Protective and pathogenic roles for mast cells during viral infections

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Mast cells (MCs) are long-lived immune cells. They are armed with preformed mediators within granules that can be instantaneously released in response to an invading pathogen, including certain viruses. At the skin and mucosae, they initiate innate immune responses and promote the development of adaptive immune responses, through cellular recruitment or antigen presentation. However, systemic MC activation may promote immune pathologies through their vasoactive proteases and biogenic amines. Recently, MC products were identified to contribute to pathologies associated with viral hemorrhagic fever, such vascular leakage and thrombocytopenia. Similar associations of MCs with disease severity have been noted for certain respiratory viral pathogens. Here we discuss the specific MC responses to viruses and their influences on functional immune outcomes during infection.

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Introduction

Mast cells (MCs) are granulated cells of hematopoietic origin. They are thought to be derived from immature precursors known as MC progenitors that circulate in the blood [1]. Mature MCs are present in nearly all tissues in the body and also line blood and lymphatic endothelium [2]. Although mature MCs are terminally differentiated, they are distinct from many other types of immune cells in their property of being able to survive for months to years [2]. They are strategically present in high densities at the host-environment interfaces such as in the skin and mucosae and express a wide-range of molecular receptors. These include pattern recognition receptors (PRRs), TLRs, complement receptors, and intracellular sensors, RIG-I and MDA-5, among others (Figure 1) [3]. These innate sensors give MCs the capability to recognize diverse classes of pathogens and multiple subclasses of viruses [2]. MCs are loaded with granules, which are packed with prestored mediators that can be released almost instantaneously upon stimulus or pathogen insult. Their dense granules consist of proteoglycans on which negatively charged carbohydrates heparin or chondroitin sulfate are held together with positively charged proteases, including chymases and tryptases [2]. Some other mediators that are present in granules include pre-stored cytokines, such as TNF, matrix metalloproteinases, carboxypeptidases and others [2]. Other mediators that are expressed by certain subsets of MCs and which maybe also pre-stored include VEGF, IL-4, IL-6, IL-8, TGFβ and certain biogenic amines including histamine, serotonin and dopamine [4,5]. The next phase of response immediately after degranulation involves *de novo* synthesis and release of various chemokines, cytokines and prostaglandins [2]. Unlike other granulocytes, MCs are also uniquely capable of replenishing their granules and responding to consecutive stimuli [2]. Therefore, MCs not only respond to pathogens directly, they also communicate with various other immune and tissue cell types via release of their mediators. Certain viruses are also thought to induce MC hyperplasia, such as hepatitis C virus (HCV) in the liver or respiratory syncytial virus (RSV) and Sendai virus in the lung [6–8]. Here we discuss MC responses to viral pathogens that can influence the initiation of an innate immune response, help shape the developing adaptive immune response for pathogen clearance, and, in some circumstances, promote immune pathology.

Heterogeneity of mast cells and implications for their antiviral responses

Although derived from common progenitors, MC heterogeneity exists. In rodents, connective tissue MCs contain tryptase and chymase that are bound to heparin, while mucosal MCs mainly contain chymase, which is bound to chondroitin sulfate [9]. Human MCs, similarly, display heterogeneity, where connective tissue MC granules contain both tryptase and chymase. However, human mucosal MCs predominantly express tryptase [9]. The tissue microenvironment in which they reside also contributes to their phenotypic differences [10]. For instance,

