

THEILER'S ENCEPHALOMYELITIS VIRUS

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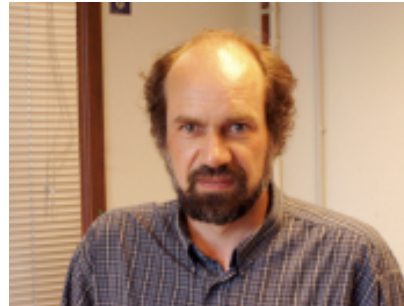
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*Theiler's murine encephalomyelitis virus (TMEV or Theiler's virus) is a murine picornavirus responsible for infections of the central nervous system. Strains of Theiler's virus have a striking ability to **persist in the central nervous system** in spite of a specific cellular and humoral immune response. Persistence of the virus is associated with a strong inflammatory response and with lesions of primary demyelination reminiscent of those found in human **multiple sclerosis**. The genome of Theiler's virus is an 8 kb-long positive strand RNA molecule (Fig. 1).*

Theiler's virus is an outstanding model to analyze the basic mechanisms of viral persistence and demyelination. i) It replicates and persists in the central nervous system in the face of a specific humoral and cellular immune response. ii) It induces chronic demyelination in mice. iii) It is a natural pathogen of the mouse and allows the experimental analysis of a natural host-pathogen interaction. iv) Its genome is cloned and can be manipulated by the tools of molecular biology. Our work aims at understanding how a virus can persist in the central nervous system of an immunocompetent host, thus evading the immune response. We analyze viral and cellular determinants of tropism and persistence. We notably analyze the interferon α/β response and the inhibition of this response by Theiler's virus, in the context of the central nervous system.

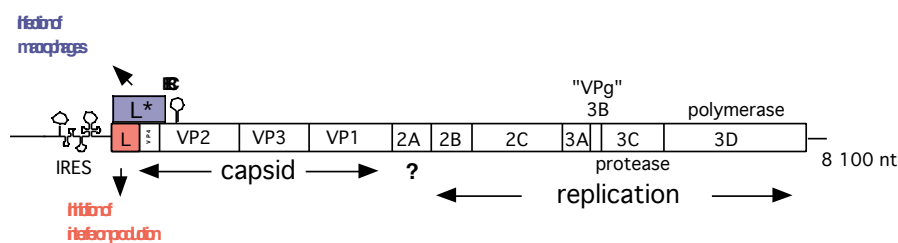


Fig. 1. Genome of Theiler's virus. A large open reading frame encodes a 2000 amino acid-long polyprotein that is cleaved, by autoproteolytic activity, into 12 mature proteins. An additional protein (L*) is encoded by an alternative open reading frame, overlapping regions L, VP4 and VP2. Translation of both ORFs is driven by an Internal Ribosome Entry Site (IRES) present in the 5' non-coding region of the genome (1). Protein L* was shown to facilitate the infection of macrophages and viral persistence (2, 3). Protein L inhibits immediate-early type-I interferon (4). The role of protein 2A is unknown (5). 60 copies of proteins VP1 to VP4 assemble to form the viral capsid. 3B (also termed VPg) is covalently linked to the 5' end of the RNA molecule during encapsidation and replication. 3C is the protease responsible for most of the cleavages occurring during polyprotein processing. 3D is the RNA-dependent RNA polymerase. Proteins 2B, 2C, 3A participate in the replication complex. A replication signal has been discovered in the VP2 coding sequence and is denoted CRE for "cis-acting replication element" (6).

Inhibition of type-I interferon production by the leader protein

V. van Pesch, S. Delhaye, and T. Michiels

The leader (L) protein encoded by Theiler's virus is a 76 amino acid-long peptide containing a zinc-binding motif. We showed recently that the L protein inhibits the production of type-I interferons (IFNs) (4). Inhibition turned out to be specific for α 4 and β interferons, interferon subtypes that are known to be activated early in response to viral infection, therefore referred to as "immediate-early interferons".

A potential target of the leader protein is IRF-3, as this cell factor is known to activate specifically the transcription of IFN- β and IFN- α 4 genes. IRF-3 is constitutively present in the cytoplasm of non-infected cells. Viral infection triggers a signaling cascade which leads to the translocation of IRF-3 to the nucleus where it activates the transcription of the IFN genes. Experiments are in progress to determine whether the leader peptide interacts with IRF-3.

Production of immediate-early interferons (α 4 and β) is generally considered to be required for transcriptional activation of the other IFN- α subtype genes. However, total IFN- α transcription appeared to be induced rather than repressed in wild-type virus-infected L929 cells, in spite of the inhibition of the α 4 and β IFNs. The IFN-alpha subtypes expressed in the absence of these early interferons are currently under identification.

