

Regulation of Anaphylactic IgG1 Antibody Production by IL-4 and IL-10

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Key Words

Anaphylaxis · IgG1 · IgE · IL-4 · IL-10

Abstract

Background: Different cytokines have been implicated in the regulation of isotype expression in primary and secondary antibody responses. The aim of this study was to assess the regulation of anaphylactic IgG1 and IgE antibodies by IL-4, IL-10 and IFN- γ at different time points of the antibody response against PI, an immunosuppressive fraction of *Ascaris suum* extract, and ovalbumin (OVA). **Methods:** Wild-type or cytokine-deficient C57BL/6 or BALB/c mice were immunized with PI or OVA in different adjuvants. Twenty days later, they were boosted with the respective antigen. IgG1 and IgE antibodies produced during primary and secondary responses were measured by passive cutaneous anaphylaxis. **Results:** PI induced low levels of anaphylactic IgG1 antibodies in the primary response and moderate levels after the antigenic booster, which were IL-4-dependent. In the absence of IL-10 and IFN- γ , PI-specific IgG1 and IgE enhanced significantly, indicating that these cytokines downregulated antibody production in primary and secondary responses. The IgG1 response to OVA in aluminium hydroxide or complete Freund's adjuvant was IL-4-dependent in the begin-

ning of the primary response. Later on, it became only partially regulated by IL-4 in C57BL/6 mice and IL-4-independent in Th2-prone BALB/c mice. In contrast, IgE antibodies depended exclusively upon IL-4 during the entire time course. **Conclusions:** These results indicate, first, that the IL-4 dependency of anaphylactic IgG1 antibody production, mainly in the secondary response, varies among mouse strains, and, second, that the nature of the antigen determines whether IL-10 and IFN- γ limit the potential to make large amounts of anaphylactic IgG1 and IgE.

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Introduction

Immunoglobulin isotype switching is known to be dependent upon different cytokines secreted mainly by subsets of CD4⁺ T cells [1–3]. Among them, IL-4 produced by Th2 cells regulates the synthesis of IgE and IgG1 antibodies [4, 5]. Substantial IgG1 responses, however, can be obtained with Th1 clones [1] and germline γ 1 transcripts are expressed in B cells after CD40 engagement, independently of IL-4 [6, 7]. In contrast to the absence of IgE, genetically IL-4-deficient mice have only a partial reduction of IgG1 levels [8]. As we demonstrated previously [9], IL-4-dependent and IL-4-independent IgG1 differ in their ability to induce mast cell degranulation. Using passive cutaneous anaphylaxis (PCA) and ELISA, the IgG1

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response of mice against two *Ascaris* components (PI and PIII) and ovalbumin (OVA) injected in complete Freund's adjuvant (CFA) was measured on day 8 of immunization. The IgG1 antibodies induced by PI were devoid of anaphylactic activity; nevertheless, IL-4^{-/-} mice produced as much IgG1 as wild-type (WT) mice when this antibody was measured by ELISA. Anaphylactic IgG1 against PI were obtained in IL-12- or IFN- γ -deficient mice, indicating that these cytokines antagonize the effect of IL-4. In contrast to PI, PIII and OVA induced both anaphylactic and nonanaphylactic IgG1 antibodies. The anaphylactic IgG1 was not detected in IL-4^{-/-} mice. The difference in the anaphylactic property of the two types of IgG1 seems to be due to a proper glycosylation of the molecules which alters the binding to the Fc γ RIII on mast cell surface [10]. In addition, the levels of the two types of IgG1 are modulated by adjuvants, aluminium hydroxide inducing mostly the anaphylactic one, quite differently from CFA [11].

The immune response against PI components is peculiar in many ways, because these proteins suppress IgE and IgG1 antibody production to PIII and unrelated antigens [12, 13]. They also profoundly impair humoral and cell-mediated immune responses, as well as cytokine synthesis to unrelated antigens via the induction of IL-4 and IL-10 [13–16].

Different subsets of Th cells have been implicated in regulating isotype expression not only in primary but also in secondary responses. Memory B cells, however, express receptors of higher affinity and are more readily activated by lower concentrations of antigen and T cells. In addition, there are differential cytokine requirements among primary and secondary B cell responses [17–19]. For example, the generation of a secondary IgE response is not dependent upon the presence of IL-4 during the primary immunization, i.e., priming can occur even when the primary response is blocked by neutralization of IL-4. However, if IL-4 is not present during primary immunization, it is required during the secondary immunization for the generation of an IgE response [4].

