Characterization of an Epstein-Barr Virus-Induced Thymidine Kinase

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Previous work from our laboratory suggested that the selective inhibition of Epstein-Barr virus (EBV) replication by 1-β-d-arabinofuranosylthymine in human lymphoid cell lines involved the induction of a new thymidine kinase (TK) able to phosphorylate the thymidine analog. We further characterized this enzyme induced in various EBV-positive cell lines after viral genome activation with a combination of sodium butyrate and 12-O-tetradecanoylphorbol-13-acetate. The following results confirmed the existence of an EBV-specific deoxypyrimidine kinase: (i) induction of EBV-related TK was connected with the appearance of viral early antigens in EBV-carrying cells; (ii) unexpected behaviors of the enzyme activity upon different fractionating treatments led to the conclusion that EBV-induced TK was extracted as a complex molecular form, larger than other known cellular or viral isozymes; (iii) enzymatic properties distinguished EBV-induced TK from host lymphoid cell isozymes but made it resemble other herpesvirus-specific deoxypyrimidine kinases, i.e., by partial inhibition by dTTP or ammonium sulfate, insensitivity to dCTP, and nonstringent specificity for normal TK substrates. Genetic evidence is required to definitively ensure that EBV-specific TK actually is virus coded in EBV-transformed human lymphoid cells.

Epstein-Barr virus (EBV) is a common human herpesvirus which causes infectious mononucleosis and has been connected with two kinds of cancer, i.e., Burkitt’s lymphoma and undifferentiated nasopharyngeal carcinoma (12). No fully permissive tissue system is available in the case of EBV. A number of continuously proliferating cell lines have been established either from Burkitt’s lymphoma explants or from B lymphocytes infected in vitro with EBV, but latency characterizes most of these EBV-carrying lymphoblastoid cells, since spontaneous virus release ability is restricted to only 1 to 10% of the cells even in the so-called producer lines. Various inducers can, however, increase the proportion of EBV-transformed cells engaged in active viral expression, and we have previously shown that both producer and latently infected lymphoblastoid lines efficiently respond to treatment with a mixture of sodium butyrate (SB) and tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) (35, 36; G. Lenoir et al., Abstr. 4th Cold Spring Harbor Meet. Herpesviruses 1979, p. 178).

A double interest led us to search for EBV-specific enzymes involved in DNA replication. First, together with the characterization of early-synthesized viral antigens (EA), such an investigation was required for understanding the basic mechanisms of virus reactivation in latent EBV-carrying cells. On a second and practical point of view, medical applications might be expected from the detection of EBV-specific thymidine kinase (TK), DNA polymerase, and DNase activities, in the same way as accurate diagnosis and efficient chemotherapy methods were developing on the basis of the immunological properties and substrate specificities of such enzymes induced by other human herpesviruses (6, 16, 17, 21, 32, 33). Our work and that of others actually revealed the existence of EBV-related DNA polymerase (1, 10, 15; 38) and DNase (9, 13, 37, 44) activities in lymphoblastoid cell lines; a relation in humans between nasopharyngeal carcinoma and the production of antibodies to EBV-specific DNase was also detected (5, 37). However, while particular TKs have been associated with herpes simplex virus (HSV) (4, 7, 22, 27), varicella-zoster virus (8, 34), and non-human herpesviruses (20, 22, 25, 27, 30, 31, 45, 46), the existence of an EBV-related TK has long been discussed. First intimated by Hampar et al. (18) and Glaser et al. (14) but not evident to Kit et al. (23), the production of a specific TK by EBV was successively favored and demigrated by Pagano and his collaborators (3, 11, 39), while our group and several others accumulated information supporting the presence of an EBV-associated TK in superinfected or chemically activated human lymphoblastoid cells transformed with the virus (29, 35, 36, 41, 43).

First observations in our laboratory concerned the selective inhibition of EBV replication by arabinofuranosylthymine (araT) in producer lymphoblastoid cells (35); some data suggested that the specific effect of the thymidine (dT) analog resulted from the conversion of araT into triphosphate form. Arabinosyl-TTP appeared as a potent inhibitor of EBV-associated DNA polymerase, and its synthesis was found to be dependent on the activity of an EBV-related TK distinguishable from the host cell isozymes (36). Biochemical studies led us to further results supporting the existence of an EBV-specific TK with proper identity, as shown by the structural and enzymatic characteristics here described.

