Purification and Properties of Epstein-Barr Virus DNase Expressed in *Escherichia coli*

MARIE-CLAUDE STOLZENBERG AND TADAMASA OOKA*

Laboratoire de Virologie Moléculaire, UM30, CNRS, Faculté de Médecine Alexis Carrel, Rue Guillaume Paradin, 69372 Lyon Cedex 08, France

Received 7 June 1989/Accepted 8 September 1989

A cDNA corresponding to the BGLF5 open reading frame of the Epstein-Barr virus (EBV) genome and coding for an early DNase was inserted into the procaryotic expression vector pKK223-3. One bacterial clone producing the expected 52-kilodalton DNase was used as a source of EBV DNase. The 52-kilodalton DNase was purified in the active form to near homogeneity by ammonium sulfate precipitation and successive chromatographies on phosphocellulose, DNA-cellulose, and gel filtration columns. The purified enzyme exhibited both exonuclease and endonuclease activities, an absolute requirement for divalent cations, an alkaline pH preference, and a typical residual activity in presence of 300 mM KCl. Moreover, the enzyme was specifically inhibited by human sera with high antibody titers to EBV early antigens. These properties are similar to those observed for EBV-induced DNase from lymphoblastoid cell extracts. In addition, the enzyme was recognized by both immunoglobulin G and A serum fractions from patients with nasopharyngeal carcinoma (NPC). From these results and previous studies which demonstrated the value of antibody titers to this viral DNase as an NPC marker, it appears that EBV-encoded DNase produced in a heterologous expression system could be used in the development of a specific and early NPC diagnosis test.

Epstein-Barr virus (EBV), an ubiquitous human herpesvirus, is the causative agent of infectious mononucleosis and is associated with numerous tumors including Burkitt's lymphoma (28), B-cell lymphomas induced in immunosuppressed people (4, 12), and nasopharyngeal carcinoma (NPC) (17). More than 95% of NPC biopsy specimens contain the EBV genome (39, 45). Furthermore, elevated titers of both immunoglobulin G (IgG) and IgA directed against viral capsid antigens (VCA) and early antigens (EA) correlate with a high risk of developing NPC and constitute a valuable marker not only for the diagnosis but also for the prognosis of the disease (18).

The EA probably include a complex involved in the synthesis of EBV DNA. In fact, EBV-induced DNA polymerase (37, 38), thymidine kinase (19, 29, 35), ribonucleotide reductase (T. Ooka, C. X. Zhang, Y. Langelier, S. Bacchetti, G. Decaussin, P. Chavrier, M. Tessier, and J. Daillie, Abstr. 12th Int. Herpesvirus Workshop, 1987, p. 97), and DNase (10, 36) have been identified and considered as EA. EBV-specific DNase appears to be important for NPC diagnosis since extensive studies have demonstrated that high antibody titers against EBV-induced DNase characterize NPC patient sera and are correlated in healthy individuals with the risk of developing NPC (7-9, 43). In our laboratory, an early DNase-encoding gene was identified and localized on the BGLF5 open reading frame (ORF) of the EBV genome (44). The corresponding cDNA sequence was expressed in vitro into a 52-kilodalton (kDa) DNase which could be inhibited by EA-positive sera and was partially resistant to 300 mM KCl, similar to the EBV-induced early DNase previously identified in lymphoblastoid cell lines (LCLs) (36, 44). However, the precise role of EBV DNase is not yet known. Several possible functions of this enzyme in virus replication have been put forward. Feighny et al. (22) suggested its intervention in cellular DNA degradation, either to provide nucleotides for EBV DNA synthesis or to

stop the host-cell metabolism. A direct action of EBV DNase on viral DNA replication has also been claimed, as suggested for other viral DNases such as herpes simplex virus (HSV) alkaline DNase (10, 13, 14, 34, 41). Like other viral nucleases, EBV DNase may contribute in many ways to viral DNA synthesis: fidelity of DNA replication, recombination, maturation of viral concatemers into unit-length DNAs (5). Further characterization of this enzyme is thus required to elucidate its role in EBV replication. For this purpose, we tried to obtain a sufficient quantity of pure DNase cleared from any other proteins possibly involved in EBV DNA synthesis.

