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Research Article

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Differences in Cyclic AMP Changes after Stimulation by Prostaglandins and Isoproterenol in Lymphocyte Subpopulations

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ABSTRACT Various lymphocyte populations have been studied for their content in cyclic adenosine 3',5'-monophosphate (cAMP) before and after stimulation by isoproterenol and prostaglandin E_1 (PGE_1). Basal cAMP levels vary among lymphocytes according to their origin: peripheral blood lymphocytes show high cAMP level while spleen and lymph node cells and thymocytes show lower levels. Thymocytes are extremely sensitive to the stimulating effects of isoproterenol and PGE_1, much more than spleen and lymph node or peripheral blood cells. Corticoresistant thymocytes are less sensitive to isoproterenol stimulation than normal thymocytes, but are significantly more sensitive than peripheral thymus-derived (T)-cells. Studies using bone-marrow-derived (B) or T cell depletion with anti-immunoglobulin-coated columns and antitheta serum (AθS) indicate that lymph node B cells synthesize more cAMP in the presence of isoproterenol than T cells. However, this difference between T and B cells has not been found in spleen cells.

INTRODUCTION

The involvement of cyclic AMP (cAMP)[1] in lymphocyte physiology has been suspected since the demonstration of the action of cAMP and various hormones, known to increase its intracellular level, on phytohemagglutinin (PHA)-induced blast transformation (1, 2). The presence of adenyl cyclase in lymphocyte membranes and stimulation of cAMP formation by human lymphocytes in vitro with prostaglandins PGE_1 and PGE_2 and catecholamines were then directly demonstrated (3–5). In keeping with these data obtained in the human, other authors showed the existence of adenyl cyclase and receptors for various hormones in mouse and rat spleen cells (6–11). Lastly, the role of cAMP in the appearance of immunocompetence in thymic-dependent (T) lymphocytes was suggested by our work (12, 13) and that of Kook and Trainin (14), indicating that cAMP might act in lieu of thymic factors in the induction of T-cell markers (such as the theta antigen) or T-cell function (such as graft versus host reaction) after incubation with T-cell precursors.

The functional significance of the hormone receptors and of the action of cyclic nucleotides (cAMP and cGMP) on the response of lymphocytes to antigens and mitogens is currently being investigated. Cyclic AMP itself and products stimulating its synthesis depress the response of normal lymphocytes to PHA (1, 2) and the cytotoxic activity of sensitized lymphocytes against allogeneic cells (6, 11, 15). Conversely, intracellular cGMP is considerably augmented after PHA stimulation (15) and cholinergic agents known to increase intracellular level of cGMP increase the cytotoxic action of sensitized lymphocytes against allogeneic cells (11). The role of cAMP and hormones stimulating its synthesis on antibody production is less clearly understood. Controversial results have been reported concerning the direct action of cAMP and dibutyryl cAMP on primary in vitro stimulation of mouse lymphocytes by sheep red blood cells (SRBC), some authors reporting inhibition of the response (16), others enhancement, at least for certain concentrations (17). Moreover, it has been shown that a large proportion of plaque-forming cells obtained from spleens of mice immunized with SRBC was retained on Sephadex bead columns coated with insolubilized hormones, such as

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[1] *Abbreviations used in this paper:* AθS, anti-theta serum; B, bone-marrow derived; BSA, bovine serum albumin; cAMP, cyclic 3',5'adenosine monophosphate; cGMP, cyclic 3',5' guanosine monophosphate; PG, prostaglandin; PHA, phytohemagglutinin; SRBC, sheep red blood cells; T, thymus-derived.

PGE_1 and histamine, suggesting the presence of receptors for histamine and PGE_1 on immunized B cells (18). In summary, cyclic nucleotides appear to interfere with immune responses, although some controversial results do not permit a definitive interpretation.

