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Preparation and characterization of the anti-idiotypic properties of rabbit anti-timothy antigen B helper factor and anti-mouse timothy IgE antisera

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Summary. Rabbit anti-T-helper factor (anti-T_{HF}) was prepared by immunization with affinity-purified timothy antigen B (AgB)-specific helper factor. The rabbit anti-T_{HF} and an anti-IgE fraction (anti-E_{id}) purified by a Sepharose-AgB≡timothy IgE affinity adsorbent were evaluated for the presence of anti-idiotypic antibodies. Both antibodies (anti-T_{HF} and anti-E_{id}), (i) specifically induce [^{3}H]-thymidine incorporation of AgB-primed T cells at optimum concentrations; (ii) specifically block antigen-induced lymphocyte proliferation when preincubated with AgB-primed T cells at an excess concentration; and (iii) specifically stimulate *in vivo* IgE formation when preincubated with AgB-primed B or T cells and injected into syngeneic *X*-irradiated recipients. Anti-T_{HF} and anti-E_{id} purified by an AgB-specific T suppressor factor (T_{SF}) affi-gel adsorbent retain their ability to specifically initiate [^{3}H]-thymidine incorporation of AgB-primed T cells.

The data indicate that both anti-T_{HF} and anti-E_{id} recognize unique determinants present on AgB-specific T-helper, T-suppressor and B cells, and suggest that the receptors on AgB-specific T and B cells share cross-reactive idiotypic determinants.

Abbreviations: AgB, antigen B; AgD, antigen D; anti-E_{id}, anti-idiotypic antibody; anti-IgEM, rabbit anti-mouse timothy IgE; anti-T_{HF}, rabbit anti-timothy antigen B helper factor; Con A, concanavalin A; CFS, cell-free supernatant; CS buffer, cacodylic-saline buffer; FCA, Freund's complete adjuvant; FCS, foetal calf serum; HEPES, N-2-hydroxyethyl-piperazine-N′-2-ethanesulphonic acid; HF, helper factor; KLH, keyhole limpet haemocyanin; NRS, normal rabbit serum; OVA, ovalbumin; Ox-AgB, photo-oxidized antigen B; PBS, phosphate-buffered saline; PCA, passive cutaneous anaphylaxis; SF, suppressor factor; T_H cells, helper T cells; T_{HF}, helper T-cell factor; T_S cells, suppressor T cells; T_{SF}, suppressor T-cell factor; WST, extract of timothy grass pollen.

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INTRODUCTION

Chemical characterization of the antigenic determinant of antigen B (AgB), the major allergen of timothy grass pollen, indicated that the flavonoid pigment quercetin represents the major part of the antigenic determinant, but that the sugar moiety (cellobiose) associated with the pigment is also involved (Malley, Baecher & Begley, 1975a,b; Malley, Begley & Forsham, 1979). This finding suggested that photo-oxidation of AgB might selectively inactivate the antigenic determinant, and leave a modified protein that would permit investigation of the relationship of B and T lymphocyte receptors in the IgE system. Initial studies with photo-oxidized AgB (Ox-AgB) demonstrated that it does not react with rabbit,

mouse, or human antibodies directed against native AgB, and that multiple immunizations (four to seven) of Ox-AgB in either alum or Freund's complete adjuvant (FCA) do not induce antibody reactive with either AgB or Ox-AgB (Malley *et al.*, 1979). On the other hand, Ox-AgB does initiate proliferation of T lymphocytes, and induces significant levels of both helper and suppressor T (T_H and T_S) cells (Malley, Begley & Forsham, 1980 Malley, Deppe & Brandt, 1981a,b). This fact raised some interesting questions regarding the nature of the T lymphocyte receptor for AgB and its relationship to the idiotype expressed by the B cell and its product.

The relationship between idiotypic determinants, V_H domains of immunoglobulin molecules, and antigen-binding receptors of T and B lymphocytes has been documented in a number of experimental systems (Bach, Green, Benacerraf & Nisonoff, 1979; Mozes & Haimovich, 1979; Eichmann, 1978; Binz & Wigzell, 1977; Rajewsky & Eichmann, 1977). This paper describes the preparation of rabbit anti-T-helper factor antibody and the characterization of the anti-idiotypic properties of anti-T_{HF} and rabbit anti-timothy mouse IgE (anti-IgE_m) antiserum depleted of its ε chain-specific (Fc-specific) antibodies. Both of these anti-idiotypic antibodies demonstrate that the receptors on AgB-specific T_H, T_S and B cells share the same idiotypic determinant.

