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Induction of Epstein-Barr Virus Lytic Cycle by Tumor-Promoting and Non-Tumor-Promoting Phorbol Esters Requires Active Protein Kinase C

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Exposure to the tiglian 12-*O*-tetradecanoylphorbol-13-acetate (TPA) represents one of the most efficient and widely used protocols for inducing Epstein-Barr virus (EBV)-infected cells from latent into lytic cycle. Since TPA is both a potent tumor promoter and a potent activator of the cellular protein kinase C (PKC), we sought to determine whether either of these activities was closely linked to EBV lytic cycle induction. A panel of TPA structural analogs, encompassing tiglians with different spectra of biological activities, was assayed on a number of EBV-positive B-lymphoid cell lines. Lytic cycle induction correlated with the capacity to activate PKC, not with tumor promoter status; some nonpromoting tiglians were as efficient as TPA in inducing lytic cycle antigen expression. We then sought more direct evidence for an involvement of PKC in the induction process. In initial experiments, 1-(5-isoquinolinyl sulphonyl)-2-methylpiperazine (H-7), the best available pharmacological inhibitor of PKC, completely blocked the induction of the lytic cycle by TPA and its active analogs. This is consistent with, but does not prove, a requirement for active PKC in the induction process, since H-7 targets PKC preferentially but also has some effects on other kinases. We therefore turned to the synthetic pseudosubstrate peptide PKC(19–36) as a means of specific PKC inhibition and to the closely related but inactive peptide PKC(19–Ser-25–36) as a control. Using the technique of scrape loading to deliver the peptides into cells of an adherent EBV-positive target line, we found that the pseudosubstrate peptide PKC(19–36) completely and specifically blocked tiglian-induced entry of the cells into the lytic cycle. The evidence both from TPA analogs and from enzyme inhibition studies therefore indicates that the pathway linking TPA treatment to lytic cycle induction involves active PKC. Interestingly, inhibition of PKC had no effect upon the spontaneous entry into lytic cycle which occurs in naturally productive cell lines, suggesting that spontaneous entry is signalled by another route.

Epstein-Barr virus (EBV) is a human herpesvirus which persists in the B-cell system in vivo as a nonproductive (latent) infection (43). There is considerable interest in the nature of this latent infection and in the mechanisms whereby latently infected cells can be activated into the virus-productive (lytic) cycle. To date, much of the evidence has come from in vitro model systems, especially from permanent lymphoblastoid cell lines (LCLs) established by EBV-induced growth transformation of cultured B lymphocytes. Most cells within an LCL culture are nonproductively infected and express only a limited number of viral proteins, namely, the nuclear antigens EBNA 1, 2, 3A, 3B, 3C, and LP and the latent membrane proteins LMP 1 and 2 (27). In some LCLs, however, a small but significant subpopulation of cells spontaneously enter the lytic cycle. The cellular controls governing entry into the lytic cycle are not well understood, although indirect evidence suggests a link with cell differentiation (4). In contrast, it is now clear from work in several laboratories that the first viral event which occurs on lytic cycle entry is the expression of the BZLF 1 protein (2, 3, 41), a transcriptional transactivator with some sequence homology and functional similarity to the c-Fos/c-Jun family of AP-1-binding proteins (11, 30).

A variety of treatments have been reported to increase the proportion of EBV-infected B cells entering lytic cycle in vitro; they range from exposure to pleiotropic agents such as halogenated pyrimidines (14) and sodium butyrate (31) to specific signals such as antibody-mediated ligation of surface immunoglobulin (40, 42). However, when examined across a range of different cell lines, the most consistently effective inducing agent is the diterpene ester (DTE) 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (45). The pathway linking TPA with EBV lytic cycle entry has recently become clearer with the identification of TPA-responsive elements (AP-1 sites) within the BZLF 1 promoter region itself (12). Since TPA treatment of human B cells can lead to the appearance of abundant AP-1-binding activity (30), these induced cellular factors are likely to play a role in the initial transactivation of BZLF 1 expression. Thereafter, the BZLF 1 protein can augment its own expression (13) and, with a second transcriptional transactivator protein, BRLF 1 (19), initiate the cascade of virus lytic cycle gene expression (11, 26).

