Cyclic AMP Effects on Antibody Formation and Their Similarities to Hormone-Mediated Events[1] (34921)

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Recent studies have revealed that 3′, 5′-cyclic adenosine monophosphate (cAMP) when administered with antigen to mice, or when added to spleen cell suspension *in vitro*, will enhance antibody formation. In view of the relationship of this observation to known events in regulation by hormones, the experimental data are presented here together with a discussion of the general nature of cAMP effects and of possible similarities between immune responses and hormone-mediated cell activations.

Materials and Methods. Antibody formation *in vivo* was assayed (1) by a determination of the number of plaque-forming cells in the spleen of 6–8-weeks-old CFW mice, 48 hr after immunization with sheep red blood cells (sRBC). Antibody formation *in vitro* was initiated and measured by using a slightly modified procedure (2) of the Mishell–Dutton technique (3). Compounds that were tested for their modifying effects on antibody formation were injected into animals, or added to cultures, at the time of administration, or additon, of antigen. Cyclic AMP (lot 68B-2580), and adenosine (lot 97B-0040) were obtained from Sigma Chemical Co., poly A:U from Miles Laboratories, N_6O_2'-dibutyril adenosine-3′,5′-cyclic phosphate disodium 8 H_2O(= DB-cAMP; lot 920266) from Calbiochem. Co., and rabbit antimouse lymphocyte serum (ALS) was donated by Ortho Research Foundation. The CFW mice employed in different tests possessed, as usual, variable "background" numbers of spleen cells forming 19S antibodies to sheep red blood cells (sRBC), and this was associated with corresponding differences in their responses to sRBC, but such variability does not affect comparisons among relative increases or decreases in responses obtained in different tests.

Results and Discussion. The ability of cAMP to enhance antibody formation *in vivo* was detected in assays on the number of antibody-forming spleen cells (AFC) 48 hr after immunization of CFW mice (Table IA) and also *in vitro* assays in which the number of AFC in sRBC-supplemented spleen cell cultures was assayed 3 or 4 days after initiation of the cultures (Table II). As indicated in Table IA, the enhancement *in vivo* was particularly pronounced when cAMP was administered in conjunction with antilymphocyte serum. Since nontoxic concentrations of ALS are known to provide stimulatory signals of their own in some systems and also can modify lymphocyte permeability, this cAMP and ALS effect may be due to a double signal or to an increased capability of cAMP to enter the cells in the presence of ALS. Dibutyril cAMP (DB-cAMP), which is less susceptible than cAMP to conversion to 5′-AMP by phosphodiesterase and enters cells more easily than cAMP (4), also enhanced *in vivo* responses after iv administration of 500 μg/mouse or more (Table IB). Noncyclic AMP had no stimulatory effects, even when given with ALS, and suppressed the stimulating effects of poly A:U (Table IC). Adenosine also produced no stimulation, in fact it caused some inhibition of the normal response as well as of poly A:U-stimu-

[1] These studies were supported by NIH Grant AM-08742 and NSF Grant B9-0301R. Theoretical considerations, based on the experimental results, catalyzed while one of us (W. B.) enjoyed the hospitality of the Department of Chemical Immunology, The Weizmann Institute of Science, with the aid of an NIH Special Fellowship.

TABLE I. The Effects of cAMP (200 μg/mouse ip), DB-cAMP (various doses iv), AMP (200 μg/mouse ip) and Adenosine (200 μg/mouse ip) on Antibody Formation to Sheep Red Blood Cells in CFW Mice, Tested in the Absence and Presence of Concommitant Administration of Poly A:U (300 μg/mouse iv).

The effects of cAMP were also tested after simultaneous administration of ALS (0.1 ml/mouse ip); five animals per group.

Treatment of spleen donors	Av no. (± SE) of AFC/10^8 nucleated spleen cells after 48 hr
A. Unimmunized controls	61.1 ± 10.4
sRBC (10^8)	642.3 ± 75.0
+ cAMP	1303.2 ± 284.9
+ cAMP + ALS	3027.5 ± 238.6
+ ALS	487.4 ± 124.6
+ poly A:U	5904.7 ± 475.8
+ poly A:U + cAMP	4040.6 ± 705.7
B. Unimmunized controls	69.0 ± 10.4
sRBC (10^8)	613.9 ± 65.7
+ DB-cAMP, 200 μg	434.0 ± 25.0
500 μg	1342.8 ± 154.2
1000 μg	1666.2 ± 85.5
DB-cAMP, 1000 μg	42.0 ± 9.1
C. Unimmunized controls	22.9 ± 4.9
sRBC (10^8)	199.3 ± 53.3
+ AMP	209.0 ± 23.4
+ AMP + ALS	123.5 ± 19.9
+ ALS	154.1 ± 33.0
+ poly A:U	619.1 ± 80.3
+ poly A:U + AMP	286.9 ± 71.6
D. Unimmunized controls	85.8 ± 11.0
sRBC (10^8)	901.1 ± 10.6
+ adenosine	564.4 ± 32.3
+ adenosine + ALS	786.7 ± 74.4
+ ALS	585.0 ± 63.9
+ poly A:U	5208.6 ± 390.6
+ poly A:U + adenosine	3025.9 ± 187.8