Although the positive and negative regulation of IgE class switching and synthesis by different cytokines and other cell surface molecules has been extensively studied [20–22], IgG1 antibodies have for many years been measured by ELISA regardless of their anaphylactic activity. Therefore, the mechanisms involved in the modulation of the synthesis of the anaphylactic type of IgG1 are not well known.

Thus, this study aimed to assess the regulation of PI-specific anaphylactic IgG1 antibodies, as compared

to IgE, by the cytokines IL-4, IL-10 and IFN- γ at different time points of the primary and secondary immune responses. Comparatively, the production of anaphylactic IgG1 and IgE antibodies was followed in IL-4-deficient mice of C57BL/6 or BALB/c background, immunized with OVA. In this immunization protocol, aluminium hydroxide gel, a Th2 inducer, or CFA that induces a Th1 response [23] were employed as adjuvant vehicles. The two mouse strains were chosen because they have been used as prototypes to investigate experimental asthma.

Materials and Methods

Animals

C57BL/6 mice with targeted disruption of the IL-4 or IL-10 gene (IL-4^{-/-} or IL-10^{-/-}), and WT C57BL/6 mice were bred in the animal house facilities of the Department of Immunology, ICB/University of São Paulo, Brazil. WT and IL-4^{-/-} BALB/c mice were obtained from the same source. Eight-week-old female mice of each type were used for immunization. Five-week-old female BALB/c mice and 3-month-old Wistar rats were used for PCA reactions. All the animals were kept under specific pathogen-free conditions. The experimental protocols were approved by the Biomedical Sciences Institute/USP Ethical Committee for Animal Research.

Antigens

OVA (grade V) was obtained from Sigma Chemical Co. (St. Louis, Mo., USA). Adult *Ascaris suum* body extract was prepared and fractionated by gel filtration under conditions previously described [13]. Fractions eluted in the first peak (PI), corresponding to high-molecular-weight components, were concentrated with Centriprep 100 (Amicon, Beverly, Mass., USA) concentrator (100 cutoff point). Protein content was measured by Lowry's method [24].

Immunization

Groups of 8 WT or IL-4^{-/-} or IL-10^{-/-} C57BL/6 mice were injected subcutaneously with 150 μ g of PI emulsified in CFA (Sigma Chemical Co.), into the base of the tail (0.2 ml/animal). Twenty days later, they were boosted intraperitoneally with 10 μ g of PI.

Groups of 6 WT or IL-4^{-/-} mice in C57BL/6 or BALB/c background were injected with 100 μ g of OVA in CFA subcutaneously or in 7.5 mg of aluminium hydroxide intraperitoneally. Twenty days later, they were boosted intraperitoneally with 5 μ g of OVA.

In vivo IFN- γ Neutralization

IL-10^{-/-} mice were given 1 mg of rat anti-IFN- γ XMG 1.2 monoclonal antibodies (MoAb) intraperitoneally on days -1 and +3 of immunization. WT mice received equivalent amounts of rat anti- β -galactosidase GL113 MoAb (isotype control).

Titration of Anaphylactic Antibodies

Plasma obtained after several days in the primary and secondary responses was quantified for IgE or IgG1 levels by PCA in rats

or mice, respectively. Only these isotypes bind to and degranulate mast cells under the conditions used in each PCA reaction, as previously described [25, 26]. The rats were shaved on the back and received intradermal (0.1 ml) injections of serial dilutions of pools of plasma from each group. After a sensitization period of 18–24 h, they were challenged intravenously with 0.25% Evan's blue solution containing 500 µg of antigen. For the IgG1 titration, aliquots of individual or pools of plasma were inactivated at 56°C for 1 h to destroy the anaphylactic activity due to IgE Abs. Two hours after the injection of 0.05 ml of plasma dilutions, the mice received intravenously 250 µg of antigen in dye solution. The PCA titers were expressed as the reciprocal of the highest dilution that gave a lesion of >5 mm in diameter in triplicate tests. Samples from WT and cytokine-deficient mice that were to be compared were injected into the same animal. Since variation of the PCA titer for the same sample is 2-fold or less, only differences above this value were considered significant when pools of plasma were titrated for IgG1 or IgE [27, 28].

Statistical Analysis

Statistical differences (significance levels of $p < 0.05$) between the logarithmically transformed individual PCA titers obtained for IgG1 in the different groups were assessed using a one-way analysis of variance followed by multiple comparisons using the Tukey method [29].