We report here the successful expression of EBV DNase in *Escherichia coli* from the cDNA sequence inserted into the vector pKK223-3. The enzyme was highly purified and compared for its enzymatic and biochemical properties with EBV-induced DNase from EBV-carrying LCLs and with HSV-encoded DNase. Since the purified DNase was specifically recognized by both IgG and IgA fractions of NPC sera, the possible use of this enzyme for a NPC early diagnosis test was examined.

MATERIALS AND METHODS

Construction and expression of recombinant plasmid. The cDNA c37 sequence (1.53 kilobases) corresponding to the BGLF5 ORF (2) was inserted into the plasmid pKK223-3 (Pharmacia, Uppsala, Sweden) under the transcriptional control of a *tac* promoter (16). The cDNA c37 was excised from a previous construction (44), and its linearized sequence was partially digested with *Bal* 31 nuclease (New England BioLabs, Inc., Beverly, Mass.) by the standard method (30) to eliminate DNA sequences flanking the cDNA coding region. cDNA c37 overhanging ends were then filled in with T4 DNA polymerase, and the resulting cDNA was ligated to the vector (Fig. 1). The recombinant plasmid was used to transform either the *lacI^r* strain of *E. coli* JM105 (induction of the *tac* promoter with isopropyl- β -D-thiogalac-

* Corresponding author.

FIG. 1. Construction of EBV DNase recombinant vector. (A) Map of B95-8 EBV genome (15) and localization of ORFs BGLF5 (122342 to 120932 base pairs [bp]) and BGLF4 (123693 to 122328 bp). Kb, Kilobases. (B) Localization of the cDNA c37 sequence on the B95-8 EBV genome (122451 to 120907 bp) and construction of EBV DNase recombinant vector by insertion of cDNA c37 into pKK223-3.

topyranoside) or the C600R5 strain (constitutive activity of the *tac* promoter).

The sequences of both extremities of the c37 insert were analyzed by the method of Maxam and Gilbert (32).

Purification of EBV DNase from *E. coli* recombinant cells. All procedures for purification were done at 0 to 4°C unless otherwise stated.

Transformed *E. coli* cells were grown overnight (optical density of ca. 2.0) in LB broth containing 50 µg of ampicillin per ml. Approximately 6 g of bacterial cells was harvested from 1.5 liters of culture and suspended in 50 ml of buffer A (50 mM Tris hydrochloride [pH 7.5], 100 mM NaCl, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride). The bacteria were lysed with lysozyme and sodium deoxycholate as described elsewhere (31). After cell lysis, solid NaCl was added up to 1 M and the suspension was gently shaken for 2 h to release the DNA-binding proteins. The DNA was then precipitated by adding 0.25 ml of a 10% (vol/vol) solution of polyethyleneimine (Miles Laboratories, Inc., Elkhart, Ind.) per g of bacteria and by diluting this mixture 1.5-fold with buffer A containing 1 M NaCl. The suspension was clarified by centrifugation at $10,000 \times g$ for 30 min, and the pellets were reextracted in a Dounce homogenizer with 1.5 ml of buffer A containing 1 M NaCl per g of bacteria. The resulting supernatants were pooled, and solid ammonium sulfate was added up to 40% saturation. Precipitated proteins were recovered by centrifugation at $10,000 \times g$ for 30 min and

washed twice with buffer A containing 40% ammonium sulfate. The protein pellet was solubilized in 2 ml of buffer B (buffer A containing 200 mM NaCl and 10% [vol/vol] glycerol) per g and then dialyzed overnight against buffer B. Insoluble material was removed by centrifugation at $10,000 \times g$ for 10 min.

(i) **Phosphocellulose chromatography.** The soluble protein extract (660 mg) was loaded onto a 40-ml column (2.6 by 7.5 cm) of phosphocellulose P11 (Whatman, Inc., Clifton, N.J.) equilibrated with buffer B. The wash fraction was discarded, and the DNase was eluted with buffer B containing 500 mM NaCl. The DNase-containing fraction was dialyzed against buffer C (buffer A containing 5 mM EDTA, 75 mM NaCl, and 10% glycerol).

(ii) **DNA-cellulose chromatography.** The preceding fraction (60 mg of protein) was applied onto a 12-ml column (1.6 by 6 cm) of single-stranded DNA-cellulose equilibrated in buffer C (denatured calf thymus DNA was adsorbed to cellulose at ca. 1.4 mg/g of cellulose as described previously [1]). Non-bound material was discarded, and the DNase was eluted with buffer C containing 500 mM NaCl. This DNase-containing fraction was concentrated to 1 ml with an Amicon ultrafiltration cell.

