

Dialyzable Leukocyte Extract Stimulates cAMP in T γ Lymphocytes

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Dialyzable leukocyte extract (DLE) from human blood leukocytes gave a 3- to 8-fold increase in the cAMP concentration after addition to purified lymphocytes. No significant change in cGMP was observed. When purified lymphocytes were separated into T cells and non-T cells by an E-AET rosette technique the cAMP response was most pronounced in T cells. When further separating T lymphocytes into T cells with Fc receptors for IgG and T cells with Fc receptors for IgM (T γ and T μ lymphocytes), the increase in cAMP following stimulation was specifically conferred to T γ lymphocytes. Our findings may support the concept that DLE activates T γ lymphocytes, leading to an increase in suppressor cell function.

Key words: cyclic AMP; cyclic GMP; monocytes; T lymphocyte subpopulations; transfer factor.

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CLINICAL ASPECTS

The present *in vitro* study demonstrated that dialyzable leukocyte extract (DLE), which contains transfer factor, can induce a significant increase in cAMP in a subpopulation of T lymphocytes with Fc receptors for IgG (T-gamma lymphocytes). There is evidence to suggest that T-gamma lymphocytes may function as suppressor cells *in vitro*.

DLE is clinically often used to increase a reduced cell-mediated immune response (cutaneous type IV reactivity). The present findings may suggest that DLE triggers a certain subpopulation of cells. Future research on T-gamma cell function during DLE therapy may give information concerning the eventual *in vivo* effect of DLE therapy.

Dialyzable leukocyte extract (DLE) from human blood leukocytes that among other substances contains transfer factor can transfer or augment delayed hypersensitivity reactions in the skin (10). DLE therapy in patients with evidence of depressed cell-mediated immune reactivity have shown promising clinical effects (7, 19, 20, 23, 25, 26). However, the chemical constitution of transfer factor in DLE and its mechanism of action is still unresolved.

Sandler et al. (16) have shown that DLE *in vitro* provokes a marked increase in cGMP, preferentially in blood monocytes, but an increased *in vitro* reactivity of monocytes has not been reported. The same authors did not see

any significant changes in lymphocyte cyclic nucleotides following incubation with DLE.

The clinical observation of increased skin reactivity and lymphokine production after DLE therapy (7, 11), and augmentation of DNA synthesis following *in vivo* (23) and *in vitro* (1) administration possibly reflect that DLE modulates the lymphocyte cyclic nucleotide system. Therefore we have been interested in looking at DLE and its influence *in vitro* on cyclic nucleotides in lymphocytes and subpopulations of these. Briefly, our results demonstrate that DLE induces a significant increase in the concentration of cAMP in T lymphocytes with Fc receptors for IgG (T γ), whereas no

effect is observed in T lymphocytes with Fc receptors for IgM (T μ).

MATERIAL AND METHODS

Purification of monocytes and lymphocyte subpopulations

Heparinized venous blood was drawn from healthy staff members, and mononuclear leukocytes were separated on Ficoll-Hypaque gradients. Monocytes were purified as earlier described (8). The monocyte suspension contained between 89% and 95% monocytes, as judged by non-specific esterase activity, and a mean of $7.4\% \pm 1.2\%$ T lymphocytes ($n = 6$).

In the non-adhering cell population from the plastic culture flasks $76.3\% \pm 3.1\%$ lymphocytes ($n = 6$) formed rosettes with 2-aminoethyl-isothiuronium bromide (AET)-treated sheep erythrocytes. The E-AET rosette-forming cells (T lymphocytes) were isolated on Ficoll-Hypaque gradients. T lymphocytes were allowed to make rosettes with ox-erythrocytes coated with either anti-ox-IgG (EA-IgG) or anti-ox-IgM (EA-IgM) thus enumerating the T γ and T μ subpopulations as described in detail elsewhere (5). Of the T lymphocytes $16.0\% \pm 1.5\%$ ($n = 5$) were T γ lymphocytes and $14.2\% \pm 3.8\%$ ($n = 5$) were T μ lymphocytes.