Figure 1
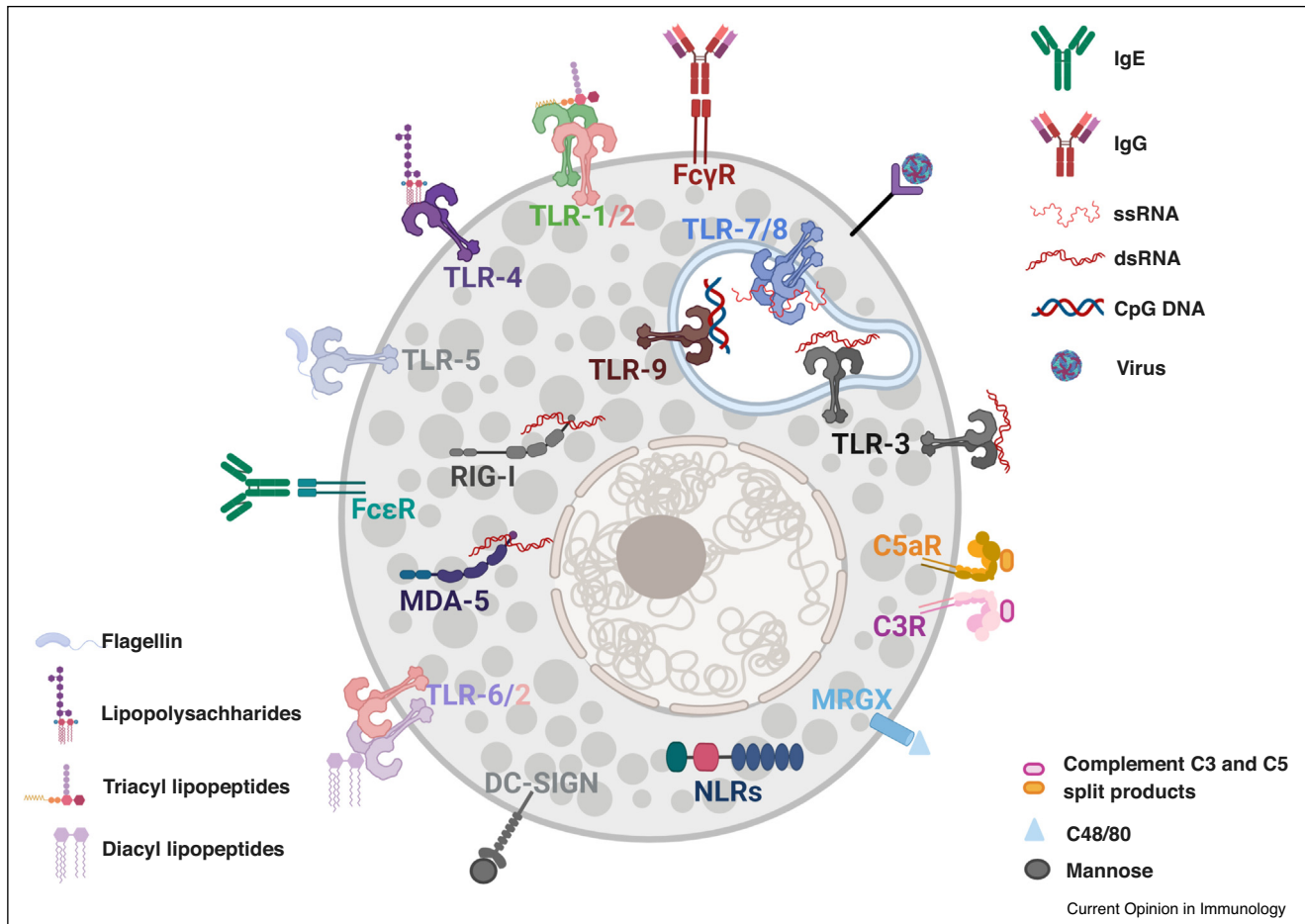
Pathogen recognition by mast cells.

MCs express a variety of membrane bound and cytosolic immunological sensors that can recognize unique pathogen associated signatures. Virus replication intermediates such as single or double-stranded RNA molecules can be recognized by membrane-bound and endosomal TLRs (TLR-3, TLR-7/8) and cytosolic RIG-I-like receptors (RIG-I, MDA-5), whereas bacterial products are recognized by TLRs 1-6 and NOD-like receptors, NLRs. TLR-9 is capable of recognizing methylated viral or bacterial DNA signatures, for instance HSV. Studies suggest that viruses like DENV may directly bind to MCs using an unknown receptor on the cell surface. MCs also express receptors for complement split products. Immunoglobulin receptors Fcγ and Fcε can interact with MCs in a unique way by cross-linking with antibodies and, thereby, increasing the magnitude of MC activation. Other receptor that could potentially be utilized by viruses include C-type lectin receptor DC-SIGN. Recently identified G-protein coupled receptor MRGX is a pseudoallergy receptor for MC-degranulation by cationic peptides and C48/80. Image was created with biorender.com.

