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SUPPRESSION OF THE BENZYLPENICILLOYL- (BPO) SPECIFIC IgE FORMATION WITH ISOLOGOUS ANTI-IDIOTYPIC ANTIBODIES IN BALB/c MICE[1]

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***In vivo* effects of actively produced or passively administered isologous anti-idiotypic antisera (aId) on the benzylpenicilloyl- (BPO) specific IgE and IgG formation in BALB/c mice have been studied. Isologous anti-BPO aId were raised in BALB/c mice by immunization with purified anti-BPO antibodies isolated from ascites induced with BPO-bovine γ-globulin in the same mouse strain. Mice producing isologous anti-BPO aId exhibited long-term suppression of BPO-specific IgE and IgG antibody responses induced by BPO-ovalbumin (BPO-OVA) in aluminum hydroxide. Simultaneously, they produced increased amounts of anti-BPO aId after each challenge with the BPO-OVA antigens. Passive administration of isologous anti-BPO aId into syngeneic mice previously sensitized with BPO-OVA caused depression of BPO-specific IgE antibody levels for 2 to 3 weeks. When anti-BPO IgE had again reached its previous level, passively administered aId had decreased to the level of untreated mice. Passive administration of anti-BPO aId also depressed the primary anti-BPO IgE formation for 2 to 3 weeks. In all these experiments the IgE antibody formation against the carrier proteins used for BPO-antigens was not affected. These results show that IgE and IgG antibodies share major idiotypic determinants and that IgE production is accessible to regulation by aId.**

The existence of identical or cross-reactive idiotypes in the immune response of different animals of the same inbred mouse strain makes it possible to attempt regulation of the antigen-specific immune response with antibodies directed against these idiotypes. Strong idiotypic cross-reactions have, for example, been observed among anti-benzoate and among anti-phenylarsonate antibodies of BALB/c and A/J mice (1, 2). They have also been observed among BALB/c myeloma proteins with antibody activity directed to the phosphorylcholine group (3, 4) and to group A streptococcal carbohydrate (5). Immune responses to these antigenic determinants have been suppressed almost completely for different lengths of time by *in vivo* administration of heterologous or homologous anti-idiotypic antisera (aId)[2] to immunized animals (5–7). However, an enhancing effect by some aId has also been reported (5).

In a number of studies it has been shown that an animal can mount an immune response against its own idiotypes (autologous aId) or against idiotypes of genetically identical animals (isologous aId) (8–11). BALB/c mice, for example, can produce antibodies (12) and helper T cells (13) recognizing the idiotypes of several myeloma proteins of BALB/c origin. Such isologous antibodies are almost entirely directed to determinants within or next to the antigen binding site (12). Animals that had been immunized with these idiotypes showed suppressed growth *in vivo* of myeloma tumors producing immunoglobulins of the corresponding idiotypes (14). Up to now, however, not much attention has been paid to the possible effects of aId on the formation of antigen-specific IgE antibodies. This particular immunoglobulin class is responsible for several allergic syndromes, and its regulation might offer possibilities for treatment of such diseases.

We have previously reported the suppression of the formation of homocytotropic anti-BPO-BGG antibodies in guinea pigs by using aId (15). Recently, we observed also that expression of phosphorylcholine-specific IgE antibodies in BALB/c mice is restricted to the T15 idiotype and that passively administered isologous anti-T15 antiserum markedly suppresses the levels of anti-phosphorylcholine IgE antibodies in these mice, as detected by passive cutaneous anaphylaxis (PCA) in the rat (16). The T15 idiotype is represented by the phosphorylcholine-specific myeloma proteins of the BALB/c tumors TEPC-15, HOPC-8, and S107.

In the present paper, *in vivo* effects of actively produced or passively administered isologous aId on the benzylpenicilloyl- (BPO) specific IgE and IgG antibody responses in BALB/c mice have been investigated. The BPO hapten is used because it is one of few chemically well-defined allergens that play an important role in human allergic diseases (17, 18). Furthermore, this model permits an evaluation of whether the aId response directed to a variety of BPO-generated idiotypes is capable of abolishing the entire response to the BPO determinant.

MATERIALS AND METHODS

Miscellaneous materials. Male and female BALB/c mice were obtained from G. L. Bomholtgård Ltd., Ry, Denmark. They were 7 to 9 weeks old when first immunized. For the production of ascites, occasionally old breeders have been used. All chemicals used were purchased from Sigma, St. Louis, Mo. $(BPO)_{42}$-bovine γ-globulin (BGG) and $(BPO)_{20}$-human serum albumin (HSA) were prepared as previously described (19). Lower substitution degrees were obtained in $(BPO)_4$-ovalbumin (OVA) and $(BPO)_9$-ascaris protein extract (Asc) by reducing the reaction time of the original procedure to 90 min. The molar ratio of BPO groups to carrier proteins was determined by the penamaldate method (20). BPO-AH-Sepharose 4B was pre-

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[1] This work was supported in part by the Swiss National Foundation for Scientific Research (Grant 3.944.78).
[2] Abbreviations used in this paper: aId, anti-idiotypic antibodies; Asc, ascaris protein extract; BPO, benzylpenicilloyl; BGG, bovine γ-globulin; OVA, ovalbumin; PCA, passive cutaneous anaphylaxis; HSA, human serum albumin.

pared by reacting 2 g AH-Sepharose 4B with 400 mg benzylpenicillin Na in 15 ml 0.025 M K_2CO_3 for 24 hr at 4°C. Crude protein extract of *Ascaris suum* (Asc) was prepared as described elsewhere (21).

