Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 7963–7967, October 1990
Medical Sciences

A monoclonal antibody that neutralizes Epstein–Barr virus, human cytomegalovirus, human herpesvirus 6, and bacteriophage T4 DNA polymerases

(antibody/viral enzyme)

CHING-HWA ANN TSAI*, MARSHALL V. WILLIAMS*†, AND RONALD GLASER*†‡

*Department of Medical Microbiology and Immunology, and †Comprehensive Cancer Center, The Ohio State University Medical Center, Columbus, OH 43210

Communicated by Leo A. Paquette, July 23, 1990 (received for review May 18, 1990)

ABSTRACT **A monoclonal antibody (mAb) designated 55H3 was produced by using chemically induced Epstein–Barr virus genome-positive B95-8 cells. mAb 55H3, which reacted with an 85- to 80-kDa polypeptide, neutralized Epstein–Barr virus-encoded DNA polymerase activity in crude extracts of chemically induced M-ABA, HR-1, and B95-8 cells, as well as the partially purified Epstein–Barr virus DNA polymerase in a dose-dependent manner. The mAb also neutralized the virus-encoded DNA polymerase activity from cells infected with human cytomegalovirus, human herpesvirus 6, and the purified bacteriophage T4 DNA polymerases. However, mAb 55H3 did not neutralize the DNA polymerase activities encoded for by herpes simplex virus types 1 and 2, the reverse transcriptase of avian myeloblastosis virus, or *Escherichia coli* DNA polymerase 1 (Klenow fragment). These results suggest that mAb 55H3 recognizes an epitope common to some herpesviruses and T4 DNA polymerases and further supports the hypothesis that these organisms are evolutionarily related.**

Members of the poxviruses, adenoviruses, and herpesviruses encode specific DNA polymerases (1–6). These DNA polymerases are not only required for replication of these viruses, but they can also be used as a target site for development of antiviral agents. While there have been advances in our understanding of the conformation of various DNA polymerases and their interaction with various substrates and with template DNA, little is known about the interaction of viral DNA polymerase with other proteins involved in the DNA replication complex.

Epstein–Barr virus (EBV), a human herpesvirus, is the etiological agent of infectious mononucleosis and is associated with African Burkitt lymphoma and nasopharyngeal carcinoma (7–11). EBV is similar to other herpesviruses in that it encodes a DNA polymerase essential for virus replication (12, 13). The EBV-encoded DNA polymerase activity has been purified from several EBV genome-positive cells, and at least four polypeptides with molecular masses of 110, 100, 55, and 49 kDa have been associated with this activity (14, 15). Although the 110-kDa polypeptide may be the DNA polymerase (14), studies have demonstrated that the 55- and 49-kDa proteins (15), as well as a 45-kDa stimulatory protein (16), are required for EBV DNA polymerase activity *in vitro*. Elucidation of these protein interactions is important not only for understanding how EBV replicates but also in determining the role of these polypeptides in diseases caused by EBV.

While sera from patients with nasopharyngeal carcinoma contain antibodies that neutralize EBV DNA polymerase, the sera also contain antibodies against the EBV DNA polymerase stimulatory protein (16–19). Similarly, although a rabbit polyclonal antibody has been developed that can inhibit EBV DNA polymerase activity (20), it is not known whether this polyclonal antibody neutralizes the DNA polymerase activity by binding to the enzyme or due to its binding to one of the associated polypeptides. Thus, further studies on the interactions of these polypeptides have been hampered, in part, from the lack of specific antibodies against the EBV-encoded DNA polymerase.

Recently, we reported the isolation of an EBV-specific monoclonal antibody (mAb), designated 55H3, that neutralizes the activity of EBV-encoded DNA polymerase (21). In a preliminary study, it was possible to demonstrate that mAb 55H3 could also neutralize a DNA polymerase activity induced in cells infected with human herpesvirus 6 (HHV6) (22). In this report, we show that this mAb neutralizes activity of the partially purified EBV DNA polymerase, the bacteriophage T4 and HHV6 DNA polymerase, and the DNA polymerase activity induced by human cytomegalovirus (HCMV). mAb 55H3 did not inhibit the DNA polymerase activity in cells infected with herpes simplex virus (HSV) types 1 or 2, purified *Escherichia coli* DNA polymerase 1, or avian myeloblastosis virus (AMV) reverse transcriptase.