MATERIALS AND METHODS

Lymphoblastoid cell cultures and EBV induction with TPA plus SB. The procedures for lymphoblastoid cell cultures and EBV induction were mainly those previously described (36). EBV-negative cells from the BJAB line and a Ramos TK− line and EBV-carrying cells from the nonproducer (latently infected) Raji and the producer P3HR-1 lines (TK+ and TK− variants) were seeded at 5 × 105 cells per ml and grown at 37°C in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, penicillin (100 U/ml), streptomycin (50 μg/ml), and amphotericin B (Fungizone; 5 μg/ml). Cell
growth was controlled up to a density of at least 2 \( \times 10^6 \) cells per ml; dilution with fresh medium was then achieved to a concentration of 0.5 \( \times 10^6 \) to 1 \( \times 10^6 \) cells per ml. TPA and SB were added at respective final concentrations of 40 ng/ml and 2 mM, and cells were harvested after a 60- to 72-hr treatment. Evaluation of the percentage of EA-positive cells was carried out by indirect immunofluorescence as described by Henle and Henle (19). Cell cultures were obligingly tested for mycoplasmas by C. Bonnisol, Institut Pasteur, Paris.

HSV-specific TK (4) was obtained from HSV type 1 (HSV-1)-infected LM TK- cells which were generously supplied by B. Jacquemond, Unité INSERM 51, Lyon, France.

Preparation of crude extracts. Cells in suspension culture were harvested by centrifugation and washed twice with phosphate-buffered saline. Cell pellets were then either immediately used or stored at -80°C. Extraction was carried out at 0 to 4°C. After suspension at concentrations of 1 \( \times 10^6 \) to 2 \( \times 10^6 \) cells per ml (5 to 12 mg protein per ml) in the extraction buffer (50 mM Tris [pH 8], 50 mM KCl, 1 mM MgCl\(_2\), 1 mM ATP, 1 mM dithiothreitol [DTT], 50 mM \( \alpha \)-aminoacapric acid [\( \alpha \)-ACA], 5% [vol/vol] glycerol), cells were sonically disrupted with a Branson sonifier at a setting for high 30-s periods. The sonically disrupted suspension was centrifuged at 105,000 \( \times g \) for 60 min, and the supernatant fluid was either immediately used for assays or divided into small fractions and stored at -80°C. The protein content of each enzyme fraction was determined by the method of Lowry et al. (28).

TK assays. Each enzyme fraction was incubated at 37°C with an equal volume of the TK substrate buffer (150 mM phosphate [pH 7.5], 20 mM MgCl\(_2\), 40 mM KCl, 1 mM DTT, 10 mM NaF, 15 mM Tris [pH 7.5]-S50, 0.5% [vol/vol] glycerol) each of Triton X-100 and Nonidet P-40, 0.5% [vol/vol] each of Triton X-100 and Nonidet P-40), frozen and thawed twice, sonicated for six 30-s periods, and centrifuged at 105,000 \( \times g \) for 60 min. A nuclear fraction was also prepared, which consisted of the high-speed supernatant fluid similarly obtained after suspension of the nuclei in buffer C and sonication.

Glycerol gradient centrifugation. For glycerol gradient centrifugation, samples of TK fractions were centrifuged in buffer C at an 189,000 \( \times g \) for 15 h in 5-m1 linear 10 to 30% [vol/vol] glycerol gradients prepared in buffer C, 250-\( \mu \)l fractions were collected, and TK activity was assayed as described above, with 10 \( \mu \)l T and overnight incubation.

Purification of TK activities by affinity chromatography on dT-Sepharose columns. The procedures for affinity chromatography purification of TK activities were essentially the same as those described by Lee and Cheng (26). Briefly, p-nitrophenylthymidine 3'-monophosphate was reduced to p-aminophenylthymidine 3'-monophosphate, which was linked to activated CH-Sepharose 4B as described by Pharmacia. TK purification was performed at room temperature on 3-m1 dT-Sepharose columns. Crude cell extract was treated with 2% streptomycin sulfate, and proteins from the supernatant fraction were slowly precipitated with ammonium sulfate. The protein pellet obtained at between 20 and 50% precipitation (about 10 mg of protein) was dissolved in 20 mM Tris (pH 7.5)-50 mM \( \alpha \)-ACA-5 mM DTT-10% [vol/vol] glycerol (5% [vol/vol] glycerol), dialyzed against the same buffer, and applied to the affinity column. Elution was performed with increasing concentrations of Tris, dT, ATP, and KCl (see Fig. 4), and 1.5-m1 fractions were collected. Bovine serum albumin (1 mg/ml) was added to protect enzyme activities in the purified fractions with very low protein concentrations. For heterogeneous TK assays, all column fractions were dialyzed against 50 mM Tris (pH 7.5)-50 mM KCl-1 mM ATP-1 mM MgCl\(_2\)-1 mM DTT-10% [vol/vol] glycerol; TK activity was then assayed by overnight incubation in the presence of 5 \( \mu \)M dT.

