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Anti-idiotype regulation of timothy grass pollen IgE antibody formation

I. *IN VIVO* INDUCTION OF SUPPRESSOR T CELLS

A. MALLEY & D. W. DRESSER *Oregon Regional Primate Research Center, Beaverton, Oregon, U.S.A. & National Institute for Medical Research, Mill Hill, London*

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Summary. Antibodies prepared against antigen B-specific T-helper factor (anti-T_{HF}) and antigen B-specific IgE (anti-IgEid) have been isolated and the anti-idiotypic properties of both antibodies were described previously. Both anti-idiotypic antibodies (anti-Id) were used to determine their influence in primary and secondary IgE responses. These studies demonstrated that both anti-Id specifically suppress timothy IgE responses, that the suppression lasts for at least 35 days, and that it is mediated by a Thy 1^+ Lyt 1^- 23^+ T cell. Attempts to induce suppressor T cells with the $F(ab)_2$ fragment of anti-T_{HF} at several concentrations (10–80 μg per day for 3 successive days) were unsuccessful. This suggests that the Fc portion may be important in presentation of anti-Id during the induction of suppressor T cells.

Abbreviations: ā-Id, anti-idiotypic antibody; anti-IgEid, Ag-B-specific anti-IgE; anti-T_{HF}, anti-T-helper factor; T_{HF}, antigen B-specific T-helper factor; T_{SF}, antigen B-specific T-suppressor factor; AgB, antigen B; WST, timothy grass pollen extract; Ox-AgB, photooxidized AgB; nRGG, normal rabbit gamma globulin; i.p., intraperitoneally; i.v., intravenously; C, complement; NRS, normal rabbit serum; T_S, suppressor T cells; NMS, normal mouse serum; PCA, passive cutaneous anaphylaxis.

Correspondence: Dr A. Malley, Oregon Regional Primate Research Center, Beaverton, Oregon 97006, U.S.A.

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INTRODUCTION

Since the first use of anti-idiotypic antibodies (ā-Id) as modulators in immune responses (Cosenza & Köhler, 1972; Hart, Wang, Pawlak & Nisonoff, 1972), the concept of idiotype-specific regulation has received considerable experimental support. Idiotype-specific interactions are not restricted to B cells, but can also affect T cells (Binz, Frischknecht, Shen & Wigzell, 1979; Eichmann, 1974; Sy, Bach, Dohi, Nisonoff, Benacerraf & Greene, 1979). The immune system can be viewed as a vast and complex intercommunicating network of cells, soluble factors, and antibodies (Jerne, 1974). The potential interactions in such a network need to be understood and how its pathways are activated is needed before various reagents (idiotypes, anti-idiotypes, etc.) can be used therapeutically in humans.

Previously, we reported the preparation and characterization of antibodies directed against antigen B-specific (AgB-specific) IgE (anti-IgEid) and affinity-purified AgB-specific helper T-cell factor (anti-T_{HF})—Malley, Brandt & Deppe, 1982. These earlier studies demonstrated that both antibodies (i) specifically induced [^{3}H]-thymidine incorporation of AgB-specific helper and suppressor T cells in the absence of antigen, (ii) initiate the secretion of antigen-specific helper and suppressor factors from the appropriate T-cell population, (iii) enhance AgB-specific IgE formation upon preincubation with B cells from timothy grass pollen

(WST)-primed mice, and (iv) do not affect normal spleen cells or spleen cells from animals primed with ovalbumin, *Ascaris suum*, or keyhole-limpet haemocyanin. Thus, anti-T_{HF} and anti-IgE antibodies appeared to be interacting with idiotypic determinants present on AgB-specific T and B cells, and both antibodies have been purified by affinity chromatography on Sepharose-T_{HF} adsorbents (Malley *et al.*, 1982).

The use of both anti-T_{HF} and anti-IgEid anti-idiotypic antibodies to influence primary and secondary anti-AgB-specific IgE responses is reported here. The data indicate that both anti-T_{HF} and anti-IgEid suppress AgB-specific IgE responses, that the suppression lasts for at least 35 days, and that it is mediated by a Thy 1^+ Lyt 1^- 23^+ T cell.

