LETTER TO THE EDITOR

EPSTEIN-BARR VIRUS NUCLEAR ANTIGEN 1 MAKES A COMPLEX DURING THE EARLY STAGE OF IMMORTALIZATION OF HUMAN LYMPHOCYTES

H. YAMAMOTO[1], H. SENPUKU[2], K. YANAGI[1]

[1]Department of Biochemistry and Cell Biology, and [2]Department of Oral Science, National Institute of Health, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162, Japan

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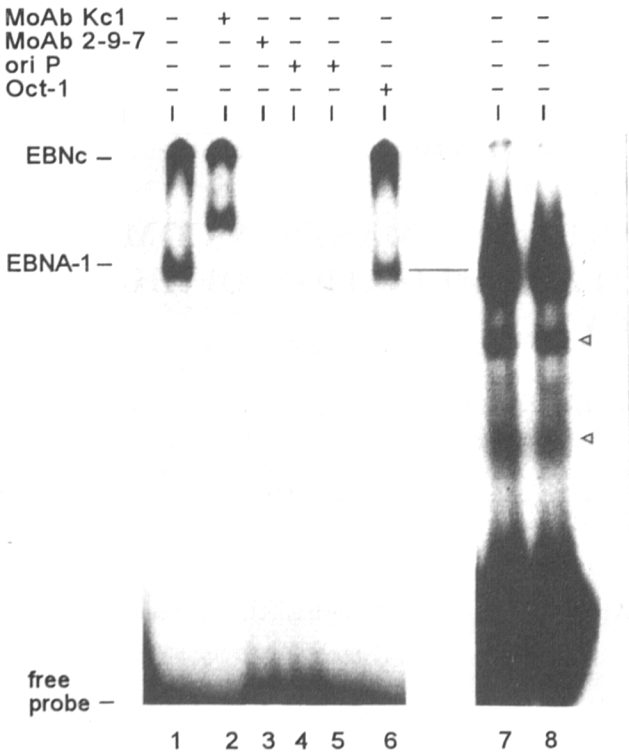
Epstein-Barr virus (EBV) is a human herpesvirus that is associated with several malignancies and diseases, including infectious mononucleosis, Burkitt's lymphoma and nasopharyngeal carcinonma. The EBV nuclear antigen 1 (EBNA-1) binds to the latent replication origin of EBV, *oriP* (*1*), and activates viral DNA replication. During latent infection of host cells, EBV episomal genomes replicate autonomously, initiating bidirectional replication from *oriP* once at each cellular S phase (*2*). This replication is absolutely dependent on EBNA-1 and presumably requires also the cellular replication apparatus (*3*). The mechanism by which EBNA-1 activates replication from *oriP* is not yet clear. EBNA-1 does not appear to have any intrinsic enzymatic activities (*4*). In addition to its role in DNA replication, EBNA-1 has been shown to activate (*5*) and repress (*6*) transcription, and govern the stable segregation of EBV episomes during cell division (*7*). These reports suggested the existence of complexes composed of EBNA-1, but they have not been identified yet.

In this study, we tried to detect the complexes in EBV-infected cells using the DNA mobility shift assay. Human lymphocytes were prepared from peripheral blood and immortalized with EBV B95-8 (*8*). EBV-infected lymphocytes were cultured in RPMI 1640 medium supplemented with 10% foetal calf serum (RPMI 1640/10% FCS medium) for 15 days, and immortalized cells, NUA-1 cells, were obtained. Then the nuclear extracts of NUA-1 cells were prepared (*9*) and subjected to DNA mobility shift assay (*9*). As shown in the figure (lane 1), the nuclear extracts of NUA-1 exhibited two slow bands; one band corresponded to EBNA-1 and the slower one was referred to as EBNc (EBNA-1 complex). In the presence of unlabelled *oriP* as a competitor, both bands derived from EBNA-1 and EBNc disappeared (lanes 4, 5). However, an unrelated DNA oligonucleotide, Oct-1, did not influence the bands of EBNA-1 and EBNc (lane 6). Furthermore, by addition of the anti-EBNA-1 monoclonal antibody (MoAb) 2-9-7 which specifically inhibited the *oriP* binding activity of EBNA-1, the bands of both EBNA-1 and EBNc disappeared (lane 3). In the presence of another MoAb against EBNA-1, Kc1, which did not inhibit the *oriP* binding activity of EBNA-1, the bands of both EBNA-1 and EBNc were present and further retarded (lane 2). These results strongly suggested that EBNc contained EBNA-1 and specifically bound to *oriP*, and its lower mobility as compared to EBNA-1 indicated that EBNc contained also some additional viral or cellular factor(s).

In another experiment, NUA-1 cells were subcultured at 3-4 day intervals and maintained in RPMI 1640/10% FCS medium for three months. Then nuclear extracts were prepared and subjected to DNA mobility shift assay. As shown in the figure (lanes 7, 8), the band of EBNA-1 was detected, however, the band of EBNc was not. The following reasons for this disappearance were considered: (i) The EBNc complex may be composed of proteins which are encoded in EBV genome or which are human gene products induced by the EBV infection. (ii) EBNc may be needed only in the early stages of the EBV infection. If a latent infection is established, the complex is not needed any more. (iii) EBNc may positively activate the expression of viral or host genes



Figure

Nuclear extracts were prepared from 1 x 10^5 NUA-1 cells and subjected to DNA mobility shift assay. *OriP*, double-stranded oligonucleotide (5'-AGATTAGGATAGCATATGCTACCCA-3', the EBNA-1 binding site is underlined) was purchased from Greiner Japan, Tokyo. *OriP* labelled with [alpha-^{32}P]dCTP (ICN, Costa Mesa, USA) at the 3'-end by Klenow fragment was used as a probe. Oct-1, a heterologous double-stranded oligonucleotide (5'-AGGATCCATGCAAATGGATCCC-3') was also from Greiner Japan.

Lanes 1-6: NUA-1 cells, obtained from human lymphocytes 15 days p.i. with EBV, were used for preparation of nuclear extracts.

Lanes 7, 8: NUA-1 cells, subcultured for additional 3 months, were used for preparation of nuclear extracts. Open triangles: non-specific bands.

needed to maintain the latent EBV infection. In order to detect these gene products, the EBV infectious stage and the cell cycle synchronization should be ensured.

Our results suggest that EBNA-1 forms a complex during the early stage of immortalization of human lymphocytes by EBV. The isolation and characterization of this complex should be done in future studies.

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