

IMMUNOPATHOGENESIS OF CORONAVIRUS INFECTIONS: IMPLICATIONS FOR SARS

Stanley Perlman^{*‡§} and Ajai A. Dandekar^{*}

Abstract | At the end of 2002, the first cases of severe acute respiratory syndrome (SARS) were reported, and in the following year, SARS resulted in considerable mortality and morbidity worldwide. SARS is caused by a novel species of coronavirus (SARS-CoV) and is the most severe coronavirus-mediated human disease that has been described so far. On the basis of similarities with other coronavirus infections, SARS might, in part, be immune mediated. As discussed in this Review, studies of animals that are infected with other coronaviruses indicate that excessive and sometimes dysregulated responses by macrophages and other pro-inflammatory cells might be particularly important in the pathogenesis of disease that is caused by infection with these viruses. It is hoped that lessons from such studies will help us to understand more about the pathogenesis of SARS in humans and to prevent or control outbreaks of SARS in the future.

Viral infection of mammals results in certain typical responses by the host immune system. These responses are initiated by the innate immune system, which recognizes ‘molecular patterns’ (such as double-stranded RNA) that are unique to pathogens. The adaptive immune system — which consists of T cells that can kill virus-infected cells and B cells that produce pathogen-specific antibodies — then proceeds to mount a response. Initiation of the adaptive and/or innate immune response results in the production of chemokines and other cytokines that induce a pro-inflammatory response and attract cells, such as neutrophils and macrophages, to sites of infection. These cells, in turn, might release cytotoxic substances, such as matrix metalloproteinases. Although these responses are crucial to clear the infection, all of these processes can cause damage to normal host tissues. Indeed, ‘side-effects’ of the immune response account for many of the signs and symptoms in human infections: for example, during infection with hepatitis B virus, hepatitis C

virus, measles virus or respiratory syncytial virus¹⁻³. Consequently, a ‘normal’ immune response often results in a transient disequilibrium of tissue homeostasis, and this is required for clearance of an infection but can contribute to disease. In this Review, we consider any immune response that results in an increase in clinical disease or tissue destruction to be immunopathological.

In many cases, immunopathogenesis is the outcome of immune dysregulation rather than of a normal response (TABLE 1). This could occur in one of three ways. First, viral infection might result in an intense inflammatory response that compromises physiological function or results in excessive destruction of host tissue. In this situation, viral infection might interfere with the normal feedback mechanisms that control inflammation, and pro-inflammatory chemokines or other cytokines might be produced in large amounts or for an excessive period. For example, induction of expression of the pro-inflammatory cytokine interleukin-6 (IL-6) is a

^{*}Interdisciplinary Program in Immunology, and Departments of Pediatrics[‡] and Microbiology[§], University of Iowa, Iowa City, Iowa 52242, USA.
Correspondence to S.P.
e-mail: stanley-perlman@uiowa.edu
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Table 1 | **Mechanisms of immunopathogenesis**

Mechanism	Description	Coronavirus example	References
Inflammatory storm	Excessive host response to pathogen occurs, resulting in either increased severity of localized disease or systemic disease; excessive response might be non-specific or induced by specific viral proteins; and manifestations might also occur as part of the 'normal' immune response required for viral clearance	MHV, FIPV, SARS-CoV	7,8,40,59
Bystander activation	T cells that are not specific for the pathogen or any host protein that is expressed at the site of inflammation are nevertheless activated (possibly by cytokines), resulting in increased tissue damage	MHV	74,111
Molecular mimicry	Pathogen and host share B- or T-cell epitopes, resulting in an autoimmune reaction in the host tissue that expresses the protein	None	–
Epitope spreading	Ongoing inflammation leads to presentation of self-epitopes, resulting in an autoimmune reaction in the host tissue that expresses the protein	MHV	69
Antibody-dependent enhancement	Antibodies specific for cell-surface glycoproteins increase virus uptake by macrophages, through cell-surface Fc receptors, resulting in disease enhancement	FIPV, possibly SARS-CoV	40,99,105

Fc receptor, receptor for immunoglobulin; FIPV, feline infectious peritonitis virus; MHV, murine hepatitis virus; SARS-CoV, severe-acute-respiratory-syndrome coronavirus.

consequence of activation of p38 mitogen-activated protein kinase (p38MAPK) by the murine coronavirus, murine hepatitis virus (MHV)⁴. Excessive production of pro-inflammatory mediators might then result in an unchecked influx of pro-inflammatory cells to the site of infection. Several of these types of cell, most notably neutrophils and macrophages, contribute to inflammation by producing toxic agents, such as reactive oxygen species, that kill both infected and normal cells at sites of infection, which would further exacerbate the response and result in immunopathological changes such as HAEMOPHAGOCYTOSIS⁵. Several of the released pro-inflammatory cytokines, such as tumour-necrosis factor (TNF), also induce apoptosis, which would result in increased tissue destruction. In addition, activated T cells that are not specific for the infecting virus or host antigens at the site of infection could traffic to sites of inflammation and contribute to tissue destruction, presumably through the production of chemokines or other cytokines. This has been shown for MHV-infected mice and is known as bystander activation (TABLE 1).

Second, direct infection of immune cells by a virus might cause increased or dysregulated production of immune mediators distinct from the aberrant production of chemokines and other cytokines previously discussed. For example, infection of mice with MHV strain 3 (MHV-3) results in production of the procoagulant prothrombinase by macrophages, leading to fulminant hepatitis and death⁶ (discussed in detail later).

Third, adaptive immune responses might become directed against host epitopes, and this would result in autoimmune reactions. Pathogen-specific antibodies or T cells might also recognize a host protein or epitope (through a process known as MOLECULAR MIMICRY). In other cases, prolonged infection and the ensuing tissue destruction might result in presentation

of host-protein-derived T- or B-cell epitopes that were previously cryptic (through a process known as EPILOPE SPREADING). The response to these epitopes might prolong the inflammatory response, with consequent tissue destruction, even after virus has been cleared. These autoimmune responses would be limited to a certain tissue or cell type by the specificity of the immune cells involved. Several such mechanisms of autoimmune immunopathogenesis have been described for models of both coronavirus infection and non-coronavirus infection; these are described in TABLE 1.

