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Effect of passively administered isologous anti-idiotypes directed against anti-carrier (ovalbumin) antibodies on the anti-hapten IgE and IgG antibody responses in BALB/c mice

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Summary. Recently we have shown that active immunization of syngenic animals with anti-ovalbumin antibodies evokes an anti-idiotypic (aId) response, which in consequence leads to suppression of the anti-hapten (benzylpenicilloyl, BPO; dinitrophenyl, DNP) IgE and IgG formation subsequently attempted by immunization with low doses of hapten-OVA conjugates. In this work we describe attempts to suppress a primary or an already established anti-hapten IgE response by passive administration of (anti-carrier) anti-idiotypes to BALB/c mice. Our results show that ongoing anti-BPO or anti-DNP IgE responses can be depressed by injection of (anti-ovalbumin) aId, provided mice were previously immunized with conjugates of the haptens with the ovalbumin (OVA) carrier. The same animals suppressed for IgE also produce less anti-hapten and anti-carrier IgG antibodies but only after 5–6 weeks following the aId injection. The primary IgE response could be blocked by treating mice with (anti-OVA) aId and antigen at the same time.

Abbreviations: Id, idiotype; aId, anti-idiotype; BPO, benzylpenicilloyl; DNP, dinitrophenyl; ASC, *Ascaris suum*; BSA, bovine serum albumin; BGG, bovine-γ-globulin; OVA, ovalbumin; CTh, carrier-specific helper T cells; CTs, carrier-specific suppressor T cells; PCA, passive cutaneous anaphylaxis; Staph. A, Staphylococcus protein A.

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INTRODUCTION

It has been established by several investigators (Ovary & Benacerraf, 1963; Rajewsky, Schirrmacher, Nase & Jerne, 1969; Mitchison, 1969; Kishimoto & Ishizaka, 1973; Blaser, Nakagawa & de Weck, 1981a) that the participation of an immunogenic carrier molecule is of crucial importance in the elicitation of a response against a hapten determinant. This so called carrier effect, also required for the establishment of an IgE antibody response, is the result of the co-operation between at least two populations of immunologically competent cells with distinct specificities for hapten or carrier antigenic determinants. Moreover two functionally opposing regulatory T cells, both specific for carrier determinants, have been described; one helps the antibody response (CTh), the other suppresses it (CTs; Blaser *et al.*, 1981a; Blaser, Geiser & de Weck, 1979; Tada, Takemori, Okumura, Noraka & Tokuhisa, 1978; Herzenberg, Tokuhisa & Herzenberg, 1980, 1981b). Furthermore, it has been demonstrated that relatively high amounts of aId induce suppressor T cells (Ts) able to suppress a primary antibody response (Eichmann, Falk & Rajewsky, 1978; Dohi & Nisonoff, 1979). It has also been shown in our group, that anti-idiotypic (aId) antibodies can suppress the formation of IgE antibodies (Blaser *et al.*, 1981a; Blaser, Nakagawa & de Weck, 1980, 1981b). In this way both initiation of IgE formation and an already established IgE antibody response could be suppressed

for different lengths of time in different antigenic systems.

Accordingly, we have undertaken attempts to suppress an anti-hapten antibody response with aId directed against anti-carrier (ovalbumin, OVA) antibodies. We have demonstrated (Blaser *et al.*, 1981a) that BALB/c mice actively producing isologous (anti-OVA) aId, following immunization with syngeneic anti-OVA antibodies, show depressed anti-benzylpenicilloyl (BPO) and anti-dinitrophenyl (DNP) IgE and IgG antibody responses, provided the aId producing mice were subsequently challenged with low doses of $(BPO)_4$-OVA or $(DNP)_8$-OVA in $Al(OH)_3$ (alum).

