Evidence for the Presence of an Oncornaviral Reverse Transcriptase in an Orbital Tumor Associated to Acute Myelomonocytic Leukemia in Children: Biochemical and Immunological Characterization of the Enzyme

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Several reports suggest that human tumors contain a DNA polymerase with biochemical properties resembling reverse transcriptases, associated to RNA tumor viruses [1–6]. However, only in a few cases, these enzymes have been characterized serologically to make it certain, that they really are of viral origin [1–4]. Such studies, in particular, the immunological characterization of reverse transcriptases from human tumors are of great importance in considering the possibility that humans may harbor viruses related to oncornaviruses which induce tumors in non-human primates, or other animal species. For the last few years, it has been our objective to purify the cellular DNA polymerases (α, β and γ) and reverse transcriptases from human tumor biopsies, and characterize them biochemically as well as immunologically [2–4,7,8].

This communication describes the purification and characterization of a reverse transcriptase from an orbital tumor of a child with acute myelomonocytic leukemia (AMML). This tumor has been reported to be associated to AMML in children in some regions of Turkey [9, 10] (see R. Miller elsewhere in this volume). The tumor consists of primitive white blood cells with supportive connective tissue, stroma, and vessels. Since these tumors were devoid of the characteristic green color, and no periosteal or other bone changes were present, Cavdar et al. [9] suggest the term granulocytic sarcoma to be more appropriate than the chloroma. The striking feature is the occurrence of ocular lesions before the onset of AMML varying from 20 days to 8 months [9].

The methods employed in the isolation and purification of reverse transcriptase from the orbital tumor are reported elsewhere [2,3]. The activities of orbital tumor reverse transcriptase at various steps of purification, using different template-primers are documented in Table 1. The single peak of activity eluted from the phosphocellulose column at 0,21 M salt concentration (Fraction V, Table 1), represents a 1340-fold purification over the enzyme activity of the crude homogenate using poly rC. $(dG)_{12-18}$ as the template-primer, and 2781-fold purification using poly rA. $(dT)_{12}$. The optimum reaction conditions for the orbital reverse transcriptase activity with poly rA. $(dT)_{12}$ as template-primer were as follows: Ionic concentrations for Mn^{++}, 0.4 mM; Mg^{++}, 0.8 mM; and KCl, 60 mM; and the pH-optimum of the reaction was found to be around 7.8.

Table 1. DNA-polymerase activities at various purification steps

Source of protein and protein content	*Template-primer: divalent cation* Activity and Purification	$(dT)_{12-18} \cdot (A)_n$		$(dG)_{12-18} \cdot (C)_n$		$(dT)_{10} \cdot (dA)_n$	
		Mn^{++}	Mg^{++}	Mn^{++}	Mg^{++}	Mn^{++}	Mg^{++}
Crude Tumor Homogenate (I) 6710 mg/90 ml	Total[2]	9248.73	7339.43	2307.07	1398.83	11827.17	3145.97
	Specific[3]	1.38 (0.12)	1.09 (0.02)	0.35 (0.08)	0.21 (0.09)	1.76	0.47
	Purification fold	1.0	1.0	1.0	1.0	1.0	1.0
Disrupted Microsomal Pellet (II) 38.0 mg/25 ml	Total	1443.17	985.72	1248.91	1061.03	1328.34	272.48
	Specific	37.98 (0.66)	25.94 (0.03)	32.87 (0.79)	27.92 (1.71)	34.56	7.17
	Purification fold	27.52	23.80	93.91	132.95	19.28	15.29
0.35 M KCl Eluate off DEAE 23 Cellulose (III) 15.84 mg/36 ml	Total	1122.46	465.24	281.49	883.58	892.64	213.0
	Specific	70.86	29.37	17.77	55.78	56.35	13.45
	Purification fold	51.35	26.94	50.77	265.62	31.96	28.67
0.07 M KCl Eluate off DEAE 52 Cellulose (IV) 0.525 mg/21 ml	Total	573.84	204.36	255.43	80.28	264.03	57.22
	Specific	1093.03	389.03	486.53	152.91	502.92	108.99
	Purification fold	792.05	357.12	1390.09	728.14	285.26	232.39
0.21 M KCl Eluate off Phospho-cellulose (V) 0.01 mg/2 ml[1]	Total	38.38	6.46	4.69	1.56	0.01	<0.01
	Specific	3838.07	646.16	469.16	156.38	1.14	<0.01
	Purification fold	2781.21	592.81	1340.46	744.67	0.64	<0.5

DNA polymerase assays were carried out at 30°C for 60 min. in a reaction mixture of 0.05 ml. which contained: 50 mM Tris/HCl pH 7.8. 60 mM KCl. 0.4 mM $MnCl_2$. or 8 mM $MgCl_2$. and 1 mM DTT. The primer-template concentration used was 50 µg/ml; other conditions are the same as described by Chandra and Steel [2]. Numbers in brackets give the endogenous incorporation.