Results

Role of IL-4 in Primary and Secondary Anaphylactic IgG1 Antibody Responses Induced by PI

Groups of WT and IL-4^{-/-} C57BL/6 mice were immunized subcutaneously with 150 µg of PI in CFA. Twenty days later, they received a booster of 10 µg of antigen intraperitoneally. Figure 1 shows the titers of PI-specific IgG1 and IgE antibodies measured by PCA in primary and secondary responses. In WT mice, a very low IgG1 response was obtained until day 20 of immunization, but the titers increased 8-fold after the antigenic booster. No IgG1 antibodies were detected in IL-4^{-/-} mice in the primary response, and a significant decrease occurred in the secondary response. In comparison, the titers of IgE were extremely low in WT mice even after booster with PI and, as expected, no antibody was detected in IL-4^{-/-} mice.

Role of IL-10 in Primary and Secondary Anaphylactic IgG1 Antibody Responses Induced by PI

Because low levels of IgG1 and IgE were produced in the primary response and the levels of IgE remained low after the booster, we investigated the role of IL-10, which is abundantly induced by PI, in the anaphylactic antibody production against this fraction. In the absence of IL-10, IgG1 levels increased in the first bleeding (8th day) of the

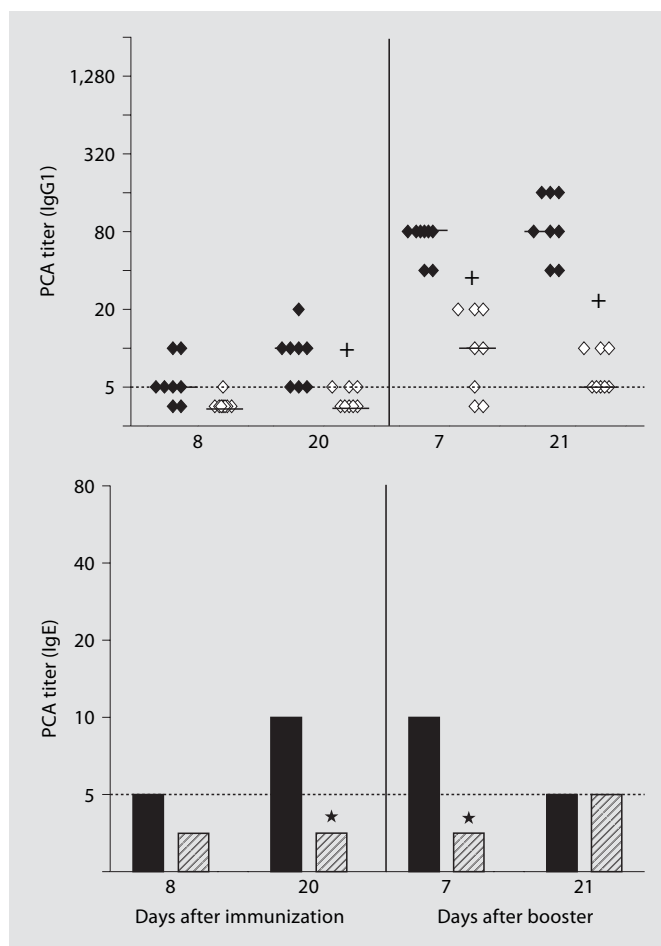


Fig. 1. Effect of IL-4 on primary and secondary anaphylactic antibody responses of C57BL/6 mice immunized with PI in CFA and boosted on day 20 with antigen. IgG1 levels in individual plasma from WT (◆) and IL-4^{-/-} (◇) mice (horizontal bars: median values), and IgE levels in pooled plasma from WT (■) and IL-4^{-/-} (▨) mice (n = 8) were measured by PCA. Dashed lines represent the detection limit of the assay. * $p < 0.05$ compared with WT mice. * Significantly different from WT mice.

primary response and at the last bleeding of the secondary response (21st day) (fig. 2). No significant change was observed in the IgE response (fig. 2).

Additive Effect of IL-10 and IFN-γ on Downregulation of PI-Specific IgG1 and IgE Antibody Production

Since IFN-γ inhibits the synthesis of IgE and anaphylactic IgG1 antibodies [4, 9, 30], we used anti-IFN-γ MoAb to neutralize this cytokine in IL-10^{-/-} mice. WT mice were treated with the isotype control MoAb (GL 113) and IL-10^{-/-} mice with XMg 1.2 MoAb 1 day before