Antibodies might also contribute to immunopathogenesis. With regard to coronaviruses, virus-specific antibody increases the uptake of several viruses by macrophages — including the feline coronavirus feline infectious peritonitis virus (FIPV) — resulting in activation of these macrophages and secretion of chemokines and other cytokines.

In several animal models of coronavirus infection, the immune system contributes considerably to the disease process — indeed, the pathology that is seen in several models is wholly, or at least partly, immune mediated. In this Review, we discuss the role of the immune system in the pathology that is seen in animals with coronavirus infections as a window onto the pathological processes that occur in humans infected with the severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV). We focus on murine and feline coronavirus infections, because an immunopathological role in disease has been most clearly documented in these settings.

SARS: a severe human coronavirus infection

In the winter of 2002–2003, SARS emerged in China and subsequently spread throughout the world. In the nine months between November 2002 and July 2003, 8,437 cases of this new disease, resulting

HAEMOPHAGOCYTOSIS
The phagocytosis of erythrocytes that results from excessive activation of macrophages. This is usually a consequence of uncontrolled activation and proliferation of T cells.

MOLECULAR MIMICRY
A mechanism for the induction of autoimmunity in which a pathogen expresses a protein or peptide that is similar to a self-protein. After the induction of a pathogen-specific immune response, a crossreactive response to self results in autoimmune pathology.

EPILOPE SPREADING
The *de novo* activation of autoreactive T cells by self-antigens that have been released after virus-specific T- or B-cell-mediated bystander damage.

ENZOOTIC VIRUS

A virus that infects animals and is endemic to a geographical locale, with only minimal changes in its incidence over time.

in 813 deaths, were reported to the World Health Organization by 29 countries. The aetiological agent of this disease, a coronavirus, was identified by several research groups in April 2003, and the virus has been named SARS-CoV after the syndrome it causes^{7,8}.

Coronaviruses are the largest of all of the RNA viruses, and they have a positive-sense, single-stranded RNA genome of 30–32 kilobases⁹ (FIG. 1). As a family, the Coronaviridae have a broad host range and cause a wide variety of gastrointestinal, respiratory and systemic diseases in animals, including infectious bronchitis in birds, a fatal disease with multi-organ involvement in felines, and enteritis in pigs, cows, turkeys and dogs. In humans, coronaviruses cause respiratory disease and, to a lesser extent, gastroenteritis. The viruses human coronavirus OC43 (HCoV-OC43) and HCoV-229E have long been known to be causative agents of the common cold, and the more recently identified agents HCoV-HKU1 and HCoV-NL63 cause more severe, although rarely fatal, infections of the upper and lower respiratory tract^{10–12}. SARS-CoV, which causes a much more severe respiratory disease, seems to be an ENZOOTIC VIRUS in Southeast Asia. Several species that might be infected, such as masked palm civets (*Paguma larvata*), are consumed as food in parts of China, and the ‘wet markets’, at which live animals are bought and sold, are likely venues for the initial crossover event to humans⁸. The 2002–2003 outbreak of SARS

in humans probably resulted from an interspecific transfer of the virus by aerosols from live, exotic animals that were infected with SARS-CoV to workers in these wet markets. Sera from masked palm civets, raccoon dogs (*Nyctereutes procyonoides*) and Chinese ferret-badgers (*Melogale moschata*) were shown to contain neutralizing antibodies that were specific for SARS-CoV, and virus that was nearly identical to the strains that were isolated from infected humans was detected in masked palm civets^{13–15}. However, a specific animal reservoir for the virus has yet to be definitively identified.

Infection of humans with SARS-CoV typically causes an influenza-like syndrome of malaise, rigors, fatigue and high fevers. In two-thirds of infected patients, the disease progresses to an atypical pneumonia, with shortness of breath and poor oxygen exchange in the alveoli. Many of these patients also develop watery diarrhoea with active virus shedding, which might increase the transmissibility of the virus. Respiratory insufficiency leading to respiratory failure is the most common cause of death among those infected with SARS-CoV⁸. Consistent with these clinical observations, the host cell-surface receptor for SARS-CoV, angiotensin-converting enzyme 2 (ACE2), is detected in the lungs and gastrointestinal tract^{16,17}. Because it binds ACE2, SARS-CoV might contribute to lung-tissue injury by a novel mechanism. ACE2 has a protective role during acute lung injury. By binding ACE2, SARS-CoV leads to the downregulation of ACE2 expression and might therefore negate the protective effect of ACE2. This mechanism of injury was shown in a mouse model using SARS-CoV spike glycoprotein, through which the virus binds ACE2, but it has not yet been confirmed in humans or animals that are infected with SARS-CoV^{18,19}. Severe cases of SARS are associated with lymphopaenia, neutrophilia, mild thrombocytopenia and coagulation defects²⁰. Haemophagocytosis, which is indicative of cytokine dysregulation, is also detected in some patients with severe disease^{21,22}.

Damage to the lungs of patients who are infected with SARS-CoV seems to occur directly, by viral destruction of alveolar and bronchial epithelial cells and macrophages, as well as indirectly, through production of immune mediators, although the exact role of these direct and indirect mechanisms remains controversial. Viral load, as determined from titres in nasopharyngeal aspirate, diminishes 10–15 days after the onset of symptoms, even though clinical disease and alveolar damage worsen, indicating that the host immune response is responsible for some of the pathology in SARS-CoV-infected patients^{23,24}. However, nasopharyngeal viral titres do not necessarily reflect viral loads in the lungs, and high concentrations of virus have been detected in several organs at autopsy, including the lungs, intestine, kidneys and brain^{25–27}.

