TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE
DEVELOPMENT IN THE POSTNATAL HUMAN THYMUS

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SUMMARY

Terminal deoxynucleotidyl transferase activity was studied in 42 whole human thymuses of different ages (2 days to 70 years). The results indicate that enzyme activity is high perinatally, falls soon after birth, rises to maximal activity early in life, and falls thereafter. Enzyme activity in homologous thymocytes of 9 thymuses ranging from 2 days to 105 months was also determined. The relative enzyme activity (thymocyte/thymus) did not remain constant with age. These results suggest that age-related changes in thymus terminal transferase activity occur which do not reflect a simple coordinate regulation of thymocyte and non-thymocyte elements.

SPECULATION

The regulation of terminal deoxynucleotidyl transferase containing cells in the thymus is an active process. Defective or altered regulation of these cells may play a role in immunodeficient and lymphoproliferative diseases.

INTRODUCTION

The function of the human thymus in the immune system is largely extrapolated from knowledge obtained as a consequence of its absence or abnormal development resulting in T-cell and severe combined immunodeficiency states (8). Its role in the regulation and expression of the immune system after birth is less clear (6,14). One approach to defining immunological function has been to identify subpopulations of pre-T and T-lymphocytes by means of various surface, cytoplasmic, and functional markers (18). One such marker is the enzyme terminal deoxynucleotidyl transferase (EC 2.7.7.31, TdT).

In the normal physiological situation, TdT has been localized to the thymus (4) and a subpopulation of bone marrow lymphocytes in all species so far examined, including man (1,12). Experiments in mouse and rat thymuses have localized the TdT-containing cells to a subpopulation of cortical thymocytes (2,16). However, the function of TdT-containing cells in the normal physiology of the human immune system has been little studied (1,12) despite its identification in the blast cells of acute lymphocytic leukemia (10), some cases of the blast crisis of chronic myelogenous leukemia (17), some non-Hodgkin's lymphomas (5), and some non-malignant viral infections (15).

The expression of TdT in these conditions and its absence in normal post-thymic T-lymphocytes suggested that regulation of cells containing the enzyme is essential to normal T-lymphocyte development. Because the thymus appears to be pivotal for normal T-cell development, we have measured TdT in normal human thymuses of different ages.

MATERIAL AND METHODS

Thymuses. Thymuses were obtained from individuals undergoing cardiac surgery (protocol approved by the University of Arizona Human Subjects Committee). Thymuses were either processed directly or frozen at -80°C in a Sorval omnimixer at half speed for 5 minutes. This suspension was brought to a concentration of 0.50% TX-100 and stirred on ice for 1 hour. The crude suspension was centrifuged at 144,000 xg for 60 minutes at 4°C in a Beckman type 40 rotor. The supernatant resulting from this procedure was used in the TdT assay.

Assay of TdT. TdT was extracted from whole thymus and thymocytes by a modification of procedures described by others (1,4,12). Tissue was suspended in 5 volumes of cold TEM buffer and homogenized at 4°C in a Sorval omnimixer at half speed for 5 minutes. This suspension was brought to a concentration of 0.50% TX-100 and stirred on ice for 1 hour. The crude suspension was centrifuged at 144,000 xg for 60 minutes at 4°C in a Beckman type 40 rotor. The supernatant resulting from this procedure was used in the TdT assay.

Assay of the enzyme was carried out at 37°C for 1 hour in a standard reaction mixture of 0.05 ml. These reaction mixtures contained a final concentration of 80 mM TRIS-HCl (pH 7.5), 40 mM KCl, 0.8 mM MnCl₂, 0.4 mM DTT, 2.50 µCi [³H]dGTP (Schwartz-Mann 15 Ci/mmol) and 1.60 µg of poly(dA). Deoxyguanosine triphosphate was added to a final concentration of 16 µM. The reaction was terminated by the addition of 15 µg bovine serum albumin and 2.0 ml of 10% trichloroacetic acid (TCA) containing 0.02 M sodium pyrophosphate. The acid insoluble material was collected on a Millipore filter (0.45 µm), washed with 5.0% TCA, dried, and counted in a liquid scintillation counter. A control reaction mixture without poly(dA) was run in every assay and activity calculated from the difference. All assays were done in triplicate and the results expressed as an average of the values. Protein concentration was measured by the method of Lowry et al. (11). Several different statistical regression models were used to obtain a mathematical description of the measurements of TdT activity by age and a best fit curve drawn. Measurements were analyzed separately by the person's sex (T. Moon, personal communication).

