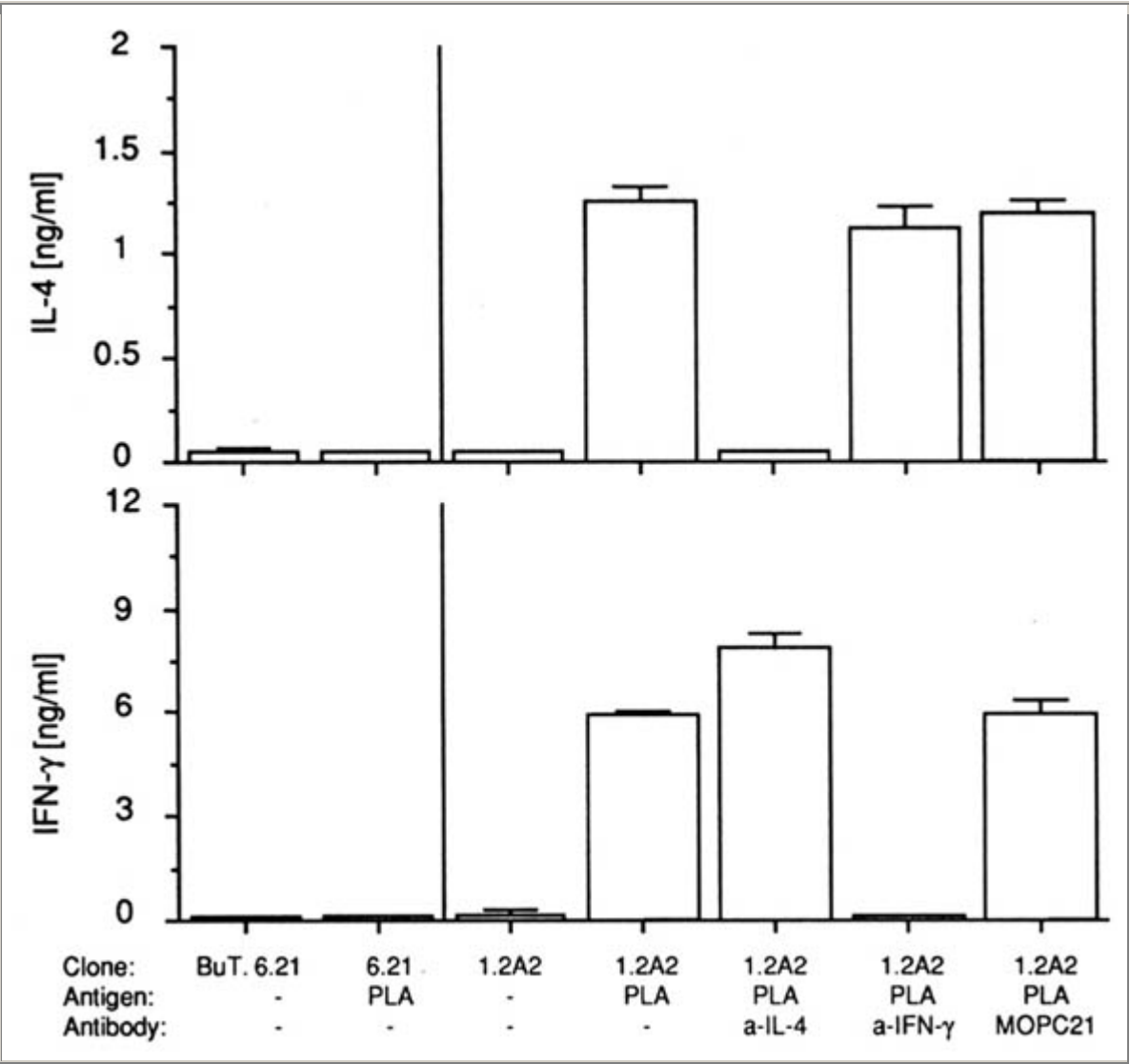
FIG. 3. T-cell–dependent, antigen-specific, and HLA-restricted production of anti-PLA antibodies. PBMCs from hyperimmune donor BU were enriched with the autologous PLA-specific T-cell clone BuT 1.2A2 or with the autologous PLA-non-specific T-cell clone BuT 6-21 and stimulated with PLA as described in the Methods section. Antibody assays were performed on day 10.

