Methylation of Marek’s Disease Virus DNA in Chicken T-lymphoblastoid Cell Lines

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SUMMARY

Methylation of Marek’s disease virus serotype 1 (MDV1) DNA in both productively and latently infected cells was examined by restriction endonuclease analyses with the isoschizomeric pair HpaII and MspI. The latent MDV1 DNA in T-lymphoblastoid cell lines established from chicken T-lymphomas was considerably methylated, whereas methylation of the virus DNA sequences was not detected in productively infected cells.

T-lymphoblastoid cell lines established from malignant lymphomas of chickens with Marek’s disease (MD) have been shown to contain multiple copies of the Marek’s disease virus serotype 1 (MDV1) genome (Nazerian & Lee, 1974; Tanaka et al., 1978; Hirai et al., 1981; Ross et al., 1981). The latent virus genomes in non-producer MD lymphoblastoid cell lines were shown to exist as closed circular DNA of full length (Tanaka et al., 1978; Hirai et al., 1981; Rziha & Bauer, 1982) or in an integrated form (Kaschka-Dierich et al., 1979). Recently, latent virus DNA was found on specific chromosomes of two MD cell lines by in situ hybridization (Hirai et al., 1986a). RNA was transcribed from limited portions of the latent virus genomes in non-producer cell lines (Silver et al., 1979; Hirai et al., 1981; A. Kanamori et al., unpublished results). In addition, an MDV1-specific cytoplasmic antigen, P-antigen, related to phosphorylated polypeptides, is the only detectable virus-specific antigen in non-producer MD cell lines (Ikuta et al., 1985) and in tumour lesions of chickens with MD (Naito et al., 1986).

Methylation of the latent virus genome may play a role in the restricted expression in MD cell lines, since the DNAs of various oncogenic viruses, including lymphotropic herpesviruses such as Epstein-Barr virus (EBV) and herpesvirus saimiri, have been shown to be highly methylated in non-producer cell lines, but not in productively infected cells or virions (for review, see Doerfler, 1981). DNA methylation may occur in either integrated virus DNA or circular plasmid DNA. This communication provides the first evidence of methylation of latent MDV1 DNA in MD cell lines.

The MD cell lines (MDCC) used were MDCC-MSB1 (Akiyama & Kato, 1974), MDCC-LS1 (Tanaka et al., 1978), and MDCC-HP1 (Powell et al., 1974). Of these, the primary MSB1 and HP1 cell lines were both reported to contain some virus-producer cells (Akiyama & Kato, 1974; Powell et al., 1974), but the cells used in the present studies, including LS1 cells, had been passaged in culture for a long time and had become non-producers, as determined by immunofluorescence tests with monoclonal antibodies against late virus-specific antigens (unpublished observations). Therefore, these cell lines were considered to contain mainly latent virus genomes. These cell lines were cultured in RPMI-1640 medium supplemented with 5% foetal calf serum at 41 °C. As a source of actively replicating MDV1 DNA, chicken embryo fibroblasts (CEF) were infected with the cell-associated BC-1 strain of MDV1. The BC-1 strain at lower passages of 16 to 23 (BC/LP) was oncogenic, while that at a higher passage of 64...
Fig. 1. Southern blot hybridization of the $^{32}$P-labelled BamHI-H or -I2 fragments of MDV1 DNA to blots containing BamHI-MspI or BamHI-HpalI digests of total DNAs extracted from productively infected cells and MD cell lines. Total DNAs extracted from infected cells or MD cell lines were doubly digested with BamHI and MspI (lane 1) or BamHI and HpalI (lane 2). The digests were electrophoresed on 1-0% agarose gel, transferred to nitrocellulose filters, and subjected to Southern blot hybridization with $^{32}$P-labelled BamHI-H (a) or BamHI-I2 (b). Each lane contains 0-1 μg of total DNA from cells productively infected with BC/LP (BL) or BC/HP (BH), or 5 μg of total cell DNA from MD cell lines MSB1 (MS), LS1 (LS) or HP1 (HP). The sizes of DNA fragments are indicated in kbp on the right side of each panel. The ladder of DNA fragments with sizes increasing by increments of 132 bp are indicated by a vertical line in (a) of this figure and the visible bands are indicated by arrowheads. The 1-3 kbp fragment in (a) is derived from BamHI-D, which has partial homology with BamHI-H.
(BC/HP) was non-oncogenic (Ikuta et al., 1983). For identification of the virus DNA sequences in MD cell lines and productively infected cells, total cell DNA of high mol. wt. was isolated and subjected to Southern blot hybridization with \(^{32}\)P-labelled cloned BamHI fragments of MDV1 DNA, as described previously (Hirai et al., 1984a, 1986b). The conditions for digestions with BamHI, HpaII and MspI were as specified by the supplier (Takara Shuzo Co., Kyoto, Japan).

