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VIRAL PERSISTENCE AND INTERFERON RESPONSE

Our work focuses on the interplay between viral infections and the immune response of the host. On one hand, we analyze the model infection of the central nervous system by Theiler's virus. This virus can escape the immune defenses of the host and provoke a persistent infection of the central nervous system. Theiler's virus infection and the resulting inflammatory response can lead to a chronic demyelinating disease considered as a model of multiple sclerosis. On the other hand, we analyze the innate immune response against viral pathogens. We focus on the analysis of the type I (IFN- α/β) and type III (IFN- λ) interferon responses which are critically important to control viral infections and to modulate the acquired immune responses.

THEILER'S VIRUS

Theiler's murine encephalomyelitis virus (TMEV or Theiler's virus) is a murine picornavirus showing a striking ability to persist in

the central nervous system of the host 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.

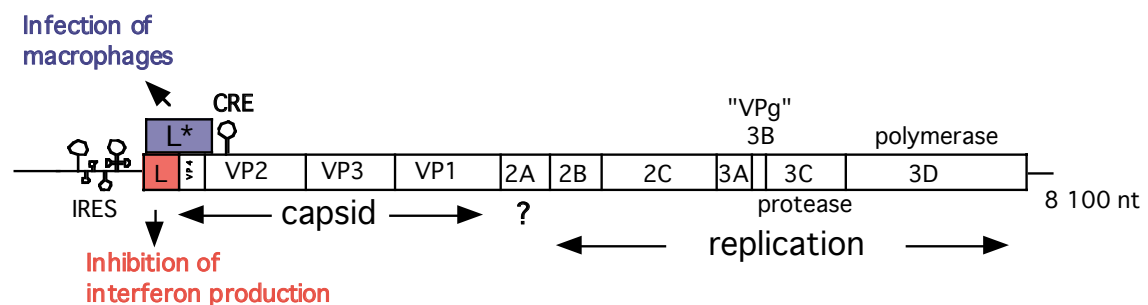


Fig. 1. Genome of Theiler's virus. A large open reading frame encodes a 2000 amino acid-long polyprotein that is cleaved, by autoproteolytic activity, to yield the viral mature proteins. Our analysis focuses on 2 proteins, L and L*, that interfere with the host innate immune responses and therefore facilitate the establishment of a persistent infection.

Our work aims at understanding how a virus can persist in the central nervous system (CNS) of an immuno-competent host, thus evading the immune response.

Recently, a novel human virus closely related to Theiler's virus was discovered and named Saffold virus. This virus is highly prevalent in the human population. Further work is needed to evaluate its virulence potential and its impact on global health.

ANALYSIS OF VIRAL PROTEINS INVOLVED IN THEILER'S VIRUS ESCAPE OF THE HOST IMMUNE RESPONSE.

F. Sorgeloos, C. Ricour, T. Michiels

Two viral proteins, namely L and L* were found to be dispensable for viral replication in cell culture but to be crucial for persistence of the virus in the central nervous system. Hence, these proteins are believed to interact with host factors in vivo and to counteract the host immune defenses.

INHIBITION OF TYPE-I IFN PRODUCTION AND ALTERATION OF NUCLEO-CYTOPLASMIC TRAFFICKING BY THE L PROTEIN

The leader (L) protein encoded by Theiler's virus is a 76 amino acid-long peptide containing a zinc-binding motif. We observed that this protein exerts pleiotropic activities in infected cells. The first activity that was detected for the L protein is its ability to inhibit the transcription of type I IFN and of chemokine genes (2, 9). This activity likely results from the fact that the L protein inhibits the dimerization of IRF-3, the main transcriptional activator of these genes.

Infection of mice deficient for the type-I interferon receptor (IFNAR^{-/-}) indicates that

IFN production inhibition by the L protein also occurs in vivo. However, the inhibition of IFN production is not absolute. If it were, the DA virus would cause a fatal encephalomyelitis in wild-type mice analogous to the disease it causes in IFNAR^{-/-} mice (6).

The L protein also interferes with nucleocytoplasmic trafficking of host proteins and mRNA. It promotes the subcellular redistribution of host nuclear and cytoplasmic proteins (4). It also represses the exportation of mRNA from the nucleus to the cytoplasm, thereby shutting-off the expression of host cell proteins. This might explain the highly toxic nature of this protein. These trafficking perturbing activities of the L protein correlate with L-mediated hyperphosphorylation of the Nup proteins that make up the nuclear pore complex (9).

In order to test whether the various activities (IFN transcriptional inhibition versus nucleocytoplasmic trafficking perturbation) of the L protein are linked, we subjected the L coding region to random mutagenesis and selected L mutants that lost toxicity. This study revealed that two domains of the L protein are critical for activity: the N-terminal zinc finger and a C-terminal domain that was named «Theilo-domain» since it is conserved in the L protein of Theiloviruses (i.e. Theiler's virus and Saffold virus) but not in the L protein of encephalomyocarditis virus (EMCV) (10).

Mutations of either the Zn-finger or of the Theilodomain abolished all reported activities of the L protein, suggesting that the various activities of the protein are linked. Current efforts are devoted to finding the master L interactor in infected cells.

INFLUENCE OF THE L* PROTEIN ON MACROPHAGE INFECTION AND VIRAL PERSISTENCE

Persistent strains of Theiler's virus produce an 18 KDal protein called L*, which is encoded by an open reading frame (ORF) overlapping the ORF coding the viral polyprotein (see Fig. 1). This 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 (3).

