The Journal of Immunology

RESEARCH ARTICLE | JULY 01 1983

Expression of terminal deoxynucleotidyl transferase in human thymus during ontogeny and development. FREE

M R Deibel, Jr; ... et. al

J Immunol (1983) 131 (1): 195–200.
https://doi.org/10.4049/jimmunol.131.1.195

Related Content

Distribution of terminal deoxynucleotidyl transferase and purine degradative and synthetic enzymes in subpopulations of human thymocytes.

J Immunol (October,1982)

Ontogeny of Terminal Deoxynucleotidyl Transferase-Positive Cells in Lymphohemopoletic Tissues of Rat and Mouse

J Immunol (September,1979)

Isolation and characterization of the Xenopus terminal deoxynucleotidyl transferase.

J Immunol (May,1994)

Downloaded from http://journals.aai.org/jimmunol/article-pdf/131/1/195/1020790/195.pdf by guest on 05 January 2023

0022-1767/83/1311-0195$02.00/0
THE JOURNAL OF IMMUNOLOGY
Copyright © 1983 by The American Association of Immunologists
Vol. 131, No. 1, July 1983
Printed in U.S.A.

EXPRESSION OF TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE IN HUMAN THYMUS DURING ONTOGENY AND DEVELOPMENT[1]

MARTIN R. DEIBEL, JR.,* LELA K. RILEY,* MARY SUE COLEMAN,[2]* MICHAEL L. CIBULL,† STEVEN A. FULLER,* AND EDWARD TODD‡

*From the Departments of *Biochemistry, †Pathology, and ‡Surgery, University of Kentucky, Lexington, KY 40536*

Expression of the enzyme terminal deoxynucleotidyl transferase (TdT) was studied in human thymus during ontogeny and development. In five fetal thymus samples, the enzyme activity was barely detectable. At birth, the terminal transferase activity remained low. Maximum expression of the enzyme activity occurred between 10 and 40 mo of age. Analysis of six other enzyme activities, adenosine kinase, deoxyadenosine kinase, AMP deaminase, dAMP deaminase, 5′ nucleotidase, and adenosine deaminase confirmed the normal status of the thymic tissue. A careful analysis of thymic architecture revealed that involution did not occur as a result of the disease process that necessitated cardiac surgery. By immunofluorescence, the TdT antigen was localized exclusively in the nucleus of cortical thymocytes. Protein immunoblotting studies indicated that human thymic terminal transferase exists as a single high m.w. species in individuals under 30 mo of age. Thereafter, a variant m.w. species is detectable. The increase in expression of this enzyme coincides with the increase observed in serum immunoglobulin levels during maturation and precedes the maximum development of the human thymus.

Terminal deoxynucleotidyl transferase (TdT)[3] is an enzyme that polymerizes *in vitro* deoxynucleoside 5′ triphosphates onto single-stranded oligodeoxynucleotides without template utilization. The unusual localization of the enzyme in mammalian immunopoietic organs during ontogeny and differentiation suggests that it plays a role in the development of the immune system.

Detailed studies of TdT antigen in individual cells have revealed that while TdT is continuously present in a major subpopulation of cortical thymocytes and a minor subpopulation of marrow prelymphocytes, transient populations of TdT-positive cells appear in a variety of immunopoietic organs (blood, lung, spleen, and liver) during ontogeny and neonatal development (1). In rodents, TdT-containing cells first arise in thymus and separately arise in marrow (2, 3). Ultimately, the protein is expressed in approximately 65% of cortical thymocytes and in less than 1% of marrow nucleated cells (4–7).

In humans, the study of TdT expression during ontogeny and development has not been extensive. Although cells that contain TdT have been shown to arise in fetal thymus and to become more numerous in the neonate, the detailed pattern of human TdT expression from the fetal stage through adolescence and middle life has not been investigated in detail. In this study, we sought to correlate TdT expression in human thymus with other biologic events known to occur in that organ as immune competence is achieved.

MATERIALS AND METHODS

Chemicals. Radioactive deoxynucleoside 5′-triphosphates and ^{125}I-protein A were obtained from New England Nuclear Corporation, Boston, MA. Nonradioactive nucleotides were purchased from Sigma Chemical Co., St. Louis, MO. Anti-calf and anti-human TdT are prepared routinely in our laboratory against homogeneous preparations of calf or human TdT (8). The antibody preparations are purified by affinity chromatography on a column of controlled pore glass linked to homogeneous TdT. All other chemicals were reagent grade from commercial sources.

Human tissues. The 36 patients (aged 1 to 561 mo) had clinically normal immune function at the time of surgery for various types of heart disease when partial thymectomies were necessary to facilitate exposure. The thymic tissues were divided; a small portion was placed in embedding medium (O.C.T., Lab Tek Products, Napierville, IL), frozen in isopentane-dry ice, and stored at −30°C for immunofluorescence. A portion was fixed in 10% neutral buffered formalin and processed by paraffin sectioning for histologic examination. A third portion was assayed for TdT activity immediately upon receipt. Five fetal thymus tissues were generously supplied to our laboratory by the Central Laboratory of Human Embryology, University of Washington, Seattle, WA (Dr. Thomas H. Shepard). Samples were immediately packed in dry ice, shipped, and assayed upon receipt. Seventeen of the thymus samples (13 postnatal and five fetal) were also used to assay for six other enzyme activities to assess the status of the thymic tissue.

Clinical review. Patient charts were reviewed for information that might have an impact upon immunologic status at the time of surgery. Factors such as sex, prematurity, birth weight, maternal abnormalities, type of heart disease and associated defects, nature and number of clinical infections, white blood cell count, percentage of circulating lymphocytes, and abnormal reactions to vaccination were assessed. Because a fragment of thymus was removed primarily to facilitate exposure at the time of cardiac surgery, the number of subjects studied represents only a small proportion of the total number of patients requiring cardiac surgery. Prospective detailed immunologic evaluations were not clinically indicated and were therefore not performed. The five fetal thymic samples were all derived from spontaneous abortions of four anatomically normal fetuses without evidence of chromosomal or other genetic defects and one fetus with presumed Potter's syndrome.