Infection of macrophages and lymphocytes is likely to be a key component in SARS-CoV-induced pathogenesis. SARS-CoV directly infects T cells, contributing

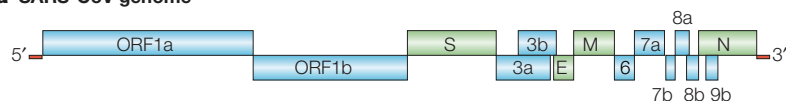
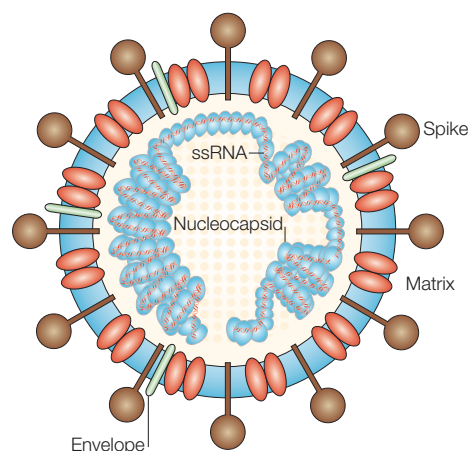
a SARS-CoV genome**b SARS-CoV virion**

Figure 1 | The severe-acute-respiratory-syndrome coronavirus genome and virion.

a | The severe-acute-respiratory-syndrome coronavirus (SARS-CoV) genome consists of 28 putative open reading frames (ORFs) in 9 mRNA transcripts. ORF1a and ORF1b, which account for about two-thirds of the genome, both encode large polyproteins. ORF1b protein is produced by a –1-base-pair ribosomal frameshift from the reading frame of ORF1a. The SARS-CoV genome encodes four structural proteins: spike (S), envelope (E), matrix (M) and nucleocapsid (N). In non-human isolates, transcription of ORF8a and ORF8b produces a single protein. **b** | A schematic representation of a SARS-CoV virion is shown. ssRNA, single-stranded RNA.

to lymphopaenia and to atrophy of the spleen and lymphoid tissue²⁵. The murine coronavirus MHV-3 also infects and destroys lymphocytes, thereby facilitating viral replication and persistence²⁸. SARS-CoV-infected lymphocytes, similar to FIPV-infected macrophages in domestic cats, might transport the virus to distant organs, resulting in systemic infection²⁵. Macrophages, both infected and uninfected, are detected in large numbers in the lungs of patients who died as a result of SARS^{22,25,29}. Although infected macrophages have been found *in vivo*, SARS-CoV causes an **ABORTIVE INFECTION** of these cells *in vitro*^{30–32}. SARS-CoV also interferes with the initiation of the innate immune response by inhibiting the expression of type I interferons (IFNs) by infected cells, including human monocyte-derived dendritic cells (DCs) and macrophages. IFN production requires the phosphorylation and dimerization of a constitutively expressed protein, IFN-regulatory factor 3 (**IRF3**). IRF3 is not activated, at least *in vitro*, after infection with SARS-CoV^{30–34}. By contrast, expression of chemokines such as CXC-chemokine ligand 10 (**CXCL10**), CC-chemokine ligand 2 (**CCL2**), CCL3, CCL5 and CCL8 is upregulated by abortively infected DCs and macrophages and might contribute to the influx of monocytes and/or macrophages that is observed in infected tissues^{30,34}; CXCL10 and CCL2 expression are also upregulated in the blood of patients with SARS³⁵. Expression of **CXCL8** (also known as IL-8), which is an attractant for neutrophils, is also upregulated in the serum of patients with SARS^{35–37}. Consistent with a role for CXCL8 in pathogenesis, severe disease is associated with an increase in the number of neutrophils in the blood^{20,38}.

Although these studies indicate that upregulation of expression of pro-inflammatory molecules contributes to the pathogenesis of SARS, increased serum concentrations of two anti-inflammatory molecules — transforming growth factor- β and prostaglandin E₂ — were detected in another study³⁹. Increased concentrations of these anti-inflammatory molecules might impair clearance of virus. The mechanism by which chemokines and other cytokines are regulated in infected patients is not known, but SARS-CoV induces the activation of p38MAPK in monocytes³⁹. The murine coronavirus, MHV, also induces p38MAPK activation, and inhibition of p38MAPK activation results in decreased production of infectious MHV⁴. Induction of p38MAPK expression by MHV results in production of IL-6 and phosphorylation of eukaryotic translation-initiation factor 4E (EIF4E), which increases cap-dependent (including MHV-specific) protein production. By analogy, it is possible that increased cytokine production by the monocytes of patients with SARS might be an untoward consequence of a mechanism that is used by the virus to increase replication in some cells⁴.

Together, these findings are consistent with models in which the immune system contributes to the SARS disease process. Understanding the immunopathogenesis of SARS could provide new insights into effective treatments for this illness.

Feline coronavirus infections

Macrophage infection and dysfunction. Feline enteric coronaviruses (FECVs) generally cause mild or asymptomatic infections, mainly of domestic cats, although cases have been reported in wild feline populations⁴⁰. Domestic cats often become persistently infected with FECV. Occasionally, these animals develop the uniformly fatal disease infectious peritonitis, which is caused by a macrophage-tropic variant of FECV that is known as FIPV. Indeed, virulent strains of FIPV replicate more efficiently in feline peritoneal macrophages *in vitro* than do avirulent strains of FECV⁴¹. However, most strains of FIPV are antigenically identical to their avirulent FECV counterparts, and the genetic changes that are responsible for the gain in virulence are not well understood.

In an elegant longitudinal study, de Groot and colleagues⁴² showed that domestic cats that were experimentally infected with FIPV developed a multiphasic disease. Initially, all animals developed fever, weight loss and lymphopaenia but could contain the infection. Total lymphocyte counts recovered with time; however, in most animals, the infection relapsed, as was shown by an increase in viral load. These increased viral burdens resulted in repeated bouts of disease, which again coincided with fever, weight loss and lymphopaenia. FIPV-infected felines develop histological evidence of **SEROSITIS** and **PYOGRANULOMATOUS VASCULITIS**. In the more common ‘wet’ form of FIP (also known as the effusive form), yellow **ASCITIC FLUID** gradually accumulates as the disease progresses; there is also a ‘dry’ form of the disease, which does not involve the accumulation of ascitic fluid. Antigen–antibody-complex formation and complement activation occur in the late stages of disease and might contribute to the production of ascitic fluid in the wet form of disease⁴³.

The clinical signs and lymphocyte depletion are postulated to be a direct consequence of the infection of macrophages and DCs by FIPV. In support of this idea, both macrophages and DCs express CD13 (also known as aminopeptidase N), the receptor for FIPV, and infection of macrophages by FIPV has been shown *in vitro*^{41,44} (FIG. 2a,b). Infected macrophages traffic throughout the body, resulting in a disseminated infection. In lymph nodes, infection of macrophages and DCs might alter the interaction of these cells with T cells so that they dampen, rather than reinforce, the FIPV-specific T-cell response. This might occur through induction of expression of IL-10 (probably produced by macrophages), which is present in increased amounts in infected lymph nodes⁴⁵. The production of IL-10 might skew the immune response away from a protective T helper 1 (T_H1)-cell response towards a non-protective T_H2-cell response, thereby diminishing the ability of immune cells to clear the virus. Regarding the lymphopaenia, lymphocyte apoptosis is commonly observed in infected lymphoid tissues, although lymphocytes are not themselves infected by the virus^{42,46,47}. So, lymphoid-cell depletion is probably mediated by a soluble factor. In support of this idea,

ABORTIVE INFECTION

An infection in which viral replication is initiated but no infectious virus is produced.

