The Selective Inhibition of Secondary IgE Antibody Response in Mice by Dibutyryl Adenosine 3′,5′-Cyclic Monophosphoric Acid and Theophylline (37499)

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Allergic reactions in humans and rodents are mediated by IgE antibody (1, 2). The precise pathway for the induction of the IgE response is unknown; however, there is evidence that under some conditions it is different from that of the thymus-derived ("T") cell dependent antibody responses of the IgG class (3) or from cellular immunity. For example, methods classically used to inhibit "T" cell-dependent immune responses, such as X-irradiation and treatment with cytostatic drugs, have been reported to be either ineffective or may even enhance IgE production in the rat (4–6). Furthermore, a regulatory mechanism may exist between IgG and IgE synthesis since 7S IgG, either actively produced or passively administered, inhibits 1 and 2° IgE production in the rabbit (7, 8). Conversely, it has been reported that in rats adoptive transfer of "T" cells, which reconstitutes "T" cell dependent immunity, depresses IgE synthesis (9). Since the control of IgE synthesis is apparently mediated by a different mechanism than most other immune responses, it is possible that suppression of its production might be achieved by a noncytostatic drug. Specific suppression of IgE production would be a valuable pharmacologic tool in treatment of allergic disease in man.

This report is the first demonstration that cAMP or theophylline, a noncytostatic drug, already in use for the treatment of allergic symptoms, inhibits 2° IgE synthesis in mice without significantly affecting IgG production. Such specificity for either the IgE or IgG response could not be demonstrated with other immunosuppressive agents.

Materials and Methods. Groups of 6 or 7 female 18–20 g SWR mice (Jackson Laboratories, Bar Harbor, Maine) were immunized with an intraperitoneal injection of 0.5 ml of a saline solution of 0.1 μg 2$\times$ crystallized ovalbumin in aluminum hydroxide. The technique of immunization and preparation of the alum adjuvant has been described by Vaz *et al.* (10). Four weeks later animals were again injected with the same antigenic preparation and sera were obtained 7 days later. Mice were treated once daily with various agents starting prior to or after the second antigen injection (see Table I for schedules). Control groups received the vehicle only. All marketed drugs were obtained from commercial sources; paramethasone from the Eli Lilly Company, N^6,O^2-dibutyryl adenosine 3′,5′-cyclic monophosphoric acid (cAMP) from the Sigma Chemical Company, and rabbit antimouse thymocyte serum (ATS) was prepared by the Freund's adjuvant immunization method previously described (11).

Homocytotrophic antibody (IgE) was assayed by passive cutaneous anaphylaxis (PCA). Aliquots of 0.05 ml of various dilutions of each sera were injected intradermally into the clipped and depilated (NAIR, Carter Products, New York, N.Y.) dorsal surface of each of 3–5 CFW 20–25 g male mice[1]. Forty-eight hours later the recipients received an iv injection of 0.2 ml of a 1% saline solution of Evans blue dye containing 1 mg/ml ovalbumin. Thirty minutes after the iv injection, the animals were sacrificed by CO_2 asphyxiation and measurements (mm^2) were made of the blueing reaction at the injection site. The titer was defined as the highest dilution of antisera which exhibited

[1] Mice obtained prior to April, 1972.

a positive blueing reaction ($> 4 \times 4$ mm). Each serum was originally assayed by injecting dilutions of 1:20, 1:60, and 1:120, and 1:480 on one side of the dorsal surface of each recipient. To confirm the IgE-like nature of the antibody, the same dilutions were heated at 60° for 90 min and then injected on the opposite side. In all cases the ability of the positive dilutions to produce a blueing reaction was lost. A generalized blueing reaction of the entire skin occurred in some recipients which received high titer sera. This phenomenon was avoided by assaying these samples at only one or two dilutions, each given in three separate recipients.

Total (IgG and IgM) and 2-mercaptoethanol (2-ME) resistant (IgG) antibodies to the immunizing antigen were assayed on representative samples in triplicate, by passive hemagglutination, using ovalbumin coated sheep erythrocytes. The antibody titer of all samples in these studies was insensitive to 2-ME. To avoid any contribution of IgE-like activity to the agglutination reaction, each serum pool was tested after heating at 60° for 90 min or treated with 2-ME, both of which are known to inactivate some IgE antibody activities (12). In all cases there was no significant effect on the IgG antibody titer by either of these treatments. Sera from nonimmunized mice did not agglutinate either ovalbumin-coated or noncoated erythrocytes.

Results. Six separate experiments were performed, each of which included several drug treated groups plus one control group which was immunized and treated with vehicle only. Sera pools made from the blood of animals undergoing each treatment schedule were separately assayed. The results of the homocytotrophic antibody (PCA) assays are given in Table I. The PCA titers and average reaction size at the 1:20 or 1:60 antisera dilution of the serum from the treated animals is given with the reactions elicited by the corresponding control group. Theophylline administered daily at 50 or 100 mg/kg from Day −2 to Day +6 (in relation to the second antigen injection) produced a dose-related inhibition of the PCA titers as well as the PCA reaction size. These data were obtained from separate experiments, each having their own control groups so as to determine whether dose-related inhibition was independent of sensitivity levels. The similarity in inhibition between the two 100 mg/kg experiments indicated that the degree of inhibition was unrelated to the amount of antibodies produced by control animals, since in both experiments the calculated inhibition of the PCA titer and reaction size was similar (84 vs 89% and 89 vs 100%) even though the control titers differed (1:50 vs 1:120, Table I).