RESULTS

Evolution of TdT Activity with Age. TdT activity in 42 (22 male and 20 female) normal whole thymuses was measured (Figure 1A). The enzyme activity was present throughout life. TdT activity is high perinatally (earliest assayed 2 days), falls soon after birth, rises to a maximum expression and then declines. A significant amount of TdT is found even in thymuses from some older individuals (oldest tested 70 years). The same basic pattern is present whether the data is expressed as activity of TdT per 0.50 gm thymus or per mg of protein.

There are slight differences in this pattern of activity between male (Figure 1B) and female (Figure 1C) thymuses. Minimum values (picomoles/mg) are reached earlier in the male than in the female (16 months versus 27 months). After the minimum value is reached, the rate of increase is faster for males and maximum activity is reached earlier than in females (36 months versus

96 months). Whether these differences are due to true differences in enzyme activity or are due to other factors (stimulators or inhibitors) will require future investigation.

Relative Activity of TdT in Thymus and Thymocytes. The activity of TdT in the whole thymus and homologous thymocytes of 9 fresh thymuses ranging from 2 days to 105 months was determined (Table 1). The relative activity (thymocyte/thymus) decreased with age (Figure 1).

TABLE 1
RELATIVE ACTIVITY OF TERMINAL TRANSFERASE
IN THYMUS AND HOMOLOGOUS THYMOCYTES*

Age	Activity*		Relative Activity (Thymocytes/Thymus)
	Thymocytes	Thymus	
2 day	8097	479	16.9
19 month	436	37	11.7
27 month	345	28	12.0
33 month	641	162	3.96
33 month	496	154	3.22
41 month	242	122	1.98
51 month	967	346	2.79
96 month	8044	2495	3.22
105 month	83	151	0.55

* picomoles dGMP incorporated/mg protein. The measurement of activity was done with fresh thymuses and thymocytes in this study.

DISCUSSION

The changes in TdT activity with age indicate either that there are rapid alterations of TdT-containing cells or that endogenous factors in the thymus contribute to the apparent activity of TdT. The decrease of relative thymocyte/thymus TdT activity with age suggests that age-related changes of TdT are not due to simple coordinate alterations of thymocyte and non-thymocyte elements. In fact, the general decrease in relative activity with age indicates either that the non-thymocyte elements are expanding more rapidly than thymocyte elements or that the amount of TdT per thymocyte is decreasing relatively faster than non-thymocyte elements. Preliminary data indicate that an age-dependent inhibitor may play a role (F. Meyskens and C. Kiefer, unpublished data). We have noted that ATP, a specific inhibitor of at least the low molecular weight TdT (15) inhibits TdT in these thymuses greater than 75% except in older thymuses (52 years, 40-60% inhibition) and thymuses at approximately the age nadir of TdT activity (4 to 12 months, approximately 50% inhibition). Whether the different effects of ATP on *in vitro* TdT activity reflects a qualitative change in TdT with age will require further studies.

The age-related differences in TdT activity seen here are another example of important changes which occur in T-lymphocytes throughout life (3,9). Peak thymic TdT activity was attained five years earlier in males as compared to females. Such a large time difference has not been reported for other physiological phenomenon and the significance of this observation remains to be defined. Studies of immunodeficiency diseases indicate that the interaction of non-thymocyte and thymocyte cells in the thymus is important during the maturational process (19). In this regard, the differences of activity of human thymus TdT with age suggest that the regulation of cells containing TdT may be an active process. Further investigations of the relationships between TdT-containing thymocytes and the non-thymocyte elements of the thymus will be required. These data may serve as one basis for studies in intrathymic lymphocyte maturation, defects of which may be related to such diseases as acute lymphocytic leukemia and immunodeficiency diseases.

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FIGURE LEGEND

Figure 1. Activity of Terminal Transferase in Thymuses of Different Ages.

- □ males, ● ○ females.
- ● picomoles dGMP incorporated per 0.50 gm thymus.
- ○ picomoles dGMP incorporated per mg protein.

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