Role(s) of Leader protein of Saffold virus

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Abstract

Objective Saffold virus (SAFV) classified in the species Thielovirus of the genus Cardiovirus is a novel human cardiovirus. The pathogenicity of SAFV in humans still remains unclear. Viruses in the genus Cardiovirus produce Leader protein, which is a small non-structural protein. Evidence that Leader protein of murine cardioviruses is a key factor in their biological activities has been provided. Therefore, the studies on the function of SAFV-L are likely to be important in order to clarify the pathogenicity of SAFV.

Methods We investigated the interferon (IFN) response in the early stage of SAFV infection by reverse transcription polymerase chain reaction and the growth kinetics of the chimera virus, SAFL/DA.

Results The study showed that SAFV-L suppresses the type I IFN response, and that the responsible region of anti-IFN activity of SAFV-L is a zinc-binding motif. Furthermore, the anti-apoptotic activity of SAFV-L was suggested from the growth kinetics analysis of SAFL/DA.

Conclusions The present study suggested that SAFV-L might be a multifunctional protein; for example, the suppression of IFN induction, anti-apoptotic effect and/or productive virus growth. (Clin. Exp. Neuroimmunol. doi: 10.1111/cen3.12109, March 2014)

Introduction

Saffold virus (SAFV), identified in 2007, was classified with Theiler-like rat virus (TRV) and Theiler’s murine encephalomyelitis virus (TMEV) in the species Thielovirus, which belongs to the genus Cardiovirus of the family Picornaviridae. Unlike TMEV and TRV, each of which is monotypic, eight genotypes of SAFV have been reported. Although several epidemiological studies have been reported, the precise relationship between SAFV and human diseases still remains unclear. Theiler’s original (TO) subgroup strains (Daniel’s strain [DA] is the representative strain) of TMEV cause a milder polioencephalomyelitis followed by virus persistence and demyelination in the spinal cords of mice. The demyelination induced by TO subgroup strains is the representative of virus-induced demyelination and serves as an excellent animal model for multiple sclerosis (MS), as its pathological features are reminiscent of MS. Viruses in the genus Cardiovirus contain Leader protein (L) coding region at the most 5'-terminus of the open reading frame. TMEV-L is a multifunctional protein that plays important roles in neurovirulence, anti-interferon (IFN) activity and viral persistence. Furthermore, TMEV-L has a pro-apoptotic activity; although, in part, it also has anti-apoptotic activity depending on the TMEV strain and the particular cell types, such as encephalomyocarditis virus (EMCV)-L. From these observations, TMEV-L should be a key factor in inducing the pathological changes found after infection of the mouse. TMEV-L has a zinc finger domain in the N-terminus, acidic domain in the middle region and S/T-rich domain in the C-terminus. SAFV-L has a partially deleted S/T-rich domain, along with a zinc-finger and an acidic domain. Therefore, the studies of the function of SAFV-L are likely to be important in order to clarify the virus’ pathogenicity. In the present study, we investigated the IFN response in the early stage of SAFV infection by reverse transcription polymerase chain reaction (RT–PCR).
Furthermore, the growth kinetics of the recombinant TMEV-DA (SAFL/DA), in which DA-L is substituted by SAFV-L in the background of DA, was analyzed.

**Methods**

**Viruses and cells**

SAFV-3 and DA viruses were prepared from the infectious cDNA clone of SAFV-3 (JPN08-404), pSAF404\(^{17}\) and the infectious cDNA clone of the DA strain of TMEV (TMEV-DA), pDAFL3,\(^{18}\) respectively. The recombinant viruses of L coding regions of SAFV-3, and TMEV-DA, DAL/SAFV and SAFL/DA, were generated by the replacement of the L coding regions of pDAFL3 and pSAF404 as described previously.\(^{19}\) SAFVLCys was the mutant disrupting zinc-binding motif, in which cysteines at amino acid (AA) residues 11 and 14 are replaced by arginines, and proline at AA residue 12 is replaced by threonine.\(^{20}\) Each design is presented in Fig. 1.

J774.1 cells, a H-2d macrophage-like cell line derived from a tumor of a female BALB/c mouse, were obtained from the Cancer Cell Repository, Tohoku University, Sendai, Japan. The cells were maintained in RPMI 1640 medium (Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA). L\(^*\)/J774 cells, the L\(^*\)-expressing J774.1 cell line, and control/J774, the control transformant with the empty vector established previously,\(^{21}\) were maintained in the same medium for J774.1.