No effect on the immune response was observed if (anti-OVA) aId producing mice were immunized with $(BPO)_9$-*Ascaris suum* (ASC; Blaser *et al.*, 1981a). In the work presented here, we have studied the effects of passively administered isologous (anti-carrier) aId on already existing anti-hapten IgE and IgG antibody responses and on the formation of antigen-specific antibodies of these immunoglobulin classes in the primary response. We show that a single injection of isologous (anti-OVA) aId can induce a long lasting suppressive effect on the ongoing hapten and OVA-carrier-specific IgE and IgG antibody responses.

MATERIALS AND METHODS

Miscellaneous materials

Male and female BALB/c mice were obtained from G. L. Bomholtgård Ltd, Ry, Denmark. They were 7–9 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).

$(BPO)_{42}$-BGG and $(BPO)_{20}$-HSA were prepared as previously described (Blaser, Nakagawa & de Weck, 1981a). Lower degrees of substitution were obtained in $(BPO)_4$-OVA and $(BPO)_9$-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 (Nakagawa, Blaser & de Weck). Low substituted (DNP)-OVA and (DNP)-ASC were prepared as described elsewhere (Eisen, 1964). Crude protein extract of Ascaris suum was obtained by the method described in (Blaser *et al.*, 1981a).

Purification of anti-OVA 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 sulphate. The precipitate was redissolved in H_2O to a 1% solution and dialysed 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 dialysed against phosphate-buffered saline (PBS), pH 7·4, and adsorbed onto a column of OVA coupled to 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-OVA antibodies was usually 0·4–0·5 mg/ml ascites. The solution was then again dialysed against PBS pH 7·4 and concentrated by ultrafiltration in an Amicon XM 100 A to 2 mg/ml. Electrophoresis in agarose gel showed one single protein band in the gamma globulin region. A single precipitation line with serum dilutions of 1:1 to 1:64 was obtained in double radial immunodiffusion against OVA. No precipitation lines were visible against normal BALB/c serum and bovine-γ-globulin (BGG). The purified anti-OVA antibody was uniformly IgG as demonstrated by specific antisera in radial immunodiffusion and in immunoelectrophoresis.

Preparation of anti-OVA Fab(t) fragment

The Fab(t) fragment was obtained by tryptic cleavage as described previously (Blaser *et al.*, 1980). 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 dodecylsulphate, only the two protein bands of the partially reduced Fab(t) corresponding to the light chain and the respective heavy chain fragment were visible next to each other.

Radioimmunoassays

For the estimation of anti-idiotypic antibodies the following assay, described in Blaser *et al.* (1980) was used. In brief, 5 μl serum was added to 200 μl of Sepharose 4B-Staphylococcus protein A (20 mg/ml; Pharmacia Uppsala) in PBS pH 8·0 containing 2 mg/ml bovine serum albumin (BSA). After 4 hr at 4°, 20 ng of the ^{125}I Fab(t) (5×10^4 c.p.m.) iodinated as indicated in a previous report (Blaser *et al.*, 1981a) was added in 10 μl of buffer, mixed and kept overnight at 4°. 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 a LKB-Wallac

80,000 gamma counter. As controls, normal mouse serum and serum of Freund's complete adjuvant (FCA) immunized mice were used.

Anti-BPO and anti-DNP antibodies of the IgG class were estimated by a similar procedure as described for estimation of aId. However, as a labelled antigen, 200 ng DNP- or BPO-^{125}I-RNase containing 10^5 c.p.m. per sample was added to the mixture of Staph-A-Sepharose and investigated serum. Estimations were repeated and made in triplicate each time.

^{125}I-ribonuclease A (RNase) conjugates

BPO-^{125}I-RNase was prepared as described previously (Ovary & Benacerraf, 1963). A slightly modified procedure was used for DNP-^{125}I-RNase, 200 μg of bovine pancreas ribonuclease A (Type III-A, Sigma, St. Louis, Mo.) was dissolved in 300 μl 0·15 M phosphate buffer at pH 7·5 and iodinated with 300 μCi Na[^{125}I] according to the chloramine T method (Blaser *et al.*, 1981a, 1980). Then 600 μl 2 M K_2CO_3 was added together with 2 mg 2, 4-dinitrobenzene sulphonic acid. The mixture was reacted for 1 hr at room temperature and purified by gel filtration on a Sephadex G-25 column (50 × 1 cm), using PBS pH 7·4 containing 2 mg BSA/ml as eluant. The product obtained had a total activity of $1·9 \times 10^8$ c.p.m., as counted in a LKB-Wallac 80,000 gamma counter.