(iii) **Gel filtration chromatography.** The concentrated DNase fraction (5.4 mg of protein) was loaded onto a column (1.6 by 80 cm; bed volume, 160 ml) of Sephadex G-100 (Pharmacia) equilibrated with buffer C containing 500 mM

NaCl. The flow rate was about 5 ml/h. Fractions of 1 ml were collected and 100 μ g of bovine serum albumin was added to each. These fractions were dialyzed overnight against 50 mM Tris hydrochloride (pH 8.0) containing 10% glycerol and then stored at -80°C before enzymatic activity assays.

Immunoblotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins was done with 8% gels as previously described (26). Protein transfer was performed on nitrocellulose (Schleicher & Schuell, Inc., Keene, N.H.) with the Sartoblot II semidry system under the specifications of the manufacturer (Sartorius). Blots were incubated with an NPC serum containing high antibody titers to EA and VCA (EA, 1,280; VCA, 1,280). The antigen-antibody complexes were then revealed with a peroxidase-conjugated antibody directed against human IgG or IgA (Nordic Immunological Laboratories). NPC serum and peroxidase-conjugated antibody were respectively diluted 1:200 and 1:1,000.

DNase activity assays. EBV-specific DNase activity was measured by using radioactive substrate as previously described (36). Briefly, in a 100- μ l final volume, 5 μ l of the gel filtration fraction described above was incubated for 1 h at 37°C with 1 μ g of ^{32}P -labeled calf thymus DNA (nick translated with the Boehringer kit and $[\alpha\text{-}^{32}\text{P}]\text{dCTP}$) in 50 mM Tris hydrochloride (pH 8.0)–1 mM dithiothreitol–3 mM MgCl_2 . The reaction was stopped by the addition of 20 mM EDTA and incubation at 65°C for 10 min. The amount of acid-insoluble material was determined by spotting 40- μ l samples of reaction mixtures onto Whatman GF/C glass fiber disks and precipitating with a 10% cold trichloroacetic acid solution. The disks were dried with ethanol, and their radioactivity was measured in a Packard scintillation counter. DNase activity is expressed as the amount (in counts per minute) of acid-soluble nucleotides released after 1 h of incubation at 37°C . Neutralization of DNase activity by NPC sera was tested by preincubating 5 μ l of the gel filtration fraction with 2 μ l of each NPC serum for 20 min at room temperature before the enzymatic reaction.

Exonuclease and endonuclease activities were detected with linear *Eco*RI-plus-*Hind*III-digested λ DNA and circular DNA (pKK223-3 or ϕ X174), respectively. A 20- μ l final assay containing 10 μ l of enzyme fraction and either 800 ng of linear DNA or 500 ng of circular DNA in 50 mM Tris hydrochloride (pH 8) was incubated for various times at 37°C in presence of different salts as indicated in Fig. 5. The digested DNA products were analyzed by electrophoresis in 1% (wt/vol) agarose gel (type II, low electroendosmosis; Sigma Chemical Co., St. Louis, Mo.). After electrophoresis, gels were stained for 10 min in buffer supplemented with 5 μ g of ethidium bromide per ml and then visualized under long-wavelength UV light.

RESULTS

Expression of EBV DNase by recombinant plasmids containing cDNA sequence. To attempt direct expression of EBV DNase in *E. coli*, instead of a fusion protein which might present altered biochemical and enzymatic properties, we inserted the cDNA sequence coding for EBV DNase into the plasmid pKK223-3 under the regulation of the *tac* promoter. The cDNA was partially digested with *Bal* 31 nuclease to remove various amounts of 5' noncoding DNA regions upstream from the initiation codon. The possible control of the *tac* promoter in *E. coli lacI^a* strains could be useful to overcome an eventual toxic effect due to constitutive expression of EBV DNase in growing cells. However, our experi-

