REVIEW

For reprint orders, please contact: reprints@futuremedicine.com

Picornavirus infection leading to immunosuppression

Matthew F Cusick^{*1}, Jane E Libbey¹ & Robert S Fujinami¹

ABSTRACT: Viruses, such as HIV, hepatitis A, poliovirus, coxsackievirus B3 and foot-and-mouth disease virus, use a variety of mechanisms to suppress the human immune system in order to evade clearance by the host. Therefore, investigating how a few changes in the viral genome of a nonlethal virus can lead to an alteration in disease, from survivable to immunosuppression and death, would provide valuable information into viral pathogenesis. In addition, we propose that gaining a better insight into how these viruses suppress an antiviral immune response could lead to viral-based therapeutics to combat specific autoimmune diseases such as multiple sclerosis and Type 1 diabetes.

Most picornaviral infections are asymptomatic. Some picornaviruses cause mild illnesses (gastro-intestinal infection, colds) and on occasion can result in more severe diseases (encephalitis). *Picornaviridae* are nonenveloped, single-stranded, positive-sense RNA viruses consisting of 17 genera to include: aphthovirus, cardiovirus, cosavirus, enterovirus (EV), erbovirus, hepatovirus, kobuvirus, parechovirus, salivirus, sapelovirus, senecavirus, tremovirus, avihepatovirus and teschovirus [1,2]. In general, the picornaviruses have a common RNA genome that consisting of viral protein (VPg) covalently linked to the 5'-untranslated region (UTR), an open reading frame (ORF) that encodes a polypeptide, from which 10–12 proteins are post-translationally cleaved by viral proteases, followed by the 3'-UTR and a poly-A tail. The proteins that are translated from the ORF are as follows: cardioviruses and aphthoviruses contain a leader (L) protein (not found in other picornaviruses) and structural proteins (1A–1D or VP4–VP1) followed by nonstructural proteins (2A–2C and 3A–3D) (reviewed in [3]).

Prominent *Picornaviridae* family members include the polioviruses, coxsackievirus B3 (CVB3), the Vilyuisk human encephalomyelitis virus (still controversial), Saffold virus (SAFV), foot-and-mouth disease virus (FMDV) and the hepatitis A virus (HAV) [3–6]. Recently, cosavirus, salivirus and SAFV have been detected in stool and respiratory samples from patients presenting with a wide range of symptoms. For example, there have been a number of reports that have identified SAFV in samples collected from patients with gastroenteritis [7–9]. SAFV, with eight genotypes isolated worldwide, is the first human cardiovirus identified, and it has been associated with causing disease [5]. However, the mechanism(s) by which SAFV may be causing disease is not known due to the virus being detected at similar frequency in both symptomatic and asymptomatic subjects. This could be due to a variety of reasons including the large number of genotypes of the virus in existence. Therefore, determining the mechanisms of viral pathogenesis is important for the development of therapeutic strategies.

The first scientific observation of virus-induced immunosuppression was made over a century ago by von Pirquet (1908), who discovered that during measles virus infection, the tuberculin skin

KEYWORDS

- autoimmune disease
- immunosuppression
- Picornavirus • T cell
- Theiler's virus

¹Department of Pathology, University of Utah, 15 North Medical Drive East, 2600 EEJMRB, Salt Lake City, UT 84112, USA

*Author for correspondence: Tel.: +1 801 585 3309; matthew.cusick@path.utah.edu

test response of immune individuals was transiently negative (reviewed in [10]). The significant complication of measles virus-induced immunosuppression is secondary infection [11,12]. Since this discovery with measles, many other viruses have been shown to cause immunosuppression, to include the extensively studied HIV.

Host immune response to picornaviruses is diverse and multifaceted due to the heterogeneity of the viruses within the family and the variety of host organisms these viruses infect. This review discusses certain examples demonstrating that picornaviruses can persist through immunosuppression. Nonpicornaviruses that have been extensively studied are discussed as a means of illustrating that a few changes in a viral genome can alter the pathogenesis of the virus in the context of certain diseases. Although examining how changes in the viral genome can lead to pathogenesis is important, we propose that gaining a better mechanistic insight into how these viruses evade host immune clearance could be put to use combating specific autoimmune diseases.

