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Short communication

Bryostatin 1 induces productive Epstein-Barr virus replication in latently infected cells: implications for use in immunocompromised patients

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Abstract. Bryostatin 1 is a novel anti-tumor agent currently undergoing clinical trial. We investigated the effect of this drug on B-lymphocyte cell lines that carry the Epstein-Barr virus and found that it induces these latently infected cells into the production of transforming virus particles over a wide range of concentrations. These results may have clinical implications, particularly with regard to the use of the drug in the immunocompromised patient.

Introduction

Bryostatin 1, a novel anti-tumor agent isolated from the marine bryozan *Bugula neritina*, is currently undergoing a phase I clinical trial under the sponsorship of the Cancer Research Campaign. The mechanism by which this agent exerts its anti-tumour effect is unknown. The drug has been shown to be a potent activator of protein kinase C (PKC) and it is suspected that this may be involved in the activity of the drug [11]. Bryostatin 1, in common with classic PKC activators (e.g. the phorbol esters), elicits a number of biological responses, including induction of differentiation, haemopoietic stimulation, platelet aggregation and immunoenhancing activity. However, unlike the phorbol esters, bryostatin 1 is not a tumour promoter and can actually inhibit the tumour-promoting activity of the phorbol esters [4]. Furthermore, bryostatin 1 has shown therapeutic activity against a number of murine tumours in vivo, including leukaemias, B-cell lymphoma, ovarian carcinoma and melanoma [6, 10]. The anti-tumour activity of bryostatin 1, together with its immunoenhancing and haemopoietic stimulatory activity, make this drug an exciting prospect.

Epstein-Barr virus (EBV) is a herpesvirus that infects humans and is present in the majority of individuals. After initial infection, the virus becomes latent, persists for life and is not normally associated with disease. EBV is, however, associated with a number of tumours, including B-cell lymphoproliferative disease, which can occur in immunocompromised individuals such as transplant recipients [1].

Phorbol esters such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) are known to induce 'productive replication' of EBV in latently infected B-lymphocytes [12]. Thus, a proportion of the cells treated with TPA express antigens associated with virion assembly and infectious, transforming EBV particles are produced. The finding that bryostatin 1 mimics biological effects displayed by TPA led us to investigate whether bryostatin 1 could, in turn, induce productive EBV replication.

Materials and methods

Cells. The latently infected B-cell lines B95-8 [8] and Mutu gI [2] were maintained at exponential growth in RPMI 1640 (Gibco/BRL) containing 10% fetal calf serum (Globepharm).

Chemical treatment of cells. 12-*O*-Tetradecanoylphorbol-13-acetate (Sigma) was dissolved in dimethyl sulphoxide (Sigma) at a concentration of 2×10^{-5} *M* and bryostatin 1 was dissolved in ethanol at a concentration of 2×10^{-5} *M* before dilution to the appropriate concentration in medium. For the chemical induction of EBV, cells were resuspended at a concentration of 3.5×10^5/ml in fresh medium. TPA or bryostatin was then added at the appropriate concentrations and the cells were incubated for 2 days. Neither vehicle used for dissolving the chemicals had any effect on the induction of EBV antigens at the concentrations used in these experiments.

Immunofluorescence analysis. Indirect immunofluorescence analysis of live cells was performed using the EBV gp340/220-specific monoclonal antibody 72A1 [5] exactly as described elsewhere [7] and the proportion

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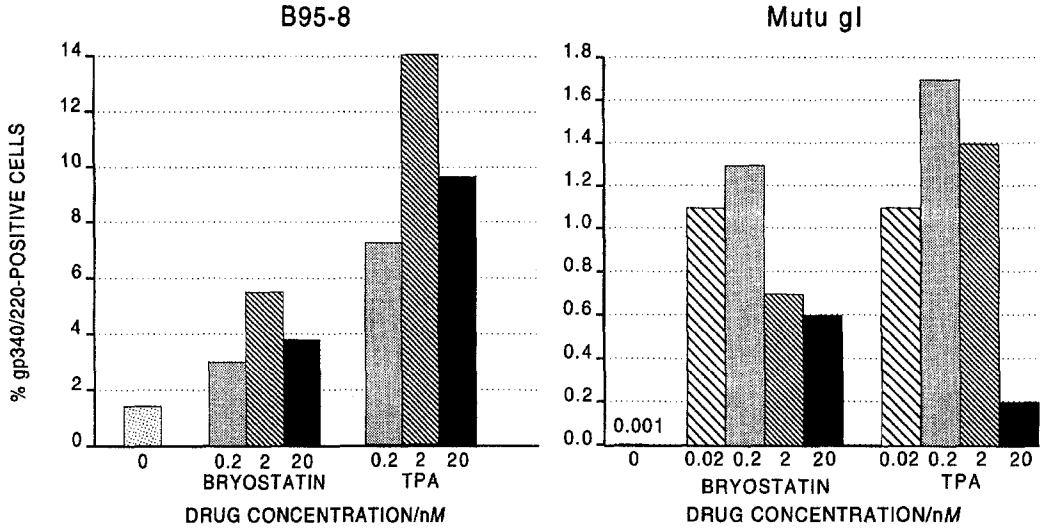