Purification of T γ and T μ was done by mixing 20 ml of T lymphocytes (2×10^6 /ml), which had been incubated in RPMI 1640 at 37°C for 60 min, with either 20 ml of EA-IgG or 20 ml of EA-IgM. The mixture was spun at 200 g for 5 min followed by incubation at 4°C for 60 min. The pellets were gently resuspended, layered on Ficoll-Hypaque and centrifuged at 800 g for 15 min. EA, which bound to either T γ or T μ cells, were lysed by incubation in 0.83% Tris-NH $_4$ Cl for 10 min. The T-T γ cell population was depleted for T γ cells as only $0.2\% \pm 0.2\%$ ($n = 5$) made rosettes with EA-IgG. In the one experiment in Fig. 4 only 2.5% of the T-T μ cell population made rosettes with EA-IgM. T γ , T μ and non-rosetting cells were washed three times in Hanks' balanced salt solution (HBSS) with 2.5% fetal calf serum (FCS). The separation and

assay procedures were performed on the same day as blood was drawn.

Preparation of dialyzable leukocyte extract (transfer factor)

Buffy-coat cells were prepared from 450 ml of blood drawn from each of eight to ten healthy, PPD-positive donors. Erythrocytes were lysed during incubation with 0.83% Tris-NH $_4$ Cl for 10 min. The leukocytes were washed twice in HBSS and counted. On average, 0.8 to 1.5×10^9 cells were obtained from each donor, 40 to 60% being granulocytes and the rest mononuclear cells. Cells from all donors were pooled and stored at -20°C . Then they were subjected to 10 cycles of freeze-thawing followed by ultrasonication for 60 sec. The disrupted leukocyte suspension was filtered through Amicon UM10 filters. The filtrate contained molecules smaller than 10,000 daltons. After a further filtration through a $0.22 \mu\text{m}$ pore-size filter, the DLE preparation was stored at -20°C until use. One unit of DLE was defined as the volume of extract from 1×10^9 leukocytes.

Determination of cAMP and cGMP

Leukocyte content of cyclic nucleotides was determined as previously described (3) using reagents from Radiochemical Centre, Amersham, England. All cell populations were resuspended in HBSS with 2.5% FCS adjusted to 15×10^6 /ml and incubated at 37°C with DLE in a final concentration of 0.025 units/ml.

RESULTS

The concentration of cyclic nucleotides in purified lymphocyte populations was followed for 2 h after addition of eight different batches of DLE. The initial cAMP level was 30.7 ± 1.6 pmol/ 10^7 cells. DLE caused a substantial increase in cAMP, lasting for 30 min and followed by a gradual decline (Fig. 1). Concomitantly, cGMP increased from 2.40 ± 0.36 pmol/ 10^7 cells to 3.75 ± 0.79 pmol after 30 min (not statistically significant; Mann-Whitney's test).