It is now admitted that lymphoid cells can be separated into two main categories: B cells, involved in antibody production, and T cells, involved in cell-mediated immunity and helper effect for antibody production. Among T cells, several pieces of evidence also argue in favor of some cellular heterogeneity, hence the new concept of T cell subsets with differences in markers and functions (19, 20). Since cyclic nucleotides appear to play a role in lymphocyte maturation and function, as discussed above, it was thought important to study cAMP metabolism in the lymphocyte subpopulations listed above. We report here data concerning the relative cAMP content of various lymphocyte subpopulations before and after stimulation by isoproterenol and PGE_1. It will be shown that T lymphocyte maturation, from the cortex thymocyte to the mature T cell, is associated with a relative loss of the capacity to respond to isoproterenol and PGE_1 by cAMP accumulation. Our data also show differences in the ability of B and T cells to be stimulated by these agents.

METHODS

Mice. In most experiments male or female C57B1/6 mice, aged 6–16 wks, were used. These mice were obtained from the Centre de Sélection et d'Elevage des Animaux de Laboratoire (C.S.E.A.L.) (Orléans, La Source, 45—France) or from Charles River (France). In some experiments male or female nude mice (C.S.E.A.L.) with the CBA background were used at the age of 5–10 wk with controls of the same breeding *nu*/+ or +/+.

Cell suspensions. All manipulations were performed in ice water after organ collection or alternatively in a cold room at 4°C, except for the incubation with PGE_1 and isoproterenol, performed at 37°C. Centrifugation was always done in a refrigerated centrifuge at 4°C. Spleen, thymus, inguinal and axillar lymph nodes were collected immediately after the animal's sacrifice by cervical dislocation, and cells were dissociated in Hanks' medium (Pasteur Institute, Paris) with a Potter homogenizer. Capsular debris and cellular aggregates were eliminated by filtration of the cell suspension through a double thickness of nylon tissue. Cells were then washed once in Hanks' medium, resuspended in the same medium, and enumerated in a hemocytometer with Turk's solution. Cell concentration was then adjusted to 10^7 cells/ml. Blood was collected on heparin in mice anesthetized with ether after heart section. In some experiments mononucleated cells were separated from red cells and polymorphs by centrifugation through a mixture of Ficoll (Pharmacia, Uppsala, Sweden) and sodium metrizoate (Nyegaard and Co., Oslo, Norway) (21), and cells thus obtained were washed twice in Hanks' medium, enumerated in Turk's solution, and then adjusted to 10^7 cells/ml. In other experiments, cells were separated from plasma by centrifugation at 200 *g;* the pellet thus obtained was resuspended in an ammonium chloride solution at 0.83% to obtain red cell lysis. Cells were then centrifuged and resuspended in Hanks' medium, the number of mononucleated cells being adjusted to 10^7 cells/ml. Lastly, in other experiments, cell suspensions devoid of nucleated cells but containing platelets were prepared by centrifuging the initial cell suspension at 100 *g* and collecting the supernate; viability tests with trypan blue were performed at the end of the cell fractionation procedure, in particular in all experiments involving long manipulations, such as separation on antibody-coated columns or AθS treatment.

Cortico-resistant thymocytes. Mice were given two injections of 10 mg hydrocortisone hemisuccinate (Roussel, Paris, France) at 24-h intervals i.p. and were sacrificed 24 or 48 h after the second injection. Cell suspensions were prepared from the thymus of these mice, as described above for normal thymus. The number of cells per thymus in steroid-treated mice was always found to be less than 5% of that observed in mice of the same age and sex not treated with steroids.

Elimination of immunoglobulin-bearing lymphocytes. Depletion of immunoglobulin (Ig)-bearing lymphocytes was performed according to the method described by Singhal and Wigzell (22), using plastic bead columns (Degalan V26 Degussa Wolfgang A. G., Hanau am Main, Germany) coated with rabbit anti-mouse Ig antiserum (Pasteur Institute, Paris). Similar columns were coated with normal rabbit serum and used as controls. Cell suspensions obtained from normal spleens or lymph nodes or spleens from nude mice were filtered on these columns (after red cell lysis by 0.83% ammonium chloride in spleen cell suspensions). Eluted cells were evaluated (*a*) for the percentage of theta-positive cells by a cytotoxicity test with trypan blue and AθS in the presence of complement (23); the AθS was prepared by injecting AKR mice with CBA thymocytes (10^7 thymocytes i.p. every week for 6–8 wk); (*b*) the percentage of cells bearing surface Ig, with anti-Ig fluorescent antiserum (Pasteur Institute, Paris) (24).