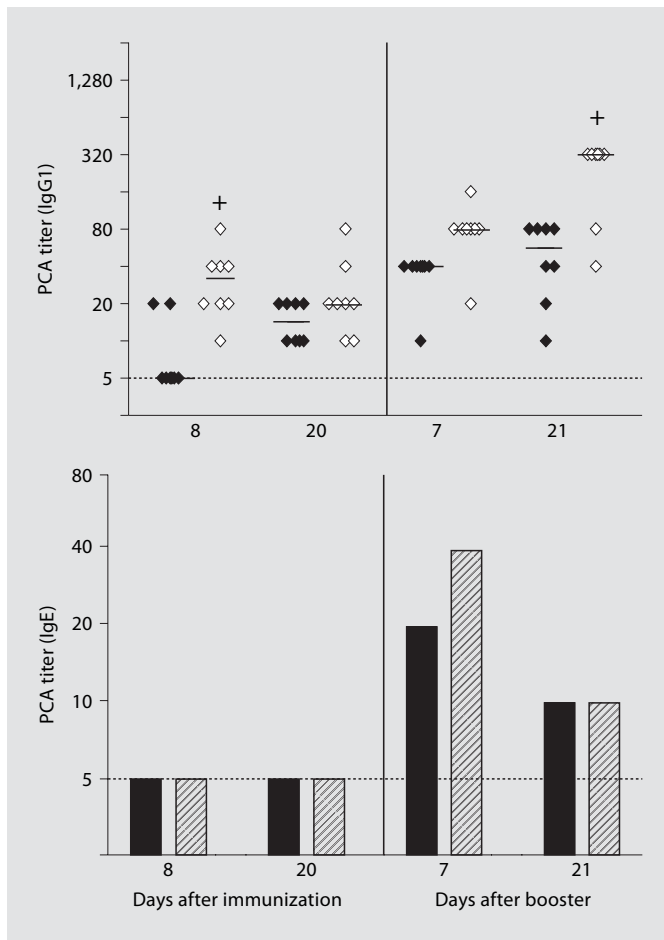


Fig. 2. Effect of IL-10 on primary and secondary anaphylactic antibody responses of C57BL/6 mice immunized with PI in CFA and boosted on day 20 with antigen. IgG1 levels in individual plasma from WT (◆) and IL-10^{-/-} (◇) mice (horizontal bars: median values), and IgE levels in pooled plasma from WT (■) and IL-10^{-/-} mice (▨) (n = 8) were measured by PCA. Dashed lines represent the detection limit of the assay. + p < 0.05 compared with WT mice.

and 3 days after immunization with PI. As shown in figure 3, IgG1 antibody levels were significantly increased in IL-10^{-/-} mice during the primary and secondary responses. IgE levels were also maintained above those of WT mice in the primary response, with a difference of 8-fold in the secondary response (fig. 3). WT mice treated with XMG 1.2 MoAb showed a slight increase in IgG1 and IgE antibody production on day 8 but not later on (data not shown).