Picornaviruses suppress the innate immune response

Suppression of the human immune system occurs following infection with the picornaviruses: EV 71 (which causes severe hand, foot and mouth disease), poliovirus, CVB3 and HAV, in that all of these picornaviruses inhibit the innate antiviral immune response in one way or another. For example, EV 71 blocks the production of type I interferon (IFN) [13], an important component of the innate antiviral immune response, from peripheral blood mononuclear cells, peritoneal macrophages and splenocytes upon infection of these cells. Mice infected with EV 71 failed to produce type I IFN in response to either poly(I:C) injection or CVB3 infection, both of which are strong inducers of type I IFN [14]. Type I IFNs are produced early in viral infection and are one factor involved in the redistribution of lymphocytes from the peripheral blood into infected tissues [15,16].

Cardioviruses, such as encephalomyocarditis (EMC) virus and Theiler's murine encephalomyelitis virus (TMEV), have an L protein zinc-finger domain. The L protein has been shown to modulate the innate immune response during acute EMCV and TMEV infections in humans and mice, respectively [17–19]. Hato *et al.* [17] demonstrated that the L protein inhibited the

type I IFN response by blocking IRF-3 dimerization and trafficking of IRF-3 from the cytoplasm to the nucleus. Mutating the L protein led to an impaired apoptotic activity in murine macrophages, suggesting that not only is the L protein important for apoptosis, but slight changes to the viral genome can have a significant effect on viral pathogenesis [20]. Although it has not yet been shown with SAFV, other rodent cardioviruses that have a close nucleotide sequence homology to SAFV, such as TMEV, have been shown to inhibit type I IFNs [21].

One cell type that serves as a bridge between the early, nonspecific (innate) immune response and the specific (adaptive) immune response are dendritic cells (DCs). DCs are professional antigen-presenting cells that activate Th cells and certain subsets of DCs are important for the innate immune response. DCs secrete IFN α upon viral infection. For example, FMDV-infected pigs had a transient lower number of IFN α -producing cells when re-stimulated *ex vivo* with the Toll-like-receptor agonist, CpG 2216, suggesting that FMDV is modulating the innate immune response [22]. In addition, IFN α production was reduced in the DC population of cells. One mechanism by which FMDV is able to block both IFN α and IFN β production is early production of FMDV viral leader protease, which cleaves elongation factor 4G, effectively inhibiting cap-dependent translation of cellular mRNA and subsequent protein synthesis [23]. Because picornaviruses, such as FMDV, are positive-sense RNA viruses, upon entry into a cell viral protein synthesis can occur even before viral replication, therefore the production of these viral proteases can skew the immune response prior to or concurrent with viral replication.

Taken together, these studies suggest that certain picornaviruses can persist in a host by suppressing the host innate immune system. However, further examination as to how viruses suppress the innate immune response is needed as we gain mechanistic insight into the innate immune system, such as the role of certain IFN-stimulated genes.

Picornaviruses causing lymphopenia

There are a variety of mechanisms by which viruses induce lymphopenia, which is the condition of having an abnormally low level of lymphocytes in the blood. For example, apoptosis of lymphocytes can be induced as a direct

result of viral infection or indirectly through the induction of cytokines. Alternatively, lymphocytes may be redistributed, through the action of type I IFNs, from the blood into virally infected tissues.

With respect to picornaviruses, poliovirus infection causes a suppression of lymphocyte stimulation [24] and CVB3, a picornavirus naturally affecting humans, has been shown to cause spleen atrophy and reduced antibody responsiveness in BALB/c mice [25]. Both of these effects may be mediated by macrophages through the production of type I IFNs. HAV infection inhibits mitosis in leukocytes [26]. Probably the most important picornavirus affecting livestock is FMDV, which infects cattle and swine. Immunosuppression by the FMDV takes the form of depletion of and lack of function of (in response to concanavalin A [conA]) T cells in the blood, lymph nodes and spleen [27,28]. Certain serotypes of FMDV cause lymphoid depletion in the spleen and thymus and severe lymphopenia in C57BL/6 mice as well [28]. Bautista *et al.* [27] provided evidence that lymphopenia and T-cell dysfunction are not the result of a productive viral infection and hypothesized that the virus was either altering lymphocyte trafficking and/or inducing apoptosis in lymphocytes through the activation of caspase 3. However, lymphopenia could be the result of a productive viral infection. Diaz-San Segundo *et al.* [28] demonstrated that FMDV can directly infect T and B cells in lymphoid compartments. Swiss White Landrace pigs inoculated with FMDV C-S8c1 had significantly fewer T cells, which corresponded to higher viremia, in comparison to control animals [28]. In addition, FMDV was demonstrated to replicate in lymphocytes *in vivo*. The levels of cellular death by apoptosis were not high enough to account for lymphocyte depletion and the lymphocytes that did survive were unable to respond to mitogen activation, suggesting that infection caused immunosuppression. However, this effect by FMDV C-S8c1 appears to be strain dependent, in that, active infection was not responsible for the lymphopenia when other strains of FMDV were used.