MCs in the lung express higher levels of FcεRI and lower levels of the MRGX receptor compared to MCs in the skin [11,12]. The diversity in MC populations may contribute to tissue specific differences in responses to various stimuli including infections and allergic reactions. Virus tissue tropism influences the immune responses generated in response to them, with certain viruses infecting through the skin while others first encounter host cells in the lung or gut mucosae. Heterogeneity of MCs in different tissues as well as heterogeneity in the maturation state of the tissue-resident MCs could result in differing responses to invading viruses.

Mast cells as immune sentinels for various types of viruses

At the host-environment interfaces such as skin and mucosae where pathogen invasion occurs, MCs are present alongside other immune cells. MCs can directly sense pathogens and as well as alert other immune cells following infection for effective containment. MCs express a variety of TLRs (TLRs 1, 2, 3, 4, 5, 6, 7, 8 and 9) [13–15] for pathogen recognition and, in the case of viruses, MCs respond to either viral RNA or DNA recognition via TLR-3 and TLR-9, respectively [2,13]. Various studies have validated that TLR signaling is activated downstream

of both viral exposure to MCs and triggering of MC TLRs with pharmacological stimuli [13,16,17]. For example, TLR-7/8 activation in MCs is also thought to explain the efficacy of the drug imiquimod, in the treatment of cutaneous tumors. Imiquimod is a specific agonist of those receptors, which are responsible for the detection of single-stranded viral RNA within endosomes [18]. Intracellular antiviral sensors, RIG-I and MDA-5 are located in the cytosol and recognize dsRNA, an important viral replication intermediate. They were shown to be activated in MCs upon exposure to viruses such as dengue virus (DENV) and influenza, resulting in the production of IFNα and TNF and, in the case of DENV, limiting virus infection in the skin [16,19]. Similarly, MC-deficient animals are more susceptible to cutaneous vaccinia virus infection and TLR activation and release of anti-microbial peptides from MCs contribute to protection [20,21]. MCs can also respond to viruses indirectly in coordination with other tissue-resident cells. For example, MC-derived TNF and IL-6 produced in response to IL-33 made by herpes simplex virus (HSV)-infected keratinocytes provided protection against cutaneous HSV infection [22,23]. RSV infection of human lung fibroblasts was also shown to indirectly alter MC phenotypes through the induction of hyaluronan-enriched extracellular matrix that induced increased expression of MC proteases [24•].

Integration of the unique activating signals received by MCs appears to induce a pathogen-specific activation program. Whereas MC responses to other stimuli such as bacteria, parasites or allergic stimulation induce a mostly Th2-associated phenotype, MCs responses to viruses are characterized by a balanced or Th1-associated phenotype, consistent with the need to clear intracellular pathogens [2]. Both type-I and type-II interferon responses are induced in MCs in response to multiple subclasses of pathogen associated molecular patterns (PAMPs) that characterize viruses, such as single-stranded and double-stranded RNA or CpG DNA [13,16,17,19,25]. MC chemokine production in response to viruses is also specific, for example, with CXCL10 and CCL4 made in response to RSV [26] and CCL5, CXCL12 and CX3CL1 being produced in response to DENV [16]. Overall, these chemokines are consistent with the need to recruit cytotoxic cells to clear viral infections. Together, these studies suggest that MCs play a protective role at the infection site and emphasize their phenotypic plasticity in response to pathogens, including viruses.

Although well characterized to induce a cytokine response, TLR signaling is generally thought to be insufficient to induce MC degranulation [27], which requires substantial calcium flux to occur in a cell, yet MC degranulation occurs *in vivo* during infection by some viral pathogens. MC degranulation directly in response to stimulation by a viral pathogen, without mediating antibodies or endogenous danger signals being required for

the response was shown first for DENV, which induces degranulation of human and rodent MCs [16]. Degranulation also occurs in response to other similar flaviviruses, such as Japanese encephalitis virus (JEV) [28•]. The receptor for DENV and other flaviviruses is not yet known, but is expected to be a surface molecule that is able to bind to and detect the virus particle without the need for replication intermediates to be produced since UV-inactivated viruses also induce degranulation [16,28•]. MC degranulation has also been observed in tissues infected with other viruses including influenza [19,29] but it is not clear if this is a direct receptor-mediated detection of virus by MCs or if endogenous host products could be required for degranulation. Host products that are known to induce degranulation and could, therefore, indirectly potentiate or induce MC degranulation during viral infection include complement split products, heat shock proteins, antibodies, and others. Indeed, virus-specific IgG can augment MC degranulation to DENV through the activating Fc receptor, FcγRIII, in a mechanism similar to the reverse arthus reaction [30] and virus-specific IgE could also theoretically induce degranulation [31–33]. Degranulation serves a sentinel function and pre-stored granule-associated products promote changes in the lymph node [34], increase edema in skin tissue [35], and activate the vascular endothelium to allow for attachment and rolling of immune cells that must enter the site of infection to aid infection clearance [36]. These are some of the ways that granule-associated products likely assist with viral clearance *in vivo*.