When the dose of theophylline was kept constant (100 mg/kg), but the treatment schedule was varied, it was found that maximum suppression of the homocytotrophic antibody production occurred only when treatment was started two days prior to the second antigen injection. Less effect was achieved when the pretreatment schedule was reduced one day or only a post-treatment schedule was used. The IgE antibody production after 1° immunization was too low to accurately quantitate using the PCA reaction. Therefore, no pharmacologic agent could be tested at the time of initial immunization. In order to determine whether the theophylline effect was related directly to inhibition of antibody production or could have been produced by an indirect effect on the PCA reaction caused by the presence of residual theophylline in the plasma, theophylline at a concentration of 10^{-4} *M* was added directly to a positive serum sample which had a titer of 1:480 with a reaction size of 100 mm^2 at 1:120. The addition of the theophylline did not effect the titer, which remained at 1:480, nor the reaction size. In other experiments, treatment was stopped two days rather than one day prior to sacrifice. Under these conditions almost maximum inhibition was observed even though 7 rather than 8 doses of drug were administered.

In order to determine if the theophylline effect might be related to its known ability to inhibit phosphodiesterase resulting in an intracellular accumulation of cAMP, dibutyryl cAMP itself was administered sc at a dose of 10 mg/kg from Day −2 to Day +6. Almost complete inhibition of homocytotrophic antibody formation occurred. This experiment was repeated three times with separate groups

TABLE I. Inhibition of 2° Antibody Response of Mice to Ovalbumin.

Drug	Daily dose (mg/kg)	Duration[a] of treatment (days)	Homocytotrophic antibody: Control (vehicle) Titer^{-1}[b]	Control (vehicle) x̄ Reaction (mm²)	Drug treated Titer^{-1}	Drug treated x̄ Reaction (mm²)	% Inhibition Titer	% Inhibition Size	Passive hemagglutination Titer $\frac{1}{\text{Log}_2}$ X Control	Passive hemagglutination Titer $\frac{1}{\text{Log}_2}$ X Treated
Theophylline	50 p.o.[f]	−2 +6	480	122[d]	120	77[d]	75	36	11.5	10.5
	100 p.o.	−2 +6	50	28[d]	7	3[d]	84 } 87	89 } 95	10.0	9.5
	100 p.o.	−2 +6	120	64[d]	13	0[d]	89 }	100 }		
cAMP	10 s.c.	−2 +6	120	90[d]	10	0[d]	90	100	9.5	9.5
	15 s.c.	+1 +6	120	90[d]	120	83[d]	0	8	7.0	6.0
Cyclophosphamide	5 p.o.	−2 +6	60	125[e]	20	35[e]	66	72	7.5	6.0
	10 p.o.	−2 +6	60	125[e]	10	4[e]	83	97	7.5	4.5
	20 p.o.	−2 +6	60	125[e]	0[c]	0[e]	>90	100	7.5	0.0
	10 p.o.	+2 +6	60	125[e]	10	27[e]	83	78	11.0	5.5
ATS	0.25 ml s.c.	−1 +2	120	90[d]	0[c]	0[d]	>90	100	9.5	7.0
	0.25 ml s.c.	+1 +4	240	48[d]	120	10[d]	50	79	7.0	4.5
Paramethasone	1.5 p.o.	−2 +6	50	28[d]	20	8[d]	60	71	10.0	6.0

[a] Relative to 2nd antigen injection given at time 0.

[b] Average titer of a serum pool from 6 to 7 mice/group assayed in at least three separate recipients at reciprocal dilutions of 20, 60, 120, 240, 480.

[c] No positive reactions at 1:10 dilution in 3 recipients.

[d] Reaction size at the 1:60 antiserum dilution.

[e] Reaction size at the 1:20 antiserum dilution.

[f] Compounds were ground in a mortar and pestle, "wetted" with 1 drop of Tween-80 and suspended in 3% cornstarch containing 5% polyethylene glycol-400. Drugs were administered by oral intubation in 0.2 ml.

of animals. The same degree of inhibition was observed in each experiment, even though the reciprocal titers of the control animals differed (240, 960, 480). As with theophylline, cAMP given after antigen (Day +1 to Day +6) was ineffective (Table I). In order to verify whether or not residual cAMP in the sera was responsible for inhibiting the reaction in the recipients, equal volumes of control serum were added to sera from animals treated with cAMP and the mixture, as well as its components, were separately assayed in the PCA test. The sera from the cAMP-treated animals did not inhibit the control reaction.