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. Our recent data suggest that the L* protein antagonizes an important effector pathway of the interferon response. Thus Theiler's virus would interfere both with the production of IFN by infected cells and with the response of cells to this cytokine. This outlines the major importance of the IFN system in the defense of the host against viral infections.

TYPE-I AND TYPE-III INTERFERONS

Interferons were the first cytokines to be identified. They were discovered about 50 years ago by Isaacs and Lindemann who observed that chick chorio-allantoic membranes developed resistance to viral infection after exposure to heat-inactivated influenza virus. Interferons are typically secreted by cells that are infected by a virus. They alert neighbouring cells about the presence of a viral infection and allow these cells to develop an anti-viral state. The interferon system represents a critical protection mechanism of the body against viral infections. In addition, interferons have anti-cancer properties and modulate the acquired immune response of the host.

Type I IFNs

P. Hermant, F. Sorgeloos, T. Michiels

Mouse and human genomes carry more than a dozen of genes coding for closely related interferon-alpha (IFN- α) subtypes. These interferons and other interferons, like IFN- β , IFN- κ , IFN- ϵ , IFN- ω , and limitin, form the type-I IFN family. In spite of important sequence divergences, all these IFNs bind the same receptor, raising the question of whether they possess specific functions.

We previously characterized the murine IFN- α family. 14 IFN- α genes were detected in the mouse genome, two of which, IFN- α 13 and IFN- α 14, were previously undescribed. In addition the mouse genome contains three IFN- α pseudogenes (5).

Ongoing work aims at understanding the specificities of the various type-I IFN subtypes.

Type-III Interferons (IFN- λ)

M. Minet, F. Sorgeloos, T. Michiels

Recently, another IFN family was described and called «type-III IFN» or «IFN- λ ». Although type III IFNs bind a receptor distinct from the type-I IFN receptor, they activate the same signal transduction pathway and upregulate the same set of genes. Thus, they are expected to exert very similar biological activities as type I IFNs. Our work addresses the question of the redundancy of these two IFN systems *in vivo*.

We observed that the relative expression of IFN- λ over that of IFN- α/β exhibited some extent of tissue specificity and was low in the brain (9). We also used a strategy based on *in vivo* expression of cloned IFN genes to compare the responses of different tissues to IFN- α and IFN- λ . Interestingly, response to IFN- λ appeared to be specific to epithelial cells, unlike response to IFN- α which occurred in most cell types and was particularly prominent in endothelial cells. Accordingly, tissues with a

high epithelial content such as intestine, skin or lungs were the most responsive to IFN- λ and expressed the higher amounts of IFN- λ receptor (9). Work performed in collaboration with M. Mordstein and P. Stäheli (Univ. of Freiburg, Germany) confirmed that IFN- λ participates to the protection of lung and intestine epitheliums (where the response to IFN- λ is prominent) against infection with several viruses such as influenza virus respiratory syncytial virus or SARS coronavirus.

Our data suggest that the IFN- λ system evolved as a specific protection of epithelia and that it might contribute to prevent viral invasion through skin and mucosal surfaces.

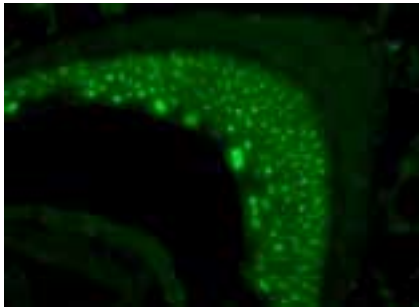


Fig. 2. Response to IFN- λ . Section of the urinary epithelium showing a strong response of epithelial cells to IFN- λ . Shown is a fluorescent labelling of the nuclear Mx1 protein, the expression of which is typically induced in response to IFN.

Production of IFN- α/β by neurons

M. Kreit, T. Michiels

In collaboration with the teams of Peter Staeheli and Friedemann Weber (Univ. Freiburg, Germany) we analyzed the cells that are responsible for type I IFN production in the central nervous system, during acute encephalitis caused by two neurotropic viruses: Theiler's virus (picornavirus) or La Crosse virus (bunyavirus).

IFN- α/β turned out to be produced by many scattered parenchymal cells and less by

cells of the inflammatory foci. Interestingly, besides some macrophages and ependymal cells, neurons turned out to be important producers of both IFN- α and IFN- β . However, not more than 3% of infected neurons expressed IFN, suggesting that some restriction to IFN production might occur in these cells (7).

All CNS cell types analyzed, including neurons, were able to respond to type I IFN by producing Mx or IRF-7. Our data show that, in vivo, in spite of the relative immune privilege of the CNS parenchyma, neurons take an active part to the antiviral defense by being both IFN producers and responders (7, 8).

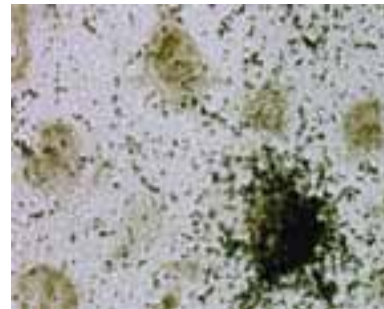


Fig. 3. Neurons can produce IFN α/β . Neuron (immunostained in brown for NeuN) producing IFN- β (detected by in situ hybridization: black dots) after infection with La Crosse virus.

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