Mast cell influence on the cellular immune response to viruses

Downstream of innate activation of MCs, the products that they release and their heightened activation state influences the local inflammatory microenvironment. In a mouse model of Newcastle virus infection, peritoneal injection of virus induced recruitment of CD8 T cells in a MC-dependent manner [37]. *In vitro*, reovirus or dsRNA analog Poly(I:C) exposure to MCs was shown to induce chemokine production that induced NK cell recruitment across trans-wells in a CXCL8-dependent fashion [38]. TLR-7 triggering within MCs by the drug imiquimod also illustrates a cellular recruitment pathway initiated by MCs in a IFN-α/β receptor 1 (IFNAR1)/MyD88-dependent mechanism that induces the production of CCL2. CCL2, in turn, led to the recruitment of plasmacytoid DCs that were required for tumor clearance [18]. DENV infection also induces a MC-dependent inflammatory response involving the recruitment of multiple subsets of conventional T cells, NKT cells and NK cells to sites of infection in the skin and draining lymph nodes [16,39••]. In DENV-infected skin, the γδT cell was one of the first T cell subsets enriched and activated following infection [39••]. MCs also appeared to form immune synapses with γδT cells at sites of infection,

which was shown to induce activation of the T cells through their TCR [39••]. The non-classical antigen presentation molecule on MCs, EPCR was essential for mediating this interaction and activation, which was in turn, important for early viral clearance from the skin [39••]. MCs have also been suggested to present antigen through classical antigen presentation, and although CD4 T cell activation is induced by co-culture of virus-exposed MCs with conventional αβT cells [39••,40], additional studies are needed to understand if MCs are important for classical antigen presentation during viral infection *in vivo*.

Mast cells as targets of viral infection

There is some evidence that MCs can be infected by certain viruses. Owing to their expression of the HIV co-receptors CCR5 and CXCR4, MCs and MC precursors can be infected by HIV and infection of MCs has been detected in humans with HIV [41,42]. As MCs are known to be long-lived and tissue resident, this has led to the suggestion that MCs could be an underappreciated reservoir for HIV. Furthermore, the potential of MCs to respond to PAMPs that are associated with virus replication such as ssRNA and dsRNA suggests that MCs can become infected by certain viruses. For DENV, our research showed that while there is limited intercellular viral replication that is sufficient to induce PRR activation, there was very limited to no production of infectious virus particles [16], which is more consistent with an incomplete replication cycle. No MCs have been reported to be infected by flaviviruses in infected human tissues. However, studies that used either a murine mastocytoma line or a human MC-like basophilic cell line *in vitro* have reported that the cells are susceptible to antibody-dependent enhancement of DENV infection [43,44], a phenomenon where IgG antibodies are able to promote the uptake of virus containing immune complexes via Fcγ receptors, leading to enhanced intracellular replication [45]. Other viruses that show limited to no replication in MCs in cell culture systems include RSV and influenza strain H1N1/A/PR8 [26,46,47].

