

HUMAN T LYMPHOCYTE "E" ROSETTE FUNCTION

I. A. Process Modulated by Intracellular Cyclic AMP*

By FRANCIS V. CHISARI AND THOMAS S. EDGINGTON

(From the Department of Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037)

The adenylyl cyclase-cyclic 3',5' adenosine monophosphate (cyclic AMP) system is capable of modulating various aspects of humoral and cellular immune responses such as antigen-induced IgE-mediated basophil histamine release (1), lymphocyte-mediated cytolysis (2), complement-mediated cytolysis (3), and hemolytic plaque formation (4, 5) to mention only a few.

Sheep red blood cell ("E") rosette formation has been demonstrated to be a specific capacity of human T lymphocytes (6). This function is depressed in a number of disease states (7-10). It is dependent on the concentration of divalent cations (6) and can be inhibited by pretreatment of the lymphocytes with iodoacetate (6), trypsin (6), azide (11), antilymphocyte serum (11), and cytochalasin B (12).

In the present study we evaluated the effect of various pharmacologic agents which elevate intracellular cyclic AMP levels on human T lymphocyte E rosette function. The investigation of this system revealed that the receptor function of human T lymphocytes necessary to form rosettes with sheep red blood cells can be diminished by such agents.

Materials and Methods

Reagents. Cholera toxin was prepared by Dr. R. A. Finkelstein (University of Texas Southwestern Medical School) and was provided by Dr. Carl Miller, NIH. Isoproterenol, aminophylline, and dibutyryl cyclic AMP were purchased from Sigma Chemical Company, St. Louis, Mo.

Lymphocyte Culture. Peripheral blood lymphocytes were isolated from heparinized venous blood from normal adults by iron-dextran (Lymphocyte Separating Reagent, Technicon Instruments Corp., Tarrytown, N. Y.) sedimentation in a magnetic field followed by lysis of erythrocytes with 0.83% NH_4Cl . The leukocyte suspension was washed three times with RPMI-1640. Absolute yield of lymphocytes was greater than 75% with 99-100% viability (trypan blue) and greater than 90% purity.

Lymphocytes ($1.0 \times 10^6/\text{ml}$) were suspended in 2-ml aliquots in 20% fetal calf serum, RPMI-1640 supplemented with penicillin (100 U/ml), streptomycin (50 mg/ml) and L-glutamine (2 mM) containing various concentrations of the pharmacologic agents under study and incubated at 37°C in a humid atmosphere containing 5% CO_2 in air. The pH was maintained at 7.25-7.35 throughout. Experiments were performed in triplicate.

E Rosette Assay. After incubation with pharmacologic agents, cells were washed three times in Puck's saline G and adjusted to a concentration of 1×10^6 viable cells per ml (viability remained greater than 95% for all conditions and yield of cells at 48 h was 75-80%).

The assay was performed exactly as described by Jondal, Holm, and Wigzell (9). 400 lymphocytes were counted from each of the triplicate determinations. Lymphocytes were scored as positive if four or more SRBC were adherent and there was definite scalloping of the lymphocyte surface. The arithmetic mean and one standard deviation were calculated. Standard deviation was less than $\pm 5\%$. Results were expressed as percent inhibition relative to the percent E rosettes formed by the same

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lymphocytes cultured simultaneously under control condition (60-75% of total lymphocytes present).

Utilizing the E rosette technique in the study of 52 normal donors the percentage of total lymphocytes which form E rosettes ($72.0\% \pm 7.5$) agreed within 1.6% with the percentage of lymphocytes lacking surface immunoglobulin ($70.4\% \pm 5.4$). Surface immunoglobulin positive lymphocytes were determined by membrane immunofluorescence following staining of viable cells for 30 min. at 0°C with fluorescein-conjugated IgG fraction of a polyvalent antisera to IgG, IgA, IgM, IgD, kappa, and lambda prepared in a single goat. The fluorescein reagent was rendered monomeric by G-200 chromatography and was titrated to give a constant percentage of positive cells over a threefold reagent concentration as described by Pernis (13).

Results

Time-Course of Inhibition of E Rosette Function. The results of four representative experiments in which E rosette function was observed to decrease following incubation of lymphocytes with various pharmacologic agents are summarized in Fig. 1. The temporal features of E rosette inhibition were

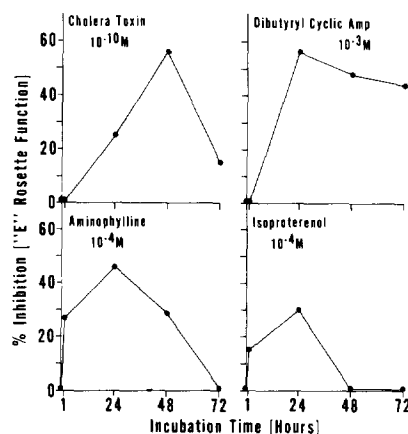


FIG. 1. Time-course of inhibitory effect of pharmacologic inhibitors of E rosette function.

characteristic and reproducible for each agent; but the absolute degree of inhibition observed with a given agent varied between donors and from day to day. Greater than 80% inhibition was never observed. None of the agents exerted a detectable effect on E rosette function within 10 min of incubation. Aminophylline and isoproterenol were decidedly inhibitory at 1 h; whereas cholera toxin and dibutyryl cyclic AMP had no effect at this time but displayed the onset of inhibitory activity (not shown) after 2 h in culture. Cholera toxin reproducibly exerted its maximal effect at 48 h whereas the other agents induced maximal inhibition, at 24 h. The relatively sustained inhibitory effect of exogenous dibutyryl cyclic AMP contrasts with the transient inhibitory effects of the other agents.