MATERIALS AND METHODS

Antigens

Timothy pollen extract (WST), purified antigen B (AgB), photooxidized antigen B (Ox-AgB), and AgB-specific T_{SF} were prepared as described elsewhere (Malley & Harris, 1967; Malley, Begley & Forsham, 1979). Ovalbumin (recrystallized three times) was purchased from ICN Pharmaceutical, Inc., Cleveland, Ohio.

Animals

(CBA/Ca × C57BL6) F_1 mice and Sprague-Dawley rats were bred under specific pathogen-free conditions at the National Institute for Medical Research (N.I.M.R.), Mill Hill, London. In most experiments, male mice 9–12 weeks old and rats weighing 200–250 g were used.

Immunization

Mice were injected intraperitoneally (i.p.) with 10 μg of WST adsorbed on 1 mg of alum on day 0. Animals given a secondary antigen challenge were treated with the same dose of WST in alum on day 21.

In some experiments mice were first injected with affinity-purified normal rabbit IgG (nRGG), anti-T_{HF}, or anti-IgEid from 0·1 to 10 μg of protein per day for 3 successive days. Twenty-four hours later these animals were primed with 10 μg of WST adsorbed on 1 mg of alum and 21 days later these animals were given a secondary antigen boost with the same dose of WST.

The induction of anti-ovalbumin IgE responses was achieved by injecting animals with 10 μg of ovalbumin adsorbed on 1 mg of alum.

Antibodies

Affinity-purified AgB-specific T_{HF} was used to immunize rabbits as described elsewhere (Brandt, Deppe & Malley, 1981). Briefly, rabbits were immunized with about 100 μg of protein in Freund's complete adjuvant (FCA), and after several boosts with the same dose of T_{HF}, animals were bled out. Rabbit anti-IgEid was separated from anti-IgE_{Fc} as described elsewhere (Malley *et al.*, 1982). Anti-T_{HF} and anti-IgEid antibodies were passed over Sepharose-normal-mouse serum and Sepharose-$F(ab)_2$ adsorbents, and finally purified by affinity chromatography on a Sepharose-T_{HF} adsorbent (Malley *et al.*, 1982).

The $F(ab)_2$ fragments of anti-T_{HF} were prepared by pepsin digestion of the intact anti-T_{HF} antibody by pH 4·2 (Williams & Chase, 1967). The digest was then passed over a protein-A-Sepharose adsorbent in phosphate-buffered saline (PBS), pH 7·2, to remove intact undigested antibodies and Fc fragments present in the digest. The effluent not adhering to the protein-A-Sepharose adsorbent was concentrated by negative pressure against PBS to a concentrate of 0·84 mg of protein/ml. The nRGG was purified from normal rabbit serum by passage of the serum over a protein-A-Sepharose adsorbent in PBS. The sample bound to the adsorbent was eluted with 3 M KSCN in PBS, pH 7·2. The eluted fraction was concentrated by negative pressure against PBS, pH 7·2, to a final concentration of 2·5 mg of protein/ml, and gave a single precipitin band against anti-normal rabbit sera and anti-rabbit IgG.

The NIM-R1, a monoclonal rat anti-mouse Thy-1 antibody, was prepared at N.I.M.R., Mill Hill, London (Chayen & Parkhouse, 1982). The goat anti-mouse Fab antisera was a gift from Dr. G. G. B. Klaus.

Cell transfers

Spleen cells from animals treated with either nRGG or anti-T_{HF} were prepared asceptically, washed in PBS, and then varying numbers of these cells injected intravenously (i.v.) into recipient animals. The recipients had been primed 20 days earlier with 10 μg of WST adsorbed on 1 mg of alum. They received a secondary boost of the same dose

of antigen 24 hr after cell transfer. In some experiments, donor cells were treated with either normal rabbit serum or anti-Thy-1 antiserum (NIM-R1) and complement (agarose-adsorbed guinea-pig serum) before the washed cells were injected into the recipients.

Enrichment of T cells

The method of Wysocki & Sato (1978) was used to obtain a population of enriched splenic T cells. Briefly, polystyrene petri dishes (Falcon 3003, BioQuest, P.O. Box 243, Cockeysville, Md) were coated with goat anti-mouse Fab antibody. Spleen cells (3×10^7 in 3 ml/dish) were incubated at 4° for 60 min. Non-adherent cells were removed by Pasteur pipette and the coated dishes washed with 7 ml of cold buffer at 4°. The combined wash and initial aliquot of non-adherent cells were washed several times in cold buffer and represent an enriched T-cell population. Analysis of the non-adherent cell population indicated that this cell population was between 2% and 5% Ig^+ when stained with a fluorescein-conjugated anti-mouse immunoglobulin serum, and more than 95% Thy 1^+ when stained with fluorescein-conjugated anti-Thy-1 antisera (NIM-R1) and examined microscopically.

