Cutting Edge: Mouse IgG1 Antibodies Comprise Two Functionally Distinct Types That Are Differentially Regulated by IL-4 and IL-12

Eliana L. Faquim-Mauro, Robert L. Coffman, Ises A. Abrahamsohn and Mahasti S. Macedo

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IL-4-dependent and -independent IgG1 Abs differ in their ability to induce mast cell degranulation as measured by passive cutaneous anaphylaxis (PCA). Mice immunized with OVA or PIII (fraction of *Ascaris suum* produced high titers of IgG1 as shown by ELISA and PCA. In contrast, another *A. suum* fraction, PI, elicited IgG1 Abs with no PCA activity. IgG1 with anaphylactic activity required IL-4, as IgG1 responses to OVA and PIII in IL-4<sup>−/−</sup> mice gave no PCA. PI-specific IgG1 was IL-4-independent, because no difference was found between the responses of IL-4<sup>−/−</sup> and IL-4<sup>+/+</sup> mice. Significant PCA reactions were elicited, however, with PI-specific IgG1 from IL-12<sup>−/−</sup> or anti-IFN-γ Ab-treated mice, although less Ab was measured by ELISA. These results indicate that one type of IgG1 has anaphylactic activity and its synthesis is IL-4-dependent, being inhibited by IL-12 or IFN-γ; the other lacks this activity and its synthesis is stimulated by IL-12 or IFN-γ. *The Journal of Immunology*, 1999, 163: 3572–3576.

IgG1 isotype switching is known to be partially dependent upon IL-4. In addition to the well-established mechanism of IgG1 induction by Th2 clones, which is mediated by IL-4 (1, 2), most Th1 clones also stimulate substantial IgG1 responses (1). The interaction of CD40 on the surface of B cells with the CD40 ligand present on activated Th cell membranes (3) or expressed in the baculovirus system (4) also results in the expression of germ-line γ1 transcripts in B cells independently of IL-4. Moreover, in vivo studies have shown that anti-IL-4 Ab treatment does not abrogate IgG1 responses (5), and IL-4-deficient mice have only a partial reduction in total and Ag-specific IgG1 Ab levels as measured by ELISA (6). These data are indicative of a mechanism of isotype switching to IgG1 that is IL-4-independent and raise the intriguing question of whether IgG1 Abs that are regulated or not by IL-4 have differing biological activities.

IgG1 and IgE are the only Ig isotypes that can elicit active and passive anaphylactic reactions in mice (7–9), through binding to FcεRII and FcεRI, respectively, on mast cells (10–12). As the ability of IgE to bind to FcεRI is lost after heating, passive cutaneous reactions induced by heat-inactivated plasma are only due to IgG1 Abs (7). In this work, the levels of specific IgG1 Abs present in plasma from wild-type (wt) (IL-4<sup>−/−</sup>) and IL-4-deficient (IL-4<sup>−/−</sup>) mice immunized with three different Ags (OVA and two components from a helminth extract) were measured by ELISA; the biological activity of those Abs was determined by passive cutaneous anaphylaxis (PCA) analysis of heat-inactivated plasma. The results showed that IgG1 Abs that display anaphylactic activity are positively regulated by IL-4 and negatively regulated by IL-12 and/or IFN-γ. In contrast, the synthesis of IgG1 Abs that lack this activity is IL-4-independent and is enhanced in the presence of IL-12 or IFN-γ.

**Materials and Methods**

**Animals**

Female age-matched BALB/c mice that had targeted disruption of the IL-4 gene (IL-4<sup>−/−</sup>) were bred and maintained at the animal house facilities of DNAX Research Institute. Mice with a targeted disruption of the IgG1 switch region backcrossed for two generations onto a BALB/c background (IgG1<sup>−/−</sup>) were bred at DNA from pairs kindly supplied by Dr. Andreas Radbruch (Institute for Genetics, University of Cologne, Cologne, Germany). Age- and sex-matched BALB/c mice were purchased from Simonsen Laboratories (Gilroy, CA) and used as wt controls. Female age-matched C57BL/6 mice that had targeted disruption of the IL-4 gene (IL-4<sup>−/−</sup>) or the IL-12 gene (IL-12<sup>−/−</sup>) were kindly provided by Dr. L. V. Rizzo (Institute of Biomedical Studies, University of São Paulo). These mice and the age- and sex-matched C57BL/6 mice (wt controls) were bred in the animal house facilities of the Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, under specific pathogen-free conditions. We used 5- to 6-wk-old female BALB/c mice and 2- to 3-mo-old Wistar rats for PCA reactions.