Association of mast cells with virus-induced pathologies

MCs have been implicated in the vascular pathologies that are characteristic of certain viral infections, which may be due to their key importance as regulatory cells of vascular endothelium (Figure 2) [34]. MCs line blood vessels *in vivo*, and make a multitude of vasoactive factors including their proteases [5]. For example, MCs have been implicated in DENV-associated vasculopathy (Figure 2). DENV not only causes a characteristic rash that is associated with early acute infection, it also can cause a more severe viral hemorrhagic fever, characterized by coagulopathy, thrombocytopenia, microvascular permeability and occasionally frank hemorrhaging and organ failure [45]. MCs were shown to be strongly activated and degranulated in both mouse models of mild and severe dengue (using immune competent and immune compromised models) and also in humans [35]. Interestingly, in human dengue patient samples from multiple cohorts, MC proteases and particularly MC chymase, have been shown to correlate with disease severity, raising the potential of their use as prognostic biomarkers of disease severity [35,48]. MC activation was also highest in severe dengue patients with secondary infections [35], potentially consistent with the ability of antibodies to enhance MC degranulation [30]. Animal models also indicated that preventing MC activation using MC stabilizing drugs, which have been employed clinically for decades in the treatment of allergic conditions and asthma may be effective in preventing dengue vascular pathologies. This was shown in rodent models and is currently being tested in humans [35,49]. In DENV-infected mice, treatment with the MC stabilizer ketotifen modified the transcriptional activation of pathways including cholesterol biosynthesis, intrinsic prothrombin signaling, complement signaling and LXR/RXR activation [49], supporting the role of MCs in regulating these pathways and the potential benefits of MC stabilization as a therapeutic strategy for viral hemorrhagic fevers.

Differential roles of various MC products have been shown to contribute to unique aspects of DENV vascular pathology. For example, thrombocytopenia is a characteristic sign of DENV disease, occurring in the majority of cases. Serotonin derived from MCs was shown to induce DENV thrombocytopenia in mouse models and to be sufficient to induce aggregation of human platelets [50••]. This activation mechanism led to platelet aggregation and uptake by macrophages resulting in platelet destruction [50••]. Interestingly, since the mechanism of platelet destruction is via an activation phenotype which leads to a cascade amplifying platelet aggregation and activation, this MC-dependent mechanism may explain why platelet replacement therapies have been unsuccessful in treating DENV-induced thrombocytopenia [51]. However, blocking of the platelet serotonin receptor 5HT2A was able to reverse the phenotype in animals and human cell culture systems [50••]. Whereas MC-derived serotonin appears to be key for thrombocytopenia, MC proteases are more important for promoting vascular permeability. Injection of MC tryptase, for example, was shown to promote vascular leakage *in vivo* to a greater extent than a similar concentration of chymase [52•]. Tryptase provided to healthy uninfected mice at approximately the same concentrations detected in severe dengue patients was sufficient to induce shock in the animals [52•]. Importantly, in humans, tryptase levels were not only shown to be higher in severe compared to mild patients, like chymase, but also were uniquely associated with the development of shock [52•]. Tryptase is able to cleave PAR receptors which are key components of endothelial tight junctions [53] and this was shown to be a contributing factor to the