The long-standing interest in TPA as an EBV-inducing agent reflects not only its efficiency of action in this particular system, but also the more widely appreciated activity of this molecule as a tumor promoter (20). Indeed, the coincidence of these two biological activities in TPA gave rise to the view that EBV induction might be used as a convenient in vitro assay for tumor-promoting function (25). It has never been clear, however, to what extent these two activities are mechanistically linked. In this context one, but not the only, route through which TPA can mediate its effects is via activation of the protein kinase C (PKC) family of cellular enzymes (34, 35). One approach to identifying those effects

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which are mediated via PKC activation has come with the isolation of DTEs which are close structural analogs of TPA but which differ in their biological properties, including their ability to activate enzymes of the PKC family (7); indeed, recent evidence from such a panel of DTEs suggests that tumor promotion and PKC activation are not necessarily linked (1). A second approach to the same question has come with the development of specific PKC inhibitors, in particular the use of synthetic peptides as pseudosubstrate inhibitors of particular PKC isoforms (18, 24). Here we have used both approaches to test whether TPA-induced activation of the EBV lytic cycle requires active PKC.

MATERIALS AND METHODS

Cell lines. A variety of EBV-positive B-lymphoid cell lines were used in the present work, including the human Burkitt's lymphoma (BL) cell lines Raji, P3HR1 and its subclones (21), and Mutu BL and its subclones (16); the virus-producing marmoset LCL B95.8 (32); and a number of LCLs established by in vitro transformation of adult human B cells with the B95.8 EBV strain. In addition, some experiments used the EBV-positive somatic cell hybrid line D98/HR1#8 (15), generated by fusion of P3HR1 and D98/AH-2, a derivative of the HeLa cell line. All cells were maintained in RPMI 1640–2 mM glutamine–100 IU of penicillin per ml–100 μg of streptomycin per ml–10% fetal calf serum, and the hybrid cell line medium was further supplemented with 100 μM thymidine.

Chemical induction of EBV lytic cycle. Stock solutions of the various DTEs used were made to as high a concentration as possible (usually 10^{-3} M) by the addition of dry acetone. They were then aliquoted and stored at −20°C. Working dilutions were made in distilled water and stored at 4°C, at which temperature they were found to be stable for at least 12 months. Preliminary dose-response experiments showed that, for each of the active inducers, a concentration of 3×10^{-8} M was sufficient for optimal induction; this concentration was therefore used throughout this work. Results obtained with partially active or inactive inducers were likewise not altered by increasing the dose above 3×10^{-8} M.

Cells in log phase of growth were washed twice in phosphate-buffered saline (PBS) and then resuspended at 0.25×10^6/ml in culture medium containing either tiglian inducer or the appropriate solvent control. Cells were harvested up to 5 days later, and lytic cycle induction was assayed as detailed below.

Assays of EBV lytic cycle induction. Immunofluorescence staining for the early antigen (EA) and late virus capsid antigen (VCA) complexes of the EBV lytic cycle was carried out by standard two-step immunofluorescence with appropriate dilutions of a human serum, EE (anti-EA titer, 1:1,000; anti-VCA titer, 1:10,000). In addition, staining for individual lytic cycle antigens was carried out by standard methods with the following monoclonal antibodies (MAbs): MAb BZ-1 for the BZLF 1 immediate-early protein (44), MAb R63 for the 85-kDa component of EA (36), MAb L2 for the BALF 4 late capsid protein (28), and MAb 72.A1 for the major late glycoprotein of gp340 (23).

Immunoblotting for lytic cycle antigens was carried out as previously described (16) by using human sera with strong reactivities against the 45- to 55-kDa EA-D complex and against the BZLF 1 protein. In each case, equal loading of tracks was checked by Ponceau staining.

PKC inhibitors. The pharmacological inhibitor of PKC, 1-(5-isoquinolinyl sulfonyl)-2-methylpiperazine (H-7) (22), was aliquoted and stored as a stock solution of 100 mM concentration at −20°C.

The synthetic pseudosubstrate peptide inhibitor of PKC, corresponding to residues 19 to 36 of the primary sequence of PKC isoforms α, βI, and βII and hereafter referred to as PKC(19–36), has the following sequence: NH_2-Arg-Phe-Ala-Arg-Lys-Gly-Ala-Leu-Arg-Gln-Lys-Asn-Val-COOH.

Substitution of a serine for the alanine residue at position 25 (underlined) abolishes the pseudosubstrate inhibitory effect; this inactive control peptide is hereafter referred to as PKC(19–Ser-25–36). Peptides, supplied by Alta Bioscience (Birmingham, United Kingdom), were synthesized by FMOC chemistry and purified by chromatography on Sephadex G-50 and equilibrated and eluted with 50 mM NH_4HCO_3.