**Antigens**

OVA grade V was obtained from Sigma (St. Louis, MO). Adult *Ascaris suum* body extract was prepared and fractionated by gel filtration in a Sephacryl S200 column as described previously (13). Fractions eluted in peak I (PI) and peak III (PIII), corresponding to high- and low-m.w. components (14), were concentrated with Centriprep 100 and 10 (Amicon, Massachusetts). Protein concentration was measured by the method of Bradford (15).

**Isotypes of IgG1**

The isotypes of IgG1 and IgG2a, produced by the two mouse lines, were determined by Western blotting as described previously (16). The mice used were of inbred strains BALB/c and C57BL/6. *Ascaris suum* worm extract was prepared and fractionated by gel filtration in a Sephacryl S200 column as described previously (13).

**Expression of Germ-Line γ1 Transcripts**

The expression of germ-line γ1 transcripts in B cells was determined by RT-PCR as described previously (16). The primers used were (A) CTT-CCG-GCA-CAT-CTT-CTA-CA and (B) TCT-ATT-GGC-GTA-GAA-GTG-ATT-GGG (16). The amplification products were separated by agarose gel electrophoresis and visualized using ethidium bromide.

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PCA activity (Fig. 2, A) was also found in the absence of IL-4 were able to mediate PCA reactions, whereas those dependent IgG1 Abs produced by IL-4 were not. Heat treatment to render IgE completely inactive in the PCA reaction. To confirm this, IgG12 heat treatment was used. In addition, to destroy any anaphylactic activity due to IgE Abs. The animals were inactivated at 56°C for 1 h described by Ovary (16). Titration curves were conducted for all samples of plasma. ANOVA followed by multiple comparisons using the Tukey method (15) was employed to compare the different groups.

**Titration of IgG1 Abs by ELISA**

Plasma obtained 8 days after immunization was tested for IgG1Abs using Ag-coated, 96-well plates and biotinylated goat anti-mouse IgG1 antisera (Southern Biotechnology Associates, Birmingham, AL). The reactions were developed with streptavidin-peroxidase conjugate (Jackson ImmunoResearch Laboratories, West Grove, PA), o-phenylenediamine, and H2O2; the plates were read at 450 nm on an automated ELISA reader (Dynatech, Chantilly, VA). Titration curves were conducted for all samples of plasma from wt, B6, IL-4−/−, and IL-4+/− mice as measured by ELISA compared with IL-4 wt mice. However, similar results to those reported here were obtained, that is, no PCA activity that develops after a short sensitization period is indeed due to anaphylactic IgG1 Abs.

**Immunization protocols**

Groups of four to five mice were injected s.c. in the base of the tail with 150 µg of OVA, PI, or PIII emulsified in 0.2 ml of CFA (Sigma). After 8 days the animals were killed by CO2 inhalation and bled from heart with a heparinized syringe. Plasma was stored at −20°C.

**Titration of IgG1 and IgE Abs by PCA**

Titration of IgG1 Abs was performed by PCA reactions in mice as described by Ovary (16). Aliquots of plasma were inactivated at 56°C for 1 h to destroy any anaphylactic activity due to IgE Abs. The animals were shaved on the back and three intradermal injections (0.05 ml) of serial dilutions of plasma were made in each side of the dorsal skin. After 2 h, mice were challenged i.v. with 0.5 ml of 0.25% Evans blue solution containing 250 µg of Ag. For the IgE titration, PCA reactions were elicited in rats according to Mota and Wong (17), using noninactivated plasma. After a sensitization period of 18–24 h, the animals were injected i.v. with 1.0 ml of dye plus Ag solution. All tests were made in triplicate, and PCA titers were expressed as the reciprocal of the highest dilution that gave a lesion of dye plus Ag solution. All tests were made in triplicate, and PCA titers were expressed as the reciprocal of the highest dilution that gave a lesion of dye plus Ag solution. ANOVA followed by multiple comparisons using the Tukey method (15) was employed to compare the different groups.