Figure 2
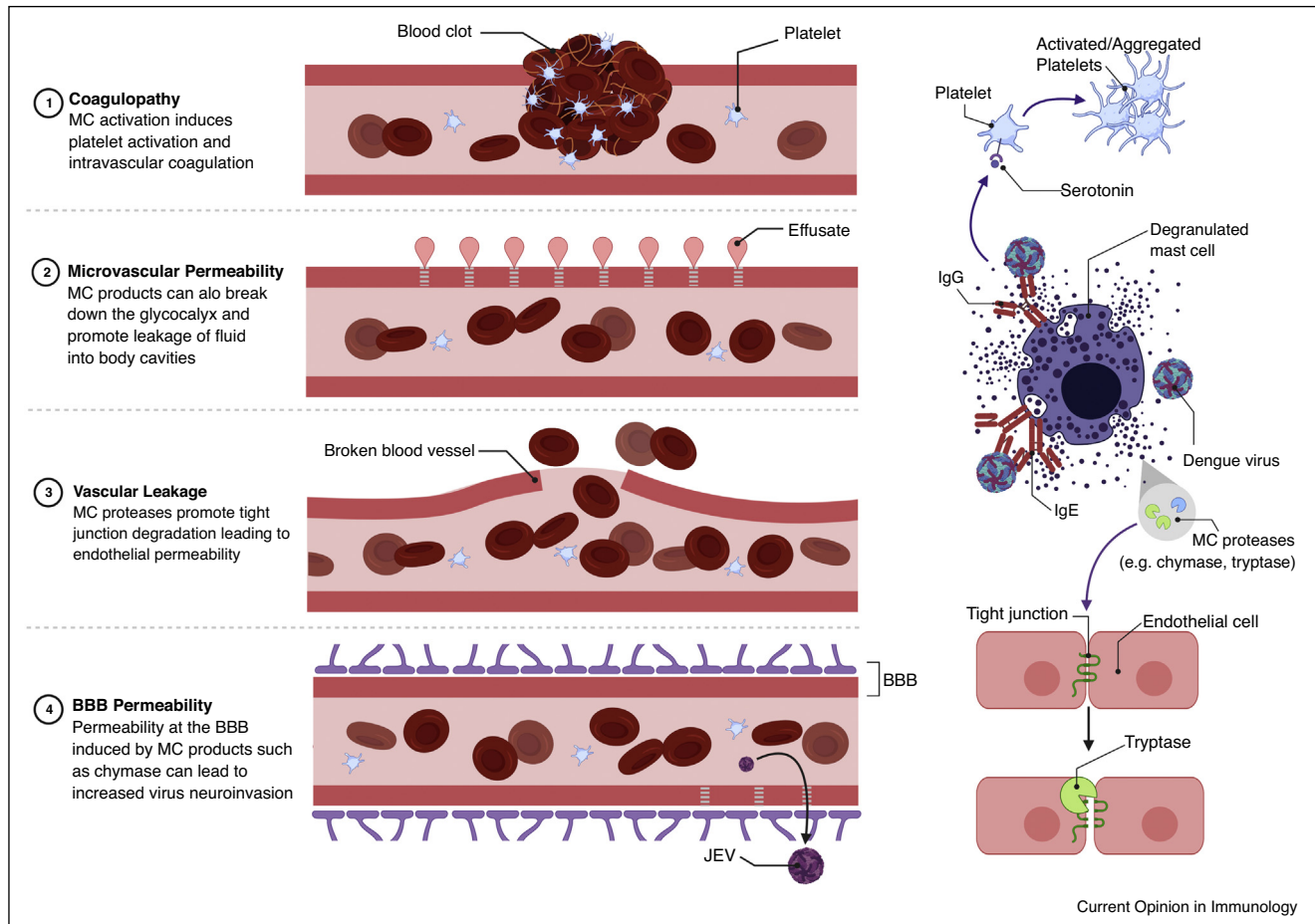
Mast cell influence on vascular pathology.
MCs can respond directly to viruses such as to DENV and degranulate. In the presence of antibodies such as IgG or IgE the magnitude of MC degranulation increases. Upon degranulation and activation, release of MC products can induce (1) coagulopathy by serotonin-mediated platelet activation, (2) microvascular permeability by shedding endothelial glycocalyx and (3) vascular leakage by breakdown of endothelial tight junctions. Tryptase is a MC-derived protease that can degrade both glycocalyx and tight junctions between endothelial cells. (4) While tryptase has a known prominent role in peripheral tissues, another MC-specific protease, chymase was identified as inducing BBB permeability during JEV infection and facilitating viral neuroinvasion. Since tryptase and chymase have different substrate specificities and may be released differentially depending on virus tissue tropism, they may play unique roles at differing tissue sites. Image was created with biorender.com.

vascular leakage that occurs during DENV infection [52*]. Other functional attributes of tryptase, such as its ability to cleave fibrin, leading to fibrin deposition and possibly intravascular coagulation, remain to be explored in the context of DENV infection. Consistent with its role in DENV vascular pathology, tryptase targeting using the drug nafamostat mesylate significantly reduced vascular permeability in animals, yet did not influence platelet levels or viremia, suggesting specificity of the pathway to vascular regulation [52*]. Interestingly, this drug has recently been used against the SARS-CoV-2 virus that causes COVID-19 disease [54]. In that context, it was used *in vitro* at high concentrations that are non-specific for tryptase and begin to effect other trypsin-like proteases [55], which may explain its mechanism in this context where tryptase was not present. However, the association of COVID-19 with clinical signs such as cutaneous manifestations including rash and intravascular coagulation warrant examining whether MCs may also play a role in its pathogenesis.