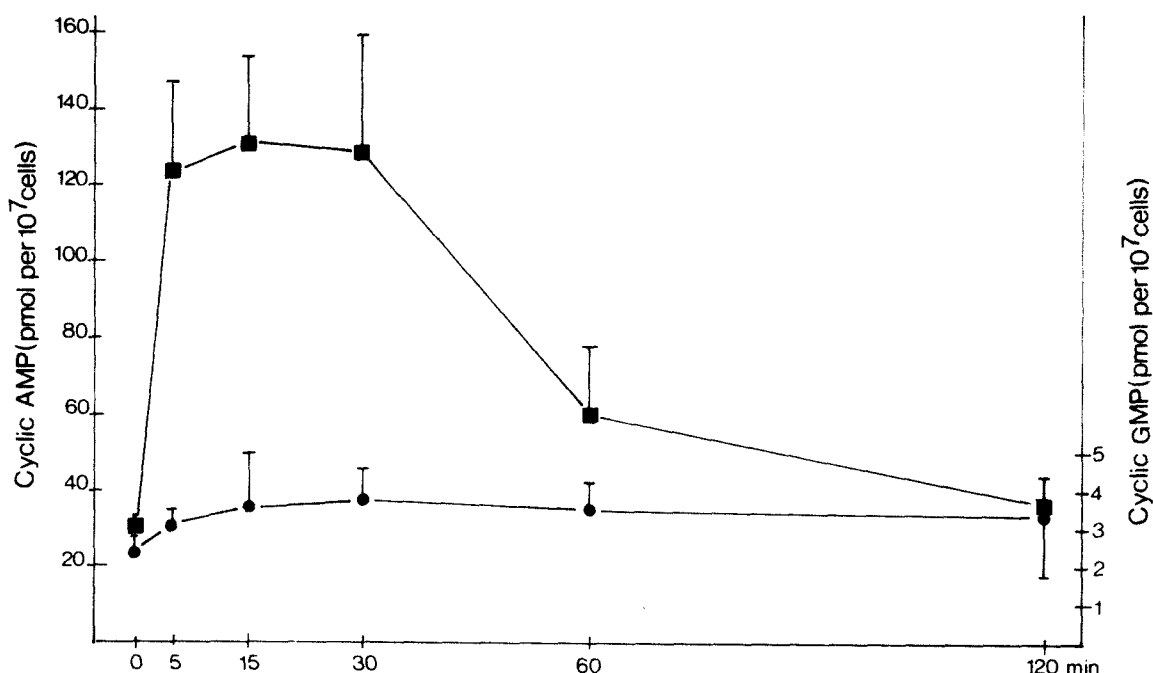


Fig. 1. Changes in cyclic nucleotides in purified lymphocytes ($76.3\% \pm 3.1\%$ being T lymphocytes) after addition of 0.025 IU/ml DLE at 0 min. Values are obtained from eight separate experiments and are expressed as mean $\pm$ SEM. Square-line: cAMP, dot-line: cGMP.

After incubation of lymphocytes with DLE for 24 and 48 h at 37°C the cAMP concentration was 14.23 ± 1.74 and 10.9 ± 1.59 pmol (four experiments), and without DLE 9.16 ± 1.06 and 10.5 ± 0.84 pmol, respectively. The concentration of cGMP did not change in the lymphocytes (results not shown).

The effect of DLE was found to be concentration-dependent. Thus, decreasing amount of DLE gave less increases in cAMP (Fig. 2).

In an attempt to identify the DLE responding cell, DLE was added to monocytes, lymphocytes, non-T lymphocytes, T lymphocytes, Ty lymphocytes and non-Ty lymphocytes. All studies were done on the same day as blood was drawn from different donors. The same batch of DLE was used in these experiments.

The effect of DLE on the cAMP concentrations in these cell populations is shown in Fig. 3. Lymphocytes and purified T lymphocytes showed nearly identical increases in cAMP, whereas non-T lymphocytes and monocytes only had a modest increase. When separating purified T cells into Ty and non-Ty lymphocytes, the increase in cAMP was found only in the Ty cell population.

In one of two similar experiments with posi-

tively selected T_{μ} lymphocytes cAMP did not increase, whereas the cAMP response in non- T_{μ} cells was high (Fig. 4), comparable to the response in Ty cells. This indicates that the separation procedure does not affect the qualitative response of cAMP in the cells.

Fig. 5 illustrates the effect of DLE on cGMP concentrations in the cell populations obtained from the same donors as in Fig. 3. DLE gave the largest increase in cGMP in monocytes, 78% above basal level, but due to a rather large variability this increase was not statistically significant. The cGMP concentrations in subpopulations of lymphocytes did not change significantly following DLE stimulation.

DISCUSSION

All our preparations of DLE were able to stimulate the formation of cAMP, but not cGMP, in human blood lymphocytes. The increase in cAMP lasted for 30 min and was followed by a gradual decline. No decreased cAMP to cGMP ratio was observed. This is in contrast to Kalmár et al. (6), who found a short-lasting increase in cAMP, but a decrease in the cAMP to cGMP ratio after 10 min incubation of lymphocytes

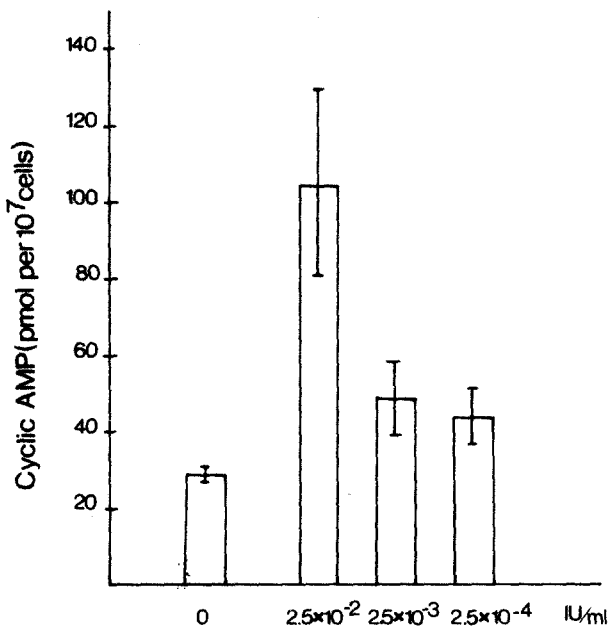


Fig. 2. Changes in cAMP in purified lymphocytes (as in Fig. 1) 5 min after addition of different amounts of DLE. Bars indicate mean $\pm$ SEM obtained from four experiments.