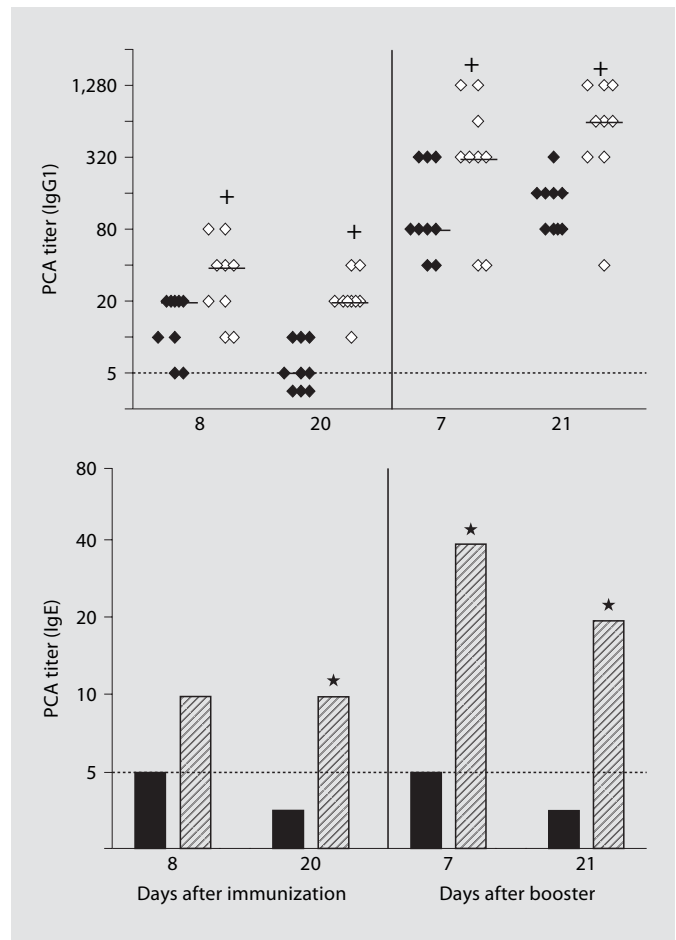


Fig. 3. Effect of IL-10 and IFN- γ on primary and secondary anaphylactic antibody responses of C57BL/6 mice immunized with PI in CFA and boosted on day 20 with antigen. IgG1 levels in individual plasma from GL 113 MoAb-treated WT (◆) and XMG 1.2 MoAb-treated IL-10^{-/-} (◇) mice (horizontal bars: median values), and IgE levels in pooled plasma from GL 113 MoAb-treated WT (■) and XMG 1.2 MoAb-treated IL-10^{-/-} mice (▨) (n = 8) were measured by PCA. Dashed lines represent the detection limit of the assay. + p < 0.05 compared with WT mice. * = Significantly different from WT mice.

Role of IL-4 in Anaphylactic IgG1 Antibody Responses Induced by OVA in C57BL/6 and BALB/c Mice

In the next experiments, OVA, a very potent antigen commonly used to induce experimental anaphylaxis, was employed to investigate the role of IL-4 in the stimulation of anaphylactic antibodies in C57BL/6 and BALB/c mice. We had previously shown in IL-10- and IFN- γ -deficient mice immunized with OVA in CFA that the levels of anaphylactic IgG1 and IgE on day 9 of immunization were similar to those obtained in WT mice [16].

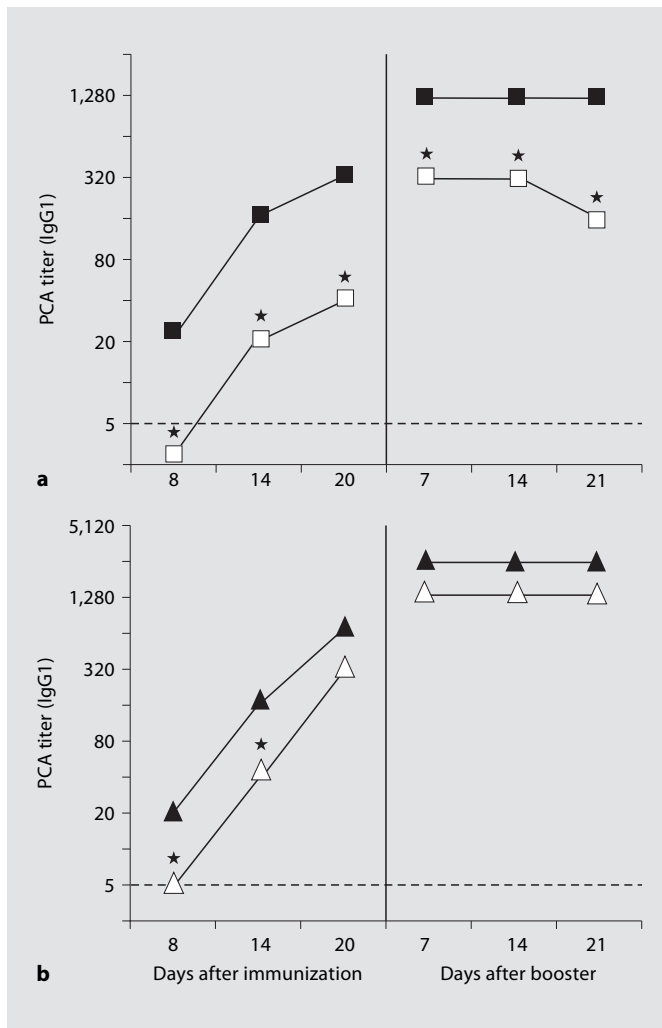


Fig. 4. Primary and secondary anaphylactic IgG1 responses in C57BL/6 (a) or BALB/c (b) mice immunized with OVA in CFA and boosted on day 20 with antigen. Pooled plasma from WT (■, ▲) or IL-4^{-/-} (□, △) mice (n = 6) was measured by PCA. Dashed lines represent the detection limit of the assay. * Significantly different from WT mice.