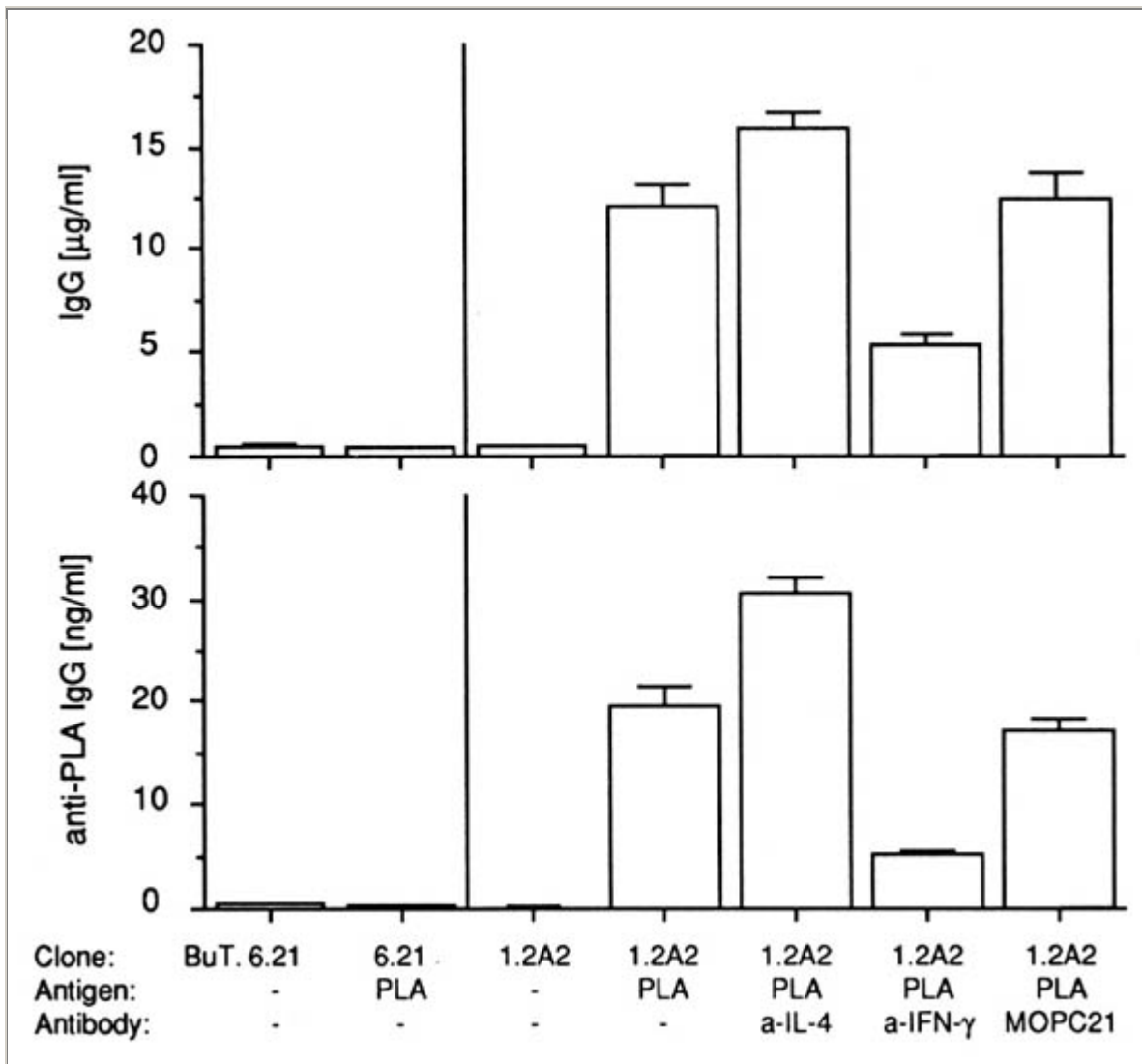


Modulation of the T-cell clone–dependent antibody production by neutralizing antibodies to IL-4 and IFN- γ