Immunizations and production of antibodies

For the production of IgE antibodies, BALB/c mice were sensitized with several injections of 10 μg $(BPO)_9$-ASC, $(BPO)_4$-OVA or $(DNP)_8$-OVA suspended in 2 mg Al $(OH)_3$. Anti-idiotypic serum was obtained by the procedure described for (anti-BPO) aId (Blaser *et al.*, 1980). Briefly, mice were immunized five times at weekly intervals with 200 μg of purified anti-OVA antibody, first suspended in 0·2 ml CFA/PBS 1:1 v/v and then in Freund's incomplete adjuvant (FIA). Of the suspensions 0·1 ml was inoculated in four portions subcutaneously and 0·1 ml intraperitoneally. The mice were challenged every month with 200 μg anti-OVA antibodies in FCA/PBS 1:1 and bled every week. Ascites containing anti-OVA antibodies were obtained by a slightly modified procedure according to Tung, Ju, Sato & Nisonoff (1976).

BALB/c mice were inoculated intraperitoneally with 0·5 mg OVA, suspended in 0·2 ml CFA/PBS 9:1. This was repeated with FIA twice in weekly intervals. After a waiting period of 4 weeks mice were challenged weekly with 0·5 mg of OVA in 0·2 ml of FCA/PBS 9:1. Usually ascites developed after 3–4 weeks and 25–30 ml of ascites were obtained in average per mouse after several tappings.

Passive cutaneous anaphylaxis

Mouse IgE was titrated by passive cutaneous anaphylaxis (PCA) in Wistar rats as described by (Ovary, Caiazza & Kojima, 1975). The sensitization period before intravenous administration of 1 mg (BPO)-polylysine, $(DNP)_{40}$-BSA, 2 mg OVA or 2 mg ASC in 1 ml of 1% Evans blue was 20–24 hr; therefore, the skin reactions were elicited almost entirely by IgE antibodies (Ovary *et al.*, 1975). The reaction was set up in duplicate and the titre was expressed as the reciprocal of antiserum dilutions yielding reactions of 5 mm diameter (end point titration). In controls, purified mouse anti-BPO IgG_1 antibodies showed no PCA reactions when challenged after 20–24 hr.

Cell transfer

Spleen cells from normal or (anti-OVA) aId suppressed BALB/c mice were teased into single cell suspensions, washed three times with Earle's balanced salt solution (EBSS) and injected into recipients irradiated with 650 rad. Usually 4×10^7 lymphocytes were injected per mouse. Subsequently, mice were immunized at the same day with 2 μg of the original antigen in alum and boosted on day 14. IgE levels were estimated by PCA titration in the rat skin.

Adoptive cell transfer

Spleen cells of BALB/c mice injected either with normal serum or (anti-OVA) aId, following immunization with antigen, were teased into single cell suspensions 6 weeks after injection of serum. B and T cells were separated according to the 'panning' technique by using antibody-coated plastic Petri dishes (Lewis & Kamin, 1980). T cells (2×10^7) were mixed with 5×10^7 spleen cells obtained from BALB/c mice primed with 1 μg carrier protein (OVA) in alum 3 weeks before cell transfer. The mixture was injected intraperitoneally into 650 rad irradiated normal BALB/c mice. The recipients were challenged on the same day and 2 weeks later with 10 μg of antigen (BPO-OVA) in alum. Formation of anti-BPO IgE antibodies was estimated by PCA titration in rat skin.