Immunofluorescence examination. Ten specimens from patients ranging in age from 4 to 145 mo were examined by indirect immunofluorescence. Six-micron sections were fixed in methanol at 4°C for 1 min and were stained by the indirect technique we described previously (9). We employed affinity column-purified anti-calf or anti-human TdT (20 μg/ml) as the primary antibody and goat anti-rabbit affinity-purified IgG-fluorescein isothiocyanate (Kirkkegaard and Perry Laboratories, Gaithersberg, MD) as the secondary antibody. Stained sections were examined with a Leitz Dialux microscope equipped for incident light excitation and fitted with an I^2 narrow band filter cube.

Histologic examination. Paraffin-embedded tissues were sectioned at 4 μm and stained with hematoxylin and eosin. The thymic sections were examined for architectural normality, including signs of involution, cortical thickness, corticomedullary ratio, cortical mitotic activity, cortical macrophage activity, and calcification of Hassall's corpuscles.

Enzyme assays. Representative cross-sectional slices of thymic lobules (0.5 g of tissue), containing constant amounts of both the cortex and medulla,

Received for publication August 16, 1982.
Accepted for publication March 15, 1983.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported by grants from the National Cancer Institute (CA 19492 to M.S.C.), the National Institute of General Medical Sciences (GM 31076 to M.R.D., Jr.).
[2] M. S. Coleman is the recipient of a Research Career Development Award from the NCI, CA 00494. Correspondence should be addressed to Dr. Coleman.
[3] Abbreviations used: TdT, terminal deoxynucleotidyl transferase; $p(dA)_{50}$, poly(deoxyadenylic acid); NP-40, Nonidet P-40.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/131/1/195/1020790/195.pdf by guest on 05 January 2023

were added to 3 vol of extraction buffer (0.25 M potassium phosphate, pH 7.2, containing 1 mM 2-mercaptoethanol). The suspension was sonicated (for 15 sec, three times), and centrifuged for 5 min at 10,000 × G. The supernatant was removed and immediately assayed for TdT activity. In several cases, thymocytes were isolated from fresh thymus tissue by standard techniques and were assayed for TdT (10, 11). Thirteen postnatal thymus samples and all fetal thymus samples were assayed for the following enzymes by using standard procedures: adenosine kinase (12, 13), deoxyadenosine kinase (12, 13), AMP deaminase (14), dAMP deaminase (14), 5′ nucleotidase (15), and adenosine deaminase (16). Adenosine deaminase was also detected by radioimmunoassay (17) and the data were kindly provided to us by Dr. Dan Wiginton.

The components of the TdT assay in a total volume of 125 μl were: 0.2 M potassium cacodylate, pH 7.5, 1 mM 2-mercaptoethanol, 8 mM $MgCl_2$, 0.01 mM poly(deoxyadenylic acid) with an average chain length of 50 residues ($p(dA)_{50}$), and 1 mM ^{3}H-dGTP (100 cpm/pmol). The reactions, in duplicate, were incubated at 35°C for 3, 6, 9, and 12-min intervals, and terminated by application of 25-μl aliquots onto GF/C glass fiber papers as described (11). One unit of enzyme activity is defined as 1 nmol of radioactive deoxynucleotide incorporated per hour. Specific activity is expressed as units of activity per milligram of protein, per gram of thymus, or per 10^8 nucleated cells. Reaction velocities were fitted by linear regression.

Protein blotting techniques. To probe for m.w. variants of TdT in the human thymus, eight sample extracts were subjected to SDS polyacrylamide gel electrophoresis. Proteins were then transferred electrophoretically to nitrocellulose paper as described by Burnette (18). After transfer, the nitrocellulose paper was incubated with 5% BSA-phosphate-buffered saline, pH 7.2 (PBS) to block protein-reactive sites on the paper, and then was incubated with antibody against TdT. After extensive washing with PBS and PBS containing 0.05% Nonidet P-40 (NP-40), the nitrocellulose was reacted with ^{125}I-labeled *Staphylococcus aureus* protein A to detect antigen-bound antibodies on the paper. After washing with PBS and PBS-0.05% NP-40, the nitrocellulose was exposed to NS-5T x-ray film (Eastman Kodak Co., Rochester, NY) for 48 hr. Standards to verify m.w. were included on the gel, transferred to nitrocellulose, and stained (0.1% amido black, 45% methanol, 10% acetic acid).

RESULTS AND DISCUSSION

The postnatal thymic tissue used for this study was obtained from patients undergoing surgery for congenital heart disease in 34 cases and rheumatic heart disease in two cases (409 and 561 mo). Tissue samples were assayed for enzyme activities within 1 hr of removal. Fetal samples were determined to be morphologically normal at the institution of origin. When thymic tissue from all age groups was extracted and assayed for terminal transferase, the pattern of expression of enzyme activity became apparent (Fig. 1). In the human fetal samples (14 to 22 wk gestation, Fig. 1*A*), TdT activity was barely detectable (0.08 to 1.2 U/mg protein). Immediately after birth, TdT activity was very low and rose to its maximum expression (17 to 45 U/mg protein, Fig. 1*B*) between 10 and 40 mo of age. The diminution in enzyme activity after 40 mo (3.3 yr) was gradual and the age data were best expressed on a logarithmic scale. Tissues from the oldest individuals tested (31 to 46 yr) demonstrated very low levels of enzyme activity (0.3 to 5.3 U/mg, Fig. 1*C*). Similar results are observed when the specific activities of TdT are expressed per gram of thymic tissue (data not shown). Of the post-birth tissue samples, 16 were female and 20 were male, and no differences in expression of TdT with sex were detected.