For determination of whether the latent MDV1 DNA in MD cell lines was methylated, DNAs from MD cell lines and productively infected cells were digested with BamHI and then with HpaII or MspI. HpaII and MspI both cleave DNA at a specific sequence, CCGG. When the internal C residue of the CCGG site is methylated, this site can be cleaved by MspI but not by HpaII. Thus the differential cleavage patterns generated by the isoschizomeric pair HpaII–MspI indicates methylated CCGG sites in a given DNA fragment of MDV1 DNA. BamHI-H and HpaII were chosen for analyses of methylated sites, since the two fragments are of similar sizes, being 5.3 and 5.0 kbp, respectively, and are located next to each other in the long internal repeat (IR\(_l\)) of the MDV1 genome (Fukuchi et al., 1984; Maotani et al., 1986, Fig. 2a). The BamHI–MspI and BamHI–HpaII cleavage patterns of oncogenic BC/LP and non-oncogenic BC/HP DNAs were compared by Southern blot hybridization with \(^{32}\)P-labelled BamHI-H (Fig. 1a). Digestion of BamHI-H with HpaII or MspI produces six fragments (Hirai et al., 1984b) and the largest fragment of 2.1 kbp contains three units of 132 bp tandem direct repeats (Maotani et al., 1986). Methylation was not detected by digestion with the HpaII–MspI pair in either BC/LP or BC/HP DNA, suggesting that methylation of the internal C residue of CCGG sites in the oncogenic strain-specific fragment BamHI-H may not be related to the oncogenicity of MDV1. In contrast to virus DNA in productively infected cells, BamHI-H of the latent virus DNA in the MD cell lines MSB1, LSI and HPI, was considerably resistant to HpaII digestion, as demonstrated by the presence of larger-sized virus DNA fragments of 2.7 and 2.8 kbp in the BamHI–HpaII digests, in addition to fragments detected in the BamHI–MspI digests. These results indicate the presence of both methylated and unmethylated BamHI-H populations in these MD cell lines. In addition, the largest fragment in the HpaII digests of DNAs from MD cell lines was smaller than the undigested BamHI-H fragment of 5.3 kbp. Therefore, BamHI-H of the latent virus DNA from these MD cell lines contains both methylated and unmethylated internal cytosine residues at five CCGG sites. The BamHI-H2 fragment of virus DNA in MD cell lines was also shown to be methylated as found for the BamHI-H fragment, whereas no methylation of the virus DNA fragment from cells productively infected with either BC/LP or BC/HP was detected from the differential cleavage patterns with the HpaII–MspI pair (Fig. 1b). Fig. 1(a) also shows that BamHI–MspI or BamHI–HpaII digestion of BC/HP DNA produced a series of bands with sizes increasing by increments of about 130 bp in the place of a 2.1 kbp fragment of BC/LP DNA. Since the 132 bp tandem direct repeats present in the 2.1 kbp fragment of BC/LP DNA were shown to be amplified in the BC/HP DNA by use of cloned fragments (Maotani et al., 1986), the present result indicates that the copy number of the 132 bp repeat varies in each BC/HP DNA molecule; the number was estimated to vary from three to about 100 units from the electrophoretic mobilities of the DNA fragments containing 132 bp tandem repeats. The copy number of the 132 bp repeat in BC/HP DNA was higher than that estimated previously (Hirai et al., 1984a), because of the enhanced resolution of fragments of small size in the present study. The BC/LP DNA used also appeared to include a few fragments containing more than three units of the 132 bp repeat as indicated by arrowheads in Fig. 1(a).