lated (5) responses (Table ID). Adenine acted essentially like adenosine except that a slight stimulation was observed following its administration in conjunction with ALS. As indicated in Table IA, the stimulating effects of cAMP were not additive or synergistic with the stimulatory effects produced by poly A:U (5). However, theophylline, a known stabilizer of cAMP (6), has an enhancing effect on the stimulation produced by poly A:U which is particularly pronounced in the presence of otherwise inactive concentrations of poly A:U (Table III). This last observation suggests a possible relationship between the stimulating effects of double-stranded synthetic polynucleotides and cAMP-mediated effects. In view of the fact that a stabilizer of cAMP, but not cAMP itself, increases the effects of poly A:U, one can suspect that the stimulation may be more dependent on the duration than on the intensity of the signal. Alternatively, there might be a difference in the ability of theophylline and cAMP to get into the cell. The more potent stimulatory activity of poly A:U, compared to that of cAMP, also may reflect a poor uptake of cAMP by cells, yet there also remains the possibility that, despite similar end-effects, the mode of action of poly A:U and cAMP may differ. In any event, the finding that cAMP influences antibody formation *in vitro* strongly suggests that

TABLE II. The Effects of cAMP on Antibody Formation *in Vitro*.

Spleen cell cultures were supplemented with 10 μg of cAMP/ml and the results shown are from assays on pools of three replicate cultures. Results of two separate experiments are shown in one of which enhancement was detectable on day 3, in the other one not until day 4.[a]

Cultures supplemented with	AFC/10^6 spleen cells in cultures on day 3	4
Nothing (controls)	5.4	—
sRBC (1% v/v)	30.1	—
+ cAMP	57.5	—
Nothing (controls)	5.6	1.8
sRBC (1% v/v)	63.1	67.9
+ cAMP	66.8	128.8

[a] Such differences are frequently encountered under *in vitro* conditions where the degree of stimulation obtained appears to be dependent on the magnitude of the unstimulated response in a given test.

TABLE III. The Effects of Theophylline (200 μg/mouse ip), Administered in the Absence or Presence of Poly A:U (different doses iv) on Antibody Formation to Sheep Red Blood Cells in CFW Mice.

Five animals per group.

Treatment of spleen donors	Av no. (± SE) of AFC/10^8 nucleated spleen cells after 48 hr
A. Unimmunized controls	36.6 ± 3.0
sRBC (10^8)	299.2 ± 42.4
+ poly A:U, 300 μg	1743.5 ± 70.4
+ theophylline	2635.8 ± 138.3
+ theophylline	277.5 ± 114.4
B. Unimmunized controls	30.3 ± 4.6
sRBC (10^8)	493.1 ± 46.7
+ poly A:U, 3 μg	346.8 ± 38.9
+ theophylline	707.0 ± 55.7
30 μg	1428.9 ± 260.3
+ theophylline	1889.8 ± 151.0
300 μg	1757.0 ± 267.5
+ theophylline	3230.7 ± 208.7

the effects of this compound are directly on cells involved in the response, rather than on other systems that may influence antibody formation.

The capacity of cyclic AMP to modify immune responses suggests that it may serve in immunological systems in a manner that is similar to its role in the mediation of signals produced by hormones interacting with appropriate target cells. In such interactions the primary recognition of the stimulator by membrane sites of the appropriate target cell (a specific event) is followed by a signal-transforming step (occurring in various cell systems) which involves the following reaction (6):

$$\text{ATP} \xrightarrow[\text{cyclase}]{\text{adenyl}} 3',5'\text{-cAMP} \xrightarrow[\text{diesterase}]{\text{phospho-}} 5'\text{-AMP}.$$

The enzyme adenyl cyclase, present in membranes (7), is activated as a result of the initial stimulator–receptor interaction (6, 8, 9) and the product of the enzyme's activity, *i.e.*, the cyclic ester, has the ability to change the activity of many intracellular systems. These include enzymes involved in energy balance, exocrine systems, and endocrine systems (6). The molecular basis for the mediation of stimuli by cAMP is only partly understood in a few of these systems, but apparently involve kinases that phosphorylate relevant enzymes and thereby change their parameters of activity (V_{max}, K_m). Of particular interest is the ability of cAMP to affect enzymes that control the phosphorylation of histones and other basic proteins (10–11) since this suggests a possible link from cAMP to the regulation of transcription of genetic information.