Scrape loading. Adherent D98/HR1#8 cells were transiently permeabilized to allow the introduction of synthetic peptides essentially as described by Morris et al. (33). A confluent monolayer of cells in a 35-mm-diameter petri dish was washed twice with PBS, and then the cells were gently scraped from the dish with a rubber policeman in the presence of a small volume (180 μl) of peptide solution (3 mg/ml in PBS). This suspension was then immediately transferred into 10 ml of PBS, and the cells were washed twice before being used for reseeding at 10^6 cells per dish. A solution of bovine serum albumin (BSA) (3 mg/ml in PBS) was used instead of peptides to control for possible effects of the scrape-loading process itself.

RESULTS

Induction of EBV lytic cycle by analogs of TPA. The structural formulae of TPA and of five key structural analogs, the sapintoxins Sap A, Sap C, and Sap D and two closely related deoxyphorbol esters, DOPP and DOPPA, are shown in Fig. 1 along with the relevant systematic chemical names. All of these compounds are β-epimers of the tiglian subgroup of DTEs (of which TPA is the prototype) and differ only in the natures and locations of their substituents on the tiglian skeleton. These particular analogs were chosen for the present work because their biological properties, especially in tumor promoter and PKC activation assays, have recently been determined (9, 10). Thus, the present panel includes compounds such as Sap D, which is almost identical to TPA in biological activity; Sap A and DOPP, which possess most of the biological activities of TPA (including PKC activation) but are not tumor promoters; DOPPA, which displays only a few of TPA's activities; and Sap C, which is essentially biologically inactive.

A number of EBV-positive B-cell lines were tested for induction of lytic cycle antigens by TPA and by each of the above analogs. A reproducible pattern of results was obtained, and representative data are shown in Fig. 2. Induction of nonproductive Mutu BL subclones (in this case subclone 88) was assayed by immunoblotting for expression of the BZLF 1 immediate-early protein and of the early EA-D antigen complex (Fig. 2A) and by immunofluorescence staining for the late VCA complex (Fig. 2B). Both assays show efficient induction not only by the tumor promoters TPA and Sap D, but also by the closely related but non-tumor-promoting tiglians Sap A and DOPP. Two other nonpromoting tiglians, DOPPA and Sap C, were inactive on this subclone. Responsiveness to induction was also monitored by immunofluorescence staining for VCA in the B95.8 cell line, in which there is already a significant level of spontaneous lytic cycle entry, and in the tightly