WT and IL-4^{-/-} mice of C57BL/6 and BALB/c strain were immunized subcutaneously with 100 µg of OVA in CFA. Three bleedings were done before they were boosted intraperitoneally on day 20 with 5 µg of OVA. The levels of IgG1 increased during the primary response, and were maintained in the secondary response for 3 weeks in both strains (fig. 4). C57BL/6 IL-4^{-/-} mice produced 8-fold less IgG1 than WT mice in the primary response and 4- to 8-fold less antibody after the antigenic booster (fig. 4a). In contrast, in BALB/c IL-4^{-/-} mice, the levels of IgG1 were lower (4-fold) only until 2 weeks of immu-

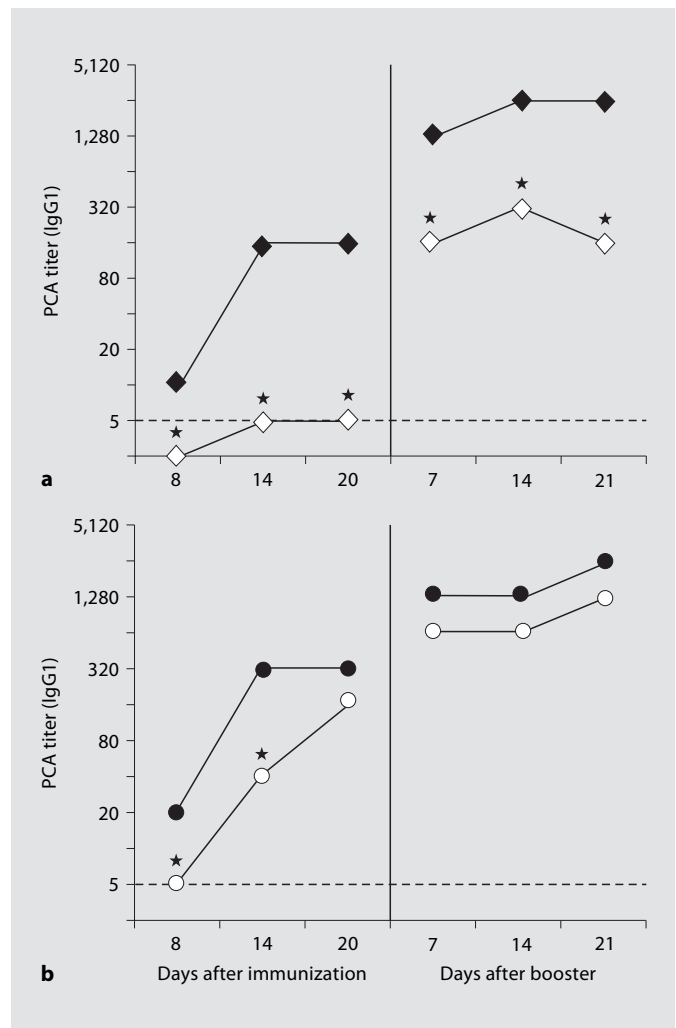


Fig. 5. Primary and secondary anaphylactic IgG1 responses in C57BL/6 (a) or BALB/c (b) mice immunized with OVA in aluminium hydroxide and boosted on day 20 with antigen. Pooled plasma from WT (◆, ●) or IL-4^{-/-} (◇, ○) mice (n = 6) was measured by PCA. Dashed lines represent the detection limit of the assay. * Significantly different from WT mice.

nization and the difference compared with WT mice was not significant later on (fig. 4b).

Other groups of mice were immunized intraperitoneally with the same amount of OVA in 7.5 mg of aluminium hydroxide and boosted as before. Once more, in IL-4-deficient C57BL/6 mice, the IgG1 levels were 32-fold lower than in WT mice before the antigenic booster and 8- to 16-fold after it (fig. 5a). The difference of 4- to 8-fold between the two groups of BALB/c mice was restricted to the first two bleedings (fig. 5b).