RESULTS

It has been demonstrated by several authors (Sirisinha & Eisen, 1971; Sakato & Eisen, 1975; Janeway & Paul,

1973) that aIds directed against antibodies from the same inbred strain can be produced by repeated immunization of animals in a syngeneic way. We have obtained isologous aId directed against anti-OVA antibodies in BALB/c mice by repeated injections of purified syngeneic antibodies (Ids). The anti-OVA antibodies used in these experiments, were isolated by adsorption of anti-OVA antibody-containing ascitic fluid, onto OVA-Sepharose column. Such fluid was induced by intraperitoneal injections of OVA, first in complete and then in incomplete adjuvant, according to the method described by Tung *et al.* (1976). The characterization of (anti-OVA) aId has been described in (Blaser *et al.*, 1981a). Depending on the time after immunization with Ids, sera containing 10–30 μg aId/ml were obtained. The reaction of (anti-OVA) aId with ^{125}I-anti-OVA-Fab(t) in a radioimmunoassay was totally inhibitable with unlabelled Fab(t) and to 30% with OVA but not with Fab(t) fragments of different myeloma proteins as already described (Blaser *et al.*, 1981a). The following studies with aId antibodies have been performed with a pool of isologous (anti-OVA) aId obtained from forty BALB/c mice. In order to study the effects of (anti-OVA) aId on the ongoing response, a group of eight BALB/c mice has first been immunized three times at 2 week intervals with $(BPO)_4$-OVA in alum. Such procedure establishes IgE titres of 320–640. Seven days after the last inoculation of antigen, four mice of this group were injected intravenously (i.v.) with 0·2 ml (anti-OVA) aId per mouse and the other four mice with normal BALB/c serum. As shown in Fig. 1, a single i.v. injection of isologous (anti-OVA) aId depressed the ongoing anti-BPO IgE antibody response to one quarter of its original level within 2 days. This effect was lasting for more than 12 weeks. An additional booster injection with antigen after 6 weeks caused no or only little increase of PCA titres. To demonstrate whether the effect of administered (anti-OVA) aId is carrier specific, groups of BALB/c mice already immunized with $(DNP)_8$-OVA or $(BPO)_9$-ASC have been treated with (anti-OVA) aId in the same way. As shown in Fig. 1, also the anti-DNP IgE antibody response induced by $(DNP)_8$-OVA can be depressed by (anti-OVA) aId, while no significant effect on the $(BPO)_9$-ASC induced anti-BPO IgE response has been obtained. Although still existing, the extent of depression of the IgE response against DNP in $(DNP)_8$-OVA immunized animals was less significant after a further booster injection with the