IL-4 and IFN- γ production, achieved after stimulation of PBMCs from donor BU supplemented with resting BuT 1.2A2 cells in the presence of 1 ng/ml of PLA, is shown in Fig. 4. The levels of IL-4 and IFN- γ secreted by this T-cell clone at this antigen concentration were similar to those observed in the culture supernatants of PWM-stimulated PBMCs and generated PLA-specific and total IgG. The addition of neutralizing anti-IL-4 antibodies during the first 3 days of culture enhanced the IFN- γ production (31%) and increased both total (32%) and PLA-specific (54%) IgG formation on day 10 (Fig. 4). Although the presence of neutralizing anti-IFN- γ did not substantially affect the IL-4 secretion, the production of both total IgG and PLA-specific IgG antibodies was suppressed (40% and 73%, respectively). This discrepancy between the two systems might be due to more effective neutralization of the IFN- γ production, since in the case of BuT 1.2A2 the cells were selectively stimulated by antigen, whereas in the mitogen-driven system potentially all T cells could be activated and deliver a helper effect to B cells. Control MOPC-21 IgG₁ did not influence either cytokine nor antibody production. Enrichment of PBMCs with the non-PLA-specific clone BuT 6.21 did not generate significant cytokine secretion or antibody production.

FIG. 4. Effect of neutralizing anti-IL-4 and anti-IFN- γ mAb on the specific T-cell clone–dependent production of cytokines (day 3) and antibodies (day 10) of autologous PBMCs isolated from hyperimmune donor BU. Antibodies to IL-4, IFN γ , and control MOPC21 IgG₁ were used at 5 ng/ml. Values represent mean results of assays performed in triplicate $\pm$ SEM.

