GENETICS

Relationship between Intracellular cAMP Level in Intact Splenocytes and Population Composition of Cell Suspension and Cycloxygenase Activity

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A maximum increment in cAMP was detected by radioimmunoassay on the 30th min of *in vitro* incubation of mouse splenocytes in nonfractionated suspension, while in fractionated T or B cells cultures the level of cAMP was the lowest. Removal of A cells from suspension or blocking of endogenous eicosanoid production alters the cAMP curve approximating it to distribution in fractionated lymphocytes. Therefore, splenic macrophages are the main regulators of intracellular cAMP level in intact splenocyte suspension, realizing their effect through cycloxygenase products.

Key Words: *splenocytes; fractionated lymphocytes; A cells; cAMP; cycloxygenase*

The adenylate cyclase system is the best studied pathway of intracellular information transfer. 3',5'-cyclic adenosine monophosphate (cAMP) catalyzed by adenylate cyclase exerts numerous effects on functional activity of lymphoid cells [12]. A high level of intracellular cAMP decreases proliferative and functional activities of T cells [7,12]. However, its increase at the early stage of B cell activation directly stimulates proliferation and antibody production [5]. Macrophages as the chief producers of prostaglandins E_2 (PGE_2) and key cytokines, such as interleukin-1 [2], contribute to immunomodulation processes at the level of the adenylate cyclase system [13]. A combination of interleukin-1 and dibutyril-cAMP activates the induction of antibody-producing cells in *nude* mice [7], although when used alone, these agents cannot modify the production of immunoglobulins in a studied system. By increasing the level of cAMP, PGE_2 stimulates the proliferation and cytokinase-dependent differentiation of CD40-activated B lymphocytes [6]. On the other hand, PGE_2 inhibits many functions of lymphoid cells, such as the effects of cytotoxic T lymphocytes and natural killer cells or mitogen-induced proliferation [8].

Therefore, the level of cAMP determines virtually the entire spectrum of cell-mediated and humoral immune reactions, and lymphocyte capacity to realize their functional activity depends on the type of cells responding by intracellular changes in the concentration of this second messenger and on the time of this response. We studied the dynamic profiles of intracellular cAMP in intact splenocytes and the contribution of individual populations to its formation.

MATERIALS AND METHODS

Experiments were carried out on a macroculture of splenocytes of adult intact CBA and (CBA×C57Bl/6) F_1 mice weighing 18-22 g bred at the Rappolovo Breeding Center of the Russian Academy of Medical Sciences.

Intact splenic cells and individual fractions were incubated for 1 h at 37°C. Splenocytes were trans-

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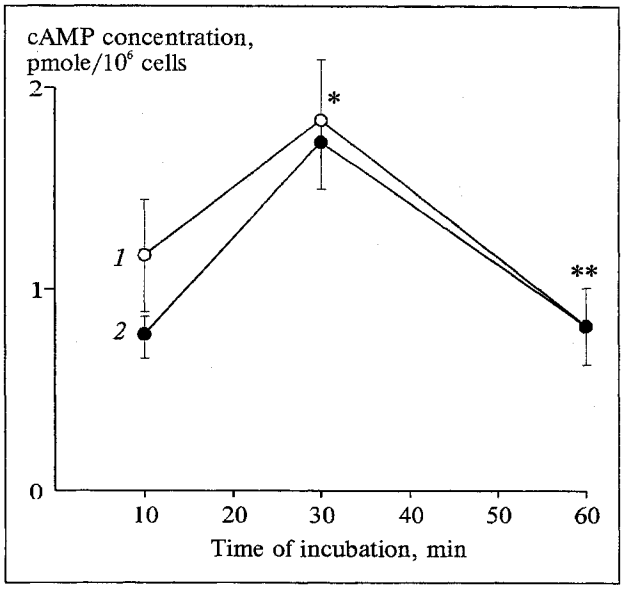

Fig. 1. Dynamic profile of cellular level of cAMP in native suspensions of intact splenocytes (2 experiments). *p0.001 *vs.* 10th and 60th min, **p<0.01 *vs.* 30th min.

ferred to culture flasks with medium 199 in a concentration of 5×10^6 nuclear cells/ml. The volume of each culture was 4 ml. Cells were collected for examination on the 10th, 30th, and 60th min of culturing.

T lymphocytes were isolated by filtering splenic suspension through a Nylon mesh (Nitron) [10]. The eluate contained 70-75% T lymphocytes and 1-6% B lymphocytes. B lymphocytes were isolated by the complement-dependent mass cytolysis test [9]. Monoclonal antibodies to murine Thy 1.2 antigens (Dia-M) were used as cytotoxic sera. Testing of fractionated suspension showed 60-80% splenocytes to be EAC-rosette forming cells (B lymphocytes) and 0-2% to carry Thy 1.2 markers (T cells).

Macrophages were removed from splenocyte suspension by negative selection (active adhesion of macrophages to glass surface, A cells) [4].

Table 1. Levels of Cellular cAMP at the 30th Minute of Incubation of Intact and Fractionated Splenocytes ($M\pm m$)

Cells	Number of specimens	cAMP level, pmole/10^6 cells
Splenocytes:		
intact (control)	12	1.73±0.08
without A cells	15	0.34±0.11*
with cycloxygenase inhibitor	12	0.69±0.10*
Splenic lymphocytes:		
T	12	0.97±0.12*
B	13	1.02±0.09*

Note. *p<0.001 *vs.* the control.

