CASE 28
RECURRENT HERPES SIMPLEX ENCEPHALITIS

The control of viral infections by type I interferons.

In addition to lymphocyte-mediated defenses against viral infection (see Cases 14 and 43), the type I interferons—IFN-α and IFN-β—provide another ubiquitous cell-mediated antiviral defense mechanism. The induction of interferons is transcriptionally regulated, and is elicited by a variety of upstream receptors. Among these, the Toll-like receptors TLR-3, TLR-7, TLR-8, and TLR-9, which all recognize viral components, play a prominent role. TLR-3 binds double-stranded RNA; TLR-7 and TLR-8 recognize single-stranded RNA; and TLR-9 binds double-stranded DNA. These TLRs are present in the membranes of endosomes, and are therefore prompted to bind nucleic acid intermediates generated during intracellular viral replication.

All these TLRs require association with the endosomal membrane protein UNC93B for signaling, but differ in the pathway components that are downstream of the receptor. TLR-7, TLR-8, and TLR-9 signal through the adaptor protein MyD88 and the serine/threonine kinases IRAK4 (IL-1 receptor-associated kinase 4; see Case 29) and IRAK1, activating the IKKα:β:γ protein kinase complex, which in turn leads to activation of the transcription factor NFκB (the mechanism of NFκB activation is shown in more detail in Fig. 23.1). Activation of TLR-7, TLR-8, and TLR-9 can also induce the interferon regulatory factor IRF-7 by an alternative signaling pathway that involves MyD88 and IRAK4 but is independent of IKK (**Fig. 28.1**). In contrast, TLR-3 signals through the adaptor protein TRIF, activating a signaling pathway that

TOPICS BEARING ON THIS CASE:

Toll-like receptor signaling

Type I interferons

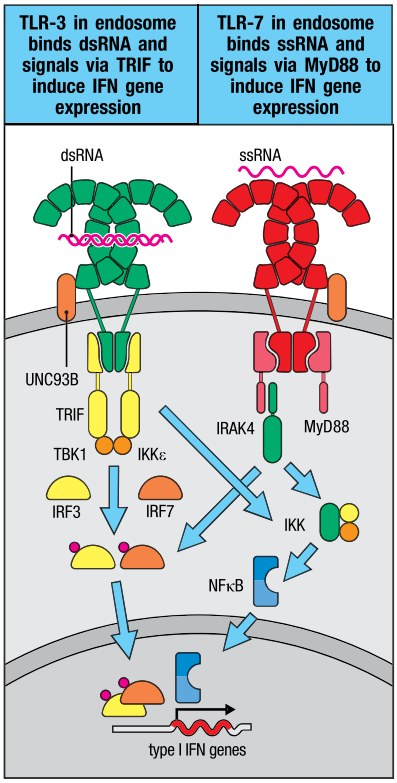

Fig. 28.1 Toll-like receptor-mediated induction of type 1 interferons in response to viral infection. Viruses induce production of type 1 interferons (IFN-α and IFN-β) by triggering activation of endosome-associated TLR-3, TLR-7, TLR-8, or TLR-9. The endoplasmic reticulum membrane protein UNC93B is essential for signaling by all these receptors. TLR-3 signaling occurs through a pathway dependent on the adaptor TRIF and primarily activates interferon-regulatory factors IRF3 and IRF7 (left panel; see text for details), whereas TLR-7 (shown here), TLR-8, and TLR-9 signal through a pathway dependent on the adaptor MyD88 that involves IRAKs and primarily activates the classical NFκB pathway. Cross-talk between the pathways means that IRF3, IRF7, and NFκB can in principle all be activated via either set of receptors.

uses the protein kinases TBK1 and IKKε and activates IRF3 and IRF7 (see Fig. 28.1). IRF3, IRF7, and NFκB can all switch on the type I interferon genes. Thus, the activation of TLRs, which recognize different viral replication intermediates, is linked to a common outcome—the production of type I interferons.

After IFN-α and IFN-β are produced and released by the virus-infected cell, they bind to their common receptor, a heterodimer of IFNαR1 and IFNαR2, on the cell surface. This results in activation of the JAK1 and TYK2 kinases and of the transcription factor ISGF3, a heterotrimeric complex of STAT1, STAT2, and IRF9. ISGF3 binds to the interferon-stimulated response element (ISRE) within the promoter of various type I-IFN-dependent genes, thereby inducing their transcription and triggering antiviral activity and destruction of the virus (Fig. 28.2).