**Results and Discussion**

In the course of the studies on the response to *Ascaris* Ags, we found that DBA/2 mice immunized with two partially purified fractions, designated PI and PIII, had differences in IgG1 PCA titers that could not be explained by the differences in specific IgG1 Ab levels obtained by ELISA (18). Synthesis of IgG1 is regulated in part by IL-4; however, it can also be made in the absence of this cytokine. To determine whether IL-4 could regulate IgG1 Abs with differing biological activities, IL-4−/− and IL-4+/− mice were immunized with OVA, PIII, or PI in CFA and bled after 8 days.

IL-4−/− mice immunized with OVA (Fig. 1A) or PIII (Fig. 1B) showed significant but decreased Ag-specific IgG1 Ab levels in the plasma by ELISA compared with IL-4 wt mice. However, the IgG1 Abs produced by IL-4−/− mice exhibited strong anaphylactic activity by PCA (titer of 40–160), whereas the IL-4−/− independent IgG1 Abs produced by IL-4−/− mice had no detectable PCA activity (Fig. 2, A and B). As expected, OVA- and PIII-specific IgE Abs were absent and IgG2a Abs were elevated in IL-4−/− mice (data not shown). Thus, IgG1 Abs made in the presence of IL-4 were able to mediate PCA reactions, whereas those made in the absence of IL-4 were not.

The validity of the above conclusion depends upon the ability of heat treatment to render IgE completely inactive in the PCA reaction. To confirm this, IgG1−/− and IgG1+/+ mice were immunized with PIII in CFA. As expected, no IgG1 Ab production could be detected by ELISA in IgG1−/− mice. These mice, however, had IgG1 levels that were comparable with IgG1+/+ mice as measured by PCA using noninactivated plasma. In contrast, no PCA activity remained in heat-inactivated plasma from IgG1−/− mice (data not shown). This finding confirms that the heat-stable PCA activity that develops after a short sensitization period is indeed due to anaphylactic IgG1 Abs.

In addition, a very interesting result was obtained when IL-4+/+ and IL-4−/− mice were immunized with PI. Both groups produced similar levels of specific IgG1, as measured by ELISA (Fig. 3A), and these Abs completely lacked anaphylactic activity by PCA (Fig. 4A). These results demonstrate that this Ag induced primarily IL-4−/−independent IgG1 Ab under the immunization conditions employed and further confirm that IL-4 only regulates the production of IgG1 Abs with the ability to induce mast cell degranulation.

Because mouse IgG2b globulin or IgG2a and IgG2b myelomas are capable of blocking IgG1-mediated PCA reactions in mice (19), we used a protein A-Sepharose column to separate IgG1 and IgG2a/IgG2b isotypes from the plasma of PI- or PIII-immunized mice. The IgG1 fraction was then used to elicit PCA reactions and similar results to those reported here were obtained, that is, no PCA activity was found in the anti-PI IgG1 fraction, whereas a PCA titer of 40 was obtained with the anti-PII IgG1 fraction. Furthermore, four serial dilutions (1/5–1/40) of plasma from nonimmunized or PI-immunized mice (containing nonanaphylactic IgG1) were mixed with the plasma of PIII-immunized mice. The IgG1 fraction was then used to elicit PCA reactions and similar results to those reported here were obtained, that is, no PCA activity was found in the anti-PI IgG1 fraction, whereas a PCA titer of 40 was obtained with the anti-PII IgG1 fraction.
These data indicate that the difference in anaphylactic activity between the two types of IgG1 is due to intrinsic properties of the Ig molecule itself and does not depend upon interaction with other plasma components or interference of IgG2a or IgG2b isotypes.

