PERSISTENT CORONAVIRUS INFECTION OF PROGENITOR OLIGODENDROCYTES

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1. INTRODUCTION

Mouse hepatitis virus (MHV) is a prototype of murine coronavirus. It can infect rodents and causes enteritis, hepatitis, and central nervous system (CNS) diseases. Infection of mouse CNS with neurovirulent MHV strains usually results in acute encephalitis followed by demyelination.\textsuperscript{1,2} If the majority of the virus can be cleared from the CNS, encephalitis then resolves; if mice survive the acute phase, demyelination develops. Although acute demyelination can be detected histologically as early as 6 days postinfection (p.i.), extensive demyelination is often not seen until 4 weeks p.i.\textsuperscript{3} However, infectious virus can no longer be isolated from the CNS at this time, although viral RNAs continue to persist in the CNS for more than one year, during which time period demyelination is concomitantly detectable.\textsuperscript{4,5} The correlation between viral RNA persistence and demyelination in the CNS suggests that viral persistence may be a prerequisite for the development of CNS demyelination. However, virtually nothing is known as to how viral persistence contributes to demyelination.

Previous studies attempted to establish an \textit{in vitro} system of glial or fibroblast cell culture for viral persistence.\textsuperscript{6,7} Unfortunately, the persistent infection established in these cells is productive, i.e., generation of infectious viruses with significant virus titers. This type of persistence does not reflect on the infection of animal CNS. Recently we established a persistent MHV infection in a progenitor rat oligodendrocyte. We showed that MHV RNAs were continuously detected in infected cells of more than 20 passages. However, no infectious virus could be isolated from these cells. This phenomenon resembles the persistent, nonproductive infection in animal CNS. To understand the molecular basis of viral persistence in host cells, we analyzed the gene expression profiles of the persistently infected cells by using DNA microarray technology and RT-PCR. We found that the expression of a substantial number of cellular genes was altered by viral persistence. Interestingly, although persistently infected progenitor cells could be induced to differentiate into mature oligodendrocytes, the number of dendrites and level of myelin basic protein were markedly reduced in persistent cells. This finding indicates

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that MHV persistence has an inhibitory effect on oligodendrocyte differentiation and dendrite outgrowth and provides the first direct evidence linking viral persistence to demyelination.

2. MATERIALS AND METHODS

2.1. Cell, Virus, and Reagents

The CG (central glial)-4 cell is a permanent, undifferentiated type 2 oligodendrocyte/astrocyte progenitor cell that was established during a primary neural cell culture derived from the brain of newborn Sprague-Dawley rat pups (1–3 days postnatal). CG-4 cell culture was maintained as described previously. MHV strain JHM was obtained from Michael Lai’s laboratory. It was propagated in mouse astrocytoma cell line DBT cells and was used throughout this study. Virus titers were determined by plaque assay as described previously.

2.2. Reverse Transcription–Polymerase Chain Reaction (RT-PCR)

The intracellular RNAs were reverse-transcribed into cDNAs by using a random hexomer oligonucleotide primer (Invitrogen, Inc.), and the cDNAs were amplified by PCR using gene specific primers as described previously. The following gene-specific primer pairs were used in PCR: 5’-BamN (5’-TAG GGA TCC ATG TCT TTT GTT CCT-3’) and 3’-EcoN515 (5’-TAG GAA TTC GGC AGA GGT CCT AG-3’) for viral nucleocapsid (N) gene; 5’-cmyc (5’-TTT CTC GAG GCC ACG ATG CCC CTC AAC GTG AGC TTC-3’) and 3’-cmyc (5’-TTT GAA TTC CCA GAG TCG CTG CTG GTG GTG GGC-3’) for c-myc gene; 5’-sox (5’-TTT CTC GAG ATG GTG CAG CAC GCC GAG-3’) and 3’-sox (5’-TTG AAT TCC ATA CGT GAA CAC CAG GTC GGA-3’) for Sox11 gene; 5’-bcl2 (5’-TTT CTC GAG GCC ACC ATG GCG CAC GAT GGG AGA ACA-3’) and 3’-bcl2 (5’-TTT GAA TTC CCT TGT GGC CCA GAT AGG CAC CCA-3’) for Bcl-2 gene; 5’-mb-actin (5’-ACC AAC TGG GAC GAT ATG GAG AAT A-3’) and 3’-mb-actin (5’-TAC GAC CAG AGG CAT ACA GGG ACA-3’) for β-actin, which was used as an internal control.

2.3. DNA Microarray Analysis

For DNA microarray analysis, mRNAs were extracted from persistent- or mock-infected CG-4 cells at passage 20 p.i. using the Qiagen RNAeasy kit according to the manufacturer’s instructions. The purity and quantity of the RNAs were determined by spectrophotometry. The levels of individual mRNA species were determined by microarray using the Affymetrix Oligo Gene Chip (U34), which detects approximately 7,000 known genes and 1,000 EST clusters. The DNA microarray analysis was carried out at the University of Iowa DNA Core facility. A 2-fold or greater difference between the test (persistently infected CG-4 cells) and the control (mock-infected CG-4 cells) was considered a significant change while any genes that are absence (below detectable level) in both test and control cells were excluded from the analysis.