IMMUNOPATHOGENESIS OF CORONAVIRUS INFECTIONS: IMPLICATIONS FOR SARS

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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 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...
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 HAEMOPOAGOCYTOSIS\(^5\). 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 death\(^6\) (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 EPITOPE 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
The severe-acute-respiratory-syndrome coronavirus genome and virion.

**a** SARS-CoV genome

![SARS-CoV genome diagram](image)

**b** SARS-CoV virion

![SARS-CoV virion diagram](image)

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 tissue\textsuperscript{35}. The murine coronavirus MHV-3 also infects and destroys lymphocytes, thereby facilitating viral replication and persistence\textsuperscript{36}. SARS-CoV-infected lymphocytes, similar to FIPV-infected macrophages in domestic cats, might transport the virus to distant organs, resulting in systemic infection\textsuperscript{37}. Macrophages, both infected and uninfected, are detected in large numbers in the lungs of patients who died as a result of SARS\textsuperscript{22,25,29}. Although infected macrophages have been found \textit{in vivo}, SARS-CoV causes an abortive infection of these cells \textit{in vitro}\textsuperscript{30–32}. SARS-CoV also interferes with the initiation of the innate immune response by inhibiting the expression of interferon (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 \textit{in vitro}, after infection with SARS-CoV\textsuperscript{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\textsuperscript{33,35}; CXCL10 and CCL2 expression are also upregulated in the blood of patients with SARS\textsuperscript{36}. Expression of CXCL8 (also known as IL-8), which is an attractant for neutrophils, is also upregulated in the serum of patients with SARS\textsuperscript{36–37}. Consistent with a role for CXCL8 in pathogenesis, severe disease is associated with an increase in the number of neutrophils in the blood\textsuperscript{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-\(\beta\) and prostaglandin E\(_2\) — were detected in another study\textsuperscript{39}. 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 monocytes\textsuperscript{40}. The murine coronavirus, MHV, also induces p38MAPK activation, and inhibition of p38MAPK activation results in decreased production of infectious MHV\textsuperscript{41}. 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 cells\textsuperscript{42}.

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**

**Macrople 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 populations\textsuperscript{43}. 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 \textit{in vitro} than do avirulent strains of FECV\textsuperscript{44}. 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 colleagues\textsuperscript{45} 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 disease\textsuperscript{46}.

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 \textit{in vitro}\textsuperscript{47,48} (Fig. 3a,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 node\textsuperscript{49}. 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\textsuperscript{2,46,47}. So, lymphoid-cell depletion is probably mediated by a soluble factor. In support of this idea,
conditioned media from peritoneal or splenic cells that were isolated from infected domestic cats and cultured in vitro induced the apoptosis of T cells. 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 FIP. 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 response.

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 detected11,12. 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 hypergammaglobulinemia. 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 Fcy receptors (receptors for IgG) (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 FIP. Antigen–antibody-complex formation and its consequences occur late in the course of disease and are predictive of a poor outcome. 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. This syndrome does not develop in FECV-infected domestic cats, possibly reflecting the ability of FIPV to replicate more efficiently in macrophages.

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 FcyRs (receptors for IgG). Some evidence indicates that this process augments the normal spike–CD13 interaction. After binding of the opsonized virions to FcyRs, 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; Tc2 cell, T helper 2 cell; TNF, tumour-necrosis factor.
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. In early studies, demyelination was thought to result from virus-mediated lysis of infected cells. 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. 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). 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.

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. 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 virus. Large influxes of activated macrophages and microglia are found in these areas of demyelination, indicating that these cells have a crucial role in the pathological process.

Both CD4+ and CD8+ T cells can mediate disease. 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. 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 activated (a process that is known as bystander activation).

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−/− mice. 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 antibody.

**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. 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 occurs 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 Fcy receptors (receptors for IgG), TCR, T-cell receptor.
Soluble factors also have a key role in MHV-JHM-induced demyelination. The cytokine IFN-γ is directly involved in clearance of virus from oligodendrocytes and is required for demyelination induced by CD8+ T cells but not by CD4+ T cells. 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.

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, 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 host. 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 disease. 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.

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 macrophages, 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 procoagulant molecule, fibrinogen-like protein 2 (FGL2; also known as fibroleukin/Fgl2).

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. Uproregulation 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 p38 MAPK and binding of the transcription factor hepatocyte nuclear factor 4α (HNF4α), which is constitutively expressed by macrophages, to the Fgl2 promoter. How the nucleocapsid protein induces HNF4α 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.
**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. 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. 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 host. 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-1β, IL-6 and CXCL8, which are secreted by infected epithelial cells in other respiratory-virus infections. 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 loss. Similarly, the early stages of renal disease are characterized by a massive infiltration of neutrophils. 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 persistent infection, with an interstitial lymphocytic nephritis in the kidneys. Similar to the MHV-infected CNS, lymphocytic infiltration and ongoing renal damage have been shown when viral loads are low or undetectable. 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, whereas in mice with MHV-mediated demyelination, clinical disease and myelin destruction increase as virus is cleared. 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. 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. 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. Consistent with this, increased concentrations of pro-inflammatory chemokines and other cytokines are present in most infected patients, and by analogy with other coronavirus infections, as well as ARDS (adult respiratory distress syndrome), 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 FIPV, and it occurs when tissue-culture cells are exposed to recombinant viral vectors that are coated with the SARS-CoV spike protein. However, this phenomenon has not been shown in most immunization studies, 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 SARS. Although SARS-CoV replicates in the lungs of mice, hamsters and domestic cats, these animals remain asymptomatic. 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. 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 cells. Mouse and rat ACE2 molecules are less-efficient receptors for SARS-CoV than is human ACE2, 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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Acknowledgements

The authors declare no competing financial interests.

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