Point mutations in picornavirus genomes alters pathogenesis & disease

Picornaviruses have been implicated as the causative agent in certain autoimmune diseases [29]. One such example is Type 1 diabetes (T1D).

T1D is an immune-mediated disease characterized by lymphocyte infiltration, especially T cells, into the islet of Langerhans leading to pancreatic β cell destruction. While the etiology of T1D is not fully elucidated, the causes are likely based on a combination of hereditary and environmental factors. Craighead *et al.* [30] demonstrated that EMC virus induced insulin-dependent diabetes mellitus (IDDM) or T1D in mice. The M variant of EMC (EMC-M) sporadically infected and destroyed pancreatic β cells in mice [31,32]. Further examination of the M variant by plaque purification led to the identification of two stable variants, EMC-D and EMC-B. EMC-D was found to induce IDDM/T1D in approximately 90% of infected mice compared with mice inoculated with the EMC-B variant, which did not acquire IDDM/T1D [33,34]. Furthermore, Jun *et al.* [35] demonstrated that the change of a single amino acid (Thr to Ala) at position 776 of the EMC polyprotein, located in the capsid protein VP1, caused diabetes in mice. Alternatively, substitution of other bases at the same or next position resulted in a loss of viral-induced diabetes.

Similarly, TMEV infection of mice via the natural route of infection (enteric) is mainly asymptomatic [36]. However, if this picornavirus gets into the CNS, it can cause acute encephalitis and a chronic demyelinating disease depending on the strain of the virus and the genetic background of the mouse [37,38]. C57BL/6 mice infected with the Daniel's (DA) strain of TMEV, a less virulent strain of TMEV, via the intracerebral route develop acute encephalitis; mice survive the acute disease and clear the virus. However, TMEV-DA-infected SJL/J mice are unable to clear the virus and subsequently present with chronic demyelinating disease, similar to multiple sclerosis (MS). A mutant of TMEV-DA was inadvertently created as a result of transcription error(s) by the T7 polymerase while using a modified full-length infectious cDNA clone of the TMEV-DA virus as template [39]. The TMEV-H101 mutant virus encodes a point mutation (Thr101Ile) in VP1. In addition, in sequencing the TMEV-H101 viral genome, there were also several nucleotide substitutions in the 5'-UTR as well as additional amino acid substitutions in the capsid protein coding region, suggesting that there are a number of perturbations in the viral genome [40]. TMEV-H101 was not detected in the CNS, but led to greater than 90% mortality by day 7 postinfection,

Figure 1. Theiler's murine encephalomyelitis virus-H101 infection ablates splenic T cells at day 3 postinfection in comparison to mock and Theiler's murine encephalomyelitis virus-Daniel's infected mice. Representative flow cytometric histograms of splenic T cells, monocytes and B cells. Procedure used to isolate and stain cells is described in Cusick *et al.* [41]. Briefly, spleens were obtained from mock, TMEV-DA- and TMEV-H101-infected animals at day 3 postinfection, cells were mechanically isolated, stained with antibodies specific for T cells (CD45⁺ CD3⁺), monocytes (CD45⁺ CD11b⁺) and B cells (CD45⁺ CD19⁺), and analyzed on a BD FACSCanto™ II flow cytometer. Gating was determined by FMO with isotype-matched immunoglobulin (black dotted line), in that, if the cells did not fluoresce at a level higher than the FMO control, then the samples were negative. Spleen cells from mock (gray line) and TMEV-DA (black line)-infected mice had T cells. Spleen cells from TMEV-H101-infected mice had a marked reduction in T cells (red line). These experiments were performed two times with at least four mice per group (data not shown). DA: Daniel's strain; FMO: Fluorescence-minus-one; TMEV: Theiler's murine encephalomyelitis virus.