and DLE. Sandler et al. (16) found a significant increase in the cGMP concentration following DLE stimulation *in vitro*, but only in monocytes. This is comparable with our results although our increase in the cGMP concentration in monocytes was not statistically significant. The same authors saw a small increase in leukocyte cAMP from 68 to 108 pmol/10⁷ cells after 5 min of DLE incubation, but they did not emphasize their observation.

The cAMP response seen with our DLE preparation was most pronounced in T lymphocytes and almost exclusively in T γ cells. A smaller increase in cAMP was also seen in non-T cells and in monocytes, but T cells were present in these cell suspensions (mean values 6.2% and 7.4%, respectively).

The pronounced effect of DLE of T γ lymphocytes may be of importance for the ex-

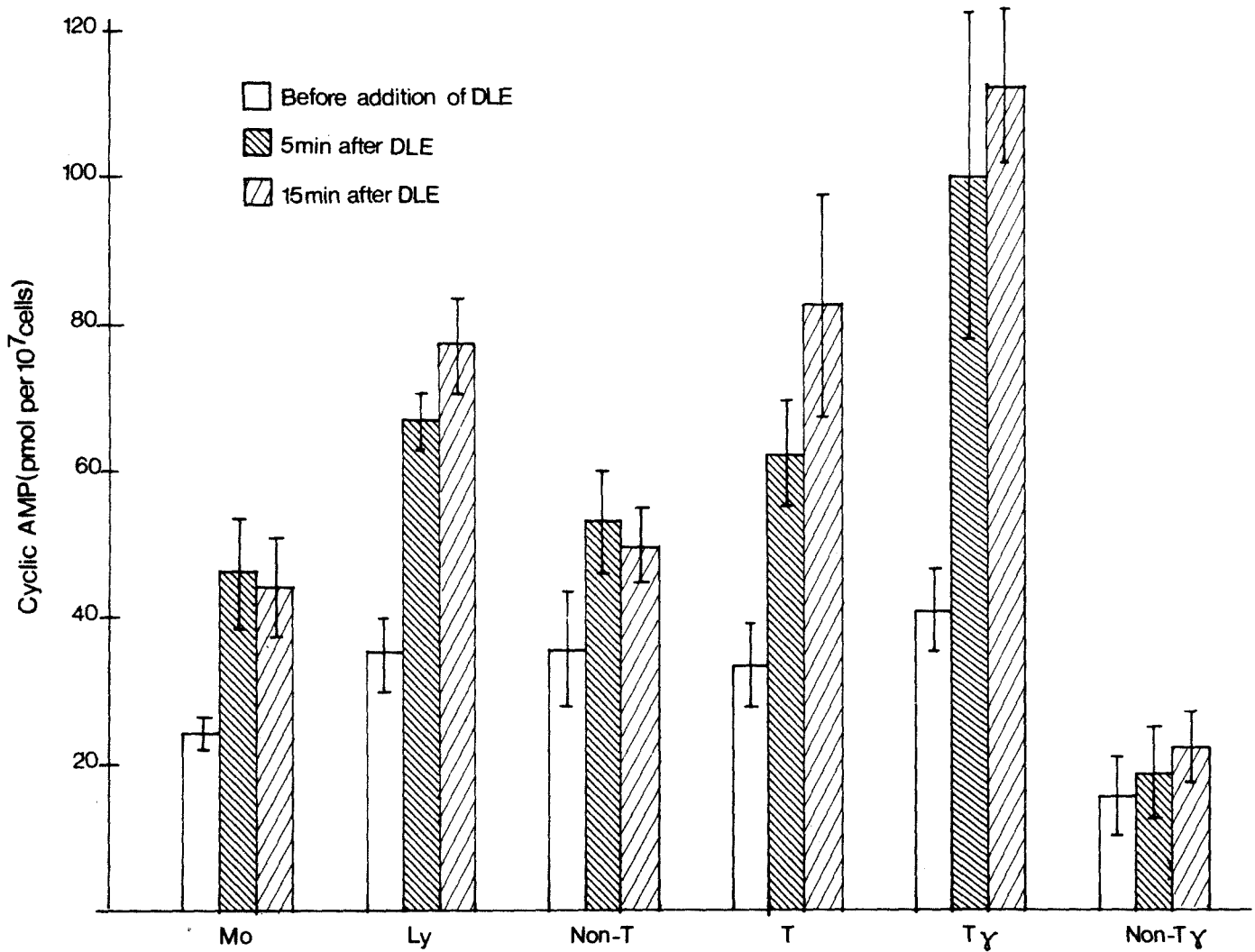


Fig. 3. Cyclic AMP measured after addition of 0.025 IU/ml DLE from the same batch to purified monocytes, lymphocytes, and subpopulations of lymphocytes (see text). Bars indicate mean $\pm$ SEM obtained from five experiments.

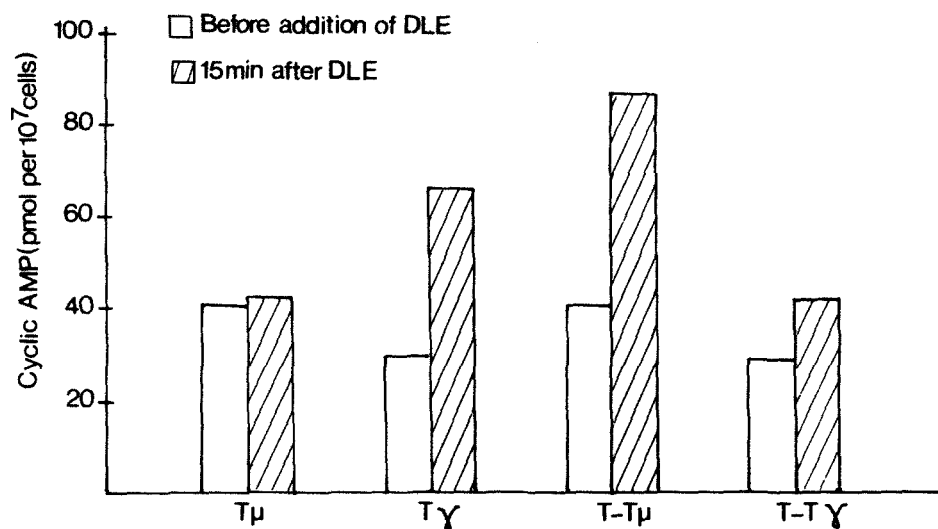


Fig. 4. Cyclic AMP measured 15 min after addition of 0.025 IU/ml DLE to purified T_γ , T_μ , and non-rosetting T lymphocytes. (Results from one representative experiment is given).