MATERIALS AND METHODS

Timothy grass pollen extract (WST), purified antigen D (AgD), AgB, and Ox-AgB were prepared as described elsewhere (Malley *et al.*, 1979; Malley & Harris, 1967). Ovalbumin (OVA), three times recrystallized, was purchased from ICN Pharmaceuticals, Inc., Cleveland, Ohio. Antigen B-specific helper factor and suppressor factor were prepared as described elsewhere (Malley *et al.*, 1981a,b).

Briefly, spleen cells from mice primed with Ox-AgB in alum were obtained 7 days after priming. A T cell-enriched population was obtained by passage of these cells over nylon wool (Julius, Simpson & Herzenberg, 1973), and the non-adherent T-cell population was cultured with an optimum concentration of Ox-AgB for 5–18 hr at 37° in 5% CO_2 in air. The cell-free supernatant (CFS) were removed by centrifugation, and AgB-specific T_{HF} was purified by affinity chromatography on Sepharose-AgD_I. The AgB-specific T_{HF} was eluted from the adsorbent with 3 M KSCN in PBS, pH 7·2. The recovered T_{HF} was applied to the Seph-AgD_I adsorbent a second time, and greater than 95% of the protein bound was eluted with KSCN. As little as three micrograms of protein of the isolated AgB-specific T_{HF} injected intravenously with a limiting number ($2{\cdot}5 \times 10^6$) of WST-primed B cells (anti-thy plus complement treated) induces a secondary IgE response with a titre of 3000 in syngeneic *X*-irradiated (650 rad) recipients.

AgB-specific T_{SF} was obtained by sonication of spleen cells from mice primed with Ox-AgB in FCA. The CFS was collected by ultracentrifugation at 20,000 r.p.m. for 20 min, and AgB-specific T_{SF} was isolated by passage twice over a Seph-AgD_I affinity adsorbent (Malley *et al.*, 1981a). Ten micrograms (10 μg of protein) of AgB-specific T_{SF} completely suppresses a secondary anti-timothy IgE response.

Immunizations

LAF_1 mice, 8–12 weeks old (The Jackson Laboratory, Bar Harbor, Maine), were immunized with WST as described elsewhere (Fairchild & Malley 1975a, 1976). For the production of T_H cells, mice were immunized once intraperitoneally with 150 μg of Ox-AgB adsorbed on 1 mg of alum, and 7 days later single cell preparations of their spleens were made as described elsewhere (Fairchild & Malley, 1976). Suppressor T cells were obtained from mice primed with 150 μg of Ox-AgB incorporated in FCA twice at 14-day intervals; the spleen cells were collected 7 days later.

Mice immunized with OVA received 10-μg injections of OVA protein adsorbed on 1 mg of alum, and the spleen cells were collected 7–10 days later.

Antisera

Rabbit anti-helper factor was prepared by immunization of albino New Zealand rabbits with 150 μg of protein of affinity-purified AgB-specific T_{HF} in FCA distributed in three to four sites along the backs of the rabbits. Each rabbit received a booster injection of the same amount of T_{HF} in FCA at 12- to 18-day intervals over the next 50 days, and the animals were exsanguinated 7 days after the last injection of T_{HF}.

The anti-IgE_m was prepared as described elsewhere (Malley, Begley & Forsham, 1977), and the specific antibody (anti-E_{id}) was separated from anti-IgE_{Fc} (heavy chain-specific) antibodies by affinity chromatography (described below).

Rabbit anti-T-helper factor and rabbit anti-E_{id} antisera were routinely passed over Sepharose-rabbit anti-mouse Ig and Sepharose-foetal calf serum

(Seph-FCS) adsorbents to remove any non-specific antibodies present in these antisera.

Lymphocyte transformation

Spleen cells or nylon wool enriched T cells from mice primed with Ox-AgB in alum or FCA, and OVA in alum, were cultured for 5 days at 37° in 5% CO_2 in air as described elsewhere (Fairchild & Malley, 1976, 1975b). Cultures received pulses of $[^3H]$-thymidine (1 μCi; 6·7 mmol/Ci, New England Nuclear, Gardena, Calif.) during the last 24 hr of culture. Upon termination of these cultures the cells were harvested by a minimultiple automatic sample harvester (Microbiological Associates, Los Angeles, Calif.), and harvested samples were counted on a Packard scintillation counter (Packard Instrument Company, Inc., Downers Grove, Ill.).