suggesting that TMEV-H101 led to a markedly different pathogenesis when compared with the parent strain TMEV-DA. To note, TMEV-H101 infection by either the intraperitoneal or intravenous routes does not cause any mortality or notable phenotype (data not shown). In addition, we recently found that TMEV-H101 specifically killed splenic T cells *in vivo* in comparison to TMEV-DA-infected mice and mock infected mice, regardless of the route of infection (Figure 1). Spleens isolated on day 3 postinfection from C57BL/6 mice infected via the intracerebral route with the H101 mutant virus had no CD45⁺ CD3⁺ T cells, compared with the spleens of mock and DA-infected mice. Furthermore, both monocytes and B cells were still detected at similar frequencies to mock infected mice, suggesting that TMEV-H101 is specifically killing splenic T cells (Figure 1). Also, other organs were screened for the presence of T cells and mice infected with TMEV-H101 had no detectable T cells by flow cytometric analysis (data not shown). One explanation for this reduced T-cell frequency in spleens is that TMEV-H101 infection induces the redistribution of T cells into other, not as yet screened, organs, a possibility which is currently being investigated.

However, we favor direct killing of T cells by TMEV-H101 instead of redistribution of T cells to other organs due to the fact that the spleen, a secondary lymphoid organ, had no detectable T cells by flow cytometric analysis. Taken together, these results provide evidence that a point mutation(s) in the viral genome alters viral-induced disease and pathogenesis.

Can we exploit viruses for therapeutic gain?

The concept of using viruses as a therapy for disease is not novel. The use of viruses as a therapy for cancer has been noted for over a hundred years due to the observations that hematological malignancy regression coincided with viral infection (reviewed in [42]). More recently, infection with measles virus, a negative-stranded RNA virus, coincided with regression in leukemia [43,44], Hodgkin's disease [45,46] and Burkitt's lymphoma [47]. A number of picornaviruses are currently being trialed as oncolytic agents: coxsackievirus (CAVATAK), poliovirus (PVS-RIPO) and Seneca Valley virus (NTX-010; oncolytic virotherapy is reviewed in [48]). Furthermore, an epitope modified TMEV has been proposed as a candidate T-cell vaccine for use as an immunotherapeutic against

tumors [49]. We are not aware of any trials currently using viruses to combat autoimmune diseases.

One of the most complete series of studies examining the role of parent and variant strains of a virus impacting an autoimmune disease were performed by the Oldstone group. Oldstone *et al.* [50,51] demonstrated that a certain strain of lymphocytic choriomeningitis virus (LCMV) was able to prevent a lethal autoimmune disease in mice. More specifically, infection of nonobese insulin-dependent diabetes mice, a spontaneous murine model of IDDM/T1D, with LCMV-Pasteur (parental strain) resulted in termination of the autoimmune response and provided lasting immunosuppression without total suppression of the immune system, suggesting that the virus was specifically targeting the cells associated with disease [51]. LCMV is an ambisense, bisegmented RNA virus, in that both the S and L RNA segments are negative-sense and in a second nonoverlapping region there are 'pseudo'-positive-sense RNA segments. Genetic reassortment of the therapeutic strain of LCMV-Pasteur and the nontherapeutic variant, LCMV Clone 13, mapped the immunosuppressive effect to the S RNA segment of LCMV, suggesting that a few differences in the S RNA segment of the viral genome can significantly alter protection against T1D [52].

Another autoimmune disease similar to T1D, in which antigen-specific activated T cells cause tissue damage, is MS [53]. In MS, it is widely accepted that myelin-specific autoreactive T cells contribute to this inflammatory demyelinating disease of the CNS [54]. One of the preclinical animal models used to study MS is the SJL/J female mouse sensitized with synthetic myelin protein(s) causing experimental autoimmune encephalomyelitis (EAE). Upon subcutaneous injection of a myelin-specific peptide, 139–151 peptide of myelin proteolipid protein (PLP_{139–151}) in the presence of adjuvant, mice develop relapsing-remitting EAE, which mirrors the most common disease course in MS patients [55]. RR-EAE results from the MHC class II (I-A^s) molecules presenting PLP_{139–151} peptides to the T-cell receptor on autoreactive CD4⁺ Th cells. The engagement of the T-cell receptor by the peptide–MHC complex is necessary for the activation of the CD4⁺ T cells, which then proliferate and secrete proinflammatory cytokines. Another common murine