Elimination of theta-positive lymphocytes. Spleen cell suspensions were submitted to the action of AθS and complement to kill theta-positive cells. Cells were incubated with AθS for 30 min at 4°C, then washed, and incubated for 45 min more at 37°C in the presence of fresh guinea pig serum. Cells were then washed again and centrifuged at 20,000 *g* for 30 min on a bovine serum albumin (BSA) gradient with two phases (35%, 10%) to eliminate dead cells, which go into the pellet, whereas viable cells remain at the interface between the two phases (25). Control cell suspensions were prepared according to the same procedure by using normal AKR serum instead of AθS. The percentage of theta-positive lymphocytes was evaluated by a cytotoxicity test with trypan blue, with the initial batch of AθS.

Study of the action of isoproterenol and PGE_1 on cAMP synthesis by lymphocytes. Isoproterenol was obtained from Sigma Chemical Co. (St. Louis, Mo.) as isoproterenol bitartrate or from Winthrop (Isuprel, Winthrop Laboratories, New York) as isoproterenol chlorhydrate. PGE_1 was given by Dr. J. Pike (Upjohn Co., Kalamazoo, Mich.). These two products were diluted to the appropriate concentration in Hanks' medium. D-L Propranolol was obtained from Sigma. The various cell suspensions, adjusted to 10^7 cells/ml, were distributed in hemolysis tubes (1 ml/tube), into which isoproterenol and PGE_1 were introduced at various concentrations. Each experiment included control tubes without drug addition. No theophylline was added. Tubes were placed in an incubator at 37°C in an atmosphere of 95% air and 5% CO_2 and removed from the incubator after various periods, according to the experiments. The incuba-

TABLE I

Effects of Isoproterenol and PGE_1 on cAMP Level in Lymphoid Cells

	No drug added*	Isoproterenol (10^{-5} M)*	No drug added‡	PGE_1 (10^{-6} M)‡
		pmol/10^7 cells		
Thymus	2.18±0.33	138.95±27.72	3.32±0.74	77.65±15.63
Spleen	4.29±0.69	23.14±4.49	3.90±0.63	24.85±3.48
Lymph nodes	9.99±1.93	31.42±4.39	8.71±1.99	27.43±3.71
Blood	27.04±4.80	49.75±5.86	16.68±3.23	32.03±4.61

Each value represents the mean of 6–10 experiments. *P* values are given in the text. Results are means±SE.

* 10 min incubation.

‡ 30 min incubation.

tion time was chosen to provide an optimal cAMP accumulation. Preliminary experiments (not shown) indicated that optimal incubation time was 10 min for isoproterenol and 30 min for PGE_1.

Cyclic AMP extraction. The tubes containing the cellular suspensions were centrifuged for 5 min at 400 *g* and the pellet was precipitated by 0.5 ml of 5% trichloracetic acid. After addition of 0.05 ml of normal HCl and centrifugation of the precipitates at 3,000 *g* for 15 min, supernates containing cAMP were collected in glass hemolysis tubes. Trichloracetic acid was extracted by ether (five consecutive extractions by 1 ml of ether) and the content of the tubes was frozen and lyophilized.

Cyclic AMP dosage. Cyclic AMP was assessed according to Gilman's competition-binding method with a binding protein isolated from bovine striated muscle (26). The recovery of added nucleotide, measured after addition of known amounts of tritiated cAMP, averaged 75%. Duplicate determinations of the same samples varied by 15%. Preincubation of the cellular extracts for 1 h at 37°C with cyclic nucleotide phosphodiesterase (Sigma) diminished cyclic AMP level more than 95%. Results were expressed in picomoles of cyclic AMP per 10^7 cells.