We observed striking fluctuations in TdT-specific activity in separate thymus samples within a given age group. To rule out the possibility that this variation was due to errors associated with the assay of terminal transferase activity, we assayed multiple cross-sectional slices of a single thymus in which areas represented by medulla and cortex were similar. We found that variation of enzyme activity was less than 15%. The stability of TdT enzymatic activity was also assessed in freshly excised human thymus tissue. A 15-g sample from an 8-mo-old infant was divided into equal portions. TdT activity was assayed under a variety of conditions (tissue storage at 4°C, −20°C, −70°C; extract storage at 4°C, −20°C, −70°C). Minor variations in enzyme activity were observed (up to 20% loss in 24 hr under the worst conditions, 4°C), but the best retention of enzyme activity occurred when the tissue was stored at −70°C (less than 5% loss of enzyme activity). These data indicate that deviations in TdT activity from a specific age group are not due to errors of measurement, but rather are derived from biologic variation. Averaging of levels of TdT expression as a function of age is represented by the dashed line in Figure 1.

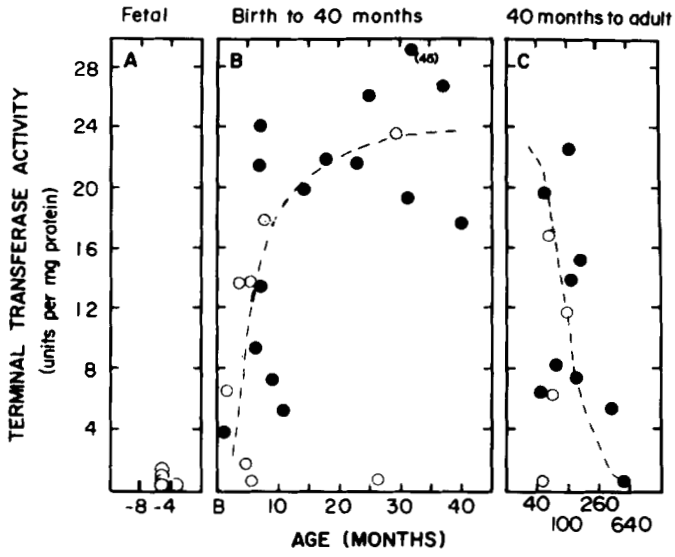

Figure 1. Expression of TdT activity in normal human thymus as a function of age. Each point represents a different patient (n = 41). *A*, data were plotted on a linear scale for fetal samples. *B*, data were plotted on a linear scale to demonstrate the rapid development of TdT activity during the initial 40 mo of life. *C*, data were plotted on a logarithmic scale to demonstrate the slow, heterogeneous decline in TdT activity observed up to 46 yr of life. Specific activities of TdT are expressed as units enzymatic activity per milligram of protein. One sample in which the TdT activity was abnormally elevated (32 mo) is indicated in parentheses. Open circles, TdT determinations for which other enzyme activities have been measured (Figs. 6 and 7).

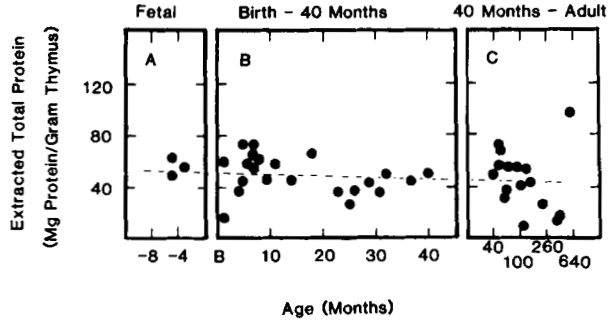

Figure 2. Protein concentrations in extracts of human thymus for which TdT values have been measured. Data points correspond to the same thymic samples depicted in Figure 1. An average protein concentration was 40 mg soluble protein/g thymus tissue.

Fluctuations in protein concentration as a function of thymic age were random and relatively invariant throughout the period studied as illustrated in Figure 2. This appears to rule out the possibility that specific activity trends in the TdT enzymatic activities were due exclusively to parallel changes in total protein concentration.

Ten thymic specimens representative of a variety of patient ages and TdT activities were examined by immunofluorescence staining for TdT antigen. All exhibited normal, bright nuclear staining of cortical thymocytes with only rare positive medullary cells (Fig. 3) in a sample from a patient of 23 mo. It was difficult to assign an absolute percentage of immunofluorescent cells because the samples were not of single cell thickness. Certainly it appeared that at least 90% of small cortical thymocytes were

Downloaded from http://journals.aai.org/jimmunol/article-pdf/131/1/195/1020790/195.pdf by guest on 05 January 2023

