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# DNA POLYMERASES OF HUMAN TONSIL AND CHICKEN BURSA: ABSENCE OF A DISTINCT B CELL SPECIFIC TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE

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SUMMARY:

The extracts obtained from chicken bursas and human tonsils, both of which are known to contain predominantly B lymphocyte precursor cells, were examined for the presence of putative B cell specific terminal deoxynucleotidyl transferase (B-TdT). Neither of the two types of tissue extracts revealed the presence of specific TdT, although the presence of DNA polymerase $\alpha$, $\beta$, and $\gamma$ could be easily demonstrated.

INTRODUCTION:

The confinement of classical terminal deoxyribonucleotidyl transferase (Bollum enzyme, abbreviated as TdT), to thymus tissue or products bearing thymus influence has been well established (1-3). Due to its organ-specific location TdT has been postulated to play a role in the process of immune response (1). Baltimore (4) outlined a tentative hypothesis suggesting that TdT might be a somatic mutagen resulting in the generation of diversified immunoglobulins. Since immunological response is elicited by both T and B lymphocytes, it was logical to assume that B lymphocytes analogous to their T counterparts should also contain this enzyme, probably during their pre-maturation period. Baltimore and colleagues have presented some as yet unconfirmed evidence that there may be a B lymphocyte specific terminal transferase in lymphoid organs such as the bursa of fabricius in chicken (5). This B cell TdT apparently differs from classical thymic TdT in that it adds only a single non complementary nucleoside triphosphate at the 3' OH

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terminus of a primer which must be hydrogen bonded to template. We have been interested in studying the relationship, if any, between TdT levels and immunodeficiencies of both B and T cell origin, which mandated a protocol for identification of various DNA polymerases including putative TdT in cells other than thymocytes (or TdT positive immature human leukemic precursor T cells).

Choice of human tonsil and chicken bursas for this purpose was based on the fact that a) both of these tissues are known to contain predominantly B precursor cells and b) some as yet unconfirmed information on the presence of unique TdT from chicken bursa (5,6) would permit a ready comparison of the results that we would obtain. A systematic study was, therefore, undertaken to identify and characterize various DNA polymerases present in these tissue extracts. Results obtained from 22 different tonsil samples and 6 different pooled bursa samples did not reveal the presence of B cell specific TdT; although DNA polymerases $\alpha$, $\beta$, $\gamma$, were identified in most of the extracts.

## MATERIALS AND METHODS:

Tonsil samples were obtained from children as well as adults undergoing tonsillectomy for chronic tonsilar enlargement. The patients were free of any gross immunological abnormalities. Chicken bursas were kindly provided by D.Y.S Choi from 18 day old chick embryos. Tissue samples were stored in 10% glycerol at -70$^0$ until use. Radioactive chemicals were purchased from Amersham Searle, Inc. Various template primers and substrates were obtained from P.L. Biochemicals. Partially purified TdT from cells of a patient with acute lymphoblastic leukemia was obtained as described (7).

### Extraction Procedure and Phosphocellular Chromatography:

Cell free extracts of individual human tonsils or chicken bursas (usually 30 embryo bursas have to be pooled) were obtained by detergent treatment of freeze-thawed samples followed by 3 one minute homogenization in Sorvall omnimixer using buffer containing 50 mM Tris. HCl (pH 7.8), 1 mM DTT,† 0.4M KCl, 0.1 mM EDTA and 0.5% NP 40. Extracts were clarified by two successive centrifugations at 1800 x g and 27,000 x g for 1 hr. The 27,000 sup (10 ml) was then mixed with 2 g of DEAE cellulose and allowed to stand for 30 min. The nucleic acid free supernatant was diluted fourfold and loaded onto a 1 x 20 cm phosphocellulose column preequilibrated with 50 mM Tris HCl (pH 7.8), 1 mM DTT, 0.1 mM EDTA and 0.01% albumin. The column was eluted with linear salt gradient containing 0-0.7 M KCl in the equilibrium buffer. A twenty microliter portion from alternate fractions was assayed for activity using the various template primers described below.

†Abbreviations used: DTT-Dithiothreitol, EDTA-Ethylenediaminetetracetic acid, NP 40 Nonidet P.40, NEM-N.ethylmaleimide, TdT-Terminal deoxynucleotidyl transferase.