Statistical tests. Student's *t* test was used in all experiments. All data represent the mean of 3–10 different experiments.

RESULTS

An increase in the cellular content of cAMP due to the effect of stimulation by isoproterenol and PGE_1 has been demonstrated in all lymphocyte populations examined, no matter what their origin, but the intensity of this increase varied considerably with the organ from which the cells were obtained.

Thymocytes. Isoproterenol (10^{-5} M) induced a considerable accumulation of cAMP in thymocytes after 10 min incubation (63-fold increase compared with control cells incubated without isoproterenol) (Table I). Fig. 1 shows the dose-response curve of cAMP cellular level as a function of isoproterenol concentration. Initially at 2.18 pmol/10^7 cells, cAMP level began to increase significantly at a concentration as low as 2.5×10^{-8} M and reached a plateau at 10^{-6} M. No difference was observed between stimulation by isoproterenol bitartrate or chlorhydrate.

PGE_1 also induced a very important accumulation of cAMP in thymocytes after 30 min incubation (Table I). Fig. 2 shows the dose-response curve of cAMP level as a function of PGE_1 concentrations. A significant increase of cAMP level was detected for a concentration as low as 10^{-9} M. At 10^{-6} M (the highest concentration examined), the increase in cAMP level was 23-fold when related to initial values.

Preincubation of thymocytes for 10 min with propranolol (10^{-5} M) before addition of isoproterenol (10^{-5} M) or PGE_1 (10^{-6} M) completely blocked isoproterenol action on cAMP accumulation but had no effect on PGE_1-induced accumulation (Table II).

Spleen cells. In the absence of any stimulation, cAMP level of spleen cells was slightly but signifi-

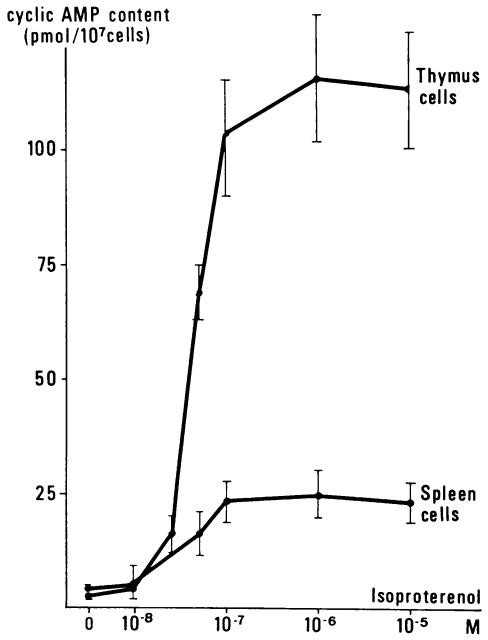

FIGURE 1 Cyclic AMP amount (mean±SE) in 10^7 thymocytes or spleen cells as a function of molar isoproterenol concentration after 10 min incubation.

cantly higher than that of thymocytes ($P < 0.01$). In the presence of isoproterenol (10^{-5} M) the cellular level of cAMP of spleen cells increased 5.3 times after a 10-min incubation (Table I). The dose-response curve (Fig. 1) of cAMP level, as a function of molar isoproterenol concentration, was not very different from that observed for thymocytes, but the level of the plateau was significantly lower than for thymocytes ($P < 0.01$). Spleen cell incubation with propranolol (10^{-5} M) 10 min before isoproterenol addition (10^{-6} M) completely suppressed isoproterenol effects on spleen cells (Table II).

After 30 min incubation, PGE_1 increased the cellular level of cAMP of spleen cells 5.6-fold (Table I). The dose-response curve of cAMP, as a function of the molar concentration of PGE_1, is shown in Fig. 2. As noted above for isoproterenol, the level reached by spleen cells at 10^{-6} M of PGE_1 was lower than that obtained for thymocytes ($P < 0.05$).