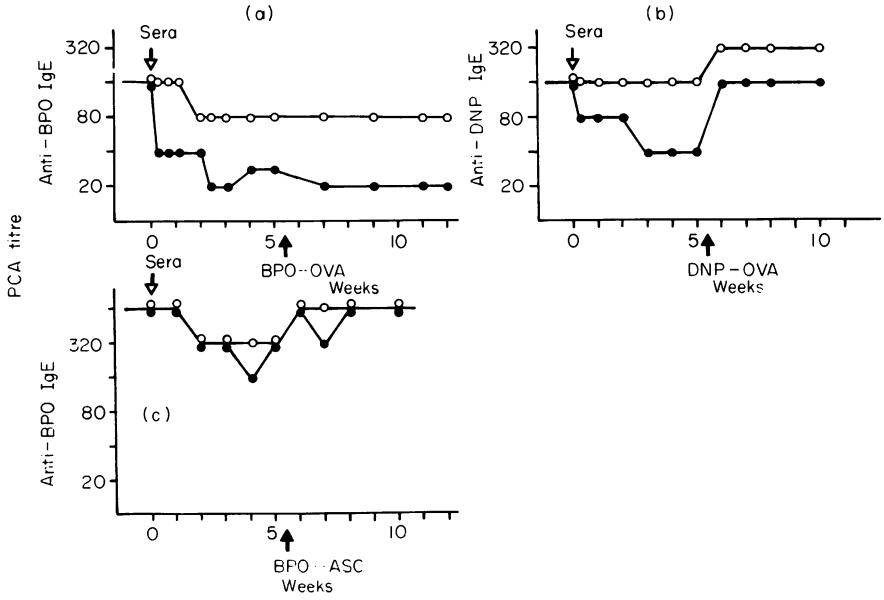

Figure 1. Anti-hapten IgE levels in BALB/c mice immunized with 10 μg $(BPO)_4$-OVA (a), $(DNP)_8$-OVA (b) or $(BPO)_9$-ASC (c) in alum after passive administration of (anti-OVA) aId (●) or normal serum (○). The closed arrow indicates the day of the fourth injection of antigen; the open arrow indicates the day of administration of sera. IgE levels are estimated by PCA titration in the rat skin.

antigen. These experiments were therefore repeated, but similar results were obtained. The IgE antibody production in controls undergoes higher fluctuation after a booster injection but it remained lower in aId treated mice and followed similar time curves as shown in Fig. 1. Only small effects of (anti-OVA) aId have been observed on the ongoing anti-OVA IgE response and no effect was obtained in $(BPO)_9$-ASC immunized animals on the anti-carrier response. As an additional control, BALB/c mice producing similar high titres of anti-BPO-OVA IgE antibodies were passively immunized with aId directed against the phosphorylcholine (PC) specific myeloma protein of the TEPC-15 tumour (Potter & Leon, 1968; Potter, 1972). In this case, as expected, no significant effects, whether on the anti-BPO nor on the anti-OVA response have been obtained (results not shown).

To see whether the suppressive effect of (anti-carrier) aId affects also the IgG response arising in the course of the immunization procedure leading to IgE antibody formation, levels of anti-hapten and anti-carrier IgG antibodies were estimated in the same experimental groups of mice as described above for IgE. The levels of IgG antibodies in BPO-OVA, DNP-OVA and BPO-ASC immunized animals have been followed by a radioimmunoassay with either BPO-^{125}I-RNase or DNP-^{125}I-RNase and Staph-A bound to Sepharose. Anti-carrier IgG antibodies have been estimated in the same way with ^{125}I-OVA or ^{125}I-ASC. It is shown in Fig. 2 that (anti-OVA) aId also affects the anti-hapten IgG response, similar to the IgE response. However, the depressive effect is less pronounced for IgG and does not become apparent before a booster injection after 5–6 weeks. That the effect on IgG is not visible immediately after aId has been administered, may be due to the half-life time of 21 days for IgG compared with 2–3 days for IgE. The effect of (anti-OVA) aId on the anti-carrier (OVA) IgG response is shown in Fig. 3. Again the depression in anti-OVA antibodies becomes visible only 5–6 weeks after administration of aId, shortly after an antigen boost triggering new antibody formation in the control group.

In order to study the regulatory mechanism of the primary immune response against a T-dependent antigen, (anti-OVA) aId has been administered to