FIG. 2. Characterization of recombinant bacterial clones producing EBV DNase. (A) Western blot analysis of protein extracts with an NPC serum (see Materials and Methods). Lanes 1 and 7, Clones without an insert and with a wrong-sense insert, respectively. The other lanes correspond to producer clones containing a right-sense insert; lanes 8 and 12, respectively, correspond to pMS4 and pMS64 clones. (B) Precise localization on BGLF5 ORF of cDNA c37 pMS4 insert (122336 to 120928 bp) and pMS64 insert (122373 to 120928 bp). The second and third ATGs are respectively positioned 10 codons (122312 bp) and 13 codons (122303 bp) downstream from the beginning of the ORF (122342 bp).

mental data (not shown) indicated that an overnight culture of *E. coli* C600R5 cells continuously expressing EBV DNase gave higher DNase yields than *E. coli* JM105 cells grown to the mid-log phase or high density before induction of DNase synthesis. Bacterial clones expressing EBV DNase in *E. coli* C600R5 are presented in Fig. 2A. On a Western blot (immunoblot) analysis of crude protein extracts, EBV DNase was specifically detected by our NPC serum as a major band present in all producing clones. The molecular size of the protein produced by clone pMS64 was around 52 kDa (lane 12), which corresponds to the size of the DNase obtained after *in vitro* transcription and translation of cDNA c37 (44). However, EBV-specific DNase produced by clones other than pMS64, in particular by clone pMS4 (lane 8), corresponded to a 50-kDa protein. To determine whether this 50-kDa DNase would result either from a cDNA c37 sequence deletion consecutive to *Bal* 31 digestion or from a degradation of the 52-kDa protein, we sequenced both extremities of the cDNAs respectively inserted in clones pMS4 and pMS64. The sequence analysis revealed a deletion of 37 nucleotides in the 5' coding region of pMS4 cDNA, while the whole cDNA coding sequence was preserved in pMS64 (Fig. 2B). Since the BGLF5 ORF contains numerous in-frame ATG codons, the 50-kDa polypeptide may be

FIG. 3. Last purification step of EBV DNase from clone pMS64. (I) Gel filtration on Sephadex G-100 column detected as UV (280 nm)-absorbing material (---) or as 300 mM KCl-resistant DNase activity (—). OD, Optical density; BSA, bovine serum albumin; OVA, ovalbumin. (II) Immunoblot analysis of different gel filtration fractions. (III) Coomassie blue staining after SDS-PAGE; lane A, purified DNase; lane B, crude protein extract.

translated from a second ATG located 10 or 13 codons downstream from the first one (Fig. 2B). The other minor bands observed on immunoblot seem to be nonspecific polypeptides since they were not detected by other NPC sera (data not shown). Since the pMS64 clone expressed high levels of a full-length DNase, we used it as a source of recombinant protein for the purification of EBV DNase.

Purification of EBV DNase from *E. coli*. The 52-kDa DNase expressed in the pMS64 clone was purified almost to homogeneity by ammonium sulfate precipitation followed by a three-step chromatography procedure: a cation-exchange column (phosphocellulose), an affinity column (DNA-cellulose), and a size exclusion column (Sephadex G-100) (see Materials and Methods). DNA-cellulose chromatography gave rise to a fraction which, after SDS-PAGE analysis, revealed seven well-separated bands including the 52-kDa DNase. This fraction was further purified on Sephadex G-100 with the same DNA-cellulose buffer. EBV-specific DNase activity resistant to 300 mM KCl was eluted as a major peak coinciding with a peak of UV-absorbing material (Fig. 3-I). On immunoblotting (Fig. 3-II), the DNase was recognized as a doublet in fractions 35 and 40. The lowest band, which represents a small percentage of the whole recombinant DNase, may correspond to a truncated or a degraded form of the protein. After Coomassie blue gel staining, one major polypeptide corresponding to EBV DNase was observed, although very minor contaminant

bands could also be detected (Fig. 3-III). The molecular mass of native DNase as evaluated from its elution volume on gel filtration corresponded to about 52 kDa, which is similar to our previous estimation from SDS-PAGE analysis.