Reagents. [\( ^3 \)H]dT was purchased from C.E.A., Paris, France. araT purchased from Calbiochem-Behring, La Jolla, Calif., was titrated by C. E. A. and purified on polyethyleneimine cellulose (Machery-Nagel, Düren, Federal Republic of Germany). Unlabeled dT, DTT, \( \alpha \)-ACA, p-nitrophenylthymidine 3'-monophosphate, Dowex 50 W (H\(^+\); 100/100 mesh), bovine serum albumin, and most active preparations of lipase (type XI from Rhizopus sp.) and hyaluronidase (type V1-S from bovine testes) were pur-
chased from Sigma Chemical Co., St. Louis, Mo. Streptomycin sulfate and deoxynucleoside triphosphates were provided by Boehringer GmbH, Mannheim, Federal Republic of Germany. Activated CH-Sepharose 4B was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. DE-81 chromatography paper was the product of Whatman Co. N,N',N'-tetra-
methylene-bisacrylamide, acrylamide monomer, and N,N,N',N'-tetramethylethylene diamine were purchased from Eastman Kodak Co., Rochester, N.Y. Common chemicals were provided by Merck, Darmstadt, Federal Republic of Germany.

RESULTS

Associated inductions of EBV-specific TK and EA. From comparative studies on different lymphoblastoid cell lines treated or not with a combination of TPA and SB, we had previously shown (36, 37) that production of EA and the appearance of new enzymatic activities (DNA polymerase, DNase, and TK) in EBV-carrying cells were both associated with viral genome activation. For further investigation, kinetic studies were performed on both the nonproducer EBV-positive Raji line and the EBV-negative Ramos line. Since, in our experiments, the level of EBV-related TK induced upon chemical treatment never exceeded 40% of the TK activity measured in normally grown TK+ cells, we used TK− variants containing 4 to 8% residual TK activity only (see below). In chemically treated Raji cell culture, the percentage of EA-positive cells was increasing for at least 3 days, while EBV-specific TK activity could attain its highest level from day 2. On the other hand, neither EA-positive cells nor TK activity increases were detected in the Ramos line (treated with the chemicals or not) or in the noninduced Raji line (Fig. 1).

Physical characteristics of EBV-specific TK. Peculiar physical characteristics of EBV-specific TK were revealed by the enzyme on different fractionation attempts from crude cell extracts.

(i) PAGE. Besides its ability to efficiently phosphorylate araT (36), EBV-related TK was first distinguished from other known cellular or viral isozymes by its very low electrophoretic mobility in 5% polyacrylamide gel (Fig. 2). Host cell TK isozymes of human lymphoblastoid lines were examined by PAGE performed on different extracts (crude supernatant fluid [105,000 × g] or cytosol and mitochondrial extracts obtained after subcellular fractionation) from EBV-positive (Raji and P3HR-1, TK+ and TK− variants) and EBV-negative (BJAB TK+ and Ramos TK−) cells. PAGE analysis of the soluble fraction from Raji, P3HR-1, or BJAB TK+ cells showed a main peak of slowly migrating (Rm = 0.2) TK activity which was absent from TK− mutants of the Raji, P3HR-1, and Ramos lines; as previously observed by Kit et al. (23), this major TK peak appeared identical with the cytosol fetal isozyme found in other mammalian cells. When the residual TK activity of human lymphoblastoid lines was investigated, two other isozymes were detected, i.e., a fast-migrating (Rm = 0.65) activity was extracted from Ramos (TK−) mitochondria and identified with the minor TK observed in P3HR-1 (TK−) mitochondria and cytosol fractions (23) (Fig. 2), while an isozyme with medium electrophoretic mobility (Rm = 0.4) was released from Raji (TK+ and TK−) mitochondria. The former (only once described [39] from Raji cells) appeared similar to the mitochondrial adult TK found in other mammalian cells, and the latter resembled the second mitochondrial isozyme detected in human HeLa and KB cells (23, 24).