In experiments to block antigen-induced proliferation, whole spleen cells or nylon wool enriched T cells cultured with antigen (AgB or OVA) were preincubated for 90 min at 37° with 750 μl of anti-T_{HF} serum, 150 μl of anti-E_{id} serum, or 750 μl of normal rabbit serum (NRS). The cells were washed three times with RPMI 1640 supplemented with N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES) buffer, 5% foetal calf serum and penicillin-streptomycin (100 u./ml). These cells were diluted to 2 ml with the same media for a final dilution of 6×10^6 cells/ml. One hundred microlitres of these cell suspensions were added to each well of a Falcon microtitre plate (Falcon Labware Division, Becton Dickinson, Oxnard, Calif.), and 5 μg of AgB (100 μl), 0·5 μg of concanavalin A (Con A, 100 μl), or 5 μg of OVA (100 μl) were added and these mixtures were cultured for 5 days as described above.

The ability of anti-T_{HF} or anti-E_{id} to induce proliferation of T_H and T_S cells was determined by culturing nylon wool non-adherent T cells from mice primed with Ox-AgB in alum or cells from mice primed with Ox-AgB in FCA and enriched for T cells by the method of Wysocki & Sato (1978).

Adoptive transfer method

Spleen cells from mice immunized with WST were either passed over nylon wool (Julius *et al.*, 1973) to provide immune T cells or were treated with rabbit anti-thy 1·2 (1:40 final dilution at 4° for 1 hr) and guinea-pig complement (1:10 final concentration)—Colorado Serum Company, Denver, Co.—to provide immune B cells.

Previous studies had demonstrated that intravenous injection of a limiting number of immune B cells ($2{\cdot}5 \times 10^6$ cells) and 4×10^6 immune T cells into *X*-irradiated (600 rad) syngeneic mice and subsequent challenge with 10 μg of WST in alum within 2 hr initiated a significant secondary IgE response. Serum IgE was measured by PCA in Sprague-Dawley rats (Fairchild & Malley, 1975a), and IgG was measured by a modified radioimmunoassay as described elsewhere (Malley *et al.*, 1980).

The ability of anti-idiotypic antibodies to block or enhance T_H or B cell activity was determined through incubation of T_H or B cells (2×10^7 cells) with 1 ml of anti-T_{HF} (diluted 1:2·5 in unsupplemented RPMI), 1 ml of anti-E_{id} (diluted 1:5 in unsupplemented RPMI) or 1 ml of NRS (diluted 1:2·5 in unsupplemented RPMI) for 90 min at 37°. The cell suspensions were washed three times in RPMI, mixed with the appropriate quantity of untreated immune T or B cells, and injected intravenously into *X*-irradiated (600 rad) syngeneic recipients.

Preparation of adsorbent

The AgD fragment of timothy grass pollen was attached to Sepharose-AH-4B (Pharmacia Fine Chemicals, Piscataway, N.J.) as described elsewhere (Malley *et al.*, 1975a,b). Antigen B-specific T_{HF} and T_{SF} were partially purified by passage of the cell-free supernatant fluids containing either T_{HF} or T_{SF} over the Sepharose-AH-AgD (Sepharose-AgD) adsorbent. Non-adherent proteins were washed off with excess 0·01 M cacodylic-saline (CS) buffer, pH 7. Bound proteins were eluted with 3 M KSCN in CS buffer. Eluted proteins were dialysed against phosphate-buffered saline and concentrated by negative pressure.

Antigen B was attached to Sepharose 4B by the method of March, Parikh & Cuatrecassas (1978). The Sepharose-AgB adsorbent was treated with 3 ml of mouse anti-AgB IgE serum diluted to 10 ml with C.S. buffer. The IgE titre of this antiserum was 1:60,000 measured by PCA in Sprague-Dawley rats. When all of the diluted anti-AgB IgE serum was on the adsorbent, the flow was stopped and this suspension was incubated at room temperature for 2 hr. The adsorbent (10 ml of packed Sepharose) was washed with 500 ml of CS buffer to remove non-specifically bound protein. The first 100 ml of this wash was recovered and reconcentrated for recovery of unbound anti-AgB IgE antibodies. The Sepharose AgB-IgE complex was next treated with 0·02 M gluteraldehyde for 60 min at room temperature to stabilize the bound IgE to the AgB. The Sepharose AgB-IgE

adsorbent was washed with 250 ml of CS buffer, 50 ml of 3 M KSCN in CS buffer, 200 ml of distilled water, and 200 ml of each of the following reagents: 0·5 M Na_2CO_3-0·5 M NaCl, pH 10; 1 M NaAc-0·5 M NaCl, pH 4·0; 2 M urea-0·5 M NaCl; and CS buffer, pH 7·0.