MATERIALS AND METHODS

Cell Lines. HR-1, B95-8, and M-ABA cells were grown in RPMI 1640 medium/10% fetal bovine serum/2 mM glutamine containing gentamicin at 25 μg/ml. Cell lines were maintained at 35°C.

EBV DNA Polymerase Neutralization Assay. The neutralization assay was done as described (21, 22). Cells (5×10^7) were treated with phorbol 12-myristate 13-acetate at 40 ng/ml and 3 mM sodium butyrate for 48 hr at 37°C to induce EBV reactivation as described (22). After low-speed centrifugation, cell pellets were resuspended in extraction buffer (1 ml of 50 mM Tris·HCl, pH 7.2/2 mM ATP/3 mM dithiothreitol/0.2 M KCl) and sonicated on a Branson Sonifier model 350 (15 pulses, microtip, output setting 4). The resulting homogenate was centrifuged in a Fisher model 235A microcentrifuge for 3 min; supernatants were used for the enzyme assays. Cell extracts containing DNA polymerase activity (10 μl) were incubated for 30 min at room temperature in an equivalent amount of water containing variable amounts of bovine serum albumin (BSA), normal mouse serum, mouse myeloma ascites fluid, or mAb 55H3. After the initial incubation, the volume of the reaction mixture was increased to 0.1 ml by adding (final concentration) 70 mM Tris·HCl, pH 8.0/0.2 M KCl/4 mM $MgCl_2$/70 μg of BSA/0.7 mM dithiothreitol/2%

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: EBV, Epstein–Barr virus; mAb, monoclonal antibody; HSV, herpes simplex virus; HHV6, human herpesvirus 6; EA, early antigen; HCMV, human cytomegalovirus; BSA, bovine serum albumin; AMV, avian myeloblastosis virus.

‡To whom reprint requests should be addressed at: Department of Medical Microbiology and Immunology, The Ohio State University Medical Center, 333 West 10th Avenue, Columbus, OH 43210.

(vol/vol) glycerol/100 μM (each) dATP, dGTP, and dCTP/1.6 μM [^{3}H]dTTP (500 μCi/nmol; 1 Ci = 37 GBq)/13 μg of activated calf thymus DNA, and the mixture was allowed to incubate an additional 30 min at 37°C. Reactions were terminated as described (21, 22). Values represent the average of triplicate assays. A unit of DNA polymerase activity was defined as the amount of enzyme required to incorporate 1 pmol of dTTP into acid-insoluble material per hr per ml under our assay conditions. The difference of enzyme activity with and without ascites fluid or purified IgG was determined by using the above assay and reported as units of DNA polymerase activity neutralized per ml of test sample.

Purification of EBV-Encoded DNA Polymerase from Chemically Induced B95-8 Cells. DNA polymerase activity was purified as described (14, 15). All purification procedures were performed at 4°C, and all buffers contained 0.2 mM phenylmethylsulfonyl fluoride and pepstatin A at 0.1 μg/ml to prevent proteolytic digestion of the enzyme. The amount of protein was determined using the Bio-Rad Coomassie blue dye-binding reagent with BSA as standard. Briefly, ≈10^9 cells were chemically induced as described. At harvesting, cells were 45–50% early antigen (EA)/virus capsid antigen positive, as determined by immunofluorescence. Cell pellets were resuspended in 400 mM Tris·HCl, pH 7.2/150 mM KCl/1 mM EDTA/10 mM 2-mercaptoethanol (buffer A) and were sonicated as described. Crude homogenates were centrifuged (60 min, 10,000 rpm in a Beckman model J2-21 in a JA20 rotor at 4°C), and the resulting supernatants were applied to a DEAE-Sephacel column (1 × 10 cm) equilibrated in buffer A. Under these conditions the DNA polymerase activity did not bind to the matrix and was eluted with buffer A. Fractions (3 ml) were collected and assayed for DNA polymerase activity by using the standard assay described. Fractions containing the enzyme activity were pooled and dialyzed overnight against buffer B [20 mM potassium phosphate, pH 7.2/100 mM KCl/1 mM EDTA/20% (vol/vol) glycerol]. The dialysate was applied to a double-stranded DNA cellulose column (1 × 8 cm) equilibrated in buffer C (buffer B contained a final KCl concentration of 150 mM). The DNA polymerase was bound to the matrix and eluted by increasing the ionic strength using a linear gradient buffer C and buffer D (buffer B contained a final KCl concentration of 800 mM). Fractions (3 ml) were collected and assayed for DNA polymerase activity. Fractions containing the EBV DNA polymerase activity were pooled and dialyzed overnight against buffer B. The dialysates were applied to a phosphocellulose column (1 × 8 cm) equilibrated in buffer C. Viral DNA polymerase was bound to the matrix and eluted by increasing the ionic strength using a linear gradient of buffers C and D. Fractions (1.5 ml) were collected, and those fractions containing viral DNA polymerase activity were pooled. KCl was added to a final concentration of 400 mM, and the sample was applied to a Blue Sepharose column (1 × 2 cm) equilibrated in buffer C. Under these conditions EBV DNA polymerase does not bind to the matrix. Fractions (3 ml) were collected and used for subsequent experiments.