Studies also support a role for MCs in the peripheral tissues and central nervous system (CNS) during viral encephalitis. Early studies in mice with the c-$kit^{W/W-v}$ mutation, which have significant defects in MCs and some other defects, such as anemia [56], were suggestive that MCs promote cellular inflammation in the brain following intracerebral inoculation of Sindbis virus [57]. For another encephalitic virus, JEV, multiple c-kit-dependent and c-kit-independent MC deficiency models supported a protective role for MCs

in the clearance of virus in peripheral organs, yet MC proteases, and specifically MC-derived chymase, contributed significantly to JEV neuroinvasion [28•]. Chymase is an angiotensin converting enzyme that also is able to induce microvascular permeability [58], although, as discussed above, to a lesser extent than a comparable concentration of tryptase [52•]. However, it is highly active at the blood brain barrier (BBB) and has been shown to promote BBB permeability during sterile inflammation [59]. In JEV infection, both peripheral and CNS-resident MCs contributed to augmented BBB permeability, resulting in increased CNS viral titers, worsened neuroseverity scores, and decreased survival [28•]. In support of the potential of MC-derived chymase as a therapeutic target for JEV infection, treatment of JEV-infected mice with the chymase inhibitor TY-51469 prevented BBB leakage, reduced disease severity, and prolonged survival [28•]. There is also limited evidence that MCs maybe involved in disease caused by other highly pathogenic viruses. For example, immune cell type profiling of the transcriptional response to Nipah virus infection in primates identified a strong MC-associated signature differentially regulated in surviving versus fatal experimental cases [60•], but the influence of MC transcriptional regulation in disease outcomes has not yet been defined.

An interesting clinical observation linking viral infection and MC-mediated disease relates to respiratory viral pathogens. For multiple Paramyxoviruses (including RSV) as well as rhinoviruses, the risk of developing allergic disease is heightened following severe viral infection [61,62]. In rodent models of RSV and Sendai infection, exposure to these viruses induced MC hyperplasia in the lungs, further suggesting a MC-mediated augmentation of allergy following infection [7,8]. Furthermore, in animals with allergic airway disease, dsRNA was shown to augment eosinophilia in a TRIF-dependent manner, which was abrogated in MC-deficient mice, supporting that TLR stimulation can potentiate MC responses in the lung, with possible implications for viral infections in allergic individuals [63]. In another example of MCs influencing virus-induced tissue remodeling, MCs were identified as potential contributors to liver fibrosis during HCV infection [64]. In that study, MCs were shown to express HLA-G, a non-classical antigen presentation molecule that is expressed in membrane-bound and soluble forms and which has been shown to induce suppressor cells while limiting NK and T cell activation. HLA-G was correlated with the extent of fibrosis in the HCV-infected liver tissues [64].

In contrast to influencing the long-term remodeling of the lungs during the respiratory viral infections discussed above, MC activation may also occur in the lungs during acute infection by certain viruses, such as H5N1 Influenza [29]. Targeting of MC activation with the MC stabilizing drug ketotifen reduced MC activation in a mouse model of H5N1 infection while also reducing lung lesions and apoptosis in the lung tissue [29]. Combination therapy with a neuraminidase inhibitor, further improved disease outcomes, suggesting the potential of targeting the MC inflammatory response along with conventional antiviral therapies [29]. MCs may also contribute to edema and lung pathology triggered by the enterovirus EV71, which causes hand foot and mouth disease. That study used a neonatal mouse model and observed increases in MCs in various target tissues including the lungs [65•], but further studies are needed to confirm this in MC-deficient models. Together, these studies indicate that respiratory viral pathogens evoke highly specific MC responses in the respiratory tract and that they can influence the long-term homeostasis of this tissue.

Summary and outlook
MCs are powerful regulatory cells of the immune, vascular and nervous systems while also serving a sentinel role for the immune system in the early detection of pathogens. The literature suggests that MCs detect and respond to multiple classes of viruses including both DNA and RNA viruses, and enveloped and non-enveloped representatives of each subtype. They achieve this breadth of response through the expression of many unique PRRs, specific receptors for pathogen products, and receptors for host danger signals that are present during infection. While usually protective, occasionally MC responses to viruses can lead to enhanced inflammation that is harmful, or to increased penetrance of virus into tissues where they would otherwise be excluded, such as the BBB. Understanding the kinetics of MC responses are central to their differing roles in early and late disease. While they are key for the early innate inflammatory response and cellular recruitment to peripheral sites of viral infections, aberrant, systemic or prolonged MC activation can lead to severe pathologies during viral infection, as MCs do in the context of other sterile inflammatory insults such as anaphylaxis or asthma. Potentially, therapeutic targeting of either MC degranulation or individual MC products could be used to prevent the specific pathologies associated with viral infections.

Author contributions
A.P.S.R and A.L.S contributed equally to all aspects of the article.

Conflict of interest statement
Nothing declared.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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