BPO-^{125}I-ribonuclease A. Two hundred micrograms of bovine pancreas ribonuclease A (Type III-A, Sigma) were dissolved in 300 μl 0.15 M phosphate buffer at pH 7.5 and iodinated with 300 μCi Na ^{125}I according to the chloramin-T method (22). Then, 600 μl 2M K_2CO_3 were added together with 2 mg penicillin G. The mixture was reacted overnight at 4°C and purified by gel filtration on a Sephadex G-25 column (50 x 1 cm), by using PBS, pH 7.4, containing 2 mg BSA/ml as eluant. A crude product containing on the average 2.2 BPO groups per molecule, as determined by penamaldate assay had a total activity of 1.9 $\times 10^8$ cpm, as counted in an LKB-Wallac 80,000 gamma counter.

Immunization. For the production of IgE antibodies, BALB/c mice were sensitized with one or several injections of 10 μg $(BPO)_9$-Asc or $(BPO)_4$-OVA suspended in 10 mg $Al(OH)_3$. Anti-idiotypic serum was obtained by a slightly modified procedure described by Sakato and Eisen (12). Briefly, mice were immunized five times at weekly intervals with 200 μg of purified anti-BPO antibody, first suspended in 0.2 ml CFA/PBS 1:1 v/v and then in IFA. Of the suspensions, 0.1 ml was inoculated in four portions subcutaneously and 0.1 ml i.p. The mice were challenged every month with 200 μg anti-BPO antibodies in CFA/PBS 1:1 and bled individually every week. The same schedule was used for immunization with T15 and M167. Ascites containing anti-BPO-BGG antibodies was obtained according to the description of Tung *et al.* (23), by inoculating per mouse 0.5 mg $(BPO)_{42}$-BGG suspended in 0.2 ml CFA/PBS 9:1 i.p. After 2 weeks the mice were challenged weekly until the production of ascites ceased. Usually ascites developed after 8 to 9 weeks, and an average of 15 ml ascites was obtained per mouse after several tappings. The total fluid from 60 mice was pooled.

Purification of anti-BPO antibodies. Ascitic fluid was diluted 1:1 v/v with 0.2 M Tris-HCl buffer, pH 8.2, and centrifuged at 8000 × G for 60 min. The supernatant was precipitated with 45% saturated ammonium sulfate. The precipitate was redissolved in H_2O to a 1% solution and dialyzed against 0.2 M Tris-HCl buffer, pH 8.2. Proteins were then mildly reduced by adding dithiothreitol to a final concentration of 10 mM and alkylated in 25 mM iodoacetamide. The reduced and alkylated protein solution was dialyzed against PBS, pH 7.4, and adsorbed onto a column of $(BPO)_{20}$-HSA coupled to Sepharose 4B or onto BPO-AH-Sepharose 4B. Desorption was performed either with 0.01 M glycine/saline at pH 2.5, collecting the eluent in 2 M Tris-HCl, pH 8.5, or with 3 M sodium thiocyanate in PBS, pH 7.8. The yield of purified anti-BPO antibodies was usually 0.4 to 0.5 mg/ml ascites. The solution was then again dialyzed against PBS, pH 7.4, and concentrated by ultrafiltration on Amicon XM.100A to 2 mg/ml. Electrophoresis in agarose gel showed one single protein band in the γ-globulin region. A single precipitation line with serum dilutions of 1:1 to 1:64 was obtained in double radial immunodiffusion against BPO-OVA. No precipitation lines were visible against normal BALB/c serum, BGG, and OVA. The purified anti-BPO antibody was uniformly IgG, as demonstrated by specific antisera in radial immunodiffusion and in immunoelectrophoresis.

Preparation of anti-BPO Fab(t) fragment. The Fab(t) fragment was obtained by tryptic cleavage as described previously (8). Separation of the fragments was performed by gel filtration on a Sephadex G-100 column in 0.05 M Tris-HCl at pH 8.0. On electrophoresis in 10% polyacrylamide gel containing sodium dodecylsulfate, only the two protein bands corresponding to the light chain and the heavy chain fragments were visible next to each other.

Radioimmunoassays. For the estimation of specific antibodies in serum, we used an assay with *Staphylococcus aureus* protein A coupled to Sepharose 4B. Protein A is known to interact at pH 8.0 with the main subclasses of IgG molecules from many species including mouse (24, 25). It is known that it binds entirely to the Fc part of IgG molecules (26).

For the estimation of aId, 5 μl serum were added to 200 μl of Sepharose-4B-protein A (20 mg/ml) (Pharmacia, Uppsala) in PBS, pH 8.0, containing 2 mg/ml BSA. After 4 hr at 4°C, 200 ng of the corresponding ^{125}I Fab(t) (4×10^5 cpm) iodinated as indicated above for BPO-^{125}I-ribonuclease were added in 10 μl buffer; they were mixed and kept overnight at 4°C. The mixture was centrifuged and washed four times with PBS, pH 8.0, containing BSA and 0.5% Tween-20. Bound radioactivity was measured in an LKB-Wallac 80,000 gamma counter.