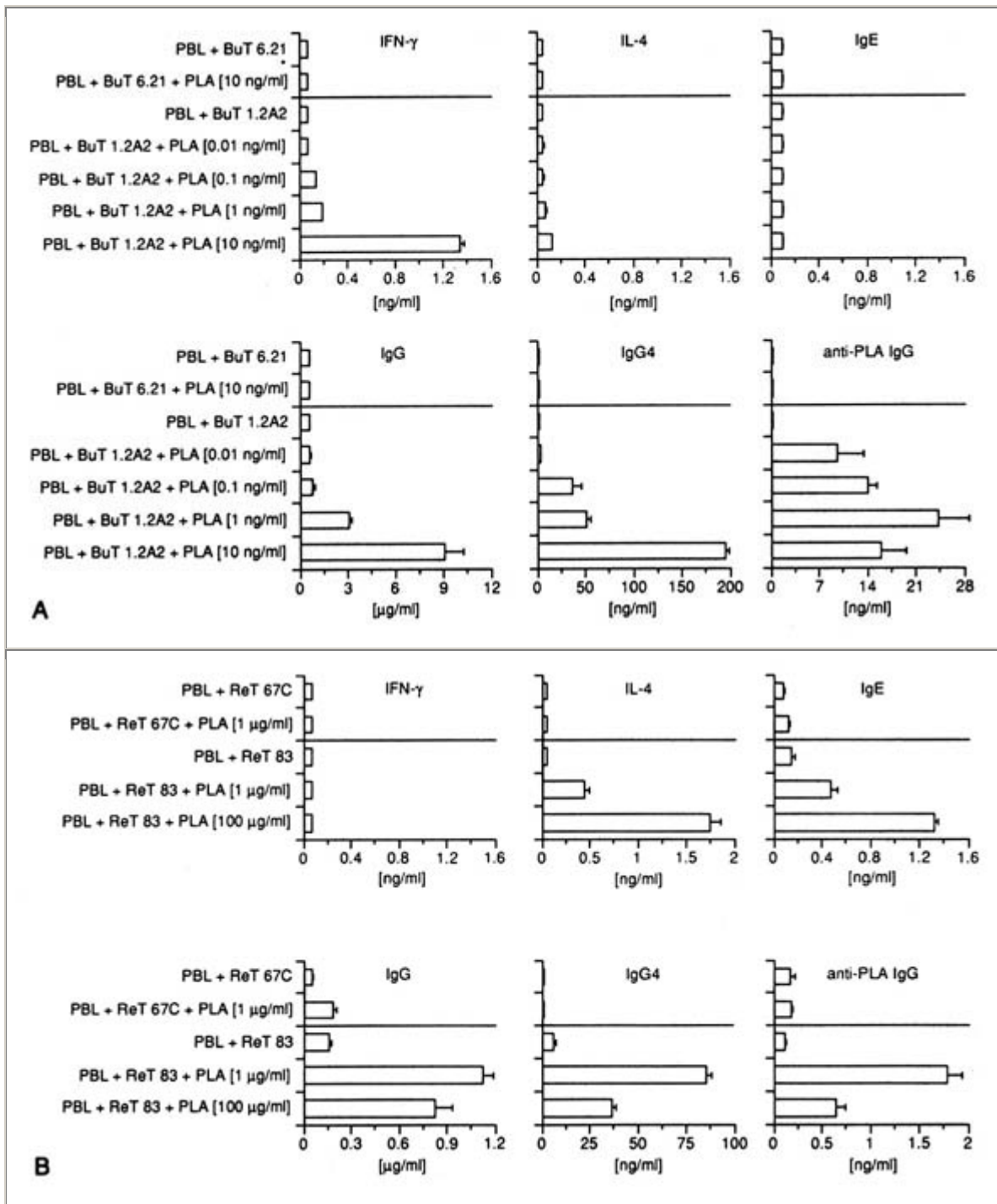




Isotype regulation by T_{H1} and T_{H2}-like T-cell clones

Although BuT 12A2 is strictly a T_{H0} clone,²⁷ PLA stimulation of BuT 1.2A2 cells in the range of 0.01 to 1000 ng/ml favored the production of IFN- γ rather than IL-4.²⁷ Fig. 5, A shows that the stimulation of BuT 1.2A2 supplemented autologous PBMCs with 0.01 to 10 ng/ml PLA resulted in PLA-specific IgG and IgG₄ synthesis. Again, the ratios of total IgG to IgG₄ (mean, 2% IgG₄) and anti-PLA IgG:IgG₄ (mean, 85% IgG₄) antibody production matched with the proportions found in the serum of the bee venom hyperimmune donor BU. IgE antibodies could not be detected at any antigen dose. The same conditions applied to the PLA-nonspecific, autologous T-cell clone. BuT 6.21 did not induce antibody synthesis of any isotype.

FIG. 5. Effect of (A) T_{H1}-like and (B) T_{H2}-like cytokine expression on total and PLA-specific antibody formation. BuT 1.2A2 and ReT 83 are PLA-specific T-cell clones from the hyperimmune donor BU and the allergic patient. RE, respectively. Clones BuT 6.21 and ReT 67C are PLA-nonspecific T-cell clones from the corresponding donors. The experiment was assayed in triplicate, and results represent mean values $\pm$ SEM.



IgE production was only possible when the clone that was used to supplement the PBMCs displayed T_{H2} characteristics. PLA stimulation of PBMCs from the allergic donor RE, supplemented with the autologous, resting, PLA-specific T-cell clone ReT 83, ²⁷ is shown in ■Fig. 5, B. Antigen-dose-dependent production of IL-4 was observed in the supernatants of PBMCs supplemented with the T_{H2} -like clone ReT 83 and stimulated with PLA, whereas little or no IFN- γ could be detected in any culture condition. Characteristically for this clone, optimal IL-4 production was obtained at 100 μ g/ml PLA. This highest IL-4 secretion correlated with maximal IgE formation. Concentrations of 1 μ g/ml of PLA induced less IL-4 and generated less IgE. In contrast, the production of PLA-specific IgG antibodies, as well as total IgG and IgG₄, was reduced at PLA doses that generated higher amounts of IL-4. Thus although IgE and IgG₄ isotypes were coexpressed, their levels were conversely regulated by the amount of IL-4. Similar results (not shown) were generated with the T-cell-depleted fraction of PBMCs, obtained after sheep red blood cell rosetting, as a source of antigen-presenting and antibody-producing cells.

Discussion

The elucidation of the mechanisms involved in the regulation of IgE versus IgG₄ production in allergic and nonallergic individuals is of particular interest to the understanding of the pathologic conditions associated with IgE-mediated hypersensitivity and IgG₄-related protective states. Two different systems to induce in vitro human anti-PLA antibody production were applied to study the roles of IL-4 and IFN- γ as regulators of the immune response to allergens. One system used the capability of PWM to nonspecifically induce immunoglobulin secretion in cultures of PBMCs, and the other used autologous resting T-cell clones that were activated by the antigen. The proportions of total and PLA-specific antibodies generated in both in vitro systems correlated with those found in the sera of the respective donors, matching the in vivo situation.