The amount of purified protein recovered was typically 60 µg/liter of bacterial culture. This low yield partially resulted from the difficulties in solubilizing the protein in nondenaturing conditions, since we observed insoluble DNase fractions at each step of the purification. Indeed, addition of polyethyleneimine to the extract first induced a coprecipitation of DNase along with the DNA. Then, about 30% of the total DNase in the ammonium sulfate fraction was found to be insoluble in phosphocellulose buffer containing 200 mM NaCl. Finally, when the phosphocellulose fraction was dialyzed against the DNA-cellulose buffer containing 75 mM NaCl, approximately 5% of the DNase became insoluble. Thus, the soluble enzyme fraction finally recovered after these steps should represent less than 65% of the DNase really produced. We could obtain up to 230 µg of purified DNase per liter under certain conditions: better bacterial growth and elimination of the ammonium sulfate precipitation step, replacing it by direct dialysis of the extract against phosphocellulose buffer.

Biochemical and enzymatic properties of purified EBV DNase. The DNase activity was first examined for its sensitivity to pH and to monovalent or divalent cations. Gel filtration fraction 33 was used for all enzymatic assays

FIG. 4. Properties of purified EBV DNase. Nuclease activity of the gel filtration fraction was tested on radioactively labeled calf thymus DNA as described in Materials and Methods. (A) pH optimum. (B) Divalent cation requirements and optimum concentrations. (C) Sensitivity to different salt concentrations.

because it corresponded to the most active fraction. Figure 4A shows an optimum DNase activity at pH 8.0, although the enzyme retained more than 95% of its activity at pH 7.0. The enzyme absolutely required divalent cations and presented optimal activity at 20 mM Mg^{2+} , which corresponds to a 4.5-fold increase compared with the value obtained at 1 mM Mg^{2+} (Fig. 4B). With Mn^{2+} , the activity was optimal at 3 mM but remained limited to about 85% of the value measured at the Mg^{2+} optimum. Although the enzyme could function without KCl or NaCl, it reached optimal activity in the presence of 50 mM of each of these salts (Fig. 4C). The enzyme activity was completely inhibited at 300 mM NaCl, while 10% residual activity was observed with the same concentration of KCl.

To further characterize the DNase, we investigated exonuclease and endonuclease activities by incubating the en-

zyme either with linear *Eco*RI-plus-*Hind*III-digested λ DNA or with circular DNA (pKK223-3 or ϕ X174). The time course of digestion is shown in Fig. 5. In the absence of KCl, Mg^{2+} and Mn^{2+} were effective in stimulating both exonuclease and endonuclease activities of the purified protein (Fig. 5A). However, the level of exonuclease activity obtained in presence of Mg^{2+} was higher than that obtained with Mn^{2+} (Fig. 5A, left). On the contrary, Mn^{2+} was more effective for the endonuclease activity (Fig. 5A, right). In the presence of Mg^{2+} , the covalently closed circular ϕ X174 DNA was partially converted to nicked open circular DNA in 30 min (Fig. 5B, left), which indicates that an internal cleavage occurred on one DNA strand. The open circular DNA form was further degraded with time (60 min).

In the presence of 300 mM KCl, EBV endonuclease was completely inhibited with either Mg^{2+} (Fig. 5B, right) or Mn^{2+} (results not shown). Only a weak exonuclease activity was still detected in the presence of 300 mM KCl and 20 mM Mg^{2+} (Fig. 5B, right).

To verify that the purified EBV DNase fraction was free of any contaminating *E. coli* nuclease, a protein extract from bacterial cells containing a nonrecombinant pKK223-3 was prepared and fractionated as described in Materials and Methods. After gel filtration chromatography, fractions presumed to contain proteins around 52 kDa were tested for endonuclease and exonuclease activities. Neither endonuclease nor exonuclease activities could be detected in any of these fractions (Fig. 5C).

NPC sera recognize EBV DNase produced by pMS64 recombinant bacteria. EBV DNase activity from pMS64 was specifically inhibited by EA-positive and VCA positive sera (85% reduction of the activity measured without serum), but not by EA-negative and VCA-positive or EA-negative and VCA-negative sera (Fig. 6, left). Such human sera did not inhibit a DNase activity obtained from a partially purified extract of pKK223-3-transformed bacteria used as negative control (Fig. 6, right). This specific neutralization of EBV-encoded DNase by EA-positive NPC sera was similar to that observed from EBV-carrying LCLs after EA induction (36). To determine whether the serological response to EBV DNase resulted from a recognition by IgG or IgA or both, we separated an NPC serum into IgG-enriched and IgG-free fractions by chromatography on protein A-Sepharose (Pharmacia). These immunoglobulins were tested against the partially purified DNase by immunoblotting. A specific response to the 52-kDa DNase was obtained from both IgG and IgA (Fig. 7B). Notice that EBV DNase both from a partially purified fraction (DNA-cellulose chromatography) and from crude extracts was specifically recognized by IgG and IgA from an NPC serum (Fig. 7A, lanes 1 and 2).