Mutation of the zinc-finger was sufficient to abolish the anti-IFN activity of the L protein, outlining the importance of this motif in the protein function. A Theiler's virus mutant bearing a mutation in the zinc-binding motif of L was dramatically impaired in its ability to persist in the central nervous system of SJL/J mice.

In contrast, interferon receptor-deficient mice were readily infected by both the wild-type and the mutant viruses. These data confirm the crucial role played by the interferon response against Theiler's virus infection and the anti-IFN role of the L protein. However, IFN production inhibition was not complete in vivo. Modulation rather than blockade of the IFN response might be viewed as a viral strategy toward long-term persistence in the host.

Influence of the L* protein on macrophage infection and viral persistence

O. van Eyll, B. Michel, and T. Michiels

In persistent strains of Theiler's virus, an 18 KDa protein called L* is encoded by an alternative open reading frame (ORF) overlapping the L-VP4-VP2 coding regions of the main ORF (see Fig. 1). In neurovirulent strains, however, the entire open reading frame is conserved but the AUG codon initiating translation of the L* ORF is replaced by an ACG codon. The L* protein was shown to enhance the infection of macrophage cell lines and to be critical for persistence of the virus in the central nervous system of the mouse (2, 3, 7).

We compared the phenotype of L* mutant viruses carrying either an AUG to ACG mutation of the initiation codon or a stop codon mutation introduced in the L* ORF. Surprisingly, viruses carrying a stop codon mutation (and thus expressing a truncated L* protein) had a dramatically impaired ability to persist in the central nervous system of the mouse while mutants bearing the AUG to ACG mutation persisted almost as well as the wild-type virus. Our data suggest that L* could be expressed from an ACG initiation codon. This would be the first example of picornavirus IRES-driven non-AUG translation initiation. In addition, it indicates that neurovirulent strains can also produce the L* protein.

Current efforts aim at characterizing the function of the L* protein in the infection of macrophages. Macrophages are indeed key players in the demyelinating disease induced by Theiler's virus, being simultaneously effectors of the immune response and targets of viral infection.

Interaction of Theiler's virus with the cell

K. Jnaoui, and T. Michiels

Despite many efforts, the cellular receptor for Theiler's virus has not yet been identified. We analyzed the interaction between the virus and the cell.

On the one hand, we obtained and analyzed a series of virus variants adapted to grow on various cell lines (8-10). Mutations responsible

for adaptation of the virus to these cells occurred in the capsid of the virus and affected amino acids contained in loops that are exposed at the surface of the virus. Such amino acids that affect the tropism of the virus in vitro most probably correspond to the residues that interact with the host receptor. The fact that optimal infection of a specific cell lines requires adaptation of the viral capsid suggest that the receptor or co-receptor for the virus is polymorphic.

On the other hand, we isolated mutant cell lines that became resistant to the entry of the GDVII strain of Theiler's virus, but which retained susceptibility to persistent strains (5). This shows that infection of a given cell lines by different strains of Theiler's virus involves at least partly distinct factors.

Persistent strains but not neurovirulent strains of Theiler's virus were reported to bind sialic acid. This interaction involves protein loops exposed at the surface of the viral capsid and in particular the EF loop of the capsid protein VP2. We confirmed this interaction for the DA virus strain. In addition, we showed that some variants of the neurovirulent GDVII strain could interact weakly with sialic acid through different capsid determinants. Subtle changes in the capsid had a dramatic impact on persistence of the DA virus or neurovirulence of the GDVII virus (9).

Thus we believe that subtle changes in the surface loops of the capsid modulate the affinity of a given capsid for the receptor present on a given cell line and thereby modulate the tropism of the virus and the pathology that it causes in the central nervous system.

Selected publications

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3. van Eyll O, Michiels T. *Non-AUG-initiated internal translation of the L* protein of Theiler's virus and importance of this protein for viral persistence.* **J Virol** 2002 ;76:10665-73.

4. van Pesch V, van Eyll O, Michiels T. *The leader protein of Theiler's virus inhibits immediate-early alpha/beta interferon production.* **J. Virol** 2001 ;75:7811-7.

5. Michiels T, Dejong V, Rodrigus R, Shaw-Jackson C. *Protein 2A is not required for Theiler's virus replication.* **J Virol** 1997;71:9549-56.

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8. Michiels T, Jarousse N, Brahic M. *Analysis of the leader and capsid coding regions of persistent and neurovirulent strains of Theiler's virus.* **Virology** 1995;214:550-8.

9. Jnaoui K, Michiels T. *Analysis of cellular mutants resistant to Theiler's virus infection: differential infection of L929 cells by persistent and neurovirulent strains.* **J Virol** 1999 ;73:7248-54.

10. Jnaoui K, Minet M, Michiels T. *Mutations that affect the tropism of DA and GDVII strains of Theiler's virus in vitro influence sialic acid binding and pathogenicity.* **J Virol.** 2002 ;76:8138-47