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Short communication

Cellular uptake of the EBV transcription factor EB1/Zta

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Abstract

A number of viral proteins have the property to penetrate into the cells when present in the extra-cellular compartment. Here, we report that the Epstein-Barr virus (EBV) transcriptional activator EB1/Zta, which is responsible for the activation of the EBV lytic replication, binds to lymphoid cells surface, is efficiently translocated and accumulates in the nucleus. The internalization of EB1/Zta is energy-dependent and shares common features with endocytosis. As the EB1/Zta was not degraded in the cells and reached the nucleus, the potential effect of its internalisation on viral reactivation was assessed.

Keywords: EBV; EB1/Zta; Protein transduction; Endocytosis

It has been reported several years ago that a number of viral proteins are able to enter into cells when exogenously added to the culture medium (Elliott and O'Hare, 1997; Frankel and Pabo, 1988; Green and Loewenstein, 1988). Recent studies have suggested that their uptake involved general endocytosis pathways, requiring the previous binding of the protein to the cell surface (Lundberg et al., 2003; Richard et al., 2003), however the way by which proteins leave endocytosis vesicles to assure their normal function in the cell remains unclear (Fittipaldi et al., 2003; Fisher et al., 2004). The Epstein-Barr Virus (EBV) EB1/Zta *trans*-activator shows homologies to members of the fos family of proteins, and is absolutely required for EBV replication. The first EBV genes expressed during the productive cycle of EBV are the immediate-early genes BZLF1 and BRLF1 (coding for EB1/Zta and R/Rta trans-activators, respectively) (Chevallier-Greco et al., 1986; Manet et al., 1989). These two proteins are transcriptional activators and induce the expression of the next tier of EBV genes (Giot et al., 1991; Kenney et al., 1989; Urier et al., 1991; for a review see Speck et al., 1997).

A critical issue concerns the modulation of EBV host's cellular environment in order to create favorable conditions for viral replication and survival. Both latent and lytic EBV proteins are able to interfere with cellular functions, and EB1/Zta has also been implicated in the regulation of some cellular factors (for a review, see Sinclair, 2003). An accumulating body of evidence suggests that EB1/Zta influences cell machinery, namely by inducing cell cycle arrest (Cayrol and Flemington, 1996; Mauser et al., 2002) and interfering with activity of cellular transcription factors (Adamson and Kenney, 1999; Adamson et al., 2000; Gutsch et al., 1994; Zhang et al., 1994). EB1/Zta protein modulates cellular immune responses (Adamson and Kenney, 2001; Cayrol and Flemington, 1995; Morrisson et al., 2001) and is able to bind directly to hIL-10 gene promoter, therefore inducing hIL-10 cytokine expression (Mahot et al., 2003). In this study, we examined the ability of EB1/Zta protein to enter lymphoid cells (both EBV-positive and EBV-negative cells) and reach their nucleus. We show that this protein is able to accumulate on the

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cell surface *via* heparane sulfate (HS) proteoglycans and to actively penetrate into cells, before being transferred into the nucleus.

To investigate the potential cellular uptake of EB1/Zta protein, 1×10^6 Raji cells (an EBV-infected B cell line) were incubated during increasing times with 1 ml serum free-RPMI medium containing 10 ng histidine-tagged recombinant EB1/Zta (His-EB1/Zta). After extensive washing, cells were fixed with paraformaldehyde (2% PFA in PBS, 20 min) and permeabilized under mild conditions (0.2% Triton in PBS, 3 min). EB1/Zta detection was performed by immunofluorescence (IF) with anti-EB1/Zta Z125 mouse monoclonal antibody (mAb) (ascitic fluid purified by protein-A sepharose beads, then diluted 1:100) (Mikaelian et al., 1993) and a secondary anti-mouse Alexa488-conjugated antibody (diluted 1:1000, Molecular Probes). Cell nuclei were