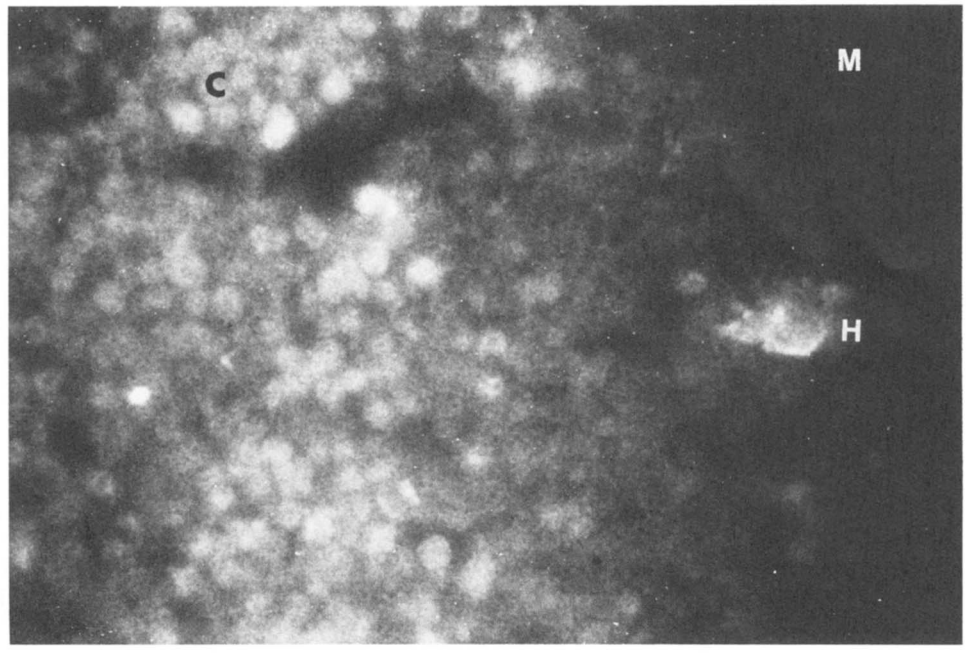

Figure 3. Photomicrograph of corticomedullary junction of thymus (23 mo, 21.6 U/mg TdT) showing the cortex (C) containing numerous small thymocytes demonstrating bright nuclear fluorescence and the medulla (M) showing a virtual absence of such cells. A Hassall's corpuscle (H), which stains nonspecifically, is seen within the medulla. Immunofluorescence ×128 (original magnification).

stained in all samples so studied. As we noted previously, enzyme activity levels cannot be directly extrapolated from percentage of cells that show positive immunofluorescence for TdT antigen (9).

Because the expression of TdT activity in human thymus was age-related, an initial concern was whether any of the samples used were derived from patients that had experienced immunologic abnormalities as a function of their disease. Patient records were examined as described above. There was no difference in any of the enzyme activities with regard to the clinical parameters examined. No patients were lymphopenic and although episodes of otitis media and upper respiratory tract infection were relatively common, they did not differ between patients with high or low levels of TdT activity. No patients had clinical courses suggestive of defects in humoral or cell-mediated immunity.

Although the morphometric studies of cortical width and the contribution of the cortex to total thymic area and volume showed a slight decrease with age, the standard deviation for the relatively small number of samples studied was quite large. Data for two such measurements (cortical thickness and the ratio of cortex to medulla) are depicted in Figure 4. Similarly, the *in vitro* enzymatic activity of TdT decreased with age but showed a marked variability when plotted against the above detailed morphometric parameters. No evidence of thymic involution as assessed by loss of cortical thymocytes, increased keriotaxis, or increased number of cortical phagocytic cells was noted. Cortical thymocyte proliferation as judged by the number of mitoses was independent of the enzyme measurements reported in this study.

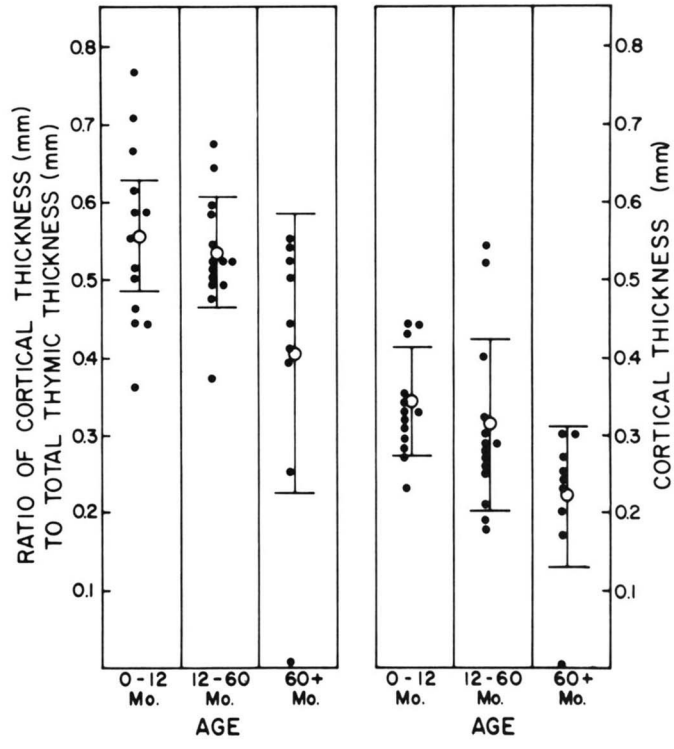

Figure 4. Statistical mean values and associated standard deviations of human thymus preparations used in this study as a function of age. Left, ratio of cortical thickness(mm) to total thymic thickness(mm). Right, cortical thickness(mm).

Our findings in this patient group upon histologic examination are consistent with other reports of normal human thymic development (19). Representative thymic slices from six patients are shown in Figure 5; the age range in sections *A* through *F* is 1 to 47 mo. The ratio of cortex to medullary areas is typical for this age progression and TdT activity is low in *A* and *B*, rises in *C* and *D*, and decreases in *E*. It is interesting to note that the TdT activity varies greatly in sections *E* and *F*, although there is no corresponding association with loss of cortex. Histologic examination of each of the thymus samples used in this study indicates that thymic involution as a consequence of the disease sequelae has not occurred.