Immunodepletion of EBV DNA Polymerase by mAb 55H3. The procedure used for immunoprecipitation of EBV-encoded DNA polymerase by the various antibodies has been described (23). Partially purified (phosphocellulose fraction) EBV DNA polymerase was used in these studies. Briefly, DNA polymerase (6.0 units, in 15 μl) was treated with various dilutions of the test reagents (12–15 mg of protein per ml) for 1 hr at 4°C. After initial incubation, a protein A-Sepharose suspension (25 μl) was added to the DNA polymerase–antibody mixture, and the mixture was then incubated with rotation at 4°C for 90 min. The mixture was centrifuged at 4°C (10 min, Fisher Microcentrifuge model 235A), and the supernatants were tested for residual DNA polymerase activity by using the standard assay. Values represent the average of triplicate assays.

Neutralization of Various DNA Polymerases by mAb 55H3. T4 DNA polymerase (cloned), *E. coli* (Klenow fragment) DNA polymerase 1 (cloned), and AMV reverse transcriptase were purchased from United States Biochemical. Virus-infected KB cells were the source of the HSV-1 (strain KOS) and HSV-2 (strain HG-52)-encoded DNA polymerases. HCMV (strain VHLE)-infected epithelial cells (HUVE) (24) were from Jim Waldman (Ohio State University) and HHV6-infected HSB2 cells were from Dharam Ablashi (National Cancer Institute). HCMV-, HHV6-, and HSV-infected cells were extracted as described. Crude cell extracts were used as a source of DNA polymerase. Neutralization assays were performed, as described, except that different reaction mixtures were used to access the activities of the various DNA polymerases. The activities of HCMV, HHV6, and HSV DNA polymerases were determined by using the reaction mixtures described for EBV. For T4 DNA polymerase activity, the reaction mixture was (in a total volume of 0.1 ml) 59 mM Tris·HCl, pH 8.0/5.9 mM $MgCl_2$/8.9 mM 2-mercaptoethanol/70 μg of BSA/13 μg of activated calf thymus DNA/29.4 μM (each) of dGTP, dCTP, and dATP/[^{3}H]dTTP (0.17 μCi/nmol)/3 units of polymerase, as defined by the manufacturer/various amounts of mAb. For *E. coli* DNA