SEROSITIS

Inflammation of the membranes that line the lungs (the pleura), the heart (the pericardium), and the abdomen (the peritoneum) and the organs within.

PYOGRANULOMATOUS VASCULITIS

A type of vasculitis (that is, inflammation of the blood vessels) that is associated with a chronic inflammatory process in which neutrophils are mixed with components of granulomas.

ASCITIC FLUID

Serous fluid that accumulates in the abdominal cavity. It might result from serositis.

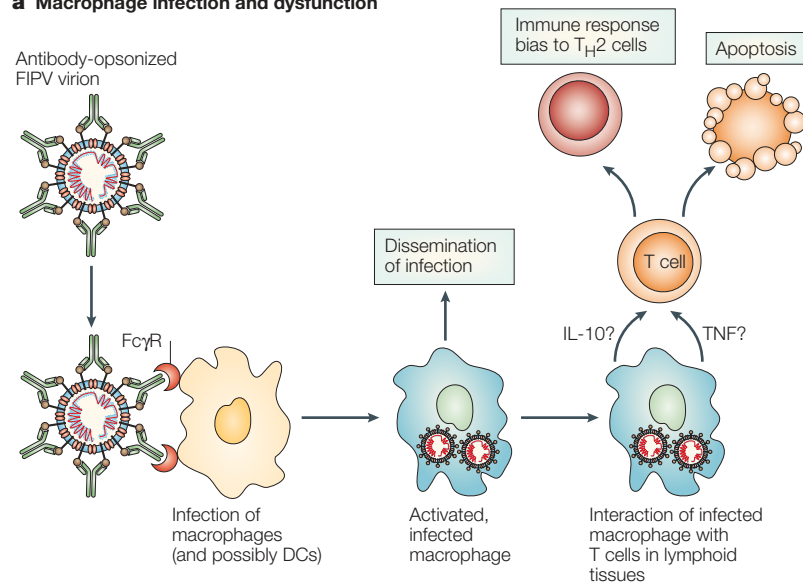
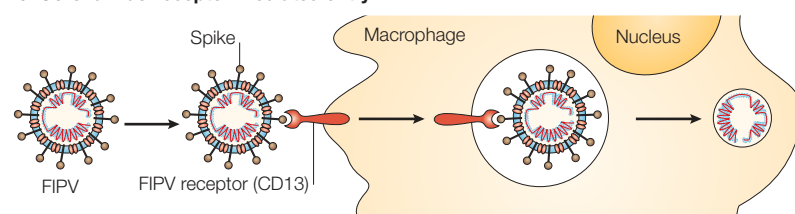
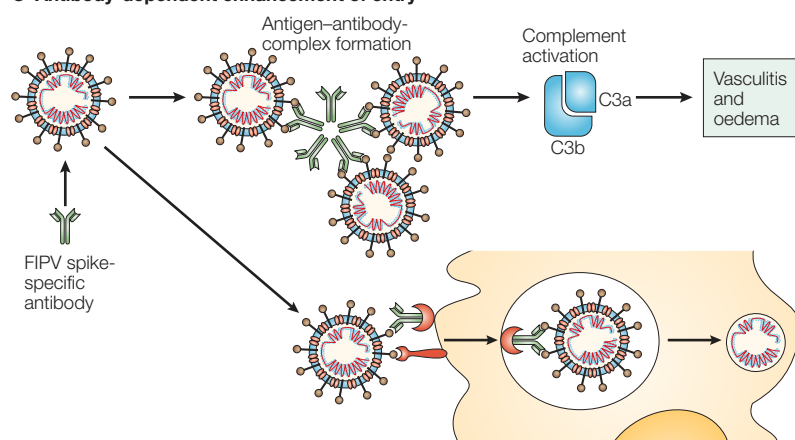
a Macrophage infection and dysfunction**b Coronavirus-receptor-mediated entry****c Antibody-dependent enhancement of entry**

Figure 2 | Macrophage infection and antibody-dependent enhancement of virus entry in infection with feline infectious peritonitis virus. **a** | Infection of macrophages and possibly dendritic cells (DCs) results in both dissemination of feline infectious peritonitis virus (FIPV) infection and dysregulation of these cells, leading to lymphocyte apoptosis. **b** | FIPV usually infects cells through the binding of the spike protein to its cellular receptor, CD13. The virus is then internalized and released into the cytoplasm. **c** | In antibody-dependent entry, specific antibodies bind the spike protein. The antibody-opsonized FIPV virions then interact with Fc γ Rs (receptors for IgG). Some evidence indicates that this process augments the normal spike-CD13 interaction. After binding of the opsonized virions to Fc γ Rs, the virus is internalized and released into the cytoplasm. Antigen-antibody complexes are also deposited in the vasculature, resulting in complement activation. Activation of complement contributes to the development of vasculitis and oedema, with death of the animal occurring soon after. C3, complement component 3; IL-10, interleukin-10; T_H2 cell, T-helper 2 cell; TNF, tumour-necrosis factor.

conditioned media from peritoneal or splenic cells that were isolated from infected domestic cats and cultured *in vitro* induced the apoptosis of T cells⁴⁶. The specific factor that is responsible for apoptosis has not yet been identified, although TNF, a factor that is implicated in lymphocyte apoptosis, is detected at increased levels in domestic cats with FIPV^{48,49}. By contrast, domestic cats that are exposed to FIPV but do not develop disease show lymphoid hyperplasia, which is consistent with the development of a protective T-cell response⁵⁰.