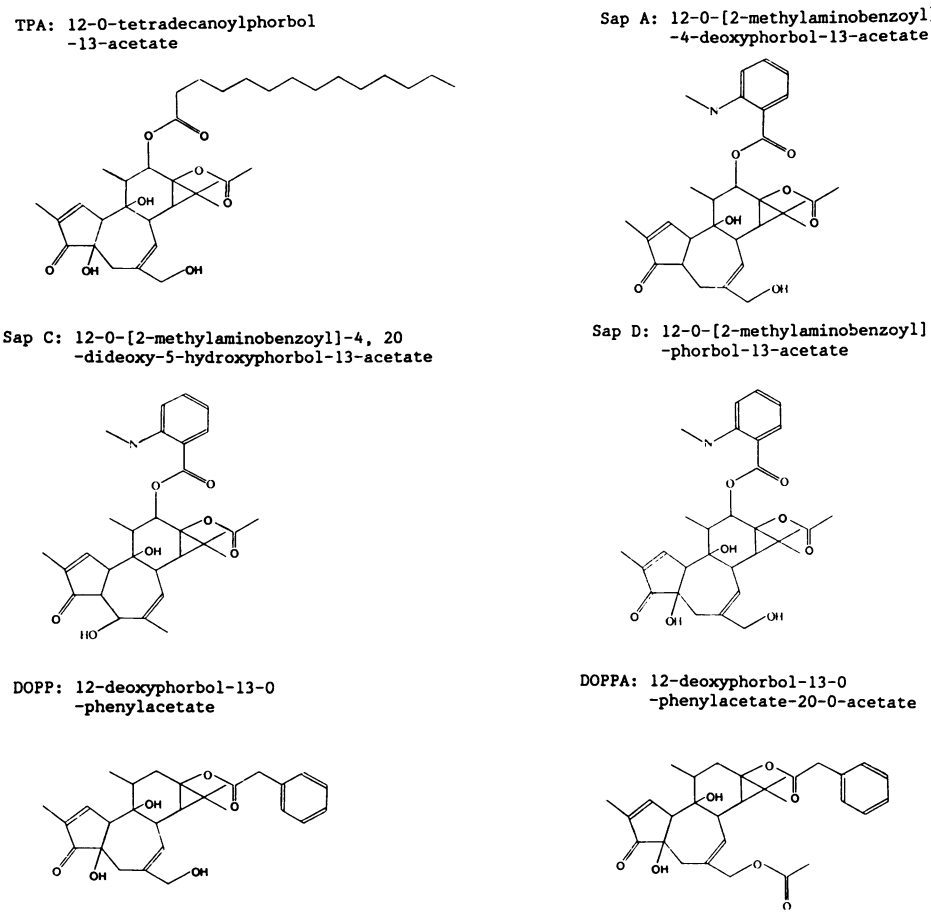

FIG. 1. Chemical structure of tiglian analogs of TPA.

latent D98/HR1#8 hybrid cell line (Fig. 2C and D). A similar pattern of results was obtained in that TPA, Sap A, Sap D, and DOPP all induced lytic cycle entry whereas Sap C was unreactive. Interestingly, DOPPA was inactive on B95.8 cells, as it was on Mutu BL cells (Fig. 2A), yet it did induce low levels of lytic cycle entry in the D98/HR1#8 cell line. Extension of these studies to the tightly latent Raji cell line and to the spontaneously productive P3HR1 line produced tiglian activity profiles like that observed for D98/HR1#8 cells (data not shown).

Effect of pharmacological PKC inhibitor H-7 on induction of EBV lytic cycle. The pharmacological inhibitor H-7 is the most effective of the isoquinoline sulphonamide class of PKC inhibitors (22). It was therefore selected for initial inhibition studies of tiglian-mediated induction of the EBV lytic cycle. We consistently found complete inhibition of such induction by H-7 at concentrations of 50 μM and greater. Figure 3A illustrates typical dose-response curves for this inhibition obtained in an experiment in which B95.8 cells were treated with TPA or with Sap A and the level of induction was monitored by VCA staining 5 days later. Cell viability in these cultures remained high throughout the experiment, indicating that the inhibition of lytic cycle induction was specific and not the result of any general cytotoxicity of the drug itself.

The effects of H-7 upon tiglian-induced lytic cycle entry in several target lines were observed. For targets such as Raji and subclones of Mutu BL which were tightly latent in the noninduced state, cultures treated with TPA or Sap A in the presence of large doses of H-7 never developed any lytic cycle antigen-positive cells. However, we reproducibly noticed that in lines which showed low levels of spontaneous lytic cycle entry, TPA- or Sap A-induced cultures showed some residual VCA expression which appeared to be H-7 resistant (e.g., B95.8 in Fig. 3A). Further studies indicated that this drug-resistant fraction indeed represented the spontaneously productive cell population. Thus, in the same experiment as reported in Fig. 3A, control B95.8 cultures either not exposed to tiglians or treated with the inactive analog Sap C maintained the same steady-state level of spontaneously VCA-positive cells even in the presence of 100 μM doses of H-7 (Fig. 3B). Resistance of spontaneous lytic cycle entry to H-7 inhibition was also observed with the P3HR1 cell line and with several spontaneously productive LCLs (data not shown).

Effect of pseudosubstrate PKC inhibitor PKC(19–36) on induction of EBV lytic cycle. Since the specificity of pharmacological inhibitors of PKC, such as H-7, is increasingly open to question (38), the final series of experiments sought to develop a more specific means of antagonizing PKC activity in EBV-infected target cells. We therefore turned to a pseudosubstrate peptide corresponding to the autoinhibitory domain of those PKC isoforms (α, βI, and βII) which are predominant in B cells. Such a peptide has been shown to specifically inhibit PKC activity in a cell-free system (24). As a means of delivering the peptide to cultured cells at a