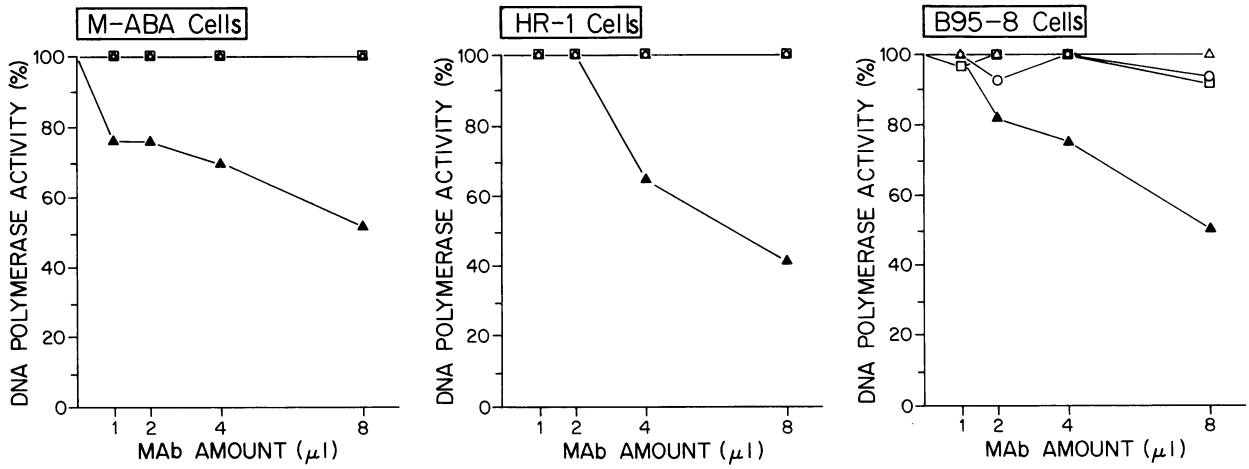

FIG. 1. Effect of mAb 55H3 (MAb) on DNA polymerase activity induced by different EBV strains. (*Left*) M-ABA cells, 25–30% EA-positive, 153 units per assay. (*Middle*) HR-1 cells, 40–45% EA-positive, 249 units per assay. (*Right*) B95-8 cells, 35–40% EA-positive, 133 units per assay. △, BSA; ○, mouse serum; □, mouse ascites; and ▲, 55H3 mAb. The difference of enzyme activity with and without ascites fluid or purified IgG was determined by using the above assay and reported as units of DNA polymerase activity neutralized per ml of test sample.

Table 1. Purification of EBV DNA polymerase from activated B95-8 cells

	Total protein, mg	Total activity, units	Specific activity, units/mg	Recovery, %	Purification, -fold
Cell extract	70.17	15,209	215.08	100	1
DEAE-Sephacel	64.35	14,638	227.47	96.25	1.06
DNA cellulose	3.948	12,600	3,191.49	82.85	14.84
Phosphocellulose	0.252	2,407	9,551.69	15.84	44.41
Blue Sepharose	0.02625	1,575	60,000.01	10.36	278.97

polymerase, the reaction mixture was (in a total volume of 0.1 ml) 70 mM Tris·HCl, pH 7.5/4 mM $MgCl_2$/1 mM dithiothreitol/2% (vol/vol) glycerol/70 μg BSA/13 μg of activated calf thymus DNA/100 μM (each) dCTP, dGTP, and dATP/1 μM [^{3}H]dTTP (2.7 μCi/nmol)/0.1 unit of polymerase as defined by the manufacturer/various amounts of mAb. For AMV reverse transcriptase, the reaction mixture was (in a total volume of 0.05 ml) 50 mM Tris·HCl, pH 8.0/10 mM dithiothreitol/0.05% (vol/vol) Triton X-100/5 μg of BSA/6 mM KCl/0.0256 unit of poly(A)·dT_{15}/25 μM [^{3}H]dTTP (6.15 μCi/nmol), 5 units of enzyme, as defined by the manufacturer/various amounts of mAb.

RESULTS

EBV DNA Polymerase Neutralization Assay. Fig. 1 shows that BSA, normal mouse serum, and mouse myeloma ascites fluid had no effect on activities of the EBV-encoded DNA polymerases expressed in the various cell lines. However, mAb 55H3 significantly inhibited DNA polymerase activities in a dose-dependent manner. These data suggest that inhibition of EBV DNA polymerase activity resulted from a specific interaction between the mAb and a polypeptide required for DNA polymerase activity under the *in vitro* conditions used in these experiments. mAb 55H3 was purified by using a protein A-Sepharose column and identified as IgG1 (21).