The purified orbital tumor reverse transcriptase showed a strong preference for the template-primers poly rA. $(dT)_{12}$, poly rC. $(dG)_{12-18}$ and poly-rC (OMe). $(dG)_{12-18}$, as seen in Table 2; whereas poly dA. $(dT)_{10}$ was clearly ineffective. These results agree with those reported for other mammalian C-type oncornavirus DNA polymerases [11]. The inability to utilize the primers $(dT)_{12-18}$ or $(dG)_{12-18}$ indicates no terminal transferase activity was present in the purified enzyme preparation. Transcription of heteropolymeric regions of a 70S RNA from R(Mu)LV, and stimulation of its utilization by addition of the primer $(dT)_{12-18}$ further support the oncogenic nature of the orbital enzyme and its similarity to other known RNA tumor virus reverse transcriptases.

Table 3 summarizes the responses of the orbital tumor and SiSV-1 reverse transcriptases when challenged with antibodies to various type-C virus DNA polymerases. The serological cross-reactivity of SiSV-1 DNA polymerase with antibodies to gibbon-ape leukemia virus (GaLV) reverse transcriptase was previously demonstrated by Sarin and Gallo [12]. As shown in Table 3, both the human orbital tumor and SiSV-1 enzymes demonstrated marked

Table 2. Primer-Template activities of the orbital tumor RNA-dependent DNA polymerase

Primer-Template	pmol ^{3}H-dNMP incorporated in 60 min. per mg protein	
Divalent cation	Mn^{2+}	Mg^{2+}
activated DNA	0.31	0.68
$(dT)_{12-18} \cdot (A)_n$	3476.11	603.07
$(dT)_{10} \cdot (dA)_n$	1.14	<0.01
$(dG)_{12-18} \cdot (C)_n$	461.01	174.92
$(dG)_{12-18} \cdot (OMeC)_n$	53.33	3.95
$(dG)_{12-18}$	<0.1	NT
RLV 70S RNA	138.22	NT
RLV 70S RNA + $(dT)_{12-18}$	262.61	NT
$(dT)_{12-18}$	<0.01	NT

Assays were carried out for 60 min. in a reaction mixture described under Table 1. The primer-template concentration used was 50 µg per ml: R(Mu)LV-70S RNA was used at a concentration of 20 µg/ml. ^{3}H-dTTP was used as the labeled substrate for activated DNA. poly rA · $(dT)_{12}$. poly dA · $(dT)_{10}$. R(Mu)LV-RNA and oligo dT. ^{3}H-dGTP was used as the labeled substrate for $(dG)_{12-18}$. poly rC · oligo dG. and Poly rC(OMe) · oligo dG. NT = not tested.

Table 3. Preliminary results of the effect of various type-C virus DNA polymerase antibodies on the reverse-transcriptase activity of the orbital tumor

Source of reverse transcriptase	IgG (µg)	Specific anti-reverse transcriptase (IgG) added: non-immune (control) IgG	anti-RLV RT IgG	anti-AMV RT IgG	anti-GaLV RT IgG
		^{3}H-dTMP incorporation (% of enzyme activity[a])			
SSV-1	8	93.4	91.2	94.5	17.4
	13	101	NT	NT	23.0
	32	101	79.0	NT	21.0
Orbital tumor from AMML patient	8	98.2	94.0	97.8	NT
	13	103	NT	NT	44.3
	32	114	93.2	NT	40.2

Orbital tumor DNA polymerase (10 µl) was incubated with 10 µl of non-immune rabbit sera IgG. or the indicated immune sera IgG at 4°C for 4 hr. before assaying for the enzyme activity. For comparison. SiSV-1 reverse transcriptase was similarly challenged with immune and non-immune sera. NT = not tested.

[a] The % of ^{3}H-TMP incorporation expresses activity. compared to that of enzyme without IgG.

inhibition with antibodies to GaLV-polymerase. The orbital tumor reverse transcriptase was not inhibited by antibodies to the DNA polymerase from two non-primate sources. avian myeloblastosis virus and Rauscher leukemia virus. at the concentrations challenged thus far. Owing to the low amounts of purified enzyme, repetition and further elaboration of the immunological data await the availability of more tumor material.

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