The anti-IgEM was separated into heavy chain-specific (anti-IgE_{Fc}) and anti-E_{id} antibodies by passage over the Sepharose AgB-IgE adsorbent. The anti-IgE_m antibodies were precipitated with ammonium sulphate as described by Campbell, Garvey, Cremer & Sussdorf (1963), and the final precipitate was dialysed extensively against CS buffer and concentrated by negative pressure. The protein concentration of the IgG preparation was determined spectrophotometrically on the basis of the extinction coefficient of rabbit IgG, $E^{1\%}_{280} = 14{\cdot}5$. Approximately 12 mg of protein (0·4 ml) of the IgG fraction of rabbit anti-IgE_m was applied to the Sepharose AgB-IgE adsorbent in CS buffer. The non-adherent protein was eluted with CS buffer, and bound protein was eluted with 3 M KSCN in CS buffer. Upon dialysis and concentration, non-adherent and eluted fractions were assayed for their ability to neutralize AgB-specific IgE or OVA-specific IgE (a gift from Schering Company, F.R.G.). The fraction eluted from the Sepharose AgB-IgE adsorbent with KSCN was equally efficient in neutralizing AgB and OVA-specific IgE antibodies, and is therefore referred to as anti-IgE_{Fc}. The fraction not adhering to the Sepharose AgB-IgE adsorbent was effective in neutralizing AgB-specific IgE but not OVA-specific IgE antibodies and is therefore referred to as anti-E_{id}.

Attempts to conjugate affinity-purified AgB-specific T_{HF} or T_{SF} to Sepharose or affi-gel 10 (Bio-Rad Laboratories, Richmond, Calif.) and to have the conjugated factor retain its ability to react with antigen or anti-idiotypic antibodies were unsuccessful. However, preincubation of AgB-specific T_{SF} with soluble antigen for 1–2 hr at 36° before coupling with affi-gel 10 provided an insolubilized AgB-specific T_{SF} adsorbent that could be used to purify anti-idiotypic antibody. Antigen B-specific T_{SF} (500 μg of protein) was incubated with 25 mg of AgD at what was calculated to be about equal molar quantities. After incubation for 60 min at 37° the solution was added to 12 g of affi-gel 10 equilibrated in 0·1 M $NaHCO_3$ (pH 8), and the suspension was stirred at room temperature for 6 hr and overnight at 4°. The suspension was centrifuged at 2500 r.p.m. for 20 min at 4° to remove the supernatant. The affi-gel matrix was washed in 0·1 M $NaHCO_3$ buffer (pH 8) and resuspended in 1 M ethanolamine (Mallinckrodt, Inc., St. Louis, M.) in 0·1 M $NaHCO_3$, pH 8. The suspension was stirred at

Table 1. Effect of pretreating ovalbumin- and photo-oxidized antigen B-primed cells with anti-antigen B idiotypic antisera upon the antigen-specific lymphocyte transformation response

Source of spleen cells	Treatment*	[^{3}H]-thymidine incorporation†		
		AgB	Con A	OVA
Ox-AgB-primed‡	NRS	47,521 ± 1125	39,758 ± 1023	1075 ± 500
	anti-T_{HF}	910 ± 310	40,150 ± 1275	1210 ± 250
	anti-E_{id}	1170 ± 450	39,927 ± 1151	975 ± 390
OVA-primed§	NRS	975 ± 250	37,521 ± 1395	32,175 ± 1161
	anti-T_{HF}	1210 ± 300	39,357 ± 1510	35,193 ± 1710
	anti-E_{id}	1157 ± 291	39,771 ± 2100	34,710 ± 1423

* Nylon wool enriched T cells from mice primed with either OVA or Ox-AgB were preincubated for 90 min at 37° with 750 μl of heat-inactivated anti-T_{HF}, NRS, or 150 μl of anti-E_{id}. The cells were washed three times with 5% FCS-supplemented RPMI 1640 media and suspended to a final volume of 2 ml.

† Cell suspensions, at a final concentration of 6×10^6 cells/ml, were cultured with 100 μl of each cell preparation and the optimal concentrations of AgB (10 μg of protein), Con A (0·5 μg of protein), and OVA (10 μg of protein). The values reported are the means of triplicate cultures ± SD and are corrected for background.