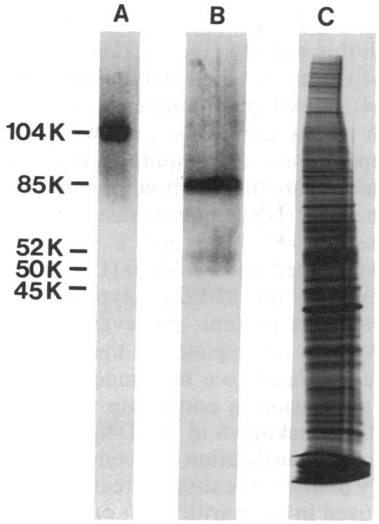

FIG. 2. SDS/PAGE of EBV-encoded DNA polymerase and T4 DNA polymerase; SDS/PAGE was performed as described (26). Stacking gel was 5% acrylamide/0.13% bisacrylamide/125 mM Tris·HCl, pH 6.8/0.1% SDS/3.3 mM *N,N,N',N'*-tetramethylethylenediamine/0.1% ammonium persulfate. Separating gel was 10% acrylamide/0.13% bisacrylamide/375 mM Tris·HCl, pH 8.8/0.1% SDS/2.2 mM *N,N,N',N'*-tetramethylethylenediamine/0.03% ammonium persulfate. Running buffer was 192 mM glycine/25 mM Tris·base/0.1% SDS. Samples were electrophoresed at 100 V until samples entered stacking gel and then at 200 V for 3–4 hr. After electrophoresis, gel proteins were silver stained by using the procedure described by Tunon and Johansson (26). Molecular mass standards were as follows: myosin, 200 kDa; phosphorylase b, 92.5 kDa; bovine serum albumin, 69 kDa; ovalbumin, 46 kDa; carbonic anhydrase, 30 kDa; and trypsin inhibitor, 21.5 kDa. Silver stainings of T4 cloned DNA polymerase (lane A), EBV DNA polymerase (Blue Sepharose preparation) (lane B), and crude cell extract (lane C) are shown.

Purification of EBV-Encoded DNA Polymerase from Chemically Induced B95-8 Cells. To determine whether inhibition of EBV DNA polymerase activity by mAb 55H3 was from the interaction between the mAb and DNA polymerase or another polypeptide required for DNA polymerase activity, EBV DNA polymerase was purified from chemically induced B95-8 cells by using conventional chromatography techniques (Table 1).

SDS/PAGE of the EBV DNA polymerase activity after Blue Sepharose chromatography, as described by Laemmli (25), demonstrated four polypeptides with molecular masses of ≈45, 50, 52, and 85 kDa by silver staining (26) (Fig. 2). Western (immuno-) blotting showed that mAb 55H3 reacted with an 85-kDa polypeptide in crude extracts of chemically induced B95-8 cells, but no reactivity was seen with any polypeptide when the partially purified DNA polymerase was used; this result was probably due to the small amounts of protein in this preparation. However, neutralization studies using the partially purified EBV-encoded DNA polymerase demonstrated that mAb 55H3 neutralized EBV DNA polymerase activity in a dose-dependent manner (Fig. 3). Conversely, BSA, mouse serum, and mouse myeloma ascites, used as controls, had no effect on the enzyme activity.

Immunodepletion of EBV DNA Polymerase by mAb 55H3. Immunodepletion studies further showed the ability of mAb 55H3 to inhibit DNA polymerase activity in the *in vitro* assay. The partially purified EBV DNA polymerase preparation was pretreated with BSA, mouse serum, mouse myeloma ascites, or mAb 55H3. Immune complexes were removed with pro-

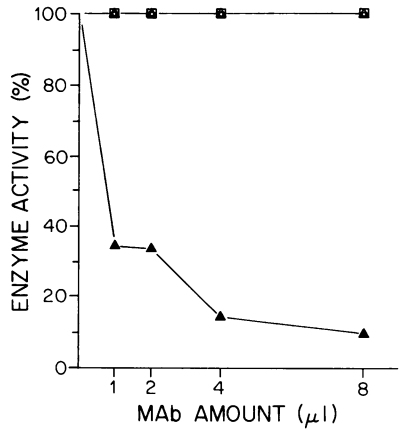

FIG. 3. Dose-dependent neutralization of purified EBV-encoded DNA polymerase by mAb 55H3 (MAb). Neutralization assays were done as described by using purified DNA polymerase (4.4 units per assay). Amount of protein in each test serum and BSA were adjusted so that equivalent amounts of protein were used in each assay (12–15 mg/ml). △, BSA; ○, mouse serum; □, mouse ascites; and ▲, mAb 55H3.

tein A-Sepharose. The preparations were then assayed for residual DNA polymerase activity. At equivalent protein concentrations, mAb 55H3 significantly reduced DNA polymerase activity, and the reduction was dose-dependent (Table 2).