Passive cutaneous anaphylaxis

The serum IgE titres were measured by passive cutaneous anaphylaxis (PCA; Moto & Wong, 1969; Fairchild & Malley, 1975) with 500 μg of WST in PBS as the antigen challenge. The PCA titre was measured in duplicate Sprague-Dawley rats and was expressed as the reciprocal of the highest dilution of serum yielding a 5 mm (diameter blueing reaction.

Affinity adsorbents

Sepharose adsorbents coupled with normal mouse serum (NMS), the $F(ab)_2$ fragments of mouse IgG, and AgB-specific T_{HF} have been described elsewhere (Malley *et al.*, 1982; Malley, Begley & Forsham, 1977). The protein-A-Sepharose adsorbent was purchased from Pharmacia, Piscataway, N.J.

RESULTS

Pretreatment of normal mice with nRGG for 3 successive days before immunization with 10 μg of protein of WST adsorbed on 1 mg of alum did not alter the normal primary or secondary response to WST (Table 1). On the other hand, pretreatment with either anti-T_{HF} or anti-IgEid led to

Table 1. Effect of pretreatment of normal mice with anti-idiotypic antibody on the primary and secondary anti-timothy IgE responses

Treatment*	Dose μg/day	Primary IgE response†		Secondary IgE response†	
		Day 7	Day 19	Day 7	Day 14
Normal rabbit IgG	10·0	50	100	800	600
Anti-T_{HF}	0·1	—‡	—	400	200
	1·0	—	—	200	100
	10·0	—	—	200	100
Anti-IgEid	0·1	—	—	400	200
	1·0	—	—	400	100
	10·0	—	—	200	100

* Affinity purified normal rabbit IgG (nRGG), anti-T_{HF}, or anti-IgEid was injected intravenously at the indicated doses for 3 successive days (−3 to −1) into six normal mice per group. All animals were challenged with 10 μg of WST adsorbed on 1 mg of alum on day 0, and given a secondary boost with the same dose of WST on day 21. Blood samples were collected on days 7 and 19 after the primary, and on days 7 and 14 following antigen challenge.

† IgE responses were measured by passive cutaneous anaphylaxis (PCA) in Sprague-Dawley rats. All titrations were done in triplicate and the values represent the reciprocal of the mean of the lowest dilution giving a 5 × 5 mm positive reaction.

‡ —, IgE response less than 50.

a profound suppression of the primary WST response and yielded a dose-related suppression of the secondary WST response (Table 1). The suppression induced by anti-T_{HF} or anti-IgEid pretreatment persisted for at least 35 days. The observed suppression could be due either to the induction of a population of suppressor T cells or by antibody feedback suppression. In experiments to distinguish between these two possibilities, it was found that cells from mice injected with nRGG and treated with either normal rabbit serum or anti-Thy 1 and C′ did not alter the secondary anti-AgB IgE response. Cells obtained from mice injected with anti-T_{HF} and treated with NRS and C′ suppressed the secondary anti-AgB IgE response, but treatment of these cells with anti-Thy 1 and C′ completely abrogated their observed suppressive effect (Table 2). This suggests that the reduction in anti-AgB IgE antibody by anti-T_{HF} treatment was mediated by a population of suppressor T (T_S) cells.

Further evidence that the observed suppression is mediated by a T_S cell population is shown in Table 3. Animals given cells that were from mice treated with nRGG and that were non-adherent to petri dishes coated with goat anti-mouse immunoglobulin did not alter a secondary anti-AgB IgE response. Animals given cells that were from mice treated with anti-T_{HF} and that were non-adherent to petri dishes coated with goat anti-mouse immunoglobulin significantly suppressed (>90%) a secondary anti-AgB IgE response, and as few as 10^6 of the enriched T cells transferred into primed recipients resulted in a 75% suppression of the secondary response.