Dose-Response Relationships. In every experiment the degree of inhibition of E. rosette function was directly related to the dose of the agent employed. Table I summarizes the data from a representative experiment. Cholera toxin was the most potent inhibitor of the group by at least five orders of magnitude. Lymphocyte viability remained greater than 95% at the concentrations of agents

TABLE I
*Effect of Pharmacologic Inhibitors on E Rosette Formation as a
 Function of Dose*

Agent	Concentration	E rosettes	Inhibition*
	<i>M</i>	%	%
Cholera toxin‡	10 ⁻¹¹	71.5 ± 4.5	2
	10 ⁻¹⁰	54.0 ± 3.6	26
	10 ⁻⁹	37.5 ± 2.5	49
	10 ⁻⁸	40.7 ± 1.7	44
DiButyryl-cyclic AMP§	10 ⁻⁶	69.3 ± 1.7	0
	10 ⁻⁵	70.3 ± 2.5	0
	10 ⁻⁴	54.5 ± 2.7	22
	10 ⁻³	42.0 ± 3.0	40
Isoproterenol§	10 ⁻⁶	69.0 ± 3.0	0
	10 ⁻⁵	62.8 ± 2.8	10
	10 ⁻⁴	50.3 ± 2.3	37
	10 ⁻³	21.5 ± 2.5	68
Aminophylline§	10 ⁻⁶	70.0 ± 4.0	0
	10 ⁻⁵	71.8 ± 5.0	0
	10 ⁻⁴	53.8 ± 4.8	19
	10 ⁻³	40.3 ± 4.7	42

* % Inhibition: 100 - % E reagent/% E control.

‡ Assayed after 48 h in culture. Control = 73.3% ± 1.7.

§ Assayed after 24 h in culture. Control = 69.3% ± 4.8.

indicated in Table I. Higher concentrations could not be evaluated because of reduced lymphocyte viability (<90%).

Potentiating Effect of Aminophylline. The potentiation of an agent (cholera toxin, isoproterenol) by the phosphodiesterase inhibitor aminophylline has been accepted as good presumptive evidence that the agent acts by stimulating adenyl cyclase and also that the effect is mediated via the cyclic AMP system (14). We therefore cultivated lymphocytes in media containing both a noninhibiting dose of aminophylline and a suboptimal dose of each agent. A pronounced synergistic effect was obtained when these agents were administered simultaneously (Table II).

Discussion

The inhibition of E rosette function by the adenyl cyclase stimulators, cholera toxin and isoproterenol, and the phosphodiesterase inhibitor, aminophylline, suggests that cyclic AMP may participate in the regulation of this lymphocyte membrane function. This hypothesis is strengthened by the observation that exogenous dibutyryl cyclic AMP exerts a sustained inhibiting influence on E rosette formation. Finally, the potentiating effect of the phosphodiesterase inhibitor in the presence of either the adenyl cyclase stimulators or dibutyryl cyclic AMP further supports this interpretation.

The temporal course of E rosette inhibition induced by different agents is in

TABLE II
*Inhibition of E Rosette Formation: Potentiation of Drug Effects
 by Aminophylline**

Agent	Concentration	E rosettes	Inhibition‡
	<i>M</i>	%	%
Aminophylline	10 ⁻⁶	68.0 ± 1.0	0
Isoproterenol	10 ⁻⁴	53.3 ± 5.0	20
" + Aminophylline	10 ⁻⁶	41.3 ± 4.7	40
Cholera toxin	10 ⁻¹⁰	53.0 ± 2.0	22
" + Aminophylline	10 ⁻⁶	47.5 ± 1.5	34
Dibutyl cyclic AMP	10 ⁻⁴	55.8 ± 4.2	18
" + Aminophylline	10 ⁻⁶	43.8 ± 3.8	35

* All conditions assayed after 24 h in culture. Control = 67.8 ± 3.8

‡ % Inhibition: 100 - % E reagent/% E control.

keeping with the rate at which each agent induces measurable changes in intracellular cyclic AMP (15, 16). The relatively sustained inhibitory effect of exogenous dibutyl cyclic AMP at 48 and 72 h suggests that E rosette function may be susceptible to inhibition for as long as intracellular cyclic AMP levels remain elevated. Likewise, the transient inhibitory effect of the other agents is consistent with the transient elevations of intracellular cyclic AMP which they evoke (15, 16).

