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In cis inhibition of antigen processing by the latency-associated nuclear antigen I of Kaposi sarcoma Herpes virus

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Abstract

Kaposi sarcoma Herpes virus (KSHV), also known as human Herpes virus 8 (HHV8), can persist as episome in target cells. The latency-associated nuclear antigen 1 (LANA-1) is a key component of the latency process, and may be a functional equivalent of the EBNA-1 protein of Epstein–Barr virus. EBNA-1 can subdue immune recognition by virtue of a long glycine and alanine-rich repeat, which interferes with the proteasomal degradation of EBNA-1 and in this way averts the presentation of antigenic peptides derived from it. LANA-1 contains a strongly acidic-repeat region of approximately 580 amino acids, which consists almost exclusively of aspartic acid, glutamine, and glutamic acid residues. The LANA-1 repeat is not similar to the EBNA-1 Gly-Ala-rich repeat. We demonstrate that this acidic region could inhibit antigen processing *in cis*. Upon transfection of expression vectors containing LANA-1-eGFP fusion genes the cells did not present an ovalbumin-derived $H2K^b$-restricted CTL epitope inserted at the carboxyl terminus of the GFP reporter. Deletion of the central acidic-repeat region of LANA-1 abolished the capacity of LANA-1 to block antigen presentation. Similar to the EBNA-1-derived Gly-Ala-rich repeat, the LANA-1 repeat does not inhibit presentation *in trans*: co-transfection of LANA-1 expression vectors does not inhibit presentation of the ova epitope from the GFP_{Ova} fusion protein. These data demonstrate for the first time that the acidic-repeat region of LANA-1 could function as an *in cis* acting inhibitor of antigen presentation. This may contribute to the immune evasion of cells latently infected by KSHV.
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1. Introduction

Kaposi sarcoma Herpes virus (KSHV) also known as human Herpes virus 8 (HHV8), is a gamma Herpes virus associated with AIDS-related lymphoproliferative disorders (Oksenhendler et al., 2002; Schulz, 2001). Like other Herpes viruses KSHV can persist for life in a latent form in infected cells (Cotter and Robertson, 2002; Spear and Longnecker, 2003; Verma and Robertson, 2003).

One of the most studied proteins implicated in Herpes virus latency is the Epstein–Barr virus (EBV) nuclear antigen 1 (EBNA-1). EBNA-1 binds to the viral origin of replication and to metaphase chromosomes (Leight and Sugden, 2000; Marechal et al., 1999; Sugden et al., 1985; Yates et al., 1985), thus allowing EBV episomal maintenance within the infected cell and equal partitioning to the daughter cells. EBNA-1 is the only viral protein present in all EBV-associated tumors. By a mechanism not totally understood yet, the Gly-Ala repeat can interfere with the proteasomal degradation and prevent cytotoxic T lymphocyte epitope generation (Levitskaya et al., 1995, 1997; Ossevoort et al., 2003). The current model explaining this phenomenon dictates that a long stretch of alanines interspaced by 1, 2 or 3 glycines would give to EBNA-1 the appropriate conformation to interact with a proteasomal component that contains a hydrophobic pocket (Sharipo et al., 2001). So far, although this 30–200 GA repeat (depending on virus isolates) confers a benefit mechanism for the virus, only two lymphocryptoviruses, RhLCV (rhesus lymphocryptovirus) and BaLCV (baboon lymphocryptovirus), have kept such a repeat even though their inhibitory effect is still unclear (Blake et al., 1999; Fogg et al., 2005).

Like EBV, KSHV infects human B cells, macrophages, endothelial cells, and epithelial cells. The infected cells are not eradicated from host cells by the immune system, suggesting that

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KSHV developed a strategy to evade the immune surveillance (Cotter and Robertson, 2002; Lan et al., 2004, 2005; Rajcani and Kudelova, 2003). During latency, KSHV persists as a multicopy circular episomal DNA in the nucleus, and here it expresses a small subset of viral genes. One of the proteins encoded by these genes is the latency-associated nuclear antigen 1, LANA-1 (also called LNA or LNA-1). This protein is the functional equivalent of EBNA-1 of Epstein–Barr virus (Bennett et al., 2005). LANA-1 is a large multi-functional nuclear protein of 1162 amino acids, expressed from ORF73, and is involved in numerous cellular processes (Garber et al., 2002). It has been reported to improve dissemination of KSHV by modulating expression of oncosuppressor proteins (Denis et al., 2000; Friborg et al., 1999; Radkov et al., 2000). The central region of LANA-1 is occupied by a long acidic sequence that can be arbitrary divided in three parts: aspartic acid/glutamic acid (DE), glutamine/glutamic acid (QE), and aspartic acid/glutamine (DQ) repeats (Borah et al., 2004). The function of the central domain is not well described yet but seems to be required for the activation of the latency-associated promoter Cp (Viejo-Borbolla et al., 2003). LANA-1 contains two nuclear localization signals (nls), one located in the N-terminal part (aa 24–30) (Piolot et al., 2001) and one in the C-terminal part (Schwam et al., 2000). LANA-1 is associated with heterochromatin during interphase and with chromosomes during mitosis (Moorman et al., 2003; Szekely et al., 1999).