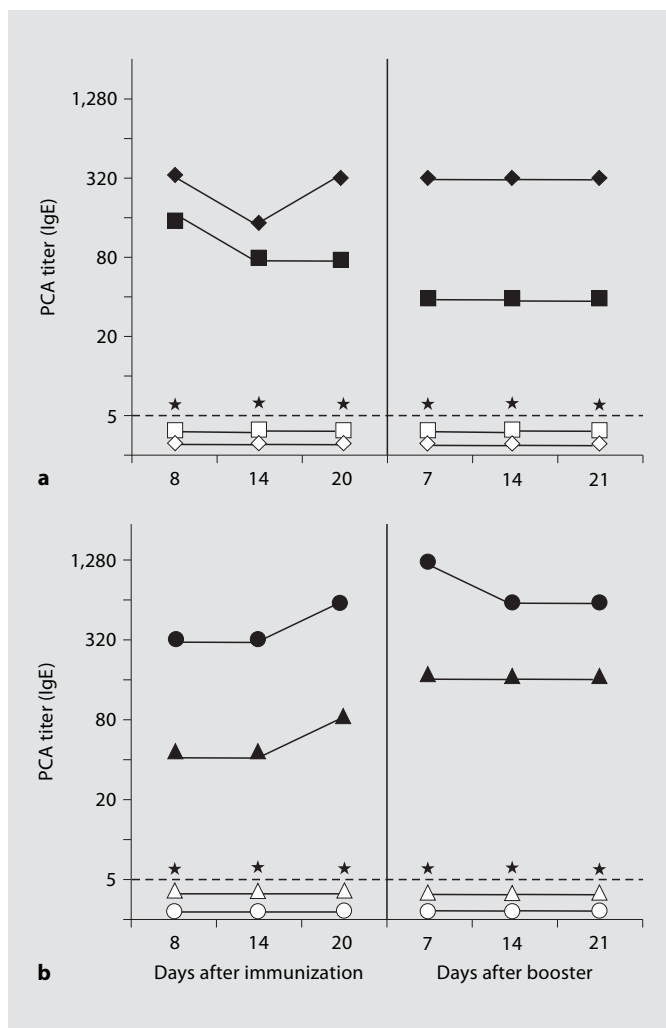


Fig. 6. Primary and secondary IgE responses in C57BL/6 (a) or BALB/c (b) mice (n = 6) immunized with OVA in adjuvant and boosted on day 20 with antigen. Pooled plasma from WT (closed symbols) or IL-4^{-/-} (open symbols) C57BL/6 mice injected with CFA (■, □) or aluminium hydroxide (◆, ◇), and from BALB/c mice injected with CFA (▲, △) or aluminium hydroxide (●, ○) was measured by PCA. Dashed lines represent the detection limit of the assay. * Significantly different from WT mice.

Comparatively, IgE antibodies were titrated in all the groups immunized with either adjuvant (fig. 6). In both strains, the levels of IgE were higher in WT mice when aluminium hydroxide was used as adjuvant vehicle. In addition, BALB/c mice produced more IgE than C57BL/6 mice. As expected, no IgE antibodies were detected in IL-4-deficient mice of both strains.

Discussion

Helminth infections are strongly associated with Th2 cells and their cytokines that stimulate large IgE antibody responses, blood and tissue eosinophilia, and intestinal mastocytosis [31, 32]. In addition, the parasites or their components have the ability to downregulate host immunity [33], leading to parasite-specific and more generalized levels of immunosuppression. In *A. suum*, we identified from the adult worm extract a high-molecular-weight fraction (PI) with strong immunosuppressive properties mediated by IL-4 and IL-10 [12–16]. In this work, we show for the first time that IL-10 induced by PI inhibits IgE and anaphylactic IgG1 antibody responses not only to bystander antigens but, together with IFN- γ , it also impairs the same responses to PI's own components. Thus, the very low titers of PI-specific anaphylactic IgG1 obtained in the primary response and the moderate secondary response were significantly enhanced in the absence of these cytokines. The apparent inability to induce IgE antibodies, even in the secondary response, was also overcome under this condition. Similar inhibition of IgE and IgG1 antibodies (measured by ELISA) by IL-10 has been reported for other antigens such as OVA and ragweed [34, 35]. The high serum levels of IgE and IgG1 obtained in IL-10^{-/-} mice sensitized and challenged with these antigens correlated well with high amounts of IL-4 and IL-5 in the bronchoalveolar lavage fluid of those injected with ragweed [35], but not with OVA [34]. In addition, lung T cells from IL-10^{-/-} mice sensitized with *Aspergillus fumigatus* antigen produced IL-4, IL-5, and also large amounts of IFN- γ compared with WT lung cells, but the presence of these cytokines in bronchoalveolar lavage fluid was dependent upon the genetic background and the route of immunization. Independent of the latter, IgE and IgG1 titers in IL-10^{-/-} mice were not different from those of WT mice [36]. It is interesting that, besides IgE and IgG1, Mäkelä et al. [34] obtained in their model of OVA sensitization in IL-10-deficient mice higher levels of IgG2a antibodies, which are known to be enhanced by IFN- γ [4, 37], but this cytokine remained unchanged. Recently, we have shown that an immunosuppressive protein isolated from the PI fraction had also an inhibitory effect on a model of experimental asthma induced by *Ascaris* allergenic components in mice, leading to the impairment of eosinophilic inflammation, airway mechanical parameters, and IgE and IgG1 antibody production. In contrast to the reduction of IL-4, IL-5, and chemokine levels, IL-10 and IFN- γ were increased in these mice [38]. All these reports together with our previous [16] and

present results indicate, therefore, that many factors, including primarily the nature of the antigen, determine whether IL-10 limits the potential to make large Th2 responses and other Th2-related immunological events, and, together with IFN- γ , to make large amounts of IgE and anaphylactic IgG1.