model of MS is TMEV infection of mice. SJL/J mice infected with TMEV-DA intracerebrally are unable to clear the virus leading to chronic demyelination in the CNS and causing an MS-like disease. Interestingly, our observation that TMEV-H101 is able to kill T cells (Figure 1) led us to test whether this variant of TMEV-DA could modulate a T-cell-mediated autoimmune disease *in vivo*. In a proof-of-concept experiment, SJL/J PLP_{139–151}-immunized mice were infected with TMEV-H101 at day 5 postimmunization with PLP_{139–151} (Figure 2). TMEV-H101-infected animals had significantly lower clinical score at days 10–13 compared with no treatment mice (Figure 2). More specifically, the cumulative disease index, an average score of the clinical score during the first attack, was significantly lower for TMEV-H101 (mean $\pm$ standard error of the mean: 9.38 ± 0.85) when compared with no treatment mice (13.8 ± 0.98) by *t*-test, $p < 0.05$. Infecting PLP_{139–151}-immunized SJL mice between relapses with TMEV-H101 aborts exacerbation (second

Figure 2. TMEV-H101 ameliorates experimental autoimmune encephalomyelitis. The materials and methods were adapted from Cusick *et al.* [56]. Briefly, SJL/J female mice were immunized with PLP_{139–151} in complete Freund's adjuvant and mice were intravenously injected with *Bordetella pertussis* at days 0 and 2. Immunized mice were infected intraperitoneally with TMEV-H101 (3×10^5 PFU/mouse, gray square) at day 5 postimmunization (arrow). PLP_{139–151}-immunized mice were used as a control (no treatment, black triangle). Mice were weighed and scored daily for clinical signs. Clinical scoring was as follows: 0, no clinical disease; 1, loss of tail tonicity; 2, presents with mild hindleg paralysis with no obvious gait disturbance; 3, mild leg paralysis with gait disturbance and paralysis; 4, hindlimbs are paralyzed; and 5, moribund or dead. Data are representative of the mean $\pm$ standard errors of the means for groups of 20 mice for TMEV-H101 and 40 mice for no treatment (control). * $p < 0.05$, paired *t*-test.

attack) of EAE [CUSICK MF, UNPUBLISHED DATA]. Importantly, we are currently investigating whether TMEV-DA, the parent strain of TMEV-H101, similarly affects EAE, which would suggest that a general viral infection eliminates T cells through type I IFN.

Taken together, these results suggest that TMEV-H101 could be an interesting candidate to combat certain T-cell-mediated autoimmune diseases. However, an important question that is currently being investigated is whether the elimination of T cells by TMEV-H101 infection is occurring through a direct or indirect mechanism.

Conclusion

In conclusion, variation(s) in viral genomes can have a variety of effects on pathogenesis. Although these variations can lead to evasion of host responses and enhance disease, this review highlighted some examples where viral variants arising in certain viruses could be used as therapeutic platforms to combat disease.

Future perspective

We hypothesize that virotherapy is a viable option for treating certain autoimmune diseases. Clinical trials of genetically altered viruses targeting cancerous cells are ongoing; therefore, these ideas can be translated over to treating autoimmune diseases in humans.

Acknowledgements

The authors would like to thank DJ Doty for technical assistance and DJ Harper for the outstanding preparation of the manuscript.

Financial & competing interests disclosure

This work was supported by NIH grants T32AI055434 (MF Cusick) and 1R01NS082102 (RS Fujinami). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

EXECUTIVE SUMMARY

Background

- Picornaviruses can cause immunosuppression.

Picornaviruses suppress the innate immune response

- One mechanism by which picornaviruses evade host clearance is by suppressing the innate immune response.

Picornaviruses causing lymphopenia

- Productive picornavirus infection can lead to lymphopenia and subsequently persistence.

Point mutations in picornavirus genomes alters pathogenesis & disease

- Point mutation(s) in the viral genome can alter viral-induced disease and pathogenesis.

Can we exploit viruses for therapeutic gain?