Interestingly, at 10^{-6} M, PGE_1 stimulated cAMP accumulation in spleen cells in the same proportion as has been shown for isoproterenol, at variance with what has been reported for thymocytes.

Lymph node cells. Lymph node cells had a basal cAMP level significantly higher than that of thymocytes ($P < 0.001$) or spleen cells ($P < 0.01$). However, the cAMP level reached by lymph node cells after 10 min incubation with isoproterenol (Table I) was significantly lower than that obtained with thymocytes under the same conditions ($P < 0.01$) and slightly higher than that reached by spleen cells (but the difference was not significant). One may note that the difference between the initial cAMP level and the level obtained after isoproterenol stimulation (10^{-5} M) after 10 min incubation was about the same for spleen and lymph node cells (respectively, 18.25 and 21.43 mol/10^7 cells), but much lower than for thymocytes (136.77 pmol/10^7 cells). PGE_1 (10^{-6} M) also stimulated cAMP accumulation by lymph node cells after 30 min incubation. PGE_1-induced accumulation was slightly higher

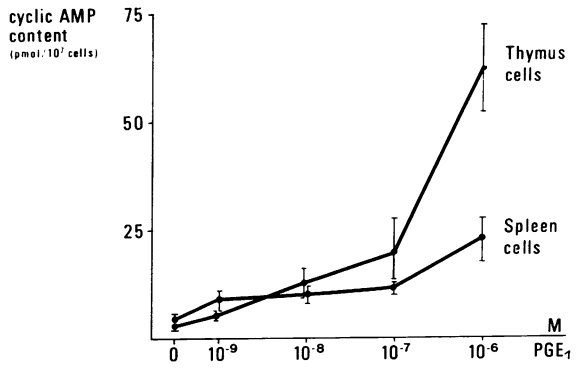

FIGURE 2 Cyclic AMP amount (mean±SE) in 10^7 thymocytes or spleen cells as a function of molar PGE_1 concentration after 30 min incubation.

(but not significantly) than that of spleen cells incubated under the same conditions, and lower than that obtained with thymocytes (but again difference was not significant).

Peripheral blood cells (Table I). The basal cAMP level of peripheral blood lymphocytes was higher than in thymus ($P < 0.001$), spleen ($P < 0.001$), or lymph node cells ($P < 0.01$). Isoproterenol (10^{-5} M) and PGE_1 (10^{-6} M) both enhanced cAMP accumulation to a similar extent. The absolute increase in cAMP level was of the same order as that observed with spleen or lymph node cells and much lower than that noted with thymocytes. It was verified that neutrophil contamination (3% in the case of centrifugation on Ficoll-sodium metrizoate mixture, 10–20% when buffy coat was used directly) did not modify the results. Platelet cAMP content, assessed on platelet-rich supernate, was found negligible in comparison to total leukocyte cAMP content before and after isoproterenol and PGE_1 stimulation.

Corticoresistant thymocytes (Table III). In the absence of any stimulation, corticoresistant thymocytes had a significantly higher basal cAMP level than normal total thymocytes measured under the same conditions on the same day ($P < 0.01$). However, isopro-

TABLE II

Effects of Propranolol (10^{-5} M) on Isoproterenol- and PGE_1-Induced cAMP Accumulation by Thymocytes and Spleen cells

	Thymocytes		Spleen cells
	Exp. 1	Exp. 2	Exp. I
		pmol/10^7 cells	
No drug added	1.98	0.35	3.5
Propranolol 10^{-5} M	1.72	0.31	4.5
Isoproterenol 10^{-5} M	78.0	82.5	10.5
Propranolol 10^{-5} M + Isoproterenol 10^{-5} M	1.70	0.31	2.8
PGE_1 10^{-6} M	68.0		
Propranolol 10^{-5} M + PGE_1 10^{-6} M	52.5		