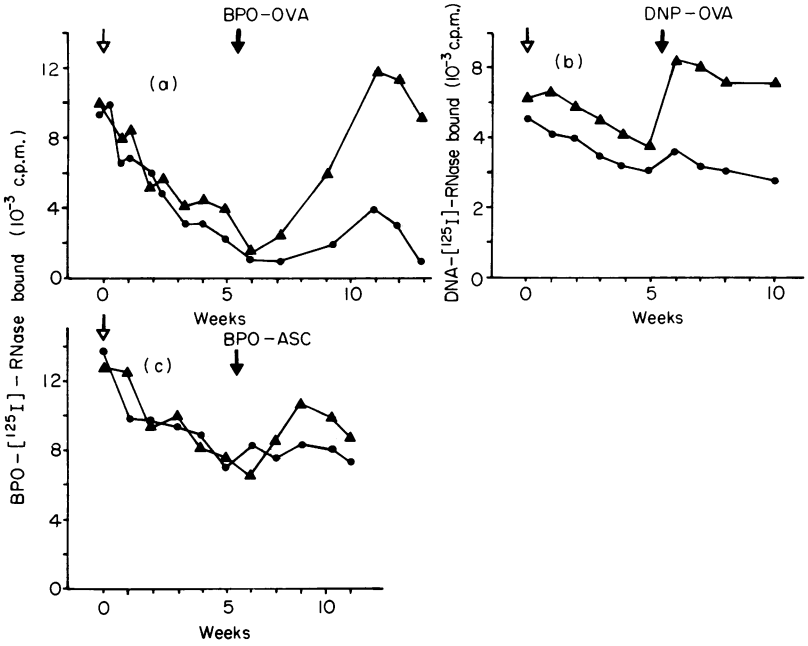

Figure 2. Anti-hapten IgG levels in BALB/c mice immunized with 2 μg $(BPO)_4$-OVA (a), $(DNP)_8$-OVA (b) or $(BPO)_9$-ASC (c) in alum, after passive administration of (anti-OVA) aId (▲) or normal serum (●). IgG levels are measured by radioimmunoassay with Staph A. The closed arrow indicates the day of the fourth immunization with antigen; the open arrow indicates the day of administration of sera.

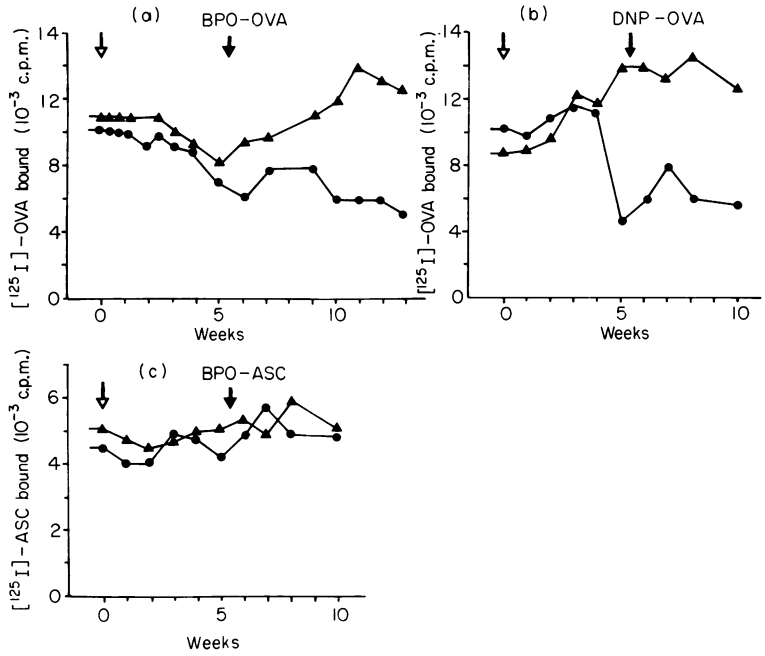

Figure 3. Anti-carrier IgG levels in BALB/c mice immunized with 10 μg $(BPO)_4$-OVA (a), $(DNP)_8$-OVA (b) or $(BPO)_9$-ASC (c) in alum, after passive administration of (anti-OVA) aId (▲) or normal serum (○). IgG levels are measured by radioimmunoassay with Staph A. The closed arrow indicates the day of the fourth immunization with antigen; the open arrow indicates the day of administration of sera.

mice before immunization with hapten-carrier conjugates in alum. Three groups of mice were primed with carrier protein alone in alum 7 days before immunization with antigen, prior to four injections of 0·4 ml (anti-OVA) aId on each following day. Control groups received 0·4 ml of normal BALB/c serum. Two and 5 weeks later the same procedure but without carrier priming was repeated. The results are shown in Fig. 4. Isologous (anti-OVA) aIds passively administered before immunization with antigen also suppress the primary anti-hapten response, provided the animals were immunized with the haptens (BPO, DNP), chemically bound to the OVA carrier. In other experiments with different schedules for aId administration, i.e. with an interval of 7 days between aId and antigen administration and without priming with carrier protein, however, we have not always been successful in depressing the primary immune response.