At present, the copy number of the 132 bp tandem direct repeat is the only known difference between oncogenic and non-oncogenic MDV1 DNAs. Therefore, methylation of the MspI or HpaII site close to the repeats in the EcoRI-1 fragment of the BamHI-H was examined by digestions of DNAs from BC/LP infected cells and MD cell lines with BamHI–MspI and BamHI–HpaII (Fig. 2). EcoRI-1–BamHI-H is cleaved to three fragments (1, 2 and 3) by MspI or HpaII (Fig. 2a). Of these, MspI-1 (or HpaII-1) of 2.1 kbp contains three copies of the 132 bp repeat (Maotani et al., 1986) and can be detected by Southern blot hybridization with \(^{32}\)P-labelled AccI-2 of EcoRI-1–BamHI-H (Fig. 2b). Using the AccI-2 as a probe, only MspI-1 and HpaII-1 of the same size were detected in the BamHI–MspI and BamHI–HpaII digests of BC/LP DNA respectively, indicating that the CCGG site at the left end of MspI-1 was not methylated.
in productively infected cells. The $^{32}$P-labelled AccI-2 was found to hybridize to two fragments of 2·1 and 2·0 kbp in BamHI-MspI digests of DNAs from MSB1 and LS1 cells and to one fragment of 2·0 kbp in BamHI-HpaII digests of HP1 cells (Fig. 2b). The 2·0 kbp fragment was estimated from its size to contain two units of the 132 bp repeat. This small number of 132 bp repeats could be characteristic of the latent virus DNA in MD cell lines (unpublished...
observations). In BamHI–HpaII digests of DNAs from MD cell lines, the Accl-2 hybridized to 2.8 and 2.7 kbp fragments of MSB1 and LS1 DNAs, and to a 2.7 kbp fragment of HP1 DNA, in addition to a small number of fragments detected in BamHI–MspI digests of these DNAs (Fig. 2b). Since the 2.7 and 2.8 kbp fragments correspond to the fused fragment of MspI-1 (2.0 and 2.1 kbp, respectively) and MspI-2 of 0.7 kb, the CCGG site at the left end of MspI-1 is methylated in a significant proportion of the DNA population. However, we could not detect any fragments larger than the 2.7 and 2.8 kbp fragments. These results indicate that the EcoRI-1–BamHI-H fragments of virus DNAs from MD cell lines are mostly methylated at one of two CCGG sites (Fig. 2a).

The methylation of virus DNA in MD tumour cells resembles that of DNAs in cell lines positive for the genomes of herpesvirus saimiri (Desrosiers et al., 1979) or EBV (Larocca & Clough, 1982), although the extent of DNA methylation varies in different cell lines. Usually, only a portion of the virus genome is expressed in these tumour or transformed cell lines and methylation of the latent virus DNA may play a role in limiting expression, possibly by its transcriptional control. Among the MD cell lines used here, limited degrees of latent MDV1 genome transcription have been demonstrated in LS1 cells by DNA-RNA reassociation kinetics (Silver et al., 1979) and in non-producer MSB1 and HP1 cells by blot hybridization with cDNA specific to virus-specific polyadenylated [poly(A)] RNA or by Northern blot hybridization (unpublished observations). The non-producer MSB1 cells contain poly(A) RNA transcribed from the region containing 132 bp direct repeats of the MDV1 DNA, whereas HP1 cells do not. However, we have no evidence to show that methylation of the virus DNA region in HP1 cells results in loss of its transcription, since some of the latent virus DNA was unmethylated. For further understanding of the relationship between virus DNA methylation and possible transcriptional control of the DNA in MD cell lines, we need to make a precise transcription map of MDV1 DNA and to compare it with the methylated sites.

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REFERENCES


Short communication


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