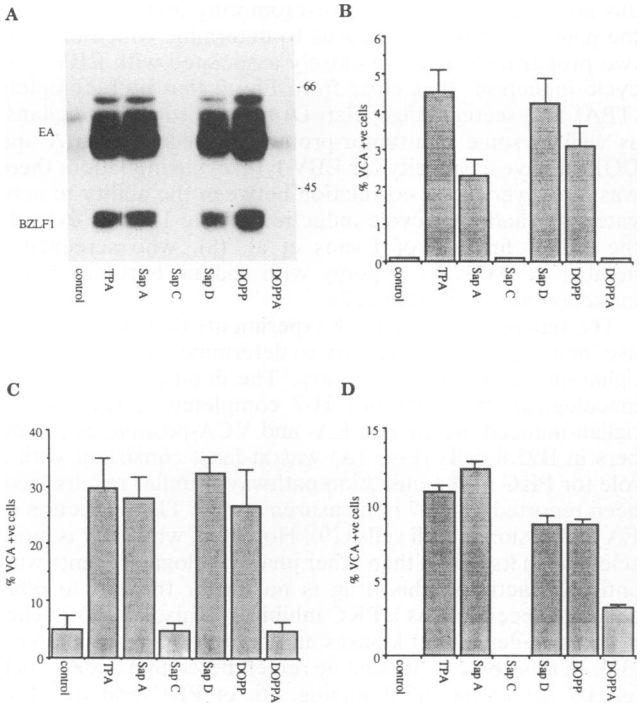

FIG. 2. Induction of EBV from latency by tiglian analogs of TPA. (A) Mutu BL cells in log phase of growth were inoculated in medium containing 3×10^{-8} M of each tiglian inducer, or solvent control, as described in Materials and Methods. Cells were harvested after 3 days for Western blotting with the human anti-EA serum EE. Molecular sizes (in kilodaltons) are shown on the right. (B) VCA staining data for induced Mutu BL cell cultures described above. (C) VCA staining data for induced B95.8 cell cultures 5 days postinduction. (D) VCA staining data for induced D98/HR1#8 cell cultures 5 days postinduction. Results in panels B, C, and D are the means of three separate immunofluorescence assays, in each of which at least 300 cells were scored for each set of culture conditions. Standard deviations are indicated as vertical bars. Results are representative of those obtained in three successive experiments.

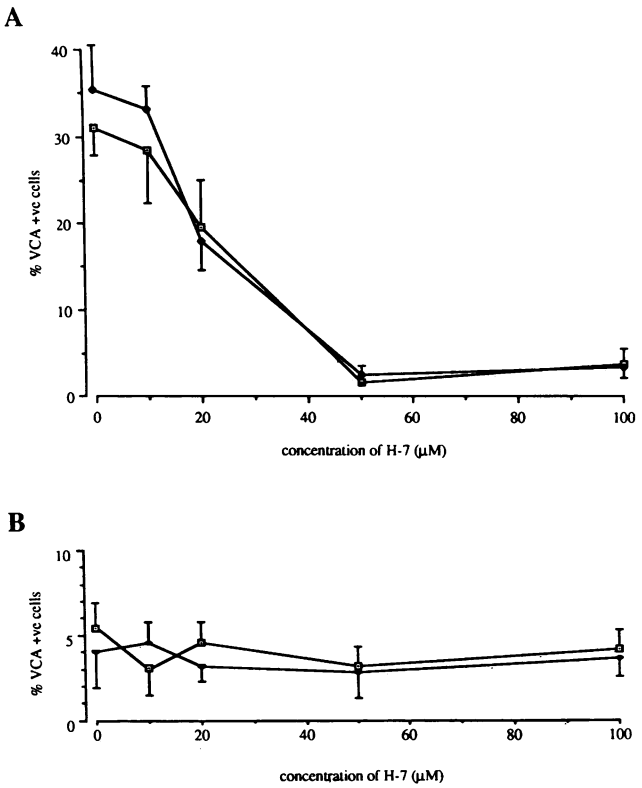

FIG. 3. (A) Effect of the pharmacological PKC inhibitor H-7 on TPA induction of lytic cycle in B95.8 cells. Mid-logarithmic-phase B95.8 cells were cultured in either 3×10^{-8} M TPA (⊡) or 3×10^{-8} M Sap A (◆) in the presence of various concentrations of H-7, and the level of VCA-positive cells was determined 5 days postinduction. (B) Effect of H-7 on spontaneous (noninduced) entry into lytic cycle of B95.8 cells. Mid-logarithmic-phase cells were cultured in the absence of tiglians (◆) or in 3×10^{-8} M Sap C (⊡) in the presence of various concentrations of H-7, and the level of VCA-positive cells was determined 5 days postinduction. Results are given as the means of three separate immunofluorescence assays, in each of which at least 300 cells were scored for each set of culture conditions. Standard deviations are indicated as vertical bars. Results are representative of those obtained in three successive experiments.

sufficiently high concentration, we chose the technique of scrape loading, which has been used previously to introduce a variety of exogenous molecules into cells (33, 37). Scrape loading requires an adherent target cell phenotype, and for this reason the experiments were carried out with the EBV-positive adherent somatic cell hybrid line D98/HR1#8, whose pattern of tiglian responsiveness had already been determined (Fig. 2D). D98/HR1#8 cells were first scrape loaded in the presence of pseudosubstrate inhibitor peptide PKC(19–36), the inactive control peptide PKC(19–Ser-25–36), or BSA as an additional control. The scrape-loaded cells were then reseeded in the presence of TPA, Sap A, or the inactive analog Sap C and were monitored for entry into lytic cycle.