Fig. 1. Induction of gp340/220 expression on the cell surface by TPA and bryostatin 1. Cells were treated with either TPA or bryostatin at the concentrations indicated. After 2 days, cells were stained for gp340/220 expression by immunofluorescence and the number of positive cells were counted by microscopy

of positive cells was determined by counting a minimum of 1000 cells using a UV microscope (Leitz).

Assay of transforming virus. The amount of EBV released from cells was quantitated by titrating cell supernatants on fetal-cord lymphocytes and assaying for transformation as previously described [9]. The virus titre was expressed as the negative log to the base 10 of the virus dilution that induced transformation in 50% of the cultures (TCD_{50}).

Results

To ascertain the effect of TPA and bryostatin on EBV productive replication, the B-cell lines B95-8 and Mutu gI, which contain EBV in a predominantly latent state, were treated with these agents. The proportion of cells undergoing productive virus replication was then quantitated by analysing expression of a 'late' EBV antigen, that is, the major membrane glycoprotein gp340/220, by indirect immunoflourescence. Ths marker was chosen because it is a component of virus particles and, hence, its expression by cells is usually concomitant with virion production. The experiment was repeated on three separate occasions using both cell lines with comparable results. The results of one representative experiment are shown in Fig. 1. The percentage of cells expressing gp340/220 was markedly increased after the addition of either TPA or bryostatin 1 at all concentrations tested. The optimal concentration of both agents was the same for each individual cell line, being around 2 n*M* for B95-8 and 0.2 n*M* for Mutu gI. The absolute number of cells induced to produce gp340/220 was lower in Mutu gI than in B95-8. Mutu gI cells have a much lower rate of 'spontaneous' production and, hence, the induction of antigen expression after the addition of drug was greater in these cells than in B95-8 cells. Hence, bryostatin 1 and TPA caused maximal inductions of 1300-fold and 1700-fold, respectively, in Mutu gI cells as compared with 5-fold and 7-fold, respectively, in B95-8 cells, with the coefficient of variation between replicate experiments being <15% in all cases.

It is normally assumed that the production of EBV late antigens is concomitant with virion production. However, to check whether this was indeed the case for bryostatin 1-treated cells, we performed a fetal-cord lymphocyte transformation assay to quantitate the release of EBV particles. B95-8 cells were treated with TPA or bryostatin 1 at a concentration of 2×10^{-9} *M*. Supernatants from these or untreated cells were then assayed for EBV. The amount of transforming virus detected in supernatant from bryostatin 1- and TPA-treated B95-8 cells was similar ($10^{2.6}$ and $10^{2.7}$ TCD_{50}/ml) and was 1 log higher than that found in supernatant from untreated cells ($10^{1.6}$ TCD_{50}/ml). Thus, the results were in line with gp340/220 staining and show that bryostatin 1 can induce the production of transforming EBV from infected cells.

Discussion

The results presented herein clearly show that bryostatin 1 is capable of inducing the production of EBV from B-lymphocytes that harbour the virus in a predominantly latent state. Our results are not in concordance with those of Gschwent et al. [3], who found that bryostatin, unlike TPA, did not induce EBV antigen synthesis in Raji cells to any significant extent. However, the concentration of drug used by these workers was higher (10^{-7} *M*) than those used in the present study and this may account for the discrepancy since, as can be seen in Fig. 1, we found that bryostatin was less effective at 2×10^{-8} *M* than at lower concentrations. Although the PKC isoenzyme compositions of these cells are not known, it is also possible that differences in PKC isoenzyme composition are responsible for the discrepency between our results and those of Gschwent et al. [3].

Our results have obvious implications for the use of bryostatin 1 as a chemotherapeutic agent, particularly in the immunocompromised patient, as these individuals are highly susceptible to EBV-associated lymphoproliferative disease [1]. Further studies are required to ascertain whether the use of bryostatin 1 increases the levels of virus present in patients.

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