- Certain viruses could be used as therapeutic agents to combat specific autoimmune diseases.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

- 1 Fauquet CM, Fargette D. International Committee on Taxonomy of Viruses and the 3,142 unassigned species. *Virol. J.* 2, 64 (2005).
- 2 International Committee on Taxonomy of Viruses. <http://ictvonline.org/Virustaxonomy.Asp>
- 3 Tapparel C, Siegrist F, Petty TJ, Kaiser L. Picornavirus and enterovirus diversity with associated human diseases. *Infect. Genet. Evol.* 14, 282–293 (2013).
- 4 Lipton HL. Human Vilyuisk encephalitis. *Rev. Med. Virol.* 18(5), 347–352 (2008).
- 5 Jones MS, Lukashov VV, Ganac RD, Schnurr DP. Discovery of a novel human picornavirus in a stool sample from a pediatric patient presenting with fever of unknown origin. *J. Clin. Microbiol.* 45(7), 2144–2150 (2007).
- 6 Nielsen AC, Bottiger B, Banner J, Hoffmann T, Nielsen LP. Serious invasive Saffold virus infections in children, 2009. *Emerg. Infect. Dis.* 18(1), 7–12 (2012).
- 7 Blinkova O, Kapoor A, Victoria J *et al.* Cardioviruses are genetically diverse and cause common enteric infections in South Asian children. *J. Virol.* 83(9), 4631–4641 (2009).
- 8 Chiu CY, Greninger AL, Chen EC *et al.* Cultivation and serological characterization of a human Theiler's-like cardiovirus associated with diarrheal disease. *J. Virol.* 84(9), 4407–4414 (2010).
- 9 Chiu CY, Greninger AL, Kanada K *et al.* Identification of cardioviruses related to Theiler's murine encephalomyelitis virus in human infections. *Proc. Natl Acad. Sci. USA* 105(37), 14124–14129 (2008).
- First report linking a cardiovirus in humans to disease.