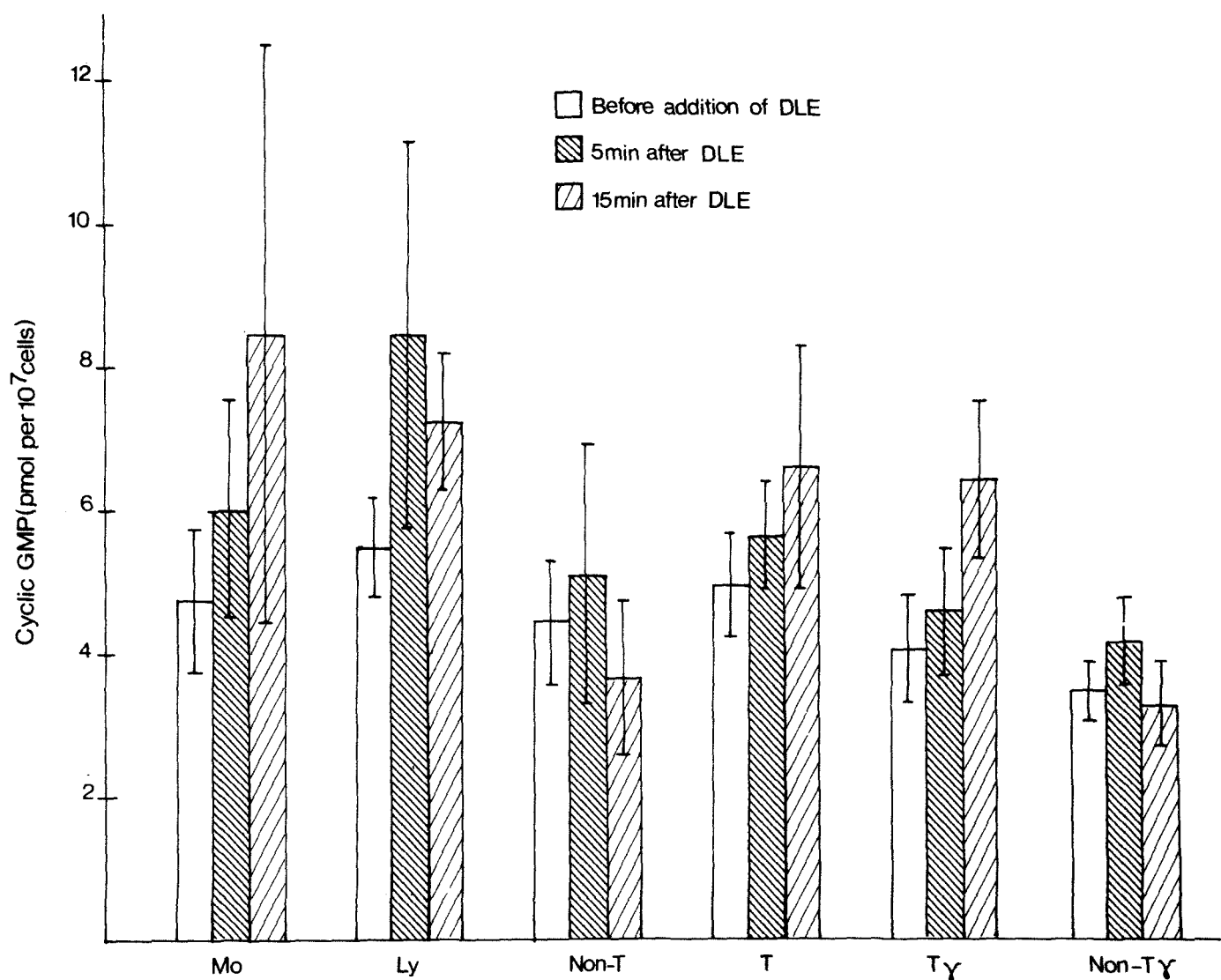


Fig. 5. Cyclic GMP measured after addition of 0.025 IU/ml DLE to purified monocytes, lymphocytes and subpopulations of lymphocytes obtained from the same donors as in Fig. 3 (see text). Bars indicate mean $\pm$ SEM obtained from five experiments.

planation of its clinical effects. T lymphocytes with Fc receptors for IgG are believed to express suppressor T cell functions (12). Suppressor T cells have regulatory functions for reaginic antibody production (22). Thus, long-term DLE therapy to patients with mycosis fungoides was paralleled by a significant fall in the concentration of IgE in serum and an increase in percentage of T lymphocytes in peripheral blood (25). In one patient with severe atopic dermatitis, high IgE level and low T cell percentage reversed following DLE administration (20). It is possible that the changes in IgE and T cells percentage are a result of the clinical improvement in these patients. However, it has recently been shown by Jarisch et al. (4) that daily DLE injections to patients with atopic dermatitis resulted in a prompt fall in serum IgE concentrations and they suggested an increased suppression of IgE production. However, the action of DLE on suppressor T cell activity was not investigated.

Although it is generally accepted that agents that increase cAMP depress various immunologic functions whereas increased cGMP enhances them (14, 15, 18, 21) the role of cAMP in the immune response remains controversial (13). Remarkably, the mitogen PHA which activates a large proportion of the lymphocyte population initially increases lymphocyte cAMP (17), comparably to our findings with DLE, whereas the PHA-induced blastogenesis can be inhibited or activated by cAMP dependent on the PHA concentration used (24). Therefore, like PHA, an increase in cellular cAMP by DLE may be the first signal of metabolic activation of the responding cell.

It has been claimed that DLE is able to transfer specifically the immune reactivity of the donor to the recipient (10). Our results indicate a non-specific activity in the DLE preparations. Dialyzable leukocyte extract is a mixture of many moieties (9) and at the present time we do not know which particular substance is responsible for the observed effect on cAMP on T γ lymphocytes. It is intriguing that a substance of low molecular weight is able to affect a subpopulation of T lymphocytes in such a pronounced way. The observation adds new in-

formation about differences between T γ and T μ lymphocytes.

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