DNA Polymerase Assays:

Basically five types of assays were performed on all samples. The specific assay component and diagnostic implications of each one are described: All the reaction mixtures contained in a final volume of 0.1 ml, 50 mM Tris · HCl, pH 7.8, 1 mM dithiothreitol, 0.01% serum albumin, 0.5 to 2.5 microgram of desired template primer, appropriate divalent cation and 10-20 μM of appropriate deoxyribonucleoside triphosphate (adjusted with tritiated substrate such that the final specific activity was 2,000 cpm per picomole of nucleotide. For thymic TdT assay, oligo $(dA)_{12-18}$ was used as a primer with $Mn^{++}$ as an effective metal ion (8) while poly (dT) · oligo $(dA)_{12-18}$ (annealed at a molar ratio of 1L1) with either $Mn^{++}$ (0.5 mM) or $Mg^{++}$ (2 to 5 mM) was used as a template primer to measure the incorporation of a mismatched deoxytriphosphate, dGTP or dCTP. This assay was used to indicate the presence of B cell specific terminal transferase (6). Poly (rA) · $(dT)_{12}$, poly (dC) · $(dG)_{12-18}$ and activated DNA were used individually for the measurement of cellular DNA polymerases utilizing $MnCl_2$ (0.5 mM), $MgCl_2$ (2.5 mM) and $MgCl_2$ (10 mM) respectively as effective divalent cations. The activated DNA directed reaction contained all the four triphosphates (unlabeled triphosphates were adjusted to 200 μM). The incubation time in all the cases was 30 minutes. Reactions were terminated by the addition of 5% trichloroacetic acid containing 0.01% sodium pyrophosphate and acid insoluble radioactivity was determined in a toluene based scintillation fluid (9).

RESULTS:

Human Tonsils:

A typical phosphocellulose chromatogram obtained from six year old tonsil tissue extracts (5 samples pooled) is presented in fig. 1. From the variety of template primers used to assay various fractions, the presence of DNA polymerase α, β, γ can be easily identified (10). However, no substrate incorporation in the presence of oligo dA primer (thymic TdT specific) could be observed. Similarly, no incorporation of dGTP or dCTP (mismatched substrate) is observed in the presence of poly (dT) · oligo (d) or poly (dT) · poly (dA) template-primer, Each activity peak was then analyzed by glycerol gradient centrifugation to obtain an approximate estimate of its molecular weight (data not shown). From this information, it appears that DNA polymerase α and γ are contained in first peak (0.3-0.38 M salt) while the second peak (0.5 M salt) was predominantly β polymerase. Response of these enzyme activities to N-ethylmaleimide and inorganic phosphate is depicted in table 1. As expected, β polymerase is inhibited in the presence of inorganic phosphate (11) and is resistant to NEM, while DNA polymerase α and γ exhibit exactly opposite response. Some

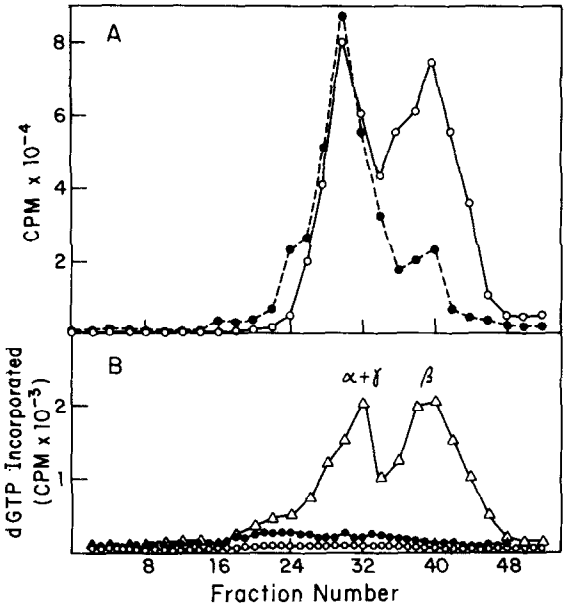


Fig. 1: Phosphocellulose chromatography of tonsil extracts: Fractions were assayed with indicated template primers as described in Materials and Methods. Frame A represents Poly (rA)·$(dT)_{12}$ directed incorporation of TTP (o———o) and Poly (dC)·$(dG)_{12-18}$ directed synthesis of DNA (●-------●). Frame B represents activated DNA directed synthesis of DNA (Δ———Δ); while closed (●———●) and open (o———o) circles represent dGTP incorporation directed by oligo $(dA)_{12-18}$ and Poly (dT)·$(dA)_{12-18}$ respectively.

variation in the levels of DNA polymerase has also been noted amongst individual tonsil samples; however, basic pattern has remained unchanged.