The absence of significant amounts of TdT activity in early postnatal thymus is not anticipated from studies of other mammalian (bovine and rodent) and avian species because in these animals, the expression of TdT is nearly maximal at the time of birth (1, 20). To confirm that the lack of TdT activity in fetal samples and immediately after birth was not due to enzyme inactivation as a consequence of *in utero* degenerative changes or tissue handling, six other enzyme activities were measured in 17 selected samples (open circles in Fig. 1). Enzymes assayed were adenosine kinase, deoxyadenosine kinase, AMP deaminase, dAMP deaminase, 5′-nucleotidase, and adenosine deaminase. Several of these enzymes are known to be particularly labile and thus are good controls for our tissue handling. The

Downloaded from http://journals.aai.org/jimmunol/article-pdf/131/1/195/1020790/195.pdf by guest on 05 January 2023

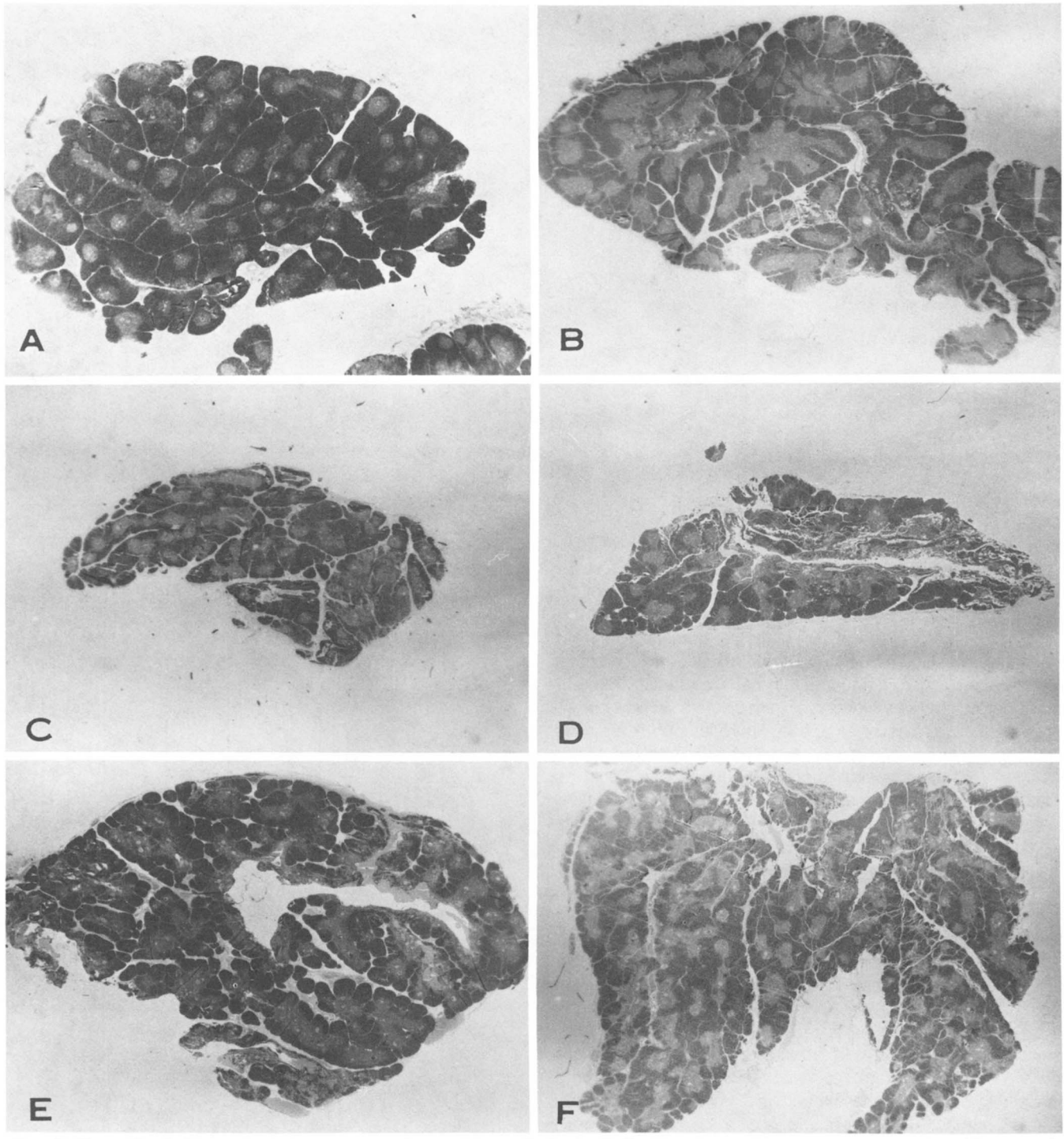

Figure 5. Slices of thymic tissue stained with hematoxylin and eosin. The magnification level is five times. These six representative cross-sections of thymus are from patients of various ages and TdT activities. *A*, 1 mo, 3.8 U/mg; *B*, 11 mo, 5.2 U/mg; *C*, 29 mo, 23.8 U/mg; *D*, 37 mo, 26.8 U/mg; *E*, 46 mo, 0.2 U/mg; *F*, 47 mo, 19.5 U/mg. A comparison of these samples illustrates the lack of correlation of enzymatic activity with thymic cortical thickness and ratio of cortex to medulla.

results are shown in Figure 6. Enzyme units were defined so that all could be plotted on the same scale. As can be seen, none of these activities appear to be particularly deficient in any group of samples. Five postnatal (1, 4, 5, 26, and 46 mo) thymic tissues that demonstrated unusually high or low TdT values were assayed for each of the above enzymes and were plotted separately (Fig. 7). Again, no unusual variation in any other enzyme activities are apparent.

When we examined the statistical mean values and associated standard deviations of all the enzymatic activities measured in this study, the pattern shown in Figure 8 emerged. Only small differences in mean values between fetal (F) and postnatal (A) samples are observed, with overlapping standard deviations for adenosine kinase, deoxyadenosine kinase, AMP deaminase, dAMP deaminase, 5′ nucleotidase, and adenosine deaminase. A radically different pattern is observed for terminal transferase. The mean values are quite different and standard deviations do not overlap for fetal and postnatal samples. The standard deviation in the postnatal group is large, representing the age-related expression of TdT activity.