DISCUSSION

In this study, the production of an active EBV DNase was achieved by using a heterologous *E. coli* expression system. The viral DNase gene was inserted under the control of a *tac* promoter usually allowing massive production of exogenous proteins (27). In our case, we only obtained 60 to 230 μ g of purified DNase per liter of culture. This low yield partially resulted from the loss of recombinant DNase in insoluble form during extraction and fractionation procedures, in particular during the steps of DNA and protein precipitations by polyethyleneimine and ammonium sulfate, respectively. A number of exogenous polypeptides expressed in *E. coli* are known either to accumulate in bacteria as inclusion bodies or to aggregate during purification, so that stringent

FIG. 5. Digestion of linear and circular DNAs by purified EBV DNase or negative control. (A) Digestion of *Eco*RI and *Hind*III λ DNA restriction fragments [(E+H) λ] or pKK223-3 DNA by purified EBV DNase in the presence of 20 mM Mg^{2+} or 3 mM Mn^{2+} . Enzymatic reactions were stopped at 5, 15, 30, or 90 min, and the samples were analyzed by electrophoresis in 1% (wt/vol) agarose. Enzyme was omitted in control assays either incubated (I) or not incubated (NI) at 37°C for 90 min. (B) Digestion with 20 mM Mg^{2+} of λ DNA restriction fragments or $\Phi X174$ DNA by EBV purified DNase in the absence (left) or presence (right) of 300 mM KCl. (C) Same digestion assays as in panel A with gel filtration fraction from pKK223-3-transformed bacteria as a negative control. OC, Open circular DNA; L, linear DNA; CCC, covalently closed circular DNA.

chemical conditions can be required for their solubilization (31). Strong denaturants such as 1% SDS were able to solubilize EBV DNase efficiently from our expression system (data not shown), but we chose a gentler treatment to preserve enzymatic activity. However, since we observed an increase of DNase solubility in strong ionic force solutions, we maintained the NaCl concentration as high as possible during purification. In such conditions, although

more than 35% of the produced DNase was lost, the recombinant protein could be purified to more than 95% homogeneity as an active enzyme.

Our purified recombinant protein presents several characteristics similar to those observed for the EBV-induced DNase from LCLs. Our and other studies previously showed that an EBV-specific DNase extracted from lymphoblastoid cells sedimented on neutral sucrose gradient with a

FIG. 6. Neutralization of EBV DNase activity by NPC serum. DNase-neutralizing activities of NPC serum (EA positive), normal serum (EA negative), and EBV-negative serum were tested on purified EBV DNase (left) and on partially purified extract (ammonium sulfate step) from pKK223-3-transformed bacteria as a negative control (right).

coefficient of 4S corresponding to a molecular mass of about 52 kDa (13, 36). Both enzymes show a preference for alkaline pH, an absolute requirement for divalent cations, and a residual activity in presence of 300 mM KCl (13, 36). No clear substrate specificity has yet been observed with either enzyme (13, 14). The recombinant DNase presents both exonuclease and endonuclease activities, which agrees with previous observations on the DNase from EBV-carrying LCLs. However, minor differences appeared between the enzymes. The divalent cation optimum concentrations are much higher for the DNase expressed in the procaryotic system than for the EBV-induced DNase derived from LCLs. Furthermore, we detected exonuclease activity in the presence of 3 mM Mn^{2+} with the recombinant enzyme, whereas EBV-specific exonuclease activity from the LCLs was found to be maximal at 0.2 mM and selectively inhibited by more than 1 mM Mn^{2+} (14). In the presence of high KCl concentrations, only exonuclease activity could be detected from the recombinant DNase, while endonuclease activity was observed from the LCL-extracted enzyme. These discrepancies might result from differences in purity between both enzyme fractions or from altered recombinant DNase properties owing to the absence of posttranslational modifications in bacteria.

The fact that EBV DNases respectively expressed in bacteria and in LCLs show main enzymatic similarities and the same antigenicity (specific recognition by EA-positive sera) brings further support to our previous conclusion that the 52-kDa EBV-specific DNase identified in LCLs must be translated from the BGLF5 ORF (44).