Table 2. Evidence of anti-idiotypic antibody induction of T cells that suppress a secondary anti-timothy IgE response

Immunization*	Donor cell treatment†	IgE response‡ Day 7	11	14
Normal rabbit IgG	NRS+C′	1600	1200	800
	anti-Thy+C′	1600	1200	800
Anti-T_{HF}	NRS+C′	200	100	100
	anti-Thy+C′	1600	1200	800

* Normal mice were injected with 10 μg of protein of either normal rabbit IgG or affinity purified anti-T_{HF} daily for 3 successive days. On day 4 the spleens were removed and single cell suspensions made.

† Cell suspensions were treated with equal volumes of either normal rabbit sera (NRS) or anti-Thy-1 sera and guinea-pig complement (C′) at a 1:3 dilution for 1 hr at 37°. Donor cells, after treatment with NRS or anti-Thy 1 and C′, were washed twice with PBS and $2\cdot5 \times 10^7$ cells were injected intravenously into six recipient mice per group. Recipients had been primed 20 days earlier with 10 μg of protein of WST on 1 mg of alum. Twenty-four hours after cell transfer, all mice received a secondary WST challenge (10 μg of protein in alum).

‡ IgE responses were measured by PCA in Sprague-Dawley rats. All titrations were done in duplicate and the values represent the reciprocal of the mean of the lowest dilution giving a 5×5 mm positive reaction.

Table 3. Titration of cells non-adherent on petri dishes coated with goat anti-mouse immunoglobulin

Immunization*	Number of cells transferred	Number of recipients†	IgE response‡ Day 7	14
Anti-T_{HF}	4×10^7	6	200	0
	10^7	6	300	0
	5×10^6	6	400	0
	10^6	6	400	0
	5×10^5	4	800	200
	10^5	4	1600	400
nRGG§	3×10^7	6	1600	400

* Animals were injected intravenously with 10 μg of protein of either anti-T_{HF} or nRGG daily for 3 successive days. Twenty-four hours later spleens were removed and applied to petri dishes coated with goat anti-mouse immunoglobulin at 4°. Non-adherent cells were recovered, washed twice with PBS, and resuspended in PBS.

† All recipients were primed 20 days earlier with 10 μg of protein of WST adsorbed on 1 mg of alum. The indicated number of cells non-adherent to the goat anti-mouse Ig plates were injected intravenously and within 24 hr all recipients were given a secondary WST challenge.

‡ IgE response was measured in duplicate by PCA in Sprague-Dawley rats. The mean titre is reported.

§ nRGG, normal rabbit gamma globulin.

Specificity of the suppression induced by anti-T_{HF} treatment is shown in Table 4. Animals treated with anti-T_{HF} and immunized with 10 μg of ovalbumin adsorbed on 1 mg of alum had primary IgE response identical to those of control animals given saline intravenously and then immunized with ovalbumin.

Table 5 compares the ability of anti-T_{HF}, anti-IgEid, and the $F(ab)_2$ fragment of anti-T_{HF} to induce T_S cells. Although anti-IgE_{id} was as effective as anti-T_{HF} in inducing T_S cells, the $F(ab)_2$ fragment of anti-T_{HF} did not significantly induce T_S cells.

Table 4. Effect of anti-T_{HF} antibody treatment on the induction of anti-ovalbumin IgE formation

Immunization*	Treatment†	Number of animals	IgE response‡ Day 7	14
Ovalbumin	None	4	800 (600–1200)	400 (100–600)
Ovalbumin	Anti-T_{HF}	4	800 (600–1200)	400 (100–600)

* All animals were immunized intraperitoneally with 10 μg of ovalbumin adsorbed on 1 mg of alum.

† Animals were injected intravenously with either saline or 10 μg of anti-T_{HF} daily for 3 successive days. On the next day all animals were immunized with ovalbumin.

‡ IgE responses were measured in duplicate by PCA in Sprague-Dawley rats. The mean titres and the range (in parenthesis) of responses are indicated.

