EFFECT OF THYMOSINE ON HUMAN LYMPHOCYTE SUBPOPULATIONS IN VITRO

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During incubation of peripheral blood lymphocytes from healthy blood donors with thymosine there is an increase in the number of high-avidity "active" lymphocytes with receptors for sheep's red blood cells and of lymphocytes with receptors for the C'3-component of complement and for the FC-fragment of immunoglobulins; the proportion of cap-forming immunoglobulin-positive lymphocytes also increases. The role of T- and B-lymphocyte subpopulations and of their precursors as target cells for the action of thymosine is discussed.

KEY WORDS: Human lymphocyte; lymphocyte subpopulations; thymosine.

Numerous investigations in vivo and in vitro have shown that most biologically active substances of the thymus and, in particular, thymosine induce the expression of T-lymphocyte markers and potentiate immunologic reactions mediated through or regulated by T-cells [4, 12, 13]. Extracts of thymus containing thymosine, of different degrees of purity, have been widely applied for the identification of immature precursors of T-cells among human peripheral blood lymphocytes [9, 12, 13]. In certain immunopathological states incubation of lymphocytes with thymosine has been shown to cause an increase in the fraction of T-lymphocytes because of a decrease in the number of "zero" lymphocytes [9]. However, the action of thymosine and of extracts of the thymus on expressions of markers characteristic both of human T- and B-lymphocytes simultaneously, and the effect of thymus extracts on differentiation of precursors of B-lymphocytes have not yet been adequately investigated. The possibility of the latter was demonstrated in experiments with mouse lymphocytes [8].

The object of the present investigation was to study the action of thymosine (fraction IV of calf thymus extract) in vitro on expression of markers for T- and B-lymphocytes.

EXPERIMENTAL METHOD

Mononuclear cells from the peripheral blood of healthy donors, separated in a Ficoll-Verografin density gradient [1], were incubated (5×10^6 cells to 1 ml) for 90 min at 37°C with 300 μg/ml of thymosine (fraction IV of calf thymus extract [5]) or without thymosine in medium containing 90% Eagle's medium and 10% pooled, inactivated group IV (AB) serum. After incubation and a single washing, the number of different types of rosette-forming cells (RFC) – lymphocytes with receptors for sheep's erythrocytes (E-RFC) [6], "active" E-RFC [15], cells with receptors for the C'3-component of complement (EAC-RFC) [3], and for the FC-fragment of immunoglobulin (EA-RFC) [2] – was determined. During counting, depending on the number of corresponding test erythrocytes adherent to lymphocytes, groups of RFC of low avidity (with 1 or 2 erythrocytes), of average avidity (with 3 to 10 erythrocytes), and of high avidity (with more than 10 erythrocytes) were distinguished. Lymphocytes with more than three erythrocytes were regarded as true RFC. The number of "zero" cells was determined by the formula: percent of "zero" cells = 100% – (% E-RFC + % EAC-RFC). The action of thymosine was assessed quantitatively through an index of sensitivity (IS): $IS = (A-B)/B$, where A is the percentage of lymphocytes of a given type of RFC after incubation with thymosine and B the percentage of the same type of RFC after incubation without thymosine.

The number of lymphocytes with built-in immunoglobulins in their membrane was determined by the method of Lobo and Horvitz [5].

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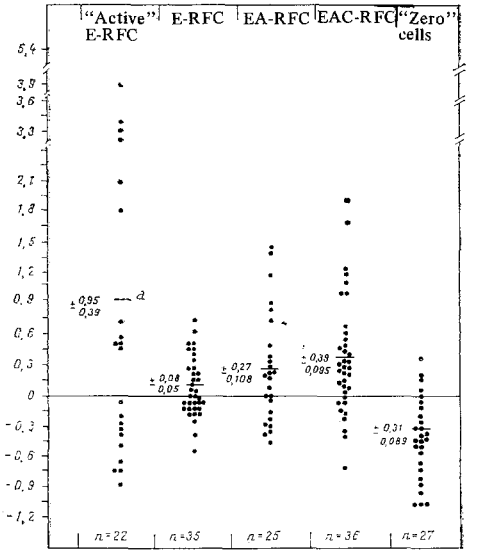

Fig. 1. Individual differences in sensitivity of lymphocyte subpopulations to thymosine: a) mean values of index of sensitivity for corresponding RFC. Ordinate, values of IS.

TABLE 1. Effect of Thymosine on Relative Percentages of Human Lymphocyte Population in Vitro

Suspension of mononuclear cells tested	Numbers of different types of RFC (X ± m, %)				
	"Active" E-RFC	E-RFC	EA-RFC	EAC-RFC	"Zero" cells
Control 1 (intact cells)	11,3±2,34	48,5±2,02	28,7±2,91	20,1±1,70	28,9±2,95
Control 2 (after incubation without thymosine)	9,9±1,50	50,1±2,42	28,8±2,07	20,3±1,65	31,0±2,55
Experiment (after incubation with thymosine)	11,9±2,46	51,6±2,20	34,8±2,88	26,0±1,90	23,1±3,02
P	>0,05	>0,05	<0,02	<0,001	<0,01
n	22	35	25	36	27

Legend. P) significance of differences by Student's paired t-test between experiment and control 2. Differences between control 1 and control 2 are not significant. n) Number of observations.

EXPERIMENTAL RESULTS

The effect of thymus extract on the lymphocyte subpopulations was marked by considerable variation in the individual sensitivity of the cells from different donors (Fig. 1). The greatest heterogeneity between lymphocytes from donors was observed in the sensitivity of "active" E-RFC to thymus extract, the least in the sensitivity of plain E-RFC.