Here we demonstrate that the latency-associated nuclear antigen-1 of KSHV can inhibit the presentation of linked antigenic peptides. In this respect it resembles EBV and MHV68 latency-associated (Bennett et al., 2005) proteins. The mechanism involved is not clear but it is evident that the long central acidic region of LANA-1 is required for this inhibition.

2. Materials and methods

2.1. Constructs

To generate the GFP_{Ova} expression vector, we introduced the codons for the Ova epitope SIINFEKL by mutagenic PCR (stratagene Kit) into the open reading frame for green fluorescent protein in the vector pRRL-CMV-GFP with the following primers: forward 5′-ACGAGCTGTACAAGAGCATAATTA-ATTTCGAAAAGCTCTAAGCGGCCGCGTC-3′; reverse 5′-GACGCGGCCGCTTTAGAGCTTTTCGAAATTAATTATGC-TCTTGTACAGCTCGTC-3′.

To check the *cis*-inhibitory effect of the LANA-1 repeat, we created a protein in which amino acids 1–1082 of LANA-1 were fused with GFP_{Ova}.

Briefly, HindIII/XcmI fragment from pCDNA3.1 myc-LANA-1 (kindly provided by Dr. Kenneth Kaye) has been inserted into pRRL-CMV-GFP_{Ova} generating a pRRL-CMV-LANA-GFP_{Ova}. The pRRL-CMV-LANAΔr-GFP_{Ova} construct was generated by deleting the central repeat region by mutagenic PCR using following primers: forward 5′-GATGACA-ATGACAATAAGGATATCTTAGAGGAGGTGGAAGAG-3′; reverse: 5′-CTCTTCCACCTCCTCTAAGATATCCTTATTGT-CATTGTCATC-3′.

2.2. Cell lines

The cell lines 293T and 911 were grown in high-glucose DMEM supplemented with 10% fetal bovine serum (Gibco BRL) and penicillin/streptomycin. B3Z hybridomas (kindly provided by Dr. Rene Toes) were grown in IMDM supplemented with 8% fetal bovine serum, penicillin/streptomycin, glutamax, β-mercaptoethanol and hygromycin B. The B3Z indicator cells (Karttunen et al., 1992) were cultured in IMDM (Gibco) containing 8% (v/v) fetal bovine serum, 100 IU of penicillin per milliliter, 100 μg of streptomycin per milliliter, 28 μM β-mercaptoethanol and 500 μg/ml hygromycin B. All cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air.

2.3. Transfection and FACS analysis

The 293T and 911 cells were modified at 70% confluency by transfection with the calcium phosphate co-precipitation technique (Graham and van der Eb, 1973). Transfection in 6-well dishes and 24-well dishes are performed with, respectively, a total of 5 and 2 μg DNA/well.

Twenty-four hours post-transfection, cells were trypsinized, resuspended in PBS, and the transfection efficiency was measured by FACS analysis(FACScan Becton-Dickinson). GFP fluorescence was detected using a 530/30 nm bandpass filter (FL1 channel) following excitation with an argon ion laser source at 488 nm. Using a forward-scatter/side-scatter representation of events, a region was defined to exclude cellular debris from the analysis. A number of events/FL1 histogram, which reflects the fluorescence intensity, was generated, and percentages of GFP-positive cells were determined in comparison to the negative control (untreated cells). Data analysis was performed using CellQuest 3.1 software (Becton-Dickinson). For each sample, 10,000 events were collected.

2.4. Localization study

The 911 cells were cultured in 6-well plates on coverslips. Forty-eight hours after transfection, cells were fixed with paraformaldehyde (PFA) 2%, Tween-20 0.5%, in PBS and subsequently washed three times with PBS/tween 0.05%. Nuclear staining was performed using propidium iodide for 15 min. Slides were analysed by Confocal Laser Scan Microscopy (CLSM, Leica DM-IRBE).

2.5. CHX-chase

To measure the rate of degradation of LANA-1, we transfected myc-tagged LANA-1 constructs. Twenty-four hours post-transfection, 293T cells were treated with 25 μg/ml of cycloheximide (CHX) to prevent further protein synthesis and in the absence or presence of the proteasome inhibitor MG132 (25 μM) (Calbiochem). Whole-cell extracts were prepared from samples harvested at different time points (0, 1, 2, 4 and 8 h). The amounts of LANA-1 and LANAΔr were determined by

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