The virtual absence of TdT activity we observe in human fetal thymus is comparable to and in agreement with a study by Janossy *et al.* (7) in which nine fetal thymuses were tested for TdT activity alone. These investigators also included seven post-

Downloaded from http://journals.aai.org/jimmunol/article-pdf/131/1/195/1020790/195.pdf by guest on 05 January 2023

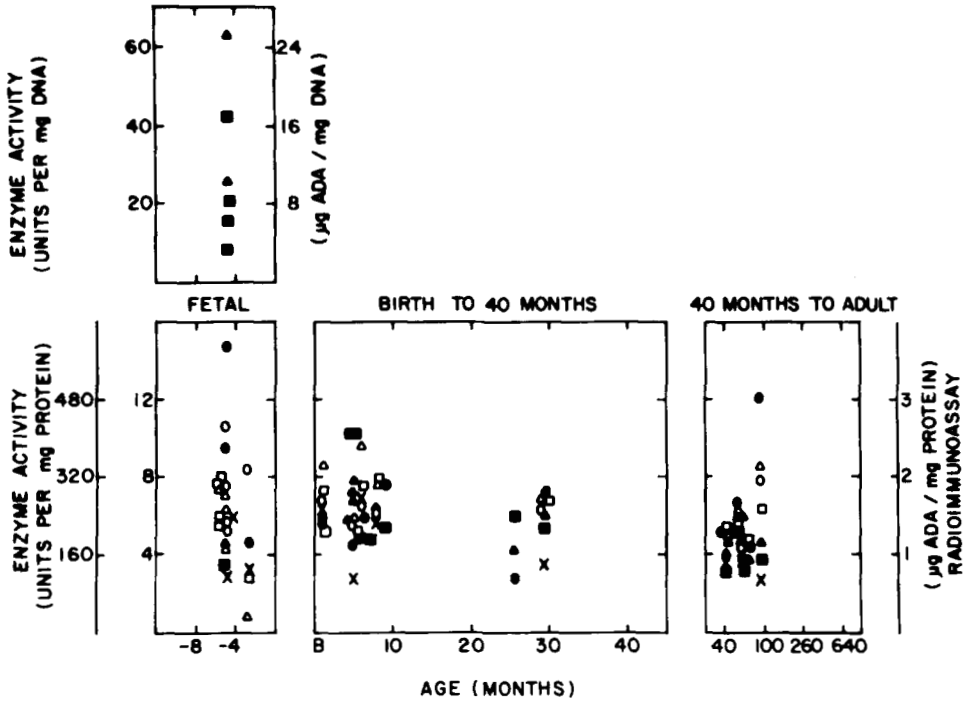

Figure 6. Representative enzymatic activities in human thymus extracts as a function of age. The human thymus preparations for which these determinations were made are shown as open circles in Figure 1. Adenosine kinase (1 U = nmol/hr; scale of 4 to 12 U/mg); deoxyadenosine kinase (1 U = nmol/hr; scale of 4 to 12 U/mg); AMP deaminase (1 U = nmol/min; scale of 4 to 12 U/mg $\times 10^{-1}$); dAMP deaminase (1 U = nmol/min; scale of 4 to 12 U/mg); 5′nucleotidase (1 U = nmol/hr; scale of 160 to 480 U/mg); adenosine deaminase (ADA) (1 U = nmol/min; scale of 4 to 12 U/mg $\times 10^{-2}$). ADA was also determined by radioimmunoassay (micrograms ADA per milligram of protein). Enzymatic activity of ADA in human fetal thymus samples was measured as a function of DNA content (U/mg of DNA $\times 10^{2}$).

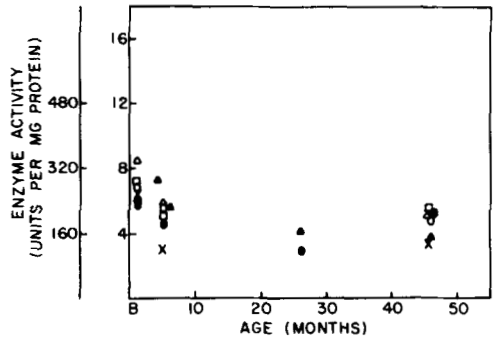

Figure 7. Representative enzymatic activities in five human thymus preparations for which TdT activities were unusually high or low (1, 4, 5, 26, and 46 mo). TdT values for these preparations are indicated as open circles in Figure 1 at the appropriate age. The enzymes assayed for this determination and their respective specific activity scales are identical to those in Figure 6.

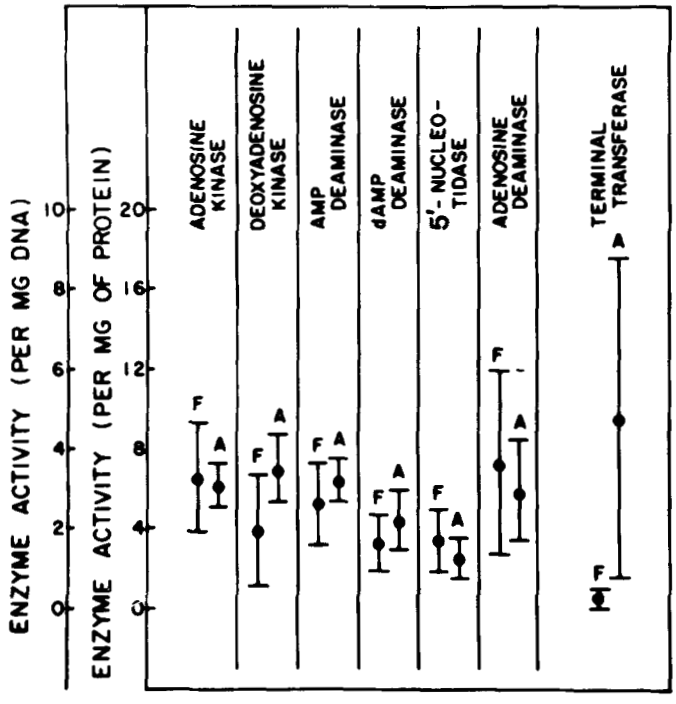

Figure 8. Statistical mean values and associated standard deviations of all enzymatic activities measured in this study. The human thymus samples utilized in these statistical measurements are shown as open circles in Figure 1. F = fetal thymus samples; A = postnatal thymus samples. Adenosine kinase (U/mg protein); deoxyadenosine kinase (U/mg protein); AMP deaminase (U/mg protein $\times 10^{-1}$); dAMP deaminase (U/mg protein); 5′-nucleotidase (U/mg protein $\times 10^{-2}$); adenosine deaminase (U/mg DNA); terminal deoxynucleotidyl transferase (U/mg protein).