Due to the very high levels of fetal TK activity present in TK+ lymphoblastoid cells, PAGE detection of EBV-specific isozyme could be ensured from TK− cells extracts only, with araT phosphorylation as a control test. Indeed, EBV-associated TK was surprisingly found to migrate hardly at all in 5% polyacrylamide gel (Rm = 0.05) in conditions where expected mobilities were observed on host cell isozymes (as described above) and on HSV-coded TK from HSV-1-infected LM TK− cells (Rm = 0.5, as shown in Fig. 2 insert). Confirmed on slab electrophoresis in a 3 to 7.5% gradient of acrylamide, the very low electrophoretic mobility of EBV-related TK activity was maintained after treatment of crude supernatant fluid (105,000 × g) with different agents such as detergents (0.5% Triton X-100 plus 0.5% Nonidet P-40), salts (2 M KCl or gentle protein precipitation with ammonium sulfate), thiol groups (200 mM β-mercaptoethanol plus 20 mM DTT), or nucleolytic enzymes (DNase I or RNase A plus T1). Subcellular fractionation (performed on induced Raji TK− cells) still resulted in the obtainment of EBV-specific

![FIG. 1. Kinetics of EA and EBV-induced thymidine kinase in Raji TK− and Ramos TK− cells.](http://jvi.asm.org/)
TK as a very slowly migrating activity, mainly recovered in the cytosol (results not shown). However, part of EBV-related TK could show a higher electrophoretic mobility than usual ($R_m = 0.3$) after crude cell extract had been incubated with either lipase or hyaluronidase (Fig. 3).

(ii) Affinity chromatography on dT-Sepharose. Affinity chromatography purification of EBV-specific TK on dT-Sepharose column was attempted on crude extracts prepared as usual from chemically induced Raji TK$^-$ cells; the major isozyme from BJAB (TK$^+$) cells and HSV-coded TK from HSV-1-infected LM TK$^-$ cells were similarly isolated for comparison. Major lymphoblastoid-cell TK and HSV-coded isozyme were almost totally adsorbed on the column, and the purified enzymes were both eluted in accordance with the literature (7, 26), the former with 200 mM Tris plus 0.1 mM dT, the latter with 300 mM Tris plus 0.2 mM dT (Fig. 4). On the other hand, EBV-specific TK could not be isolated from the bulk of crude extract proteins which were not retained on the affinity gel (the minor adsorbed activity apparently purified from induced Raji TK$^-$ cells did not efficiently phosphorylate araT and was not identified with EBV-related isozyme; it might be contaminating cellular activity, since it showed properties of 0.2-$R_m$ TK just like both peaks previously retained by the same column on BJAB extract purification).

(iii) Other partial fractionation assays. On 10 to 30% glycerol gradient centrifugation, EBV-associated TK behaved differently from host cell isozymes (Fig. 5). The latter were recovered as single peaks in the lighter half of the gradient, 0.2- and 0.4-$R_m$ isozymes sedimenting identically and faster than 0.65-$R_m$ TK, as described in the literature (23). On the other hand, EBV-specific activity was found to spread through the heavier three-quarters of the gradient, the highest levels being distributed in the middle and bottom fractions.

Two other peculiar behaviors characterized EBV-related TK (results not shown). The enzyme was almost totally sedimented on further centrifugation of the supernatant fluid (105,000 × g) for 16 h at 200,000 × g, and it was excluded from Biogel A 0.5-m columns in conditions under which elution of host cell major isozyme was delayed as expected from the molecular size range (70,000 to 90,000 daltons) of known TKs.

All the preceding data associate EBV-specific TK activity with a heterogeneous protein fraction differing in physical structure from other described isozymes. As such a situation might prevent EBV-related TK from binding to affinity chromatography columns, further attempts were made to get the enzyme as a homogeneous and smaller active molecular form. Ionic or nonionic detergents or both (deoxycholate, sodium dodecyl sulfate, Triton X-100, Nonidet P-40) were added to the extraction buffer before cell disruption, but,
once more, no utilizable effect was obtained in different extraction conditions.