TABLE III

Effects of Isoproterenol on cAMP Level in Normal and Corticoresistant Thymocytes

	No drug added	Isoproterenol (10^{-5} M)	
	pmol/10⁷ cells		
Normal thymocytes	2.18±0.31	138.95±27.72	
	$P < 0.05$		$P < 0.05$
Corticoresistant thymocytes	6.99±1.62	59.75±5.91	

Each value represents the mean ± SE of six experiments.

terenol-induced increase was significantly lower ($P < 0.05$) than that observed with normal thymocytes, although higher than that obtained with spleen ($P < 0.01$) or lymph node cells ($P < 0.01$) under the same conditions. No difference was seen between normal and corticoresistant thymocytes in their response to PGE_1.

B or T cell-enriched lymph node or spleen cells. Table IV shows the average content of spleen cells in Ig-bearing lymphocytes and theta-positive cells after passage on columns coated with rabbit anti-mouse Ig antibodies or normal rabbit serum and the amount of cAMP obtained from these cells in the presence or absence of isoproterenol. Spleen cell suspensions depleted in Ig-bearing cells showed less isoproterenol-induced cAMP accumulation than cells passed on normal rabbit serum-coated columns. The basal cAMP level of these cells (in the absence of any stimulation) was not modified by the column treatment.

Cyclic AMP levels were then studied in the presence or absence of isoproterenol in spleen cells treated by AΘS and complement, and thus depleted in theta-positive cells (cytotoxic index between 0 and 8%) or in control spleen cells treated with normal AKR serum (cytotoxic index between 15 and 24%). One can observe in Table V a slight (but not significant) increase in cAMP-isoproterenol-induced accumulation by spleen cells depleted of theta-positive cells, but no difference in basal cAMP levels.

Table VI shows results of similar experiments performed with lymph node cells passed on anti-Ig antibody-coated columns and columns coated with normal rabbit serum. One may note a significant decrease ($P < 0.05$) of the isoproterenol-induced cAMP accumulation in B-cell-depleted lymph node cell suspensions. Basal cAMP level in these experiments was not modified by column treatment. One should emphasize that the number of cells recovered was not always high enough to allow evaluation of the percentage of Ig-bearing cells, but the cell suspensions have always been proved completely depleted of Ig-positive cells when tested.

Nude spleen cells (Table VII). Isoproterenol and PGE_1-induced cAMP accumulation in nude spleen cells was found identical to that induced in *nu*/+ or +/+ control spleen cells. Basal level of cAMP was found higher in nude mice than in normal spleen cells from C57B1/6 mice, but similar levels have been found in control *nu*/+ and +/+ mice.

Nude spleen cells completely depleted of Ig-bearing cells by passage through rabbit anti-mouse Ig-coated columns responded to isoproterenol like nude cells passed through normal rabbit serum-coated columns.

DISCUSSION

Recent studies have shown that various stimulants of the adenyl cyclase system, such as prostaglandins (PGE_1 or PGE_2)and catecholamines, stimulate cAMP accumulation in mouse lymphocytes (6, 7, 10). Our experiments confirm this finding for isoproterenol and PGE_1 and show that this property also extends to thymus, lymph node, and peripheral blood lymphocytes.

TABLE IV

Effects of Isoproterenol on Spleen Cells Passed through Anti-Ig-Coated Columns or Normal Rabbit Serum-Coated Columns

	Θ-positive cells (extreme values)	Ig-bearing cells (extreme values)	cAMP mean±SE: without isoproterenol	cAMP mean±SE: with isoproterenol (10^{-5} M)	
	%	%	*pmol/10⁷ cells*		
Anti-Ig serum	73–81	≤2	5.73±1.19	18.83±4.76	
			$P > 0.05$		$P > 0.05$
Normal serum	28–38	35–40	5.28±0.60	20.83±2.28	

Experimental values were obtained from four experiments.