These experiments clearly showed specific inhibition of tiglian-induced lytic cycle entry by the pseudosubstrate inhibitor peptide PKC(19–36). For example, as illustrated by the stained-cell preparations in Fig. 4, TPA treatment of D98/HR1#8 cells induced both BZLF 1 and VCA expression in a significant proportion of cells within 24 h in cultures scrape loaded in the presence of the inactive control peptide PKC(19–Ser-25–36). However, the induction of both lytic cycle antigens appeared to be blocked in cultures scrape loaded in the presence of the pseudosubstrate peptide (PKC(19–36). The quantitative results from one such experiment are presented in Fig. 5, for which lytic cycle entry was monitored in terms of BZLF 1 expression (Fig. 5A) and of VCA expression (Fig. 5B). These results clearly illustrate both the efficiency and the specificity of the pseudosubstrate peptide's inhibitory effect.

DISCUSSION

The cellular controls governing entry of EBV-infected cells into lytic cycle are poorly defined. At present, the most accessible means of studying this question in vitro is via latently infected B-cell lines, and the most consistently efficient means of inducing lytic cycle entry in such lines is via the tiglian TPA. Here we have used two independent approaches to examine the cellular pathway through which TPA induces EBV lytic cycle gene expression. The evidence both from the use of biologically characterized TPA analogs and from the use of specific pseudosubstrate peptide inhib-

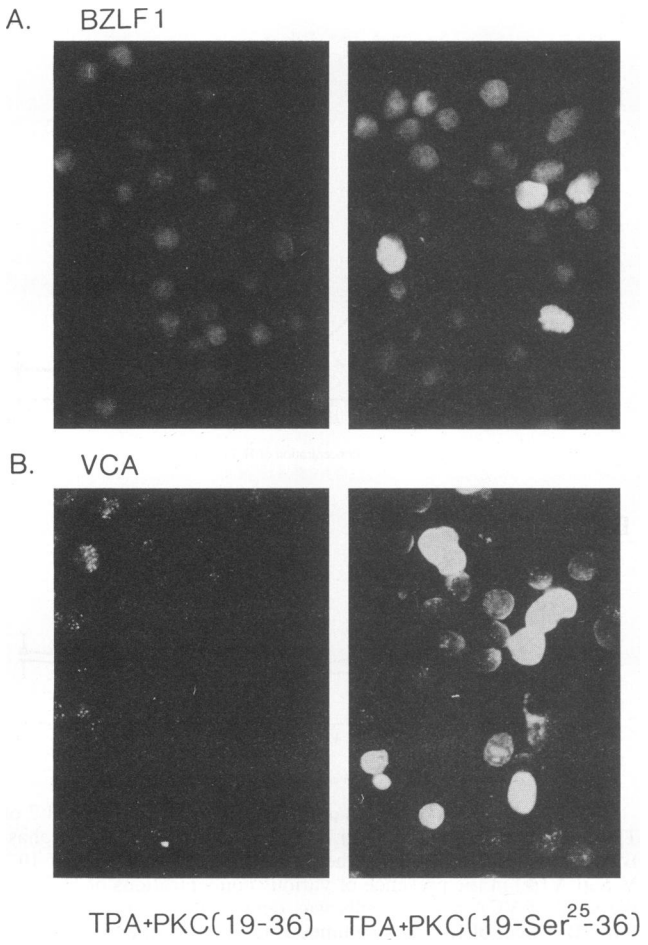

FIG. 4. Effects of the PKC inhibitor peptide PKC(19–36) and the inactive control peptide PKC(19–Ser-25–36) on induction of the immediate-early BZLF 1 protein and the late VCA complex. Peptides were introduced into mid-logarithmic-phase D98/HR1#8 cells by transient permeabilization as described in Materials and Methods. Cells were then plated out in medium containing 3×10^{-8} M TPA, and after 24 h the cells were stained for BZLF 1 by using MAb BZ-1 (A) and for VCA by using a standard human serum (B).

itors strongly suggests that this induction requires activation of PKC.