IL-12 and IL-4 are characteristic of Th1- and Th2-dominated responses, respectively, and many of the biological activities of IL-12 are reciprocal to those of IL-4. Immunization with the PI Ag constituted a good model to examine the IL-12-exerted negative control over the synthesis of anaphylactic-type IgG1, as PI normally induces IgG1 Abs with absent or minimal anaphylactic activity. After immunization with PI, the PCA titers obtained with plasma from IL-12−/− mice were at least 8-fold higher than those obtained in IL-12+/+ mice (Fig. 4B), indicating that IL-12 was involved in the down-regulation of anaphylactic-type IgG1 Abs. Indeed, in the absence of IL-12, mice produced IgG1 PCA titers to PI that were comparable with the anti-PII PCA titers (data not shown). Despite the substantial increase in anaphylactic-type IgG1 Abs to PI in IL-12−/− mice, the levels of PI-specific IgG1 Abs, as shown by ELISA, were lower in IL-12−/− mice (Fig. 3B), indicating that the production of IgG1 Abs that lack anaphylactic activity is impaired in IL-12−/− mice. These data suggest that IL-12 can positively regulate the synthesis of the IL-4-independent IgG1 Abs. The production of anaphylactic-type IgG1 Abs to PI in IL-12−/− mice also excludes the possibility that PI could not leak from the vascular lumen into the Ab-sensitized dermal tissue due to its high m.w. and therefore would be unable to activate the local mast cells and elicit a skin reaction.

Both immunomodulating activities of IL-12, as an inducer and inhibitor of IgG1 Ab production, have been determined previously in different immunization protocols. When IL-12 was injected together with a polyclonal stimulant (anti-IgD Abs), a suppression of all major isotypes, but mainly of IgG1 and IgE, was observed (20). McKnight et al. (21), using aqueous Ag or Ag adsorbed to alum plus Bordetella pertussis as an adjuvant, reported strongly suppressed IgG1 and modestly enhanced IgG2a Ab responses upon administration of IL-12 to trinitrophenyl-keyhole limpet hemocyanin-immunized mice. In contrast, IL-12 plus alum promoted a major increase in the IgG1 Ab response to recombinant HIV-1 glycoprotein 120, a molecule shown to be weakly immunogenic in mice (22). Administration of IL-12 to CBA/J mice together with protein Ags adsorbed to alum primarily up-regulated the synthesis of IgG2a, IgG2b, and IgG3 Abs but also increased (3- to 5-fold) the synthesis of Ag-specific IgG1 Abs, whereas that of IgE was
on their ability or inability to activate the complement system by the classical pathway. We are currently investigating whether the nonanaphylactic type IgG1 could “fix” complement. Mota and Perini (27) also demonstrated that anti-OVA IgG1 Abs from mouse hyperimmune serum can differ in their affinity for protein A and in their PCA activity in mice and rats, indicating structural differences in the Fc fragments.

Slight differences in the Fc portion of human IgE molecules have been also described (28, 29). Using a human e-specific RT-PCR strategy, several alternatively spliced human e mRNAs were found that encode two membrane and four secreted forms of e proteins (30, 31). We have tried the same approach using RNA isolated from lymph node cells obtained after 6, 7, or 8 days of immunization with PI or PIII. However, we failed to detect any different alternative 3′ γ1 splices in these preparations. Because IgG1−/− mice immunized with PIII did not produce any type of IgG1, confirming the existence of only one Cγ1 gene, and because, for the moment, just one spliced γ1 mRNA seems to be generated, we would suggest that the difference between the anaphylactic- and the nonanaphylactic-type IgG1 might be posttranslational, probably at the glycosylation level. Aglycosylated mouse IgG2a (32) and human IgG3 Abs (33) have been shown to loose their binding capacity to human FcγRIII, indicating that carbohydrate chains might also be important for IgG1 Ab binding to and activation of mast cells through these receptors.

As demonstrated in this work, the synthesis of anaphylactic and nonanaphylactic type IgG1 primarily depends upon the nature of the Ag, because OVA and PIII injected in CFA induced both types of Ab, whereas PI induced almost only the nonanaphylactic type. However, we have observed that the proportion of the two types of IgG1 can be altered by the adjuvant, because OVA administered in aluminum hydroxide, for example, stimulated mostly the anaphylactic type. When IL-4 was neutralized by anti-IL-4 mAb in mice immunized with this adjuvant, no IgG1 Abs could be detected by PCA reactions and by ELISA (Faquim-Mauro, E.L., and M. S. Macedo, manuscript in preparation).

In conclusion, we propose that mouse IgG1 comprises two functionally distinct and independently regulated types of Ab: one has anaphylactic activity and its synthesis is completely IL-4-dependent, being inhibited by IL-12 and/or IFN-γ; the other lacks anaphylactic activity and its synthesis is IL-4-independent and impaired in IL-12- or IFN-γ-deprived mice.

References