Anti-BPO antibodies of the IgG class were estimated by a similar procedure. However, as a labeled antigen, 200 ng BPO-^{125}I-ribonuclease A containing 10^5 cpm per sample were added to the mixture of Sepharose-protein A and investigated serum. The amount of IgG antibodies was estimated with a calibration curve obtained from dilutions of purified anti-BPO IgG antibodies. Estimations were repeated and made in triplicate each time.

Passive cutaneous anaphylaxis (PCA). Mouse IgE was titrated by PCA in Wistar rats as described (27). The sensitization period before intracutaneous administration of 1 mg BPO-polylysine, 2 mg OVA, or 2 mg Asc in 1 ml of 1% Evans blue was 20 to 24 hr; therefore, the skin reactions were elicited almost entirely by IgE antibodies (27). The reaction was set up in duplicate, and the titer was expressed as the reciprocal of antiserum dilutions yielding reactions of 5 mm diameter (end point titration).

Tumor lines. The mouse plasmacytomas TEPC-15 and MOPC-167 were generously provided by Dr. Michael Potter, National Institute of Health. Ascitic fluid was collected by paracentesis of BALB/c mice that had been inoculated i.p. with 0.5 ml of crude ascites four weeks previously (28).

RESULTS

Production and characteristics of anti-BPO aId. BALB/c mice were immunized with purified syngeneic anti-BPO antibodies obtained from BPO-BGG/CFA-induced ascites. Contamination of an anti-BPO antibody preparation with BPO-HSA was less than 8×10^{-4} percent (w/w) after desorption from a (^{14}C-$BPO)_{20}$-HSA-Sepharose column. Such a small amount does not influence the radioimmunoassay for the detection of aId by ^{125}I-anti-BPO-Fab(t). Moreover, no difference was obtained when mice were immunized with anti-BPO antibody preparations either purified on Sepharose-columns with BPO-HSA or with BPO-1,6-diaminohexyl groups. The latter molecule is monovalent and is neither immunogenic nor does it influence the aId radioimmunoassay.

After repeated immunizations with anti-BPO antibodies, individual sera were collected weekly, and the levels of aId were estimated by using a radioimmunoassay with ^{125}I-anti-BPO-Fab(t) and protein A bound to Sepharose 4B. Highest levels of aId were obtained 7 to 8 weeks after the first immunization; a booster injection at day 79 again raised the aId levels (Fig. 1). All mice immunized with 200 μg anti-BPO antibodies produced aId.

By using the radioimmunoassay for aId, radioactivity bound

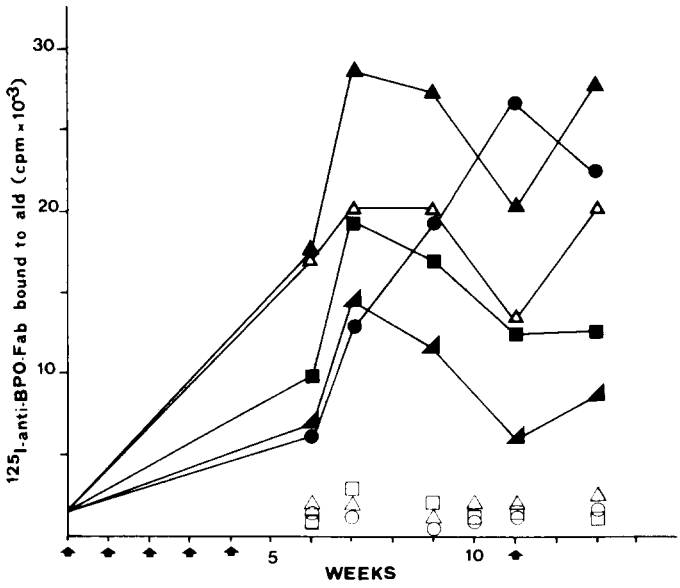

Figure 1. Estimation by radioimmunoassay with ^{125}I-anti-BPO Fab(t) and Protein A-Sepharose of isologous anti-BPO aId levels in antisera of five repeatedly immunized BALB/c mice (◢, ■, ▲, ●, △). Sera of individual mice were taken every week. (↑) indicates days of immunization with 200 μg purified anti-BPO antibodies. As controls, sera of untreated BALB/c mice (□), isologous anti-T15 (○), and anti-M167 (△) antisera were used.

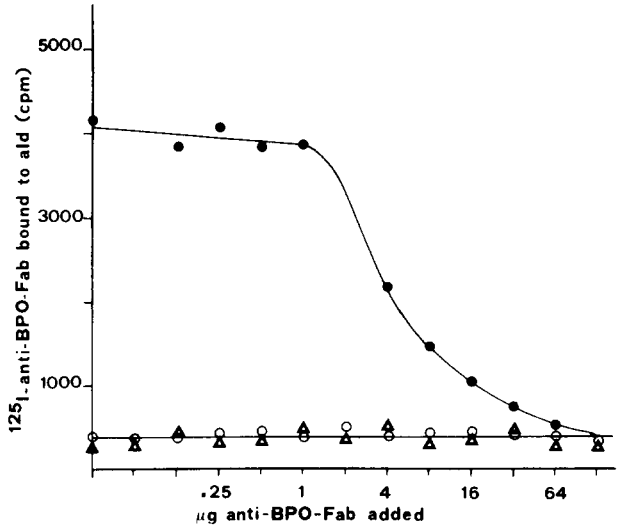

Figure 2. Specificity for anti-BPO Fab(t) of isologous antisera obtained by immunization of BALB/c mice with purified anti-BPO antibodies (●) or M167 myeloma protein (△) and of normal BALB/c serum (○). Antisera were reacted with ^{125}I-anti-BPO-Fab(t) in a radioimmunoassay with Protein A-Sepharose and increasing amounts of unlabeled anti-BPO-Fab(t) were added. Inhibition is shown only in anti-BPO aId antiserum but not in normal serum or anti-M167 antiserum.