‡ Animals were immunized with 150 μg of Ox-AgB adsorbed on 1 mg of alum. Spleens were collected 7 days later, and 2×10^8 cells were passed over nylon wool columns.

§ Animals were immunized with 10 μg of OVA adsorbed on 1 mg of alum. Spleens were collected 7 days later, and 2×10^8 cells were passed over nylon wool columns.

room temperature for 1 hr and then washed three times in CS buffer. The washed adsorbent was treated with 3 M KSCN in CS buffer for 30 min to remove AgD_l bound to the T_{SF} and any free or weakly bound proteins. The excess KSCN was removed from the T_{SF} affi-gel adsorbent by extensive washing in CS buffer and stored at 4° until ready for use.

Foetal calf serum (Grand Island Biological Company, Santa Clara, Calif.) was attached to Sepharose 4B by the method of March *et al.* (1978). The Sepharose-FCS adsorbent was used to evaluate the specificity of anti-T_{HF} and anti-E_{id} binding to an AgB-specific T_{SF} affinity adsorbent.

RESULTS

The possibility that anti-T_{HF} serum contained anti-idiotypic antibody activity was examined with T cells from mice primed with Ox-AgB in alum preincubated with NRS, anti-T_{HF}, or anti-E_{id} for 90 min at room temperature. Preincubation of AgB-specific T_H cells with NRS did not reduce the ability of these cells to respond to either AgB or Con A. Preincubation of these cells with either anti-T_{HF} or anti-E_{id} completely blocked their ability to respond with AgB, but did not reduce the level of [^{3}H]-thymidine incorporation of these cells by Con A. Specificity of the effect of anti-T_{HF} and anti-E_{id} was demonstrated by the failure of these antibodies to block either Con A or OVA-induced proliferation responses with OVA/al-primed cell (Table 1). Spleen cells from non-immunized mice pretreated with either anti-T_{HF} or anti-E_{id} did not respond when cultured with AgB or OVA, but gave normal levels of [^{3}H]-thymidine incorporation with Con A (data not shown).

The direct proliferative effect of anti-T_{HF} and anti-E_{id} antisera upon AgB-specific T_H and T_S cells is shown in Fig. 1. Nylon wool non-adherent T cells from mice primed with Ox-AgB in alum provided a source of T_H cells. T cells enriched by depleting B cells on petri dishes coated with goat anti-mouse Ig (provided by Dr Gerald B. Klaus, National Institute for Medical Research, Mill Hill, London) from mice primed with Ox-AgB in FCA provided a source of T_S cells. Both T_H and T_S populations contained less than 5% cells that stained with fluorescein anti-mouse Ig (Microbiological Associates, Los Angeles, Calif.). Both T_H and T_S cells show significant levels of [^{3}H]-thymidine incorporation at concentrations of anti-T_{HF} and anti-E_{id} that are twenty to fifty times lower than that used to block

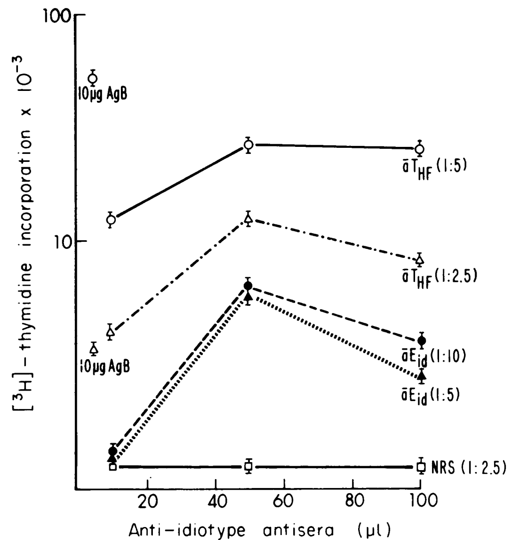

Figure 1. Antigen B-specific helper T (T_H) cells (○——○); ●– – –●) and suppressor T cells (△– – – – –△; ▲– – – – –▲) were cultured with varying amounts of anti-T helper factor antiserum, anti-E_{id} antiserum, or normal rabbit serum (□——□). The AgB-induced [^{3}H]-thymidine incorporation of the same T_H and T_S cell population is presented. Each point represents the mean of at least three cultures, and the bars (|) represent the variation of individual cultures from the mean.