**Enzymatic characteristics of EBV-specific TK.** As detected from a comparative study on electrophoretically parted activities (Table 1), enzymatic properties also discriminated EBV-related TK from host cell isozymes. The latter appeared the same in human lymphoblastoid lines as in other described mammalian cells (23, 24, 26, 27). The 0.2-RRm TK was characterized by great specificity towards the usual substrates dT and ATP and by strong inhibition in the presence of a relatively low dTTP concentration; the 0.4-RRm isozyme (from Raji mitochondria) resembled the major cell TK, though it seemed less sensitive to dTTP and more inhibited by ammonium sulfate. On the other hand, 0.65-RRm mitochondrial activity (from Ramos cells) could use phosphate donors and acceptors respectively distinct from ATP and dT, and it was efficiently inhibited by dCTP and partly resistant to ammonium sulfate. As for EBV-specific TK, non-stringent substrate specificity clearly distinguished it from both 0.2- and 0.4-RRm cellular isozymes. The enzyme also differed from 0.65-RRm mitochondrial activity since it showed insensitivity to 1 mM dCTP, much better affinity with araT, greater sensitivity to ammonium sulfate, and higher resistance to dTTP. From these enzymatic properties, EBV-related TK resembled other known herpesvirus-specific isozymes (4, 7, 8, 20, 22, 25, 30, 31, 46). It must be noticed that EBV-associated dT-phosphorylating activity could be assigned neither to contaminating mycoplasmal TK (see Materials and Methods) nor to any nucleoside phosphotransferase since it was sensitive to dTTP, active in the presence of NaF, and unable to use AMP as a phosphate donor (Table 1; results not shown).

**DISCUSSION**

This report gives evidence of the appearance of a new TK in EBV-transformed human lymphoblastoid TK- cells after treatment with combined TPA and SB. Not to be confused with either mycoplasmal TK or cellular nucleoside phosphotransferase activities, the enzyme could not be identified with any known host cell TK; on the basis of its specific properties and constant association with EBV-genome expression, it appeared rather as a virus-induced protein, possibly one component of the EA complex.

![FIG. 3. Effects of lipase or hyaluronidase on the electrophoretic mobility of EBV-related TK. Disk PAGE analyses were performed as usual after 50-μl samples of induced Raji TK- cell extract had been preincubated at 37°C for 2 h with 30 μl of buffer either pure (A) or containing about 2,500 U of lipase (B) or hyaluronidase (C). The latter were purified preparations from Sigma (see Materials and Methods) and were not found to significantly alter the level of TK activity in our extracts.](image)

![FIG. 4. Elution patterns of major lymphoblastoid-cell and EBV-related TKs from dT-Sepharose affinity chromatography columns. Details of the procedure are in Materials and Methods. Major lymphoblastoid-cell TK was purified from BJAB cells, and EBV-related TK was purified from induced Raji TK- cells. HSV-specific TK purification (from HSV-1-infected LM TK- cells) was performed under the same conditions to control the TK-binding efficiency of dT-Sepharose. Elution buffers all contained 10% (vol/vol) glycerol and 5 mM DTT, with added 50 mM ε-ACA and 20 mM Tris (pH 7.5) for buffer 1, 50 mM ε-ACA and 200 mM Tris (pH 7.5) for buffer 2, 200 mM Tris (pH 7.5) and 0.1 mM dT for buffer 3a, 300 mM Tris (pH 7.5) and 0.2 mM dT for buffer 3b, and 500 mM Tris (pH 7.5), 0.3 mM dT, 0.2 mM ATP, and 500 mM KCl for buffer 4.](image)
comparison of our results with other data raises discussion on several points.

Our experience emphasized two possible explanations of the conflict concerning the existence of an EBV-specific TK. Peculiarity of the biological system may first be involved. Expression of the viral genome is restricted in EBV-carrying human lymphoblastoid cell lines, and activation of EBV replication, by cell treatments with either chemical inducers or superinfecting virus, would result from complex and not experimentally controlled processes, as suggested by the frank and unexplained variations found on comparing the levels and kinetics of EA production and EBV-related TK induction obtained by the authors (3, 29, 41; see above). If EBV-specific TK synthesis actually depends on a variably expressed viral gene as proposed by Kit et al. (23), it is not surprising that, too hazardous in the presence of major host cell isozyme (3, 11, 36, 39), the detection of EBV-specific TK could be assured from TK− mutant cell lines (29, 36, 41, 43). Another source of difficulty in identifying EBV-related TK lies in the peculiar structural form of the extracted enzyme. Roubal and Klein, after performing 5% PAGE on the crude soluble fraction of Raji TK− cells superinfected with P3HR-1 virus, reported a precipitation of EBV-associated TK on top of the stacking gel (41). Thus our similar results should not be attributed to any artifact connected with our viral activation procedure using TPA and SB; neither should lymphoblastoid cell origin be involved, since EBV-related TK showed the same electrophoretic behavior in our experiments whether it had been extracted from the Raji or the P3HR-1 line (Fig. 2).