Despite little amino acid sequence homology between EBV and HSV DNases (21, 33), both enzymes closely resemble each other: alkaline optimum pH, divalent cation

FIG. 7. Immunodetection of EBV DNase by IgG and IgA fractions of an NPC serum. (A) Specific recognition of the DNase by an NPC serum. NPC serum (EA and VCA positive) and EBV-negative serum (EA and VCA negative) were incubated with the DNA-cellulose fraction of pMS64 (lanes 1) or with bacterial crude extracts from either pMS64 (lanes 2) or the negative control (lanes 3). The complexes were revealed with peroxidase-conjugated antibodies respectively directed against human IgA or IgG. (B) Immunodetection of the DNase (DNA-cellulose fraction) with IgG-enriched and IgG-free fractions from an NPC serum. Blotted DNase was incubated with either the flowthrough (IgG-free) or the retained (IgG-enriched) fraction from a protein A-Sepharose chromatography of the NPC serum. The complexes were revealed as in panel A.

requirement, and coexistence of exonuclease and endonuclease activities without apparent specificity toward DNA sequences (3, 23–25). The presence of similar early DNase activities in HSV- and EBV-infected cells reinforces the well-extended idea of the importance of these enzymes in viral DNA synthesis. HSV alkaline DNase is certainly essential for virus replication since (i) the enzyme was copurified with the HSV major DNA-binding protein and HSV DNA polymerase (42) and (ii) viral mutants for the alkaline DNase expression were inefficient in producing extracellular mature virions (34; S. Weller, R. Seghatolslami, E. Carmichael, and D. Rowse, Abstr. 13th Int. Herpesvirus workshop, 1988, p. 129). A recent study on protein complexes binding to the cleavage sites of the HSV genome has revealed the presence of an HSV alkaline DNase (11). The investigators suggested that viral DNase in connection with other site-directed proteins is responsible for the cleavage of nonmature DNA forms (concatemers or circles) into unit-length DNA molecules (11). Such an action would explain why cells infected with viruses bearing a mutated alkaline DNase gene were just able to produce empty capsids but not infectious virions (Weller, et al., Abstr. 13th Int. Herpesvirus Workshop, 1988). A similar action of EBV DNase in viral replication (maturation of viral DNA replication intermediates) could be considered. To test this possibility, the presence of EBV DNase until the end of DNA synthesis has to be verified. This requires specific antibodies to EBV DNase and cells (such as the Akata cell line) in which the viral cycle can be synchronized (K. Takada, N. Shimizu, and Y. Ono, Abstr. 13th Int. Herpesvirus Workshop, 1988, p. 377). Another possible function of EBV DNase is to inhibit cellular metabolism to the advantage of virion production. The fact that the appearance of EA and particularly of EBV DNase is followed by cellular breakdown led some investigators to claim that EBV DNase could be responsible for the degradation of host-cell DNA (22). In such a case, EBV DNase may promote viral replication in two ways: providing nucleotides from degraded cellular DNA and allowing the requisition of host enzymes necessary to viral DNA synthesis (22). However, no direct action of EBV DNase on cellular DNA has yet been demonstrated. To elucidate this question, the expression of a functional EBV-specific DNase should be attempted by transfection of EBV-negative LCLs with the EBV DNase gene.

One purpose of the present study was to ensure the value of EBV recombinant DNase for NPC diagnosis tests. Seroprevalence studies have already shown that antibody titers to EBV-induced DNase activity allow NPC early diagnosis (6, 8, 9, 41, 43) and also prognosis of the disease, especially in monitoring the success of treatments of NPC patients (7, 40). In all these epidemiological studies, EBV DNase neutralizing assays were performed with LCL crude extracts as a source of viral DNase. Since the IgA class antibodies against EA represent today the best epidemiological and clinical marker (18, 20), we investigated which immunoglobulin class would react with a purified fraction of the recombinant DNase. The data presented here clearly demonstrate that the viral DNase expressed in *E. coli* is specifically recognized by EA-specific antibodies from an NPC serum, and not only by the IgG but also by the IgA. Thus, purified recombinant EBV DNase appears to be a good candidate for developing very specific and practical tests (enzyme-linked immunosorbent assay type) to improve the control of NPC.

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