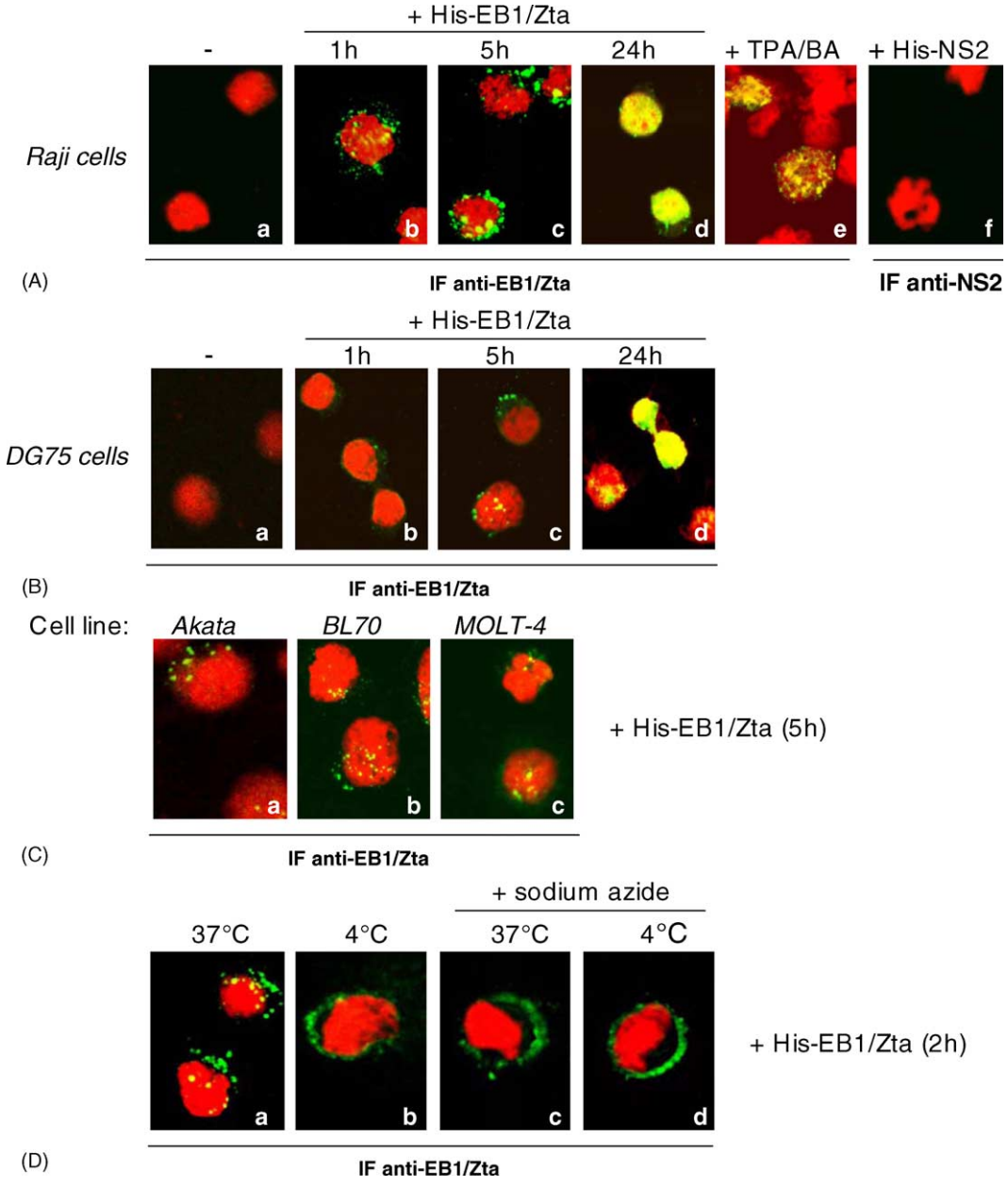

Fig. 1. Cellular uptake of EB1/Zta recombinant protein. (A) Raji cells were incubated without any protein (a), with purified recombinant his-tagged EB1/Zta for 1, 5, and 24 h (resp. b, c, d), with 20 ng/ml TPA and 3 mM BA (24 h incubation) as positive control (e) or with an unrelated his-tagged viral protein (His-NS2) (f). EB1/Zta and NS2 were detected by IF, using purified specific mAbs (resp. Z125 and anti-NS2) (green signal). Laser scanning confocal microscopy was performed on an MRC600 (Bio-Rad) and pictures were obtained at magnification 2. (B) DG 75 cells were incubated without (a) or with His-EB1/Zta for 1, 5, and 24 h (resp. b, c, d), and analyzed as in A (magnification 2). (C) Akata (a), BL 70 (b) and MOLT-4 cells (c) were incubated for 5 h with His-EB1/Zta, and analyzed as in (A) (magnification 2.5). (D) Raji cells were incubated with the His-EB1/Zta at 37 °C (a), 4 °C (b), with sodium azide at 37 °C (c) and 4 °C (d), and analyzed as in (A) (magnification 2.5).