DISCUSSION

At the concentrations used, both anti-idiotypic antibodies (anti-T_{HF} and anti-IgEid) completely suppressed primary anti-AgB IgE responses, and up to 75% of the secondary responses in recipients immunized with WST absorbed on alum (Table 1). Suppression induced by the anti-idiotypic antibodies was fairly long lived, lasting more than 35 days. It was mediated by a T-cell population, as indicated by abrogation of suppression through treatment of cells from animals given anti-idiotypic antibody with anti-Thy-1 antiserum and complement, and the enrichment of the suppressor cell populations in the fraction not adhering to petri dishes coated with anti-mouse immunoglobulin (Tables 2 and 3). The failure of mice treated with anti-idiotypic antibody before immunization with ovalbumin to yield antibody titres different from those of control mice (treated with saline or nRGG) not only indicates the specificity of anti-idiotype-induced suppressor T cells, but also argues

Table 5. *In vivo* induction of suppressor T cells by anti-idiotypic antibody

Immunization*	No. of cells transferred	No. of recipients†	IgE response‡ Day 7	14
Anti-T_{HF}	3×10^7	15	200 (100–300)	0
Anti-IgEid	5×10^7	6	200 (100–300)	0
Anti-T_{HF} $(Fab)_2$§	5×10^7	8	1200 (1000–1600)	600 (400–800)
Anti-T_{HF}	3×10^7¶	6	1600	800 (400–1200)
nRGG**	$2{\cdot}5 \times 10^7$	12	1600	800 (400–1200)

* Animals injected intravenously daily for 3 successive days with 10 μg anti-T_{HF}, 50 μg anti-Eid, or 80 μg of anti-T_{HF} $(Fab)_2$. On day 4 spleens were removed, single-cell suspension prepared, and the indicated number of viable cells injected into recipients.

† Recipients were primed with 10 μg of WST in alum 20 days earlier, and all animals were given a secondary challenge within 24 hr after receiving cell transfers.

‡ IgE response of each recipient group was measured in duplicate in Sprague-Dawley rats. The mean responses and the range of responses is indicated.

§ Pepsin digest of anti-T_{HF} and passed over a Sepharose-protein A adsorbent.

¶ Anti-Thy 1 and C′ treated.

** Normal rabbit IgG eluted from Sepharose-protein A adsorbent and injected into donor mice at 10 μg of protein per day for 3 successive days.

against the suppression being a feedback mechanism induced by anti-immunoglobulin (Uhr & Möller, 1968; Fitch, 1975; Hetzelberger & Eichmann, 1978)—Table 4.

The failure of the $F(ab)_2$ fragment of anti-T_{HF} to induce a suppressor cell population could reflect a difference in the half-lives of the intact antibody in the circulation and of the fragment. We unsuccessfully tried to overcome this potential problem by using up to eight times (80 μg of protein) more $F(ab)_2$ fragment than the intact (10 μg of protein) anti-idiotypic antibody. The $F(ab)_2$ fragment of anti-T_{HF} retained its full combining-site activity as demonstrated by its ability to completely block the antigen-induced PCA activity of sera from mice having an AgB-specific IgE titre of 30,000 (data not shown). Others have similarly reported that $F(ab)_2$ fragments of anti-idiotypic antibodies are ineffective immunosuppressants (Fitch, 1975; Forni & Pernis, 1975; Sidman & Unanue, 1976; Pierce & Klinman, 1977; Moretta, Mingari & Romanzi, 1978). Recently several investigators suggested that B cells may play an important role in activating T-cell function (Zubler, Cantor, Benacerraf & Germain, 1980b; Zubler, Benacerraf & Germain, 1980a; L'age-Stehr, Teichmann, Gershon & Cantor, 1980; Black & Herzenberg, 1979). The failure of the $F(ab)_2$ fragment of anti-T_{HF} to induce a suppressor cell population may indicate that either macrophages or B-cell processing via their Fc receptors is needed. Alternatively, T cells with Fc receptors (Setcavage & Kim, 1980) may play an important role in the induction of suppressor T cells.

Characterization of the suppressor cell induced by anti-idiotypic antibody indicates that it is a Thy 1^+ Lyt 1^- 23^+ T cell. Preliminary studies have demonstrated that factors extracted from spleen cells treated with anti-T_{HF} antibody and non-adherent to petri dishes coated with goat anti-mouse immunoglobulin binds to both antigen (Sepharose-antigen D) and idiotype-positive affinity adsorbents (Sepharose antigen B-specific suppressor factor) indicating that both Ts_1 and Ts_2 suppressor cells are induced.

Additional experiments to characterize the suppressor T-cell population and to evaluate the role of B cells and macrophages during induction of regulatory T cells by anti-idiotypic antibody are in progress.

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