Antibody-dependent enhancement of FIPV infection. As disease progresses, FIPV-infected macrophages are deposited in the endothelium of small blood vessels in organs such as the liver, spleen and kidneys, and GRANULOMAS subsequently form at these sites. Granulomas consist of monocytes and/or macrophages, B cells and CD4⁺ T cells, with only small amounts of virus detected^{51,52}. These pyogranulomatous lesions are associated with severe damage to the endothelium and are responsible for many of the manifestations of disease, such as liver and renal disease, in infected domestic cats.

Another manifestation of FIP is B-cell hyperplasia with associated hypergammaglobulinaemia. Although the aetiology of B-cell hyperplasia is not known, it most probably results from aberrant cytokine production by infected macrophages. Neutralizing antibodies that are raised during FIPV infection and other coronavirus infections are mainly directed against spike protein. In the case of FIPV, however, the presence of these antibodies does not provide sterilizing immunity. Instead, these antibodies opsonize virus particles and facilitate their entry to monocytes and/or macrophages through Fc γ receptors (receptors for IgG)⁴⁰ (FIG. 2a,c). Furthermore, antigen-antibody complexes are deposited in the blood-vessel walls. These complexes activate complement, leading to vasculitis and oedema, and might thereby contribute to the development of the wet form of FIPV⁴⁰. Antigen-antibody-complex formation and its consequences occur late in the course of disease and are predictive of a poor outcome⁵³. Indeed, the humoral response that develops in FECV-immune domestic cats does not protect animals against FIPV infection and might contribute to a particularly fulminant disease known as early death syndrome. This 'enhanced' form of disease has not been documented for animals that are naturally infected but has been shown for domestic cats that are passively or actively immunized against FIPV. Administration of spike-protein-specific antibodies to uninfected domestic cats or active immunization of domestic cats with recombinant vaccinia virus that expresses spike protein results in an accelerated disease course after infection with FIPV^{54,55}. This syndrome does not develop in FECV-infected domestic cats, possibly reflecting the ability of FIPV to replicate more efficiently in macrophages. This accelerated pathogenesis of FIPV in immunized animals provides strong support for the idea that the immune response is an important contributing factor to disease.

GRANULOMA

A collection of modified macrophages that resemble epithelial cells. This is usually surrounded by a layer of lymphocytes that often includes multinucleated giant cells. Granuloma formation is a chronic inflammatory response that is initiated by various infectious and non-infectious agents.

Murine coronavirus infections

Immune-mediated demyelination: the result of an excessive immune response? Several strains of MHV cause acute and chronic neurological diseases in susceptible mice and rats. The JHM and A59 strains of MHV cause demyelination; this infection has been intensively studied because it is a useful model of the human disease multiple sclerosis^{56–58}. In early studies, demyelination was thought to result from virus-mediated lysis of infected cells^{59,60}. However, it is now clear that myelin destruction is largely immune mediated (FIG. 3). Accordingly, mice that receive sub-lethal doses of irradiation or are congenitally immunodeficient (such as mice with severe combined immunodeficiency (SCID) or mice that are deficient in recombination-activating gene 1 (*Rag1*) activity) do not develop demyelination after infection with MHV-JHM^{61–64}. Both *Rag1*^{-/-} and SCID mice lack T and B cells but have normal numbers of macrophages and natural killer cells.

In one model of infection, an attenuated variant of MHV-JHM (MHV-JHM 2.2-V-1) with a tropism for oligodendrocytes is used to inoculate susceptible mice (usually C57BL/6 or BALB/c mice)^{61,65–68}. These mice develop signs of demyelination, including hind-limb paralysis and gait disturbances, by 7 days after infection. The virus is cleared by 12–14 days after infection, but the neurological deficits persist. MHV-JHM infection of the central nervous system (CNS) results in

a large infiltration of B cells and CD4⁺ and CD8⁺ T cells. MHV-JHM-specific CD8⁺ T cells are required for initial clearance of virus, which occurs through perforin- and IFN- γ -dependent pathways, whereas MHV-JHM-specific antibody is required to prevent reactivation of virus^{61,65–67}. However, the influx of these T cells also seems to set up a destructive cascade of cytokine production that results in demyelination. These T cells are mainly directed against MHV-JHM antigens, although in one report, the authors identified myelin-specific CD4⁺ T cells in the CNS of the infected rat (a possible example of epitope spreading)⁶⁹.

Histological evidence of demyelination is present in the spinal cords of MHV-JHM-infected mice, beginning at ~5 days after infection, with maximal myelin destruction occurring at 14–21 days after infection. Most tissue destruction occurs when infectious virus has been completely or partially cleared^{70,71}. Viral antigens are not detected in areas of demyelination, whereas they are found in adjacent white matter that appears to be normal, indicating that myelin destruction is a direct consequence of clearance of virus⁷². Large influxes of activated macrophages and microglia are found in these areas of demyelination⁷⁰, indicating that these cells have a crucial role in the pathological process.

Both CD4⁺ and CD8⁺ T cells can mediate disease^{64,72}. Mice that have defective MHC class I or class II expression and therefore lack CD8⁺ or CD4⁺ T cells, respectively, develop demyelination when infected with MHV-JHM or MHV-A59 (REFS 64,73). Other studies have used adoptive transfer of MHV-JHM-specific immune cells to infected *Rag1*^{-/-} mice; in the absence of transferred cells, these mice do not develop demyelination after infection with MHV-JHM, but transfer of splenocytes results in robust demyelination. Infected *Rag1*^{-/-} mice that are reconstituted with either CD4⁺ or CD8⁺ T cells from a mouse that is immune to infection with MHV-JHM also develop demyelination. Demyelination can also be induced by CD8⁺ T cells that do not recognize viral or CNS antigens, if these T cells are sufficiently activated⁷⁴ (a process that is known as bystander activation) (TABLE 1). Accordingly, MHV-JHM-infected *Rag1*^{-/-} mice that expressed a single T-cell receptor specific for an epitope encoded by another virus (lymphocytic choriomeningitis virus) developed demyelination, but this only occurred when the transgenic T cells were activated by cognate antigen.

Although the antiviral T-cell response is crucial for MHV-JHM- or MHV-A59-induced demyelination in normal mice, MHV-JHM-specific antibodies (in the absence of any transferred T cells) can mediate demyelination in infected *Rag1*^{-/-} mice⁷⁵. Virus-specific T cells and antibodies activate macrophages and/or microglia, resulting in their migration into the white matter of the CNS and, subsequently, in demyelination. The activation and migration of macrophages and microglia might be the most crucial process for demyelination, because virus-encoded expression of a single macrophage attractant, CCL2, is sufficient to induce demyelination in MHV-JHM-infected *Rag1*^{-/-} mice in the absence of transferred T cells or antibody⁷⁶.