natal thymus samples (5 to 7 yr). In agreement with our data, they detected less than one unit per 10^8 cells in fetal thymus but high levels of TdT activity (an average of 57 units per 10^8 cells) in juvenile thymus (5 to 12 yr). The postnatal TdT value compares with an average of 60 units per 10^8 cells in the current study. Because only a few postnatal samples were included and TdT activity was not calculated as units per milligram of protein, the age-related expression of TdT activity was not apparent in the earlier study (7).

When TdT antigen was examined by protein blot analysis, we were able to detect as small an amount as 1 ng of TdT in crude extract samples. The enzymatic activity of TdT in these samples correlates with the intensity of antigen detected. The samples in lanes 1 to 3, Figure 9, have three- to fivefold less activity than the samples in lanes 4 to 6. Of the six representative thymus samples shown, four have a single polypeptide species of 60,000 m.w. (Fig. 9, lanes 1–4), which corresponds to the m.w. of TdT detected *in situ* in calf thymus extracts and a number of human leukemic cell types. In the two samples from older individuals (32 and 107 mo), an additional m.w. species was observed at 44,700 (Fig. 9, lanes 5 and 6). Results from other studies suggested that TdT is synthesized as a high m.w. species (m.w. = 60,000) that can be proteolytically cleaved to smaller, kinetically indistinguishable molecular variants *in vitro* (21, 22). We cannot discern if this cleaved form of TdT in the thymus samples of these older individuals is due to *in vivo* proteolytic processing of the enzyme in the aging thymus or if high levels of proteases present in these older thymuses readily cleave the enzyme upon cellular disruption.

In the aggregate, the data we generated indicate that TdT activity is expressed in an age-related manner in human thymus.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/131/1/195/1020790/195.pdf by guest on 05 January 2023

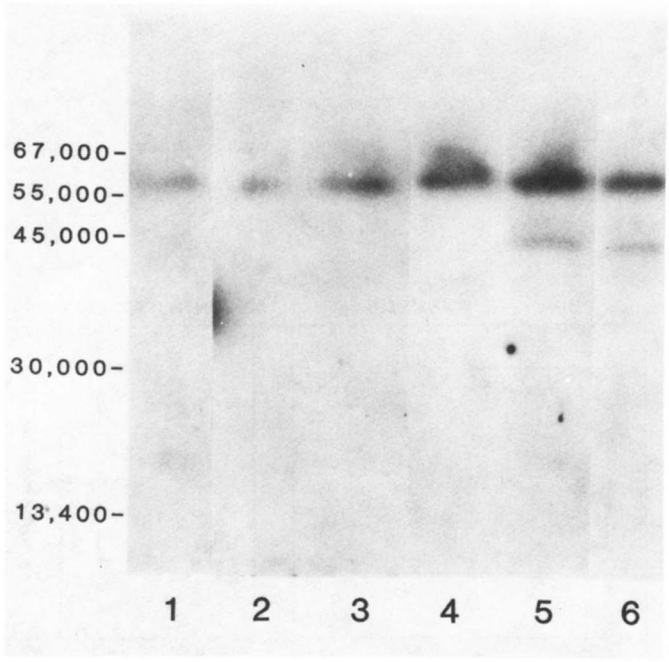

Figure 9. Protein blot analysis of human thymus samples. Lanes represent patients of various ages at the time thymectomies were performed. Lane 1, 1 mo; lane 2, 5 mo; lane 3, 5 mo; lane 4, 7 mo; lane 5, 32 mo; and lane 6, 107 mo.

TABLE I

Terminal transferase activity and antigen in thymus tissue from various sources during periods of maximum expression

Source	Maximum Expression	Activity (U/g)[a]	Activity (U/10^8 Cells)[b]	Percent Antigen Content[c]
Calf	<6 mo	2000	20–30	ND[d]
Human	10–30 mo	1500	30–60	>60
Rat	5–7 wk	2000	20–30	>60
Mouse	<8 wk	400	4–6	>60
Chicken	2–10 wk	500	5–8	>60

[a] One unit of TdT activity is defined as 1 nmol of dNTP incorporated per hour at 35°C.
[b] Total population of nucleated cells represented.
[c] Determined by immunofluorescence, percentage of cortical thymocytes.
[d] Not determined.

Careful examination of the tissues used showed that normal architecture existed in all the samples. Detection of several other labile enzymatic activities attests to the careful fashion in which the tissues were handled. The data we obtained for human thymus, however, differ from those observed in other mammalian and avian species (Table I). All other species tested show a dramatic rise in TdT activity during the late stage of fetal development followed by only a small increase after birth. Both mouse and chicken thymus have much lower levels of TdT activity than other species tested, but a similar proportion of cortical thymocytes (60 to 80%) demonstrate TdT antigen by immunofluorescence detection. In the very early postnatal period of rodents, transient populations of TdT-positive cells appear in blood, liver, and spleen. These transient cells may be indications of the migration of precursor populations out of primary lymphoid organs into secondary sites (2).

The pattern of TdT expression in humans we report here coincides with the increase observed in serum immunoglobulin levels during maturation (23) and precedes the maximum development of human thymus (10 yr) as measured by the mass of cortex and medulla (19). It is interesting that the human infant is immunocompetent before the maximum rise in TdT activity in thymus. Whether TdT is involved at the molecular level in DNA rearrangement events that appear to occur during the acquisition of immunologic diversification is an intriguing and as yet unsolved question in the biology of this enzyme (24, 25).