From all our data, large molecular size of the enzyme complex should apparently be considered a main characteristic distinguishing EBV-associated TK from the major soluble and mitochondrial host cell isozymes. This property should be connected with our failure in isolating EBV-related TK by very different methods efficiently used for the fractionation of various cellular and viral isozymes. Although we experimented on several dissociating agents and extraction conditions, only lipase and hyaluronidase were able to liberate part of the EBV-related TK from its large molecular form so that active enzyme could enter the 5% polyacrylamide gel and migrate at 0.3 Rm. Such an electrophoretic mobility is similar to that of (0.37 Rm) initially attributed by Chen et al. to EBV-specific TK from superinfected Raji cells (3), but identifying these last two TK activities with each other would now be purely speculative, owing to insufficient characterization of these apparently unstable enzymatic forms. Since simple protein aggregate should be easily disrupted solely by increasing the ionic strength, only rather sophisticated models can be proposed at present to account for the unexpected behavior of EBV-associated TK in our experiments of electrophoresis, sedimentation, chemical and physical dissociation, and affinity chromatography. EBV-related TK might enter into some multienzyme complex similar to the complexes including TK and other deoxyribonucleotide-synthesizing enzymes during active mammalian DNA replication (40, 47), or it could resemble membrane enzymes characterized by lipid-dependent activity and difficult solubilization from vesicular lipoprotein complexes (42).

As detailed above, enzymatic properties revealed by roughly purified fractions allowed us to further distinguish

TABLE 1. Enzymatic properties of EBV-specific TK and host human lymphoblastoid cell isozymes

<table>
<thead>
<tr>
<th>TK isozyme*</th>
<th>Rm</th>
<th>+0.1 mM dTTP</th>
<th>+1 mM dTTP</th>
<th>+100 mM (NH4)2SO4</th>
<th>With CTP instead of ATP</th>
<th>With uraT instead of dT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoblastoid cell</td>
<td>Soluble</td>
<td>0.2</td>
<td>30</td>
<td>94</td>
<td>31</td>
<td>7</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>0.4</td>
<td>76</td>
<td>90</td>
<td>7</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>0.65</td>
<td>57</td>
<td>45</td>
<td>79</td>
<td>43</td>
<td>6</td>
</tr>
<tr>
<td>EBV specific</td>
<td>0.05</td>
<td>86</td>
<td>108</td>
<td>47</td>
<td>48</td>
<td>57</td>
</tr>
</tbody>
</table>

* Tests were performed on electrophoretically parted TKs, as described in Materials and Methods.

a Expressed as a percentage of the control value (100%).
EBV-related TK from known lymphoblastoid host cell isozymes. Few data have been published concerning enzymatic characterization of EBV-associated TK from either chemically induced or virus-superinfected cells. Among the properties we found similar to those of other herpesvirus-specific TKs, limited sensitiveness to inhibition by 0.1 mM dTTP and broad substrate specificity towards phosphate donor and acceptor have at least been confirmed by other authors (29, 41, 43).

Since late viral capsid antigen production is possible after superinfection but not after chemical activation of Raji cells, our results (Fig. 1) support observations by McGabhann et al. (29) that EBV-related TK appearance in stimulated EBV-positive cells actually is constantly connected with EA synthesis. Moreover, EBV-associated TK has been detected early after the beginning of cell activation by superinfection (3, 41) as well as by chemical treatment (Fig. 1). On the other hand, while good immunological specificity was described for human antibodies to other herpesvirus-induced TKs (17, 21), we got evidence that the main immunoglobulin G fraction from nasopharyngeal carcinoma patient serum efficiently neutralizes EBV-induced TK but not major host cell TK (data to be published). Thus, EBV-specific TK may likely be identified as an early viral antigenic protein, possibly an element of the EA complex.

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LITERATURE CITED