The case of Mercédès Mondego: relapsing fever and lateralized seizures.

High fever and right-sided seizures.

Mercédès was born at term after an uneventful pregnancy. Her parents are immigrants from a small village in French Guiana. At birth, Mercédès was of normal weight and length, and she grew normally, reaching her milestones according to the calendar. At the age of 6 months, she spiked a high fever (39.7°C) accompanied by vomiting and right hemiclonic seizures. She was immediately brought to the emergency room, where the seizures were treated with diazepam.

Initial analysis of the cerebrospinal fluid (CSF) was normal. However, electroencephalographic (EEG) tracing carried out at day 3 revealed spike-waves in the left temporal lobe. Cerebral magnetic resonance imaging (MRI) showed hyperintensity of the signal in the left temporal lobe. Results of a repeat CSF analysis at day 5 suggested viral meningoencephalitis, with 154 cells μl^{-1} (normal less than 3 cells μl^{-1}), 94% of which were lymphocytes, and an increase in protein concentration (155 mg dl^{-1}; normal 20–50 mg dl^{-1}). Using polymerase chain reaction (PCR), the CSF tested positive for herpes simplex virus type 1 (HSV-1) DNA, confirming an HSV-1 infection.

Relapse of encephalitis.

Mercédès was treated with intravenous acyclovir for 3 weeks and gradually recovered. No significant infections were recorded in the following months. One month after the episode, she had detectable anti-HSV-1 antibodies. At 14 months of age, she was immunized against measles, mumps, and rubella (MMR) without adverse consequences, and was able to mount a protective antibody response. Mercédès began daycare at 2 years old and continued to do well, both in her growth and her development. She suffered from the common viral infections of early childhood, especially during the winter, but the frequency of infections was no different from that of her peers. At the age of 3 years 4 months, she again developed high fever (39.4°C), right-sided tonic-clonic seizures, paralysis of the right face and

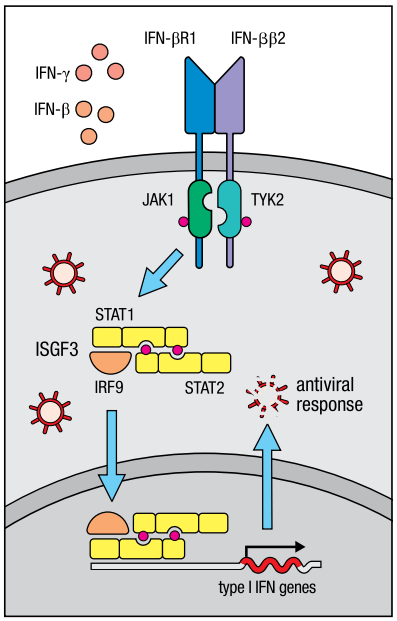

Fig. 28.2 Cellular responses to type I interferons. Type I interferons bind to the IFN-α receptor (IFNαR), which is composed of two different chains. This receptor triggers activation of Janus kinases JAK1 and TYK2 and formation of the transcription factor ISGF3 (a heterotrimer of STAT1, STAT2, and IRF9), which drives the expression of type I IFN-dependent genes that encode proteins that mediate the antiviral response.

arm (brachio-facial paralysis), and photophobia (an aversion to light). CSF analysis revealed a recurrence of meningoencephalitis (180 cells μl^{-1}, with 97% lymphocytes; 62 mg dl^{-1} protein), and PCR again confirmed that there was HSV-1 in the CSF. A brain MRI revealed a new lesion in the left parietal lobe and in the left thalamus. Mercédès was treated with intravenous acyclovir for 3 weeks, with progressive clinical improvement. Genetic testing for possible causes of relapsing herpes simplex encephalitis (HSE) revealed a homozygous single-nucleotide deletion in exon 1 of the *UNC93B* gene. Mercédès is now 5 years old and the paralysis of the right face and arm remain. She has not suffered other episodes of severe viral infection.

Recurrent herpes simplex encephalitis.