to the aId in these sera was 3 to 5 times higher than those obtained with normal sera. The idiotype-anti-idiotype reaction was fully inhibitable with 65 μg anti-BPO-Fab(t), and inhibition of 20 to 70% was achieved with 10 mM BPO-epsilon-aminocaproic acid. No reaction with anti-BPO-Fab(t) was observed in normal serum and anti-M167 antiserum (Fig. 2). Conversely, anti-BPO aId antiserum did not react with T15 or M167 Fab(t) fragments. Figure 3 shows that the reaction between aId and ^{125}I-labeled anti-BPO-Fab(t) was not inhibitable by the Fab(t) fragments of M167 and T15. Isologous anti-T15 and anti-M167 antisera showed the same inhibition curves with their Fab(t) fragments as demonstrated by Sakato and Eisen (12). These results are therefore not shown here. In addition, 70 μg of purified anti-BPO-Fab(t) failed to inhibit in the M167 system but showed inhibition of 30% of the reaction anti-T15 with T15-^{125}I-Fab(t). These various experiments suggest that isologous antiserum against anti-BPO antibodies is directed to the idiotypic region. Since the idiotype-aId reaction is partially blocked by BPO-epsilon-aminocaproic acid, we suggest that the idiotypic region of the anti-BPO antibodies is mostly within or next to the antigen combining site of these molecules. Similar findings were made by Sakato and Eisen (12) for idiotypic determinants in myeloma proteins. With the radioimmunoassay used, aId antisera could usually be diluted to at least 1/512 (i.e., aId contained in 0.01 μl antiserum is still detectable) before reaching control levels (end point titration).

Effects of i.v. administered isologous anti-BPO aId on the BPO-specific IgE formation. Two groups of four animals, which were primed with 10 μg ascaris protein extract suspended in $Al(OH)_3$ one week earlier were injected i.v. with either 0.1 ml of normal BALB/c serum or 0.1 ml of aId. One day later, they were immunized with $(BPO)_9$-Asc in aluminum hydroxide. Sera of these mice were pooled, and the level of anti-BPO IgE was estimated by PCA in rats. As shown in Figure 4, the group that

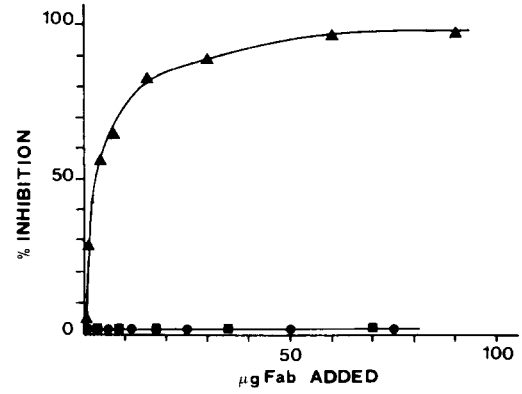

Figure 3. Specificity of isologous antiserum obtained by immunization of BALB/c mice with purified anti-BPO antibodies. The reaction of the antiserum and ^{125}I-labeled anti-BPO-Fab(t) is inhibitable with unlabeled anti-BPO-Fab(t) (▲) but not with Fab(t) of T15 (●) or M167 (■) myeloma proteins.

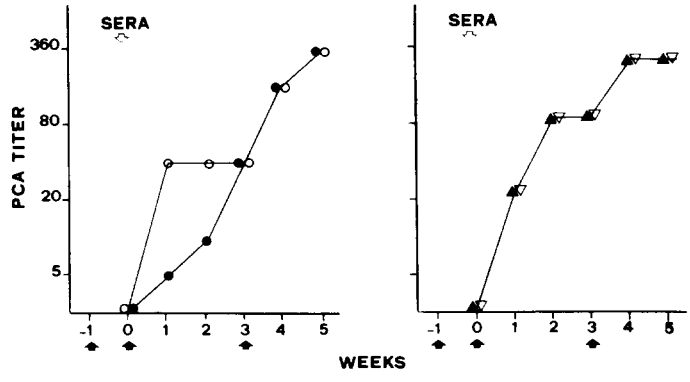

Figure 4. Effect of passively administered isologous anti-BPO aId on the primary IgE response. Compared to carrier-primed BALB/c mice treated with normal serum (○), mice injected with anti-BPO aId (●) show depressed anti-BPO IgE antibody formation after immunization with $(BPO)_9$-Asc in aluminum hydroxide (↑). Titers of anti-Asc IgE antibodies are similar in both of the two groups (▽, ▲).