Since there is a significant delay between the time when cyclic AMP levels rise (15, 16) and E rosette function falls, it is likely that diminished E rosette function is a secondary or tertiary event mediated by changes in intracellular cyclic AMP. Increased intracellular cyclic AMP may decrease synthesis of a discrete receptor for sheep erythrocytes, decrease incorporation of this receptor into the plasma membrane, or lead to increased shedding of receptor from the lymphocyte surface. Precise evaluation of these hypotheses must await the isolation and purification of this putative receptor.

Summary

The capacity of normal human T lymphocytes to form rosettes with sheep red blood cells can be inhibited by drugs or agents which induce elevations in intracellular levels of cyclic AMP. The effect is early in the presence of agents which elicit rapid elevations in intracellular cyclic AMP (isoproterenol, aminophylline) and occurs later in the presence of cholera toxin which induces a delayed increase in endogenous cyclic AMP. Dibutyl cyclic AMP is inhibitory, and the effects of dibutyl cyclic AMP and the adenylyl cyclase stimulators are potentiated by inhibition of phosphodiesterase. These data provide substantial evidence that elevation of intracellular cyclic AMP diminishes E rosette function of lymphocytes.

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References

1. Lichtenstein, L. M., C. S. Henney, H. R. Bourne, and W. B. Greenough, III. 1973. Effects of cholera toxin on *in vitro* models of immediate and delayed hypersensitivity. *J. Clin. Invest.* **52**:691.
2. Henney, C. S., H. R. Bourne, and L. M. Lichtenstein. 1972. The role of cyclic 3',5' adenosine monophosphate in the specific cytolytic activity of lymphocytes. *J. Immunol.* **108**:1526.
3. Kaliner, M., and K. F. Austen. 1973. Adenosine 3',5' monophosphate: inhibition of complement-mediated cytotoxicity. *Science (Wash. D. C.)*. **183**:659.
4. Melmon, K. L., H. R. Bourne, Y. Weinstein, G. M. Shearer, J. Kram, and S. Bauminger. 1974. Hemolytic plaque formation by leukocytes *in vitro*: control by vasoactive hormones. *J. Clin. Invest.* **53**:13.
5. Chisari, F. V., R. S. Northrup, and L. C. Chen. The modulating effect of cholera enterotoxin on the immune response. *J. Immunol.* In press.
6. Jondal, M., G. Holm, and H. Wigzell. 1972. Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. *J. Exp. Med.* **136**:207.
7. Wybran, J., and H. H. Fudenberg. 1973. Thymus-derived rosette-forming cells in various human disease states: cancer, lymphoma, bacterial and viral infections, and other disease states. *J. Clin. Invest.* **52**:1026.
8. DeHoratius, R. J., R. G. Strickland, and R. C. Williams, Jr. 1974. T and B lymphocytes in acute and chronic hepatitis. *Clin. Immunol. Immunopathol.* **2**:353.
9. Messner, R. P., F. D. Lindström, and R. C. Williams, Jr. 1973. Peripheral blood lymphocyte cell surface markers during the course of systemic lupus erythematosus. *J. Clin. Invest.* **52**:3046.
10. Aiuti, F., V. Lacava, J. A. Garofalo, R. D'Amelio, and C. D'Asero. 1973. Surface markers on human lymphocytes: studies of normal subjects and of patients with primary immunodeficiencies. *Clin. Exp. Immunol.* **15**:43.
11. Bentwich, Z., S. D. Douglas, F. P. Siegal, and H. G. Kunkel. 1973. Human lymphocyte—sheep erythrocyte rosette formation: Some characteristics of the interaction. *Clin. Immunol. Immunopathol.* **1**:511.
12. Kersey, J. H., D. J. Hom, and P. Buttrick. 1974. Human T lymphocyte receptors for sheep erythrocytes: conditions for binding including inhibition by cytochalasin B. *J. Immunol.* **112**:862.
13. Pernis, B., L. Forni, and L. Amante. 1970. Immunoglobulin spots on the surface of rabbit lymphocytes. *J. Exp. Med.* **132**:1001.
14. Robison, G. A., R. W. Butcher, and E. W. Sutherland. 1971. Cyclic AMP and hormone actions. *In Cyclic AMP*. Academic Press, Inc., New York 41.
15. Bourne, H. R., R. I. Lehrer, L. M. Lichtenstein, G. Weissman, and R. Zurier. 1973. Effects of cholera enterotoxin on adenosine 3',5' monophosphate and neutrophil function: comparison with other compounds which stimulate leukocyte adenyl cyclase. *J. Clin. Invest.* **52**:698.
16. Zurier, R. B., G. Weissmann, S. Hoffstein, S. Kammerman, and H. H. Tai. 1974. Mechanisms of lysosomal enzyme release from human leukocytes. II. Effects of cAMP and cGMP, autonomic agonists and agents which affect microtubule function. *J. Clin. Invest.* **53**:297.