Cell viability assessed in the Trypan Blue test was 98%.

The level of intracellular cAMP in T or B cell populations and in nonfractionated splenocyte suspension and suspension from which macrophages were removed was radioimmunoassayed using standard KC-ACP-N-3 kit (Izotop). The measurements were carried out at the 10th, 30th, and 60th min of cul-turing. For this, theophyllin, a phosphodiesterase inhibitor (3 mM) was added to studied lymphocyte macrocultures after incubation, after which the cells were precipitated by centrifuging (300 rpm, 15 min), and the precipitate was resuspended in 1 ml 6% $HClO_4$ and left overnight at 4°C for more complete extraction of nucleotides. After deproteinization and neutralization with KOH, perchlorate extracts were diluted to a final volume of 2 ml (2×10^7 cells), in which cyclic nucleotides were measured. Radioactivity was measured in a Beta-2 counter and a ZhS-7 dioxane scintillator.

The cycloxygenase inhibitor sodium diclophenak (Pliva) in a concentration of 0.015 mg/ml [11] was added to splenocyte macroculture at the beginning of incubation.

Results were processed using Student's t test [3].

RESULTS

Two curves in Fig. 1 show the reproducibility of results of studies of cAMP concentrations in a native splenocyte suspension during 1 h. Under standard conditions of culturing, splenic immunocompetent cells possess a characteristic cAMP profile with the peak on the 30th min. The results are statistically significant in comparison with the 10th and 60th min of incubation (Fig. 1).

Removal of A cells from native splenocyte suspension by active adhesion onto glass surface modifies the curve, reflecting the accumulation of intracellular cAMP. cAMP level during the 30th min is the minimal, but not the maximum, and its values change significantly in comparison with the 10th or 60th min of incubation (Fig. 2). Splenocytes exposed to cycloxygenase inhibitor also change the pattern of cAMP accumulation and repeat the profile of the curve representing cultures without A cells.

T and B lymphocytes constitute 90-95% of splenic cells, and therefore we evaluated the profiles of intracellular cAMP for individual lymphocyte populations of an intact organ. Figure 2 shows that the level of cAMP is low in T and B lymphocytes only during the 30th min of incubation. Analysis of changes in cAMP level showed that the main shifts caused by activity of splenic macrophages are formed by this time. The decrease in intracellular cAMP on the 30th

min of incubation in suspensions without macrophages or in those blocked with sodium diclophenak and in suspensions of fractionated T or B lymphocytes is statistically significant in comparison with intact splenocytes (Table 1).

Time fluctuations in intracellular cAMP level in intact splenocytes are regulated by A cell cycloxygenase products, and their removal or macrophagal blocking of eicosanoid production changes the level of cAMP and, presumably, functional activity of T and B cells in intact spleen. The mechanism determining cAMP increase on the 30th min of incubation directly depends on cycloxygenase activity and apparently on the level of PGE_2, the main transmitter of eicosanoid macrophages [2]. These results agree with numerous published reports about PGE_2 capacity to activate adenylate cyclase [6,13].

Thus, intact splenic macrophages regulate the cell-to-cell noncognant interactions and modulate cAMP production in a short-term macroculture. Studies of cell composition of secondary immunocompetent organs forming the immune response will show us the true picture of normal key second messengers, such as cAMP. It is particularly important for studies of mechanisms of immunomodulating effects of hormones, autocoids, and cytokines, because the results of experiments on fractionated cells alone are formal and do not reflect the physiology of cell-to-cell relations.

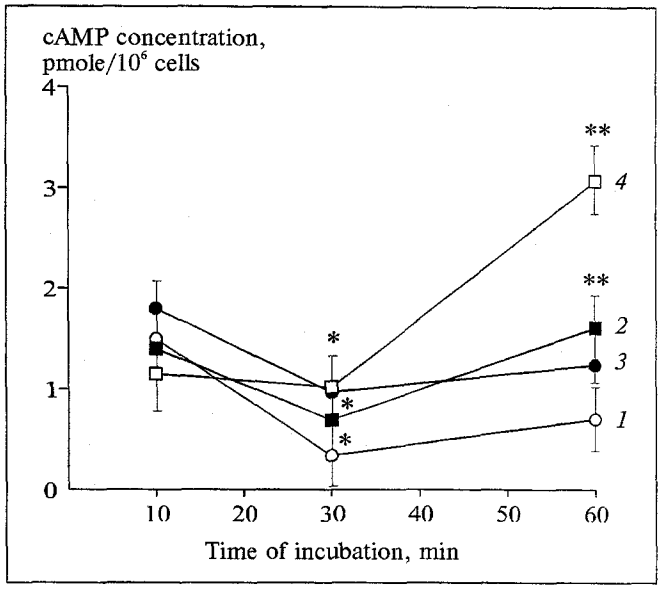

Fig. 2. Dynamic profile of cellular cAMP level in suspensions of fractionated splenocytes and cells treated with cycloxygenase inhibitor. *1*) splenocytes lacking A cells; *2*) splenocytes with cycloxygenase inhibitor; *3*) T lymphocytes eluted from Nylon fibers; *4*) B lymphocytes after immune cytolysis of T cells. $p<0.001$: *vs. 10th, **vs. 30th min.

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