The panel of tiglians used in the present work (Fig. 1) are all closely related structurally and differ only in their substitutions on the tiglian skeleton. Indeed, the biologically inactive molecule Sap C differs from its active counterpart Sap A only in the position of a single hydroxyl group. Appropriate negative-control analogs of this kind have not been widely used in biological studies with DTEs. Thus, a commonly used inactive TPA analog, 4-α-phorbol, is in fact an α-epimer of the tiglian subgroup of DTEs and thus structurally quite different from TPA, because epimerization at position 4 on the tiglian skeleton changes the conformation of the entire diterpene entity (6). We were further attracted to the panel of tiglians diagrammed in Fig. 1 because each had been tested in a variety of biological assays, including PKC activation and tumor-promoting function (9, 10), and the panel encompassed molecules with novel combinations of activities, which are summarized in Table 1. Most interesting was the fact that PKC activation did not segregate with tumor-promoting ability; screening the panel therefore allowed us to determine which of these two properties was more closely associated with EBV lytic cycle induction. It is clear from Fig. 2 that both complete (TPA) and second-stage (Sap D) tumor-promoting tiglians, as well as some non-tumor-promoting analogs (Sap A and DOPP), have a capacity for EBV lytic cycle induction; there was, however, good correlation between the ability to activate PKC and lytic cycle induction (Table 1). This extends the earlier findings of Evans et al. (8), who screened a number of DTEs for synergy with sodium butyrate in EA induction assays in Raji cells.

The remaining two sets of experiments then attempted to use inhibitors of PKC activity to determine their effect upon tiglian-induced lytic cycle entry. The finding that the pharmacological PKC inhibitor H-7 completely abrogated the tiglian-induced increase in EA- and VCA-positive cell numbers in B95.8 cells (Fig. 3A) was at least consistent with a role for PKC in the induction pathway. Similar results have been reported for H-7 in measurements of TPA induction of EA expression in Raji cells (29). However, while H-7 is more selective in its action than other pharmacological agents with anti-PKC activity, this drug is no longer thought to offer absolute specificity as a PKC inhibitor; it also affects cyclic-nucleotide-dependent kinases and myosin light-chain kinase (38). In this context, it must be remembered that agents such as H-7 act at the ATP-binding site of PKC and that this domain is strongly conserved among many other protein kinases (17).

For this reason, we chose to adopt a different strategy for PKC inhibition which offered greater specificity. It is known that the PKC molecule contains a pseudosubstrate prototope in its N-terminal conserved domain. This part of the regulatory domain can engage the active site with greater avidity than natural substrates and can therefore efficiently autoregulate enzymic activity. A synthetic peptide corresponding to this region, designated PKC(19–36), potently and specifically inhibits PKC but has very little, if any, effect on other known kinases (24). Furthermore, mutation of a single residue, Ala-25 to Ser-25, in the above peptide is sufficient to abrogate inhibitory activity completely, thereby providing a relevant specificity control when the pseudosubstrate inhibitor peptide is used in a biological system. Previous work has demonstrated the applicability of the pseudosubstrate inhibitor approach in a cell culture situation, using microinjection as a method of peptide delivery into cells (39). Here we used scrape loading as an alternative, more convenient delivery system; this method exploits the transient leakiness of cell membranes on physical removal of cells from their substratum and has been shown to be capable of introducing a variety of sizes of molecule into cultured cells (33, 37). The experiments were made possible through the availability of an adherent EBV-positive cell line, the P3HR1-epithelial cell hybrid D98/HR1#8, which showed the same inducibility into lytic cycle by tiglians as did the suspension cultures of various B-lymphoid cell lines (Fig. 2). Our data (Fig. 4 and 5) showed specific inhibition of tiglian-induced lytic cycle entry in D98/HR1#8 cells by the pseudosubstrate inhibitor peptide PKC(19–36) but not by the control peptide PKC(19–Ser-25–36). This clearly demonstrates that active PKC is required in the induction process.