Chicken Bursa:

The DNA polymerase pattern obtained from bursal extracts is shown in fig. 2. Similar to tonsil extracts, the presence of DNA polymerase α, β and γ can be easily noticed from the template primer response pattern of various phosphocellulose fractions. There is also an absence of enzyme activity that would catalyze terminal addition of nucleoside triphosphates (oligo dA dependent

TABLE 1

Effect of N-ethylmaleimide and inorganic phosphate on various DNA polymerases from human tonsil and chicken bursa

| Template primer | Substrate | DNA polymerase from Human Tonsil α+γ | Human Tonsil β | Chick Embryo Bursa α+γ | Chick Embryo Bursa β |
|---|---|---|---|---|---|
| | | cpm incorporation/hr | | | |
| None | TTP or dGTP | 120 | 150 | 105 | 128 |
| $rAdT_{12-18}$ | TTP | 70,230 | 30,800 | 6,000 | 8,200 |
| + NEM (5mM)+ | TTP | 2,220 | 26,560 | 388 | 6,700 |
| + K-$PO_4$ (2 mM) | TTP | 72,400 | 10,620 | 6,325 | 3,555 |
| Activ. DNA | dGTP | 2,500 | 2,800 | 1,800 | 1,288 |
| + NEM (5 mM)† | dGTP | 320 | 2,750 | 150 | 1,150 |
| Poly dT·oligo dA | dGTP | - | - | - | 250 |
| + NEM (5 mM)+ | dGTP | - | - | - | 200 |
| + K-$PO_4$ (2 mM) | dGTP | - | - | - | 120 |

† Enzyme was preincubated for 15 minutes with NEM. Assay conditions are described in materials and methods.

synthesis). An occasional incorporation of a mismatched substrate (in this case dGTP) directed by poly dT · oligo dA was observed which appears to be catalyzed by DNA polymerase β in the presence of $Mn^{++}$ ions (fig. 2). This activity, however, was less than 0.1% of the activity seen with the correct substrate by β polymerase and exhibited identical response to NEM and inorganic phosphate (Table 1). Thus, we conclude that it is not a distinct enzyme activity, but is indeed DNA polymerase β.

DISCUSSION:

The molecular mechanism involved in the process of immunoglobulin diversity continues to be a challenging problem. Based on the exclusive presence of TdT in the thymocytes and T lymphocyte precursor cells, Baltimore proposed an interesting hypothesis (4 that wou ain

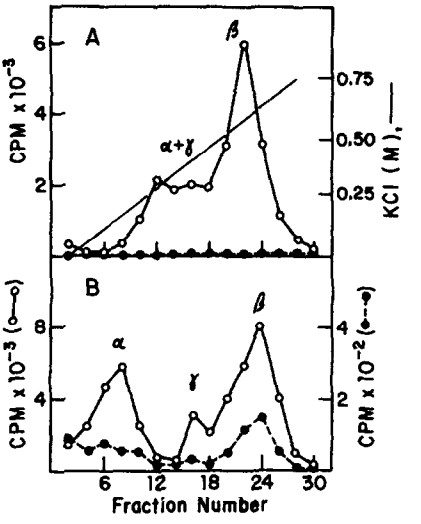


Fig. 2: A phosphocellulose chromatogram of extracts obtained from chicken bursa: Assays were carried out with various template primers and reaction mixtures as described in Materials and Methods. Frame A shows Poly (rA)·$(dT)_{12}$ directed (o———o) and oligo (dA) primed (•———•) synthesis. Frame B depicts Poly (dC)·$(dG)_{12-18}$ directed synthesis (o———o). The closed circles (•-------•) indicate incorporation of dGTP in the presence of Poly (dT)·$(dA)_{12-18}$.

the immunoglobulin diversity with TdT playing a pivotal role as somatic mutagen. This hypothesis, as well as our own desire to know status of TdT in immunodeficiencies, prompted us to search for similar enzymes in B precursor cells. Since tonsil in human and bursa of Fabricius in chicken are known to be a good source of precursor B cells, we examined the extracts of these two organs for the presence of B cell specific terminal transferase like enzyme. This study required identification of a spectrum of DNA polymerases that are known to be present in vertebrates (10).

Examination of a majority of tonsil extracts revealed that DNA polymerase α, β and γ are present. We have occasionally observed presence of thymic TdT in some tonsil samples (12); however, it appears to be thymic

TdT presumably due to infiltrating T precursor cells responding to local antigenic challenge. There was, however, no evidence of enzyme activity that will incorporate mismatched nucleotide.

In the case of chicken bursas, analogous to tonsil extracts, three known DNA polymerases have been identified. These enzymes have also been identified in chick embryo extracts (13-14) and appear to represent normal avian polymerases. The thymic TdT was absent in these extracts but in some samples DNA polymerase β appear to catalyze small amount of mismatched nucleotide incorporation. Whether this activity is inherent property of bursal β polymerase is not known at present. This observation certainly confirms the original report of Baltimore and colleagues, that mismatched nucleotide addition activity coelutes with that of β polymerase upon phosphocellulose chromatography (5), but disagrees with their later (corrected?) observation demonstrating presence of a distinct enzymatic activity in bursal extracts (6).

Further studies are in progress to clarify and characterize β polymerase from this source.

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