Acknowledgments. We are grateful to Dr. Thomas Roszman for his critical reading of the manuscript.

REFERENCES

1. Gregoire, K. E., I. Goldschneider, R. W. Barton, and F. J. Bollum. 1979. Ontogeny of terminal deoxynucleotidyl-transferase-positive cells in lymphohemopoietic tissues of rat and mouse. J. Immunol. 123:1347.
2. Sasaki, R., F. J. Bollum, and I. Goldschneider. 1980. Transient populations of terminal transferase-positive (TdT+) cells in juvenile rats and mice. J. Immunol. 125:2501.
3. Bollum, F. J., and I. Goldschneider. 1980. Terminal deoxynucleotidyl transferase and lymphocyte differentiation. *In* Membranes, Receptors and the Immune Response. H. Kohler and E. P. Cohen, eds. Alan R. Liss, Inc., New York. P. 189.
4. Coleman, M. S., J. J. Hutton, P. Desimone, and F. J. Bollum. 1974. Terminal deoxynucleotidyl transferase in human leukemia. Proc. Natl. Acad. Sci. USA 71:4404.
5. Coleman, M. S., J. J. Hutton, and F. J. Bollum. 1974. Terminal deoxynucleotidyl transferase and DNA polymerase in classes of cells from rat thymus. Biochem. Biophys. Res. Commun. 58:1104.
6. Goldschneider, I. 1982. Ontogeny of terminal deoxynucleotidyl transferase containing lymphocytes in rats and mice. *In* Terminal Transferase in Immunobiology and Leukemia. U. Bertazzoni and F. J. Bollum, eds. Adv. Exp. Med. Biol. 145:115.
7. Janossy, G., J. A. Thomas, F. J. Bollum, *et al.* 1980. The human thymic microenvironment: an immunohistological study. J. Immunol. 125:202.
8. Deibel, M. R., Jr., M. S. Coleman, K. Acree, and J. J. Hutton. 1981. Biochemical and immunological properties of human terminal deoxynucleotidyl transferase purified from blasts of acute lymphoblastic and chronic myelogenous leukemia. J. Clin. Invest. 67:725.
9. Cibull, M. L., M. S. Coleman, O. Nelson, J. J. Hutton, D. Gordon, and F. J. Bollum. 1982. Evaluation of methods of detecting terminal deoxynucleotidyl transferase in human hematologic malignancies. Am. J. Clin. Pathol. 77:420.
10. Coleman, M. S., and J. J. Hutton. 1977. Effects of antineoplastic and immunosuppressant drugs on deoxyribonucleic acid-polymerizing enzymes of rat thymus and spleen. Mol. Pharmacol. 13:122.
11. Coleman, M. S. 1977. Terminal deoxynucleotidyl transferase: characterization of extraction and assay conditions from human and calf tissue. Arch. Biochem. Biophys. 182:525.
12. Ives, D. H., J. P. Durham, and V. S. Tucker. 1969. Rapid determination of nucleoside kinase and nucleotidase activities with tritium-labeled substrates. Anal. Biochem. 28:192.
13. Deibel, M. R., Jr., M. S. Coleman, and J. J. Hutton. 1981. Effect of adenosine deaminase inhibitors on the apparent rate of phosphorylation of deoxyadenosine. Biochem. Med. 25:288.
14. Yun, S.-L., and C. H. Suetter. 1978. Human erythrocyte 5′AMP aminohydrolase. J. Biol. Chem. 253:404.
15. Rowe, M., C. G. de Gast, T. A. E. Platts-Mills, G. L. Asherson, A. D. B. Webster, and S. M. Johnson. 1979. 5′-nucleotidase of B and T lymphocytes isolated from human peripheral blood. Clin. Exp. Immunol. 36:97.
16. Coleman, M. S., and J. J. Hutton. 1975. Micromethod for quantitation of adenosine deaminase activity in cells from peripheral blood. Biochem. Med. 13:46.
17. Wiginton, D., M. S. Coleman, and J. J. Hutton. 1981. Purification, characterization and radioimmunoassay of adenosine deaminase from human leukemic granulocytes. Biochem. J. 195:389.
18. Burnette, W. N. 1981. Western blotting: electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated Protein A. Anal. Biochem. 112:195.
19. Hammar, J. A. 1936. Die normal morphologische thymusforschung in letzten vierteljahrhundert. Leipzig. Barth.
20. Sugimoto, M., and F. J. Bollum. 1979. Terminal deoxynucleotidyl transferase (TdT) in chick embryo lymphoid tissues. J. Immunol. 122:392.
21. Chang, L. M. S., P. Plevani, and F. J. Bollum. 1982. Proteolytic degradation of calf thymus terminal deoxynucleotidyl transferase. J. Biol. Chem. 257:5700.
22. Coleman, M. S., and M. R. Deibel, Jr. 1982. Terminal deoxynucleotidyl transferase. *In* Enzymes of Nucleic Acid Synthesis and Processing. S. T. Jacob, ed. CRC Press, Inc., Boca Raton, FL. P. 93.
23. Alford, C. A., Jr. 1971. Immunoglobulin determinations in the diagnosis of fetal infection. Pediatr. Clin. North Am. 18:99.
24. Bollum, F. J. 1975. Terminal deoxynucleotidyl transferase: source of immunological diversity? Karl August Forster Lectures, 14 Verlag Akad. Wiss. Lit. Mainz, Steiner, Weisbaden.
25. Alt, F. W., and D. Baltimore. 1982. Joining of immunoglobulin heavy chain gene segments: implications from a chromosome with evidence of three D-J fusions. Proc. Natl. Acad. Sci. USA 79:4118.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/131/1/195/1020790/195.pdf by guest on 05 January 2023