It is possible that (anti-OVA) aId can suppress directly the B cells producing anti-carrier antibodies, but it seems unlikely that B cells producing anti-hapten antibody can be directly affected by (anti-carrier) aId. However to demonstrate that the effect of carrier-related aId is mediated at a cellular level, we have performed cell transfer experiments. Spleen cells (4×10^7) of mice passively suppressed by (anti-OVA) aId, or normal spleen cells have been injected into BALB/c mice irradiated with 650 rad. Subsequently one group of these animals has been immunized with $(DNP)_8$-OVA, the other with $(DNP)_9$-ASC in alum. Depression of anti-DNP IgE has been observed only in the $(DNP)_8$-OVA immunized group of mice, compared with the control group which was treated with normal spleen cells. Only slight depression of the anti-OVA IgE response has been achieved, as shown in Table 1. No effect on the anti-DNP IgE response has been observed in DNP-ASC immunized animals.

On the other hand we have not been able to transfer the suppressive state by isolated T cells. A mixture of 2×10^7 T cells from suppressed or normal animals has been injected together with 5×10^7 total spleen cells of carrier primed animals into sublethally irradiated (650 rad) mice, which were subsequently immunized with

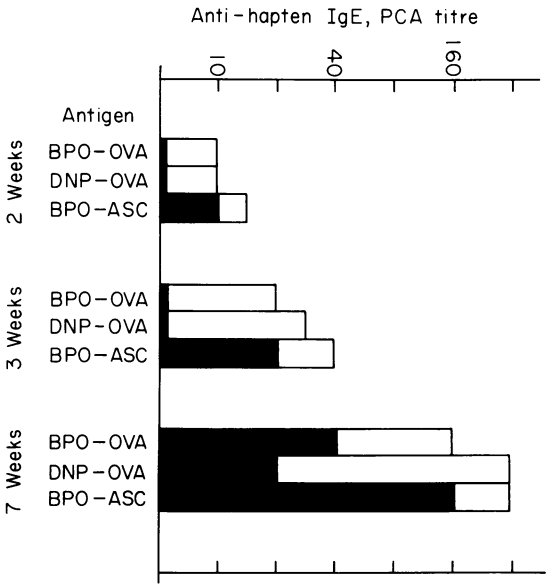

Figure 4. Effect of (anti-OVA) aId on the primary anti-hapten IgE antibody response in BALB/c mice. Carrier primed mice were injected four times at daily intervals with 0·4 ml (anti-OVA) aId immediately following immunization with hapten-carrier conjugates in alum. Boosts of antigen were given after 2 and 5 weeks. (□) Anti-hapten IgE titres of the control groups; (■) titres of the (anti-OVA) aId treated group of animals.

Table 1. PCA titres of 650 rad irradiated recipients after transfer of spleen cells from BALB/c mice with ongoing anti-BPO-OVA or anti-BPO-ASC IgE responses, which were treated with (anti-OVA) aId or normal serum (NS), respectively

Treatment of donors	Antigen	PCA titre in recipients (groups of four animals)	
		Anti-DNP	Anti-carrier
(Anti-OVA) aId	DNP-OVA	10	40
NS	DNP-OVA	160	80
(Anti-OVA) aId	DNP-ASC	80	80
NS	DNP-ASC	80	100

Recipients were immunized with 2 μg antigen in alum on days 0 and 14.

$(BPO)_4$-OVA in alum on days 0 and 14. Spleens have been taken 10–13 weeks after aId injection. The anti-BPO IgE response in the group of mice which received T cells from suppressed animals has not been significantly different from the group which received normal T cells (results not shown). This could indicate that the suppression of the IgE response by passive administration of carrier-related aId was not due to Ts cells, at least not in a later stage of the response.