TABLE V

Action of Isoproterenol on Spleen Cells after Elimination of Theta-Positive Cells by AΘS and Complement

Spleen cell treatment	Θ-positive cells (extreme values)	cAMP mean±SE		
		without isoproterenol	with isoproterenol (10^{-6} M)	
	%	*pmol/10⁷ cells*		
AΘS + complement	0–8	3.63±1.52	18.66±3.06	
		$P > 0.05$		$P > 0.05$
Normal AKR serum + complement	15–24	3.73±0.32	15.41±2.70	

Experimental values were obtained from three experiments.

One should note, however, that theoretically isoproterenol and PGE_1-induced increases in cellular cAMP level could be due to decrease in degradation as well as to increase in synthesis, although the former hypothesis is unlikely. Stimulation of cAMP accumulation by isoproterenol is specifically blocked by propranolol, suggesting that isoproterenol-induced stimulation involves lymphocyte beta receptors, as previously shown by Hadden, Hadden, and Middleton (27).

We have found important differences in basal cAMP level, as well as in cAMP accumulation after stimulation by isoproterenol and PGE_1, according to the lymphocyte cell population considered. Peripheral blood lymphocytes show higher basal cAMP levels than lymph node and spleen cells, which in turn show higher levels than thymocytes. These results are in keeping with the work of others in the human (3–5) and in rodents (6–11), although strict comparisons cannot be made because none of these studies included a simultaneous evaluation of cAMP levels in these different lymphocyte populations. Thus, studying the human peripheral blood cells, Smith, Steiner, Newberry, and Parker (3), and Mendelsohn, Multer, and Boone (5) found basal cAMP levels ranging from 10–70 pmol/10^7 cells. Studying rat spleen cells, Strom et al. (11) found basal cAMP levels of 2 pmol/10^7 cells, whereas Lichtenstein, Henney, Bourne, and Greenough (10) found basal levels of 6.9 pmol/10^7 cells in mouse spleen cells. Lastly, Whitfield, MacManus, Braceland, and Gillan (8) reported levels of 2 pmol/10^7 cells in rat thymocytes.

In our work, cAMP accumulation after stimulation by isoproterenol and PGE_1 varied according to the lymphocyte population studied. Thus, normal thymocytes, the cAMP content of which is very low in absence of stimulation, are extremely sensitive to stimulation by isoproterenol and PGE_1 and relatively more sensitive to isoproterenol than to PGE_1. Lymph node, spleen, and peripheral blood cells, which have higher initial values of cAMP than thymocytes, are less sensitive to isoproterenol and PGE_1-induced stimulation, but are equally sensitive to both agents. Other works have shown similar increase in cAMP levels after isoproterenol or PGE_1 stimulation in spleen and in peripheral blood cells. Studying human peripheral blood lymphocytes, Smith et al. (3), Mendelsohn et al. (5) and Bourne, Lehrer, Lichtenstein, Weissmann, and Zurier (4) have reported 1.5–3-fold stimulation with isoproterenol and 3–10-fold stimulation with PGE_1. In rat

TABLE VI

Action of Isoproterenol on Lymph Node Cells after Passage on Plastic Bead Columns Coated with Anti-Ig or Normal Rabbit Serum

	Θ-positive cells (extreme values)	cAMP mean±SE		
		without isoproterenol	with isoproterenol (10^{-6} M)	
	%	*pmol/10⁷ cells*		
Anti-Ig serum	87–92	3.98±0.69	26.16±8.91	
		$P > 0.05$		$P < 0.05$
Normal serum	52–55	4.10±0.85	35.33±9.81	

Experimental values were obtained from three separate experiments. Student's *t* test was applied to differences in cAMP values obtained in each experiment after stimulation by isoproterenol in lymph node cells passed through anti-Ig-coated columns, compared to control lymph node cells passed through normal serum-coated columns.