**RT–PCR**

Total RNA was extracted from 1 × 10\(^6\) cells of mock-infected cells and HeLa cells infected with SAFV-3 at a multiplicity of infection (MOI) of 5 PFU per cell (3, 6 and 9 h postinfection [p.i.]). Total RNA was reverse transcribed using oligo dT(20) primer and ReverTraAce reverse transcriptase (TOYOBO, Osaka, Japan). First stranded cDNA was amplified by PCR (30 cycles) using KOD plus Neo DNA polymerase (TOYOBO). The primer pairs used were as follows:

\[5'\text{-TTGTGCTTCTCCACTACG-3'} \text{(forward)} \text{ and } 5'\text{-CTGTAAGTGCTGTTAATGAAG-3'} \text{(reverse) for IFN-\(\beta\), and } 5'\text{-GTAATGATCAGTCAACGGGGAC-3'} \text{(forward)} \text{ and } 5'\text{-CCAGCAAGCTTGCAACCTTAACCA-3'} \text{(reverse) for hypoxanthine guanine phosphoribosyltransferase (HPRT).}

**Kinetics of virus growth**

The kinetics of virus growth of SAFL/DA chimera virus in J774.1 and L\(^*\)/J774 were analyzed as previously described.\(^{22}\) The cells were seeded at a density of 1 × 10\(^6\) cells in a 35-mm dish. After 24 h, the cells were infected with each virus at an MOI of 5 PFU per cell. After virus adsorption at 37\(^\circ\)C for 60 min, the cells were washed twice with Dulbecco’s phosphate buffered saline (PBS), and incubated at 37\(^\circ\)C in RPMI1640 with 1% fetal calf serum up to 48 h. The virus lysates were collected at 0, 3, 6, 12, 24 and 48 h p.i., and titrated by a standard plaque assay on BHK-21 cells.

**Results**

Recently, we found that IFN-\(\beta\) was induced significantly by SAFV infection in HeLa-N cells, which were maintained at the Department of Biochemistry,
Kanazawa Medical University School of Medicine, Ishikawa, Japan, but it was induced weakly in HeLa-R cells, which were derived from RIKEN, Ibaraki, Japan. However, IFN-α is induced in both cells without the virus infection (A. I. Ai Shimizu, T. H. Toshiki Himeda, T. O. Takako Okuwa, Y. M. Yasushi Muraki, Y. O. Yoshiro Ohara, unpublished data). Therefore, we analyzed the induction of IFN-β after the infection of various viruses in HeLa-N cells by RT–PCR. The data of the electrophoresis of the RT–PCR products are shown in Fig. 2. As expected, the transcripts of IFN-β in the cells infected with SAFV were induced gradually from 6 h p.i. Furthermore, the induction of IFN-β in the cells infected with SAFVLcys, which is the mutant disrupting zinc-binding motif, was significantly higher than that in the cells infected with SAFV at 9 h p.i. The data showed that SAFV-L suppressed the IFN response through the zinc binding-motif as well as TMEV-L.20 Interestingly, the transcripts of IFN-β in the cells infected with DAL/SAFV were further increased from those in the cells infected with SAFVLcys at 6 h p.i. It was suggested that the ability of SAFV-L to suppress the IFN induction might be higher than that of TMEV-L. Therefore, we next studied the induction of IFN-β in SAFL/DA and DA viruses. The transcripts of IFN-β in the cells infected with DA were clearly induced at 6 h p.i. In contrast, the induction of IFN-β in the cells infected with SAFL/DA was clearly lower than those at 6 and 9 h p.i. From the aforementioned results, it was strongly suggested that the ability of SAFV-L to suppress the IFN response is higher than that of DA-L in HeLa-N cells.

It has been reported that TMEV-L is required for virus growth in J774 macrophage cells.15 Then, we wonder whether SAFV-L is also required for virus growth. The genome structure of L and L* coding regions of TMEV or SAFV is shown in Fig. 3. TMEV-L has another initiation codon, AUG (Fig. 3a, black box), 13 nt downstream of the polyprotein’s initiation codon. From this AUG, the protein named L* is synthesized out-of-frame with the polyprotein. In contrast, SAFV-L has non-initiation codon ACG (Fig. 3b, black box), not AUG at the corresponding location, and in addition, there are four stop codons in the frame for L* in the L coding region. Therefore, so-called L* is never synthesized in SAFL/DA. TMEV-L* is also reported to be required for virus growth in J774 cells,21 as TMEV-L* inhibits the TMEV-L induced apoptosis with localization to mitochondria in order to help the virus replication by delaying the cell death.11,23 Therefore, we analyzed the growth kinetics of SAFL/DA in J774 and L*/J774 cells, which is a clone of J774 cells expressing L* stably.21 Previously, we have reported that DAL*-1, which lacks the expression of L*, did not grow in J774 cells.21 In contrast, the growth kinetics of SAFL/DA in J774 cells showed the clear virus replication at 12 h p.i. after the eclipse period at 6 h p.i., although the titers were low (Fig. 4). A similar tendency is observed in L*/J774 cells. The data showed that L* is not required for the replication of SAFL/DA in J774 cells.

**Discussion**

TMEV-L and EMCV-L show pro-apoptotic and the anti-apoptotic activity depending on the conditions of infection, as described in the Introduction. SAFV-L might show anti-apoptotic activity in J774 cells infected with SAFL/DA, as DAL*-1 did not grow in macrophage cells as a result of the apoptotic cell death induced by TMEV-L.8 Otherwise, the higher ability of SAFV-L to suppress IFN response might help the replication of SAFL/DA in J774 cells. From these results, it is speculated that SAFV-L is a multifunctional protein, and that SAFL/DA could persist.
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SAFV-L could be multifunctional; for example, the suppression of IFN induction, anti-apoptotic effect and/or productive virus growth.

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References