Neutralization of Various DNA Polymerases by mAb 55H3. Recent studies demonstrated that various DNA polymerases from both prokaryotic and eukaryotic organisms exhibit extensive amino acid homology and have highly conserved functional domains (27–33). To determine whether mAb 55H3 could neutralize DNA polymerases from various organisms, neutralization studies were done with purified T4 DNA polymerase (Fig. 2), *E. coli* DNA polymerase 1 (Klenow fragment), AMV reverse transcriptase, and the DNA polymerases from crude cell extracts of cells infected with HSV-1 and HSV-2, HCMV, and HHV6 (Table 3). mAb 55H3 inhibited the DNA polymerase activity in cell extracts from cells infected with HCMV and HHV6; similarly, mAb 55H3 inhibited T4 DNA polymerase activity. The inhibition of these DNA polymerase activities by mAb 55H3 depended on the amount of antibody used. mAb 55H3 did not neutralize activities of AMV reverse transcriptase or *E. coli* DNA polymerase, and it did not inhibit the DNA polymerase activities in cells infected with HSV types 1 or 2. The lack of neutralizing activity to HSV-1 and HSV-2 DNA polymerases was not from hydrolysis of the antibody in the crude cell extracts because similar results were seen with partially purified enzymes (data not shown).

DISCUSSION

Studies have demonstrated that strain differences exist among isolates of EBV (34, 35). However, the importance of these strain differences in virus replication and in the ability of EBV to cause disease is unknown. Because mAb 55H3 was produced using phorbol 12-myristate 13-acetate-induced B95-8 cells (21), our initial approach was to determine (*i*) whether mAb 55H3 could neutralize activity of the EBV-encoded DNA polymerase induced in various EBV genome-positive producer cell lines and (*ii*) whether the neutralization activity was due to immunoglobulin (IgG) rather than to some nonspecific inhibitor present in ascites fluid; these were established positively in an earlier study (21).

The data presented here suggest that mAb 55H3 specifically neutralizes DNA polymerase activity induced by several herpesviruses and by T4 by its ability to recognize a common epitope on these polypeptides. This premise is supported by the data showing that mAb 55H3 neutralized the purified T4 DNA polymerase. Although we have not identified this epitope, analysis of sequence data of the five functional domains common to the herpesviruses and T4 indicate that EBV, HCMV, and T4, but not HSV, contain an arginine residue at the 3′ end of domain IV, the domain proposed to be involved with DNA primase interaction. If this is the epitope recognized by mAb 55H3, it could explain how this mAb neutralizes DNA polymerase activity of HCMV, EBV, HHV6, and T4, but not of HSV. However, regardless of the epitope recognized by mAb 55H3, these data support the hypothesis that T4 and certain animal virus DNA polymerases are evolutionarily related (27–33).

Table 2. Dose-dependent immunodepletion of purified EBV DNA polymerase

Protein added, μg	DNA polymerase activity, units*				
	BSA	Protein A + BSA	Mouse sera	Mouse ascites	mAb 55H3
15	593 ± 1	486 ± 16	534 ± 17	419 ± 1	310 ± 1
30	546 ± 18	447 ± 2	545 ± 1	712 ± 1	201 ± 1
60	554 ± 1	457 ± 1	399 ± 1	560 ± 29	58 ± 1
120	757 ± 1	518 ± 6	279 ± 34	493 ± 12	10 ± 1

*Values are reported as units of DNA polymerase activity neutralized per ml of serum. Values represent an average of at least two determinations ± SD.