proliferation of T_H cells (Table 1). Although similar experiments have been repeated at least six times with the identical pattern of results, the data from a single experiment is presented (Fig. 1) to permit comparison of the AgB-induced and anti-idiotype-induced proliferation of the same T_H and T_S cell populations. Incubation of T_H or T_S cell populations with the appropriate anti-lyt 1 or -lyt 2 antisera (a gift from Dr. D. Taplow, Fred Hutchinson Cancer Center, Seattle, Washington) and guinea-pig complement reduces the level of [^{3}H]-thymidine incorporation to background. For the T_H cell population the optimum concentration of anti-T_{HF} (50 μl at 1:5 dilution) antiserum induced 50% of the level of [^{3}H]-thymidine incorporated by the same cell population with the optimum concentration of AgB. The anti-E_{id} was able to initiate proliferation of T_H cells at a much lower concentration, but the degree of proliferation was much lower (6500 c.p.m.) than that attained with either AgB or anti-T_{HF} serum. In contrast, both anti-T_{HF} and anti-E_{id} antisera gave higher levels of [^{3}H]-thymidine incorporation with T_S cells than did soluble AgB (Fig. 1). Normal rabbit serum tested over a concentration range from 1:2·5 to 1:10 did not induce levels of [^{3}H]-thymidine incorpo-

ration significantly higher than background (cells plus medium only). In addition, normal spleen cells, or cells from OVA-primed mice, cultured with either anti-T_{HF} or anti-E_{id} over a wide concentration range (1:2·5 to 1:20) did not induce levels of [^{3}H]-thymidine incorporation above background.

Since anti-T_{HF} and anti-E_{id} initiated significant levels of [^{3}H]-thymidine incorporation with AgB-specific T_H cells, we decided to determine if the action of anti-T_HF and anti-E_{id} antisera upon either T_H cells or immune B cells (primed with WST and treated with anti-thy 1·2 and complement) would enhance the secondary AgB-specific IgE response in *X*-irradiated recipients (Table 2). Immune T cells (non-adherent to nylon wool) from WST-primed mice mixed with immune B cells at a limiting ratio gave a good secondary IgE response. Preincubation of either T_H or B cells with NRS did not alter the secondary IgE response in the irradiated recipients. On the other hand, preincubation of either T_H or B cells with anti-T_{HF} or anti-E_{id} antisera significantly enhanced the secondary IgE response in irradiated recipients. The level of enhanced antibody formation obtained by pretreatment with anti-E_{id} was slightly less than that achieved with anti-T_{HF} antiserum. Incubation of anti-T_{HF} or anti-E_{id} with OVA-primed T cells (nylon wool non-adherent) or B cells (anti-thy and complement) did not alter their immune response in *X*-irradiated syngeneic recipients. The AgB-specific IgG antibody response in all of the groups tested was less than the sensitivity of the radioimmunoassay (100 ng/ml).

Table 2. Enhancement of the antigen B-specific IgE response by pretreatment of immune cell populations with anti-idiotype.

Treatment*	Cell population† treated	Ratio of cells given recipients‡	IgE response§
		T:B	
None	—	4×10^6:$2{\cdot}5\times10^6$	600
NRS	T		600
anti-T_{HF}	T	4×10^6:$2{\cdot}5\times10^6$	2800
anti-E_{id}	T		1600
NRS	B		600
anti-T_{HF}	B	4×10^6:$2{\cdot}5\times10^6$	3200
anti-E_{id}	B		1600

* Cell populations (T or B cells) were treated with 1·5 ml of heat-activated NRS or anti-T_{HF} and 1·0 ml of anti-E_{id} per 2×10^7 cells. These mixtures were rocked at 37° for 90 min and centrifuged at 1000 r.p.m. for 10 min; the supernatant was discarded. The cell pellets were resuspended in PBS and washed two more times with PBS before being resuspended to a final volume of 2×10^7 per ml.

† Spleen cells from mice immunized with 10 μg of protein of WST adsorbed on 1 mg of alum at 21-day intervals were collected on day 28. Single cell suspensions were prepared; portions of these cells (2×10^8 cells) were passed over nylon wool columns and represent immune T cells (not adherent to nylon wool). The remaining cells, treated with anti-thy 1·2 (1:10) and complement (1:10), represent immune B cells.

‡ Syngeneic recipients were *X*-irradiated (600 rad) and received intravenous injections of 4×10^6 T cells and $2{\cdot}5\times10^6$ B cells. All recipients were challenged within 2 hr of this injection with 10 μg of WST.

§ Passive cutaneous anaphylaxis responses were determined in duplicate in Sprague-Dawley rats. The values represent the mean reactions observed in at least two rats. At least three animals per group made up the serum pool tested, and blood was collected 7, 10 and 14 days after cell transfers. The titre reported represents the mean titre of the maximum response (day 7) observed.