DISCUSSION

According to the results reported here and to those described in a previous publication (Blaser *et al.*, 1981a), we suggest that anti-OVA IgG and IgE antibodies of BALB/c mice bear Ids common to most or all individuals of this strain. Common Ids have been observed up to now only in immune responses against hapten or molecules with repetitive structures, such as carbohydrates and synthetic co-polymers. It is not yet established whether the phenomenon is unique to OVA or whether other high molecular proteins also evoke antibodies with common Ids. We have shown that a primary, as well as an ongoing but relatively early anti-hapten IgE response can be regulated by isologous carrier-related (anti-OVA) aId, provided OVA protein conjugates of these haptens were used for immunization. The aId antibodies were obtained by immunization of syngeneic animals with anti-OVA antibodies from a pool of many donors. Such collection of Ids might generate aId possessing enough cross-reactivities to react with a major portion of the antibodies of the total response. Mice which showed depressed IgE antibody formation were also depressed for anti-hapten IgG antibodies. However, this was only apparent 5–6 weeks after administration of aId following a further antigen boost. This may be mainly the result of the different half-life times for IgE (3 days) and IgG (21 days), to exclusively T-dependent regulation of IgE in contrast to IgG, or that the IgE response is less heterogenous in its idiotypic pattern.

The effect of (anti-carrier) aId on the ongoing anti-hapten IgE response showed similar extents and time curves in repeated experiments. In some cases, a certain increase of IgE titre after an additional booster injection with antigen was observed at which, however, IgE levels in aId treated mice always remained lower than in controls. Such fluctuations in the IgE responses may appear if not in all animals, in a group responding to aId treatment and antigen injection to the same extent. Such individual differences may then be reflected in serum pools of only four animals. Depression of anti-hapten IgE levels upon administration of (anti-carrier) aId was observed in all experiments for a long period of time. In ascaris-

conjugate immunized animals, identical values in the control and the (anti-OVA) aId-treated group were measured.

The IgE response against the OVA carrier was not significantly suppressed, although a certain effect could always be observed. Our explanation for this is that anti-OVA antibodies for the production of aId were raised against the native protein. On the other hand, for antigen immunization chemically coupled hapten-OVA conjugates were used. These conjugations may alter the native OVA molecule in such a way that new antigenic determinants can be formed. Antibodies against these may then cross-react with the native OVA. In addition, stepwise PCA titrations may be less sensitive than the RIA used for IgG. Taken together, this could explain that in (anti-carrier) aId trated mice only less significant suppressive effects on the anti-OVA IgE antibody and little effects on the anti-OVA IgG formation were observed.

The mechanism of suppression of anti-hapten IgE and IgG formation by carrier-related (anti-OVA) aId is not yet clear. However, such aId may not directly affect the anti-hapten antibody producing B cell. The suppressive effect could rather be the result of either depletion of CTh or alternatively induction of CTs. Possible regulatory mechanisms involving carrier-specific T cells have been described by several authors (Herzenberg *et al.*, 1980b; Herzenberg, Black & Herzenberg, 1980a; Tada, Taniguchi & Okumura, 1977). It remains to be clarified whether treatment with (anti-carrier) aId activates the same suppressive pathways if administered before injection of antigen or in an already established response. The transfer experiments demonstrate that regulation acts at a cellular basis. That we were not able to transfer suppression by T cells 10–13 weeks after aId administration in an established response may indicate that aIds could be effective for rather short periods only. Experiments to study the regulatory effects of repeated (anti-OVA) aId administration over a long period of time were not undertaken.

Although IgE synthesis seems more susceptible to regulation by aId, passive administration of carrier-related aId also affects the formation of IgG antibodies. From this we assume that IgE and T-dependent IgG responses are regulated in principle at early stages of the response by the same mechanisms. Regulation of IgE antibody formation, which is strongly T-cell-dependent, provides an excellent model for the study of regulation by complementary Ids. Such knowledge might finally help in providing possible new treatments (immunoglobulin therapy) for some allergic diseases.

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