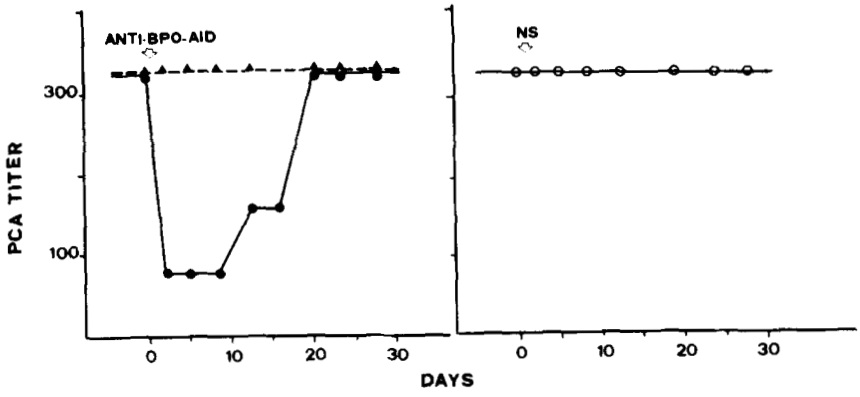

Figure 5. Effect of passively administered isologous anti-BPO aId on the ongoing IgE response induced with $(BPO)_4$-OVA. BALB/c mice injected with anti-BPO aId show depressed anti-BPO IgE antibody formation for 2 to 3 weeks (●). No effect is observed on the anti-OVA IgE antibody formation (▲), compared to BALB/c mice that observed normal serum (○).

had received normal BALB/c serum developed an IgE titer of 40 within 7 days, whereas animals administered with isologous aId showed delayed formation of anti-BPO IgE. Both groups of immunized mice showed the same anti-BPO IgE titer 3 weeks after administration of either aId or normal serum. No influence of passively administered anti-BPO aId was observed on the carrier- (OVA) specific IgE response.

With regard to practical conditions in allergic diseases, it would be more relevant to suppress IgE responses that are already established. Therefore, either isologous anti-BPO aId or normal BALB/c serum was administered i.v. into two groups of four BALB/c mice that were producing anti-BPO-OVA IgE at a titer of 320. The group that received normal serum did not show any alteration of the ongoing anti-BPO IgE level, but the group that had received anti-BPO aId showed a decrease of anti-BPO IgE to one-fourth of the original level (Fig. 5). This reduced titer of 80 was observed one day after injection. BPO-specific IgE levels remained depressed for 2 to 3 weeks and then spontaneously reached the initial PCA titer of 320 again. In all these experiments, levels of IgE antibodies against the carrier protein (OVA) were not affected (Fig. 5). To determine whether the increase of anti-BPO IgE in the 3rd week is due to exhaustion of aId in serum, we estimated the content of aId over the period after administration of the aId antiserum. Figure 6 shows that aId has disappeared from the serum at the same time when BPO-specific IgE has reached its original level.

It might be argued that experiments showing depressed IgE titers after passive administration of aId reflect an inhibition of antibodies rather than real suppression of specific IgE formation. To exclude this possibility, BALB/c anti-BPO IgE antiserum with a titer of 320 was mixed with various volumes of either anti-BPO aId, normal BALB/c serum, or saline. After 1 hr incubation at room temperature, these mixtures were injected in several dilutions into a rat skin. Mixtures up to equal amounts of anti-BPO aId showed no difference in PCA titers compared with normal serum or saline.

Suppression of the anti-BPO response in mice actively producing aId. It has been demonstrated that T cells also carry idiotypes and aId that have been obtained by immunization with primed T lymphocytes (29). As shown by A. F. Geczy *et al.* (15), serum of guinea pigs immunized with syngeneic anti-BPO-BGG antibodies is capable of suppressing BPO-specific T cell proliferation *in vivo*. Therefore, in the present study it was investigated whether active immunization with purified anti-BPO antibodies would lead to suppression of formation of antibodies directed to the BPO determinant. Mice actively producing anti-BPO aId were immunized with $(BPO)_4$-OVA in aluminum hydroxide gel 3 months after their last challenge with purified anti-BPO immunoglobulins. It was found that

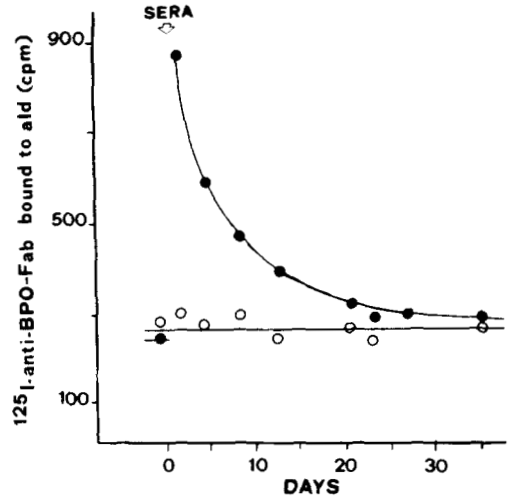

Figure 6. Decrease with time of passively administered anti-BPO aId as estimated by radioimmunoassay with ^{125}I-anti-BPO Fab(t) and Protein A-Sepharose. After 2 to 3 weeks BALB/c mice injected with anti-BPO aId (●) show the same levels for aId as mice that received normal BALB/c serum (○).

these anti-BPO aId producers could not develop high anti-BPO IgE titers for more than 6 months despite five injections of BPO-OVA. However, they produced IgE directed to the carrier protein (OVA) at the same level as control mice (Fig. 7). The group of control mice that did not produce anti-BPO aId detectable in serum, formed IgE antibodies specific to the BPO group and to the OVA carrier. After a third and a fourth booster injection, PCA titers of 640 for anti-BPO and 320 for anti-OVA were obtained in the group of control mice. Producers of aId showed titers of 40 for anti-BPO IgE and of 320 for anti-OVA IgE antibodies.