Table 3. Neutralization of DNA polymerases and reverse transcriptase activity by mAb 55H3

Enzyme source	Polymerase activity*		Activity remaining, %
	BSA	55H3	
HSV-1	2.65 ± 0.72	2.05 ± 0.54	77.4†
HSV-2	4.05 ± 0.46	3.43 ± 0.72	84.7†
HCMV	4.25 ± 0.53	1.04 ± 0.24	24.5
HHV6	0.17 ± 0.03	0.03 ± 0.01	17.6
T4	31.35 ± 2.31	10.04 ± 1.64	32.0
E. coli	4.21 ± 0.14	4.20 ± 0.12	99.8
AMV	74.49 ± 6.58	63.78 ± 4.81	85.6†

*nmol of dTTP incorporated per hr per ml. Values represent the average of at least two determinations ± SD.
†The level of neutralization did not significantly differ from that seen by using mouse IgG or myeloma ascites (data not shown).

Several antigen complexes have been identified in EBV-infected cells. The EA complex, which is induced in cells undergoing abortive or permissive replication of EBV, is composed of a diffuse component and a restrictive component (36). The EA complex has been of interest because elevated antibody titers to this complex are found in patients with EBV-associated diseases (7–11), and recent studies have demonstrated that EBV DNA polymerase activity is associated with part of the diffuse component of EA (15). Attempts to purify EBV DNA polymerase from chemically induced HR-1 and Raji cells have shown that at least four polypeptides with molecular masses of 110, 100, 55, and 49 kDa are associated with this activity (14, 15). The 110-kDa polypeptide is thought to represent the catalytic portion of the DNA polymerase (14), based upon sequence data of the B95-8 virus, although recent studies have shown that the 55- and 49-kDa polypeptides are also required for activity (15, 37). Our results demonstrate that the predominant polypeptide in our partially purified DNA polymerase preparation has a molecular mass of ≈85 kDa, and a polypeptide of this molecular mass reacted with mAb 55H3 in crude extracts. This fact suggests that this 85-kDa polypeptide may be EBV DNA polymerase. At present, however, we do not know whether the apparent discrepancy in the molecular mass of the polymerase is related to a host modification (reflecting differences in expression in cotton-top tamarin and human cells) or from the breakdown of the DNA polymerase polypeptide during our purification procedures. We view the latter possibility unlikely because protease inhibitors were in all the buffers used in the purification protocol and because the preparation retains DNA polymerase activity. However, results of this study suggest that mAb 55H3 could be useful for examining evolutionary relationships between the herpesviruses and other eukaryotic and prokaryotic viruses, for determining how other polypeptides necessary for EBV replication interact with the EBV DNA polymerase, and in determining the role(s) of these polypeptides in diseases associated with EBV.

This work was supported, in part, by Ohio State University Comprehensive Cancer Center Grant CA-16058, Grant DE-06866 from the National Institute for Dental Research (M.V.W.), and Grant ES-00163 from the National Institute for Environmental Health Science (M.V.W.), Research Career Development Award.

1. Moss, B. & Cooper, N. (1982) *J. Virol.* **43**, 673–678.
2. Nagata, K., Guggenheimer, R. A. & Hurwitz, J. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6177–6181.