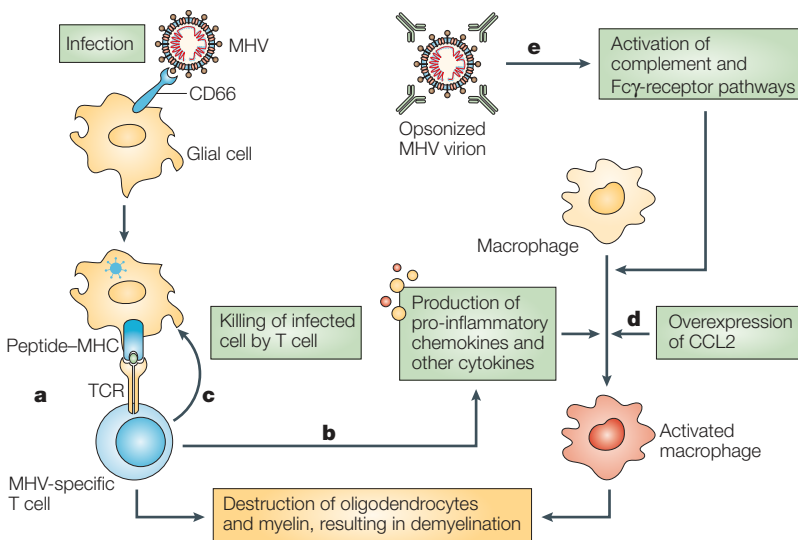


Figure 3 | Mechanisms of immune-mediated demyelination in infection with murine hepatitis virus. In immunocompetent mice, infection of glial cells (that is, astrocytes, microglia and oligodendrocytes) results in migration of T cells into the central nervous system (a). Myelin destruction is mediated by CD4⁺ and CD8⁺ T cells, and these cells activate macrophages by the production of cytokines (b) or kill infected cells directly (c), both of which result in demyelination. In recombination-activating gene 1 (*Rag1*)^{-/-} mice, which lack T and B cells, two additional mechanisms of demyelination have been elucidated. *Rag1*^{-/-} mice do not develop demyelination when infected with the JHM strain of murine hepatitis virus (MHV-JHM); as occurs in immunocompetent mice, demyelination develops after the adoptive transfer of MHV-JHM-specific T cells. However, demyelination also results if infected *Rag1*^{-/-} mice are infected with a recombinant MHV-JHM expressing the macrophage attractant CC-chemokine ligand 2 (CCL2) (d), presumably by direct activation of macrophages. Similarly, exogenous delivery of neutralizing MHV-JHM-specific antibody (e) results in macrophage activation and demyelination; this process depends on activation through complement and activating Fc γ receptors (receptors for IgG). TCR, T-cell receptor.

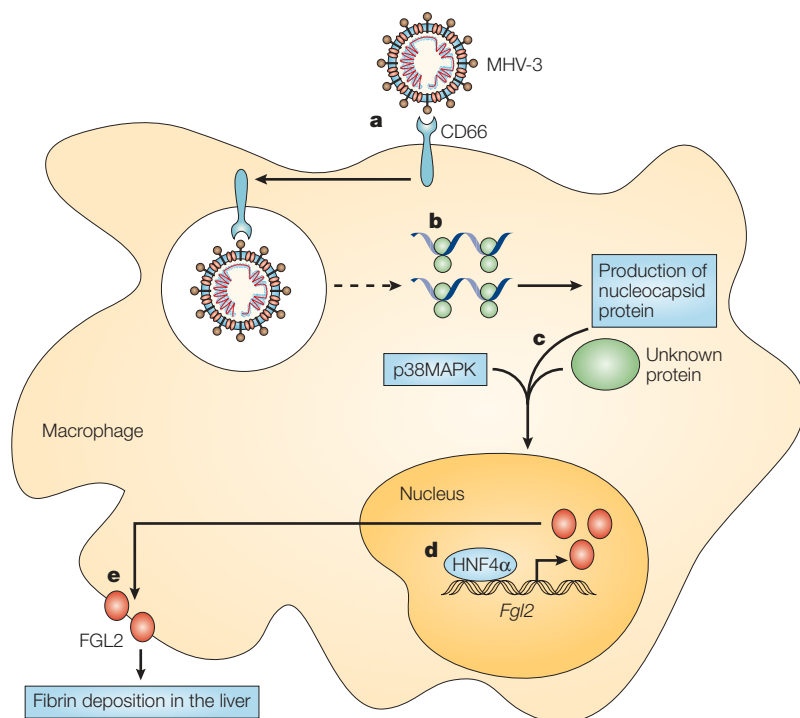


Figure 4 | Infection with murine hepatitis virus strain 3 results in upregulation of expression of fibrinogen-like protein 2. Murine hepatitis virus strain 3 (MHV-3) becomes internalized after binding macrophages through its receptor, CD66 (a). Subsequent to internalization, virions are uncoated and begin to replicate. As part of the replication process (b), the nucleocapsid protein is synthesized. Subsequently, a signalling pathway involving p38 mitogen-activated protein kinase (p38MAPK) activation, as well as the nucleocapsid protein and other unknown host factors, is initiated (c), resulting, ultimately, in binding of the transcription factor hepatocyte nuclear factor 4 α (HNF4 α) to the gene that encodes fibrinogen-like protein 2 (FGL2) (d), which is a prothrombinase. The FGL2 protein that is produced then translocates to the cell surface (e), where it induces fibrin deposition and, consequently, acute liver necrosis.

Soluble factors also have a key role in MHV-JHM-induced demyelination. The cytokine IFN- γ is directly involved in clearance of virus from oligodendrocytes⁶⁷ and is required for demyelination induced by CD8⁺ T cells but not by CD4⁺ T cells^{61,72,77,78}. Several studies have shown that certain chemokines contribute to maximal viral clearance and demyelination (but none has been shown to be required for either). Chemokines such as CCL4, CCL5, CXCL9 and CXCL10 are crucial for lymphocyte and macrophage infiltration into the MHV-JHM-infected CNS, and they positively reinforce the milieu in which demyelination takes place^{79–82}.