Comparison of the results of statistical analysis of the data for percentages of lymphocytes in cell suspensions incubated with or without thymosine (Table 1) shows that, in the group of donors investigated, thymosine significantly increased the numbers of EA-RFC and EAC-RFC and reduced the number of "zero" cells. No statistically significant differences were found between the numbers of E-RFC and "active" E-RFC between the control and experiment. However, as is clear from Fig. 2, thymosine considerably increased the number of high-avidity RFC among the "active" E-RFC. No changes were found in the avidity of the cells among E-RFC under the influence of thymosine. The increase in the number of EAC-RFC was accompanied by an increase in the number of RFC with high and average avidity. The number of the EA-RFC subpopulation was increased by thymosine almost entirely on account of high-avidity rosettes; under these circumstances the number of low-avidity rosettes showed a corresponding statistically significant decrease. The results illustrated in Fig. 3 demonstrate that thymosine did not change the number of immunoglobulin-positive cells, in agreement with observations made by other workers [14], but the number of cap-forming lymphocytes increased statistically significantly. The results described above for the change in avidity of "active" E-RFC confirm previous findings [14] on induction of expression of markers of mature human T-lymphocytes in vitro by thymosine.

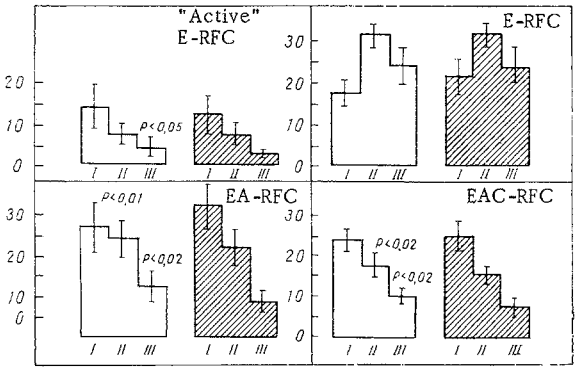

Fig. 2

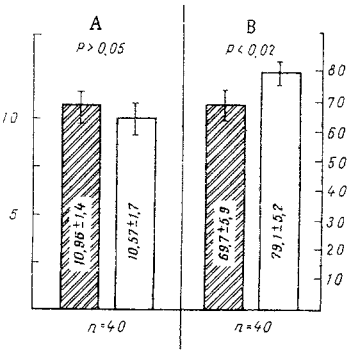

Fig. 3

Fig. 2. Changes in avidity of RFC after incubation with thymosine in vitro. Unshaded columns represent number of RFC after incubation with thymosine, shaded columns number of RFC in control. Value of P is shown for statistically significant differences (by Student's paired t-test) value of P shown above corresponding column. Ordinate, percentage of cells forming rosettes, respectively, with: I) low, II) average, and III) high avidity.

Fig. 3. Effect of thymosine on immunoglobulin-positive lymphocytes. A) Relative percentage of immunoglobulin-positive cells among lymphocytes after incubation with thymosine (unshaded column) and in control (shaded column); B) relative percentage of cap-forming cells among immunoglobulin-positive lymphocytes after incubation with thymosine (unshaded column) and in control (shaded column).

Thymosine also was found to have an action on expression of receptors for FC-fragment of immunoglobulins. In the method of EA rosette-formation used in these experiments, unlike in that suggested by Morretta et al., [7], no T-cells with FC-receptors were found. This showed that fraction IV of thymus extract induces differentiation of B-cells. This suggestion is confirmed by the increased mobility of the immunoglobulin receptors under the influence of thymosine [10] and the increase in expression of the C'3-receptor [8]. However, the possibility cannot be ruled out that the increase in the number of EAC-RFC took place because of an increase in the number of T-cells carrying C'3-receptors, for it has recently been shown that about 4% of T-cells in human peripheral blood carried these receptors [11].

The width of action of thymus extract thus revealed may be due both to the pleiotropic action of thymosine and to the presence of additional substances, such as "universal immunopoietic polypeptide" [10], in fraction IV of thymus extract.

LITERATURE CITED

1. B. B. Kheifets and V. A. Abalakin, Lab. Delo, No. 10. 579 (1973).
2. S. M. Shergin, M. I. Musatov, and V. S. Kozhevnikov, Lab. Delo, No. 3, 62 (1978).
3. U. Galili and M. Schlesinger, J. Immunol., 115, 827 (1975).
4. J. A. Hooper, M. C. McDaniel, G. B. Thurman, et al., Ann. N.Y. Acad. Sci., 249, 125 (1975).
5. P. I. Lobo and D. A. Horvitz, J. Immunol., 117, 936 (1976).
6. N. F. Mendes, M. E. A. Tolnai, N. P. A. Silveira, et al., J. Immunol., 111, 860 (1973).
7. L. Morretta, M. Ferrarini, A. Morretta, et al., J. Immunol., 117, 2171 (1976).
8. M. R. Scheid, G. Goldstein, U. Hammerling, et al., Ann. N.Y. Acad. Sci., 249, 531 (1975).
9. M. A. Scheinberg, E. S. Cathcard, and A. L. Goldstein, Lancet, 2, 424 (1975).
10. C. I. Sidman and E. R. Unanue, Nature, 257, 149 (1975).
11. J. Stobo, J. Lab. Clin. Med., 91, 9 (1978).
12. J. L. Touraine, F. Touraine, G. S. Incefy, et al., Ann. N.Y. Acad. Sci., 249, 335 (1975).
13. J. E. Vogel, G. S. Incefy and R. A. Good, Proc. Natl. Acad. Sci. USA, 72, 1174 (1975).
14. J. Wybran, A. S. Levin, H. H. Fudenberg, et al., Ann. N.Y. Acad. Sci., 249, 300 (1975).
15. D. T. Y. Yu, J. Immunol., 115, 91 (1975).