The predominant expression of the T_{H1}-related cytokines characteristic for PBMC stimulation with PWM, IFN- γ and IL-2, increased the production of IgG₄ antibody but not IgE. Increasing the IL-4 to IFN- γ ratio by the addition of IL-4 decreased IgG₄ antibody production but induced the coexpression of polyclonal IgE. In the T-cell clone-dependent system, the expressed cytokine pattern was a characteristic property of the particular T-cell clone used. As in the mitogen-driven system, total and PLA-specific IgG₄ antibody production were enhanced by low IL-4 to IFN- γ ratios and declined with higher IL-4 to IFN- γ quotients. With T_{H2}-like clones, such as ReT 83, IgE and IgG₄ were both secreted. In this case the levels of IL-4 correlated directly with IgE but inversely with IgG₄ secretion. These data suggest that although both IgE and IgG₄ production are promoted by IL-4, elevated amounts of this cytokine are detrimental to IgG₄ production.

IL-4 was found to induce simultaneously both human IgE and IgG₄ in polyclonal systems.¹⁰⁻¹⁴ These results, which were also confirmed in our laboratory, apparently conflict with the fact that high serum levels of allergen-specific IgE in atopic individuals generally do not correlate with elevated allergen-specific IgG₄ levels. Moreover, successful immunotherapy of allergic patients generally decreases the IgE serum levels, whereas the IgG₄ titers rise.^{20, 23-25} Thus it is difficult to attribute the preferential expression of IgE in allergic and IgG₄ in nonallergic individuals exclusively to IL-4. Rather, both IL-4 and IFN- γ , in concert with other factors, may contribute to the in vivo formation of IgE and IgG₄.³³⁻³⁵

In contrast to the T-cell clone-dependent system, polyclonal activation of mononuclear cells with high doses of IL-4¹¹⁻¹³ does not require antigen-specific or MHC-restricted T-cell-B-cell interaction. Moreover, neither antigen-mediated nor MHC-restricted interactions are involved in the activation of purified B cells by IL-4 plus activated CD4 + T-cell clones.³⁴ These T cells could be replaced by anti-CD40 to generate IL-4-dependent IgE and IgG₄ formation,¹⁴ although the activation pathways may be different, because anti-CD40 alone (in contrast to activated CD4 + T cells) could not induce the expression of germline transcripts.¹⁴ It has been shown that IFN- γ , which blocks IL-4-induced IgE production by PBMCs or purified B cells cocultured with activated T-cell clones, failed to inhibit anti-CD40-induced IgG₄ and IgE synthesis.¹⁴ Different pathways of T-cell-mediated B-cell activation might explain different isotype expression.

Recently, we have demonstrated that the in vitro expressed cytokine pattern of PLA-specific T-cell clones depends on the concentration of antigen added to the culture.^{27, 28} Particularly in clones derived from hyperimmune, nonallergic individuals, the ratio of IL-4 to IFN- γ tends to decline with increased antigen doses, whereas clones derived from allergic donors had an increased IL-4 to IFN- γ quotient under the same conditions.²⁸ In this report we have shown that the regulation of IgE and IgG₄ secretion in response to PLA is governed by the ratio of IL-4 to IFN- γ production. IL-4 is required for the switch of both IgE and IgG₄,^{11, 12, 34-37} while IFN- γ inhibits switching and the expression of IgE.³⁶⁻³⁹ However, in cell populations containing isotype-committed B cells, such as PBMCs from bee sting-sensitized individuals, IL-4 can nonspecifically suppress Ig secretion.^{37, 38} On the other hand, it has been demonstrated that mice with homozygous disruption of the IL-4 gene lack the capability to mount IgE responses, although they were still able to produce IgG₁ antibodies.³⁹ In mice IgG₁ is claimed to be the counterpart to human IgG₄. Thus the induction of both IgE and IgG₄ production requires IL-4, but the relative amounts of IL-4 to IFN- γ may decisively favor the production of one Ig class. In vivo the dose of allergen injected by a bee sting may be roughly constant. However, each individual expresses his or her own particular HLA phenotype, which exhibits a different affinity to PLA-derived peptides. These differences in affinity might determine the density of class II-peptide complexes displayed on the surface of the antigen-presenting cell and the number of contact sites to the T cell. This may influence the total strength of T-cell activation and subsequent cytokine synthesis, similar to the way that the allergen dose acts in vitro.²⁸ Furthermore, environmental or genetic conditions, particularly MHC expression,^{28, 38-42} that favor high IL-4 to IFN- γ ratios may enhance IgE production and simultaneously decrease IgG₄ production and vice versa.

Antigen-driven in vitro systems are closely related to physiologic conditions and are important tools with which to study multifactor regulated immune responses, such as hypersensitivity to allergens. This may be of special interest for a better understanding and more successful application of clinical hyposensitization.

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