Collectively, these data indicate that myelin destruction is immune mediated. Virus-specific T cells mediate myelin destruction in immunologically intact mice, but in some circumstances, demyelination clearly occurs in their absence. A common feature in all forms of demyelination is the activation and migration of macrophages and/or microglia into the white matter, and this process is sufficient, in the absence of T or B cells, for myelin destruction in MHV-JHM-infected mice. As occurs in FIPV-infected domestic cats, macrophages and microglia can be infected by MHV-JHM^{71,83,84}, but there are no data indicating that direct infection of

macrophages is a cause of the immune dysregulation that is observed in these animals. Instead, a generalized, excessive, but perhaps appropriate, response by both infected and uninfected macrophages seems to be crucial for demyelination.

MHV-3-mediated lethal hepatitis: induction of expression of a novel prothrombinase. MHV-3 causes various diseases, with the outcome dependent on the strain, age and immune status of the mouse host⁸⁵. Semi-susceptible strains, such as C3H mice and F₁ crosses of resistant and susceptible strains, become persistently infected, which manifests mainly as neurological disease: ependymitis, encephalitis and hydrocephalus are hallmarks of this disease⁵⁹. MHV-3-infected C3H mice also develop a chronic thrombotic vasculitis, with viral antigen detected in endothelia. The pathogenesis of this disease is not well understood, but it is likely to be immune mediated. MHV-3, unlike the neurotropic MHV-JHM and MHV-A59 strains, infects T and B cells. This infection is largely non-productive but results in lymphocyte death, perhaps by apoptosis, with consequent cellular and humoral immunosuppression. This immunosuppression is an important contributory factor to the persistence of MHV-3 (REF. 28).

Infection of susceptible strains of mice with MHV-3 results in an acute hepatitis, with death occurring a few days after inoculation. The receptor for MHV-3, CD66 (also known as CEACAM1), is expressed by macrophages⁸⁶, and infection of these cells has a central role in the pathogenesis of the liver failure that is seen in these animals. Macrophages from susceptible mouse strains that are infected with MHV-3 upregulate the production of several pro-inflammatory molecules both *in vitro* and *in vivo*, including a transmembrane pro-coagulant molecule, fibrinogen-like protein 2 (FGL2; also known as fibroleukin)^{6,87–89} (FIG. 4). Expression of FGL2 results in cleavage of prothrombin to thrombin, which initiates the coagulation cascade that begins with fibrin deposition. This deposition of fibrin throughout the hepatic sinusoids and venous system results in inadequate perfusion or lack of perfusion to the liver and accelerates the necrosis that is caused by the direct cytotoxicity of MHV-3 to hepatocytes.

This upregulation of expression of the prothrombinase FGL2 occurs only in macrophages, monocytes and, to a lesser extent, endothelial cells in the liver of susceptible strains of mice, even though MHV-3 can also replicate in the liver-resident macrophages of resistant strains of mice. Upregulation of FGL2 expression by macrophages and monocytes correlates with the severity of liver disease considerably better than do viral titres. Expression of FGL2 depends on activation of p38MAPK, expression of the MHV-3 nucleocapsid protein, and binding of the transcription factor hepatocyte nuclear factor 4 α (HNF4 α), which is constitutively expressed by macrophages, to the *Fgl2* promoter^{90,91}. How the nucleocapsid protein induces HNF4 α binding to the promoter of *Fgl2* is unknown; similarly, the factors that abrogate upregulation of FGL2 expression in resistant strains of mice are also unknown.

Table 2 | **Does SARS have an immunopathological component?**

Criterion	Evidence in SARS-CoV infection	Precedent in other coronavirus infections?
Worse disease with decrease in viral load	Controversial; viral titres, measured in nasopharyngeal-aspirate samples, decrease as clinical disease worsens ^{24,36} ; but high viral loads have been detected in lungs and immune cells after death ²⁵⁻²⁷	MHV-induced demyelination increases as virus is cleared ⁷¹ ; MHV-3-induced hepatitis correlates with macrophage activation and not viral load ⁸⁷ ; and IBV-induced nephritis is detected in chickens with very low viral loads ⁹²
Macrophage or DC infection	Infection is abortive but induces expression of pro-inflammatory mediators ^{30,32,34}	MHV and FIPV productively infect macrophages ^{40,59}
Macrophage infiltration into sites of inflammation	Macrophages are present in large numbers in infected lungs ^{22,29}	In MHV infection, macrophages infiltrate the CNS coincident with demyelination (thought to be the final effector cell) ^{62,76} ; and in FIPV infection, macrophages are the main cell type in granulomas and are crucial for pathogenesis ^{40,43,52}
High concentration of pro-inflammatory mediators in serum or at site of infection	Controversial; anti-inflammatory mediators might contribute to delayed viral clearance ^{7,39}	MHV-3-induced FGL2 expression is crucial for liver necrosis; in MHV-JHM-infected mice, IFN- γ is required for CD8 ⁺ T-cell-mediated responses ^{61,77,89} ; and in FIPV infection, increased cytokine concentrations are present in blood and tissues during exacerbation of disease ^{49,112,113}
Inhibition of type I IFN induction in infected cells	Shown using isolated macrophages, DCs and fibroblasts ^{30,32-34}	MHV does not induce type I IFN expression ^{114,115}
Lymphopaenia and neutrophilia	Present in most severe cases ^{8,38} ; and lymphocytic infection has been detected ²⁵	In FIPV infection, lymphopaenia is present during clinical relapses ⁴² ; and in MHV-3 infection, lymphopaenia is present and lymphocytic infection has been detected ²⁸
Haemophagocytosis	Present in severe cases ^{21,22}	Not reported

CNS, central nervous system; DC, dendritic cell; FGL2, fibrinogen-like protein 2; FIPV, feline infectious peritonitis virus; IBV, avian infectious bronchitis virus; IFN, interferon; MHV, murine hepatitis virus; MHV-JHM, MHV strain JHM; SARS, severe acute respiratory syndrome; SARS-CoV, SARS coronavirus.