HSV-1 is a double-stranded DNA virus, and is typically associated with infection of the oral mucosa (causing oral ulcers) or of the eye (causing conjunctivitis and keratitis). After replication at the initial site of infection, the virus is transported through sensory neurons to the trigeminal nerves and ganglia, where it establishes a latent infection. Reactivation of the virus manifests as herpes labialis (cold sores) in about 30% of the infected population. HSV-1 infection is very common in the general population; about 85% of adults have detectable antibodies against HSV-1.

Although HSV-1 infection is usually benign, on rare occasions the virus invades the brain, causing herpes simplex encephalitis (HSE), which affects between two and four individuals in 1 million each year in the United States. The virus invades the brain through the olfactory tract and trigeminal nerves, and infects both neuronal and glial cells, causing a necrotizing encephalitis. The temporal and parietal lobes are typical targets.

For a long time HSE was thought to be simply an acquired disease, but it has recently been shown to follow a pattern of Mendelian inheritance in some families. Various genetic defects have been shown to cause HSE, all of which result from defects in the production of, or the response to, type I interferons.

Although *MyD88*-deficient mice are prone to HSE, patients with MyD88 or IRAK4 deficiency suffer from recurrent infections with pyogenic bacteria but do not show increased susceptibility to HSE or to other viral infections (see Case 29), even if their fibroblasts fail to produce type I interferons in response to stimulation of TLR-7, TLR-8, or TLR-9. This indicates that these IRAK4-dependent TLR-mediated interferon responses are redundant for protective immunity to HSV-1 (and other viruses) *in vivo* in humans.

In contrast, defects along the TLR-3 signaling pathway are associated with increased susceptibility to recurrent HSE. Although HSV-1 is a DNA virus, double-stranded RNA is generated during its replication, and it is this RNA intermediate that is recognized by endosomal TLR-3. Genetic defects in *TLR3*, *UNC93B*, *TRAF3*, *TRIF*, *TBK1*, and *IRF3* have been identified among patients with HSE. Interestingly, these patients do not show disseminated HSV-1 disease, nor do they show increased susceptibility to other viral infections, suggesting that HSE results from the inability of cells of the central nervous system to respond to HSV-1 through TLR3-dependent

mechanisms. This hypothesis has been confirmed by generating induced pluripotent stem cells (iPSCs) from fibroblasts of controls and of patients with genetic defects associated with HSE. The iPSCs were allowed to differentiate into primary neurons and were then infected with HSV-1. The neurons derived from patients with the genetic defects exhibited enhanced viral replication while producing significantly lower amounts of IFN-α, IFN-β, and IFN-λ as compared to HSV-1-infected neurons from healthy controls. Pre-treatment of patient neurons with IFN-α2b rescued the cellular response to HSV-1 infection. Altogether, these results demonstrate that genetic defects of the TLR-3 signaling pathway compromise the neuronal cellular response to HSV-1 infection.

HSE can also result from genetic defects in the transcription factor STAT1. In addition to activation by the type I interferon receptor, STAT1 can be activated via a different receptor that responds to the cytokine IFN-γ (which does not have antiviral activity but which promotes T_H1 activation of macrophages, among other functions). Activation of this receptor leads to formation of the gamma-activated factor (GAF), a dimer of STAT-1, that promotes transcription of IFN-γ-dependent genes. Three genetic forms of STAT1 deficiency are known. Autosomal recessive, null mutations of the *STAT1* gene result in increased susceptibility to severe viral disease (including HSE) and to mycobacterial infection (resulting from the failure of macrophage activation), because the formation of both ISGF3 and GAF is impaired. In contrast, dominant-negative, heterozygous mutations of *STAT1* increase susceptibility to mycobacterial disease but not to severe viral infections (and hence do not cause HSE). In fact, in patients with heterozygous, dominant-negative mutations of *STAT1*, the formation of sufficient amounts of ISGF3 is still possible and, consequently, type I interferon-dependent anti-viral responses are not or are only marginally impaired. Finally, heterozygous gain-of-function mutations of *STAT1* cause chronic mucocutaneous candidiasis, often associated with other infections and with autoimmunity (see Case 52).

Questions.

1 *Why do patients with TLR-3 signaling defects present increased susceptibility to HSE and not to other viral infections that generate double-stranded RNA intermediates during viral replication?*

2 *How might we take advantage of the identification of genetic defects leading to HSE to help in the treatment of the patients?*