TABLE VII

Action of Isoproterenol on Spleen Cells from Nude Mice and nu/+ or +/+ Mice from the Same Breeding

	cAMP		
	without isoproterenol	with isoproterenol (10^{-6} M)	
	pmol/10^7 cells		
Nude spleen cells	9±1.5	67±5.7	
	$P > 0.05$		$P > 0.05$
(+/+) and (*nu*/+) spleen cells	7.9±1.3	68.3±4.9	

Experimental values (means±SE) were obtained from three experiments.

spleen, Strom et al. (11) found 2-fold stimulation with isoproterenol and 2–4-fold stimulation with PGE_1. Also in keeping with our results, one may mention the results of Whitfield et al. (8), finding a 45–70-fold increase in thymocyte cAMP level after PGE_1 stimulation, with the Gilman assay for the evaluation of cellular content in cAMP level. However, the same authors, in previous works (9, 28), using a different method for cAMP determination (measurement of newly formed cAMP from tritiated adenine), had found a less important stimulation by epinephrine (two-fold) and PGE_1 (three-fold). This discrepancy with our results and their own previous findings is probably explained by differences in cAMP evaluation techniques. One may lastly mention that, as in our work, Makman (7) found that basal and catecholamine-stimulated adenyl cyclase activity of mouse or rat thymic cells was appreciably greater than that of splenic or mesenteric node cells in the same animal. However, the differences were less important than ours, perhaps because of differences in techniques for cAMP evaluation, as these authors measured adenylcyclase activity of cell homogenates.

Differences in cAMP levels between thymocytes and lymphoid cells of other origins may be explained by a difference in adenyl cyclase quantity as suggested by Makman (7) or by differences in the number and nature of membrane receptors for isoproterenol and PGE_1. Anyhow, both hypotheses imply differences in membrane structure, which is reminiscent of other membrane differences in dealing with surface antigens, namely presence of the TL antigen on thymocytes and not on T cells from lymph nodes, spleen, or blood (29), and the presence of more theta antigen on cortical thymocytes than on peripheral T cells (20, 29).

Our results concerning cortico-resistant thymocytes are in favor of a continuous maturation from cortical thymocytes to peripheral T cells with an intermediate stage represented by medullar thymocytes, suggested by the intermediate average response of cortico-resistant thymocytes to isoproterenol and PGE_1, between that of total thymocytes and that of peripheral lymphocytes. However, steroid-sensitive lymphocytes could not be studied directly and inferences on the steroid-sensitive population is thus an extrapolation: one cannot exclude the possibility that steroids do alter the function of thymocytes surviving steroid treatment in spite of the 48-h lag time between the last steroid injection and cAMP measurement. Finally, the appearance of immunocompetence in T cells might thus be associated with changes in other membrane structures, such as adenyl cyclase and hormone receptors as well as the fully documented TL or theta antigen changes mentioned above.

Our data also put forward differences between B and T cells in isoproterenol-induced cAMP accumulation, B cells being better stimulated than T cells. Thus, B cell-depleted lymph node cells respond less well to isoproterenol than normal lymph node cells. However, such a difference between B and T cells was not found in the spleen. Spleen cell suspensions deprived of T cells by AθS treatment or deprived of B cells by passage through anti-Ig-coated columns showed basal cAMP levels and cAMP increases after isoproterenol stimulation similar to those of normal spleen, including both B and T cells. Moreover, nude spleen cells, which do not contain T cells, respond to isoproterenol like normal spleen cells. This discrepancy between spleen and lymph node cells may be due to the presence in the spleen and not in lymph nodes of a large proportion of relatively immature cells which do not bear surface Ig or theta antigen ("nul" cells). These nul cells are not eliminated by passage through anti-Ig serum-coated columns or AθS treatment and thus contaminate the pure T or B cell population obtained by these methods. One may thus note that after passage through anti-Ig serum-coated columns, spleen cells still contained 25% theta-negative cells, whereas the percentage is lowered to 10% for lymph node cells.

Finally, the physiological significance of isoproterenol and PGE_1 receptors is still a matter of speculation. It is possible, however, that they play a role in the regulation of antigen-induced differentiation and antibody synthesis. The differences we have found between B and T

cell subpopulations are still difficult to interpret but might have some biological significance in this context.

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