The same serum samples have been used to estimate the BPO-specific IgG response obtained in aId producers or controls after low-dose immunization with 10 μg $(BPO)_4$-OVA in aluminum hydroxide gel. For this, the described radioimmunoassay with Sepharose-4B-bound protein A, which is selective for IgG, and (BPO)-^{125}I-ribonuclease A as a labeled antigen was performed. In Figure 8, it is shown that anti-BPO antibodies of the IgG class appear in aId producers soon after immunization with $(BPO)_4$-OVA in aluminum hydroxide, whereas control mice show anti-BPO IgG antibodies only after the second boost. Therefore, in contrast to the IgE antibody formation, the production of IgG in the primary response appears slightly enhanced in aId producers. However, 2 weeks after the third booster injection, the control mice overtake the aId producers and show 3 to 6 times higher amounts of anti-BPO IgG anti-

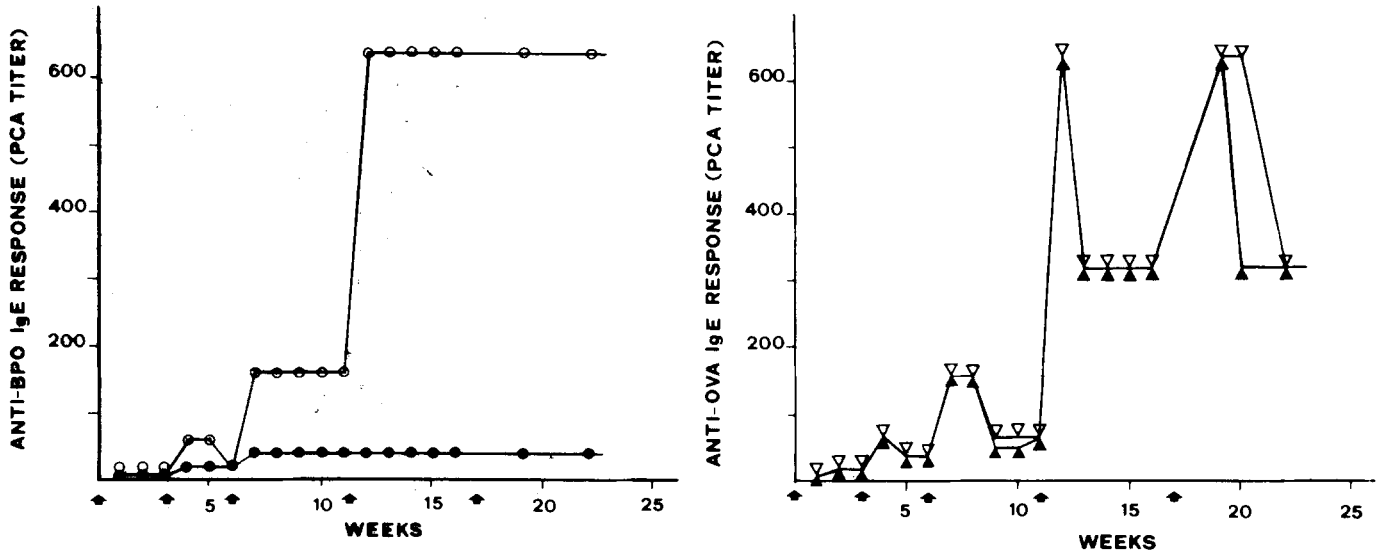

Figure 7. Formation of anti-BPO IgE antibodies in BALB/c mice upon immunization with $(BPO)_4$-OVA in aluminum hydroxide. Mice actively producing anti-BPO aId (●) suppress the formation of anti-BPO IqE antibodies, whereas age-matched control mice (○) show anti-BPO IgE titers up to 640. Both groups show the same titers for IgE antibodies against the carrier protein (OVA) (▽, ▲).

bodies than the latter. In contrast to the IgE production, IgG antibodies remained at a level of 300 μg/ml throughout.

In order to examine whether suppression of cross-reactive IgE and IgG idiotypes is related to the production of aId, the levels of aId were assessed in the serum of aId producers and controls during the course of immunization with $(BPO)_4$-OVA on the same serum samples as used for determination of anti-BPO IgE and IgG antibodies. No significant amounts of aId were detected by radioimmunoassay in the serum of control mice that produce high titers of anti-BPO IgE and IgG (Fig. 9). However, mice that had first been immunized with purified anti-BPO antibodies and that suppressed the anti-BPO IgE response, still produced aId 3 months after their last challenge with anti-BPO antibodies. Moreover, repeated immunization with $(BPO)_4$-OVA seemed to boost aId production.