- 10 Silverstein AM, Clemens Freiherr von Pirquet: explaining immune complex disease in 1906. *Nat. Immunol.* 1(6), 453–455 (2000).
- 11 Beckford AP, Kaschula RO, Stephen C. Factors associated with fatal cases of measles. A retrospective autopsy study. *S. Afr. Med. J.* 68(12), 858–863 (1985).
- 12 Shanks GD, Lee SE, Howard A, Brundage JF. Extreme mortality after first introduction of measles virus to the polynesian island of Rotuma, 1911. *Am. J. Epidemiol.* 173(10), 1211–1222 (2011).
- 13 Lei X, Sun Z, Liu X, Jin Q, He B, Wang J. Cleavage of the adaptor protein TRIF by enterovirus 71 3C inhibits antiviral responses mediated by Toll-like receptor 3. *J. Virol.* 85(17), 8811–8818 (2011).
- **The picornavirus enterovirus 71 specifically blocks type I interferon production.**
- 14 Lee YP, Wang YF, Wang JR, Huang SW, Yu CK. Enterovirus 71 blocks selectively type I interferon production through the 3C viral protein in mice. *J. Med. Virol.* 84(11), 1779–1789 (2012).
- 15 Selin LK, Lin MY, Kraemer KA *et al.* Attrition of T cell memory: selective loss of LCMV epitope-specific memory CD8 T cells following infections with heterologous viruses. *Immunity* 11(6), 733–742 (1999).
- 16 McNally JM, Zarozinski CC, Lin MY, Brehm MA, Chen HD, Welsh RM. Attrition of bystander CD8 T cells during virus-induced T-cell and interferon responses. *J. Virol.* 75(13), 5965–5976 (2001).
- 17 Hato SV, Ricour C, Schulte BM *et al.* The mengovirus leader protein blocks interferon-alpha/beta gene transcription and inhibits activation of interferon regulatory factor 3. *Cell. Microbiol.* 9(12), 2921–2930 (2007).
- 18 Paul S, Michiels T. Cardiovirus leader proteins are functionally interchangeable and have evolved to adapt to virus replication fitness. *J. Gen. Virol.* 87(Pt 5), 1237–1246 (2006).
- 19 Ricour C, Delhay S, Hato SV *et al.* Inhibition of mRNA export and dimerization of interferon regulatory factor 3 by Theiler's virus leader protein. *J. Gen. Virol.* 90(Pt 1), 177–186 (2009).
- 20 Son KN, Liang Z, Lipton HL. Mutation of the Theiler's virus leader protein zinc-finger domain impairs apoptotic activity in murine macrophages. *Virus Res* 177(2), 222–225 (2013).
- 21 Himeda T, Ohara Y. Saffold virus, a novel human cardiovirus with unknown pathogenicity. *J. Virol.* 86(3), 1292–1296 (2012).
- 22 Nfon CK, Ferman GS, Toka FN, Gregg DA, Golde WT. Interferon-alpha production by swine dendritic cells is inhibited during acute infection with foot-and-mouth disease virus. *Viral Immunol.* 21(1), 68–77 (2008).
- **Foot-and-mouth disease virus (FMDV), a picornavirus, causes lymphopenia and inhibition of T-cell function during acute infection.**
- 23 Devaney MA, Vakharia VN, Lloyd RE, Ehrenfeld E, Grubman MJ. Leader protein of foot-and-mouth disease virus is required for cleavage of the p220 component of the cap-binding protein complex. *J. Virol.* 62(11), 4407–4409 (1988).
- 24 Van Loon AM, Van Der Logt JT, Van Der Veen J. Poliovirus-induced suppression of lymphocyte stimulation: a macrophage-mediated effect. *Immunology* 37(1), 135–143 (1979).
- 25 Bendinelli M, Matteucci D, Toniolo A, Patane AM, Pistillo MP. Impairment of immunocompetent mouse spleen cell functions by infection with coxsackievirus B3. *J. Infect. Dis.* 146(6), 797–805 (1982).
- **One of the first examples of viral infection contributing to an autoimmune disease.**
- 26 Mella B, Lang DJ. Leukocyte mitosis: suppression *in vitro* associated with acute infectious hepatitis. *Science* 155(3758), 80–81 (1967).
- 27 Bautista EM, Ferman GS, Golde WT. Induction of lymphopenia and inhibition of T cell function during acute infection of swine with foot and mouth disease virus (FMDV). *Vet. Immunol. Immunopathol.* 92(1–2), 61–73 (2003).
- 28 Diaz-San Segundo F, Salguero FJ, De Avila A, De Marco MM, Sanchez-Martin MA, Sevilla N. Selective lymphocyte depletion during the early stage of the immune response to foot-and-mouth disease virus infection in swine. *J. Virol.* 80(5), 2369–2379 (2006).
- 29 Cusick MF, Libbey JE, Fujinami RS. Molecular mimicry as a mechanism of autoimmune disease. *Clin. Rev. Allergy Immunol.* 42(1), 102–111 (2012).
- 30 Craighead JE, McLane MF. Diabetes mellitus: induction in mice by encephalomyocarditis virus. *Science* 162(3856), 913–914 (1968).
- 31 Ross ME, Onodera T, Brown KS, Notkins AL. Virus-induced diabetes mellitus. IV. Genetic and environmental factors influencing the development of diabetes after infection with the M variant of encephalomyocarditis virus. *Diabetes* 25(3), 190–197 (1976).
- 32 Yoon J, Onodera T, Notkins AL. Virus-induced diabetes mellitus: VIII. Passage of encephalomyocarditis virus and severity of diabetes in susceptible and resistant strains of mice. *J. Gen. Virol.* 37(2), 225–232 (1977).
- 33 Yoon JW, McClintock PR, Onodera T, Notkins AL. Virus-induced diabetes mellitus. XVIII. Inhibition by a nondiabetogenic variant of encephalomyocarditis virus. *J. Exp. Med.* 152(4), 878–892 (1980).
- 34 Ray UR, Aulakh GS, Schubert M, McClintock PR, Yoon JW, Notkins AL. Virus-induced diabetes mellitus. XXV. Difference in the RNA fingerprints of diabetogenic and non-diabetogenic variants of encephalomyocarditis virus. *J. Gen. Virol.* 64 (Pt 4), 947–950 (1983).
- 35 Jun HS, Kang Y, Notkins AL, Yoon JW. Gain or loss of diabetogenicity resulting from a single point mutation in recombinant encephalomyocarditis virus. *J. Virol.* 71(12), 9782–9785 (1997).
- 36 Libbey JE, Fujinami RS. Neurotropic viral infections leading to epilepsy: focus on Theiler's murine encephalomyelitis virus. *Future Virol.* 6(11), 1339–1350 (2011).
- 37 Tsunoda I, Fujinami RS. Two models for multiple sclerosis: experimental allergic encephalomyelitis and Theiler's murine encephalomyelitis virus. *J. Neuropathol. Exp. Neurol.* 55(6), 673–686 (1996).
- 38 Kirkman NJ, Libbey JE, Wilcox KS, White HS, Fujinami RS. Innate but not adaptive immune responses contribute to behavioral seizures following viral infection. *Epilepsia* 51(3), 454–464 (2010).
- 39 Zurbriggen A, Thomas C, Yamada M, Roos RP, Fujinami RS. Direct evidence of a role for amino acid 101 of VP-1 in central nervous system disease in Theiler's murine encephalomyelitis virus infection. *J. Virol.* 65(4), 1929–1937 (1991).
- 40 Tsunoda I, McCright IJ, Kuang LQ, Zurbriggen A, Fujinami RS. Hydrocephalus in mice infected with a Theiler's murine encephalomyelitis virus variant. *J. Neuropathol. Exp. Neurol.* 56(12), 1302–1313 (1997).
- 41 Cusick MF, Libbey JE, Patel DC, Doty DJ, Fujinami RS. Infiltrating macrophages are key to the development of seizures following virus infection. *J. Virol.* 87(3), 1849–1860 (2013).
- 42 Kelly E, Russell SJ. History of oncolytic viruses: genesis to genetic engineering. *Mol. Ther.* 15(4), 651–659 (2007).