To define the role of IL-4 in controlling the synthesis of anaphylactic IgG1 during the primary and secondary responses, we used PI and OVA as antigens, two different adjuvant vehicles and IL-4-deficient mice in two different genetic backgrounds. At first glance, the difference between the IgG1 response obtained against PI and OVA in C57BL/6 mice immunized with CFA (fig. 1 vs. fig. 4a) is a clear example of what was mentioned above, i.e., the nature of the antigen solely can define the magnitude of the response, based mostly upon the profile of the cytokines it induces. The same applies to the IgE response. Altogether, the results show that IL-4 is crucial for the synthesis of anaphylactic IgG1 induced by PI, as previously reported [9]. In the response against OVA, the same is true at the beginning (day 8) of the primary response, but later on there is only a partial dependency upon this cytokine in C57BL/6 mice. BALB/c mice that have a genetically determined default Th cell differentiation towards Th2 [39] made IL-4-independent OVA-specific anaphylactic IgG1 antibodies late in the primary, and during the whole secondary response. These results suggest that other Th2 cytokines may compensate for the absence of IL-4 in this mouse strain. Among them, IL-13 shares many biological functions with IL-4 and cross-competes for IL-4R α , recruiting this chain into its receptor complex to increase binding affinity and initiate signaling transduction [40]. While IL-4- and IL-4/IL-13-deficient mice are severely impaired in their ability to mount an IgG1 response to immunization with OVA and alum, IL-13-/- mice develop a normal IgG1 response to antigen challenge [41]. Therefore, it is unlikely that IL-13 may replace IL-4 in BALB/c mice or act in combination with IL-4 in C57BL/6 mice. More recently, IL-21, another Th2 cytokine, has been shown to have the ability to trigger T cell and NK cell activation, and to promote B cell proliferation and apoptosis [42]. IL-21R-deficient mice immunized with OVA or KLH have low levels of total and antigen-specific IgG1 and high levels of circulating IgE, indicating that this cytokine may differentially influence IgE and IgG1 switch recombination [43]. In addition, IL-21 enhanced IgG1 production from splenic cells stimulated with LPS and IL-4, and increased the generation of mIgG1+ B cells in LPS-stimulated B cells in an STAT-6-independent fashion [44]. IL-21 itself also induced the

secretion of IgG4, analogous to murine IgG1, from mitogen-stimulated human PBMC [45]. Taken together, these results indicate that there is an IL-21-dependent pathway for IgG1 production, and, as suggested previously by others [37], this pathway may be involved in the residual IgG1 response obtained in IL-4-/- C57BL/6 mice. Recently, it was demonstrated in BALB/c mice that IL-21 expression is regulated independently of other Th2 cell cytokines (IL-4, IL-5 and IL-13) by the Th cell subset-specific action of the transcription factors NFATc2 and T-bet [46]. These findings suggest that the regulation of IgG1 production against a given antigen, like OVA, by IL-4 and/or IL-21 might be dissociated in time in different mouse strains, such as we observed in BALB/c and C57BL/6 mice. In addition, the nature of antigens, like PI and OVA, might determine in a given strain whether IgG1 responses are more or less dependent upon one of these cytokines or downregulated by cytokines like IL-10 and IFN- γ .

Another interesting finding in our present results is that only the IgE response fully represents the Th2- and Th1-inducing nature of aluminium hydroxide and CFA, respectively. Thus, in both mouse strains, IgE levels were higher in the primary and secondary responses when aluminium hydroxide was used as an adjuvant vehicle. Th2-prone BALB/c mice did also produce more IgE antibodies than C57BL/6 mice. The same was not true for anaphylactic IgG1 antibodies, because their levels did not vary significantly between C57BL/6 and BALB/c mice, neither was a difference obtained between CFA- or aluminium hydroxide-injected mice. These results may probably be due to the fact that IgE is exclusively dependent upon IL-4 [4] (fig. 6), and anaphylactic IgG1 depends upon other cytokine(s) late in the primary and also in the secondary response. All these discrepancies should be of concern when analyzing the results obtained in different experimental asthma models, since it has been demonstrated that IgE and anaphylactic IgG1 antibodies can induce or potentiate pulmonary eosinophilic inflammation and airway hyperreactivity [47–50].

Acknowledgements

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