3. Gibbs, J. S., Chiou, H. C., Hall, J. D., Mount, D. W., Retondo, M. J., Weller, S. K. & Coen, D. M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7969–7973.
4. Quinn, J. P. & McGeoch, D. J. (1985) *Nucleic Acids Res.* **13,** 8143–8163.
5. Davison, A. J. & Scott, J. E. (1986) *J. Gen. Virol.* **67,** 1759–1816.
6. Kouzarides, A., Bankier, A. T., Satchwell, S. C., Tomlinson, P. & Barrell, B. G. (1987) *J. Virol.* **61,** 125–133.
7. zur Hausen, H., Schulte-Holthausen, H., Klein, G., Henle, W., Henle, G., Clifford, P. & Santesson, L. (1970) *Nature (London)* **228,** 1056–1058.
8. Henle, G., Henle, W., Klein, G., Gunven, P., Clifford, P., Morrow, R. H. & Ziegler, J. L. (1971) *J. Natl. Cancer Inst.* **46,** 861–871.
9. Nonoyama, M., Huang, C. H., Pagano, J. S., Klein, G. & Singh, S. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 3265–3268.
10. Henle, W., Henle, G., Gunven, P., Klein, G., Clifford, P. & Singh, S. (1973) *J. Natl. Cancer Inst.* **50,** 1163–1173.
11. Henle, W., Ho, H. C., Henle, G. & Kwan, H. C. (1973) *J. Natl. Cancer Inst.* **51,** 361–369.
12. Miller, R. L., Glaser, R. & Rapp, F. (1977) *Virology* **76,** 494–502.
13. Goodman, S. R., Przyna, C. & Benz, W. D. (1978) *J. Biol. Chem.* **253,** 8617–8628.
14. Kallin, B., Sternas, L., Saemundssen, A. K., Luka, J., Jornvall, H., Eriksson, B., Tao, P. Z. & Klein, G. (1985) *J. Virol.* **54,** 561–568.
15. Li, J. S., Zhou, B. S., Dutschman, G. E., Grill, S. P., Tan, R. S. & Cheng, Y. C. (1987) *J. Virol.* **61,** 2947–2949.
16. Chiou, J. F., Li, J. K. K. & Cheng, Y. C. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5728–5731.
17. Cheng, Y. C., Chen, J. Y., Glaser, R. & Henle, W. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 6162–6165.
18. Glaser, R., Tan, R. S., Cheng, Y. C., Moeschberger, M., Holliday, J., Yao, T. H., Wu, H. Y., Mu, M. Y., Li, Y. W., Hu, Y. H., Wu, B., Cai, W. M. & Clausen, K. (1983) in *Nasopharyngeal Carcinoma: Current Concepts* (Univ. Malaysia Press, Kuala Lumpur, Malaysia), pp. 103–113.
19. Tan, R. S., Li, J. S., Gill, S. P., Nutter, L. M. & Cheng, Y. C. (1986) *Cancer Res.* **46,** 5024–5028.
20. Petit, R. G., Leung, K. & Shaw, J. E. (1987) *J. Virol.* **61,** 3331–3334.
21. Tsai, C. H., Williams, M. V. & Glaser, R. (1990) *Intervirology* **31,** 215–222.
22. Williams, M. V., Ablashi, D. V., Salahuddin, S. Z. & Glaser, R. (1989) *Virology* **173,** 223–230.
23. Gallo, M., Dorsky, D., Crumpacker, C. & Parris, D. S. (1989) *J. Virol.* **63,** 5023–5029.
24. Waldman, W. J., Sneddon, J. M., Stephens, R. E. & Roberts, W. (1989) *J. Med. Virol.* **28,** 223–230.
25. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
26. Tunon, P. & Johansson, K. E. (1984) *J. Biochem. Biophys. Methods* **9,** 171–179.
27. Earl, P. L., Jones, E. V. & Moss, B. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3659–3663.
28. Bernad, A., Zaballos, A., Salas, M. & Blanco, L. (1987) *EMBO J.* **6,** 4219–4225.
29. Larder, B. A., Kemp, S. D. & Darby, G. (1987) *EMBO J.* **6,** 169–175.
30. Knopf, C. W. & Weisshart, K. (1988) *Biochim. Biophys. Acta* **951,** 298–314.
31. Wong, S. W., Wahl, A. F., Yuan, P. M., Arai, N., Pearson, B. E., Arai, K., Korn, D., Hunkapiller, M. W. & Wang, T. S. F. (1988) *EMBO J.* **7,** 37–47.
32. Wang, T. S. F., Wong, S. & Korn, D. (1989) *FASEB J.* **3,** 14–21.
33. Spicer, E. S., Ruch, J., Fung, C., Reha-Krantz, L. J., Karam, J. D. & Konigsberg, W. H. (1988) *J. Biol. Chem.* **263,** 7478–7486.
34. Kieff, E., Dambaugh, T., Heller, M., King, W., Cheung, A., van Santen, V., Hummel, M., Beisel, C., Fennewald, S., Hennessy, H. & Heiniman, T. (1982) *J. Infect. Dis.* **146,** 506–517.
35. Tarr, K. & Glaser, R. (1989) *J. Med. Virol.* **29,** 46–52.
36. Henle, G., Henle, W. & Klein, G. (1971) *Int. J. Cancer* **8,** 272–282.
37. Baer, R., Bankier, A. T., Biggin, M. D., Deininger, P. L., Farrel, P. J., Gibson, T. J., Hatfull, G., Hudson, G. S., Satchwell, S. C., Tuffnell, P. S. & Barrell, B. G. (1984) *Nature (London)* **310,** 207–211.