Cyclophosphamide, ATS and to a lesser extent, paramethasone, all inhibited homocytotrophic antibody production when treatment was started before the 2nd antigen injection (Table I). Cyclophosphamide and ATS also inhibited homocytotrophic antibody when treatment was started 2 days after antigen administration. This regimen, however, was less effective than when the pretreatment schedule was used (Table I).

Although all drugs used were capable of inhibiting homocytotrophic antibody production, only cyclophosphamide, paramethasone and ATS also suppressed IgG antibody in the same animals. Both responses were affected about equally by these agents. For example, cyclophosphamide at 5, 10, and 20 mg/kg given from Day −2 to Day +6 inhibited the PCA reaction (66, 83 and 100%) and the hemagglutination titer to approximately the same extent (63, 87, and 100%). Conversely, theophylline and cAMP had a selective effect on the homocytotrophic antibody response since they did not effect IgG production. For example, theophylline (100 mg/kg) and cAMP (10 mg/kg) each given for 8 days had essentially no effect on IgG antibody production in the same animals in which the PCA titers had been 90% suppressed.

All data in Table I was obtained from pooled samples; therefore, experiments were performed to ascertain whether pooled samples accurately reflected the effect of drug on individual responses. Mice were treated with vehicle or 100 mg/kg of theophylline from Day −1 to Day +6, and the sera pools, as well as the individual samples comprising the pools, were assayed separately. The results given in Table II represents the average PCA titers of 6 sera and their respective pool, each assayed in at least three recipients. These experiments indicated that the serum pools accurately reflected the group response, and confirmed the earlier experiments indicating that theophylline inhibited IgE antibody production without significant effect on the IgG response.

Discussion. There have been several re-

TABLE II. Antibody Content of "Pool" of Six Serums Compared With Average Titer of the Individual Samples.

Treatment	Passive cutaneous anaphylaxis		Passive hemagglutination
	Reciprocal titer[a]	$\bar{x}$ Reaction (mm^2) 1:20 + 1:40	Titer $\left(\frac{1}{\text{Log}_2}\right)$
Vehicle			
Pool	160	64	10
Individual	120 ± 25	48 ± 13	8.8 ± 0.7
Theophylline (100 mg/kg p.o. Day −1 +6)			
Pool	80	23	9
Individual	56 ± 14[b]	29 ± 11	7.8 ± 0.9

[a] Average titer of the 6 serums each assayed in at least 3 mice at dilutions of 1:20, 1:40, 1:80, 1:160.

[b] $p < 0.01$ compared with controls.

ported examples of pharmacological specificity in suppressing the immune response. In all cases selectivity has been achieved by taking advantage of the cellular kinetics of a particular response. For example, antilymphocyte antibody (ALS) is specific for the "T" cell since this cell type does not divide rapidly, recirculates, and can be destroyed by direct cytotoxicity or cytophilia induced by ALS in circulation (13, 14). Similarly, the antimetabolite 6-mercaptopurine can selectively suppress IgG and not IgM production since the IgG response is more dependent upon rapid cell division (15). Our results demonstrate that in the mouse, theophylline and cAMP selectively inhibit a 2° IgE response without significant effect on IgG production. This effect is not comparable to the action of cyclophosphamide which has been reported to be specific for both "T" and "B" antigen sensitive cells (16, 17) and in our system inhibited the IgE and the ovalbumin ["T" cell dependent (18)] IgG responses to an equal extent. Similarly, the glucocorticosteriod used in our experiments inhibited both types of antibody synthesis possibly by an effect on a common cell type ("B") (19). This is in accord with the report that one of the differences between IgG and IgE production are "helper cell" requirements (20).

The mouse was used in our experiments, since it has been shown that all animals of certain inbred strains are capable of making IgE antibody, thus making this model useful for testing the effects of pharmacological agents.

A unique feature of theophylline as compared to other immunosuppressive agents is the fact that, as a phosphodiesterase inhibitor, it leads to intracellular cAMP accumulation. That this is the mechanism by which it selectively inhibits IgE antibody production is suggested by a similar effect induced by parenteral administration of cAMP itself. It has been shown that the induction of an IgE response has different cellular requirements than for IgG (20). Our data support this concept, since the IgE response can be separately inhibited. The mechanism by which cAMP exerts its selective effect is not known, however, it has been reported to enhance or depress (dependent upon doses used) "T" cell dependent cellular and humoral responses *in vitro* and *in vivo* (21). Whether cAMP inhibits IgE production by modulating "T" cell functions or by selectively inhibiting IgE secretion, can only be ascertained when additional data is available pertaining to the precise pathways of IgE production.

Summary. The effect of various agents on the *in vivo* anamnestic IgE and IgG responses of mice to alum suspensions of ovalbumin was determined. Dibutyryl cAMP and theophylline inhibited IgE but not IgG synthesis, whereas other immunosuppressive drugs inhibited both antibody classes equally. Previous reports have suggested separate pathways for the synthesis of IgG and IgE, and this report suggests that the IgE response can be selectively inhibited.

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