DISCUSSION

Anti-BPO IgE and IgG antibodies of BALB/c mice share idiotypic determinants with BPO-specific antibodies obtained from ascites of the same inbred strain. Therefore, variable regions of IgE and IgG antibodies may be coded for by the same V_H gene, as was previously shown for IgM and IgA antibodies (30). This is apparent mainly from the fact that i) anti-BPO aId obtained from anti-BPO IgG antibodies depress formation of anti-BPO IgE antibodies. The IgE response to the antigen carrier (OVA) is not affected. ii) ^{125}I-labeled anti-BPO Fab(t) from purified anti-BPO ascites antibodies reacts in a radioimmunoassay with isologous antisera obtained after syngeneic immunization with anti-BPO antibodies. There was no reaction of the anti-BPO Fab(t) with anti-M167 and anti-T15 (BALB/c myeloma proteins specific for the phosphorylcholine determinant) antisera obtained by the same immunization schedule. Labeled Fab(t) fragments of M167 and T15 proteins also failed to react with sera obtained by immunization with anti-BPO antibodies. The reaction between anti-BPO aId and ^{125}I-anti-BPO Fab(t) was not inhibitable by Fab(t) fragments of M167 and T15. iii) The reaction of anti-BPO Fab(t) with anti-BPO aId was inhibited with BPO-ε-aminocaproic acid. These various arguments lead to the conclusion that depression of the anti-BPO antibody response is achieved by aId and not by anti-isotypic or anti-allotypic antibodies. It also appears unlikely for theoretical reasons that syngeneic immunization would evoke anti-isotypic and/or anti-allotypic antibodies.

Upon a single passive administration of isologous anti-BPO aId, the appearance of BPO-specific IgE antibodies was delayed in the primary response (Fig. 4) and temporarily depressed in an ongoing anti-BPO-OVA IgE response (Fig. 5). In contrast, administration of serum from untreated BALB/c mice had no effect on the anti-BPO IgE response in control animals. Two to 3 weeks after passive administration of aId, the IgE response again reached the original level, and aId activity was no longer detectable in serum. In an additional experiment, there was no change of the PCA titer observed when anti-BPO IgE antiserum was preincubated with anti-BPO aId. This is evidence that aId acts on IgE antibody synthesis and not merely by inhibition of the PCA reaction through forming idiotype-aId complexes. Also, a number of facts described in the literature indicate clearly that aId are capable of acting at the cellular level (31–33). For example, T cells can recognize idiotypes (13); lymphoid cells and lymphoblasts carry idiotypes similar to those of immunoglobulins (29, 34) and are therefore recognized by aId. This can take place also for IgE responses, as recently shown by A. F. Geczy *et al.* (15). Cell transfer experiments and repeated administration of aId over a longer period may clarify this point, and ongoing experiments in our laboratory support the cellular basis of aId action for IgE.

Transient reversible suppression of IgE response could also be observed if anti-BPO IgG antibody is administered before or shortly after primary immunization. However, it appears (35) that only antiserum that had been raised by immunization with the same hapten-carrier conjugate has an immunosuppressive effect. This may indicate that suppression by isologous aId is induced by a mechanism other than administration of anti-conjugate antibodies.

Mice actively producing aId show long-term effects on the production of cross-reactive idiotypes of the IgE class. BALB/c mice immunized with purified isologous anti-BPO antibodies demonstrated suppression of BPO-specific IgE antibody formation for more than 6 months. Appearance of new idiotypes was not detected in the IgE class, since the IgE antibody titers remained very low throughout, but it cannot be excluded for other immunoglobulin classes. Particularly, the

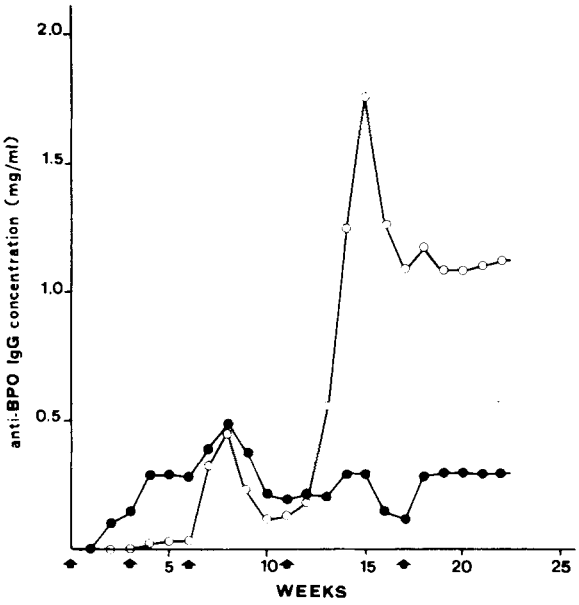

Figure 8. Formation of BPO-specific IgG antibodies in BALB/c mice upon immunization with $(BPO)_4$-OVA in $Al(OH)_3$. Levels were estimated by radioimmunoassay with (BPO)-^{125}I-ribonuclease and Protein A-Sepharose. Amounts were obtained from an anti-BPO IgG standard curve. The formation of anti-BPO IgG antibodies is depressed 11 weeks after the first immunization with $(BPO)_4$-OVA, but slightly enhanced during the first 7 weeks in mice producing anti-BPO aId (●). Age-matched control mice (○) show increased anti-BPO IgG antibody formation after 11 weeks. Values in the curve are means of triplicates.