- 43 Pasquinucci G. Possible effect of measles on leukaemia. *Lancet* 1(7690), 136 (1971).
- **Proof-of-concept study demonstrating that a virus could inhibit an autoimmune disease.**
- 44 Gross S. Measles and leukaemia. *Lancet* 1(7695), 397–398 (1971).
- 45 Zygiert Z. Hodgkin's disease: remissions after measles. *Lancet* 1(7699), 593 (1971).
- 46 Taqi AM, Abdurrahman MB, Yakubu AM, Fleming AF. Regression of Hodgkin's disease after measles. *Lancet* 1(8229), 1112 (1981).
- 47 Bluming AZ, Ziegler JL. Regression of Burkitt's lymphoma in association with measles infection. *Lancet* 2(7715), 105–106 (1971).
- 48 Russell SJ, Peng KW, Bell JC. Oncolytic virotherapy. *Nat. Biotechnol.* 30(7), 658–670 (2012).
- 49 Pavelko KD, Girtman MA, Mitsunaga Y *et al.* Theiler's murine encephalomyelitis virus as a vaccine candidate for immunotherapy. *PLoS ONE* 6(5), e20217 (2011).
- 50 Oldstone MB. Prevention of Type I diabetes in nonobese diabetic mice by virus infection. *Science* 239(4839), 500–502 (1988).
- 51 Oldstone MB. Viruses as therapeutic agents. I. Treatment of nonobese insulin-dependent diabetes mice with virus prevents insulin-dependent diabetes mellitus while maintaining general immune competence. *J. Exp. Med.* 171(6), 2077–2089 (1990).
- 52 Oldstone MB, Ahmed R, Salvato M. Viruses as therapeutic agents. II. Viral reassortants map prevention of insulin-dependent diabetes mellitus to the small RNA of lymphocytic choriomeningitis virus. *J. Exp. Med.* 171(6), 2091–2100 (1990).
- 53 Von Herrath MG, Fujinami RS, Whitton JL. Microorganisms and autoimmunity: making the barren field fertile? *Nat. Rev. Microbiol.* 1(2), 151–157 (2003).
- 54 Kawamura K, McLaughlin KA, Weissert R, Forsthuber TG. Myelin-reactive type B T cells and T cells specific for low-affinity MHC-binding myelin peptides escape tolerance in HLA-DR transgenic mice. *J. Immunol.* 181(5), 3202–3211 (2008).
- 55 Tuohy VK, Lu Z, Sobel RA, Laursen RA, Lees MB. Identification of an encephalitogenic determinant of myelin proteolipid protein for SJL mice. *J. Immunol.* 142(5), 1523–1527 (1989).
- 56 Cusick MF, Libbey JE, Trede NS, Eckels DD, Fujinami RS. Human T cell expansion and experimental autoimmune encephalomyelitis inhibited by lenalidomide, a small molecule discovered in a zebrafish screen. *J. Neuroimmunol.* 244(1–2), 35–44 (2012).