As in the case of hormones, immune responses, specifically antibody formation, also involve interactions among several cell types and also can be divided into several sequential steps reminiscent of those discussed for hormone action (12). Thus the activating encounter between an immunogen and antigen-reactive stem-cells of potentially antibody-forming cell populations also is a specific event that relies on a recognition of the antigen by receptor sites. The intracellular events triggered by this first step, the multiplication of activated cells, their differentiation, and their possible interactions with still other cells, probably all involve general mechanisms common to all participating lymphocytes regardless of their differential inherent capacities for the potential or actual formation of immunoglobulins. The final

event, however, the formation of a specific immunoglobulin by the cells at the end of the chain of events leading to antibody formation, is again specific since the products of different activated immunoglobulin synthesizing cell populations can differ in their structure and activity. Thus the superficial parallels between hormone-mediated activation and antigen-mediated activations are striking and the observation that cAMP can modify both systems suggests that these parallels are not merely spurious.

There are additional parallels; for example immune responses are dependent on immunogen concentrations, so that at very high concentrations nonresponsiveness rather than responsiveness will result (12). Similarly, a typical feature of some of the hormonal systems involving cAMP is the occurrence of a sharp peak in activity at a certain dosage with lesser activity at higher dosages (13). Vasopressin represents an extreme example where, at certain dose level, a shift from diuretic to antidiuretic effects is obtained (14).

It remains to be determined at which of several stages in antibody production (12) cAMP might be involved. Does it mediate signals in the first encounter between an immunogen and primary antigen-handling cells, which appear to be unique members of the macrophage population? Does it mediate signals produced by the inactivation of antigen with antigen-reactive stem-cells of lymphocyte?[2] Does it play a role in the antigen-dependent shift (15, 16) from "nonperforming" memory cells to "performing," *i.e.*, antibody-forming cells? Does it influence the level of performance of activated antibody forming cells? Or does it play a role at all these known cellular stages in antibody formation? Does cAMP mediate the signals that trigger, in sensitized lymphocytes, cell transformation and the formation of effector substances (17) that play a major role in phenomena of delayed hypersensitivity?[3] The answers to such questions should come from an analysis of cAMP effects on different cellular stages in the antibody response *in vitro,* from studies on the stimulating effects of cAMP when injected into animals at different times after antigen administration, from a determination of adenyl cyclase levels in membranes of stimulated and nonstimulated lymphocytes, and from a determination of cAMP levels in immunocompetent cells following the exposure of animals or tissues to known nonspecific stimulators, including synthetic polynucleotides (15), phytohemagglutinins (17-18), and calcitonine (19).

Recent data have shown that antibody formation can be enhanced or suppressed by alterations of Ca^{2+} levels *in vivo* (19) or *in vitro* (Ishizuka and Braun, unpublished data), the direction of such changes depending on the time of Ca^{2+} alterations in relation to the time of antigen administration. These observations may also be pertinent to any consideration of similarities between immunological and hormonal events since it is known that cAMP effects can be mimicked by an elevation of Ca^{2+} levels in several of the hormonal systems examined in this respect (20). Finally, the similarities between events in cellular systems involved in immune responses and in hormone-mediated responses are also indicated by the finding that an elevation of Ca^{2+} levels can lead to an enhanced transformation of thymocytes, *i.e.*, of cells participating in immune responses, and that the same enhancement can be achieved by several peptide hormones and minute amounts of cAMP (21, 22).

[2] Recent data showing that the administration of cAMP 24 hr after antigen produces more stimulation than cAMP given at time of antigen administration suggest that lymphocytes are particularly affected.

[3] Recent studies by Hirschhorn *et al.* (23) suggest that the answer to this question may be in the affirmative. They noted that low concentrations of cAMP (but not of adenosine or its derivatives) produced a stimulation of lymphocytes, as measured by uridine and thymidine incorporation, and suggest that cAMP might regulate a rearragement of the intracellular vacuolar system, similar to changes produced after phytohemagglutinin (PHA) stimulation. However, they also observed an as yet unexplainable inhibition of PHA stimulation by cAMP and other adenosine derivatives (R. Hirschhorn, J. Grossman, and G. Weissmann, in press).

Summary. Cyclic AMP enhances antibody formation *in vivo* and *in vitro* as judged by tests in which sheep red blood cells served as antigen and the effects were measured by determining the early rate of increases in the number of antibody-forming spleen cells of CFW mice. Furthermore, theophylline, a known stabilizer of cAMP, was found to enhance the stimulatory effects of poly A:U on antibody formation. These observations form the basis for a discussion of apparent parallels between immunological events and known events in hormone-controlled activations of cells.

Thanks for technical assistance are due to M.-J. Rega. Special thanks are due to Dr. K. Hirschhorn who first suggested to one of us (W. B.) that it may be interesting to determine the effect of cAMP on antibody formation.

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Received Jan. 28, 1970. P.S.E.B.M., 1970, Vol. 134.