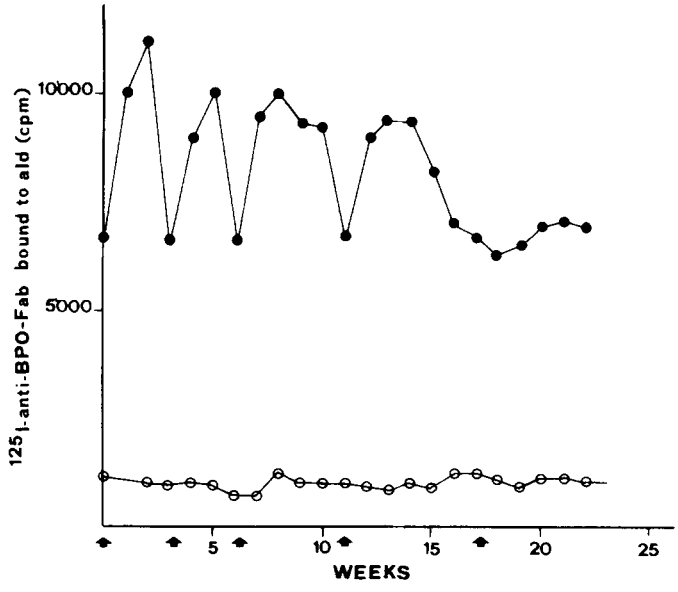

Figure 9. Amounts of anti-BPO aId estimated in sera of BALB/c mice by radioimmunoassay with ^{125}I-anti-BPO Fab(t) and Protein A-Sepharose. Mice were immunized seven times with purified anti-BPO antibodies and received 3 months later multiple injections of $(BPO)_4$-OVA in $Al(OH)_3$. Animals producing isologous anti-BPO aId show increased levels of anti-BPO aId after each challenge with $(BPO)_4$-OVA (●), whereas age-matched control mice (○) show no significant levels of anti-BPO aId. (↑) indicates the days of immunization with $(BPO)_4$-OVA in aluminum hydroxide gel.

production of IgG antibodies is only partially depressed. In contrast to the production of IgE antibodies, low-dose immunization with $(BPO)_4$-OVA in aluminum hydroxide does not initiate anti-BPO IgG antibodies in control mice before the third injection (Fig. 8). This may be due to the adjuvant, which does not initiate IgG production very efficiently. On the other hand, mice producing anti-BPO aId show an apparent helper effect on the primary IgG antibody formation, since these mice produce significant amounts of anti-BPO IgG after the second injection. After 11 weeks, however, the IgG production in aId producers appears to be depressed, whereas control mice start to produce anti-BPO IgG antibodies in amounts up to 1.8 mg/ml of serum. This late depression of IgG antibody formation in aId producers could be explained by the fact that the anti-BPO antibodies that were used to evoke aId are obtained from ascites after 9 weeks of immunization and may therefore contain mainly "late" and high specific major idiotypes; aId to reduced and mildly alkylated ascites antibodies are possibly not able to suppress the "early" and less specific minor idiotypes. On the other hand, the apparent helper effect on the primary IgG response cannot be fully explained at this time. It might be a priming effect of BPO-HSA contaminants in the anti-BPO antibody preparation or different effects of aId on the T/B cell cooperation at various stages of the immune response.

Although mice hyperimmunized with purified isologous anti-BPO antibodies show suppressed idiotype formation, increased amounts of aId occur in serum after each challenge with $(BPO)_4$-OVA antigen. $(BPO)_4$-OVA might induce limited amounts of highly immunogenic idiotypes stimulating helper cells for aId production by B cells; alternatively, antigen-idiotype complexes might foster the production of aId (36).

Suppression of the IgE antibody formation by aId may operate through mechanisms similar to those governing allotype suppression. However, it may be of greater therapeutical value for the treatment of allergic diseases, since allotype suppression must be initiated in newborn individuals. Thus, idiotype suppression can be applied in animals with an ongoing antigen-specific immune response. The production of immunoglobulin of one specificity but of all classes will in principle be suppressed, whereas the immune responses to other antigenic determinants will not be affected. According to the experiments reported here, the IgE response appears especially susceptible to suppression by aId. Although a thorough analysis of the heterogeneity of the idiotypic response of BALB/c mice to the BPO determinant is still pending, and although the precise molecular and cellular mechanisms leading to aId suppression are not yet fully understood, we have shown that the BPO-specific IgE response in mice is accessible to aId regulation. Therefore, further knowledge of idiotype aId regulation will probably be applicable to the regulation of IgE in allergic diseases. Finally, the question arises from this work as to whether the aId themselves can induce allergic reactions, since the antibody binds to the combining site of IgE molecules. In that case, passive administration of aId could elicit undesired side effects, and active production of aId might be the cause of recurrent allergic phenomena, such as chronic urticaria, by some autoimmune mechanisms. Indeed, elicitation of Prausnitz-Küstner (P-K) reactions with aId have been obtained (Nakagawa T., Blaser K. and de Weck A.L., manuscript submitted), but the amounts of aId injected to obtain IgE depression have not caused systemic reactions.

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