Table 3. Purification of anti-idiotypic antibodies on an antigen B-specific T suppressor factor affi-gel affinity adsorbent

Sample	Adsorbent	Fraction*	Volume tested (μl)	[^{3}H]-thymidine incorporation† (c.p.m. ± SD)
Anti-T_{HF} (1:5)	Seph-FCS	Column pass	10	13,160 ± 875
			50	28,190 ± 2045
			100	21,200 ± 2127
Anti-T_{HF} (1:5)	Seph-FCS	Eluate	10	322 ± 362
			50	590 ± 319
			100	965 ± 475
Normal rabbit serum (1:5)	Affi-gel	Eluate	10	375 ± 290
			50	497 ± 418
			100	921 ± 510
Anti-T_{HF} (1:5)	Affi-gel T_{SF}	Eluate	10	26,009 ± 2153
			50	36,240 ± 1741
			100	29,959 ± 1915
Anti-T_{HF} (1:5)	Affi-gel T_{SF}	Column pass	10	435 ± 275
			50	543 ± 295
			100	595 ± 280
Anti-E_{id} (1:5)	Affi-gel T_{SF}	Eluate	10	8975 ± 1201
			50	15,375 ± 975
			100	11,811 ± 917
Anti-E_{id} (1:5)	Affi-gel T_{SF}	Column pass	10	515 ± 195
			50	621 ± 250
			100	710 ± 235

* One- to five-millitre portions of anti-T_{HF}, anti-E_{id}, or normal rabbit antiserum were applied to 10- to 15-ml packed volumes of the indicated affinity adsorbents. The column pass (non-adherent) and eluate (adherent) fractions were dialysed against PBS, pH 7·2, and concentrated by negative pressure back to their original volume.

† Cell suspensions, at a final concentration of 6×10^6/ml, were cultured with 10 μl and the indicated volumes of antisera. RPMI 1640 medium supplemented with 5% foetal calf serum was added to each well for a final volume of 200 μl/well. The values reported represent the means of triplicate cultures ± SD and are corrected for background.

Purification of anti-idiotypic antibodies by affinity chromatography was achieved with an AgB-specific T_{SF} affi-gel adsorbent (affi-gel-T_{SF}). Fractions of anti-T_{HF} or anti-E_{id} eluted from the affi-gel-T_{SF} with KSCN in PBS (pH 7·2) retained their ability to initiate [^{3}H]-thymidine incorporation when cultured with spleen cells from mice primed with either WST or Ox-AgB in alum (Table 3). These same fractions were inactive (background levels of [^{3}H]-thymidine incorporation) in cultures with normal spleen cells (data not shown). The eluate fractions of NRS (1:5) applied to affi-gel-T_{SF} adsorbent was also inactive. The anti-idiotypic antibodies present in anti-T_{HF} antiserum did not bind non-specifically to either a Sepharose-FCS adsorbent (Table 3) or an affi-gel adsorbent saturated with 1 M ethanolamine (data not shown).

DISCUSSION

The preparation of antisera against purified proteins isolated by various techniques is often used to characterize unknown molecules or cell surface antigens. Feldmann and co-workers prepared rabbit and mouse antisera against a purified suppressor factor (SF) preparation (Feldmann, Howie & Kontiainen, 1979), and they demonstrated that these antisera recognize different structures. The rabbit anti-SF recognizes a common determinant present on SF of any strain or antigen specificity. The mouse anti-SF reacts only with SF or helper factor induced with their antigen, keyhole limpet haemocyanin (KLH). Thus, this latter antiserum appears to be anti-idiotypic. Rabbit antiserum made against AgB-specific T_{HF} (anti-T_{HF}) does

not seem to be reactive against determinants common with other T_H cells. Specificity of the anti-T_{HF} antibody for AgB-specific T cells is demonstrated by the ability of anti-T_{HF} antibody to block AgB-induced lymphocyte transformation of Ox-AgB-primed cells, but not the response of these cells with Con A (Table 1). On the other hand, anti-T_{HF} antibody does not block the proliferative response of OVA-primed cells cultured with OVA (Table 1). A number of investigators (Bach *et al.*, 1979; Moses & Haimovich, 1979; Cosenza, Augustin & Julius, 1976; Bottomley, Mathieson & Mosier, 1978; Yamamoto, Nonaka & Katz, 1979) have demonstrated that B-cell products (antibodies) possess cross-reacting idiotypes with T-cell products (soluble SF and HF). Similarly, a rabbit antiserum directed against AgB-specific IgE (anti-E_{id}) depleted of its ε chain-specific antibodies blocks the proliferation of Ox-AgB-primed cells with AgB, but does not affect OVA-primed cells cultured with OVA (Table 1).