Avian coronavirus infections

Role of robust innate immune response in acute respiratory disease. Avian infectious bronchitis virus (IBV) causes marked respiratory disease, especially in young chickens^{92,93}. Similar to SARS-CoV, IBV also infects organs other than the respiratory tract. IBV replicates in the gastrointestinal tract, but infection of the gut does not usually result in clinically evident disease. IBV also infects the kidneys, and some strains of virus cause severe nephritis, which results in a high rate of mortality^{92,93}. At present, there is no evidence that IBV infects macrophages. It is clear that virus-induced cytolytic destruction accounts for many of the pathological changes that are observed in this infection. However, there are indications that an immunopathogenic component contributes to IBV-induced disease.

Much of the respiratory disease that is observed in young chickens with severe clinical signs is a result of mucosal thickening and excessive production of a thick mucus in the airways, which asphyxiates the infected host⁹². Although it has not been proven for IBV-infected chickens, it is probable that this excessive production of mucus is mediated by several cytokines, such as IL-1 β , IL-6 and CXCL8, which are secreted by infected epithelial cells in other respiratory-virus infections⁹⁴. CXCL8 is an attractant for neutrophils, which are one of the main cellular components of the nasal exudates that are found in infected chickens. Neutrophil depletion by

5-fluorouracil has been shown to reduce the thickness of the nasal exudate, thereby diminishing epithelial-cell damage and cilia loss⁹⁵. Similarly, the early stages of renal disease are characterized by a massive infiltration of neutrophils⁹⁶. Collectively, these observations indicate that the cellular innate immune response to the virus is an important factor in the development of severe disease.

Chronic lymphocytic nephritis. Under certain conditions (depending on age at time of inoculation, and strain of IBV and of chicken), IBV causes a persistent infection, with an interstitial lymphocytic nephritis in the kidneys^{92,93,97}. Similar to the MHV-infected CNS, lymphocytic infiltration and ongoing renal damage have been shown when viral loads are low or undetectable⁹⁷. The precise roles of persistent virus and the host immune response in IBV-induced renal disease are not known, but by analogy with other coronavirus infections, it is probable that the chronic nephritis that is observed in these animals is partially immune mediated.

An immunopathogenic component in SARS?

Several features of SARS indicate, but do not prove, that the host immune response contributes to disease; these have been described in detail in previous sections and are summarized in TABLE 2. However, as also outlined in this Review, the pathogenesis of

several animal coronaviruses includes an immune-mediated component. It is therefore reasonable to suggest that similar mechanisms occur in SARS. Clearly, it is crucial to determine the extent to which the pathological changes that are observed in SARS result from direct destruction by virus compared with immune-mediated elimination of infected cells. In FIPV-infected domestic cats, increased viral replication initiates a cascade of events that leads to injury to the immune system, as well as to several organs^{42,43}, whereas in mice with MHV-mediated demyelination, clinical disease and myelin destruction increase as virus is cleared^{70,71,76}. In patients with SARS, virus is detected in the lungs and in immune cells at the time of death, indicating that virus directly causes pulmonary and immune-system injury^{25–27}. However, the kinetics of viral clearance from sites of infection in individual patients need to be established before the role of the host immune response in the disease process can be fully evaluated.

Several features that are common to animals infected with FIPV or MHV and to patients infected with SARS-CoV are consistent with immunopathological disease. These include the propensity of virus to infect macrophages and DCs, and the presence of increased, and perhaps pathological, systemic concentrations of chemokines and other cytokines. In animals infected with MHV or FIPV, activated macrophages are present at sites of inflammation and participate in tissue destruction^{40,62,70}. Activated macrophages are also present in the lungs of SARS-CoV-infected individuals. SARS-CoV-infected macrophages and DCs express increased amounts of pro-inflammatory cytokines^{30,32,34}. Consistent with this, increased concentrations of pro-inflammatory chemokines and other cytokines are present in most infected patients^{35–37,39}, and by analogy with other coronavirus infections, as well with ARDS (adult respiratory distress syndrome)⁹⁸, expression of these pro-inflammatory mediators might contribute to disease.

Another immunopathological mechanism, antibody-dependent enhancement of disease, is observed in immunized domestic cats after challenge with FIPV⁵⁵, and it occurs when tissue-culture cells are exposed to recombinant viral vectors that are coated with the SARS-CoV spike protein⁹⁹. However, this phenomenon has not been shown in most immunization studies⁷, and it needs to be confirmed using infectious SARS-CoV.

Future directions

Fortunately, the world has not witnessed a re-emergence of SARS since 2003. To be prepared for any outbreak that might occur in the future, it is crucial to understand the pathogenesis of this disease. *In vitro* studies will be useful for investigating how SARS-CoV modifies gene expression in primary target cells, such as macrophages, DCs, lymphocytes and pulmonary epithelial cells. However, many of the outstanding issues that have been discussed in this Review will be answered only in the context of SARS-CoV-infected animals or humans. Particularly in the absence of any resurgence of disease in humans, it will be most important to develop an animal model that accurately reproduces the human infection.

Current animal models of coronavirus infection are useful for testing vaccines and antiviral drugs, but they do not reproduce the pulmonary or immune-system disease that is observed in individuals with SARS⁷. Although SARS-CoV replicates in the lungs of mice, hamsters and domestic cats, these animals remain asymptomatic^{100–103}. Initial reports indicated that cynomolgus macaques (*Macaca fascicularis*) and ferrets develop clinically evident respiratory disease and would therefore be useful animal models for studying SARS; however, these results have not been reproducible^{101,104–108}. Only an animal model will allow investigators to determine the relationship of viral load to disease outcome, as well as to evaluate fully the role of infection and dysfunction of macrophages and lymphocytes in the disease process. In the case of another human coronavirus, HCoV-229E, development of a mouse model of infection required transgenic expression of the human host-cell receptor (CD13), disruption of the innate immune response of the mouse and adaptation of the virus to growth in CD13-expressing mouse cells¹⁰⁹. Mouse and rat ACE2 molecules are less-efficient receptors for SARS-CoV than is human ACE2 (REF 110), and the development of a useful murine model of SARS will probably require transgenic expression of human ACE2. However, by analogy with the mouse model of HCoV-229E infection, the development of a transgenic mouse might be only the first step towards developing a useful murine model. The knowledge gained from the study of an animal model will facilitate the development of specific therapies that are designed to minimize pulmonary disease and optimize the anti-SARS-CoV immune response, whether it be excessive (but not necessarily dysregulated), suppressed or both.

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Competing interests statement

The authors declare no competing financial interests.

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