Work from several laboratories suggests that TPA induces EBV lytic cycle entry by changing the activities of cellular AP-1-binding proteins which regulate expression of the immediate-early gene BZLF 1 (12, 30); we postulate that this change is initiated via PKC activation at the membrane. In

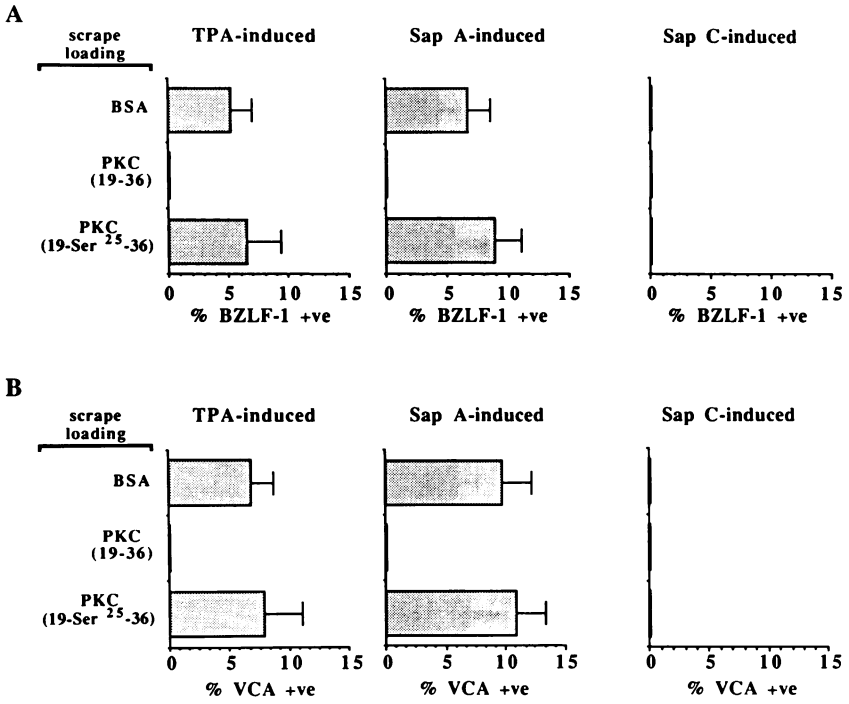

FIG. 5. Effects of the PKC inhibitor peptide PKC(19–36) and the inactive control peptide PKC(19–Ser-25–36) on induction of BZLF 1 (A) and of VCA (B) expression in D98/HR1#8 cells by TPA, Sap A, and Sap C. Results are given as the means of three separate immunofluorescence assays, in each of which at least 300 cells were scored for each set of culture conditions. Standard deviations are indicated as bars.

this context, it is interesting that another membrane signal capable of inducing lytic cycle in EBV-positive B cells, namely, ligation of surface immunoglobulin, has been shown to be transmitted via phosphatidylinositol breakdown, again leading to PKC activation (5), so that the two inducing pathways may converge at that point. However, not all routes of entry into the lytic cycle appear to be PKC linked. One of the most interesting observations to come from the present work was the apparent insensitivity of spontaneous lytic cycle entry to inhibition even by such a potent kinase inhibitor as H-7 (Fig. 3B). This clearly distinguishes spontaneous lytic cycle entry from that induced by tiglians, even though both pathways channel through BZLF 1 as the first EBV lytic cycle protein to be expressed. We also noted, using a large panel of in vitro-transformed LCLs as target lines in the induction experiments described above, that spontaneous productivity and responsiveness to tiglian induction behaved as independent variables (data not shown), again suggesting that the two pathways of entry into the lytic cycle are under separate controls. If spontaneous entry depends upon a prior movement of the cell along the B-cell differentiation pathway, as earlier studies suggest (4), then such differentiation may itself lead to a change in the balance of cellular transcription factors necessary for immediate-early viral gene expression; this spontaneous change must be independent of PKC-mediated signalling.

TABLE 1. Biological properties of TPA and its tiglian analogs[a]

Tiglian	Tumor promoter	Platelet aggregant	Lymphocyte mitogen	Skin irritant	PKC agonist	EBV lytic cycle inducer
TPA	+	+	+	+	+	+
Sap A	−	+	+	+	+	+
Sap C	−	−	−	−	−	−
Sap D	(+)[b]	+	+	+	+	+
DOPP	−	+	+	+	+	+
DOPPA	−	−	−	+	(+)[c]	(+)[d]

[a] Summary of data from reference 9 and from present work. +, active; −, inactive; (+), partially active.

[b] Sap D, in contrast to TPA, is not a complete tumor-promoting agent, but a second-stage promoter only.

[c] DOPPA, in contrast to the other active tiglians listed here, activates one of the PKC β isoforms only (10).

[d] DOPPA, in contrast to the other active tiglians listed here, induces the EBV lytic cycle in many but not all cell lines tested, and the levels of lytic cycle induction are always relatively low.

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