Additional evidence that anti-T_{HF} and anti-E_{id} antibodies are anti-idiotypic is the direct stimulation of both T_H and T_S cells with optimal concentrations of these antisera (Fig. 1). The level of [^{3}H]-thymidine incorporation of T_H cells with anti-T_{HF} is about 50% of the level of stimulation of these same cells with AgB. On the other hand, the level of [^{3}H]-thymidine incorporation of T_H cells with anti-E_{id} is only 12% of AgB-induced proliferation. Both anti-T_{HF} and anti-E_{id} induce a higher level of [^{3}H]-thymidine incorporation than AgB with the same T_S population (Fig. 1). In fact, T_S cells from mice injected three or four times with Ox-AgB in FCA do not show levels of [^{3}H]-thymidine incorporation, with either Ox-AgB or AgB, significantly higher than those presented in Fig. 1. The differences observed between AgB and anti-idiotype-induced [^{3}H]-thymidine incorporation of T_S cells may reflect a difference in the mechanisms of initiation of proliferation with these reagents, or perhaps AgB with a molecular weight of 12,000 to 14,000 requires some processing by macrophages before it can become an effective proliferative agent of T_S cells. The ability of excess anti-idiotypic antibody to block proliferation of T_H cells, and at much lower concentrations to initiate [^{3}H]-thymidine incorporation, undoubtedly reflects the dose–response characteristics of T-cell activation. Such effects have been reported by many investigators studying both antigen- and antibody-induced proliferation of lymphocytes (Ling & Kay, 1975; Loor, 1977).

With either AgB-specific T_H or T_S cells the anti-T_{HF} antibody induces a higher level of proliferation than the anti-E_{id} antibody. This activity might be explained by differences in the avidity of the two antibodies for the idiotypic determinant, an unlikely possibility since the amount of anti-E_{id} needed to either block AgB-induced proliferation or induce proliferation with T_H or T_S cells is less than the amount of anti-T_{HF} needed. Another possibility is that these differences reflect the degrees of cross-reactivity between the idiotypes expressed on B cells and T_H and T_S cells. Thus, one might expect the B-cell receptor to express a larger repertoire of idiotypes than either T_H or T_S cells. However, the dose–response curve for the direct stimulation of T_H or T_S cells with anti-E_{id} is narrower than that observed with anti-T_{HF}. A third related explanation of the observed reactivity between anti-T_{HF} and anti-E_{id} antibodies is that the idiotype on B cells may be directed against a product coded for by a V-J region of the DNA responsible for IgE formation, and that the idiotypic determinant encompasses part of both V and J regions, while the idiotype expressed on T cells may be directed against a product coded for by the V region only. Clarification of these differences will require further study.

Enhanced antibody responses by treatment *in vivo* or by direct treatment of lymphocyte populations with anti-idiotypic antibodies have been reported by Eichmann (1978) and other investigators (Eichmann & Rajewsky, 1975; Bona, Hooghe, Cozenane, Leguern & Paul, 1979). The results presented in Table 2 show that pretreatment of either nylon wool non-adherent T cells or immune B cells (anti-thy 1·2 and complement treated) with either anti-T_{HF} or anti-E_{id} *in vitro* enhances the IgE response in syngeneic *X*-irradiated recipients. Specificity of this enhancement was determined by the failure of anti-T_{HF} or anti-E_{id} pretreatment of T cells (nylon wool non-adherent) or B cells (anti-thy 1·2 and complement) from mice primed with ovalbumin.

The cross-reactivity of these sera with the receptors on T_S cells has been demonstrated not only by the direct stimulation of T_S cells cultured with anti-T_{HF} and anti-E_{id} (Fig. 1), but also by the purification of both anti-idiotypic antibodies on an affi-gel-T_{SF} adsorbent (Table 3). Our data indicate that the idiotypic determinant(s) expressed on AgB-specific T_H and T_S cells and B cells are closely related. Questions regarding whether a single idiotype, or several cross-reacting idiotypes, are expressed on AgB-specific cell surface receptors must await the development of monoclonal anti-idiotypic reagents and purified T_H and T_S cell lines. Characterization of the idiotypic determinant(s)

expressed by AgB-specific T_{HF} and T_{SF} is currently under investigation.

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